2023

Fungicide Sensitivity in *P. Tritici-Repentis* Diverse Population and Phenotyping of Spelt Wheat for Multiple Diseases

Zunera Shabbir
South Dakota State University, zunera.shabbir@jacks.sdstate.edu

FUNGICIDE SENSITIVITY IN *P. TRITICI-REPENTIS* DIVERSE POPULATION AND PHENOTYPING OF SPELT WHEAT FOR MULTIPLE DISEASES

BY

ZUNERA SHABBIR

A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Plant Science

South Dakota State University

2023

DISSERTATION ACCEPTANCE PAGE
Zunera Shabbir

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Shaukat Ali
Advisor Date

David Wright
Department Head Date

Nicole Lounsbery, PhD
Director, Graduate School Date

This dissertation is dedicated to my dear father Mr. Rana Gulzar Ahmed Shabbir (late), my dear mother Rani Shabana Shabbir, my most supportive husband Muhammad Wahab Yasir and my beloved kids Zarwa and Zamin.

ACKNOWLEDGEMENTS

First, I would like to say thanks to the creator of this universe who has everything in control and who gave me courage and patience, the Almighty God. He who led me to this path while guarding me throughout this journey. I would like to express my appreciation to all the people who have been a big support system throughout this journey with me.

I would like to thank my esteemed supervisor Dr. Shaukat Ali for his invaluable supervision, support and help during my PhD degree. I am incredibly grateful to him for taking me as his graduate student and giving me an opportunity to work in a small grains plant pathology lab. My Ph. D. would not have been possible without his support, help, encouragement, and continuous guidance. He helped me in every feasible way he could and has always cared about me. I truly respect his hard work and considerate nature. I have learned all the pathology work from him which will help me in the future. My gratitude extends to the Higher Education Commission, Pakistan and South Dakota Wheat Commission, SD for the funding opportunity to undertake my studies at the Department of Agronomy, Horticulture and Plant Science at South Dakota State University. Additionally, I would like to express gratitude to Dr. Febina Mathew for her treasured support which was influential in shaping my experimental methods and critiquing my results. I also thank Dr. Sunish Sehgal for his mentorship and support during my research. I would also like to say thanks to Dr. Timothy Hansen for being my graduate faculty representative and attending all the committee meetings and exams.

A special thanks to all instructors/faculty members at South Dakota State University, especially Dr. Marie Langham for investing her enormous efforts and precious time to shape me academically. Also, I appreciate SDSU Plant Science staff for all their help and

support to accomplish this work. I would like to thank Dr Liu Zhaohui at North Dakota State University (NDSU) for giving me training for the molecular part of this study and allowing me to work in his laboratory. I would also like to thank Mr. Gongjun for his help and guidance in his laboratory at NDSU.

I would like to thank all my fellows, colleagues, and friends – Ruchika, Navreet, Harsimar, Jyoti, Jinfeng, and Rami Al-Tameemi for a cherished time spent together in the graduate area, and in social settings. I owe a big thanks to all the people from whom I have learned as little as a single word.

I would like to say a special thanks to one of the Pakistani family living here in Brookings who always treated me as a member of their family and have always been supportive and helpful. Thanks to brother Mubashir and sister Hajra and their lovely kids for making me a part of their family here abroad when I was away from my family.

My appreciation also goes out to my family for their encouragement and support all through my studies. A big thanks to my mother for always loving me, believing in me, and sending me prayers all the time. My sisters, my brothers, and all the family members who have been connected via phone calls give me courage and hope. I owe a big thanks to my husband Muhammad Wahab Yasir who have been with me in all the tough times and always believed in me. Thanks for your unconditional love and support. And finally, our little bundle of joy, my daughter Zarwa who has always put a smile on my face. Though she really gave us tough days and nights during this time, but at the end it's all worth it. Thanks for coming into our life and making us complete, Zarwa.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

FUNGICIDE SENSITIVITY IN *P. TRITICI-REPENTIS* DIVERSE POPULATION AND PHENOTYPING OF SPELT WHEAT FOR MULTIPLE DISEASES

ZUNERA SHABBIR

2023

Pyraclostrobin, a QoI fungicide, is extensively used in the management of fungal leaf spot diseases. In this study, 215 *P. tritici-repentis* isolates collected from wheat fields in South Dakota were evaluated for their sensitivity to Pyraclostrobin. Of 215 isolates, 48 isolates (22%) exhibited insensitivity based on G143A mutation and EC_{50} values using spore germination assay. Our results indicate reduced sensitivity to insensitivity to pyraclostrobin in some isolates suggesting regular monitoring of the *P. tritici-repentis* population to QoI fungicides is essential to track the evolution of insensitive population. Spelt wheat (*Triticum spelta* L.) is an ancient cereal that is gaining small grain growers' attention under an organic setup due to its high protein content, high fiber, and other health benefits. In this study, two hundred-eight spelt accessions from twenty countries were screened against leaf spot diseases and leaf rust at the seedling stage in the greenhouse. The genotypes exhibited varying responses ranging from susceptible to moderately susceptible, moderately resistant, and resistant. Spelt accessions resistant to leaf rust and leaf spots will be further evaluated for the agronomic traits in the field, and results will be shared with the growers to assist in selection of superior spelt genotypes. ToxA, a necrotrophic effector has been identified from the fungus *Pyrenophora tritici-repentis* that causes tan spot and was later identified from *Parastagonospora nodorum and P. avenaria*

tritici. Recently, the *ToxA* gene has been identified in the fungus *Bipolaris sorokiniana* causing spot blotch in Australia and the winter wheat region of southcentral Texas. In this study, we have tested if *B. sorokiniana* population of South Dakota is having *ToxA* gene (BS *ToxA*) like *P. tritici-repentis* and *P. nodorum*. 53.5% (n=68) of 127 isolates amplified the *ToxA* gene. Further, the culture filtrates of isolates with the *ToxA* gene produced necrosis symptoms in ToxA sensitive wheat cultivars. Our results indicate that there is a diverse population of *B. sorokiniana* prevalent on wheat in South Dakota. To our knowledge, this is the first report of ToxA (BS ToxA) producing *B. sorokiniana* isolates in South Dakota.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the third staple food worldwide that provides one-fifth of the calories and 20% of the protein. It covered about 221.86 million hectares worldwide in 2020/2021, accounting for a total production of 775.82 million metric tons (MMT) (USDA 2020). In the United States, wheat was grown on 14.87 million hectares in 2020/2021 and the production was estimated to be 49.69 million metric tons. The United States Department of Agriculture (USDA) estimated world wheat production at 763.5 (MMT) in 2019/2020 projected to be 775.8 MMT in 2020/2021(USDA 2021). In the United States, wheat production was estimated at 52.6 MMT in 2019/2020 projected to be 49.7 MMT in 2020/2021. In 2020, the total wheat planted area in the United States has been estimated at 44.3 million acres, which is down 2 % from 2019 to 30.6 million acres. This represents the lowest area of wheat planted since records began in 1919 (USDA, 2020).

Spelt wheat (*Triticum spelta* L.) was a major cereal for centuries in Europe before it was replaced by higher yielding wheat varieties (Riesen et al. 1986). For the last few years, spelt has started gaining the attention of plant breeders in the United States due to its superior performance under marginal growth conditions and agronomic advantages. Spelt is well adapted to cool and wet conditions of higher altitudes and therefore termed as a 'robust' cereal (Rüegger and Winzeler 1993). Spelt wheat differs from bread wheat in several morphological characters like height, long ears with tight glumes and brittle rachis. Spelt grains are long shaped with a high single grain weight (Winzeler et al. 1993). Spelt and wheat in general face many production challenges, among which viral, bacterial, and fungal diseases are the most prominent.

Several diseases affect the growth and production of wheat in various parts of the world. In the USA, several bacterial, viral, and fungal diseases occur in wheat growing areas. Among them, fungal diseases are the most commonly occurring. Favorable conditions (temperature, humidity etc.) give rise to many fungal diseases including stripe and leaf rust, Septoria leaf spot, tan spot, spot blotch and Fusarium head blight. These fungal disease can be devastating and also damage plants and crops, resulting in yield losses and food production (Almeida et al. 2019). Plant pathologists and breeders are making efforts to screen new breeding materials for sources of resistance to fungal diseases as global food security could be compromised by these fungal diseases in wheat.

For the management of tan spot, wheat varieties with moderate resistance to tan spot are recommended along with good agronomic practices and use of fungicides (SDSU extension report 2018). Crop rotation to a non-host crop (other than wheatgrass, bromegrass, or rye) is also recommended and found to be a highly effective management strategy to control this disease. Also promoting residue decomposition reduces the inoculum. Fungicides are labeled for management of tan spot and should be applied according to the manufacturers protocol. Scout and consider factors such as varietal susceptibility, forecasted weather, length of rotation away from wheat, and yield potential prior to fungicide application.

P. tritici-repentis isolates produce one or more of the three host selective effectors (previously known as host-selective toxins =HSTs), which are Ptr ToxA, Ptr ToxB, and Ptr ToxC (Ciuffetti et al. 1998). Ptr ToxA and Ptr ToxB are proteinaceous while Ptr ToxC is not a protein, non-ionic molecule. Based on the reaction on differential lines, these isolates are classified into eight races. This reaction is determined by their

corresponding HST. There are four hexaploid wheat differential lines; Salamouni (resistant to all currently identified races and three toxins), Glenlea (necrotic reaction to Ptr ToxA; susceptible to race 1, 2, 7, and 8), 6B635 (chlorotic reaction to Ptr ToxC and susceptible to race 1, race 3, 6, and 8) and 6B622 (Chlorotic reaction to Ptr ToxB). Race 1 (Ptr ToxA and Ptr ToxC), Race 2 (Ptr ToxA), Race 3 (Ptr ToxC), Race 4 (none), Race 5 (Ptr ToxB), Race 6 (Ptr ToxB and Ptr ToxC), Race 7 (Ptr ToxA and Ptr ToxB), Race 8 (Ptr ToxA, Ptr ToxB, and Ptr ToxC).

The main objectives of the study are:

1. Characterization of *P. tritici-repentis* diverse population for fungicide sensitivity in South Dakota
2. Study the reaction of spelt wheat *(Triticum spelta* L.*)* germplasm against leaf spots and leaf rust.
3. Evaluation of presence of *ToxA* gene *(*BS *ToxA*) in South Dakota *Bipolaris sorokiniana* population

CHAPTER 1

Literature review

Spelt wheat:

Wheat is an annual grass belonging to the family *Poaceae* (*Gramineae*), tribe *Triticae*. The cultivated wheats presently are the diploid *T. monococcum* (Einkorn wheat; 2n=14, AA), the tetraploids *T. dicoccum* (emmer wheat) and *T. durum* (pasta wheat or hard wheat) (2n=28, AABB), and the hexaploid *T. aestivum* (soft wheat or bread wheat) and *T. spelta* (spelt) (2n=42, AABBDD). Around 95% of the wheat grown is bread wheat and the remaining 5% is pasta wheat. Pasta wheat is more adapted to the dry Mediterranean climate than bread wheat. Wheat species including spelt, einkorn, and emmer are still grown in Spain, Turkey, the Balkans, and the Indian subcontinent. Hulled wheats are called farro in Italy and spelt wheat still be grown in Europe, especially in Alpine areas (Arendt and Zannini 2013; Szabó and Hammer 1996).

Spelt wheat *(Triticum aestivum* ssp. *spelta*) is a hulled grain that belongs to the species of bread wheat (*Triticum aestivum* ssp. *aestivum*) (Escarnot et al. 2012). Spelt is getting popularity among users as it is considered as a “healthy alternative” to bread wheat. The processing and cultivation of spelt are comparatively expensive than other cereals because it does not give high yield. There are several nutritional and agronomic advantages of spelt wheat over common wheat which have brought attention to the researchers (Starzyńska-Janiszewska et al. 2019). Spelt is grown in organic farms because it has lower agronomic requirements as compared to bread wheat and can be grown in areas with less favorable soil and climatic conditions. The products made up of spelt wheat are suitable for those having wheat sensitivity as they are easily digestible, and they also have very good flavor and aroma (Frakolaki et al. 2018).

History of Spelt wheat:

Spelt is a hybrid of the tetraploid *T. dicoccum* (emmer wheat) and diploid *Aegilops tauschii* (goat grass). It has high carbohydrate content, which is why Romans called it the 'marching grain.' In ancient Rome, spelt was known to have been attributed to some ritualized significance in Roman weddings. Spelt was cultivated in different parts of Switzerland, Austria, Germany, France and the southern low countries in the middle ages (Bakels 2005). In the 9th century AD, spelt became a major crop in the Europe, because it is husked, more adaptable to cold climates and is also suitable for storage (Newfield 2013). Between the 12th and 19th century, spelt was one of the major cereals in Southern Germany, Austria, and Switzerland (Gradmann 1901). Spelt was introduced for the first time in the United States in the 1890s. Ohio grows about 10 times more spelt than any other state which is between 100,000 and 200,000 acres annually. The Ohio State University released a variety, named 'Champ' in 1986 (Ohio Agronomy Guide - 12th Edition. 1987). In the 20th century, spelt was replaced by bread wheat in almost all areas where it was still grown (Sugár et al. 2019). Spelt has become a common wheat substitute for making artisanal loaves of bread, several baking items, pastas, and flakes from the 21st century. According to (Longin and Würschum 2014) spelt has been grown on more than 100,000 ha in Europe, primarily in Southern Germany, Austria, and Switzerland, with a potential of growing market.

Genetic makeup of Spelt wheat:

Spelt wheat is a hexaploid wheat derived from a hybrid of the tetraploid *T. dicoccum* (emmer wheat) and diploid *Aegilops tauschii* (goat grass). The hexaploid wheat has been divided into two categories *aestivum* and *spelta* based on morphological spike

characteristics (Abrouk et al. 2018). The *Q* locus on wheat chromosome arm 5AL is a major contributor of spike morphology (Abrouk et al. 2018; Huskins 1946; Sears 1954; Simons et al. 2006). (Simons et al. 2006) reported that *Q* codes for an Arabidopsis *APETALA2 (AP2)* gene that impacts the rachis fragility, glume shape, and spike length. The dominant *Q* allele of bread wheat indicates higher transcript levels which gives rise to compact spikes with free-threshing seeds. In European spelt, the recessive *q* allele has been found. This *q* allele is primarily responsible for the pyramidal spikes with a brittle rachis and hulled grain in spelt (Luo et al. 2000). Both the alleles differ from each other by one polymorphism in the open reading frame and five conserved polymorphisms in the promoter region (Simons et al. 2006). Some spelt accessions from Asia have been reported to carry the *Q* allele also found in bread wheat (Luo et al. 2000) with which we can say that *Q* has a regulatory function and expression level of *Q* control the spike morphology, depends on the genetic background. In wheat the domestication-related spike characteristics are controlled by approximately 20 loci (Dvorak et al. 2012; Faris et al. 2014; Katkout et al. 2014). Based on the morphological or genetic differences the hexaploid wheat has been separated into subspecies.

Yet (Abrouk et al. 2018) reported a different situation for spelt. They performed whole-genome marker analysis which did not result in a separation of a common spelt from the bread wheat gene pool. Asian spelt was identical to bread wheat based on the molecular analysis, whereas the Central European and Iberian spelt formed two different groups (Abrouk et al. 2018).

Benefits of Spelt wheat:

Spelt wheat is a whole grain which has all three key edible parts, the bran, the germ, and the endosperm. It is protected by an inedible husk that protects the kernel from sunlight, pests, and diseases. As compared to bread wheat, it has more fiber and a higher concentration of minerals, including magnesium, iron, and zinc phosphorus and niacin (vitamin B-3) (Piergiovanni et al. 1997). Spelt is an excellent source of carbohydrates and dietary fiber. It is often used in Farro, a mixture of diverse types of wheat that is becoming increasingly popular in some parts of Europe and North America. The mild, nutty flavor makes it popular to use in place of rice in pilaf, risotto, and side dishes. In Germany and Austria, they use spelt flour to make bread and cakes (Future 50 Foods, pdf) German's call spelt "dinkle" and use the grain as animal feed, in bread, and for brewing. Ground spelt is used as an alternative to oats and barley in animal feed as its nutritional value is same as that of oats. In addition, spelt hulls have as much nutritional value as grain. De-hulled spelt can be consumed by humans and is a popular fiber source in Europe. In the past decade, the demand for spelt has increased significantly in the U.S. food market. Spelt is being used for a wide variety of products, from pasta and high fiber cereals to an alternative baking flour. There are several health benefits of spelt. Consuming spelt may improve the cholesterol levels, reduce blood pressure, aid in digestion, provide better heart health, help in weight management, and reduce risk of diabetes (Medical News Today article 2018). Spelt is not a cure for celiac diseases but it is good for people with gluten sensitivity, yet it still contains gluten. People experiencing any allergies to wheat have reported that they were able to tolerate spelt made products. Spelt is commonly used as a substitute for wheat flour in breads, pasta, cookies, crackers, breakfast cereal, cakes, muffins, mixes for breads, pancakes, and waffles, and in animal

feedstuffs. Having high protein content, spelt makes high quality bread. Europeans use spelt in the production of beer, gin, and vodka (Neeson et al. 2011).

Diseases in spelt wheat:

Spelt has large leaf mass, grows fast and vigorously, and if it is grown in fall, it can out-compete spring weeds. This makes it have potential to compete well against weeds. Spelt is believed to have a higher nutritive value in comparison with common wheat, due to its higher resistance to unfavorable environmental factors as well as lower fertilization and soil demands (Suchowilska et al. 2010). (Fernandez et al. 2014) reported low *P. nodorum* level in spelt wheat that concurred with a report by (Arseniuk et al. 1991). According to (Arseniuk et al. 1991) winter spelt was more resistant to *P. nodorum* than spring wheat. Singh et al. (2006) identified spelt wheat lines with resistance to the leaf spot pathogens including *Pyrenophora tritici-repentis, Phaeosphaeria norodum* and *Mycosphaerella graminicola*. Spelt wheat was found to have lower leaf spot severity and leaf spot severity was positively associated with common wheat and durum wheat when tested by Fernandez et al. 2014. With this we can say that spelt is thought to be less susceptible to most pests and diseases which affect common wheat while going through literature. However, these diseases can be reduced by crop rotation and not planting spelt after another cereal crop. Spelt has tolerance to different abiotic and biotic stresses while in a study by Chrpová et al., 2021 there was a high genetic variability found to FHB and grain DON contamination in both heritage and modern European spelt varieties and no variety was found to be resistant (Chrpová et al. 2021).

Besides several diseases spelt can still be somehow resistant to the pathogens as the spelt grain is tightly covered with the lemma and palea and this offers natural protection

against pests and diseases. This may make the species more resistant to pathogens (Kordan et al. 2007; Winnicki and Żuk-Gołaszewska 2018).

Importance of Spelt and potential use in the organic setup:

Spelt is a combination of several nutrients which are beneficial for health. It is a major source of vitamin B2, niacin, dietary fiber, and zinc. This combination of nutrients is good for migraine headache, atherosclerosis, and diabetes. As spelt is an excellent source of riboflavin (vitamin B2), an essential nutrient for energy production within cells, it can be a cure for migraine headache sufferers. Being rich in niacin, spelt can also be beneficial against cardiovascular risk factors. High fiber diet is recommended for diabetes patient thus spelt can also be useful to reduce or control diabetes levels (source: world's healthiest foods). Nowadays people are more interested and looking towards alternative foods which are grown under organic setup and have tremendous health benefits as well. Spelt is gaining small grain growers attention under an organic setup due to its high nutritional values and health benefits (Campbell 1997). Spelt has also been particularly important and helpful for livestock. In animal feed hull fiber is particularly useful for cattle as it aids in the digestion of the feed and reduces acidosis problems. Spelt increased the milk production in dairy cows when fed and spelt fed cows also had highest weight in a study (Ingalls et al. 1963).

Spelt gained popularity in the 20th century when people looked more towards the organic farming and health consciousness raised (Winnicki and Żuk-Gołaszewska 2018). As compared to bread wheat, spelt does not require intensive fertilization or pest protection which makes it more suitable for organic farming (Winnicki and Żuk-Gołaszewska 2018; Wolfe et al. 2008). Demand of spelt wheat has gradually increased in last 20 years due to

increasing scientific evidence of it being high in nutrition as well as more resistant to diseases (Wang et al. 2021). There is a huge demand for organically produced grains but challenges like poor yields and market irregularities are hindering the expansion of industry. Spelt crop has also been grazed by organic farmers during the growing season which may also reduce grain yields (Neeson et al. 2008). Yields of organically grown spelt are lower (2-3 tons) than that of bread wheat (4-5 tons) under ideal growing conditions (Neeson et al. 2011). Spelt can out-perform wheat under sub-optimal growing conditions and is better at utilizing nutrients when grown in a low input system. This has suggested that spelts plays a bigger role in Australian organic grain rotations (Neeson et al. 2008).

Leaf spot and leaf rust diseases of wheat:

Along with several biotic stresses, leaf spot diseases are a major production limiting factor to wheat. In 2019, it was reported that the estimated wheat yield loss range at a global level was 21.5% due to emerging pests and diseases (Savary et al. 2019). Out of 31 pest and pathogens reported in wheat, several fungal diseases like leaf rust, Fusarium head blight, Septoria leaf blotch, stripe rust, spot blotch, tan spot, and powdery mildew cause severe losses and also cause alterations in chemical properties and quality (Simón et al. 2021). Major fungal leaf spot diseases like tan spot, Stagonospora nodorum blotch (SNB), Septoria tritici blotch (STB), and Spot blotch are commonly occurring in the United States and reduce the yield. In North America, the complex of foliar diseases that includes tan spot caused by *P. tritici-repentis* (anamorph *Drechslera tritici-repentis*) has increased over the last several decades due to wheat culture in the same field year after year and a shift toward conservation tillage practices that leave crop residue on the soil

surface. In addition to tan spot, this complex includes diseases such as Septoria tritici blotch (*Mycosphaerella graminicola*) (asexual stage: *Septoria tritici*), spot blotch (*Cochliobolus sativus*) (asexual stage: *Bipolaris sorokiniana*), and Stagnospora nodorum blotch (*Phaeosphaeria nodorum*) (asexual stage: *Parastagonospora nodorum*) (Wegulo et al. 2011a).

In North Dakota, these diseases have the potential to reduce test weight and yield by 50 percent (McMullen and Adhikari 2009). Of these diseases, tan spot disease is the most common and important leaf spot disease in all wheat classes reported in South Dakota and in North Dakota. Tan spot is an important disease of wheat caused by necrotrophic fungus *P. tritici-repentis*. The disease occurs worldwide in all wheat grown regions and where other alternative susceptible host crops are grown. In spring and summer, the disease develops on wheat on both the upper and lower surfaces of leaves. The fungus also can infect wheat spikes and eventually the kernels, causing a disease known as red smudge. Red smudge symptoms are the result of prolonged wet periods and high humidity during kernel development (McMullen and Adhikari 2009).

On susceptible wheat genotypes, the fungus produces two different kinds of symptoms: necrosis and chlorosis (Lamari et al. 2003). Fungal isolates have been grouped into eight races based on their ability to induce necrosis and/or chlorosis (Lamari et al. 2003) on differentials Glenlea, 6B662, 6B365, and Salamouni. Out of these eight races, first five races observed in the USA (Ali et al. 2010; Ali and Francl 2003). SNB initially causes water soaked small chlorotic lesions on the lower leaves of the plant while initial symptoms of STB develop on the lower leaves as chlorotic streaks and expand into irregular brown lesions (McMullen and Adhikari 2009). Spot blotch caused by *Bipolaris*

sorokiniana (teleomorph *Cochliobolus sativus*) is one of the most concerning diseases. Additionally, the fungus can cause common root rot, seedling blight, and black point of wheat and barley (Acharya et al. 2011; Chowdhury et al. 2013; Kumar et al. 2002; McDonald et al. 2018; Sharma and Duveiller 2006; Wu et al. 2021). The morphological variability in *B. sorokiniana* isolates have been reported but not much information is available on its aggressiveness (Chand et al. 2003). *B. sorokiniana* has extensive distribution in wheat growing regions and it causes more severe symptoms in warmer and humid parts of the world (Chowdhury et al. 2013). The average losses in yield due to spot blotch reported by Duveiller et al. (2005) ranges between 17-55% (Duveiller et al. 2005). In South Asia, grain yield loss due to spot blotch was ranged from 25% to 43% in 2005 (Sharma and Duveiller 2006). In Nepal, under rice wheat cropping system, spot blight severity reported to be 100% and 70% in 2004 and 2005 respectively (Sharma et al. 2007). (Mehta 1998) reported that in severe conditions the pathogen can cause 100% yield losses in Latin America. Another leaf spot causing necrotrophic ascomycete pathogen *Parastagonospora nodorum* (teleomorph: *Phaeosphaeria nodorum*), previously known as *Septoria nodorum* and *Stagonospora nodorum* (teleomorph: *Leptosphaeria nodorum*) and causes Septoria nodorum blotch (SNB) on bread (*Triticum aestivum*) and durum (*Triticum turgidum*) wheat. The fungus produces necrotic spots with chlorotic halo on leaves called SNB and discoloration of the glume tissue on the spikes called glume blotch. High yield losses due to SNB go hand in hand with a reduction in the size, number, and quality of the grains. In contrast to other leaf spot pathogens, *P. nodorum* can infect the seed directly, reduces the germination rate of the seed and causes lesions

and damping of coleoptiles in the event of severe infection (Bennett et al. 2007; Hafez et al. 2020; Shah et al. 1995).

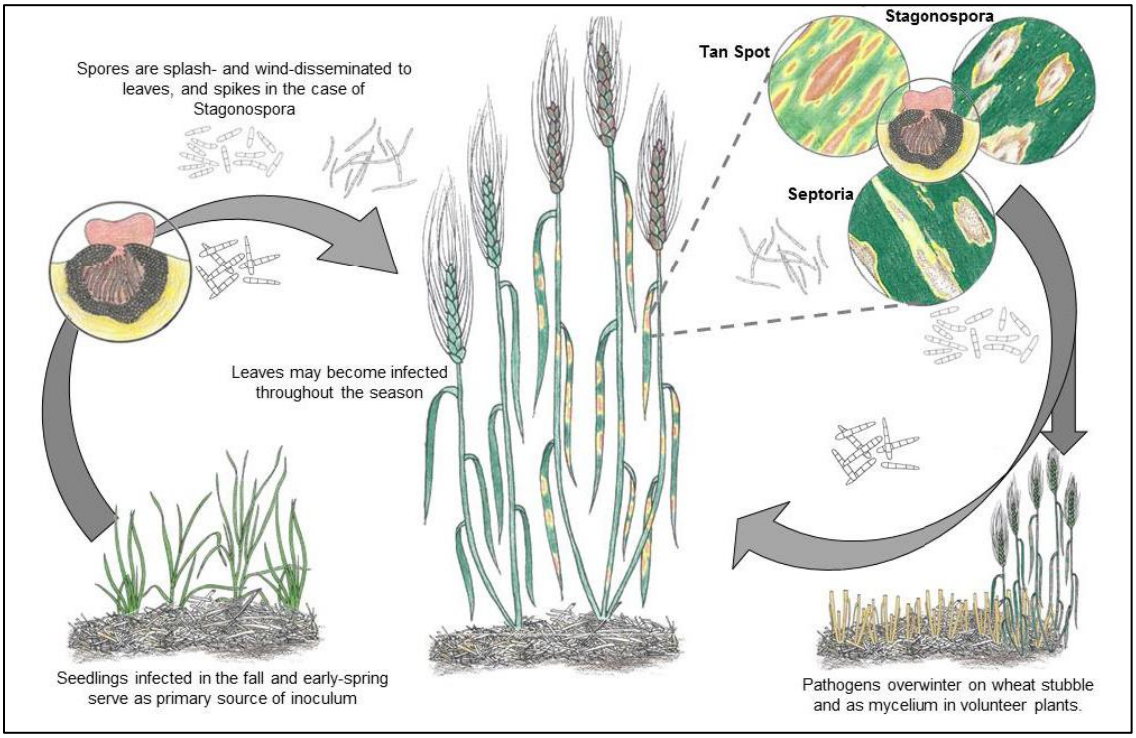

Figure 1-1: Leaf spot diseases cycle (https://ohioline.osu.edu/factsheet/plpath-cer-07)

Tan spot of wheat caused by *P. tritici- repentis*:

Tan spot caused by *Pyrenophora tritici-repentis* (Died.) is an economically important disease in the wheat-growing regions worldwide (Duveiller et al. 2005). Yield losses due to tan spot ranges from 10% to 15% but may reach up to 50% during epidemic years (Rees et al. 1981; Shabeer and Bockus 1988). This devastating disease of wheat in South Dakota can cause an estimated yield loss of about 5 percent which can go up to 30 percent in individual fields (Byamukama and Bugingo, 2018). The disease reduces total yield, grain weight, number of grains per head, total biomass, and grain quality (Fernandez et al. 1994; Shabeer and Bockus 1988).

Tan spot is more prevalent under no-tillage and wheat-on-wheat fields as the fungal pathogen survives on the wheat stubble (Conway 1996; Faris et al. 2013; Krupinsky et al. 2007; Krupinsky et al. 2002; McMullen and Lamey 1994). Wherever wheat is cultivated, tan spot is usually reported there (Faris et al. 2013). On susceptible wheat genotypes, the fungus produces two distinct symptoms in its host; necrosis and chlorosis (Lamari et al. 2003). *P. tritici-repentis* isolates are grouped into eight races based on their ability to induce necrosis and/or chlorosis (Lamari et al. 2003) on differentials Glenlea, 6B662, 6B365, and Salamouni.

Host range of *P. tritici-repentis*:

P. tritici-repentis infects all species of wheat including tetraploid and hexaploid species (Faris et al. 2013). It also infects other grass species along with wheat e.g. oats (*Avena sativa*), barley (*Hordeum vulgare*), rye (*Secale cereale*), triticale and many other grasses including *Agropyron fragile* subsp. *sibiricum* (Siberian wheatgrass), *Andropogon gerardii* var. *paucipilus* (sand bluestem), *Bromus biebersteinii* (meadow brome), *Festuca ovina* (sheep fescue), *Koeleria pyramidata* (June grass), *Schizachyrium scoparium* (little bluestem), *Setaria viridis* (green foxtail), *Stipa comata* (needle-and-thread), and *Thinopyrum ponticum* (tall wheatgrass) (Ali and Francl 2003; De Wolf et al. 1998; Krupinsky 1992).

History of tan spot disease:

The fungal pathogen *P. tritici-repentis* was described for the first time in 1823 and was considered a saprophyte in wheat crops around the world which causes minimal to acute spots (Faris et al. 2013; Hosford 1982). In 1900s, it was identified in the USA, Europe and Japan (Faris et al. 2013; Nisikado 1928). In 1902, *P. tritici-repentis* was formerly

described as *Pleospora trichostoma,* isolated from *Agropyron repens,* and later it was renamed as *Pleospora tritici-repentis* by Diedicke. In 1923, Drechsler used the name *Pyrenophora tritici-repentis* to describe the tan spot pathogen. In 1960s, Shoemaker (1959) and Wehmeyer (1949) argued whether to call the fungus *Pyrenophora* or pleospora. what. Later, they agreed on the nomenclature of the fungus and presently, the teleomorphic nomenclature of the fungus is *Pyrenophora tritici-repentis* and the anamorphic of the fungus is *Drechslera tritici-repentis* (De Wolf et al. 1998). In the 1970s, tan spot epidemic began in several countries including Canada, the USA, Australia, and the southern cone of America (Rees et al. 1981; Tekauz 1976). In central and South America, tan spot has been reported in Paraguay, Brazil, Bolivia, Uruguay, Mexico, Argentina (Kohli et al. 1992). In Central Asia, the disease was discovered in the 1980s has spread throughout Central Asia (Khasanov 1988). Friesen et al. (2006) reported that *P. tritici-repentis* has attained *ToxA* gene through horizontal gene transfer from *Stagonospora nodorum* which has allowed this pathogen to produce the host-selective toxin (HST) known as Ptr ToxA.

Disease cycle of *P. tritici-repentis*:

P. tritici repentis overwinter as psuedothecia which is a pinhead-sized structure develops on wheat straw. The pseudothecia are single-loculed fruiting bodies that discharge ascospores in the spring and early summer and these ascospores act as the primary source of inoculum. The ascospores have a cell containing 4-5 septa in the center (Ellis and Waller 1976). Favorable conditions like moisture and temperature ranging between 15°C to 28°C favor the primary infection by the ascospores gives 78% spore germination on susceptible varieties and 65% spore germination on resistant varieties. However, the

percentage rises to 95% after 6 hours for both varieties (Larez et al. 1986). Ascospores can move a short distance of nearly 6 inches with the help of wind and rain splash (De Wolf et al. 1998). After 1-2 weeks post-infection, the plants develop necrotic symptoms on the leaves. The primary infection rises an additional set of secondary spores known as conidia. The conidia can move at greater distance than the ascospores and with the help of wind dispersal and rain splash infect other wheat plants. Conditions like leaf wetness, high relative humidity, and temperatures above 10°C favors the secondary infection (Rees and Platz 1979; Sharma et al. 2003).

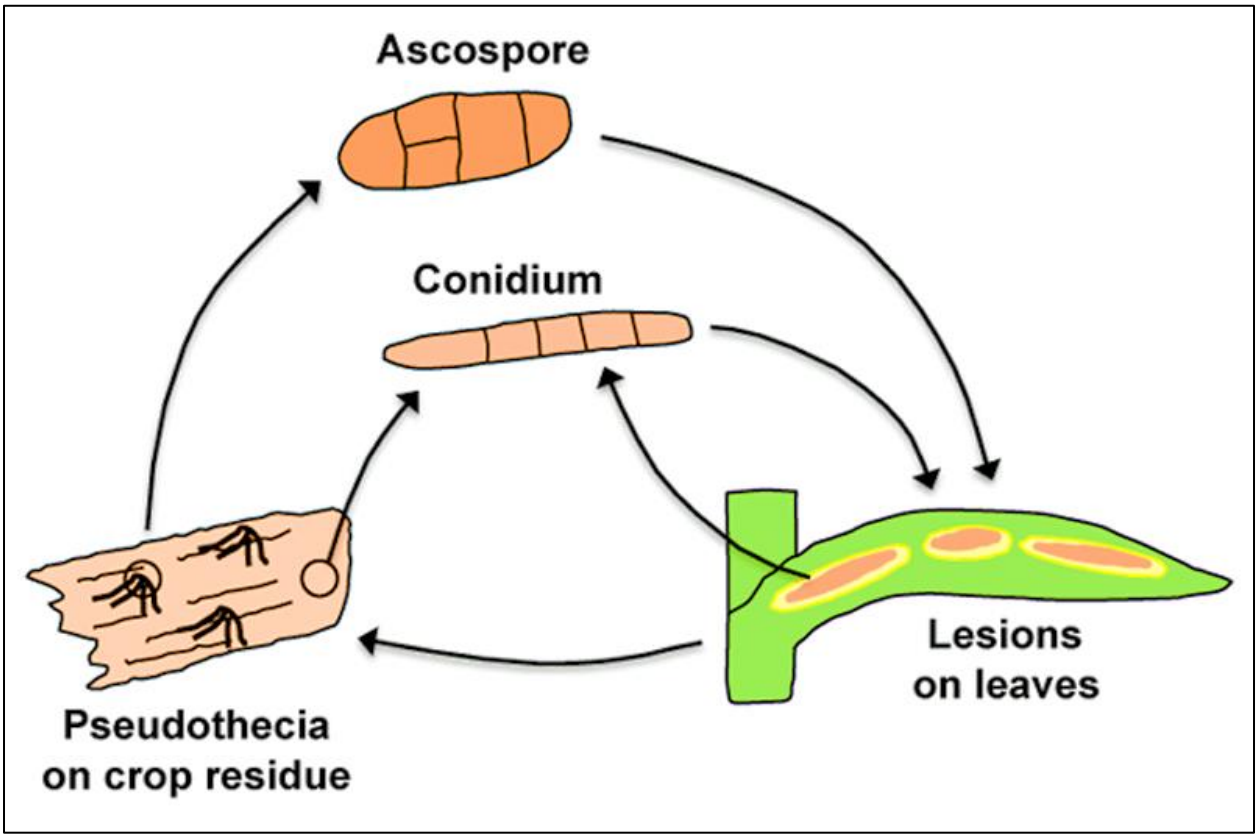

Figure 1-2: Disease cycle of tan spot

(https://www.apsnet.org/edcenter/disandpath/fungalasco/pdlessons/Pages/TanSpot.aspx)

Figure 1-3: (L-R) Conidia with and without germ tube

Tan spot infection and disease symptoms:

The conidia or ascospores gives rise to the germ tube which later produces the appressorium from which the penetration peg develops. This process requires favorable weather conditions including high humidity (78-100%) and above 10°C temperatures (Hosford et al. 1987). The fungus directly penetrates the epidermal cells due to the penetration peg in three hours. After the penetration, the penetration peg gives rise to the intracellular vesicle, but this is not always the case since the hyphae can penetrate through the epidermis. The hyphae expand to the mesophyll layers and damage the organelles which therefore develop into the distinct tan spot symptoms of brown lens shaped necrotic lesions surrounded by a chlorotic halo (De Wolf et al. 1998; Dushnicky et al. 1996; Hosford 1982; Larez et al. 1986).

(Lamari and Bernier 1989) described that the tan spot disease develops on both the lower and upper leaves with necrotic or chlorotic symptoms. Both the symptoms are under independent genetic control as each *P. tritici repentis* race gives different symptoms. The main diagnostic symptom of tan spot is the “eye shaped” dark spot located in the center of the yellow lesion which differentiates it from other wheat fungal leaf spotting diseases like Septoria tritici blotch and Stagonospora nodorum blotch (De Wolf et al. 1998;

Lamari and Bernier 1989). The disease can cause reduction in the yield as it reduces the photosynthetic area thus affecting grain quality and quantity due to reduced grain filling, lower test weight, kernel shriveling, and reduced kernel numbers per head (De Wolf et al. 1998; Shabeer and Bockus 1988). *P. tritici repentis* also infects the seed in the same way as for leaves (De Wolf et al. 1998). During grain filling period, it causes grain red smudge symptom. Seeds from infected spikelet have a reddish discoloration symptom (red smudge) (Bergstrom and Schilder 1998; Fernandez et al. 1994). This suggests that the fungus can be seed-borne (source of inoculum) but in the Great Plains, the disease is mostly stubble-borne.

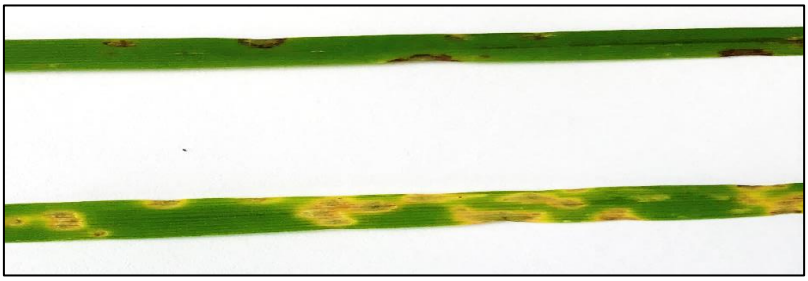

Figure 1-4: Wheat leaves with resistant reaction (top) showing the presence of small brown to black spots without chlorosis or tan necrosis and susceptible (bottom) showing well-defined lesion borders and the small brown to black spots (necrotic lesions surrounded by the chlorotic halo.

Tan spot management:

Tan spot, caused by a homothallic ascomycete parasitic fungus *P. tritici- repentis (Ptr)* (Died) Shoem., is economically important disease. The disease can cause yield losses ranges from 5 - 55% (Hosford 1982; Shabeer and Bockus 1988; Singh et al. 2007). This devastating disease of wheat in South Dakota can cause an estimated yield loss of about 5 percent which can go up to 30 percent in individual fields (Byamukama and Bugingo,

2018). For the management of tan spot, stubble management, fungicide application, crop rotations, and use of resistant cultivars is recommended.

Spot blotch of wheat caused by *Bipolaris sorokiniana*:

Spot blotch caused by *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*) is one of the most concerning diseases in wheat. The fungus is also a causal agent of common root rot, seedling blight, and black point of wheat and barley (Acharya et al. 2011; Chowdhury et al. 2013; Kumar et al. 2002; McDonald et al. 2018; Sharma and Duveiller 2006; Wu et al. 2021). The morphological and physiological variability in *B. sorokiniana* isolates have been reported but not much information is available on its aggressiveness (Chand et al. 2003). *B. sorokiniana* has extensive distribution in wheat growing regions and it causes more severe symptoms in warmer and humid parts of the world (Chowdhury et al. 2013). The average losses in yield due to spot blotch ranges between 15-20% on average (Acharya et al. 2011) but in susceptible genotypes it can go up. Mehta (1998) reported that in severe conditions the pathogen can cause 100% yield losses in Latin America. In *Bipolaris*, the conidia look fucoid, straight, or curved and germinating by one germ tube from each end (bipolar germination). This specific kind of conidial germination was one of the major reasons grouping it into new genera " *Bipolaris*" (Shoemaker 1959).

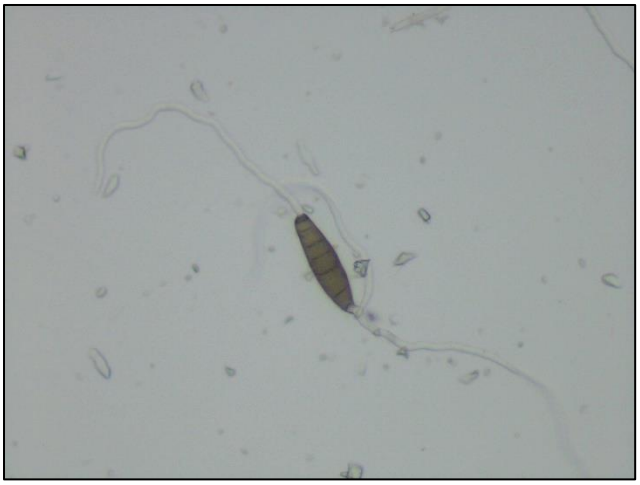

Figure 1-5: *B. sorokiniana* conidia germination having one germ tube at each end (bipolar germination)

Host range and symptoms of spot blotch:

The pathogen affects wheat and barley, but it can also affect a variety of hosts including several wild and cultivated Poaceae members. Rye and oats are rarely infected by this pathogen (Kumar et al. 2002; Steffenson et al. 2014; Zillinsky 1983). During initial infection, the disease is generally characterized by small dark brown lesions extending to1-2 mm long without chlorotic margin (Chand et al. 2003; Duveiller and Garcia Altamirano 2000). Dark brown necrotic lesions on roots, crowns, and leaves can be seen on susceptible genotypes whereas resistant genotypes show a small necrotic dark spot. The susceptible genotypes develop a distinct oval to elongated light to dark brown blotches that extend and merge quickly and kills the leaves (Chand et al. 2003; Duveiller and Dubin 2002). Humid conditions act as a favorable environment for the pathogen to cause disease. The pathogen also causes diseases like common root rot, foot rot, and black point on grains of wheat (Chand et al. 2003; Duveiller and Dubin 2002; Duveiller and Garcia Altamirano 2000; Duveiller and Gilchrist 1994; Hudec and Muchová 2009).

Figure 1-6: Resistant genotype leaves (top two) showing small necrotic dark spots versus the susceptible genotype leaves (bottom two) showing elongated light to dark brown blotches

Distribution and yield loss caused by spot blotch:

Spot blotch (SB) is one of the destructive fungal diseases occurring everywhere wheat is grown and affect several small gains as well (Chowdhury et al. 2013; Duveiller and Dubin 2002; Duveiller and Garcia Altamirano 2000; Duveiller and Gilchrist 1994; Duveiller et al. 2005; Sharma and Duveiller 2006; Sharma et al. 2007). The pathogen has been distributed extensively yet is more damaging in warm and humid regions (Chowdhury et al. 2013). Average yield losses due to spot blotch are ranged between 15-20% and can go up to 70% in susceptible genotypes and in the field condition, the disease is more devastating when the plants are exposed to high temperature and long humid or fogy hours (Acharya et al. 2011; Gurung et al. 2012; Sharma and Duveiller 2006; Sharma et al. 2007; Sharma et al. 2003; Siddique et al. 2006; Wu et al. 2021). Mehta (1998) reported that in severe conditions the pathogen can cause 100% yield losses in Latin America.

Disease cycle of spot blotch:

B. sorokiniana is a saprophyte and survives primarily as thick-walled conidia (Acharya et al. 2011). The mycelium from infected seed, conidia in the soil, stubble or conidia on the kernel surface acts as the primary inoculum in transmitting the disease (Neupane et al. 2010). When the conidia are stable and the pathogen finds a favorable environment, the infection starts. The conidia attach to the plant tissue by excreting the mucilaginous substratum. The conidia germinate in between 4 and 6 hours after inoculation (Han et al. 2010). The fungus then penetrates the cell by forming an outgrowth penetration peg developed from a specialized structure called an appressorium or directly through stomata. The infection pegs pierces through the cuticle, epidermal and parenchyma cells in leaf tissue, and the outer and inner cortex of root tissue. Once it is established, the pathogen reproduces itself in the multiple cycles to causes multiple infections within the same season (Acharya et al. 2011; Han et al. 2010). Like several seed-transmitted diseases, spot blotch more severely damages lower leaves and progresses from the lower to upper parts of the plants (Acharya et al. 2011)

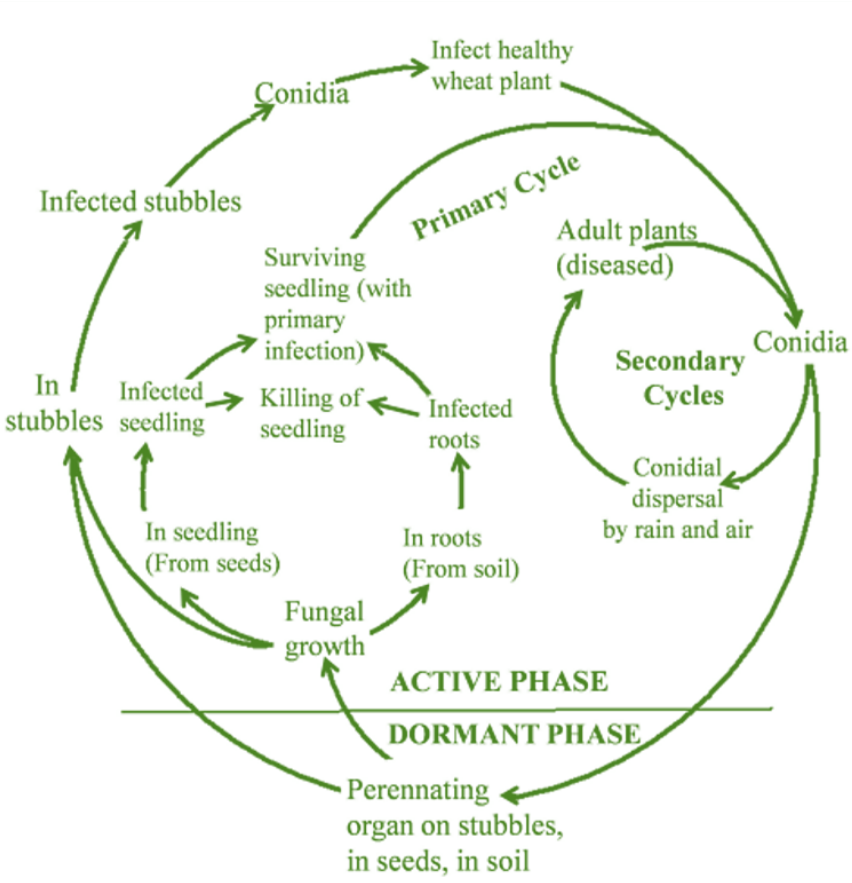

Figure 1-7: Spot blotch life cycle (Acharya et al. 2011)

Spot blotch management:

To manage spot blotch of wheat caused by *B. sorokiniana* several methods can be used like cultural practices, use of fungicide and host resistance. Developing wheat cultivars resistant to spot blotch is the most economical and sustainable disease management strategy (Chowdhury et al. 2013; Kumar et al. 2016; Vasistha et al. 2016). According to (Conner et al. 1996), rotation with two or more years of flax (*Linum usitatissimum*) as a break crop, reduced the amount of viable inoculum of *B. sorokiniana* in Canada where common root rot existed in the soil. Mixed wheat cultivars with different levels of resistance to spot blotch can also be used for the management of spot blotch (Sharma and

Dubin 1996). Avoiding zero tillage and stubble retaining is another measure taken by the farmers that can control the crown rot and common root rot disease (Kumar et al. 2002).

Leaf rust of wheat:

Leaf rust caused by *Puccinia triticina* Eriks is a significant yield limiting disease of wheat worldwide, especially in the U.S. Great Plains region. The fungus is heteroecious and requires a telial/uredinial host and an alternative (pycnial/aecial) host to complete the full life cycle (Bolton et al. 2008). Leaf rust infects the leaves and obstructs the photosynthetic efficiency of diseased plants. The preferred method to reduce leaf rust losses is genetic resistance and there are 80 leaf rust resistance (Lr) genes have been designated in wheat (McIntosh et al. 2014) and more identified genes are also added (McIntosh et al. 2019). Most Lr genes confer race-specific resistance in a gene-for-gene manner. Thus, the resistance against leaf rust can be qualitative, race specific which is called seedling resistance or/and it can be adult plant resistance which is polygenic and race nonspecific. The fungus *Puccinia triticina* can spread thousands of kilometers from the initial infection site through its urediniospores, and cause disease. Uredinia normally appear on the upper leaf surface and are brown in color and circular in shape. Leaf rust fungus is identified as an important pathogen in wheat production all over the world causing significant yield losses over large geographical areas (Kolmer 2005).

Yield losses caused by leaf rust:

Leaf rust causes significant yield losses all around the world where wheat is grown (Bolton et al. 2008). The losses ranged between 1 to 20% (USDA agricultural research services). During 2020, leaf rust was the second most important disease in Kansas state, causing yield loss of 2.8% to 13% in various parts of the state or 8.5 million bushels of

the winter wheat (Kansas cooperative plant disease survey report, 2020). Yield losses are attributed to a smaller number of kernels along with lower kernel weight (Bolton et al. 2008). Early infection can result in weak plants and poor root and tiller development.

Host Range of *Puccinia triticina*:

The primary host of *Puccinia triticina.* is wheat. The pathogen also infect wheat tetraploid species including durum wheat (*Triticum turgidum* subsp. *durum*), wild emmer wheat (*Triticum turgidum* subsp. *dicoccoides*), cultivated emmer wheat (*Triticum turgidum* subsp. *dicoccon*) and triticale (*X. triticosecale*) (Bolton et al. 2008). The sexual spore stages of *P. triticina* are hosted by *Thalictrum speciosissimum*. In North America, the fungal infections depend upon the asexual spores (Levine and Hildreth 1957)**.**

Life cycle of *Puccinia triticina*:

Wheat leaf rust caused by *Puccinia triticina* is macrocyclic; having five spore stages and heteroecious; having two different hosts. The primary host for this pathogen is wheat, where urediniospores, teliospores, and basidiospores are produced. The alternate host is *Thalictrum speciosissimum* which produces pycniospores and aeciospores. The primary infection on wheat is caused by aeciospores (from alternate host) or urediniospores (from volunteer grasses). After the infection has started, urediniospores start to develop which act as a source of secondary inoculum under the favorable conditions of 10-25ºC and enough water on the leaf surface. During unfavorable conditions, fungus produces teliospores, which act as dormant spores for overwintering. Via meiosis teliospores gives rise to basidiospores. Basidiospores are the final spores to be produced on primary host and are carried by wind to the nearby alternate host (*Thalictrum speciosissimum*). Infection leads to sexual spores - pycniospores (male spores) and receptive hyphae

(female spores), followed by fertilization and development of dikaryotic hyphae. This leads to aeciospores, which are wind born and once they infect the primary host (wheat) again, the life cycle of the fungus is complete (Bolton et al. 2008).

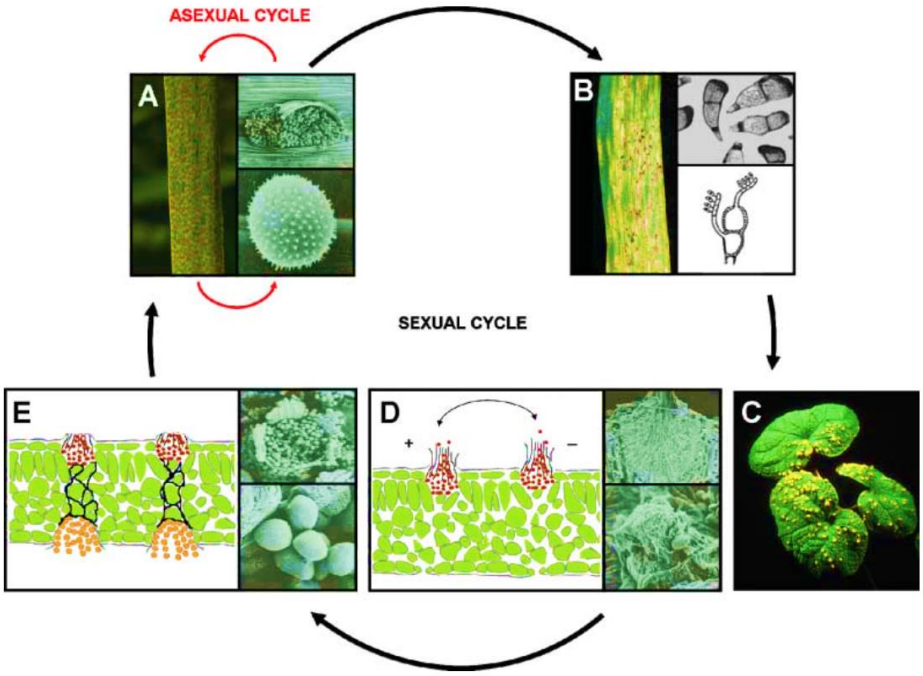

Figure 1-8: Life cycle of wheat leaf rust caused by *Puccinia triticina* (Bolton et al. 2008)

Types of resistance and resistance genes:

The genetic resistance given by rust resistance genes has been characterized as seedling resistance and adult plant resistance (APR). Seedling resistance is monogenic, race-specific, vertically controlled by major genes and is hypersensitive in nature. On the contrary, adult plant resistance (APR) is polygenic, race-nonspecific, horizontally controlled by minor genes and non-hypersensitive, slow rusting or partial and durable in nature. However, most *Lr* genes confer major, seedling or race-specific resistance and follow the gene-for-gene concept, leading to a hypersensitive response (HR) or

programmed cell death. A small number of APR genes, such as *Lr34* and *Lr46*, are very important for breeding because they have been shown to confer durable, long-term resistance in different environments and against diverse fungus pathotypes (Aktar-Uz-Zaman et al. 2017). (Wu et al. 2020a) stated that more than 100 wheat *Lr* genes have been discovered till now and 78 of them have been named. Most of these resistance genes are effective through all wheat growth stages against leaf rust and are easy to identify in the seedling stage, known as all-stage resistance (ASR). The race-specific APR have hypersensitive reaction around the rust pathogen infection sites such as, *Lr12*, *Lr13*, *Lr22a*, *Lr22b*, *Lr35*, *Lr37*, *Lr48* and *Lr49* (Bansal et al. 2008; Hiebert et al. 2007; Seah et al. 2001). The non-race-specific APR is controlled by non-hypersensitive genes such as, *Lr34*, *Lr46*, *Lr67*, and *Lr68* (Dyck 1991; Herrera-Foessel et al. 2011; Herrera-Foessel et al. 2012; Singh et al. 1998).

Use of fungicide to control wheat diseases and fungicide resistance:

Fungicides, herbicides, and insecticides are all pesticides which are used in plant protection. A fungicide is a specific type of pesticide that controls fungal disease by inhibiting or killing the fungus causing the disease. The decision to apply fungicide to a crop depends on the cost of fungicide and the price of wheat. The fungicide must be present on the plant surface before the disease symptoms to ensure the effectiveness of fungicide (true only for non-systemic fungicide). The ratio of cost to benefit must be considered at the time of application of the fungicide, as low wheat prices or high application costs may counteract any benefit of fungicide even when disease levels are high (Wegulo et al. 2011b). Fungicide resistance occurs when a pathogen population has reduced sensitivity or is no longer sensitive to the fungicide that was used to control the

same pathogen. Rigorous use of fungicides has led to the insensitivity to many fungicide groups including benzimidazoles, DMI's, and strobilurins in diseases such as powdery mildew, scald (*Rhynchosporium secalis*) and net blotch (*Pyrenophora teres*) (Jorgensen 2008). Fungicide Resistance Action Committee (FRAC) has recommended reducing the exposure of pathogens to fungicides and avoiding the use of fungicides when pathogen populations are already well-established in a crop. Along with that, exchanging fungicides with different modes of action or mixing different fungicides and using the doses that are effective in killing the pathogen are also suggested to limit pathogen insensitivity to fungicides. To avoid fungicide insensitivity, it is important to monitor the pathogen population regularly. Monitoring is especially important for new chemical groups to determine baseline sensitivity to find changes in pathogen sensitivity as soon as they appear and help in the development of anti-resistance management strategies. If field resistance is known to one member of the fungicide group, then cross resistance to other fungicide group members will be present (FRAC 2021). Degree of cross resistance can differ between group members and pathogen species or even within the species. Fungicide resistance is spreading widely, and the effectiveness of fungicides has also been affected. The extensive use of fungicides with the same chemical composition results in mutations to occur (Brent & Hollomon 2007). Fungicide resistance comes in dual nature: quantitative or polygenic and qualitative or monogenic resistance. Quantitative resistance affects several fungicides with different modes of actions and is a result of inadequate intracellular fungicide concentrations by enzymatic degradation of antifungal compounds, fungicide secretion by plasma membrane-localized efflux transporters and utilization of alternative metabolic pathways (Del Sorbo et al. 2000).

Qualitative resistance arises from mutations in genes encoding fungicide targets (Ishii et al. 2001).

Pathogen Insensitivity/resistance to fungicides:

To maintain high yields, wheat growers rely on the availability of effective fungicides (Jorgensen 2008). Rigorous use of fungicides has given rise to the development of insensitivity over time to several fungicide groups including benzimidazoles, DMI's, and strobilurins in diseases such as powdery mildew, scald (*Rhynchosporium secalis)* and net blotch (*Pyrenophora teres*). To control cereal diseases the mostly used fungicides are triazoles, strobilurins, morpholines, carboxamides, and chlorothalonil (Jorgensen 2008). Fungicide Resistance Action Committee (FRAC) has proposed a general principle to limit pathogen insensitivity to fungicides suggesting to reduce the exposure of pathogens to fungicides, avoiding the use of fungicides when pathogen populations are already well-established in a crop, alternating fungicides with different modes of action or mixing different fungicides to be applied, and using doses of the fungicide that are effective in killing the pathogen population as opposed to using multiple small doses that increase insensitive individuals in the population (FRAC 1998). These principles are usually difficult to follow but at the very least measures should be taken to limit the contact of fungicides with pathogen populations (Jorgensen 2008).

To avoid fungicide insensitivity, regular monitoring of pathogen populations is necessary. Monitoring is especially critical for new chemical groups to determine baseline sensitivity, so that changes in pathogen sensitivity can be discovered as soon as they appear, as well as aiding in the development of proper anti-resistance management strategies (Bayles et al. 2001).

Types of resistance:

Two different kinds of resistance occur when fungicides interact with plant pathogens; qualitative and quantitative. Qualitative resistance is disruptive or discrete and comes from the modification of a single major gene. It is mutation based and much higher concentrations/doses of fungicide are needed to control. Quantitative resistance is multi-step or continuous and occurs due to the usage of below threshold level fungicides (Brent & Holloman 2007).

Classification of fungicides:

The Fungicide Resistance Action Committee (FRAC) has developed a code of numbers and letters that can be used to distinguish the different fungicide groups based on their mode of action. This code is known as the FRAC Code and is included on the fungicide labels. These codes are: FRAC 1 including MBC fungicides (methyl benzimidazole carbamates). These can be used as both seed and foliar-applied products and inhibit tubulin biosynthesis. FRAC 3 includes DMI (Demethylation inhibitors) fungicides which are applied preventively or as early-infection treatments. They cause abnormal fungal growth and cell death. FRAC 7 consists of SDHI (Succinate dehydrogenase inhibitors) fungicides which are used as seed treatments or foliar fungicides and inhibit respiration in target fungi. FRAC 11 includes QoI (Quinone outside inhibitors) or strobilurins fungicides which inhibit the fungal mitochondrial respiration and can be used as seed treatments or foliar fungicides. All these fungicides have systemic properties and high resistance risk. FRAC M includes multi-site activity fungicides having broad spectrum activity and are approved for use on field crops include

inorganic compounds (copper and sulfur), dithiocarbamates (mancozeb), and chloronitriles (chlorothalonil).

Fungicide Groups and Mode of Action:

FRAC has determined the risk level of fungicide resistance developing to each of the fungicide groups. FRAC code 1, known as methyl benzimidazole carbamates (MBC), has a substantial risk of fungicide resistance development. FRAC code 3, known as demethylation inhibitors (DMI includes triazoles), has a medium risk. FRAC code 7, known as succinate dehydrogenase inhibitors (SDHI), has a medium to high risk. FRAC code 11, known as quinone outside inhibitors (Qol includes strobilurins), has a high risk. And FRAC code M5, known as choloronitriles, has a minimal risk. The FRAC code is used on most fungicide labels. The FRAC code refers to fungicides that have the same site-specific mode of action and share the same resistance problems across members of the group (cross-resistance). FRAC groups are currently numbered from 1 to 46 in order of their introduction to the marketplace. FRAC groups and mode of action subgroups are mostly the same. There are several groups of fungicides which are grouped based on similarities in chemical structure and mode of action. Site-specific fungicides target one point in one metabolic pathway in a pathogen or against a single enzyme or protein needed by the fungus. They have curative action and pose a high resistant risk. These include cell division, sterol synthesis, or nucleic acid (DNA and or RNA) synthesis. Their activity may be reduced by single or multiple-gene mutations. The MBC (benzimidazole), PA (phenylamide), and QoI (strobilurin) groups are subject to single-gene resistance and carry a high resistance risk. Other groups with site-specific modes of action include the Dicarboximides and DMIs (sterol demethylation inhibitors), but

resistance to these fungicides appears to involve slower shifts toward insensitivity because of multiple-gene involvement. Many of the site-specific fungicides also have systemic mobility. However, systemic mobility is not necessary for resistance development. Resistance problems have developed in the dicarboximide group and with dodine which are protectant fungicides. Multi-site fungicides interfere with many metabolic processes of the fungus and are usually protectant fungicides. Once taken up by fungal cells, multisite inhibitors act on processes such as general enzyme activity that disrupt numerous cell functions. Numerous mutations affecting many sites in the fungus would be necessary for resistance to develop. Typically, these fungicides inhibit spore germination and must be applied before infection occurs. Multi-site fungicides form a chemical barrier between the plant and fungus. The risk of resistance to these fungicides is low.

Sensitivity to Demethylation inhibitors (DMIs) or Triazoles:

Triazole fungicides are curative and systemic and move through the xylem of the plant. They slow down the pathogen growth by hindering sterol biosynthesis (Wegulo et al. 2012). They are effective against early fungal infection and are able to redistribute within the plant (Hewitt 1998). Triazoles have been used to manage Septoria leaf blotch in cereals but due to several point mutations in the pathogen population, the efficacy of many triazole fungicides such as epoxiconazole and prothioconazole have reduced significantly in different countries like France, U.K, and Denmark (Thygesen 2006). For more than 25 years triazoles have been used to control cereal diseases, especially in Europe during the 1980’s to control powdery mildew. Registration of a fungicide

propiconazole to control powdery mildew of barley was restricted in 1996, after the widespread of insensitivity to the fungicide (Jorgensen 2008).

Studies were performed by (Campbell and Crous 2002; Louw and Holz 1996; Peever and Milgroom 1992) and various other studies on fungicide sensitivity in *Pyrenophora teres* which is a causal agent of net blotch of barley and have demonstrated variation in genetic sensitivity to fungicide as well as differences in the efficacy of various triazole fungicides. (Machado et al. 2017) reported the sensitivity levels to triazoles for the first time in Brazil for a collection of Fusarium isolates from a regional survey of barley crops. In their study the *F. graminearum* isolates were less sensitive than *F. meridionale* to tebuconazole, but both were similarly sensitive to metconazole. (Spolti et al. 2014) demonstrated that most the *F. graminearum* isolates from the sampling population infecting wheat spikes in New York were sensitive to the triazoles and suggested the presence of an isolate that was significantly less sensitive to tebuconazole, highly aggressive, and toxigenic. As compared to other triazoles, Mefentrifluconazole is a new triazole with a novel isopropanol unit, more flexible in its structure and can bind even if the active site of the molecular target (CYP51 enzyme) is changed (Klink et al. 2021). No changes in sensitivity of *Z. tritici* field populations were observed between 1999 and 2020, while a significant shift towards decreasing sensitivity has been determined for the older triazole fungicides (Klink et al. 2021).

Sensitivity to Quinone outside inhibitors (QoI) or Strobilurins:

Strobilurins, or quinone outside inhibitors (QoI), function as respiration inhibitors, affecting the quinone binding site of the mitochondrial gene, cytochrome *b*, located in complex III (Sierotzki et al. 2007). QoI fungicides has the ability to bind with quinone

outside binding site (Qo) in cytochrome b of the fungi that inhibits mitochondrial respiration (Von Jagow and Link 1986). This prevents the electron transfer between cytochrome b and c1 which leads to energy deficiency in fungal cells by ceasing ATP production and that results in fungal death (Fisher and Meunier 2008; Hu et al. 2017). Most common QoIs used in agricultural disease management are pyraclostrobin and azoxystrobin (Bartlett et al. 2002) which are preventative as well as protective, acting to inhibit spore germination and early infection, and are considered to be locally systemic (Butzen et al. 2005). The evolution of population insensitivity to a QoI fungicide is due to selection pressure of the fungicide on the population, recurrent mutation, recombination and migration of the pathogen (Gisi et al. 2002). According to (Chang et al. 2007) in Canada the use of strobilurins has been limited due to fungicide insensitivity concern and they should only be used when rotated with other fungicides. QoI fungicides were introduced into the cereal market for the first time in 1996 (Sierotzki et al. 2007). QoI fungicide sensitivity was first time observed in Northern Germany in 1998 in wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*) (Heaney et al. 2000). QoI insensitivity in Septoria tritici blotch (*M. graminicola*) isolates in the UK and Ireland was reported in 2002 (Fraaije et al. 2003). In 2003 fungicide insensitivity in *P. tritici-repentis* isolates was reported for the first time (FRAC, 2002). By 2004, insensitivity in *P. tritici-repentis* was observed in field populations (Sierotzki et al. 2007). QoI fungicides has the ability to bind with quinone binding site (Qo) in cytochrome b of the fungi that inhibits mitochondrial respiration (Von Jagow and Link 1986). This prevents the electron transfer between cytochrome b and c1 which leads to energy deficiency in fungal cells by ceasing ATP production and that results in fungal death (Fisher and Meunier 2008; Hu et al.

2017). Different amino acid substitutions in the pathogen population provide different levels of insensitivity (Fisher and Meunier 2001). There are three mutations (G143A, G137R and F129L) found in the cytochrome b gene based on SNPs (single nucleotide polymorphisms). The mutations involved in providing resistance could be at either the G143 or F129 location (Patel et al. 2011). The most common mutation is the substitution of glycine to alanine at amino acid position 143 and is referred as G143A (Sierotzki et al. 2007). This substitution provides high levels of insensitivity to QoI fungicides as compared to any other mutations. F129L mutation is the one, in which an amino acid is changed from phenylalanine to leucine at position 129. This mutation was first time reported in *Pyricularia grisea* (Sacc) and *Pythium aphanidermatum* (Farman 2001; Sierotzki et al. 2007). The level of insensitivity given by F129L is lower than that of G143A. The G143A mutation gives complete insensitivity, making QoI fungicides useless in controlling the disease (Gisi et al. 2002). The F129L and G137R mutations give partial resistance, resulting in reduced sensitivity of the pathogen to QoI fungicides (Kim et al. 2003; Pasche et al. 2004; Sierotzki et al. 2007). QoI fungicides insensitivity have been reported in several crop pathogens across Europe and Asia including *Blumeria graminis* f. sp. *tritici (Sierotzki et al. 2000), Microdochium nivale (Walker et al. 2009), Microdochium majus (Walker et al. 2009), Mycosphaerella graminicola (Amand et al. 2003), Botrytis cinerea* (Banno et al. 2009), *Alternaria alternata* (Ma et al. 2003), *Colletotrichum graminicola* (Avila-Adame et al. 2003), *Pyricularia grisea (Kim et al. 2003; Vincelli and Dixon 2002), Pythium aphanidermatum, Podosphaera fusca, Pseudoperonospora cubensis* (Ishii et al. 2001), *Pyrenophora teres* (Sierotzki et al. 2007) and *Mycosphaerella fijiensis, Plasmopara viticola, Blumeria graminis* f. sp. *hordei*

and *Venturia inaequalis*. (Patel et al. 2011) have documented QoI insensitivity in *P. tritici-repentis* and *P. teres* where they reported four *P. tritici-repentis* isolates containing the GCT codon (G143A mutation) and one three isolates containing the TTA, TTG or CTC SNPs at F129L site. Two *P. teres* isolates also had the TTA and TTG SNPs.

Pyraclostrobin sensitivity:

Strobilurin sensitivity has already been reported in several pathogens. Sensitivity of *P. tritici-repentis* populations to pyraclostrobin has been reported by (Patel et al. 2012). Pyraclostrobin is the most used active ingredient in the United States and provide protection against leaf spot diseases for more than 7 years. In *P. tritici-repentis* isolates from Europe mutation of glycine 137 to arginine was also found. Both G137R and F129L mutations confer the same level of insensitivity to azoxystrobin in *P. tritici-repentis* (Patel et al. 2012; Sierotzki et al. 2007). Baseline sensitivity of pathogen *Botryosphaeria. dothidea* to pyraclostrobin has been assessed for 97 isolates collected in Shandong Province of China reporting that pyraclostrobin provided greater than 80% control efficacy against apple ring rot disease when applied as a therapeutic or preventive fungicide (Fan et al. 2019). In a study by (Liang et al. 2015), baseline sensitivity of pyraclostrobin was established based on effective concentration for 50% inhibition of mycelial growth (EC_{50}) values of 153 isolates of *Sclerotinia. sclerotiorum* collected from five provinces of China and toxicity of alternative oxidase inhibitor salicylhydroxamic acid (SHAM) to *S. sclerotiorum* was also determined. Sensitivity of 120 *Valsa. mali* isolates causing apple tree Valsa canker in China to pyraclostrobin was detected by (Feng et al. 2020) and showed a moderate risk to pyraclostrobin suggesting that it could be used as an alternative fungicide to control AVC in the field in China. (Ali et al. 2020)

reported majority of *Colletotrichum* isolates causing anthracnose fruit rot disease collected in 2019 were not inhibited by pyraclostrobin, suggesting a growing resistance issue with the QoI fungicides.

Literature cited

Abrouk, M., Stritt, C., Müller, T., Keller, B., Roulin, A. C., and Krattinger, S. G. 2018. High-throughput genotyping of the spelt gene pool reveals patterns of agricultural history in Europe. https://doi.org/10.1101/481424

Acharya, K., Dutta, A. K., and Pradhan, P. 2011. '*Bipolaris sorokiniana*'(Sacc.) Shoem.: The most destructive wheat fungal pathogen in the warmer areas. Australian Journal of Crop Science 5:1064-1071.

Aktar-Uz-Zaman, M., Tuhina-Khatun, M., Hanafi, M. M., and Sahebi, M. 2017. Genetic analysis of rust resistance genes in global wheat cultivars: an overview. Biotechnology & Biotechnological Equipment 31:431-445.

Ali, M. E., Hudson, O., Waliullah, S., Cook, J., and Brannen, P. M. 2020. Sensitivity of Colletotrichum isolates collected from strawberries in Georgia to pyraclostrobin, a quinone outside inhibitor (QoI) fungicide. Plant Health Progress 21:69-70.

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

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

Almeida, F., Rodrigues, M. L., and Coelho, C. 2019. The still underestimated problem of fungal diseases worldwide. Frontiers in Microbiology 10:214.

Amand, O., Calay, F., Coquillart, L., Legat, T., Bodson, B., Moreau, J.-M., and Maraite, H. 2003. First detection of resistance to QoI fungicides in Mycosphaerella graminicola on winter wheat in Belgium. Communications in Agricultural and Applied Biological Sciences 68:519-531.

Arendt, E., and Zannini, E. 2013. Wheat and other Triticum grains. Cereal grains for the food and beverage industries. Elsevier (1): pp.1-66.

Arseniuk, E., Fried, P., Winzeler, H., and Czembor, H. 1991. Comparison of resistance of triticale, wheat and spelt to Septoria nodorum blotch at the seedling and adult plant stages. Euphytica 55:43-48.

Avila-Adame, C., Olaya, G., and Köller, W. 2003. Characterization of *Colletotrichum graminicola* isolates resistant to strobilurin-related QoI fungicides. Plant Disease 87:1426-1432.

Bakels, C. C. 2005. Crops produced in the southern Netherlands and northern France during the early medieval period: a comparison. Vegetation History and Archaeobotany 14:394-399.

Banno, S., Yamashita, K., Fukumori, F., Okada, K., Uekusa, H., Takagaki, M., Kimura, M., and Fujimura, M. 2009. Characterization of QoI resistance in *Botrytis cinerea* and identification of two types of mitochondrial cytochrome b gene. Plant Pathology 58:120-129.

Bansal, U. K., Hayden, M. J., Venkata, B., Khanna, R., Saini, R., and Bariana, H. S. 2008. Genetic mapping of adult plant leaf rust resistance genes Lr48 and Lr49 in common wheat. Theoretical and Applied Genetics 117:307-312.

Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M., and Parr-Dobrzanski, B. 2002. The strobilurin fungicides. Pest Management Science: formerly Pesticide Science 58:649-662.

Bayles, R., Burnett, F., Napier, B., and Home Grown Cereals Authority , L. 2001. Designing fungicide programmes to minimise the risk of resistance in wheat and barley mildew and wheat yellow rust. HGCA Project Report.

Bennett, R. S., Milgroom, M. G., Sainudiin, R., Cunfer, B. M., and Bergstrom, G. C. 2007. Relative contribution of seed-transmitted inoculum to foliar populations of *Phaeosphaeria nodorum*. Phytopathology 97:584-591.

Bergstrom, G., and Schilder, A. 1998. Seed pathology of tan spot. Pages 364-368 in: Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot. Proc. Int. Workshop, CIMMYT, El Batan, Mexico.

Bolton, M. D., Kolmer, J. A., and Garvin, D. F. 2008. Wheat leaf rust caused by *Puccinia triticina*.. Molecular Plant Pathology 9:563-575.

Brent, K. J., and Holloman, D. W., 2007. Fungicide resistance in crop pathogens: How can it be managed? FRAC Monograph 1, 2nd edition, Brussels: FRAC. https://doi.org/D/1995/2537/1

Bugingo, C., Byamukama, E., and Ali, S. 2018. Tan Spot of Wheat. Retrieved 09/2018 https://extension.sdstate.edu/sites/default/files/2018-12/03-2008-2018.pdf

Butzen, S., Marcon, A., Mcinnes, B., and Schuh, W. 2005. Asian soybean rust: fungicide application technology. Crop Insights 15:5.

Campbell, G., and Crous, P. 2002. Fungicide sensitivity of South African net-and spot-type isolates of *Pyrenophora teres* to ergosterol biosynthesis inhibitors. Australasian Plant Pathology 31:151-155.

Campbell, K. G. 1997. Spelt: agronomy, genetics, and breeding. Plant Breeding Reviews 15:187-214.

Chand, R., Pandey, S., Singh, H., Kumar, S., and Joshi, A. 2003. Variability and its probable cause in natural populations of spot blotch pathogen *Bipolaris sorokiniana* of wheat (*T. aestivum* L.) in India Journal of Plant Diseases and Protection:27-35.

Chang, K., Ahmed, H., Hwang, S., Gossen, B., Strelkov, S., Blade, S., and Turnbull, G. 2007. Sensitivity of field populations of *Ascochyta rabiei* to chlorothalonil, mancozeb and pyraclostrobin fungicides and effect of strobilurin fungicides on the progress of ascochyta blight of chickpea. Canadian Journal of Plant Science 87:937-944.

Chowdhury, A. K., Singh, G., Tyagi, B. S., Ojha, A., Dhar, T., and Bhattacharya, P. M. 2013. Spot blotch disease of wheat–a new thrust area for sustaining productivity. Journal of Cereal Research 5.

Chrpová, J., Grausgruber, H., Weyermann, V., Buerstmayr, M., Palicová, J., Kozová, J., Trávníčková, M., Nguyen, Q. T., Amores, J. E. M., and Buerstmayr, H. 2021. Resistance of Winter Spelt Wheat [*Triticum aestivum* subsp. spelta (L.) Thell.] to Fusarium Head Blight. Frontiers in Plant Science 12, p. 661484.

Ciuffetti, L., Francl, L., Ballance, G., Bockus, W., Lamari, L., Meinhardt, S., and Rasmussen, J. 1998. Standardization of toxin nomenclature in the *Pyrenophora*

tritici-repentis/wheat interaction. Canadian Journal of Plant Pathology 20:421-424.

Conner, R. L., Duczek, L. J., Kozub, G. C., and Kuzyk, A. D. 1996. Influence of crop rotation on common root rot of wheat and barley. Canadian Journal of Plant Pathology 18:247-254.

Conway, K. E. 1996. An overview of the influence of sustainable agricultural systems on plant diseases. Crop protection 15:223-228.

De Wolf, E., Effertz, R., Ali, S., and Francl, L. 1998. Vistas of tan spot research. Canadian Journal of Plant Pathology. 20:349-444.

Del Sorbo, G., Schoonbeek, H.-j., and De Waard, M. A. 2000. Fungal transporters involved in efflux of natural toxic compounds and fungicides. Fungal Genetics and Biology 30:1-15.

Dushnicky, L., Ballance, G., Sumner, M., and MacGregor, A. 1996. Penetration and infection of susceptible and resistant wheat cultivars by a necrosis toxin-producing isolate of *Pyrenophora tritici-repentis*. Canadian Journal of Plant Pathology 18:392-402.

Duveiller, E., and Gilchrist, L. 1994. Production constraints due to *Bipolaris sorokiniana* in wheat: current situation and future prospects. in: Wheat in Warm Area, Rice-Wheat Farming Systems. Dinajpur (Bangladesh). 13-15 Feb 1993.

Duveiller, E., and Garcia Altamirano, I. 2000. Pathogenicity of *Bipolaris sorokiniana* isolates from wheat roots, leaves and grains in Mexico. Plant Pathology 49:235-242.

Duveiller, E., and Dubin, H. 2002. Helminthosporium leaf blights: spot blotch and tan spot. Bread Wheat: Improvement and Production, Plant Production and Protection Series 30:285-299.

Duveiller, E., Kandel, Y., Sharma, R., and Shrestha, S. 2005. Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. Phytopathology 95:248-256.

Dvorak, J., Deal, K. R., Luo, M.-C., You, F. M., von Borstel, K., and Dehghani, H. 2012. The origin of spelt and free-threshing hexaploid wheat. Journal of Heredity 103:426-441.

Dyck, P. 1991. Genetics of adult-plant leaf rust resistance in 'Chinese Spring'and 'Sturdy'wheats. Crop Science 31:309-311.

El Harrak, A., Mergoum, M., and Saadaoui, E. 1997. Major foliar diseases of triticale in Morocco. Pages 150-156 in: Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot. Proc. Int. Workshop, CIMMYT, El Batan, Mexico.

Ellis, M., and Waller, J. 1976. *Pyrenophora tritici repentis* (conidial state: *Drechslera tritici repentis*). CMI Descr Pathog Fungi Bact Commonw Mycol Inst.

Escarnot, E., Jacquemin, J.-M., Agneessens, R., and Paquot, M. 2012. Comparative study of the content and profiles of macronutrients in spelt and wheat, a review. Biotechnology, Agronomy, Society and Environment, 16(2):243-256.

Fan, K., Wang, J., Fu, L., Zhang, G. F., Wu, H. B., Feng, C., and Qu, J. L. 2019. Baseline sensitivity and control efficacy of pyraclostrobin against *Botryosphaeria dothidea* isolates in China. Plant disease 103:1458-1463.

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

Faris, J. D., Zhang, Z., and Chao, S. 2014. Map-based analysis of the tenacious glume gene Tg-B1 of wild emmer and its role in wheat domestication. Gene 542:198-208.

Farman, M. 2001. The molecular basis of field resistance to QoI fungicides in *Pyricularia grisea*. Phytopathology 91:S110.

Feng, H., Wang, S., Liu, Z., Miao, J., Zhou, M., and Huang, L. 2020. Baseline sensitivity and resistance risk assessment of *Valsa mali* to pyraclostrobin. Phytopathology Research 2:1-8.

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

Fernandez, M., Fox, S., Hucl, P., and Singh, A. 2014. Leaf spotting reaction of spring common, durum and spelt wheat, and Kamut under organic management in western Canada. Canadian Journal of Plant Science 94:929-935.

Fisher, N., and Meunier, B. 2001. Effects of mutations in mitochondrial cytochrome b in yeast and man: deficiency, compensation and disease. European Journal of Biochemistry 268:1155-1162.

Fisher, N., and Meunier, B. 2008. Molecular basis of resistance to cytochrome bc 1 inhibitors. FEMS yeast research 8:183-192.

Fraaije, B., Lucas, J., Clark, W., and Burnett, F. 2003. QoI resistance development in populations of cereal pathogens in the UK. Pages 689-694 in: The BCPC

International Congress: Crop Science and Technology, Volumes 1 and 2. Proceedings of an international congress held at the SECC, Glasgow, Scotland, UK, 10-12 November 2003 British Crop Protection Council.

Frakolaki, G., Giannou, V., Topakas, E., and Tzia, C. 2018. Chemical characterization and breadmaking potential of spelt versus wheat flour. Journal of Cereal Science 79:50-56.

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

Fungicide Resistance Action Committee. 1998. 1998 status report and recommended fungicide resistance management guidelines. FRAC: Brussels.

Fungicide Resistance Action Committee. 2002. http://www.frac.info/frac/index.htm [12 February 2014].

Fungicide Resistance Action Committee (FRAC). 2021. FRAC Code List © * 2021: Fungal control agents sorted by cross resistance pattern and mode of action. https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2021--final.pdf?sfvrsn=f7ec499a_2

Gisi, U., Sierotzki, H., Cook, A., and McCaffery, A. 2002. Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides. Pest Management Science 58:859-867.

Gradmann, R. 1901. Der Dinkel und die Alamannen. Württembergisches Jahrb Stat Landeskd 1901:103-158.

Gurung, S., Sharma, R. C., Duveiller, E., and Shrestha, S. M. 2012. Comparative analyses of spot blotch and tan spot epidemics on wheat under optimum and late sowing period in South Asia. European journal of plant pathology 134:257-266.

Hafez, M., Gourlie, R., Despins, T., Turkington, T. K., Friesen, T. L., and Aboukhaddour, R. 2020. *Parastagonospora nodorum* and related species in Western Canada: Genetic variability and effector genes. Phytopathology® 110:1946-1958.

Han, Q., Huang, L., Buchenauer, H., Wang, C., and Kang, Z. 2010. Cytological study of wheat spike infection by *Bipolaris sorokiniana*. Journal of phytopathology 158:22-29.

Heaney, S., Hall, A., Davies, S., and Olaya, G. 2000. Resistance to fungicides in the Qol-STAR cross-resistance group: current perspectives. Pages 755-762 in: The BCPC Conference: Pests and diseases, Volume 2. Proceedings of an international conference held at the Brighton Hilton Metropole Hotel, Brighton, UK, 13-16 November 2000 British Crop Protection Council.

Herrera-Foessel, S. A., Lagudah, E. S., Huerta-Espino, J., Hayden, M. J., Bariana, H. S., Singh, D., and Singh, R. P. 2011. New slow-rusting leaf rust and stripe rust resistance genes Lr67 and Yr46 in wheat are pleiotropic or closely linked. Theoretical and Applied Genetics 122:239-249.

Herrera-Foessel, S. A., Singh, R. P., Huerta-Espino, J., Rosewarne, G. M., Periyannan, S. K., Viccars, L., Calvo-Salazar, V., Lan, C., and Lagudah, E. S. 2012. Lr68: a new gene conferring slow rusting resistance to leaf rust in wheat. Theoretical and Applied Genetics 124:1475-1486.

Hewitt, H. G. 1998. Fungicides in crop protection. Cab International. pp 221.

Hiebert, C. W., Thomas, J. B., Somers, D. J., McCallum, B. D., and Fox, S. L. 2007. Microsatellite mapping of adult-plant leaf rust resistance gene Lr22a in wheat. Theoretical and Applied Genetics 115:877-884.

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

Hosford, R. M. 1982. Tan Spot of Wheat and Related Diseases Workshop, July 14-15, 1981, North Dakota State University, Fargo, North Dakota. North Dakota Agricultural Experiment Station, North Dakota State University.

Hu, X., Dai, D., Wang, H., and Zhang, C. 2017. Rapid on-site evaluation of the development of resistance to quinone outside inhibitors in *Botrytis cinerea*. Scientific reports 7:1-9.

Hudec, K., and Muchová, D. 2009. Correlation between black point symptoms and fungal infestation and seedling viability of wheat kernels. Plant Protection Science 44:138-146.

Huskins, C. L. 1946. Fatuoid, speltoid and related mutations of oats and wheat. The Botanical Review 12:457-514.

Ingalls, J., Morgan, D., Thomas, J., and Huffman, C. 1963. Nutritive value of spelt (*Triticum Sativum* Spelta) for dairy cattle. Journal of Dairy Science 46:1085-1088.

Ishii, H., Fraaije, B., Sugiyama, T., Noguchi, K., Nishimura, K., Takeda, T., Amano, T., and Hollomon, D. 2001. Occurrence and molecular characterization of strobilurin

resistance in cucumber powdery mildew and downy mildew. Phytopathology 91:1166-1171.

Jorgensen, L. N. 2008. Resistance situation with fungicides in cereals. Zemdirbyste-Agriculture 95:373-378.

Kansas cooperative plant disease survey report. 2020. Preliminary 2020 Kansas wheat disease loss estimates. https://agriculture.ks.gov/docs/default-source/pp-plant-disease/wheat-disease-report-2020.pdf?sfvrsn=f90590c1_0

Katkout, M., Kishii, M., Kawaura, K., Mishina, K., Sakuma, S., Umeda, K., Takumi, S., Nitta, M., Nasuda, S., and Ogihara, Y. 2014. QTL analysis of genetic loci affecting domestication-related spike characters in common wheat. Genes & genetic systems 89:121-131.

Khasanov, B. 1988. Yellow leaf spot in cereals caused by *Pyrenophora tritici-repentis*(DIED) Drechs. Mikologiya i fitopatologiya 22:78-83.

Kim, Y.-S., Dixon, E. W., Vincelli, P., and Farman, M. L. 2003. Field resistance to strobilurin (QoI) fungicides in *Pyricularia grisea* caused by mutations in the mitochondrial cytochrome b gene. Phytopathology 93:891-900.

Klink, H., Verreet, J.-A., Hasler, M., and Birr, T. 2021. Will Triazoles Still Be of Importance in Disease Control of *Zymoseptoria tritici* in the Future? Agronomy 11:933.

Kohli, M., Mehta, Y., and Diaz de Ackermann, M. 1992. Spread of Tan spot in the Southern Cone region of South America.

Kolmer, J. A. 2005. Tracking wheat rust on a continental scale. Current opinion in plant biology 8:441-449.

Kordan, B., Laszczak-Dawid, A., Nietupski, M., and Zuk-Golaszewska, K. 2007. Wplyw formy przechowywania pszenicy orkisz [*Triticum spelta* L.] na rozwoj wolka zbozowego [Sitophilus granarius L.]. Progress in Plant Protection 47:263-266.

Krupinsky, J. 1992. Grass hosts of *Pyrenophora tritici-repentis*. Plant disease 76:92-95.

Krupinsky, J., Halvorson, A., Tanaka, D., and Merrill, S. 2007. Nitrogen and tillage effects on wheat leaf spot diseases in the northern Great Plains.

Krupinsky, J. M., Bailey, K. L., McMullen, M. P., Gossen, B. D., and Turkington, T. K. 2002. Managing plant disease risk in diversified cropping systems.Agronomy Journal. 94 (4):198-209.

Kumar, J., Schäfer, P., Hückelhoven, R., Langen, G., Baltruschat, H., Stein, E., Nagarajan, S., and Kogel, K. H. 2002. *Bipolaris sorokiniana*, a cereal pathogen of global concern: cytological and molecular approaches towards better control. Molecular Plant Pathology 3:185-195.

Kumar, S., Archak, S., Tyagi, R., Kumar, J., Vk, V., Jacob, S. R., Srinivasan, K., Radhamani, J., Parimalan, R., and Sivaswamy, M. 2016. Evaluation of 19,460 wheat accessions conserved in the Indian national genebank to identify new sources of resistance to rust and spot blotch diseases. PLoS One 11:e0167702.

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

Lamari, L., Strelkov, S., Yahyaoui, A., Orabi, J., and Smith, R. 2003. The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity

confirms a one-to-one relationship in tan spot of wheat. Phytopathology 93:391-396.

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

Levine, M., and Hildreth, R. 1957. A natural occurrence of the aecial stage of *Puccinia rubigo-vera* var tritici in the united-states. Phytopathology. 47(2): 110-111 Amer Phytopathological Soc 3340 Pilot Knob Road, St PAUL, MN 55121.

Liang, H.-J., Di, Y.-L., Li, J.-L., You, H., and Zhu, F.-X. 2015. Baseline sensitivity of pyraclostrobin and toxicity of SHAM to S*clerotinia sclerotiorum*. Plant Disease 99:267-273.

Longin, C. F. H., and Würschum, T. 2014. Genetic variability, heritability and correlation among agronomic and disease resistance traits in a diversity panel and elite breeding material of spelt wheat. Plant Breeding 133:459-464.

Louw, J., Crous, PW, and Holz, G. 1996. Relative importance of the barley net blotch pathogens *Pyrenophora teres* f. teres (net-type) and *P. teres* f. maculata (spot-type) in South Africa. African Plant Protection 2:89-95.

Luo, M., Yang, Z., and Dvořák, J. 2000. The Q locus of Iranian and European spelt wheat. Theoretical and Applied Genetics 100:602-606.

Ma, Z., Felts, D., and Michailides, T. J. 2003. Resistance to azoxystrobin in Alternaria isolates from pistachio in California. Pesticide Biochemistry and Physiology 77:66-74.

Machado, F. J., Nicolli, C. P., Möller, P. A., Arruda, R., Ward, T. J., and Del Ponte, E. M. 2017. Differential triazole sensitivity among members of the *Fusarium graminearum* species complex infecting barley grains in Brazil. Tropical Plant Pathology 42:197-202.

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

McIntosh, R., Dubcovsky, J., Rogers, W., Xia, X., and Raupp, W. 2019. Morphological and Physiological Traits. Annual Wheat Newsletter:98.

McIntosh, R., Dubcovsky, J., Rogers, J. W., Morris, C., Appels, R., and Xia, X. 2014. Catalogue of gene symbols for wheat: 2013-14 Supplement. Annual Wheat Newsletter 58.

McMullen, M., and Lamey, A. 1994. Crop disease control in conservation tillage cropping systems. Crop Residue Management to Reduce Erosion and Improve Soil Quality: Northern Great Plains, Conservation Research Report:60-65.

McMullen, M., and Adhikari, T. 2009. Fungal Leaf Spot Diseases of Wheat: tan spot, stagonospora nodorum blotch and septoria tritici blotch.

Mehta, Y. 1998. Constraints on the integrated management of spot blotch of wheat. Helminthosporium Blight of Wheat: Spot Blotch and Tan Spot:18-27.

Neeson, R., Evans, J., Burnett, V., Luckett, D., Wellings, C., Taylor, H., Raman, H., Van Meeuwen, E., and Bowden, P. 2008. Optimising the quality and yield of spelt under organic production in SE Australia. in: Proc. 14th Australian Society of Agronomy Conference'. Adelaide, SA, Australia.

Neeson, R. 2011. Organic spelt production. NSW Government fact sheet. https: www.industry.nsw.gov.au/publications

Neupane, A., Sharma, R., Duveiller, E., and Shrestha, S. 2010. Sources of *Cochliobolus sativus* inoculum causing spot blotch under warm wheat growing conditions in South Asia. Cereal Research Communications 38:541-549.

Newfield, T. P. 2013. The contours, frequency and causation of subsistence crises in Carolingian Europe (750-950 CE). Pages 117-172 in: Crisis alimentarias en la edad media: Modelos, explicaciones y representaciones Milenio.

Nisikado, Y. 1928. Preliminary Notes on Yellow Spot Disease of Wheat Caused by *Helminthosporium Tritici-vulgaris* Nisikado. Berichte des Ohara Instituts für landwirtschaftliche Forschungen 4:103-109.

Pasche, J., Wharam, C., and Gudmestad, N. 2004. Shift in sensitivity of *Alternaria solani* in response to QoI fungicides. Plant Disease 88:181-187.

Patel, J. S., Gudmestad, N. C., Meinhardt, S., and Adhikari, T. B. 2012. Pyraclostrobin sensitivity of baseline and fungicide exposed isolates of *Pyrenophora tritici-repentis*. Crop Protection 34:37-41.

Patel, J. S., Meinhardt, S. W., Sierotzki, H., Stammler, G., Gudmestad, N. C., and Adhikari, T. B. 2011. A two-step molecular detection method for *Pyrenophora tritici-repentis* isolates insensitive to QoI fungicides. Plant Disease 95:1558-1564.

Peever, T. L., and Milgroom, M. G. 1992. Inheritance of triadimenol resistance in *Pyrenophora teres*. Phytopathology 82:821-828.

Piergiovanni, A., Rizzi, R., Pannacciulli, E., and Gatta, C. D. 1997. Mineral composition in hulled wheat grains: a comparison between emmer (*Triticum dicoccon*

Schrank) and spelt (*T. spelta* L.) accessions. International journal of food sciences and nutrition 48:381-386.

Rees, R., and Platz, G. 1979. The occurrence and control of yellow spot of wheat in north-eastern Australia. Australian Journal of Experimental Agriculture 19:369-372.

Rees, R., Mayer, R., and Platz, G. 1981. Yield losses in wheat from yellow spot: a disease-loss relationship derived from single tillers. Australian Journal of Agricultural Research 32:851-859.

Riesen, T., Winzeler, H., Rüegger, A., and Fried, P. 1986. The effect of glumes on fungal infection of germinating seed of spelt (*Triticum spelta* L.) in comparison to wheat (*Triticum aestivum* L.). Journal of Phytopathology 115:318-324.

Rüegger, A., and Winzeler, H. 1993. Performance of spelt (*Triticum spelta* L.) and wheat (*Triticum aestivum* L.) at two different seeding rates and nitrogen levels under contrasting environmental conditions. Journal of Agronomy and Crop Science 170:289-295.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., and Nelson, A. 2019. The global burden of pathogens and pests on major food crops. Nature ecology & evolution 3:430-439.

Seah, S., Bariana, H., Jahier, J., Sivasithamparam, K., and Lagudah, E. 2001. The introgressed segment carrying rust resistance genes Yr17, Lr37 and Sr38 in wheat can be assayed by a cloned disease resistance gene-like sequence. Theoretical and Applied Genetics 102:600-605.

Sears, E. R. 1954. The aneuploids of common wheat. University of Missouri, College of Agriculture, Agricultural Experiment Station.

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

Shah, D., Bergstrom, G., and Ueng, P. 1995. Initiation of Septoria nodorum blotch epidemics in winter wheat by seedborne Stagonospora nodorum. Phytopathology 85:452-457.

Sharma, R., and Dubin, H. 1996. Effect of wheat cultivar mixtures on spot blotch (*Bipolaris sorokiniana*) and grain yield. Field Crops Research 48:95-101.

Sharma, R., and Duveiller, E. 2006. Spot blotch continues to cause substantial grain yield reductions under resource-limited farming conditions. Journal of Phytopathology 154:482-488.

Sharma, R., Shrestha, S., and Duveiller, E. 2003. Incidence of *Bipolaris sorokiniana* and *Pyrenophora tritici-repentis* on wheat in the lowlands of Nepal. Pages 122-127 in: Proceedings of Fourth International Wheat Tan Spot and Spot Blotch Workshop, Bemidji, Minnesota, USA, 21-24 July, 2002 Agricultural Experiment Station, North Dakota State University.

Sharma, R., Duveiller, E., and Jacquemin, J. 2007. Microsatellite markers associated with spot blotch resistance in spring wheat. Journal of Phytopathology 155:316-319.

Sharp, E., Sally, B., and McNeal, F. 1976. Effect of Pyrenophora wheat leaf blight on the thousand kernel weight of 30 spring wheat cultivars. Plant Disease Reporter 60:135-138.

Shoemaker, R. 1959. Nomenclature of Drechslera and Bipolaris, grass parasites segregated from 'Helminthosporium'. Canadian Journal of Botany 37:879-887.

Siddique, A., Hossain, M., Duveiller, E., and Sharma, R. 2006. Progress in wheat resistance to spot blotch in Bangladesh. Journal of Phytopathology 154:16-22.

Sierotzki, H., Parisi, S., Steinfeld, U., Tenzer, I., Poirey, S., and Gisi, U. 2000. Mode of resistance to respiration inhibitors at the cytochrome bc1 enzyme complex of *Mycosphaerella fijiensis* field isolates. Pest Management Science: formerly Pesticide Science 56:833-841.

Sierotzki, H., Frey, R., Wullschleger, J., Palermo, S., Karlin, S., Godwin, J., and Gisi, U. 2007. Cytochrome b gene sequence and structure of *Pyrenophora teres* and *P. tritici-repentis* and implications for QoI resistance. Pest Management Science: formerly Pesticide Science 63:225-233.

Simón, M. R., Börner, A., and Struik, P. C. 2021. Fungal Wheat Diseases: Etiology, Breeding, and Integrated Management. Frontiers in Plant Science 12:498.

Simons, K. J., Fellers, J. P., Trick, H. N., Zhang, Z., Tai, Y.-S., Gill, B. S., and Faris, J. D. 2006. Molecular characterization of the major wheat domestication gene Q. Genetics 172:547-555.

Singh, P., Mergoum, M., and Hughes, G. 2007. Variation in virulence to wheat in *Pyrenophora tritici-repentis* population from Saskatchewan, Canada, from 2000 to 2002. Canadian Journal of Plant Pathology 29:166-171.

Singh, R., Mujeeb-Kazi, A., and Huerta-Espino, J. 1998. Lr46: A gene conferring slow-rusting resistance to leaf rust in wheat. Phytopathology 88:890-894.

Spelt. Ohio Agronomy Guide - 12th Edition. 1987. Ohio Cooperative Extension Service. The Ohio State University, Columbus, OH.

Spolti, P., Del Ponte, E. M., Dong, Y., Cummings, J. A., and Bergstrom, G. C. 2014. Triazole sensitivity in a contemporary population of *Fusarium graminearum* from New York wheat and competitiveness of a tebuconazole-resistant isolate. Plant Disease 98:607-613.

Starzyńska-Janiszewska, A., Stodolak, B., Socha, R., Mickowska, B., and Wywrocka-Gurgul, A. 2019. Spelt wheat tempe as a value-added whole-grain food product. LWT 113:108250.

Steffenson, B., O'Boyle, P., Brooks, W., Barnett, M., Berger, G., Stromberg, E., Maroof, S., Liu, S., and Griffey, C. 2014. Mapping net blotch resistance in 'Nomini'and CIho 2291 barley. Crop Science 54(6):2596-2602.

Suchowilska, E., Kandler, W., Sulyok, M., Wiwart, M., and Krska, R. 2010. Mycotoxin profiles in the grain of *Triticum monococcum*, *Triticum dicoccum* and *Triticum spelta* after head infection with *Fusarium culmorum*. Journal of the Science of Food and Agriculture 90:556-565.

Sugár, E., Fodor, N., Sándor, R., Bónis, P., Vida, G., and Árendás, T. 2019. Spelt Wheat: An Alternative for Sustainable Plant Production at Low N-Levels. Sustainability 11:6726.

Szabó, A., and Hammer, K. 1996. Notes on the taxonomy of farro: *Triticum monococcum*, *T. dicoccon* and *T. spelta*. Hulled wheats, promoting the conservation and used of underutilized and neglected crops. IPGRI, Rome:2-40.

Tekauz, A. 1976. Distribution, severity, and relative importance of leaf spot diseases of wheat in western Canada in 1974. Can. Plant Dis. Surv 56:36-40.

USDA Agricultural research services. Retrieved 01/11/2021. https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/cereal-rusts/wheat-leaf-rust/

Vasistha, N. K., Balasubramaniam, A., Mishra, V. K., Chand, R., Srinivasa, J., Yadav, P. S., and Joshi, A. K. 2016. Enhancing spot blotch resistance in wheat by marker-aided backcross breeding. Euphytica 207:119-133.

Vincelli, P., and Dixon, E. 2002. Resistance to QoI (strobilurin-like) fungicides in isolates of *Pyricularia grisea* from perennial ryegrass. Plant Disease 86:235-240.

Von Jagow, G., and Link, T. A. 1986. [24] Use of specific inhibitors on the mitochondrial bc1 complex. Methods in Enzymology 126:253-271.

Walker, A. S., Auclair, C., Gredt, M., and Leroux, P. 2009. First occurrence of resistance to strobilurin fungicides in *Microdochium nivale* and *Microdochium majus* from French naturally infected wheat grains. Pest Management Science: formerly Pesticide Science 65:906-915.

Wang, J., Baranski, M., Korkut, R., Kalee, H.A., Wood, L., Bilsborrow, P., Janovska, D., Leifert, A., Winter, S., Willson, A. and Barkla, B., 2021. Performance of modern and traditional spelt wheat (Triticum spelta) varieties in rain-fed and irrigated, organic and conventional production systems in a semi-arid environment; results from exploratory field experiments in Crete, Greece. Agronomy11(5): 890.Wegulo, S., Klien, R., and Harveson, R. 2011a. Tan spot of cereals. Plant Health Instructor. DOI: 10.1094/PHI-I-2011-0426-01

Wegulo, S., Stevens, J., Zwingman, M., and Baenziger, P. 2012. Yield Response to Foliar Fungicide Application in Winter Wheat, In: Fungicides for Plant and Animal Diseases. InTech, DOI: 10.5772/25716.

Wegulo, S. N., Zwingman, M. V., Breathnach, J. A., and Baenziger, P. S. 2011b. Economic returns from fungicide application to control foliar fungal diseases in winter wheat. Crop Protection 30:685-692.

Wehmeyer, L.E. (1949) Studies in the genus Pleospora. Mycologia 41, 565–593

Winnicki, T., and Żuk-Gołaszewska, K. 2018. Agronomic and economic characteristics of common wheat and spelt production in an organic farming system. Acta Scientiarum Polonorum Agricultura 16:247-254.

Winzeler, H., Schmid, J., and Winzeler, M. 1993. Analysis of the yield potential and yield components of F 1 and F 2 hybrids of crosses between wheat (*Triticum aestivum* L.) and spelt (*Triticum spelta* L.). Euphytica 74:211-218.

Wolfe, M., Baresel, J., Desclaux, D., Goldringer, I., Hoad, S., Kovacs, G., Löschenberger, F., Miedaner, T., Østergård, H., and Van Bueren, E. L. 2008. Developments in breeding cereals for organic agriculture. Euphytica 163:323-346.

Wu, H., Kang, Z., Li, X., Li, Y., Li, Y., Wang, S., and Liu, D. 2020a. Identification of wheat leaf rust resistance genes in Chinese wheat cultivars and the improved germplasms. Plant Disease 104:2669-2680.

Wu, L., He, X., Lozano, N., Zhang, X., and Singh, P. K. 2021. ToxA, a significant virulence factor involved in wheat spot blotch disease, exists in the Mexican population of *Bipolaris sorokiniana*. Tropical Plant Pathology 46:201-206.

Zillinsky, F. J. 1983. Common diseases of small grain cereals. A guide to identification. CIMMYT. pp. 141.

CHAPTER 2

Characterization of *P. tritici-repentis* diverse population for fungicide sensitivity

Abstract:

Pyraclostrobin, a quinone outside inhibitor (QoI) fungicide, is extensively used in the management of fungal leaf spot diseases. QoI fungicides target the quinone outside (Qo) binding site in the cytochrome *b* gene that inhibits fungal mitochondrial respiration. The QoI-insensitive fungal isolates are known to have three mutations in the cytochrome b gene, such as G143A, F129L, G137R. Tan spot caused by the fungus *P. tritici-repentis (Ptr)* is a significant disease in wheat in South Dakota. Many QoIs including pyraclostrobin, azoxystrobin and picoxystrobin fungicides have been used for tan spot management for several years in South Dakota which warrants the monitoring of *P. tritici-repentis* population sensitivity to QoI fungicides. In this study, two hundred and fifteen *Ptr* isolates collected from wheat fields in South Dakota were evaluated for their sensitivity to Pyraclostrobin. Of 215 isolates, 47 isolates (22.9%) exhibited insensitivity based on G143A mutation and EC_{50} values using spore germination assay. Our results indicate reduced sensitivity to insensitivity to pyraclostrobin in some isolates suggesting regular monitoring of the *Ptr* population to QoI fungicides is essential to track the evolution of insensitive population. The fungicide pyraclostrobin seems to be an effective fungicide when used with another fungicide with different chemistry to maintain its effectiveness to manage tan spot of wheat. Our results will provide stakeholders with information to consider chemical applications integrating fungicides belonging to different modes of action to manage wheat tan spot.

Introduction

Quinone outside inhibitor (QoI) fungicides have been extensively used to treat fungal diseases in agricultural crops. As the most effective class of fungicide, they have been used to wide range of crops, fruits, and vegetables such as cereals, vines, pome fruits, cucurbits, tomatoes, and potatoes. These fungicides are used against various fungal diseases like powdery mildew, downy mildew, leaf spot, tan spot (Balba 2007). Most common QoIs used in agricultural disease management are pyraclostrobin and azoxystrobin (Bartlett et al. 2002). QoI fungicides has the ability to bind with quinone binding site (Qo) in cytochrome b of the fungi that inhibits mitochondrial respiration (Von Jagow and Link 1986). This prevents the electron transfer between cytochrome b and c1 which leads to energy deficiency in fungal cells by ceasing ATP production and that results in fungal death (Fisher and Meunier 2008; Hu et al. 2017). QoIs have become the crucial part of disease management in agricultural crops due to their efficacy and wide range application. Strains highly resistant to QoIs have been reported for different target pathogens, such as *Podosphaera fusca* (Fernández-Ortuño et al. 2006), *Venturia inaequalis* (Köller et al. 2004), *Plasmopara viticola*, *Erysiphe necator* (Baudoin et al. 2008), *B. cinerea* (Zhang et al. 2011) and *P. tritici-repentis* (Sierotzki et al. 2007; Sautu and Carmona 2022).*Pyrenophora tritici-repentis* is an important cereal fungal pathogen which cause tan spot or yellow on cultivated wheat. It is a significant disease observed all over the wheat growing countries including Europe, North America and Australia and results in decreased kernel weight and number of grains per head (Figueroa et al. 2018). *P. tritici-repentis* displays a complex race structure and at least eight races have been

described and designated (Lamari et al. 2003). The races are distinct from each other based on three host selective toxins (HSTs; Ptr ToxA, B, and C) production. Each race is distinguished by the expression of one or a combination of these toxins. Sensitivity of wheat to a single HST appears to be sufficient for *P. tritici-repentis* to cause disease. Thus, each of these toxins can be pathogenic and cause yield loss (Ciuffetti et al. 2010). However, the worldwide impact of the disease is difficult to assess because of a lack of available data.

QoI fungicide resistance has been reported in many pathogens since its commercial application in 1996 (Sierotzki et al. 2007). After two years of its commercialization, first reported case of QoI resistance was observed in Germany, where wheat powdery mildew (*Blumeria graminis*) showed tolerance to fungicide application (Heaney et al. 2000). Ptr insensitivity has been observed in field population. Cytochrome b mutations were responsible for QoI resistance in Ptr as repeated application of same fungicide can make pathogen species to become insensitive, especially site specific fungicides such as quinone outside inhibitors (QoI) (Brasseur et al. 1996; Ma and Michailides 2005). The most common mutation is the replacement of glycine by alanine at amino acid position 143, referred to as G143A. It is therefore important to reduce the amount of fungicide applied to fields. This can help in limiting the fungicide exposure to pathogens which leads to insensitivity. Although studies on fungicide insensitivity in *P. tritici-repentis* are limited, fungicide insensitivity in this pathogen has been observed. (Patel et al. 2012; Reimann and Deising 2005). The Ptr populations used in this study were collected from the fields of South Dakota.

Fungicide resistance is the genetic adjustment of individual pathogens in population to survive/resist the plant protection treatments that eventually affect crop productivity and yield quality. Continuous research and development is necessary to introduce new classes of fungicides and disease management practices or otherwise existing techniques can eventually lead to poor yields and reduced crop quality (Leadbeater 2014). Development of host resistant cultivars and application of chemical fungicides are two methods most implied in wheat crops. But due to rapid evolution of pathogen population, efficacy of both methods has significantly decreased. Excessive application of fungicides increases the rate of pathogen evolution, thus most species become resistant to plant protection techniques (McDonald et al. 2019a).

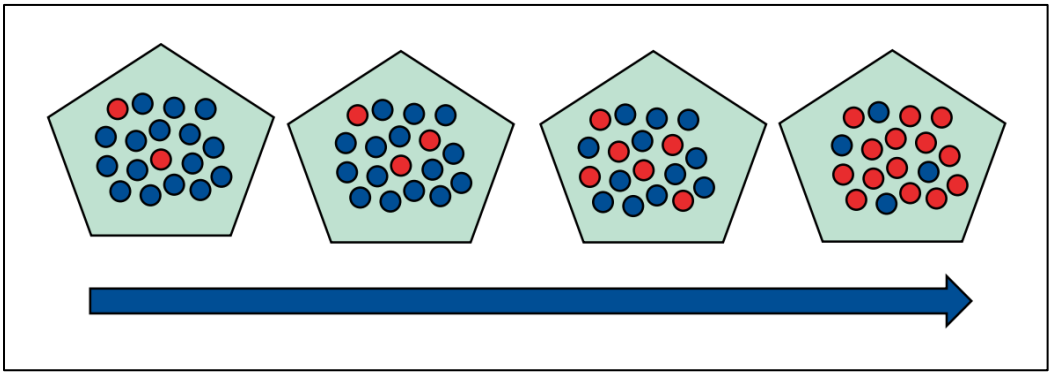

Figure 2-1: Illustration of how resistant isolates develop and increase because of selection pressure from repeated applications of fungicides (SDSU extension report 2019)

Keeping in view of the above concerns, this study was designed to identify the insensitive Ptr isolates to QoI fungicides using molecular techniques. The objective of the study was:

- Characterizing *Pyrenophora tritici repentis* isolates for fungicide sensitivity in South Dakota using site specific primers and restriction enzyme digestion.

- Sensitivity assessment of *Pyrenophora tritici repentis* isolates on fungicide amended media (spore germination and mycelial growth assays)
- Test insensitive *Pyrenophora tritici repentis* isolates if present on selected wheat varieties in-vivo.

Materials and Methods

Isolates used in study:

215 *P. tritici-repentis* isolates recovered from the wheat leaves samples collected from commercial and experimental research plots during multiple years (2012 –2020) i.e., 2012 (n=14), 2013 (n=31), 2014 (n=41), 2015 (n=6), 2018 (n=13), 2019 (n=48), 2020 (n=45), unknown (n=15), Pti2 (n=2) in South Dakota (SD). To achieve our objectives, genomic DNA of seven mutants and three wild types of *P. tritici-repentis* received from Mr. Regula Bernhard, Switzerland was also included as checks in this study. The isolates were recovered from wheat by (Abdullah 2017; Kaur 2016); and Dr. Ali (Appendix Table 2.1). Out of 215 isolates, ten isolates did not yield enough DNA and the experiment was conducted with 205 isolates (Appendix Table 2.1).

Genomic DNA Isolation:

Fresh cultures of 205 *Ptr* isolates (Appendix table 2.1) were obtained by growing them individually on V8-PDA medium, using their frozen dry plugs stored at -20°C. The Pti2 isolate was used as a control as it was collected back in the 60's and is thought to be never exposed to the fungicides. Therefore, we used Pti2 as a baseline isolate. The mycelia of each isolate were scraped from the agar surface with a sterile scalpel and placed in a 2-ml microfuge tube. The garnet grains and a bead were added to the fungal mycelia (50-100mg) in a 2-ml tube. 500 µl lysis buffer (400mM Tris-HCl [pH 8.0], 60

mM EDTA [pH 8.0], 150 mM NaCl, 10% Sodium dodecyl sulfate, ddH_20) was added to the 2-ml tube and the fungal mycelia was homogenized using a FastPrep instrument. The tubes were then left at room temperature for 10 minutes. In each tube, 150 µl of freshly prepared solution III (3M Potassium acetate, 1.15% Glacial acetic acid, ddH_20) was added and mixed by brief vortex and spun at 14,000 rpm for 1 minute with a microcentrifuge. The supernatant (approximately 500 µl) was transferred to a new Eppendorf tube and an equal amount of isopropyl alcohol was added and mixed by brief inversion and the tubes were centrifuged at 14,000 rpm for 2 minutes. The supernatant was discarded, and the resultant DNA pellet was washed in 300 µl of 70% ethanol. The tubes were spun again at 10,000 rpm for 1 minute and the supernatant was discarded. The DNA pellet was airdried for 10 minutes and dissolved in 50 µl of 1XTE or ddH2O. DNA concentration was normalized to 30-50 ng/µl using a Nano drop machine.

Genotyping of Ptr isolates for Ptr ToxA and Ptr ToxB genes:

The isolates were genotyped for Ptr ToxA and Ptr ToxB genes using the Ptr ToxA and Ptr ToxB specific primers developed by (Andrie et al. 2007a) (table 1). The primers were ordered from Integrated DNA Technologies, Coralville, IA. PCR for specific markers were performed in 20 µl volume including 2 µl genomic DNA (30ng/µl), 1 µl of each primer (10mM), 2 µl dNTP (200 µM), 2 µl 10x buffer, 1.2 µl of $MgCl_2$ 0.2 µl 10 U/ml Taq Polymerase and 10.6 µl of molecular biology grade water. PCR reagents and solutions were ordered from Midwest Scientific, Valley Park, MO. PCR reaction was conducted in a S-1000 thermal cycler (BioRad, USA). The thermal cycler conditions were set to 95°C for 3 min followed by 34 cycles of 95°C for 30 s, 60°C for 20 s, and 72°C for 35 s and a final extension of 72°C for 5 min. PCR products were

electrophoresed on 1.5% agarose gels and scored with reference to 100 bp ladder (New United Kingdom Biolabs, Ipswich, MA, 01938, USA).

Table 1: Ptr ToxA and Ptr ToxB specific primers by (Andrie et al. 2007a)

Primer name	Sequence (5′→3′)
TA51F (Tox A)	GCG TTC TAT CCT CGT ACT TC
TA52R (Tox A)	GCA TTC TCC AAT TTT CAC G
TB71F (Tox B)	GCT ACT TGC TGT GGC TAT C
TB60R (Tox B)	ACT AAC GTC CTC CAC TTT G

Primer amplification and restriction enzyme digestion for G143A and F129L mutations:

The set of primers were used to detect SNP at G143A and F129 sites in *P. tritici-repentis* isolates (Table 2). The primers were ordered from Integrated DNA Technologies, Coralville, IA. The G143 codon GGT was changed to GCT through PCR using two pairs of primers: FG143 and RG143. PCR reagents and solutions were purchased from Midwest Scientific (Valley Park, MO). The PCR mixture was prepared by adding 10.6 µl of H2O, 2.0 µl of 10× buffer, 1.2 µl of MgCl2, 2.0 µl of dNTP, 2.0 µl of primer (1 µl each of forward and reverse primer), 0.2 µl of Taq polymerase, and 2.0 µl of template DNA (30 ng/µl). All PCR reactions were run in a Bio-Rad T100 Thermal Cycler. The thermal cycler conditions were set to 40 cycles of 95°C for 30 s, 58°C for 20 s, and 72°C for 30 s. The TTC codon was changed to TTA, TTG, and CTC at the F129 site using the pair of primer FF129 and RF129. The conditions for the second PCR were same as mentioned previously, except the annealing temperature for amplifying a partial cyt b gene covering the CTC (F129L) codon was 62°C. The PCR products were digested using

three different restriction enzymes: *Fnu4HI, BsaJI*, and *MnlI* (New United Kingdom Biolabs Inc., MA.) according to the manufacturer's protocol to identify each mutation (GTC, TTA or TTG, and CTC). Restriction enzyme *Fnu4HI* detected the GTC mutation while *MnlI* detected the CTC mutation. *BsaJI* detected both the TTA and TTG mutations (Patel et al., 2012).

Table 2: Specific set of primers to amplify the region of cyt *b* gene at G143 and F129 site

Primer name	Sequence (5′→3′)	Feature
FG143	5′ GCA GCT TTA GCC CTT GGT AA 3′ covering G143 site	PCR product
RG143	5′ CTG CGC TAT TTT TAA TAT AGG TTC CTG 3 covering G143 site	PCR product
FF129	5′ AGG GTA TCT TTA ACT TGA CAC CAA TAA TT 3′ covering F129 site	PCR product
RF129	5′ TTC CAA GAC TAT TTG AGG AAC TAC TTG 3′ covering F129 site	PCR product

Gel Electrophoresis:

1.5% agarose gel in 0.5× Tris-borate-EDTA (90 mM Tris, 90 mM boric acid, and 0.1 mM EDTA) was used to separate the PCR products and stained with ethidium bromide. 2% agarose gel was used only to diagnose the GCT codon at the G143A site. Photographs were taken in Alpha Innotech FluorChem Transilluminator (Alpha Innotech Corp., San Leandro, CA).

Petri plate mycelial growth assay:

The isolates that had shown fungicide insensitivity from the PCR and restriction enzyme digestion were further tested using a Petri plate method (Figure 2-2). Ten isolates were chosen randomly and were tested on salicylhydroxamic acid (SHAM) + fungicide amended 2% water agar media. The media was prepared by dissolving 10g of agar in

500ml of distilled water and was autoclaved at chamber temperatures of 121°C for 30 minutes. To prepare stock solution of SHAM, we followed the protocol of (Pasche et al. 2004). A stock solution (100,000 mg/ml) was prepared by adding 0.1g of SHAM to 1ml of 99.9% methanol and the mixture was warmed at 37°C for 8-10 minutes to dissolve the SHAM. Six different concentrations of pyraclostrobin 0, 0.001, 0.002, 0.004, 0.008, 0.016 mg/ml were prepared by serial dilutions (Patel et al. 2012). The media was thereafter cooled down in a water bath at 35°C for 15 minutes. When it was cool enough, the fungicide and SHAM was added to it. The fungicide was dissolved in the media and the fungicide-media mixture was poured on sterile Petri plates and left to solidify in a running laminar flow hood for 24 hours. Non-fungicide amended plates were also prepared as checks. The plugs of the Ptr isolates were placed in the center of the solidified fungicide amended plates and the control plates and wrapped in aluminum foil to grow in the dark at room temperature. The plates were assessed after 7 days of incubation under the dark. Growth was assessed by measuring the radial growth of the isolates on the plates using a ruler. Four diagonal measurements were done, and the average was recorded. These measurements were contrasted with the control plates for each of the isolates for 7dpi.

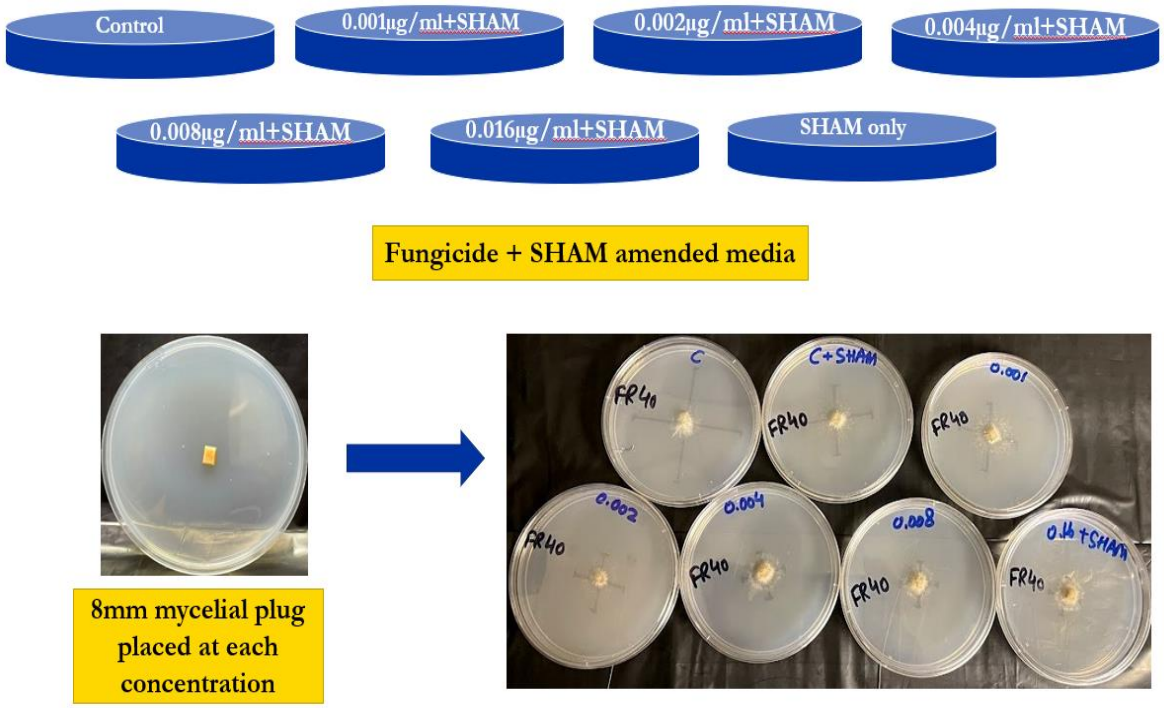

Figure 2-2: Isolate FR 40 plugs plated on different concentrations of pyraclostrobin 0, 0.001, 0.002, 0.004, 0.008, 0.016 mg/ml + SHAM amended media

Petri plate spore germination assay:

The sensitivity of the same ten *P. tritici repentis* isolates was tested using spore germination assays on water agar medium to determine the fungicide dose that can inhibit 50% growth (Pasche et al. 2004; Patel et al. 2012; Wise et al. 2008). Out of 47 Ptr isolates that were found insensitive, ten isolates were selected randomly and were grown for 5-6 days at room temperature in the dark on V8-PDA using dry plugs stored at -20°C. After six days about 30 ml of distilled sterilized water was added into each plate, and the mycelial growth was knocked down with the help of a flamed sterile test tube bottom. The plates were incubated under fluorescent light for 24 hours to induce conidiophores and then in the dark at 16°C for 24 hours to induce conidia development. After that,

about 30 ml of distilled sterile water was added to each plate, and conidia were collected with a flamed loop wired needle and spore suspension was adjusted to 3000 spores/ml (Ali and Francl, 2003). Six different concentrations of pyraclostrobin i.e., 0, 0.001, 0.002, 0.004, 0.008, 0.016 mg/ml were prepared by serial dilutions. A stock solution (100,000 mg/ml) was prepared by adding 0.1g of SHAM to one ml of 99.9% methanol and the mixture was warmed at 37C for 8-10 minutes to dissolve the SHAM. The media was thereafter cooled down in a water bath at 35°C for 15 minutes. When it was cool enough, the fungicide and SHAM was added to it. The fungicide was dissolved in the media and the fungicide-media mixture was poured on sterile petri plates and left to solidify in a running laminar flow hood for 24 hours. Three hundred microliters of spore suspension for individual isolates were pipetted onto SHAM + fungicide-amended water agar media and spread using a sterile glass spreader. The petri plates were incubated at 22-23°C for 4 hours under light to determine percentage spore germination. A spore was considered non-germinated if the germ tube was shorter than the conidia itself or had no germ tube at all. The spore was considered germinated if the germ tube was of same length as the conidia or longer than that. The percentage germination was evaluated for each isolate and for each fungicide concentration treatment by taking the mean number of conidia germinated for two replicates. The EC50 values were also calculated for the isolates. The data was regressed against the logarithm of the fungicide concentration, and the EC50 value was determined by interpolation of the 50% intercept. There were two replicates of each of the isolates for the fungicide at all concentrations plus the non-fungicide amended control for each of the isolates. The study utilizes a complete randomized design for the spore germination study.

Results

Genotyping of Ptr isolates for Ptr ToxA and Ptr ToxB genes:

As expected, the positive controls (Pti2 for race 1 and DW5 for race 5) amplified bands of 585bp and 295bp corresponding to the Ptr ToxA and Ptr ToxB, thus the validating primers and the PCR procedure (Fig 2-3). Out of 205 Ptr isolates genotyped for Ptr ToxA and Ptr ToxB genes, 197 isolates amplified Ptr ToxA showing bands of 585bp while the remaining eight isolates lacked Ptr ToxA. Out of these eight isolates, only two isolates DW7 and WW-1 amplified Ptr ToxB showing bands of 295bp. There were six isolates found which were lacking in both Ptr ToxA and Ptr ToxB. The genotypic data suggest the isolates that harbor Ptr ToxA could be race 1 or race 2. The two isolates harboring ToxB could be race 5. And the six isolates lacking in both Ptr ToxA and Ptr ToxB potentially could be race 3 or race 4 as both races do not carry any of these genes.

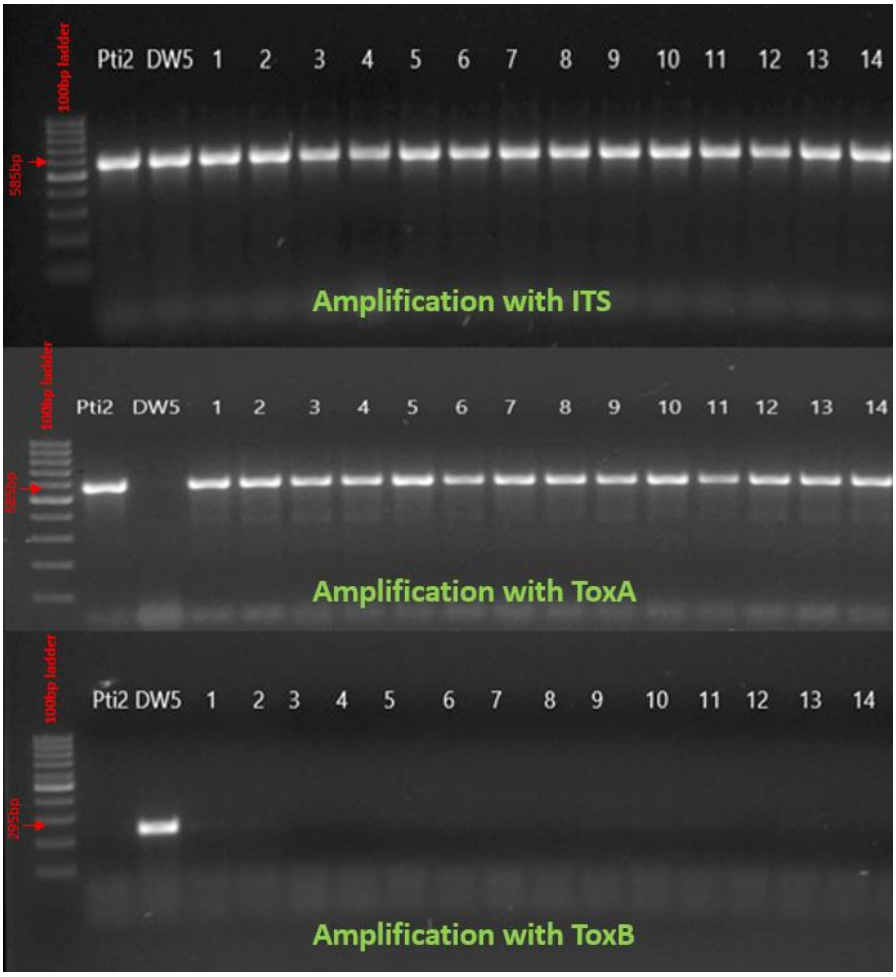

Figure 2-3: Gel image of the *P. tritici-repentis* isolates showing the presence of *ToxA* and absence of *ToxB*. In the first row, lane 1 represents 100 bp ladder, lane 2 represents Pti2 as a control, lane 3 represents DW5 as a control, and lane 4-17 represents the Ptr isolates all are amplified using ITS primer for the confirmation of fungal DNA. In the second row, lane 1 represents 100 bp ladder, lane 2 represents Pti2 as a control, lane 3 represents DW5 as a control, and lane 4-17 represents the Ptr isolates all are amplified using *ToxA* primer showing the presence of Ptr *ToxA*. In the third row, lane 1 represents 100 bp ladder, lane 2 represents Pti2 as a control, lane 3 represents DW5 as a control, and lane 4-17 represents the Ptr isolates all are amplified using *ToxB* primer showing the absence of *Ptr ToxB*.

Testing QoI-sensitive, -reduced-sensitive, and insensitive isolates of *P. tritici-repentis:*

We evaluated the 205 isolates of *P. tritici-repentis* to look for the presence of mutant codons at G143A and F129L sites responsible for the insensitivity or reduced sensitivity to fungicides. Of the 205 *P. tritici-repentis* isolates genomic DNA, 47 isolates (2012 = 1, 2014 = 14, 2018 = 1, 2019 = 6, 2020 = 22, unknown year = 3) had a codon change at G143A. Digested DNA of 47 isolates had a DNA band identical to the control containing the GCT codon (G143A mutation; Fig 2-4). The digested wild-type genomic DNA (GGT at G143) showed two fragments of 165 and 131 bp and digested mutant DNA (GCT at G143A) showed 165 and 103 bp when digested with *Fnu*4HI. We did not find the isolates to be identical to the controls containing the TTA and TTG SNPs or the CTC mutation with *Bsa*JI at F129L. Digested wild-type DNA (TTC at F129) showed DNA bands of 418 and 166 bp while the digested mutant (TTA and TTG at F129L) DNA exhibited only a 584-bp DNA band which was not observed in any isolate. The digested wild-type sequence (TTC at F129) showed band with 538 bp whereas the sequence with a codon change from TTC to CTC was expected of 425 and 114 bp in a 1.5% agarose gel when treated with *Mnl*I which was also not observed. The remaining isolates had DNA bands identical to the control (Pti2) and wild type isolates (DNA from Switzerland) and had no mutations. According to (Patel et al. 2011) there are four SNPs to be responsible for a change in sensitivity to the QoI fungicides. GCT, was responsible for the G143A mutation and for the F129L mutation, any one of three SNPs (TTA, TTG, and CTC) were responsible. To detect these four SNPs in the cyt b gene, we used already designed primers from sequences of the cyt b used by Patel et al. (2011). The primer G143 has

been covered with a length of 296 bp whereas the primer F129 of the cyt b gene has been covered with a length of 584 bp.

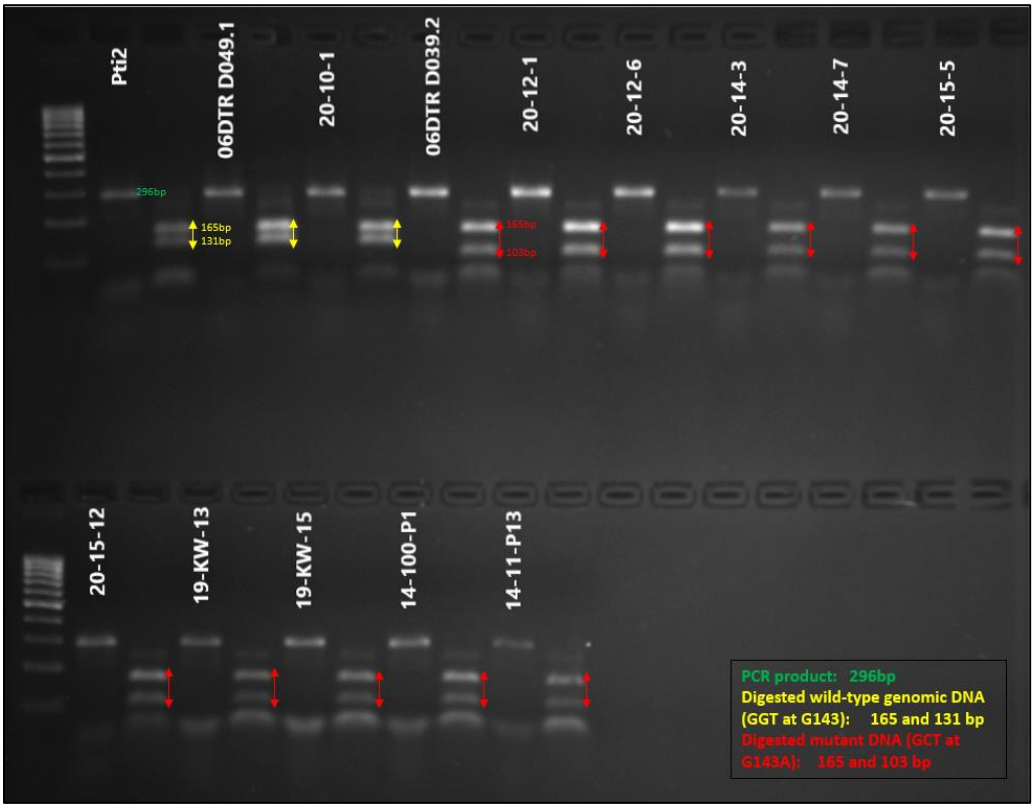

Figure 2-4: DNA banding pattern of wild-type and mutant clones of *Pyrenophora tritici-repentis* isolates digested with restriction enzyme *Fnu4HI*. In upper row, lane 1 represents 100 bp ladder, lane 2, 4 & 6 represents DNA of wild-type isolates (Pti2, 06 DTR D049.1 (isolate from Switzerland) and 20-10-1) without restriction enzyme treatment and lane 3, 5 & 7 represents DNA of wild-type isolates ((Pti2, 06 DTR D049.1 (isolate from Switzerland) and 20-10-1) treated with restriction enzyme *Fnu4HI*. Lane 8, 10, 12, 14, 16 & 18 represents DNA of mutant isolates without restriction enzyme treatment and lane 9, 11, 13, 15, 17 & 19 represents DNA of mutant isolates with restriction enzyme *Fnu4HI* treatment. Similarly, in the second row, lane 1 represents 100 bp ladder, lane 2, 4, 6, 8, and 10 represents DNA of mutant isolates without restriction

enzyme treatment and lane 3, 5, 7, 9 and 11 represents DNA of mutant isolates with restriction enzyme *Fnu4HI* treatment.

Pyraclostrobin sensitivity tests based on mycelial growth assay:

Out of the 47 isolates that showed insensitivity in the PCR and restriction enzyme digestion screening, we tested randomly chosen ten isolates on SHAM + fungicide amended 2% water agar medium using six different concentrations of pyraclostrobin 0, 0.001, 0.002, 0.004, 0.008, 0.016 mg/ml (Table 5). In Control, we found mycelial/radial growth to be highest in all the isolates as compared to the other plates (Fig 2-5 & 2-6). SHAM was found to inhibit mycelial growth as we can see reduced radial growth in the plates amended with SHAM because SHAM acts as an inhibitor of the enzyme, blocking the uninhibited flow of electrons through AOX located on the mitochondrial membrane. The results did not indicate complete stoppage of mycelial growth in the treated plates (Fig 13). The reason is that strobilurins act as preventatives by effectively killing the germinating spores. For many fungal pathogens, the germinating spore is more sensitive to QoI fungicides than is the mycelium (Vincelli 2002). It suggests that mycelial stages of pathogen *P. tritici-repentis* cannot be controlled by strobilurins. As strobilurins/QoI fungicides target spore germination to achieve efficacy, assessment of spore germination is the best approach for sensitivity studies in chemicals that use this mode of action (Olaya et al. 1998). The EC50 values of the potentially insensitive isolates to pyraclostrobin ranged from 0.5690 to 1.341 mg/ml while the EC50 value for Pti2 was 0.00058 mg/ml (Fig 2-7, Table 3 & 5).

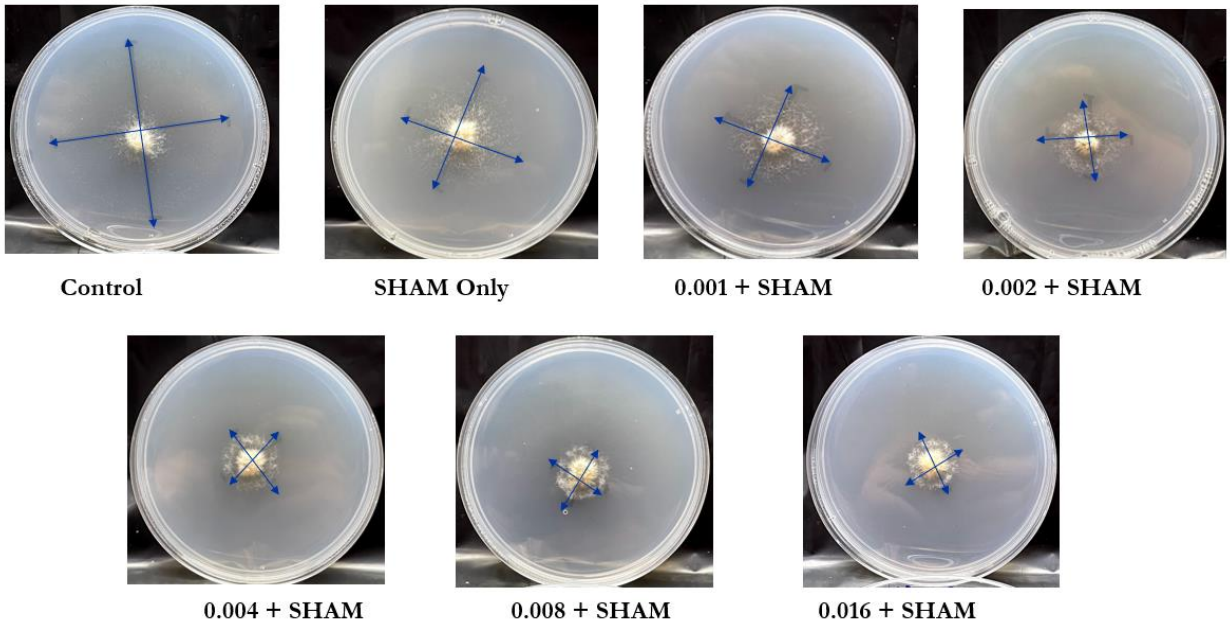

Figure 2-5: Pyraclostrobin sensitivity tests based on mycelial growth assay. Isolate 19-KW-15 grown at different concentrations + SHAM amended media and control.

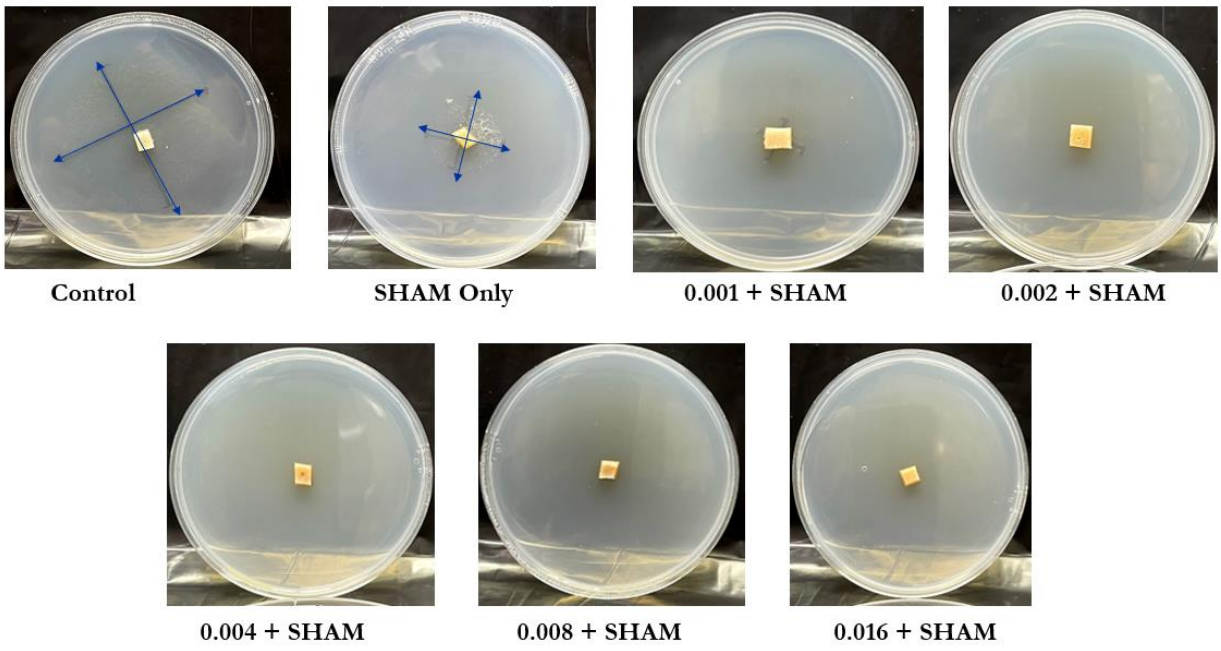

Figure 2-6: Pyraclostrobin sensitivity tests based on mycelial growth assay. Isolate Pti2 grown at different concentrations + SHAM amended media and control.

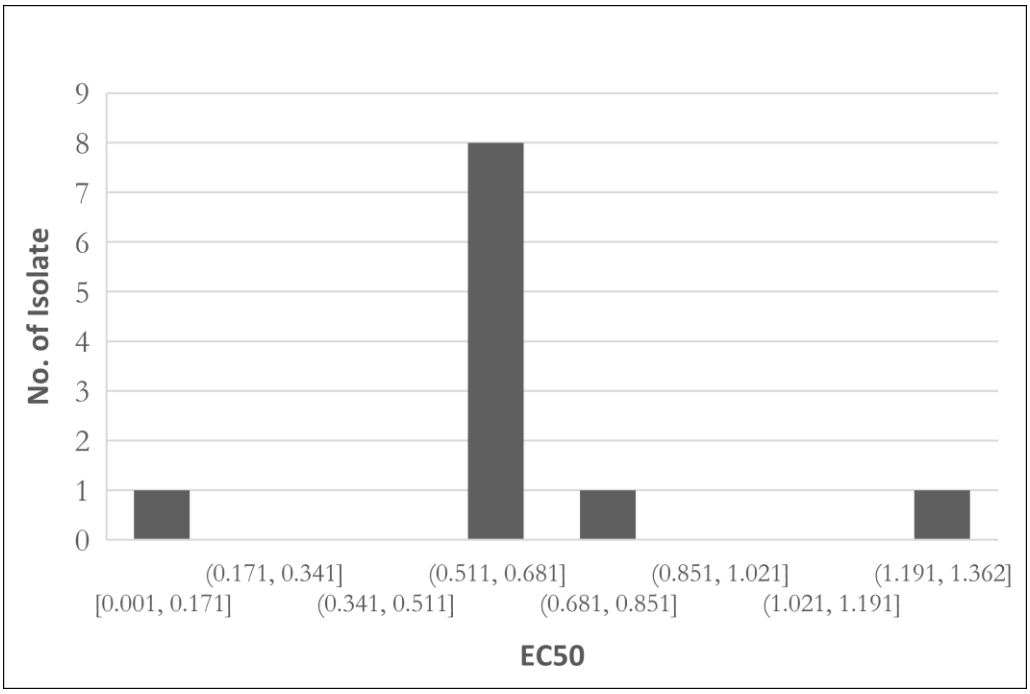

Figure 2-7: Frequency distribution of the effective concentration at which percent radial growth was inhibited (EC50) for the ten isolates

Table 3: Anova table of the effective concentration at which percent radial growth was inhibited (EC50) for the ten isolates

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Isolate	10	3.7647	0.37647	4417.2	< 2.2e-16 ***
Residuals	33	0.0028	0.00009		

Pyraclostrobin sensitivity tests based on spore germination assay:

As strobilurins/QoI fungicides target spore germination to achieve efficacy, spore germination assay was performed on ten insensitive isolates with G143A mutation. For each replicate, a total of 50 conidia were counted (Fig 2-8). The percentage germination was evaluated for each isolate and for each fungicide concentration treatment by taking

the mean number of conidia germinated for two replicates. The EC50 values of the potentially insensitive isolates to pyraclostrobin ranged from 0.0413 to 0.0885 mg/ml while the EC50 value for Pti2 was 0.00535 mg/ml (Fig 2-9, Table 4 & 5). There was a significant difference between the EC50 of Pti2 and mean EC50 value of potentially insensitive isolates in this study. This indicates that the isolates used in the study have shifted to reduced sensitivity or insensitivity.

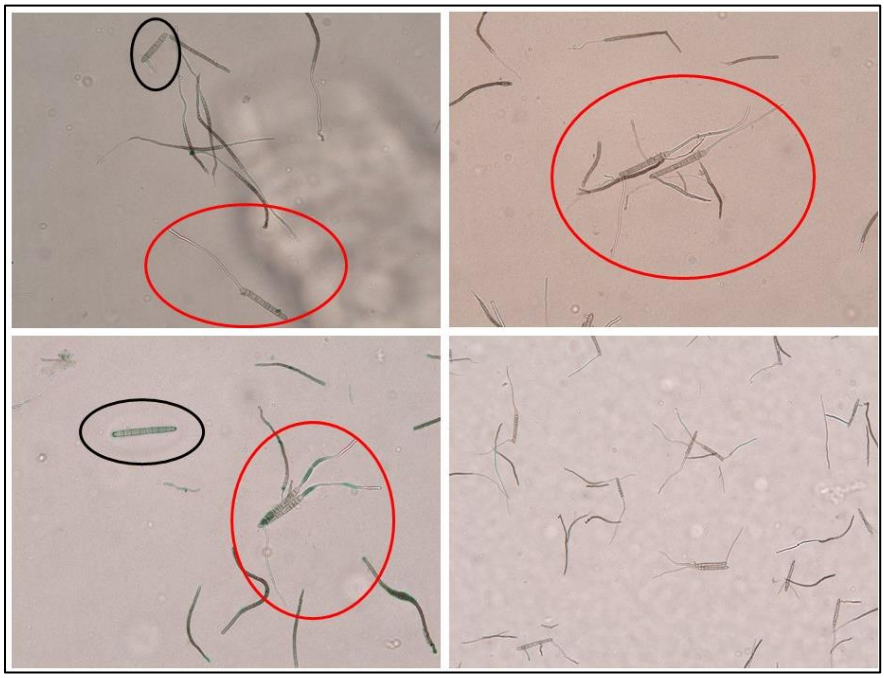

Figure 2-8: Fifty germinated (encircled red) and fifty non-germinated (encircled black) spores counted under the microscope on the fungicide amended water agar plates. At least one germ tube with the same length as the spore or more than one germ tube, irrespective of germ tube length, was considered as germinated conidium

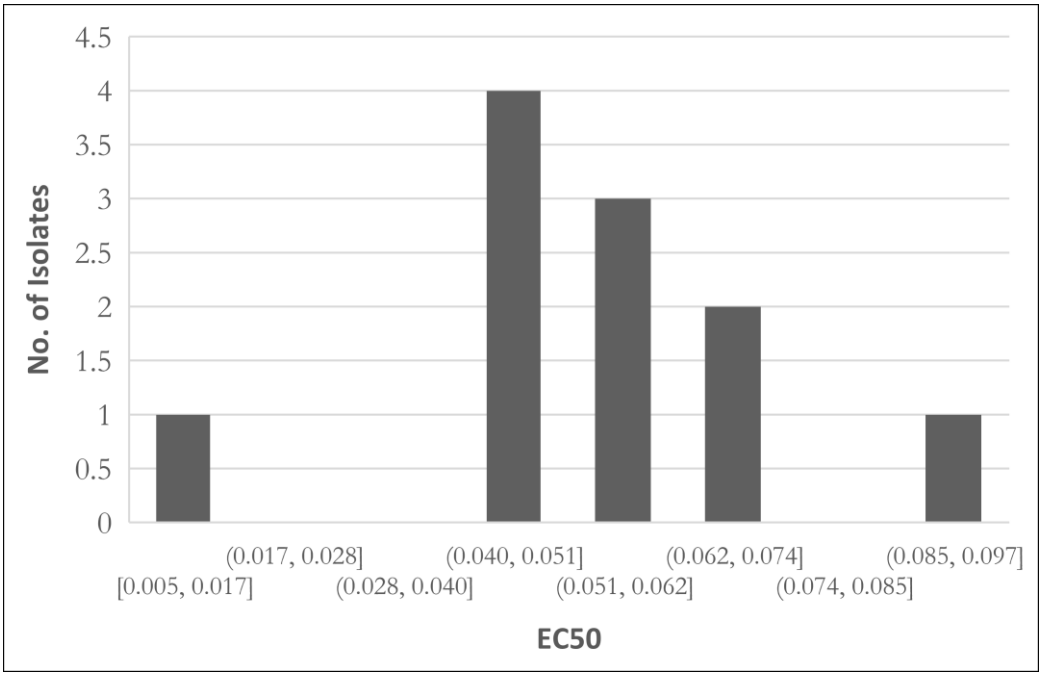

Figure 2-9: Frequency distribution of the effective concentration at which 50% of conidia germination was inhibited (EC50) for the ten isolates

Table 4: Anova table of the effective concentration at which 50% of conidia germination was inhibited (EC50) for the ten isolates

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Isolate	10	0.019137	0.001914	172.61	< 2.2e-16 ***
Residuals	33	0.000366	1.11E-05		

Table 5: Pyraclostrobin assessment of EC50 values (effective fungicide concentration that inhibited spore germination by 50%) of ten insensitive *P. tritici-repentis* isolates using spore germination assay and mycelial growth assay and presence and absence of G143A mutation.

Name of isolate	Year	G143A (GCT)*	F129L (TTA or TTG) *	F129L (CTC)*	EC50 mg/l** Mycelial/radial growth	EC50 mg/l** Spore germination
Pti2	1973	No	No	No	0.0005895	0.005357
20-14-3	2020	Yes	No	No	0.6565	0.04144
14-100-P1 (FR 66)	2014	Yes	No	No	0.6743	0.05714
19-KW-15 (FR 40)	2019	Yes	No	No	0.5690	0.08850
19-KW-13 (FR39)	2019	Yes	No	No	0.7215	0.05021
20-12-1	2020	Yes	No	No	1.341	0.05541
20-15-5	2020	Yes	No	No	0.6637	0.06617
20-12-6	2020	Yes	No	No	0.6633	0.06587
20-14-7	2020	Yes	No	No	0.6503	0.04387
20-15-12	2020	Yes	No	No	0.6084	0.05595
14-11-P13 (FR72)	2014	Yes	No	No	0.6733	0.04136

* Nucleotide substitution and responsible codon for mutation

** EC50 (50% effective concentration) values for Pyraclostrobin and isolates of *P. tritici-repentis*

Discussion:

When a pathogen population is no longer sensitive or has reduced sensitivity to the fungicide that has been used to control the same pathogen is called fungicide sensitivity or insensitivity. Measuring the fungicide sensitivity and finding shift in the baseline values is the first and crucial step. Fungicide sensitivity or insensitivity in several crops has been reported and is also attaining attention in North America. To evaluate this, limited research has been conducted in South Dakota to characterize *P. tritici-repentis* diverse population for fungicide sensitivity. We have found 47 isolates out of 205 isolates, digested DNA which had a DNA band identical to the control containing the GCT codon (G143A mutation). The two isolates 19-KW-13 and 19-KW-15 with G143 A mutation and the mycelial growth and spore germination on fungicide amended media had higher EC50 values for these two isolates which confirms that the isolates were resistant to the fungicide pyraclostrobin. These two isolates were collected from the field of South Dakota in 2019 which were sprayed with the same fungicide pyraclostrobin, and the farmer observed the disease in the sprayed field. The results comply with what farmers reported and further confirm that the isolates are found to be resistant to pyraclostrobin.

In a study by (Sautua and Carmona 2021), 82 Argentinian Ptr isolates from fields of uncontrol tan spot were assessed for in vitro sensitivity to three QoI fungicides i.e. azoxystrobin, trifloxystrobin, and pyraclostrobin. All 82 isolates were found to be highly resistant to the three QoI fungicides evaluated. The G143A substitution was confirmed in all isolates and found responsible for the strobilurin fungicide resistance in *Ptr*. The study represents the first report of QoI resistance in *Ptr* and the associated mutations in South

America. In this study, 205 isolates of *Ptr* were evaluated to look for the presence of mutant codons at G143A and F129L sites responsible for the insensitivity or reduced sensitivity to fungicides. Out of 205 isolates, digested DNA of 47 isolates had a DNA band identical to the control containing the GCT codon (G143A mutation). Out of the 47 isolates that showed insensitivity under the PCR and restriction enzyme digestion, ten isolates were selected randomly and tested on SHAM + fungicide amended 2% water agar media using six different concentrations of pyraclostrobin for spore gemination assay and mycelial growth assay. The EC50 values of the potentially insensitive isolates to pyraclostrobin ranged from 0.0413 to 0.0885 mg/ml while the EC50 value for Pti2 was 0.00535 mg/ml. There was a significant difference between the EC50 of Pti2 and mean EC50 value of potentially insensitive isolates in this study. This indicates that the isolates used in the study have shifted to reduced sensitivity or insensitivity.

(Patel et al. 2011) identified four highly QoI-insensitive isolates (GCT codon at G143A) and -reduced-sensitive isolates (TTA or TTG and CTC at F129L) of *P. tritici-repentis,* one with the CTC codon which had high EC50 value (100 mg/liter) like the GCT codon in isolates of *P. tritici-repentis*. They could not identify QoI-reduced sensitive isolates, collected in 2007 and 2009 from North Dakota, as those do not have the codon GCT at G143A or any of the three codons TTA, TTG, and CTC at F129L of the cyt *b* gene. In our findings, out of 205 *P. tritici-repentis* isolates genomic DNA, 47 isolates had a codon change at G143A. Digested DNA of 47 isolates had a DNA band identical to the control containing the GCT codon. The digested wild-type genomic DNA (GGT at G143) showed two fragments of 165 and 131 bp and digested mutant DNA (GCT at G143A) showed 165 and 103 bp when digested with *Fnu*4HI. We did not find the isolates to be

identical to the controls containing the TTA and TTG SNPs or the CTC mutation with *Bsa*JI at F129L.

Fungicide when used in premixes such as QoI + DMI tremendously controlled tan spot for many years (Wegulo et al. 2011a). From Argentina (Annone 1997) to Europe, azoxystrobin a derivative of strobilurin was very effective when used for the first years. QOI resistance was detected first time in 2003 in Europe (MacLean et al. 2017; Sierotzki et al. 2007). (Sierotzki 2015) reported that isolates from Europe were containing F129L mutation and were reduced sensitive which provides low resistance level than G143A mutation. The resistance level then increased and currently G143A mutation is prevalent in *P. tritici repentis* (FRAC 2021). In Brazil, fungicide applications were failed to control tan spot due to reduced sensitivity of the isolates to QOI and DMI fungicides (Tonin et al. 2017).

Ten isolates out of 47 insensitive isolates evaluated in this study were considered highly resistant to pyraclostrobin while the EC50 values for each of these isolates differed. For the mycelial growth assay, the EC50 values of the potentially insensitive isolates to pyraclostrobin ranged from 0.5690 to 1.341 mg/ml while the EC50 value for Pti2 was 0.00058 mg/ml. Similarly, for the spore germination assay, the EC50 values of the potentially insensitive isolates to pyraclostrobin ranged from 0.0413 to 0.0885 mg/ml while the EC50 value for Pti2 was 0.00535 mg/ml. The higher EC50 values for these isolates in agreement with (Sautua and Carmona 2021) as they also reported pyraclostrobin to completely inhibit conidial germination in all isolates at the same concentration, while trifloxystrobin showed intermediate percentages of inhibition. Similarly, Chitolina et al. (2021) reported that pyraclostrobin was more efficient than

azoxystrobin in reducing conidial germination of *Alternaria alternata*. Matsuzaki et al. (2020) reported a mean baseline EC50 value for azoxystrobin and pyraclostrobin as 0.058 µg/ml and 0.0039 µg/ml, respectively, based on the mycelial growth inhibition method. Pyraclostrobin has been reported to be most effective fungicide among all the QOI fungicides tested for *P. tritici repentis* (Tonin et al. 2017). Pyraclostrobin had the highest in vitro inhibition of spore germination in *Corynespora cassiicola*, with the lowest EC50 values (Teramoto et al. 2017). In previous studies the baseline sensitivity of Ptr populations to QoI fungicides have been evaluated. Based on the spore germination assays, baseline EC50 values for pyraclostrobin ranged from 0.0012 to 0.0024 µg/ml (Patel et al. 2012) and from 0.00001 to 0.04 µg/ml (MacLean et al. 2017). These studies have confirmed that EC50 values associated with QoI sensitivity in *P. tritici repentis* are <0.01 µg/ml. These in vitro results were also confirmed by the detection of the G143A mutation in the *cytb* gene in insensitive isolates in our study. The germinating spores are more sensitive than mycelium to strobilurin fungicides in general. QoI fungicides are significant and effective in killing the germinating spores (Vincelli 2002). They are not curatives, but they act more strongly to kill the germination spores hence considered as "protective molecules." They inhibit spore germination and thus prevent fungal penetration into the host during the initial stages of epidemics. Therefore, spore germination inhibition assay Is more effective to evaluate fungicides that are used with preventive actions.

According to USDA-NASS assessments, in South Dakota only a few acres of corn and soybean are treated with a fungicide as compared to wheat. The three classes of fungicides or mixtures i.e., FRAC code 11 (QoI, strobilurin), FRAC Code 7 (SDHI) and

FRAC code 3 (DMI, including triazoles) are the most frequently used fungicides and there is possibility that fungicide resistant pathogen population may exist but in low numbers to be detected or to cause total fungicide failure at this time. The continued use of the same modes of action over time may have led to fungicide resistance development in the state. To our knowledge, this is the first-time fungicide resistance has been observed in Ptr population prevalent in wheat in South Dakota.

Based on reaction of the four differential genotypes, Glenlea (Ptr Tox A), 6B662 (Ptr Tox B), 6B365 (Ptr Tox C), and Salamouni (no toxin) to inoculation and their ability to produce single or a combination of the three known host-selective toxins, *P. tritici repentis* isolates are classified into eight races (Ciuffetti et al. 2010; Lamari and Strelkov 2010). The presence of the *ToxA* gene and absence of the *ToxB* gene shows that the isolates evaluated either belong to race 1 or race 2. In our results, out of 205 Ptr isolates genotyped for Ptr ToxA and Ptr ToxB genes, 197 isolates amplified Ptr ToxA showing bands of 585bp while the remaining eight isolates lacked Ptr ToxA. Out of these eight isolates, only two isolates DW7 and WW-1 amplified Ptr ToxB showing bands of 295bp. There were six isolates found which were lacking in both Ptr ToxA and Ptr ToxB. The genotypic data suggest the isolates that harbor Ptr ToxA could be race 1 or race 2. The two isolates harboring ToxB could be race 5. And the six isolates lacking in both Ptr ToxA and Ptr ToxB potentially could be race 3 or race 4 as both races do not carry any of these genes. (Sautua and Carmona 2021) characterized 82 Ptr isolates and the ToxA gene was amplified in all the isolates but not the ToxB gene. Another study by (Moreno et al. 2015) reports presence of Tox A gene in 57 Ptr isolates out of 65 from Argentina. Abdullah et al. 2017 has reported the presence of races 1, 2, and 3 with race 1 to be the

most prevalent race in the isolates from Latvia, Lithuania, and Romania. According to them, a diverse fungal population was prevalent in three countries based on their phenotypic evaluation. They grouped 63% of the isolates under race 1 as they produced necrosis and chlorosis on Glenlea and 6B365, respectively and neither symptom on 6B662 and Salamouni. They found race 1 (48%) and race 3 (20%) to be the most prevalent races in Lithuania followed by race 4 (8%) while 80% of the isolates from Romania were grouped as race 1, 17% as race 2, and 2% as race 4. In contrast, Ali et al. (2010) characterized *P. tritici-repentis* isolates from Arkansas and reported most of the isolates lacked in ToxA. In our findings, 197 out of 205 Ptr isolates (96%) genotyped for Ptr ToxA and Ptr ToxB genes amplified Ptr ToxA. Race 1 has already been a predominant race in Algeria, Czech Republic, Canada, USA, and South America (Benslimane et al., 2011; Ali et al., 2004; Sarova et al., 2005; Aboudkhaddour et al., 2013; Lamari and Bernier, 1989; Singh and Hughes, 2006; Ali and Francl, 2003; Engle et al., 2006; Ali and Francl, 2002; Gamba et al., 2012; Moreno et al., 2008). Favorable weather conditions and travel of fungal spores through wind in the wheat growing regions where Ptr populations have already been identified is the reason Tox A population is so stable. The isolates used in this study were collected from the wheat growing regions from where Ptr race 1 has already been identified. In conclusion, our findings suggest that QoI-resistance is present in South Dakota *P. tritici repentis* populations causing tan spot of wheat, as the population tested found to be composed of pyraclostrobin (strobilurin) resistant individuals, and the G143A mutation is present in 23% of the isolates collected from wheat. Our results suggest that regular monitoring of the *P. tritici-repentis* population to QoI fungicides is essential to track the evolution of

insensitive population. These results will provide stakeholders with information to consider chemical applications integrating fungicides belonging to different modes of action to manage wheat tan spot. Also, the majority of *P. tritici-repentis* isolates harbored Ptr ToxA genes in our study as has been observed in the previous studies from various countries indicate Ptr ToxA carrying isolates still dominant population.

Literature cited

Abdullah, S., Sehgal, S. K., Jin, Y., Turnipseed, B., and Ali, S. 2017. Insights into tan spot and stem rust resistance and susceptibility by studying the pre-green revolution global collection of wheat. The Plant Pathology journal 33:125.

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

Ali, S. and Francl, L. J. 2002. Race structure of *Pyrenophora tritici-repentis* isolates obtained from wheat in South America. *Plant Protect. Sci*. 38(Special Issue 2):302-304.

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

Ali, S., Sarova, J. and Waukulinsky, W. 2004. *Pyrenophora tritici-repentis* races in the Czech Republic and Poland. *Phytopathology* 93(Suppl):S3 (Abstract).

Andrie, R. A., Pandelova, L., and Ciuffetti, L. A. 2007a. A combination of phenotypic and genotypic characterization strengthens *Pyrenophora tritici-repentis* race identification. Phytopathology 97:694-701.

Annone, J. 1997. Tan spot of wheat in Argentina: importance and disease management strategies. Spot Blotch and Tan Spot:339.

Balba, H. 2007. Review of strobilurin fungicide chemicals. Journal of Environmental Science and Health Part B 42:441-451.

Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M., and Parr-Dobrzanski, B. 2002. The strobilurin fungicides. Pest Management Science: formerly Pesticide Science 58:649-662.

Baudoin, A., Olaya, G., Delmotte, F., Colcol, J. F., and Sierotzki, H. 2008. QoI resistance of *Plasmopara viticola* and *Erysiphe necator* in the mid-Atlantic United States. Plant Health Progress 9:25.

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

Brasseur, G., Saribaş, A. S., and Daldal, F. 1996. A compilation of mutations located in the cytochrome b subunit of the bacterial and mitochondrial bc1 complex. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1275:61-69.

Chitolina, G. M., Silva-Junior, G. J., Feichtenberger, E., Pereira, R. G., and Amorim, L. 2021. Distribution of Alternaria alternata isolates with resistance to quinone outside inhibitor (QoI) fungicides in Brazilian orchards of tangerines and their hybrids. Crop Protection 141:105493.

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

Engle, J. S., Madden, L. V. and Lipps, P. E. 2006. Distribution and pathogenic characterization of *Pyrenophora tritici-repentis* and *Stagonospora nodorum* in Ohio. *Phytopathology* 96:1355-1362.

Fernández-Ortuño, D., Pérez-García, A., López-Ruiz, F., Romero, D., De Vicente, A., and Torés, J. 2006. Occurrence and distribution of resistance to QoI fungicides in populations of *Podosphaera fusca* in south central Spain. European Journal of Plant Pathology 115:215-222.

Figueroa, M., Hammond-Kosack, K. E., and Solomon, P. S. 2018. A review of wheat diseases—a field perspective. Molecular Plant Pathology 19:1523-1536.

Fisher, N., and Meunier, B. 2008. Molecular basis of resistance to cytochrome bc 1 inhibitors. FEMS yeast research 8:183-192.

Gamba, F. M., Strelkov, S. E. and Lamari, L. 2012. Virulence of *Pyrenophora tritici-repentis* in the southern cone region of South America. *Can. J. Plant Pathol*. 34:545-550.

Heaney, S., Hall, A., Davies, S., and Olaya, G. 2000. Resistance to fungicides in the QoI-STAR cross-resistance group: current perspectives. Pages 755-762 in: The BCPC Conference: Pests and diseases, Volume 2. Proceedings of an international conference held at the Brighton Hilton Metropole Hotel, Brighton, UK, 13-16 November 2000 British Crop Protection Council.

Hu, X., Dai, D., Wang, H., and Zhang, C. 2017. Rapid on-site evaluation of the development of resistance to quinone outside inhibitors in *Botrytis cinerea*. Scientific reports 7:1-9.

Kaur, Navjot, "Root Rot Pathogens of Wheat in South Dakota and Their Affect on Seed Germination and Seedling Blight in Spring Wheat Cultivars" (2016). *Electronic Theses and Dissertations*. 1117. https://openprairie.sdstate.edu/etd/1117

Köller, W., Parker, D., Turechek, W., Avila-Adame, C., and Cronshaw, K. 2004. A two-phase resistance response of *Venturia inaequalis* populations to the QoI fungicides kresoxim-methyl and trifloxystrobin. Plant Disease 88:537-544.

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

Lamari, L., Strelkov, S., Yahyaoui, A., Orabi, J., and Smith, R. 2003. The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. Phytopathology 93:391-396.Lamari, L., and Strelkov, S. E. 2010. Minireview/Minisynthèse The wheat/*Pyrenophora tritici-repentis* interaction: progress towards an understanding of tan spot disease. Canadian Journal of Plant Pathology 32:4-10.

Leadbeater, A. 2014. Plant health management: fungicides and antibiotics.

Ma, Z., and Michailides, T. J. 2005. Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. Crop Protection 24:853-863.

MacLean, D. E., Aboukhaddour, R., Tran, V. A., Askarian, H., Strelkov, S. E., Turkington, T. K., and Kutcher, H. R. 2017. Race characterization of *Pyrenophora tritici-repentis* and sensitivity to propiconazole and pyraclostrobin fungicides. Canadian Journal of Plant Pathology 39:433-443.

Matsuzaki, Y., Kiguchi, S., Suemoto, H., and Iwahashi, F. 2020. Antifungal activity of metyltetraprole against the existing QoI-resistant isolates of various plant pathogenic fungi: Metyltetraprole against QoI-R isolates. Pest Management Science 76:1743-1750.

McDonald, M. C., Renkin, M., Spackman, M., Orchard, B., Croll, D., Solomon, P. S., and Milgate, A. 2019a. Rapid parallel evolution of azole fungicide resistance in Australian populations of the wheat pathogen *Zymoseptoria tritici*. Applied and Environmental Microbiology 85(4):e01908-18.

Moreno, M. V., Stenglein, S. A., Balatti, P. A. and Perelló, A. E. 2008. Pathogenic and molecular variability among isolates of *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat in Argentina. *Eur. J. Plant Pathol*. 122:239-252.

Moreno, M. V., Stenglein, S., and Perelló, A. E. 2015. Distribution of races and Tox genes in *Pyrenophora tritici-repentis* isolates from wheat in Argentina. Tropical plant pathology 40:141-146.

Olaya, G., Zheng, D., and Köller, W. 1998. Differential responses of germinating *Venturia inaequalis* conidia to kresoxim-methyl. Pesticide Science 54:230-236.

Pasche, J., Wharam, C., and Gudmestad, N. 2004. Shift in sensitivity of *Alternaria solani* in response to QoI fungicides. Plant Disease 88:181-187.

Patel, J. S., Gudmestad, N. C., Meinhardt, S., and Adhikari, T. B. 2012. Pyraclostrobin sensitivity of baseline and fungicide exposed isolates of *Pyrenophora tritici-repentis*. Crop Protection 34:37-41.

Patel, J. S., Meinhardt, S. W., Sierotzki, H., Stammler, G., Gudmestad, N. C., and Adhikari, T. B. 2011. A two-step molecular detection method for *Pyrenophora tritici-repentis* isolates insensitive to QoI fungicides. Plant Dsease 95:1558-1564.

Reimann, S., and Deising, H. B. 2005. Inhibition of efflux transporter-mediated fungicide resistance in *Pyrenophora tritici-repentis* by a derivative of 4′-hydroxyflavone and enhancement of fungicide activity. Applied and Environmental Microbiology 71:3269-3275.

Sarova, J., Hanzalová, A. and Bartoš, P. 2005. Races of *Pyrenophora tritici-repentis* in Czech Republic. *Acta Agrobot*. 58:73-78.

Sautua, F. J., and Carmona, M. A. 2021. Detection and characterization of QoI resistance in *Pyrenophora tritici-repentis* populations causing tan spot of wheat in Argentina. Plant Pathology 70:2125-2136.

Sierotzki, H. 2015. Respiration inhibitors: complex III. Pages 119-143 in: Fungicide resistance in plant pathogens. Springer.

Sierotzki, H., Frey, R., Wullschleger, J., Palermo, S., Karlin, S., Godwin, J., and Gisi, U. 2007. Cytochrome b gene sequence and structure of *Pyrenophora teres* and *P. tritici-repentis* and implications for QoI resistance. Pest Management Science: formerly Pesticide Science 63:225-233.

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

Teramoto, A., Meyer, M. C., Suassuna, N. D., and Cunha, M. G. d. 2017. In vitro sensitivity of *Corynespora cassiicola* isolated from soybean to fungicides and field chemical control of target spot. Summa Phytopathologica 43:281-289.

Tonin, R. B., Reis, E. M., and Avozani, A. 2017. Reduction in the in vitro sensitivity of *Drechslera tritici-repentis*, isolated from wheat, to strobilurin and triazole fungicides. Summa Phytopathologica 43:20-25.

Vincelli, P. 2002. QoI (strobilurin) fungicides: benefits and risks. The Plant Health Instructor:63-65.

Von Jagow, G., and Link, T. A. 1986. [24] Use of specific inhibitors on the mitochondrial bc1 complex. Methods in enzymology 126:253-271.

Wegulo, S., Klien, R., and Harveson, R. 2011a. Tan spot of cereals. Plant Health Instr., http://dx. doi. org/10 1094.

Wise, K., Bradley, C., Pasche, J., Gudmestad, N., Dugan, F., and Chen, W. 2008. Baseline sensitivity of *Ascochyta rabiei* to azoxystrobin, pyraclostrobin, and boscalid. Plant Disease 92:295-300.

Zhang, C.-q., Liu, Y.-h., Ding, L., and Zhu, G.-n. 2011. Shift of sensitivity of *Botrytis cinerea* to azoxystrobin in greenhouse vegetables before and after exposure to the fungicide. Phytoparasitica 39:293-302.

CHAPTER 3

Reaction of spelt wheat *(Triticum spelta* L.) germplasm against leaf spot and leaf rust

Abstract

Spelt wheat (*Triticum spelta* L.) is an ancient cereal that is gaining small grain growers' attention under an organic setup due to its high protein content, high fiber, and other health benefits. Spelt wheat, like common wheat, also succumbs to leaf diseases. Major fungal leaf spot diseases, tan spot, spot blotch, Septoria nodorum blotch, and rusts, are commonly occur in the U.S. Great Plains region and contribute towards limiting the wheat yield. Identification of disease resistant spelt genotypes could be valuable for spelt production under organic management. In this study, two hundred-eight spelt accessions from twenty countries were screened against tan spot race 1, race 5, Ptr ToxA, spot blotch, and leaf rust at the seedling stage in the greenhouse. The genotypes exhibited varying responses ranging from susceptible to moderately susceptible and moderately resistant to resistant.

Out of 195 genotypes, 9.2% (n=18) were rated susceptible and 28.2% (n=55) were rated moderately susceptible to tan spot race 1, whereas 14.8% (n=29) genotypes were rated moderately resistant, and 47.6% (n=93) genotypes were resistant to tan spot race 1. 47.6% (n = 93) and 76.5% (n = 157) of the genotypes were resistant to tan spot race 1 and race 5, respectively. 96.6% (n = 201) were insensitive to Ptr ToxA. Of these 96.6% ToxA insensitive genotypes, 9.2% (n = 18) were susceptible to race 1 and 47.6 % (n = 93) were resistant to race 1. For spot blotch, the resistance level was lower as compared to tan spot as only 15.2% (n = 27) genotypes showed resistant reaction. For leaf rust, only 10.3% (n

=20) genotypes were resistant. Spelt accessions resistant to leaf rust and leaf spots will be further evaluated for the agronomic traits in the field and results will be shared with the growers to assist in selection of superior spelt genotypes.

Introduction

Spelt wheat *(Triticum spelta L.*) is a hulled grain that belongs to the species of bread wheat (*Triticum aestivum* spp. *aestivum*) (Escarnot et al. 2012). Spelt is getting popularity among consumers as it is considered as a "healthy alternative" to bread wheat. The processing and cultivation of spelt are comparatively expensive than wheat because of low yield potential. There are several nutritional and agronomic advantages of spelt wheat over common wheat which have brought attention to the researchers (Starzyńska-Janiszewska et al. 2019). Spelt is an alternative crop, which grows without any special soil related and climatic demands and has the potential for low input production and adaptation to harsh ecological conditions and resistance to diseases (Sugár et al. 2019). The products made up of spelt wheat are suitable for those having wheat sensitivity as they are easily digestible, and they also have good flavor and aroma (Frakolaki et al. 2018). Spelt is believed to have a higher nutritive value in comparison with common wheat and better adoptability to unfavorable environmental factors as well as lower fertilization and soil demands (Suchowilska et al. 2010). Spelt wheat is different from the common wheat in many ways as reported by (Campbell 1997; Kohajdová and Karovicova 2008; Onishi et al. 2006).

In the US Great Plains, several foliar diseases occur including Septoria tritici blotch (*Mycosphaerella graminicola*) (asexual stage: *Zymoseptoria tritici*), tan spot (*Pyrenophora tritici-repentis;* asexual stage*: Drechslera tritici-repentis*), spot blotch

(*Bipolaris sorokiniana;* asexual stage*: Biploris sorokiniana*), and Septoria nodorum blotch (*Parastagonospora nodorum*) (Wegulo et al. 2011a). Tan spot caused by *P. tritici-repentis* has increased over the last several decades due to wheat monoculture year after year and a shift toward conservation tillage practices that leave crop residue, a source of primary inoculum, on the soil surface. Tan spot is an important disease of wheat in South Dakota and the region (SDSU extension report 2018). The disease occurs worldwide in all major wheat grown regions and has the widest host range (De Wolf et al. 1998). In spring and summer, the disease develops on wheat on both the upper and lower surfaces of leaves. On susceptible genotypes, well-defined necrotic lesions surrounded by the chlorotic halo and a pin head size black spot in the center can be seen while on resistant genotypes, there are small brown to black spots without chlorosis or necrosis. The fungus also can infect wheat spikes, causing red smudge. Yield losses due to *P. tritici-repentis* range from 5% to 10% (Singh et al. 2010) whereas under favorable conditions yield losses can be as high as 50% (Hosford 1982; Shabeer and Bockus 1988).

Spot blotch caused by *B. sorokiniana* is one of the most concerning diseases. The fungus is also a causal agent of common root rot, seedling blight, and black point of wheat and barley (Acharya et al. 2011; Chowdhury et al. 2013; Kumar et al. 2002; McDonald et al. 2018; Sharma and Duveiller 2006; Wu et al. 2021). The pathogen affects wheat and barley, but it can also affect a variety of hosts including several wild and cultivated Poaceae members. Rye and oats are rarely infected by this pathogen (Kumar et al. 2002; Steffenson et al. 2014; Zillinsky 1983). During initial infection, the disease is generally characterized by small dark brown lesions extending to1-2 mm long without chlorotic margin (Chand et al. 2003; Duveiller and Garcia Altamirano 2000). Dark brown necrotic

lesions on roots, crowns, and leaves can be seen on susceptible genotypes whereas resistant genotypes show a very small necrotic dark spot. The susceptible genotypes develop a distinct oval to elongated light to dark brown blotches that extend and merge quickly and kills the leaves (Chand et al. 2003; Duveiller and Dubin 2002). Humid and warm conditions act as a favorable environment for the pathogen to cause disease. The losses in yield due to spot blotch ranges between 15-20% on average (Acharya et al. 2011) but in susceptible genotypes it can go up. Spot blotch severity went up to 70-100% in Nepal according to Sharma et al. (2007). Mehta (1998) reported that in severe conditions the pathogen can cause 100% yield losses in Latin America.

Leaf rust fungus is identified as an important pathogen in wheat production all over the world causing significant yield losses over large geographical areas (Kolmer 2005). The losses ranged between 1 to 20% (USDA agricultural research services).

The fungus *Puccinia triticina* spores can spread thousands of kilometers from the initial infection site through its urediniospores, and cause disease. Uredinia normally appear on the upper leaf surface and are brown in color and circular in shape.

Spelt wheat has a large leaf mass, grows faster and more vigorously than wheat. Also, if it is planted in fall, it can out-compete spring weeds so that it has the potential to compete well against weeds (Szewczyk et al. 2013). Fernandez et al. (2014) and Arseniuk et al. (1991) reported low SNB (*P. nodorum*) level in spelt wheat. According to Arseniuk et al. (1991) winter spelt was more resistant to *P. nodorum* than spring wheat. Singh et al. (2006) identified spelt wheat lines with resistance to the leaf spot pathogens *P. tritici-repentis*, *L. norodum* and *M. graminicola*, and to races 2, 3, and 5 of *P. tritici-repentis* and toxins Ptr ToxA and Ptr ToxB. Spelt wheat with lower leaf spot severity as compared

to common wheat and durum wheat was observed by Fernandez et al. (2014). Based on the literature available, we can say that spelt is less susceptible to most pests and diseases which affect common wheat. However, these diseases can be reduced by crop rotation and not planting spelt after another cereal crop.

In this study, we investigated a diverse panel of 208 spelt accessions, obtained from USDA National Small Grain Collection (NSGC), Aberdeen, Idaho against leaf spot and leaf rust at the seedlings stage in greenhouse.

Materials & Methods

Plant Materials:

Two hundred and eight spelt wheat accessions from twenty countries belonging to 4 continents Africa, Asia, Europe, North America (Figure 3-1) were obtained from USDA National Small Grain Collection (NSGC), Aberdeen, Idaho (Appendix table 2.3). Wheat differential genotypes Glenlea and Salamouni were included in the experiment as tan spot susceptible, Ptr ToxA sensitive and tan spot resistant, and Ptr ToxA insensitive checks, respectively. Also, wheat genotype 6B662 was included as susceptible check to race 5. Two-week old seedlings of all 208 spelt wheat genotypes including checks were grown in 1.5” x 8.25” containers (Hummert International, Earth City, MO USA) filled with Sungro professional growing mix (770 Silver Street Agawam, MA, USA) and tested for their reaction to tan spot with *P. tritici-repentis* race 1, and sensitivity to Ptr ToxA, *P. tritici-repentis* race 5, and spot blotch with ToxA sensitive and ToxA insensitive isolates, and leaf rust. Nine seedlings (three seedlings/cone) of each genotype were evaluated individually for reaction to tan spot, Ptr ToxA, spot blotch and leaf rust. The seedlings were watered and fertilized as needed. The seedlings were kept in a greenhouse at 21-

22°C with 16 hours photoperiod until the experiment was terminated.

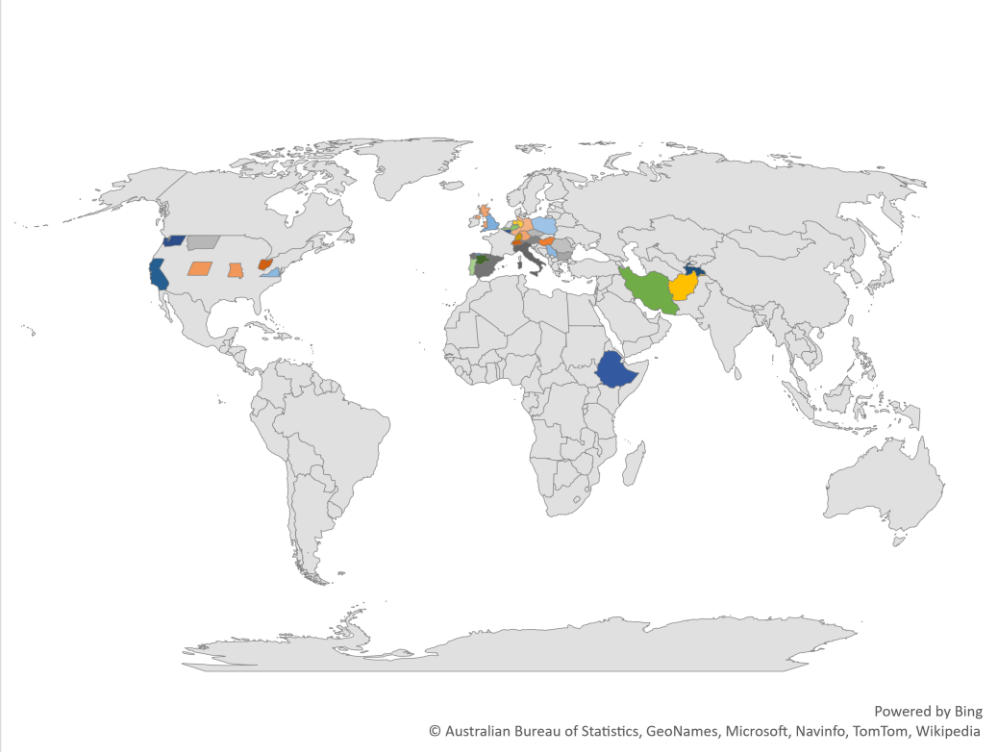

Figure 3-1: Geographic distribution of 208 Spelt wheat accessions used in this study.

Screening for tan spot and spot blotch:

Inoculum preparation, plants inoculation and disease rating:

P. tritici-repentis isolates Pti2 and DW7 were used as inoculum to screen the germplasm for race 1 and race 5, respectively throughout the experimentation. For spot blotch screening, *B. sorokiniana* isolate BS-10-97 (containing BS+ toxin) was used as inoculum. A fresh culture of each of the isolate was initiated by plating the isolate dry plugs stored at -20^{o}C on V8PDA (V8 Juice=150 ml; $CaCO_3$ =3 g; potato dextrose agar=10 g; agar=10 g; distilled water=850 ml) to prepare spore suspension as described by (Ali and Francl 2001). For tan spot, one dry plug was placed in the center of each fresh V8-PDA plate

(ten plates for each isolate), wrapped with aluminum foil paper, and incubated for 5 days at room temperature. After 5 days when the *P. tritici-repentis* cultures had grown approximately 3 cm from the center, about 30 ml of distilled sterilized water was added into each plate and the mycelial growth was knocked down with the help of a flamed sterile test tube bottom. The plates were incubated under fluorescent grow lights for 24 hours at room temperature (21-22 ^{o}C) and then in the dark at 16^{o}C for 24 hours to induce conidiophores and conidia development, respectively. Thereafter, about 30 ml of distilled sterile water was added to each plate, and conidia were collected with a loop wired needle and spore suspension was adjusted to 3000 spores/ml prior to inoculations. For spot blotch, one dry plug of *B. sorokiniana* isolate BS-10-97 was placed in the center of each fresh V-8PDA plate (ten plates) and was grown under 12 hours light and dark cycle for 5-6 days. After 6 days, 30 ml of distilled sterile water was added to each plate, and conidia were collected the same way as in Ptr with a loop wired needle and spore suspension was adjusted to 3000 spores/ml prior to inoculations (Fig 3-2).

Two-week old seedlings of each of genotype were inoculated with the race 1, race 5 and *B. sorokiniana* spore suspensions individually with a CO_2 pressurized hand sprayer (Power Sprayer, Prevail, Chicago Aerosol, 1300 E. North Street, Coal City, IL60416) and placed in humidity chambers at 100% humidity for 24 hours for infection initiation. The seedlings were then moved from the humidity chambers to the greenhouse bench. The seedlings were rated eight- and ten-days post-inoculations for tan spot and spot blotch, respectively. The seedlings were rated for disease reactions using a 1 to 5 rating scale where lesion type 1-2 is resistant to moderately resistant, and 3-5 is moderately susceptible to susceptible (Lamari and Bernier, 1989).

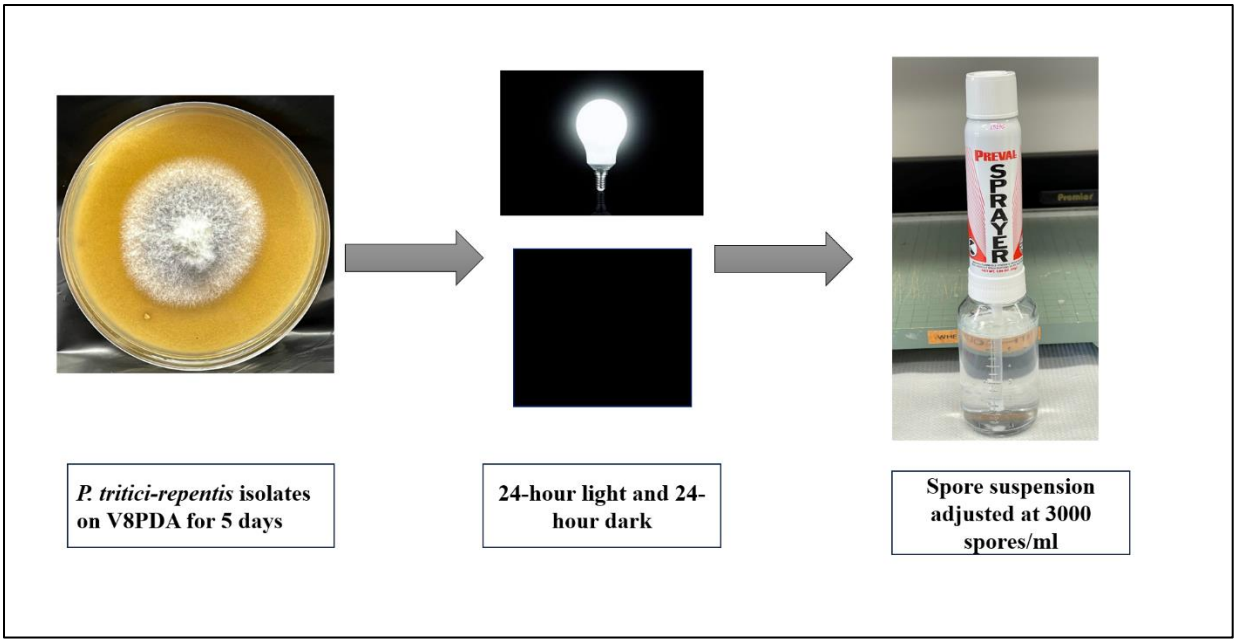

Figure 3-2: Inoculum preparatin for inoculations

Toxin Bioassays:

All two hundred and eight spelt wheat accessions were tested for their reaction to Ptr ToxA, Ptr ToxB and BS ToxA (Appendix Table 2.3). Three fully expanded first leaves of each genotype were infiltrated with culture filtrates of Ptr ToxA and Ptr ToxB (kindly provided by Dr. Timothy Friesen, ARS-USDA, Fargo, ND) using a needle-less syringe prior to inoculation with race 1 and race 5 at two- leaf-stage following the method of (Faris et al. 1996). The leaves were examined 72 hours' post-toxin infiltration for necrosis (Ptr Tox A) and chlorosis (Ptr Tox B) development and rated as "+" (toxin sensitive) and "-" (insensitive). Tan spot wheat differentials Glenlea (Ptr ToxA sensitive) and Salamouni (insensitive) were included as controls in the experiment.

Screening for leaf rust:

Seedlings of 208 spelt wheat genotypes were grown in 1.5" x 8.25" containers (Hummert International, USA) and screened against leaf rust at two-leaf stage seedling in the

greenhouse. Each genotype was planted in three cones (three seeds/cone), and each cone with three seedlings was considered as one replication. The cultivars Bolls and Baart were used as a leaf rust resistant check and a susceptible check, respectively. The inoculum was prepared by mixing urediniospores in Soltrol oil and suspended in a capsule. The inoculum was sprayed on the seedlings using atomizer. The plants were placed in a dew chamber at 100% humidity for 24 hours. After 24h of incubation, plants were returned to the greenhouse. The seedlings were assessed for infection type using a 0 – 4 (0: nearly immune; 1: very resistant; 2: moderately resistant; 3: moderately resistant to moderately susceptible; and 4: very susceptible) ten days post-inoculation. The inoculum was kindly provided by Dr. Robert Bowden (USDA-ARS, Manhattan, KS).

Results

Reaction of spelt wheat genotypes to *P. tritici-repentis* race 1 and Ptr ToxA:

We screened 208 spelt wheat genotypes with tan spot (race 1). However, 13 genotypes were removed due to the data for less than six seedlings. We evaluated 195 genotypes and the reactions ranged from resistant to susceptible. Out of 195 genotypes, 9.2% (n=18) were rated susceptible and 28.4% (n=55) were rated moderately susceptible to tan spot race 1, whereas 14.8% (n=29) genotypes were rated moderately resistant, and 47.6% (n=93) genotypes were resistant to tan spot race 1. (Figure 3-2, 3-4, appendix table 2.1). As expected, the cultivars Salamouni and Glenlea developed resistance and susceptible reaction, respectively. The same 208 genotypes were also screened for reaction to Ptr ToxA; where only 3.3% (n=7) of the genotypes exhibited necrosis in the toxin infiltrated leaf area and were rated sensitive (Figure 3-5). The rest 96.6% (n=201) were rated as toxin insensitive as they did not develop necrosis symptoms. The cultivars Salamouni and

Glenlea were insensitive and sensitive reaction, respectively when infiltrated with Ptr ToxA.

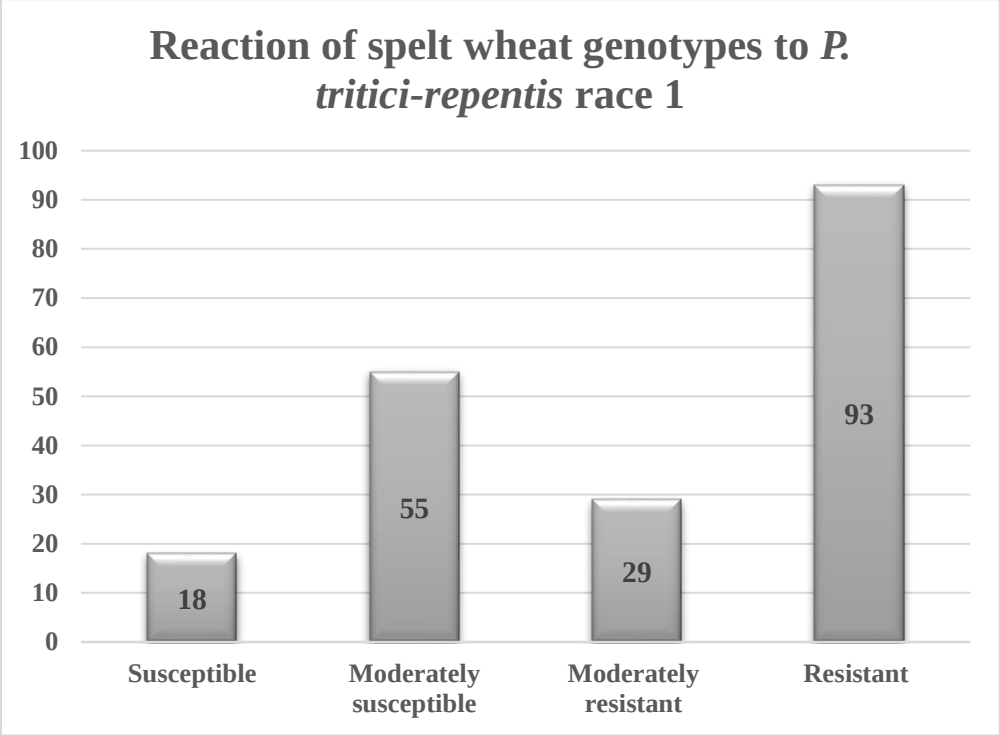

Figure 3-3: Reaction of spelt wheat genotypes to *P. tritici-repentis* race 1.

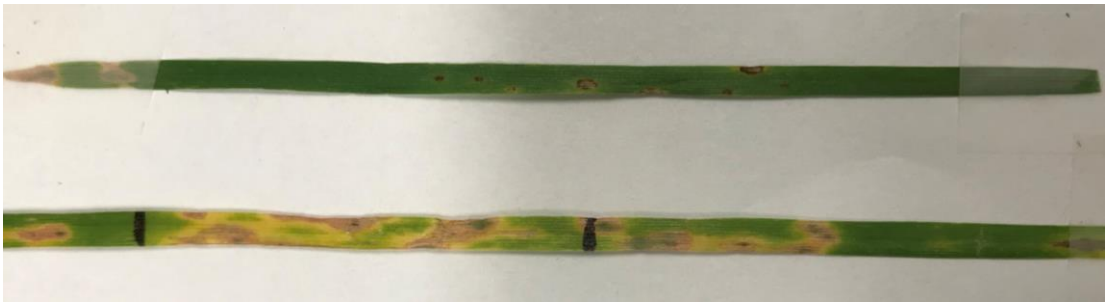

Figure 3-4: Spelt wheat genotype PI 290513 (top) inoculated with *P. tritici-repentis* race 1 exhibiting resistant reaction (small brown to black spots without chlorosis or tan necrosis) while genotype PI 290516 (bottom) with susceptible reaction (tan necrotic lesions surrounded by the chlorotic halo) when inoculated with *P. tritici-repentis* race 1

Figure 3-5: Spelt wheat genotype PI 572915 (top) sensitive to Ptr Tox A and PI 306553 (bottom) insensitive to Ptr Tox A.

Reaction of spelt wheat genotypes to *P. tritici-repentis* race 5 and Ptr ToxB:

Out of 208 spelt wheat genotypes 205 were screened for their reaction to tan spot race 5. 76.5% (n= 157) of the evaluated genotypes exhibited resistance to tan spot, whereas 4.8% (n=10) were moderately resistant. 14.1% (n=29) genotypes were moderately susceptible to tan spot race 5 and 4.3% (n=9) were susceptible (Figure 3-6 & 3-7 appendix table 2.1). As expected, the cultivars Salamouni and 6B662 developed resistance and susceptible reaction, respectively. The same genotypes were also screened for reaction to Ptr ToxB; where only 8.5% (n=18) of the genotypes exhibited chlorosis in the toxin infiltrated leaf area and were rated sensitive (Figure 3-8). The other 91.3% (n=190) were rated as toxin insensitive as they did not develop chlorosis symptoms. The cultivar Salamouni developed insensitive whereas cultivar 6B662 developed sensitive reaction when infiltrated with Ptr ToxB.

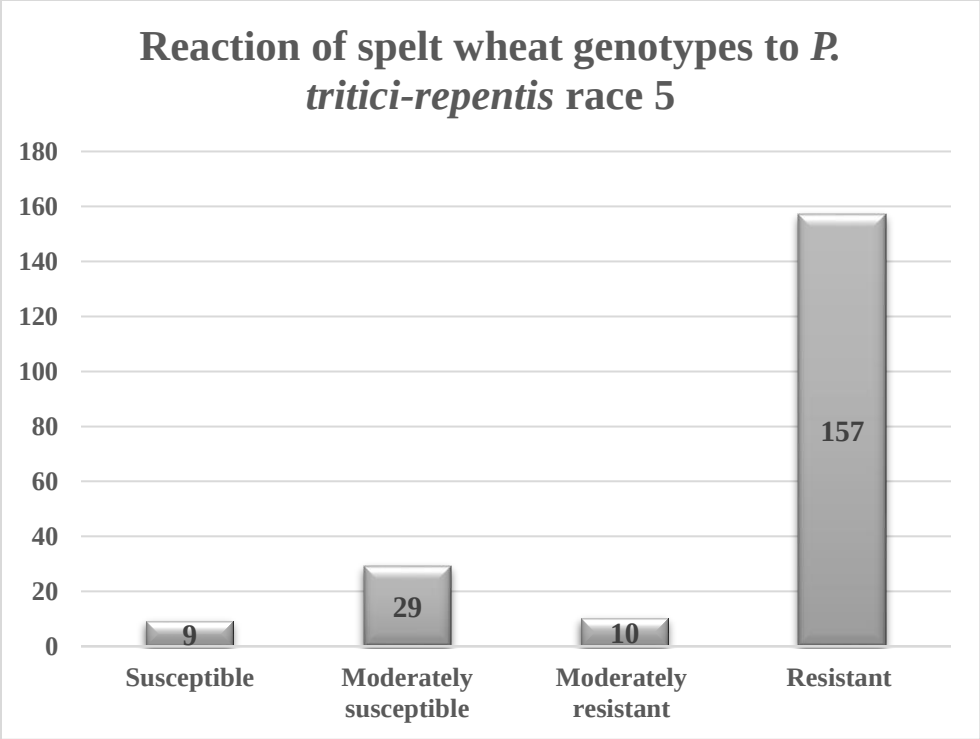

Figure 3-6: Reaction of spelt wheat genotypes to ***P. tritici-repentis*** **race 5**

Figure 3-7: Spelt wheat genotype PI 591901 (top) and genotype PI 306553 (bottom) inoculated with *P. tritici-repentis* race 5 showing resistant reaction and susceptible reaction, respectively.

Figure 3-8: Spelt wheat genotype PI 295060 (top) insensitive to Ptr Tox B and PI 290514 (bottom) sensitive to Ptr Tox B.

Reaction of spelt wheat genotypes to spot blotch:

We screened 208 spelt wheat genotypes with spot blotch isolate BS-10-97 and removed 31 genotypes having disease data of less than six seedlings. Out of 177 genotypes, 36.1% (n=64) were rated susceptible and 33.8% (n=60) were rated moderately susceptible to spot blotch, whereas 14.6% (n=26) genotypes were rated moderately resistant, and 15.2% (n=27) genotypes were resistant. (Figure 3-9, 3-10, appendix table 2.1). As expected, the cultivars Salamouni and Glenlea developed resistance and susceptible reaction, respectively. The same genotypes were also screened for reaction to BS ToxA; where only 3% (n=6) of the genotypes exhibited necrosis in the toxin infiltrated leaf area and were rated sensitive (Figure 3-11). The other 97% (n=202) were rated as toxin insensitive as they did not develop necrosis symptoms. The cultivar Salamouni did develop necrosis (insensitive) whereas cultivar Glenlea developed necrosis (sensitive) when infiltrated with BS ToxA.

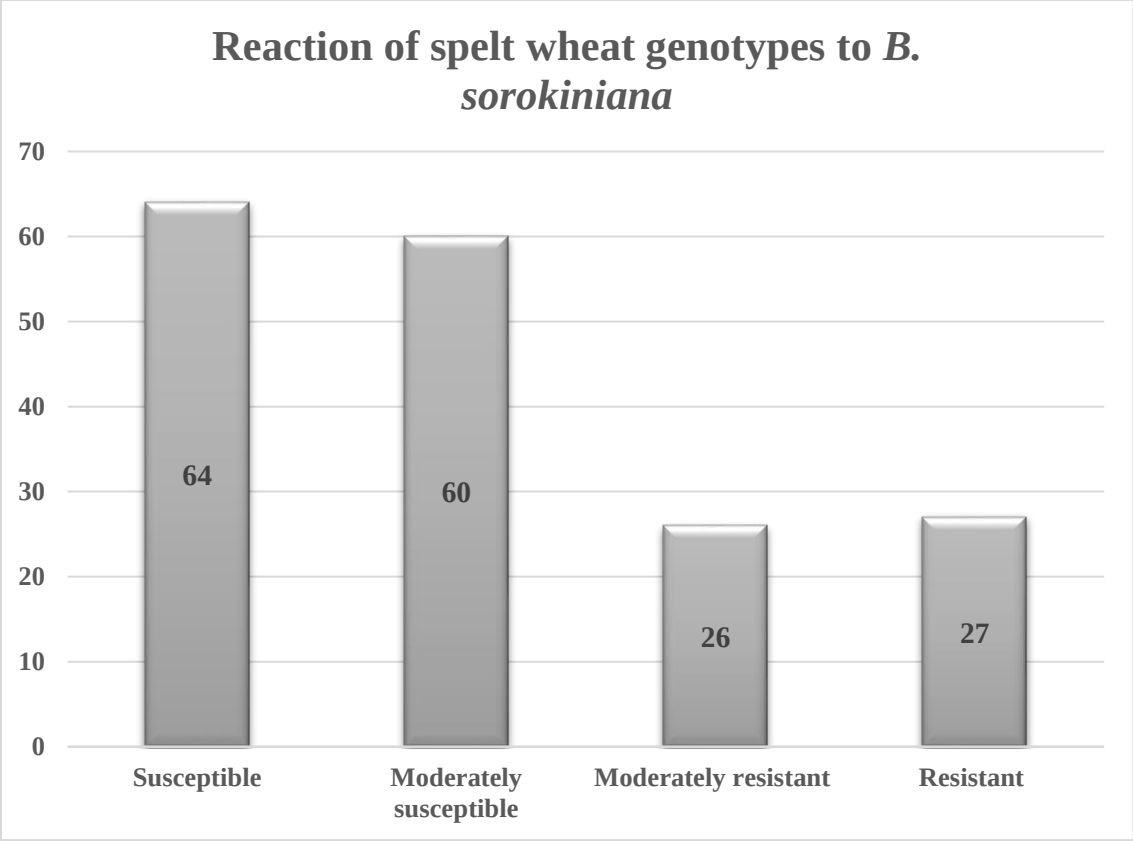

Figure 3-9: Reaction of spelt wheat genotypes to *B. sorokiniana*

Figure 3-10: Spelt wheat genotype PI 191394 (top) and genotype PI 674998 (bottom) inoculated with *B. sorokiniana* with small necrotic dark spots (resistant) and with elongated light to dark brown blotches (susceptible) symptoms.

Figure 3-11: Spelt wheat genotype PI 348111 (top) insensitive to BS ToxA and PI 348060 (bottom) sensitive to BS ToxA.

Reaction of spelt wheat genotypes to leaf rust:

We screened 208 spelt wheat genotypes and removed 14 genotypes with disease data of less than six seedlings. We evaluated 194 genotypes which varied in their reaction to leaf rust ranging from 51% susceptible (n =99), to 27.3% moderately susceptible (53), 11.3% moderately resistant (22), and 10.3% resistant (20) (Figure 3-12, 3-13, appendix table 2.1). As expected, the cultivars Bolls and Baart developed resistance and susceptible reaction, respectively.

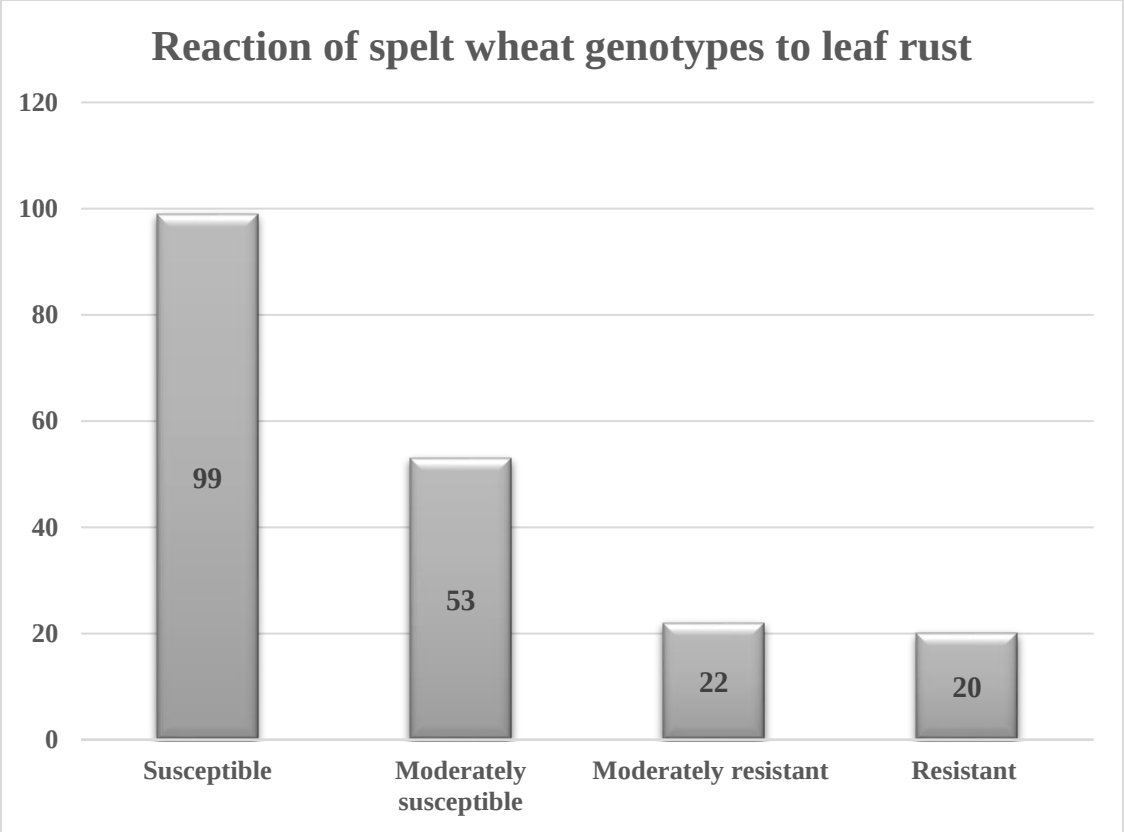

Figure 3-12: Reaction of spelt wheat genotypes to leaf rust.

Figure 3-13: Spelt wheat genotype PI 295060 (top) and genotype PI 191100 (bottom) inoculated with *Puccinia triticina.* exhibiting resistant reaction and susceptible reaction, respectively

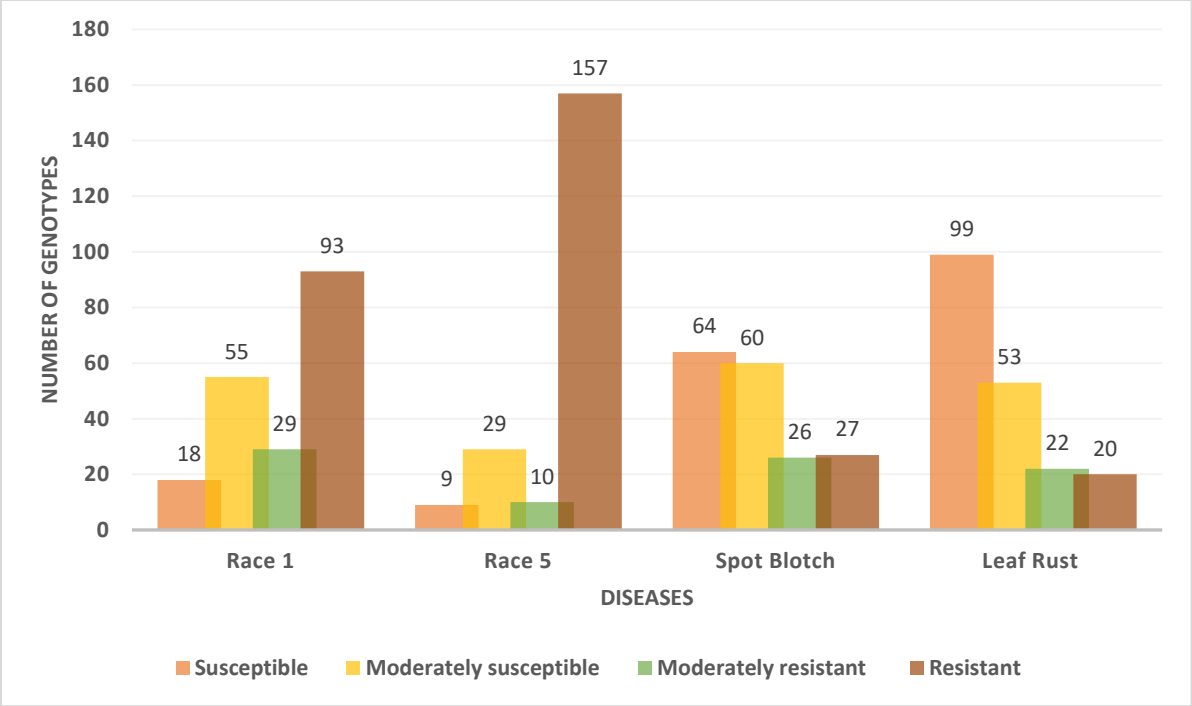

Figure 3-14: Phenotypic response of Spelt wheat against Tan spot race 1, race 5, spot blotch and leaf rust

The genotypes exhibited varying responses ranging from susceptible to moderately susceptible and moderately resistant to resistant (Fig 3-14). 16 multi-disease resistant genotypes (tan spot race 1 &5, spot blotch and leaf rust) were identified in the study (table 6). Resistant spelt accessions can further be evaluated for the agronomic traits in the field and that can aid in selection of superior spelt genotypes.

Table 6: Genotypes identified with multi disease resistance

Accession #	Country of origin	Ptr ToxA	Lesion Type	Race 1	Ptr ToxB	Lesion Type	Race 5	Spot blotch lesion type	Spot blotch	Leaf rust
PI 346853	Argentina	–	1	R	–	1	R	2	MR	R
348109 TR16ID	Germany	–	1	R	–	1	R	2.5	MR	MR
348279 TR16ID	Germany,	–	1	R	–	1	R	2.2	MR	MR
PI 355641	Germany	–	1	R	–	1	R	1.5	R	R
PI 290513	Hungary	–	1	R	–	1.4	R	2	MR	R
PI 290515	Hungary	–	1	R	–	1	R	2	MR	MR
PI 355652	Switzerland	–	1	R	–	1	R	1.3	R	R
PI 355668	Switzerland	–	1	R	–	1.8	R	1.9	R	MR
591898 TR13ID	Switzerland	–	1	R	–	1	R	2.1	MR	MR
347986 TR16ID	Switzerland	–	1	R	–	1	R	1.5	R	MR
PI 355638	Switzerland	–	1.1	R	–	1	R	2.1	MR	MR
PI 191394	Ethiopia	–	1.1	R	–	1	R	1.1	R	MR
PI 355616	Switzerland	–	1.1	R	–	1	R	1.3	R	R
PI 615238	Belgium	–	1.3	R	–	1.3	R	2.3	MR	MR
PI 347990	Switzerland	–	1.5	R	–	1	R	2.1	MR	MR
Cltr 15071 TR16ID	United States	–	1.8	R	–	1	R	1.4	R	MR

Discussion

In this study, we evaluated 208 spelt wheat accessions obtained from NSGC against tan spot (*P. tritici-repentis* race 1 and race 5) and Ptr ToxA, 117 against spot blotch, and 194 for leaf rust. We observed that 47.6% of the genotypes were resistant to tan spot (*P. tritici-repentis* race 1) (Fig 3-3) and 96.6% were insensitive to Ptr ToxA (Fig 3-5). Similarly, 76.5% of the genotypes were resistant to tan spot (*P. tritici-repentis* race 5) (Fig 3-6) and 91.5% were insensitive to Ptr ToxB (Fig 3-8). For spot blotch, the resistance level was low as compared to Ptr as only 15.2% genotypes showed resistant reaction (Fig 3-9). For leaf rust, we found only 10.3% genotypes that gave resistant reaction (Fig 3-12).

In a study by (Goriewa-Duba et al. 2020) wheat leaves were more affected by *Puccinia triticina* (Leaf rust) as compared to *Blumeria graminis* f. sp. *tritici*. They reported in 2017, the parental lines of *T. spelta* did not exhibit leaf rust. Similarly, most of the lines were also free of leaf rust symptoms in 2018. Mostly mild disease symptoms were noted in bread wheat in both years of the experiment. Leaf rust was observed in several bread wheat lines while only one wheat-spelt line was characterized by high susceptibility in both years of their study. They concluded that *Triticum spelta* can be a valuable donor of resistance genes that can be used in the pyramidization process. In our study, around 90% of the spelt accessions screened against leaf rust were susceptible. The reason behind this can be the variety of germplasm from different countries showing their response to leaf rust. We had genotypes from twenty countries belonging to 4 continents Africa, Asia, Europe, North America and all the genotypes showed varying responses.

In a study by (Abdullah et al. 2017) pre-epidemic cultivars of bread wheat and durum wheat were evaluated against stem rust and tan spot (*P. tritici-repentis* race 1). Their results did not show any correlation between stem rust susceptibility and tan spot resistance as well as the role of Ptr ToxA in the disease development. Their data suggests that resistance/susceptibility to stem rust is not likely associated with an increased susceptibility to tan spot. In our findings, there was no correlation between toxin sensitivity and susceptibility in spelt wheat against tan spot and leaf rust as in other studies (rye, durum, bread wheat).

In a study by Laribi et al. (2022) a collection of *P. tritici-repentis* isolates from Tunisia was tested for phenotypic race classification based on virulence on a host differential set and for the presence of the NE genes ToxA, ToxB, and toxb. The race composition in Tunisia was generally consistent with what has been reported elsewhere in North Africa. Race 7 was predominant in Algeria, whereas race 5 (47%) was most common in Morocco. In our findings, most of the genotypes were susceptible to race 1 as compared to race 5 based on the phenotypic characterization of the genotypes.

According to Arseniuk et al. (1991) winter spelt was more resistant to *P. nodorum* than spring wheat. In our finding, spelt wheat was only resistant to tan spot race 5 while susceptible to tan spot race 1, spot blotch and leaf rust. Singh et al. (2006) identified spelt wheat lines with resistance to the LS pathogens *P. tritici-repentis*, *L. norodum* and *M. graminicola*, and to all *P. tritici-repentis* races screened against. Spelt wheat was found to have lower leaf spot severity and leaf spot severity was positively associated with common wheat and durum wheat when tested by Fernandez et al. (2014). In our study, the genotypes exhibited varying responses ranging from susceptible to moderately

susceptible and moderately resistant to resistant. Sixteen multi-disease resistant genotypes (tan spot race 1 &5, spot blotch and leaf rust) were identified in the study (table 6). Resistant spelt accessions can further be evaluated for the agronomic traits in the field and that can aid in selection of superior spelt genotypes.

Literature Cited

Abdullah, S., Sehgal, S. K., Jin, Y., Turnipseed, B., and Ali, S. 2017. Insights into tan spot and stem rust resistance and susceptibility by studying the pre-green revolution global collection of wheat. The plant pathology journal 33:125.

Acharya, K., Dutta, A. K., and Pradhan, P. 2011. '*Bipolaris sorokiniana*'(Sacc.) Shoem.: The most destructive wheat fungal pathogen in the warmer areas. Australian Journal of Crop Science 5:1064-1071.

Ali, S., and Francl, L. 2001. Recovery of *Pyrenophora tritici-repentis* from barley and reaction of 12 cultivars to five races and two host-specific toxins. Plant disease 85:580-584.

Arseniuk, E., Fried, P., Winzeler, H., and Czembor, H. 1991. Comparison of resistance of triticale, wheat and spelt to Septoria nodorum blotch at the seedling and adult plant stages. Euphytica 55:43-48.

Bugingo. C., Byamukama. E., Ali. S. 2018. Tan spot of wheat. SDSU Extension. September 2018. http://igrow.org/agronomy/wheat/ tan spot of wheat/ https://extension.sdstate.edu/sites/default/files/2018-12/03-2008-2018.pdf

Campbell, K. G. 1997. Spelt: agronomy, genetics, and breeding. Plant Breeding Reviews 15:187-214.

Chand, R., Pandey, S., Singh, H., Kumar, S., and Joshi, A. 2003. Variability and its probable cause in natural populations of spot blotch pathogen *Bipolaris sorokiniana* of wheat (*T. aestivum* L.) in India/Variabilität und ihre wahrscheinliche Ursache in natürlichen Populationen des Erregers von Blattflecken (*Bipolaris sorokiniana*) an Weizen (*Triticum aestivum* L.) in Indien. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection:27-35.

Chowdhury, A. K., Singh, G., Tyagi, B. S., Ojha, A., Dhar, T., and Bhattacharya, P. M. 2013. Spot blotch disease of wheat–a new thrust area for sustaining productivity. Journal of Cereal Research 5.

De Wolf, E., Effertz, R., Ali, S., and Francl, L. 1998. Vistas of tan spot research. Canadian journal of Plant Pathology 20(4):349-444.

Duveiller, E., and Garcia Altamirano, I. 2000. Pathogenicity of *Bipolaris sorokiniana* isolates from wheat roots, leaves and grains in Mexico. Plant Pathology 49:235-242.

Duveiller, E., and Dubin, H. 2002. Helminthosporium leaf blights: spot blotch and tan spot. Bread Wheat: Improvement and Production, Plant Production and Protection Series 30:285-299.

Escarnot, E., Jacquemin, J.-M., Agneessens, R., and Paquot, M. 2012. Comparative study of the content and profiles of macronutrients in spelt and wheat, a review. BASE.

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

Fernandez, M., Fox, S., Hucl, P., and Singh, A. 2014. Leaf spotting reaction of spring common, durum and spelt wheat, and Kamut under organic management in western Canada. Canadian Journal of Plant Science 94:929-935.

Frakolaki, G., Giannou, V., Topakas, E., and Tzia, C. 2018. Chemical characterization and breadmaking potential of spelt versus wheat flour. Journal of Cereal Science 79:50-56.

Goriewa-Duba, K., Duba, A., Suchowilska, E., and Wiwart, M. 2020. An Analysis of the Genetic Diversity of Bread Wheat x Spelt Breeding Lines in Terms of Their Resistance to Powdery Mildew and Leaf Rust. Agronomy 10:658.

Gurung, S., Short, D., and Adhikari, T. 2013. Global population structure and migration patterns suggest significant population differentiation among isolates of *Pyrenophora tritici-repentis*. Fungal Genetics and Biology 52:32-41.

Hosford, R. M. 1982. Tan Spot of Wheat and Related Diseases Workshop, July 14-15, 1981, North Dakota Agricultural Experiment Station, North Dakota State University, Fargo, ND58105.

Kohajdová, Z., and Karovicova, J. 2008. Nutritional value and baking application of spelt wheat. Acta Scientiarum Polonorum Technologia Alimentaria 7:5-14.

Kolmer, J. A. 2005. Tracking wheat rust on a continental scale. Current opinion in plant biology 8:441-449.

Kumar, J., Schäfer, P., Hückelhoven, R., Langen, G., Baltruschat, H., Stein, E., Nagarajan, S., and Kogel, K. H. 2002. *Bipolaris sorokiniana*, a cereal pathogen of global concern: cytological and molecular approaches towards better control. Molecular Plant Pathology 3:185-195.

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

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

Mehta, Y. 1998. Constraints on the integrated management of spot blotch of wheat. Helminthosporium Blight of Wheat: Spot Blotch and Tan Spot:18-27.

Onishi, I., Hongo, A., Sasakuma, T., Kawahara, T., Kato, K., and Miura, H. 2006. Variation and segregation for rachis fragility in spelt wheat, *Triticum spelta* L. Genetic Resources and Crop Evolution 53:985-992.

Szewczyk, B. F. 2013. The influence of morphological features of spelt wheat (*Triticum aestivum* ssp. spelta) and common wheat (*Triticum aestivum* ssp. vulgare) varieties on the competitiveness against weeds in organic farming system. Journal of Food, Agriculture & Environment 11:416-421.

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

Sharma, R., and Duveiller, E. 2006. Spot blotch continues to cause substantial grain yield reductions under resource-limited farming conditions. Journal of Phytopathology 154:482-488.

Sharma, R., Duveiller, E., and Jacquemin, J. 2007. Microsatellite markers associated with spot blotch resistance in spring wheat. Journal of Phytopathology 155:316-319.

Singh, P., Singh, R., Duveiller, E., Mergoum, M., Adhikari, T., and Elias, E. 2010. Genetics of wheat–*Pyrenophora tritici-repentis* interactions. Euphytica 171:1-13.

Starzyńska-Janiszewska, A., Stodolak, B., Socha, R., Mickowska, B., and Wywrocka-Gurgul, A. 2019. Spelt wheat tempe as a value-added whole-grain food product. LWT 113:108250.

Steffenson, B., O'Boyle, P., Brooks, W., Barnett, M., Berger, G., Stromberg, E., Maroof, S., Liu, S., and Griffey, C. 2014. Mapping net blotch resistance in 'Nomini'and CIho 2291 barley. Crop Science 54:2596-2602.

Suchowilska, E., Kandler, W., Sulyok, M., Wiwart, M., and Krska, R. 2010. Mycotoxin profiles in the grain of *Triticum monococcum*, *Triticum dicoccum* and *Triticum spelta* after head infection with *Fusarium culmorum*. Journal of the Science of Food and Agriculture 90:556-565.

Sugár, E., Fodor, N., Sándor, R., Bónis, P., Vida, G., and Árendás, T. 2019. Spelt wheat: An alternative for sustainable plant production at low N-levels. Sustainability 11:6726.

Wegulo, S., Klien, R., and Harveson, R. 2011a. Tan spot of cereals. Plant Health Instructor. DOI: 10.1094/PHI-I-2011-0426-01.

Wu, L., He, X., Lozano, N., Zhang, X., and Singh, P. K. 2021. ToxA, a significant virulence factor involved in wheat spot blotch disease, exists in the Mexican population of *Bipolaris sorokiniana*. Tropical Plant Pathology 46:201-206.

Zillinsky, F. J. 1983. Common diseases of small grain cereals. A guide to identification. Common diseases of small grain cereals. A guide to identification. CIMMYT.

CHAPTER 4

EVALUATION OF PRESENCE OF TOXA GENE IN SOUTH DAKOTA *BIPOLARIS SOROKINIANA* POPULATION

Abstract

Many necrotrophic leaf spot pathogens produce host selective effectors (toxins), which are associated with disease development. The fungi *Pyrenophora tritici-repentis, Parastagonospora nodorum* and *Bipolaris sorokiniana* cause tan spot, septoria nodorum blotch (SNB), and spot blotch, respectively in wheat and they impact crop production in the US. *P. tritici-repentis* and *P. nodorum* are known to produce a host-selective effector ToxA which is associated with tan spot and SNB disease development, however, recently, ToxA was reported from *Bipolaris sorokiniana* isolates collected from Texas and may be associated with spot blotch development. In this study, 127 *B. sorokiniana* isolates recovered from wheat in South Dakota were genotyped if they carry *ToxA* gene. Further, culture filtrates of two *B. sorokiniana* isolates of each with and without *ToxA* gene were infiltrated for their reaction into leaves of two *P. tritici repentis* ToxA sensitive (Boost and Forefront) and two *P. tritici repentis* ToxA insensitive (Linkert and Prevail) hard red spring wheat varieties. 53.5% (n=68) of 127 isolates amplified the *ToxA* gene. Further, the culture filtrates of isolates with the *ToxA* gene produced necrosis symptoms in ToxA sensitive wheat cultivars. The culture filtrates of the isolates lacking in the *ToxA* gene did not produce any symptoms in all four wheat cultivars. Our results indicate that a diverse population of *B. sorokiniana* prevalent on wheat in South Dakota. Developing ToxA insensitive wheat cultivars may help in the management of all three-

leaf spot diseases tan spot, SNB, and spot blotch and hence reduce their impact on wheat productivity.

Introduction

Spot blotch caused by *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*) is one of the most concerning diseases in wheat. The fungus can also cause common root rot, seedling blight, and black point of wheat and barley (Acharya et al. 2011; Chowdhury et al. 2013; Kumar et al. 2002; McDonald et al. 2018; Sharma and Duveiller 2006; Wu et al. 2021). The morphological and physiological variability in *B. sorokiniana* isolates have been reported (Chand et al. 2003). *B. sorokiniana* has extensive distribution in wheat growing regions and it causes severe symptoms in warmer and humid parts of the world (Chowdhury et al. 2013). The average losses in yield due to spot blotch ranges between 15-20% on average (Acharya et al. 2011) but in susceptible genotypes it can go up. Spot blotch severity has been reported ranging 70-100% in Nepal (Sharma et al. 2007). (Mehta 1998) reported that in severe conditions the disease can cause 100% yield losses in Latin America. In *Bipolaris*, the conidia look fucoid, straight, or curved and germinate by one germ tube from each end (bipolar germination). This specific kind of conidia germination was one of the major reasons after the naming and grouping it into new genera (Shoemaker 1959).

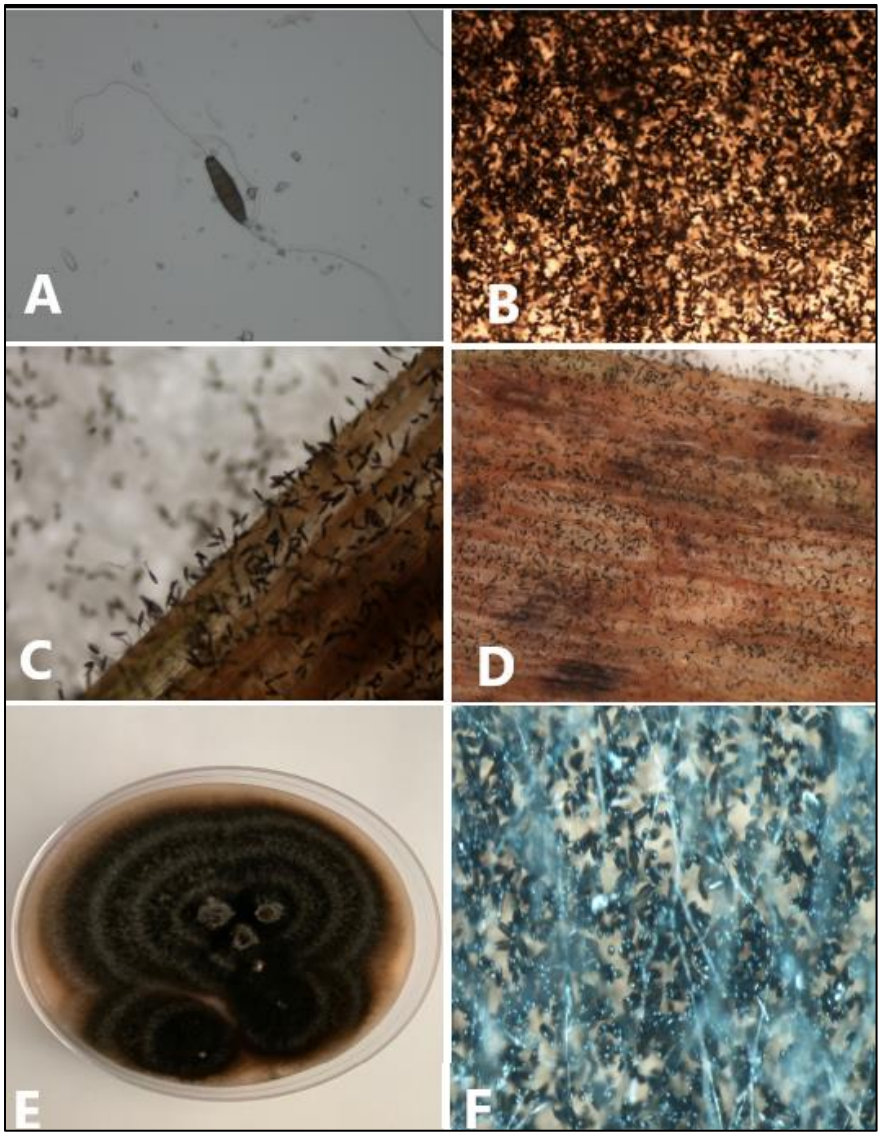

Figure 4-1: *Bipolaris sorokiniana* germinated single spore. (B) *B. sorokiniana* spores germinating on V8-PDA plate. (C) Spores emerging from leaf inoculated with *B. sorokiniana* isolate. (D) Microscopic image of spring wheat leaf surface containing spores. (E) *B. sorokiniana* isolate growing on V8-PDA. (F) Mycelia and *B. sorokiniana* spores

B. sorokiniana produces the pathogenicity factors that includes the phytotoxins and hydrolytic enzymes inducing necrosis in plant tissues (Han et al. 2010). The necrotrophic

effector ToxA is the fungal pathogenicity factor in wheat genotypes along with a corresponding susceptible factor ToxA sensitivity gene *Tsn1* which was identified in wheat (Ciuffetti et al. 1998): (Faris et al. 2010). *P. tritici-repentis* acquired *ToxA* gene by horizontal gene transfer from *Parastagonospora nodorum*, which led to the development of a highly virulent pathogen that causes tan spot all over the world (Friesen et al. 2006). ToxA was then observed in *Phaeosphaeria avenaria* f. sp. *tritici*, and it showed that the interspecific hybridization between this pathogen and its sister species and *P. nodorum* was the important factor in transferring ToxA (McDonald et al. 2012). Now ToxA has been identified in a fourth fungal pathogen, *B. sorokiniana*, from many countries including the USA (Friesen et al. 2018), Mexico (Wu et al. 2020b), Australia (McDonald et al. 2018), and India (Navathe et al. 2020). All three wheat pathogens (*P. nodorum, P. tritici-repentis*, and *B. sorokiniana*) have a ~ 14 kb regions surrounding *ToxA* and their comparative analysis provides evidence that the *ToxA* region is flanked by terminal inverted repeats. This entire transposon feature may enable a horizontal gene transfer between fungal genomes (McDonald et al. 2019b). In our study, we tested if *B. sorokiniana* population in wheat from South Dakota harbor *ToxA* gene (BS *ToxA*) like *P. tritici-repentis* and *P. nodorum*. We also screened hard red spring wheat for their reaction to spot blotch.

Materials and Methods

Isolates used in the study:

127 *B. sorokiniana* isolates were recovered from the wheat leaves and root samples collected from commercial and experimental research plots from 2012 through 2020 in South Dakota (Kaur et al. 2016 and Dr. Ali-unpublished) (Appendix table 2.2).

Molecular Characterization of *B. sorokiniana* isolates for *ToxA* gene:

Isolate storage and DNA extraction:

All fungal isolates taken out of the freezer were grown on V8-PDA at 22ºC under a 12-h light and 12-h dark cycle for 5-7 days. For long term storage the mycelia of each isolate were cut into 0.3-0.5cm plugs from the agar surface with a sterile blade and dried in the running laminar flow hood for 24 hours. Dried mycelial plugs were stored in a 2-ml microfuge tube and frozen at -20°C. for DNA extraction, mycelia for each isolate were scraped off from petri plate and stored at -20°C in a 2-ml microfuge tube. The garnet grains and a bead were added to the fungal mycelia (50-100mg) in a 2-ml tube. 500 µl lysis buffer (400mM Tris-HCl [pH 8.0], 60 mM EDTA [pH 8.0], 150 mM NaCl, 1% Sodium dodecyl sulfate) was added to the 2-ml tube and the fungal mycelia was homogenized using a FastPrep instrument. The tubes were then left at room temperature for 10 minutes. In each tube, 150 µl of freshly prepared solution III was added and mixed by brief vortex and spun at 14,000 rpm for 1 minute with a microcentrifuge. The supernatant (approximately 500 µl) was transferred to a new Eppendorf tube and an equal amount of isopropyl alcohol was added and mixed by brief inversion and the tubes were centrifuged at 14,000 rpm for 2 minutes. The supernatant was discarded, and the resultant DNA pellet was washed in 300 µl of 70% ethanol. The tubes were spun again at 10,000 rpm for 1 minute and the supernatant was discarded. The DNA pellet was air dried for 10 minutes and dissolved in 50 µl of 1x TE or ddH2O. DNA concentration was normalized to 30-100 ng/µl using a Nano drop machine.

PCR Amplification:

All isolates were genotyped for the presence or absence of the *ToxA* gene using *ToxA* (*Ptr ToxA*) specific primers (226bp upstream and 161 bp downstream) (Andrie et al. 2007b). ITS primers were also used as a positive control as ITS1 and 4 are by far the most used in differentiating and identifying a species because they amplify the highly variable ITS region (White et al. 1990). The volume of the PCR reaction mixture was 20 µl; 2 µl genomic DNA (25ng/ µl), 1 µl of each primer, 2 µl dNTP (1 mM), 2 µl 10 x thermophol buffer, 0.2 µl 10 U/ml taq polymerase and 11.8 µl water. PCR reaction was conducted in a S-1000 thermal cycler (BioRad, USA) using amplification steps of 95°C for 3 minutes, followed by 30 cycles of 95°C for 30 seconds, 60°C for 30 seconds and 72°C for 35 seconds with final extension of 72°C for 5 minutes. The amplified products were electrophoresed on 1.5% agarose gels and scored with reference to 1 Kb ladder (New United Kingdom Biolabs, USA). Known *P. tritici repentis* isolates, Pti2 (Ptr race 1) and 86-124 (Ptr race 2) were used as positive control as both carrying *ToxA* gene. DW7 (Ptr Race 5) was also included as a negative control for the confirmation as it lacks *ToxA* gene and contains *ToxB* gene.

Gel Electrophoresis:

PCR products were electrophoresed on 1.5% agarose gels and scored with reference to 100 bp ladder (New United Kingdom Biolabs, Ipswich, MA, 01938, USA). Photographs were taken in Alpha Innotech FluorChem Transilluminator (Alpha Innotech Corp., San Leandro, CA).

Infiltration of *B. sorokiniana* culture filtrate:

Based on the findings of molecular characterization using *ToxA* specific primer, Twelve *B. sorokiniana* isolates with *ToxA* (n = 6; *ToxA* +) and without *ToxA* (n=6; *ToxA* -) were

selected randomly and prepared their culture filtrate. Mycelial plugs of the isolates were grown on V8-PDA for 5-7 days and plugs were transferred to 125 ml flasks containing 50ml Fries medium (Jahani et al. 2014). The flasks were covered with aluminum foil and plugs were grown in the dark for 3 weeks. Culture filtrates were obtained by filtering the content of through Whatman filter paper # 1 and 0.47µm membrane (Jahani et al. 2014). Eighteen spring wheat varieties along with the differentials Glenlea (Ptr ToxA sensitive) and Salamouni (Ptr ToxA insensitive) were infiltrated with the culture filtrate at two-leaf-stage seedlings and their reaction was observed after 3 to 5 days. The undiluted culture filtrates of four isolates, two isolates *ToxA*+ (BS-10-114 and BS-10-97) and *ToxA*- (BS-10-77 and BS-10-116) were infiltrated to the spring wheat cultivars seedlings using a 1ml needleless syringe to verify the presence of BS *ToxA* in *B. sorokiniana* culture filtrates. All 18 spring wheat varieties and checks were also infiltrated with distilled sterile water, Fries medium and Ptr ToxA, along with the culture filtrate from BS ToxA (Fig 4-2).

Figure 4-2: Infiltrating the leaves of hard spring wheat variety Boost with culture filtrate of *Bipolaris sorokiniana* ToxA+ and ToxA- isolates.

Spore production and Inoculation:

The isolates with *ToxA* (BS-10-114 and BS-10-97) and Without *ToxA* (BS-10-77 and BS-10-116) were grown on V8-PDA for 5-7 days. One dry plug of *B. sorokiniana* isolate BS-10-97 and BS-10-77 were placed in the center of each fresh V-8PDA plate (ten plates) and was grown under 12 hours light and dark cycle for 5-6 days. After 6 days, 30 ml of distilled sterile water was added to each plate, and conidia were collected the same way as in Ptr with a loop wired needle and spore suspension was adjusted to 3000 spores/ml prior to inoculations. A drop of Tween-20 (polyoxyethylene-20 sorbitan monolaurate (Sigma-Aldrich, St. Louis, MO, USA) was added to spore suspension as a dispersing agent. All 18 hard red spring wheat cultivars and the differential cultivars Glenlea and Salamouni were inoculated with the 3000 spores/ml spore suspension on a fully expanded third leaf of the plants. The inoculated plants were placed in the humidity chamber at 100% humidity for 24 hours. The humidifiers were set to release mist for 15 seconds every 10 minutes to maintain a humid environment for disease development. The plants were then transferred to greenhouse set at 24°C /16°C (day/night) temperature and 12-hour light photoperiod until the disease was rated ten days after inoculation. The infection responses of each genotype against the pathogen was assessed based on the five-class (1–5) rating scale used by (Lamari and Bernier 1989). Infection responses were based on the presence of necrosis and chlorosis and relative size of lesions observed on the third leaves of the seedlings.

Results

Molecular Characterization of *ToxA* from *B. sorokiniana* isolates:

We investigated the presence of *ToxA* in *Bipolaris sorokiniana* affecting the wheat population in South Dakota. Based on molecular characterization of the *B. sorokiniana* isolates using *ToxA* gene specific primer, the presence of the *ToxA* gene was revealed in 68 out of the 127 *B. sorokiniana* isolates (53.5%) (Fig 4-3, 4-4 & 4-5, Table 1). 56 isolates out of 127 (44%) collected during multiple years (2010-2020) had *ToxA* gene.

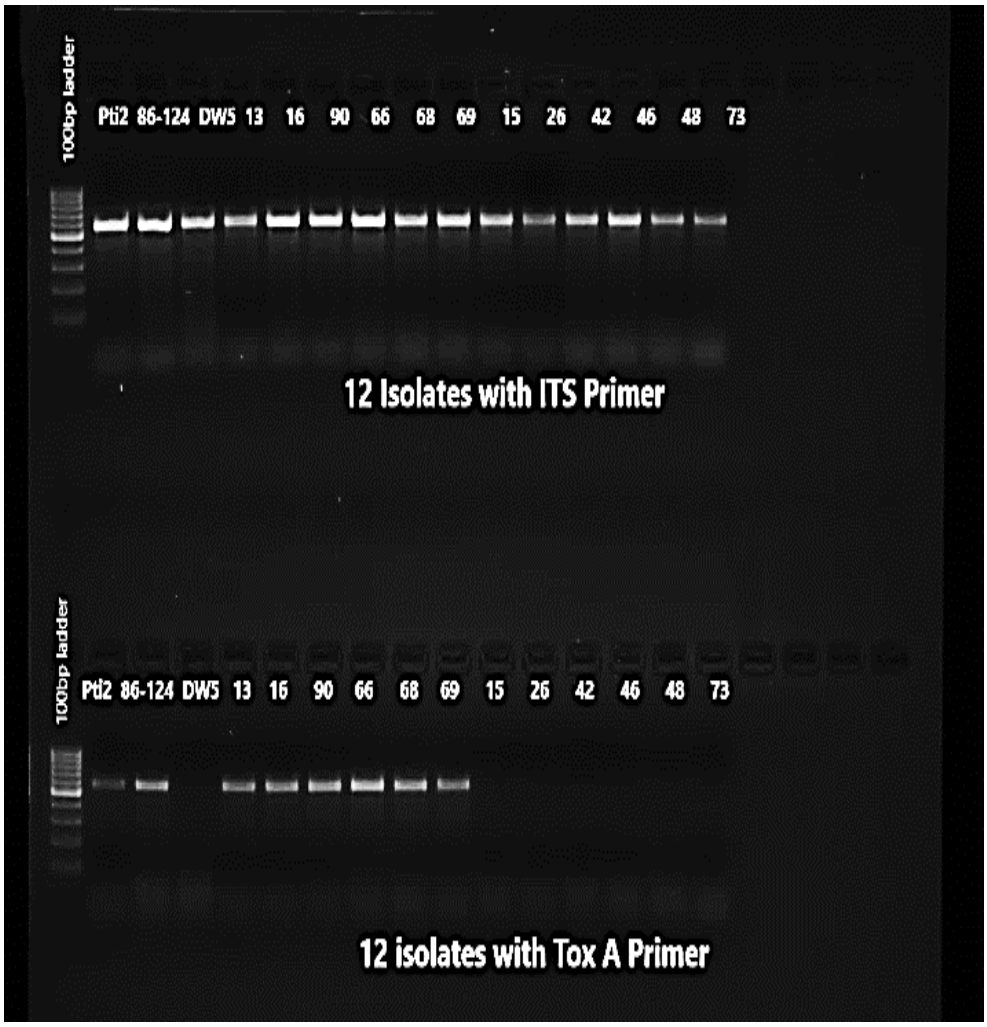

Figure 4-3: Amplification of 12 *Bipolaris sorokiniana* isolates with ITS and *ToxA* gene primers. The isolates possessing *ToxA* gene amplified at 500bp whereas the isolates lacking Tox A did not amplified. PCR products were separated in a 1.5% agarose gel and stained with ethidium bromide.

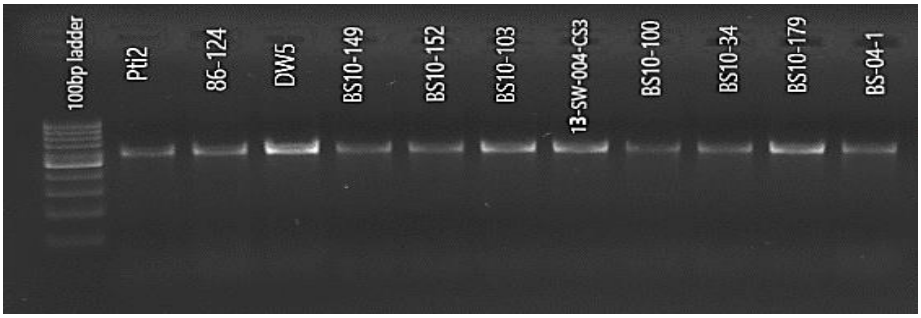

Figure 4-4: Polymerase Chain Reaction of ITS from *Bipolaris sorokiniana* isolates confirming the presence of fungal DNA.

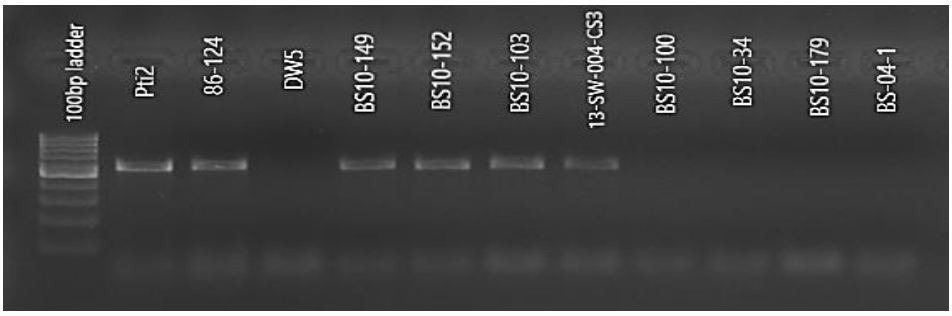

Figure 4-5: Polymerase chain Reaction of *ToxA* gene from four *Bipolaris sorokiniana* isolates (BS10-149 to 13-SW-004-CS3) confirming presence of *BS ToxA* yielding 585bp bands and four *B. sorokiniana* isolates (BS10-100 to BS-04-1) lacking *BS ToxA*. *Pyrenophora tritici-repentis* isolates, Pti2 (race 1) and 86-124 (race 2) with *ToxA* gene and isolate DW5 (race 5) without *ToxA* gene used as the positive and negative control, respectively.

Culture filtrate assay:

The culture filtrates of the two *ToxA*+ isolates produced necrosis symptoms on Glenlea, while the two *ToxA*- isolates did not produce these symptoms. Salamouni was found insensitive to the isolates with *ToxA*+ or without *ToxA*-. As expected, infiltration with Ptr

ToxA and filtrates of (BS-10-114 and BS-10-97) induced necrosis in Glenlea, whereas the Fries medium did not cause any symptoms (Fig 4-7).

The cultivars that were sensitive to Ptr ToxA were also infiltrated with BS isolates (BS-10-114 and BS-10-97) filtrates. The culture filtrates were infiltrated for their response into leaves of two *ToxA* sensitive ('Boost' and 'Forefront') and two *ToxA* insensitive ('Linkert' and 'Prevail') hard spring wheat cultivars at two-leaf-stage seedlings (Fig 4-6). All 18 spring wheat varieties were infiltrated with distilled sterile water, Fries medium and Ptr *ToxA* in the study.

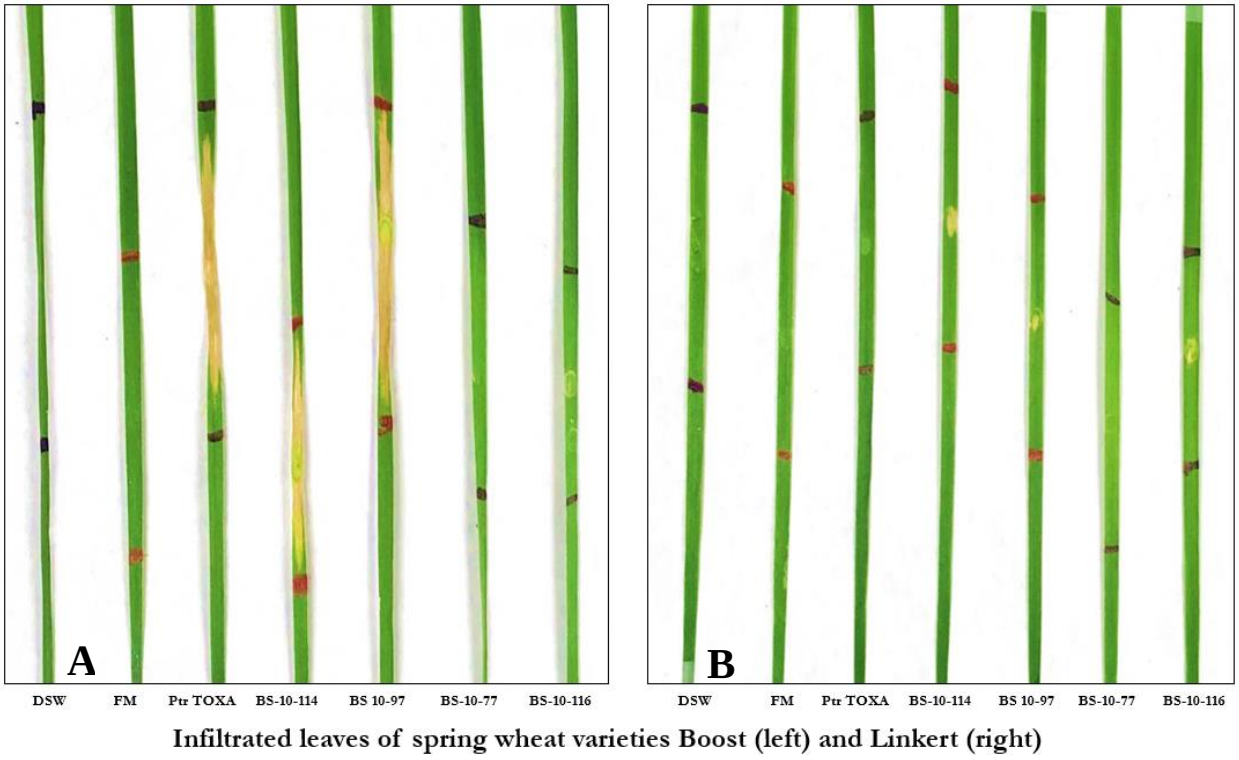

Figure 4-6: (L-R). Hard red spring (HRS) wheat cultivar 'Boost' (*Ptr* ToxA sensitive) infiltrated with distilled sterile water, Friese medium, Tox A, culture filtrate of *B. sorokiniana* isolate "BS-10 -97" and isolate "BS-10-114" with ToxA gene (exhibiting necrosis), culture filtrate of *B. sorokiniana* isolate "BS-10 -77" and

isolate "BS-10-116" without *ToxA* gene. B. (L-R). Hard red spring (HRS) wheat cultivar 'Linkert' (Ptr ToxA insensitive) infiltrated with distilled sterile water, Friese medium, ToxA, culture filtrate of *B. sorokiniana* isolate "BS-10 -97" and isolate "BS-10-114" with ToxA gene (not exhibiting necrosis), culture filtrate of *B. sorokiniana* isolate "BS-10 -77" and isolate "BS-10-116" without ToxA gene.

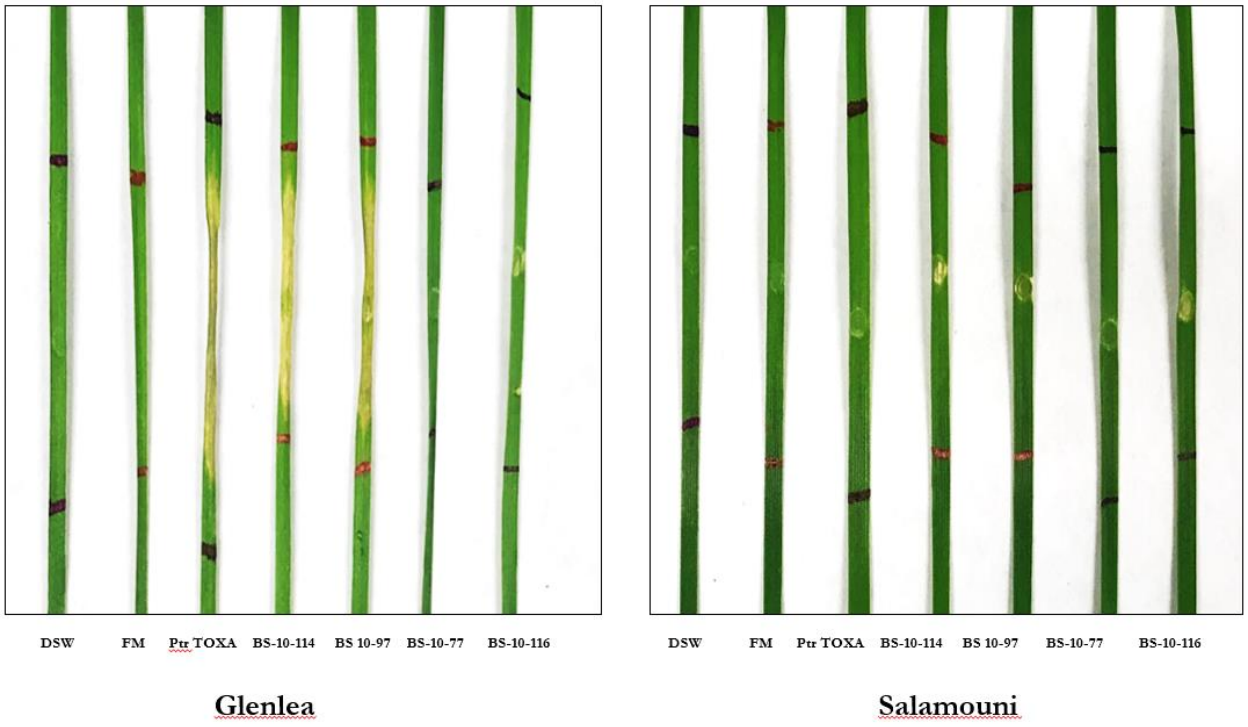

Figure 4-7: BS isolates filtrates reaction on Glenlea and Salamouni

Inoculation of *Bipolaris sorokiniana* spore suspension on wheat cultivars:

Eighteen hard red spring wheat varieties along with cultivars Glenlea and Salamouni as checks were inoculated with the spore suspension of the two isolates containing and lacking Tox A. The inoculations were performed using the Tox A (+) isolate BS-10-97 and Tox A (–) isolate BS-10-116 at three different times to evaluate the susceptibility. In the first inoculations, 83% of the spring wheat varieties were moderately susceptible to

susceptible (MS-S) when inoculated with Tox A (+) isolate BS-10-97. Whereas 94% of the spring wheat varieties were moderately resistant to resistant (MR-R) when inoculated with Tox A (–) isolate BS-10-116. The second time the inoculations were performed was in the month of June when the temperature in the greenhouse gets slightly higher than usual. There were 88% of the spring wheat varieties to be moderately susceptible to susceptible (MS-S) when inoculated with Tox A (+) isolate BS-10-97. 61% of the spring wheat varieties were moderately susceptible to susceptible (MS-S) when inoculated with Tox A (–) isolate BS-10-116. This high number of susceptibilities can be attributed to the temperature change. Genotypes Boost, Forefront, and six others were found to be susceptible to Tox A (+) isolate BS-10-97 and were also sensitive to the culture filtrate.

Figure 4-8: Leaves of Glenlea (left) and Salamouni (right) inoculated with Tox A (+) isolate BS-10-97

Discussion

In this study, we explored the occurrence of ToxA in 127 *B. sorokiniana* isolates collected during a period of 2010 to 2020 in South Dakota. The isolates were recovered from wheat roots and leaves samples collected from commercial and experimental field plots. In this study, we identified 68 (53.5%) isolates with *ToxA* gene and have designated them as ToxA+ isolates. In an independent study by Wu et al. (2021) 196

isolates of *B. sorokiniana*, collected from wide geographical locations, prone to spot blotch epidemic regions in Mexico, were characterized for *ToxA* gene. Only twenty isolates harbored *ToxA* gene, indicating that such isolates were present in most of the Mexican *B. sorokiniana* populations.

(Navathe et al. 2020) reported presence of ToxA+ isolates from India in 70% of the isolates tested. Similarly, *ToxA* was found in 86.7% of the isolates from USA (Friesen et al. 2018). In Australia, ToxA was found in 34.2% which was lower as compared to other findings the USA and India, as well as to the nearly 100% occurrence of *ToxA* in Australian *P. nodroum* and *P. tritici-repentis* populations (Antoni et al. 2010; McDonald et al. 2018; McDonald et al. 2013). In our study, we found the frequency of occurrence of *ToxA* to be 53.5%. These results are less than those reported from USA and India and greater than those reported from Mexico and Australia. The lower percentage of occurrence of ToxA+ suggests that the isolates evaluated do not carry *Tox A gene.* In warm, humid regions of the world, spot blotch has been a major problem on wheat and a little is known about how the pathogen is infecting the host. (Friesen et al. 2018) evaluated three accessions for ToxA sensitivity and found that all three accessions were sensitive to purified ToxA showing that these accessions carry *Tsn1*. This is significant because it shows that *B. sorokiniana* isolates in the regions where spot blotch has major economic impact may already harbor ToxA.

Previously, this disease was not of a major concern in the United States or Canada. However, numerous distinct or coalescing necrotic lesions were identified on the popular hard red winter wheat cultivar Duster at a wheat breeding nursery in Castroville, TX (Friesen et al. 2018). The lower percentages from Mexican *B. sorokiniana* isolates

(10.2%) indicates that majority of the isolates do not carry *ToxA* and might have not been subjected to strong selection against the susceptible gene *Tsn1* in wheat. It is believed that *P. tritici-repentis, P. nodorum,* and *B. sorokiniana* are differentially effective under different environmental conditions. To avoid the impact of this virulence factor, present in all three major wheat pathogens, it is suggested to select against *Tsn1* as it has never been shown to contribute positively to wheat.

Based on the effects of ToxA on the fungal pathogenicity of *B. sorokiniana*, *P. tritici-repentis*, and *P. nodorum*, it is recommended to investigate its prevalence in global populations of the three pathogens, especially in the wheat production regions. Our results indicate that there is a diverse population of *B. sorokiniana* prevalent on wheat in South Dakota. Developing ToxA, insensitive wheat cultivars may help in the management of all three-leaf spot diseases tan spot, SNB, and spot blotch and hence reduce their impact on wheat productivity. Further, to our knowledge, this is the first report of *ToxA* (BS *ToxA*) producing *B. sorokiniana* isolates from South Dakota.

Literature Cited

Acharya, K., Dutta, A. K., and Pradhan, P. 2011. '*Bipolaris sorokiniana*'(Sacc.) Shoem.: The most destructive wheat fungal pathogen in the warmer areas. Australian Journal of Crop Science 5:1064-1071.

Andrie, R. M., Pandelova, I., and Ciuffetti, L. M. 2007b. A combination of phenotypic and genotypic characterization strengthens *Pyrenophora tritici-repentis* race identification. Phytopathology 97:694-701.

Antoni, E. A., Rybak, K., Tucker, M. P., Hane, J. K., Solomon, P. S., Drenth, A., Shankar, M., and Oliver, R. P. 2010. Ubiquity of ToxA and absence of ToxB in

Australian populations of *Pyrenophora tritici-repentis*. Australasian Plant Pathology 39:63-68.

Chand, R., Pandey, S., Singh, H., Kumar, S., and Joshi, A. 2003. Variability and its probable cause in natural populations of spot blotch pathogen *Bipolaris sorokiniana* of wheat (*T. aestivum* L.) in India/Variabilität und ihre wahrscheinliche Ursache in natürlichen Populationen des Erregers von Blattflecken (*Bipolaris sorokiniana*) an Weizen (*Triticum aestivum* L.) in Indien. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection:27-35.

Chowdhury, A. K., Singh, G., Tyagi, B. S., Ojha, A., Dhar, T., and Bhattacharya, P. M. 2013. Spot blotch disease of wheat–a new thrust area for sustaining productivity. Journal of Cereal Research 5.

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

Faris, J. D., Zhang, Z., Lu, H., Lu, S., Reddy, L., Cloutier, S., Fellers, J. P., Meinhardt, S. W., Rasmussen, J. B., and Xu, S. S. 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proceedings of the National Academy of Sciences 107:13544-13549.

Friesen, T., Holmes, D., Bowden, R., and Faris, J. 2018. ToxA is present in the US *Bipolaris sorokiniana* population and is a significant virulence factor on wheat harboring Tsn1. Plant disease 102:2446-2452.

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

Han, Q., Huang, L., Buchenauer, H., Wang, C., and Kang, Z. 2010. Cytological study of wheat spike infection by *Bipolaris sorokiniana*. Journal of phytopathology 158:22-29.

Jahani, M., Aggarwal, R., Gupta, S., Sharma, S., and Dureja, P. 2014. Purification and characterization of a novel toxin from *Bipolaris sorokiniana*, causing spot blotch of wheat and analysis of variability in the pathogen. Cereal research communications 42:252-261.

Kaur, N. 2016. Root rot pathogens of wheat in South Dakota and their affect on seed germination and seedling blight in spring wheat cultivars. South Dakota State University.

Kumar, J., Schäfer, P., Hückelhoven, R., Langen, G., Baltruschat, H., Stein, E., Nagarajan, S., and Kogel, K. H. 2002. *Bipolaris sorokiniana*, a cereal pathogen of global concern: cytological and molecular approaches towards better control. Molecular Plant Pathology 3:185-195.

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

McDonald, M. C., Razavi, M., Friesen, T. L., Brunner, P. C., and McDonald, B. A. 2012. Phylogenetic and population genetic analyses of *Phaeosphaeria nodorum* and its

close relatives indicate cryptic species and an origin in the Fertile Crescent. Fungal Genetics and Biology 49:882-895.

McDonald, M. C., Oliver, R. P., Friesen, T. L., Brunner, P. C., and McDonald, B. A. 2013. Global diversity and distribution of three necrotrophic effectors in *Phaeosphaeria nodorum* and related species. New Phytologist 199:241-251.

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

McDonald, M. C., Taranto, A. P., Hill, E., Schwessinger, B., Liu, Z., Simpfendorfer, S., Milgate, A., and Solomon, P. S. 2019b. Transposon-mediated horizontal transfer of the host-specific virulence protein ToxA between three fungal wheat pathogens. MBio 10:e01515-01519.

Mehta, Y. 1998. Constraints on the integrated management of spot blotch of wheat. Helminthosporium Blight of Wheat: Spot Blotch and Tan Spot:18-27.

Navathe, S., Yadav, P. S., Chand, R., Mishra, V. K., Vasistha, N. K., Meher, P. K., Joshi, A. K., and Gupta, P. K. 2020. ToxA–Tsn1 interaction for spot blotch susceptibility in Indian wheat: An example of inverse gene-for-gene relationship. Plant disease 104:71-81.

Sharma, R., and Duveiller, E. 2006. Spot blotch continues to cause substantial grain yield reductions under resource-limited farming conditions. Journal of Phytopathology 154:482-488.

Sharma, R., Duveiller, E., and Jacquemin, J. 2007. Microsatellite markers associated with spot blotch resistance in spring wheat. Journal of Phytopathology 155:316-319.

Shoemaker, R. 1959. Nomenclature of Drechslera and Bipolaris, grass parasites segregated from ‘Helminthosporium’. Canadian Journal of Botany 37:879-887.

White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18:315-322.

Wu, L., He, X., Lozano, N., Zhang, X., and Singh, P. K. 2020b. ToxA, a significant virulence factor involved in wheat spot blotch disease, exists in the Mexican population of *Bipolaris sorokiniana*. Tropical Plant Pathology:1-6.

Wu, L., He, X., Lozano, N., Zhang, X., and Singh, P. K. 2021. ToxA, a significant virulence factor involved in wheat spot blotch disease, exists in the Mexican population of *Bipolaris sorokiniana*. Tropical Plant Pathology 46:201-206.

CHAPTER 5

GENERAL CONCLUSION AND RECOMMENDATIONS

This study provides information on fungicide sensitivity in *P. tritici-repentis* in wheat in South Dakota. We characterize *P. tritici-repentis* diverse population for fungicide sensitivity. 47 of 205 isolates were identified as insensitive to fungicide pyraclostrobin, based on mutation G143A in the cytochrome b gene, spore germination. The EC50 values for the insensitive isolates also compliment the molecular findings. The two isolates 19-KW-13 and 19-KW-15 were with G143 A mutation and the mycelial growth and spore germination results also provided higher EC50 values for these two isolates which confirms that the isolates were resistant to the fungicide pyraclostrobin. The two isolates were collected from a wheat field in South Dakota in 2019 which were sprayed with the same fungicide pyraclostrobin, and the farmer observed high disease levels in the sprayed field. The results comply with what farmer observed and further confirm that the isolates were resistant to pyraclostrobin. The results indicate reduced sensitivity to insensitivity to pyraclostrobin in 23% isolates suggesting regular monitoring of the *P. tritici-repentis* population to QoI fungicides is essential to avoid any potential disease epidemic. These results will provide stakeholders with information to consider chemical applications integrating fungicides belonging to different modes of action to manage wheat tan spot. Two hundred and eight spelt wheat accessions from twenty countries belonging to four continents Africa, Asia, Europe, North America obtained from USDA National Small Grain Collection (NSGC), Aberdeen, Idaho were screened against tan spot (*P. tritici-repentis* race 1 and race 5) and Ptr ToxA, spot blotch, and leaf rust. 47.6% of the genotypes were resistant to tan spot (*P. tritici-*

repentis race 1) and 96.6% were insensitive to Ptr ToxA. Similarly, 76.5% of the genotypes were resistant to tan spot (*P. tritici-repentis* race 5) and 91.5% were insensitive to Ptr ToxB. For spot blotch, the resistance level was low as compared to Ptr as only 15.2% genotypes showed resistant reaction. For leaf rust, we found only 10.3% genotypes that gave resistant reaction. The genotypes exhibited varying responses ranging from susceptible to moderately susceptible and moderately resistant to resistant. Sixteen multi-disease resistant genotypes (tan spot race 1 &5, spot blotch and leaf rust) have been identified. These resistant spelt accessions can further be evaluated for the agronomic traits in the field and that can aid in selection of superior spelt genotypes. . In this study, 127 *B. sorokiniana* isolates recovered from wheat in South Dakota were genotyped if they carry *ToxA* gene. Further, culture filtrates of two *B. sorokiniana* isolates of each with and without *ToxA* gene were infiltrated for their reaction into leaves of two *P. tritici repentis* ToxA sensitive (Boost and Forefront) and two *P. tritici repentis* ToxA insensitive (Linkert and Prevail) spring wheat varieties. 53.5% (n=68) of 127 isolates amplified the *ToxA* gene. Further, the culture filtrates of isolates with the *ToxA* gene produced necrosis symptoms in ToxA sensitive wheat cultivars. The culture filtrates of the isolates lacking in the *ToxA* gene did not produce any symptoms in all four wheat cultivars. Our results indicate that a diverse population of *B. sorokiniana* prevalent on wheat in South Dakota. Developing ToxA insensitive wheat cultivars may help in the management of all three-leaf spot diseases tan spot, SNB, and spot blotch and hence reduce their impact on wheat productivity (Repetition). To our knowledge, this is the first report of *ToxA* (BS *ToxA*) producing *B. sorokiniana* isolates from South Dakota.

APPENDIX

Appendix Table 2.1. *Pyrenophora tritici-repentis* isolates characterized for Ptr *ToxA*, Ptr *ToxB*, and fungicide sensitivity (Presence +, Absence ─)

S. No.	Isolate	Year	ITS	ToxA	ToxB	G143A mutation	F129L mutation
1	12-55-SN5-P5	2012	+	+	─	─	─
2	12-S26-N27-P21	2012	+	+	─	─	─
3	12-55-SN5-P1	2012	+	+	─	─	─
4	12-52-P4	2012	+	+	─	─	─
5	12-51-P9	2012	+	+	─	─	─
6	12-S14-P5	2012	+	+	─	─	─
7	12-NERF-17	2012	+	+	─	─	─
8	12-NERF-P2	2012	+	+	─	─	─
9	12-51-P2	2012	+	+	─	─	─
10	12-52-P3	2012	+	+	─	─	─
11	12-53-P12	2012	+	+	─	─	─
12	12-53-P6	2012	+	+	─	─	─
13	13-104-P4	2013	+	+	─	─	─
14	13-109-SN2(B6)-P5	2013	+	+	─	─	─
15	13-103-P3	2013	+	+	─	─	─
16	13-106-P7	2013	+	+	─	─	─
17	13-105-P6	2013	+	+	─	─	─
18	12-SN27-28-P17	2012	+	+	─	─	─

S. No.	Isolate	Year	ITS	ToxA	ToxB	G143A mutation	F129L mutation
19	19-KE-01	2019	+	+	–	–	–
20	19-KE-02	2019	+	+	–	–	–
21	19-KE-03	2019	+	+	–	–	–
22	19-KE-04	2019	+	+	–	–	–
23	19-KE-05	2019	+	+	–	–	–
24	19-KE-06	2019	+	+	–	–	–
25	19-KE-07	2019	+	+	–	–	–
26	19-KE-08	2019	+	+	–	–	–
27	19-KE-09	2019	+	+	–	–	–
28	19-KE-10	2019	+	+	–	–	–
29	19-KW-1	2019	+	+	–	–	–
30	19-KW-2	2019	+	+	–	–	–
31	19-KW-3	2019	+	+	–	–	–
32	19-KW-4	2019	+	+	–	–	–
33	19-KW-5	2019	+	+	–	–	–
34	19-KW-6	2019	+	+	–	+	–
35	19-KW-7	2019	+	+	–	+	–
36	19-KW-13	2019	+	+	–	+	–
37	19-KW-15	2019	+	+	–	+	–
38	19-KW-16	2019	+	+	–	–	–
39	19-KW-20	2019	+	+	–	–	–
40	19-KW-22	2019	+	+	–	–	–

S. No.	Isolate	Year	ITS	ToxA	ToxB	G143A mutation	F129L mutation
41	19-KW-26	2019	+	+	–	–	–
42	18-AUR-01	2018	+	+	–	–	–
43	18-VOL-02	2018	+	+	–	–	–
44	18-VOL-09	2018	+	+	–	–	–
45	18-VOL-10	2018	+	+	–	–	–
46	18-SEL-01	2018	+	+	–	–	–
47	18-SEL-02	2018	+	+	–	–	–
48	18-SEL-03	2018	+	+	–	+	–
49	Pti2	1973	+	+	–	–	–
50	14-29-P2	2014	+	+	–	+	–
51	14-04-P11	2014	+	+	–	–	–
52	14-13-P13	2014	+	+	–	+	–
53	14-22-P14	2014	+	+	–	+	–
54	14-29-P21	2014	+	+	–	+	–
55	Pti2	1973	+	+	–	–	–
56	12-S35-SN48	2012	+	+	–	+	–
57	14-29-P10	2014	+	+	–	+	–
58	14-100-P1	2014	+	+	–	+	–
59	14-22-P1	2014	+	+	–	+	–
60	13-02-P2	2013	+	+	–	–	–
61	Sohaila-24	Unknown	+	+	–	–	–
62	Sohaila-48	Unknown	+	+	–	–	–

S. No.	Isolate	Year	ITS	ToxA	ToxB	G143A mutation	F129L mutation
63	Across B 10	Unknown	+	–	–	–	–
64	14-11-P13	2014	+	–	–	+	–
65	331-9	Unknown	+	+	–	–	–
66	DW7	Unknown	+	–	+	–	–
67	WW-1	Unknown	+	–	+	–	–
68	WW-3	Unknown	+	+	–	+	–
69	WW-7	Unknown	+	+	–	–	–
70	WW-9	Unknown	+	+	–	–	–
71	HRSW-1	Unknown	+	+	–	–	–
72	HRSW-2	Unknown	+	+	–	–	–
73	HRSW-3	Unknown	+	+	–	–	–
74	Cordgrass-2	Unknown	+	+	–	–	–
75	WW-101	Unknown	+	–	–	+	–
76	Ptr-18-1	2018	+	+	–	–	–
77	WW-105	Unknown	+	+	–	+	–
78	19-HA-1	2019	+	+	–	–	–
79	19-HA-2	2019	+	+	–	–	–
80	19-HA-3	2019	+	+	–	–	–
81	19-HA-7	2019	+	+	–	–	–
82	19-HAW-1	2019	+	+	–	–	–
83	19-HAW-2	2019	+	+	–	–	–
84	19-HAW-6	2019	+	+	–	–	–

S. No.	Isolate	Year	ITS	ToxA	ToxB	G143A mutation	F129L mutation
85	19-Br-2	2019	+	+	—	—	—
86	19-Br-3	2019	+	+	—	—	—
87	19-AU-1	2019	+	+	—	—	—
88	19-AU-2	2019	+	+	—	—	—
89	19-AU-3	2019	+	+	—	—	—
90	19-AU-7	2019	+	+	—	—	—
91	19-AU-8	2019	+	+	—	—	—
92	19-SEL-1	2019	+	+	—	+	—
93	19-SEL-2	2019	+	+	—	—	—
94	19-SEL-3	2019	+	+	—	—	—
95	19-HAW-2	2019	+	+	—	—	—
96	19-VOL-1	2019	+	—	—	—	—
97	19-VOL-2	2019	+	+	—	—	—
98	19-VOL-6	2019	+	+	—	—	—
99	19-VOL-17	2019	+	+	—	—	—
100	19-VOL-16	2019	+	+	—	—	—
101	19-AU-15	2019	+	+	—	—	—
102	14-01-P1	2014	+	+	—	—	—
103	14-01-P3	2014	+	+	—	+	—
104	14-03-P1	2014	+	+	—	—	—
105	14-03-P5	2014	+	+	—	+	—
106	14-04-P6	2014	+	+	—	+	—

S. No.	Isolate	Year	ITS	ToxA	ToxB	G143A mutation	F129L mutation
107	14-04-P8	2014	+	+	—	+	—
108	14-05-P1	2014	+	+	—	—	—
109	14-05-P3	2014	+	+	—	—	—
110	14-06-P2	2014	+	+	—	—	—
111	14-06-P11	2014	+	+	—	—	—
112	14-9-P1	2014	+	+	—	—	—
113	14-11-P4	2014	+	+	—	—	—
114	14-13-P3	2014	+	+	—	—	—
115	14-14-P7	2014	+	+	—	—	—
116	14-15-15	2014	+	+	—	—	—
117	14-16-P4	2014	+	+	—	—	—
118	14-16-P8	2014	+	+	—	—	—
119	14-28-P5	2014	+	+	—	—	—
120	14-29-P3	2014	+	+	—	+	—
121	14-29-P8	2014	+	+	—	+	—
122	14-44-P1	2014	+	+	—	—	—
123	14-44-P2	2014	+	+	—	—	—
124	14-45-P3	2014	+	+	—	—	—
125	14-45-P7	2014	+	+	—	—	—
126	14-49-P1-1	2014	+	+	—	—	—
127	14-49-P3-4	2014	+	+	—	—	—
128	14-49-2-4	2014	+	+	—	—	—

S. No.	Isolate	Year	ITS	ToxA	ToxB	G143A mutation	F129L mutation
129	14-SN-P1-1	2014	+	+	—	—	—
130	14-50-P2-3	2014	+	+	—	—	—
131	14-NE	2014	+	—	—	—	—
132	14-100-P2-4	2014	+	+	—	—	—
133	15-01-P1	2015	+	+	—	—	—
134	15-1-P3	2015	+	+	—	—	—
135	15-01-P5	2015	+	+	—	—	—
136	15-01-P8	2015	+	+	—	—	—
137	L15-002-8	2015	+	+	—	—	—
138	L15-002-4	2015	+	+	—	—	—
139	13-SE-012-P2-2	2013	+	+	—	—	—
140	13-NW-012-P1-1	2013	+	+	—	—	—
141	13-NE-017-P6-3	2013	+	+	—	—	—
142	13-NE-017-P1-2	2013	+	+	—	—	—
143	13-NE-22-P2-1	2013	+	—	—	—	—
144	13-NW-019-P2-7	2013	+	+	—	—	—
145	13-NW-24-P1-2	2013	+	+	—	—	—
146	13-SE-012-P1-4	2013	+	+	—	—	—

S. No.	Isolate	Year	ITS	ToxA	ToxB	G143A mutation	F129L mutation
147	13-NW-023-P1-7	2013	+	+	–	–	–
148	13-NE-017-P4-2	2013	+	+	–	–	–
149	13-SE-012-P23	2013	+	+	–	–	–
150	13-NE-018-P1-3	2013	+	+	–	–	–
151	13-NE-018-P3-4	2013	+	+	–	–	–
152	13-3-P1-1	2013	+	+	–	–	–
153	13-3-P1-2	2013	+	+	–	–	–
154	13-3-P2-3	2013	+	+	–	–	–
155	13-3-P5-4	2013	+	+	–	–	–
156	13-NE-18-P1-1	2013	+	+	–	–	–
157	13-103-P4-55	2013	+	+	–	–	–
158	13-103-P4-1	2013	+	+	–	–	–
159	13-103-P4-5	2013	+	+	–	–	–
160	13-103-4-58	2013	+	+	–	–	–
161	20-10-1	2020	+	+	–	–	–
162	20-10-2	2020	+	+	–	–	–
163	20-10-3	2020	+	+	–	–	–
164	20-10-4	2020	+	+	–	–	–
165	20-10-5	2020	+	+	–	–	–
166	20-10-6	2020	+	+	–	–	–

S. No.	Isolate	Year	ITS	ToxA	ToxB	G143A mutation	F129L mutation
167	20-10-7	2020	+	+	—	—	—
168	20-10-8	2020	+	+	—	—	—
169	20-10-9	2020	+	+	—	—	—
170	20-10-10	2020	+	+	—	—	—
171	20-10-11	2020	+	+	—	—	—
172	20-10-12	2020	+	+	—	—	—
173	20-10-13	2020	+	+	—	—	—
174	20-12-1	2020	+	+	—	+	—
175	20-12-2	2020	+	+	—	+	—
176	20-12-3	2020	+	+	—	—	—
177	20-12-4	2020	+	+	—	—	—
178	20-12-5	2020	+	+	—	+	—
179	20-12-6	2020	+	+	—	+	—
180	20-15-1	2020	+	+	—	—	—
181	20-15-2	2020	+	+	—	—	—
182	20-15-3	2020	+	+	—	+	—
183	20-15-4	2020	+	+	—	+	—
184	20-15-5	2020	+	+	—	+	—
185	20-15-6	2020	+	+	—	+	—
186	20-15-7	2020	+	+	—	+	—
187	20-15-8	2020	+	+	—	+	—
188	20-15-9	2020	+	+	—	+	—

S. No.	Isolate	Year	ITS	ToxA	ToxB	G143A mutation	F129L mutation
189	20-15-10	2020	+	+	—	—	—
190	20-15-11	2020	+	+	—	+	—
191	20-15-12	2020	+	+	—	+	—
192	20-15-13	2020	+	+	—	—	—
193	20-14-1	2020	+	+	—	+	—
194	20-14-2	2020	+	+	—	—	—
195	20-14-3	2020	+	+	—	+	—
196	20-14-4	2020	+	+	—	+	—
197	20-14-5	2020	+	+	—	—	—
198	20-14-6	2020	+	+	—	+	—
199	20-14-7	2020	+	+	—	+	—
200	20-14-8	2020	+	+	—	+	—
201	20-14-9	2020	+	+	—	+	—
202	20-14-10	2020	+	+	—	+	—
203	20-14-11	2020	+	+	—	—	—
204	20-14-12	2020	+	+	—	—	—
205	20-14-13	2020	+	+	—	+	—

Appendix Table 2.2: 127 *Bipolaris sorokiniana* isolates characterized for Bs*ToxA* gene.

S. No.	BS ISOLATES	ToxA Presence/Absence
1	BS10-77	Absent
2	BS10-117	Absent
3	BS10-140	Present
4	BS10-151	Present
5	BS10-98	Absent
6	BS10-97	Present
7	BS10-94	Present
8	BS10-91	Present
9	BS10-81	Absent
10	BS10-79	Present
11	BS10-61	Present
12	BS10-116	Absent
13	BS10-149	Present
14	BS10-118	Absent
15	BS10-100	Absent
16	BS10-152	Present
17	BS10-103	Present
18	BS10-132	Absent
19	BS10-85	Absent
20	BS10-102	Absent
21	BS10-80	Absent

S. No.	BS ISOLATES	ToxA Presence/Absence
22	BS10-105	Present
23	BS10-111	Present
24	BS10-114	Present
25	BS10-104	Absent
26	BS10-34	Absent
27	BS10-46	Absent
28	BS10-36	Absent
29	BS10-175	Present
30	BS10-179	Absent
31	BS10-178	Present
32	BS10-144	Absent
33	BS10-173	Present
34	BS10-44	Absent
35	BS10-176	Absent
36	BS10-154	Present
37	BS10-159	Absent
38	BS10-177	Absent
39	BS10-166	Present
40	BS10-162	Absent
41	BS-09-3	Present
42	BS-04-1	Absent
43	BS-09-7	Absent
44	BS10-60	Absent

S. No.	BS ISOLATES	ToxA Presence/Absence
45	BS-04	Absent
46	BS10-133	Absent
47	BS10-45	Present
48	BS10-46	Absent
49	BS10-90	Absent
50	BS10-89	Absent
51	BS10-99	Present
52	BS10-112	Present
53	BS10-86	Present
54	BS10-71	Present
55	BS10-131	Absent
56	BS10-37	Present
57	BS10-47	Absent
58	BS-09-6	Present
59	BS-12-5	Absent
60	BS-12-01	Present
61	BS-12-75	Present
62	BS-12-65	Absent
63	BS-12-13	Present
64	BS-12-70	Absent
65	BS-12-30	Present
66	BS-12-9	Present
67	BS-12-22	Absent

S. No.	BS ISOLATES	ToxA Presence/Absence
68	BS-12-43	Present
69	BS-12-34	Present
70	BS-12-50	Present
71	BS-12-60	Present
72	BS-12-38	Absent
73	BS-12-18	Absent
74	BS-12-55	Present
75	BS-12-46	Present
76	BS-12-40	Present
77	BS-12-80	Present
78	BS-12-26	Present
79	18-NEF-CS-01	Absent
80	18-NEF-CS-02	Absent
81	14-39 BS	Absent
82	14-102-DR/CS-4	Absent
83	BS-10-26	Absent
84	BS-10-3L	Absent
85	BS-10-2L	Present
86	14-102-DR/CS-5	Absent
87	14-11-CS-1	Present
88	14-11-CS-2	Present
89	13-NE-020-1 CS	Present
90	13-SW-004-CS3	Present

S. No.	BS ISOLATES	ToxA Presence/Absence
91	13-N-020-4CS	Present
92	BS-10-124	Absent
93	BS-04-01	Absent
94	N1	Present
95	N2	Absent
96	N3	Absent
97	N4	Absent
98	N5	Absent
99	N6	Present
100	N7	Absent
101	N8	Absent
102	N9	Present
103	N10	Present
104	N11	Absent
105	N12	Absent
106	N13	Absent
107	N14	Present
108	N15	Present
109	N16	Present
110	N17	Present
111	N18	Present
112	N19	Present
113	20-4-BS-A	Present

S. No.	BS ISOLATES	ToxA Presence/Absence
114	20-4-BS-B	Present
115	20-4-BS-C	Present
116	20-4-BS-D	Absent
117	20-2-BS-1	Present
118	20-2-BS-2	Present
119	20-2-BS-3	Present
120	20-2-BS-4	Present
121	20-4-BS-1	Present
122	20-4-BS-2	Absent
123	20-4-BS-3	Present
124	20-4-BS-4	Absent
125	20-4-BS-5	Present
126	20-4-BS-6	Present
127	20-13-BS-1	Present

APPENDIX Table 2.3: Disease reaction of 210 spelt wheat accessions to Tan spot Race 1 Ptr ToxA, Race 5 Ptr ToxB, Spot blotch and Leaf rust

S. No.	Accession #	Country	Ptr Tox A	Lesion Type	Race 1	Ptr Tox B	Lesion Type	Race 5	Spot blotch	BS ToxA	Spot blotch lesion type	Leaf rust
1	CItr 14088	Unknown	–	4	S	–	1.4	R	4	–	S	MR
2	PI 168681	United States	–	1.8	R	+	3.4	MS	2.5	–	MR	MR
3	PI 168682	United States	–	1.1	R	–	1.6	R	4	–	S	S
4	PI 191100	Spain	–	1.5	R	–	1.1	R	3.4	–	MS	S
5	PI 191393	Ethiopia	–	2.1	MR	–	2.1	MR	2.4	–	MR	S
6	PI 191394	Ethiopia	–	1.1	R	–	1	R	1.1	–	R	MR
7	PI 221419	Serbia	–	3.9	MS	–	4.1	S	2	–	MR	MS
8	PI 225271	Iran	–	2.3	MR	–	1.8	R	2	–	MR	S
9	PI 272529	Hungary	–	4	S	–	3.5	MS	4	–	S	MS
10	PI 272574	Hungary	–	2.6	MR	–	1.8	R	3.2	–	MS	S
11	PI 272578	Hungary	–	1.6	R	–	1	R	1.3	–	R	S
12	PI 290513	Hungary	–	1	R	–	1.4	R	2	–	MR	R
13	PI 290514	Hungary	–	2	MR	+	4	S	3.3	–	MS	R
14	PI 290515	Hungary	–	1	R	–	1	R	2	–	MR	MR
15	PI 290516	Hungary	–	4.3	S	–	2.3	MR	3.6	–	MS	MS
16	PI 295056	Bulgaria	–	2.1	MR	–	3.5	MS	3.4	–	MS	S
17	PI 295060	Bulgaria	–	3.6	MS	–	3.1	MS	3.6	–	MS	R

S. No.	Accession #	Country	Ptr Tox A	Lesion Type	Race 1	Ptr Tox B	Lesion Type	Race 5	Spot blotch	BS ToxA	Spot blotch lesion type	Leaf rust
18	PI 295062	Bulgaria	–	3.7	MS	+	2.4	MR	3.7	–	MS	S
19	PI 295063	Bulgaria	–	3.9	M	–	4	S	4	–	S	MS
20	PI 306553	Romania	–	4	S	–	4.4	S	4.2	–	S	S
21	PI 306556	Romania	–	2.1	MR	–	1.8	R	4	–	S	S
22	PI 323438	Austria	–	3.3	MS	–	3	MS	3.6	–	MS	R
23	PI 330559	United Kingdom	–	4	S	–	1.3	R	4	–	S	MS
24	PI 338366	Belgium	–	2	MR	+	1	R	4	–	S	S
25	PI 346853	Argentina	–	1	R	–	1	R	2	–	MR	R
26	PI 347851	Switzerland	–	2.6	MR	–	1	R	3.2	–	MS	R
27	PI 347861	Switzerland	–	1.2	R	–	1	R	3.9	–	MS	MR
28	PI 347875	Switzerland	–	1	R	–	1.1	R	1.3	–	R	MS
29	PI 347891	Switzerland	–	3.9	MS	–	1.6	R	3.9	–	MS	MS
30	PI 347909	Switzerland	–	4	S	–	1.3	R	4	–	S	MR
31	PI 347986	Switzerland	–	1.4	R	–	1	R		–		MS
32	PI 347990	Switzerland	–	1.5	R	–	1	R	2.1	–	MR	MR

S. No.	Accession #	Country	Ptr Tox A	Lesion Type	Race 1	Ptr Tox B	Lesion Type	Race 5	Spot blotch	BS ToxA	Spot blotch lesion type	Leaf rust
33	PI 348022	Germany	–	2.1	MR	–	1.1	R	3.9	–	MS	S
34	PI 348026	Germany	–	3.4	MS	–	1	R	3	–	MS	S
35	PI 348033	Germany	–	2.8	MR	–	1.6	R	4	–	S	S
36	PI 348046	Germany	–	3.9	MS	–	1.1	R	3.9	–	MS	S
37	PI 348052	Germany	–	4	S	–	1	R	3	–	MS	R
38	PI 348062	Germany	–	2.7	MR	–	1.3	R	3.7	–	MS	MS
39	PI 348067	Germany	+	2.5	MR	–	3	MS	4	–	S	MS
40	PI 348072	Germany	–	3.8	MS	–	1	R	4	–	S	S
41	PI 348078	Germany	–	1	R	–	1	R	3.8	–	MS	R
42	PI 348081	Germany	–	1.6	R	–	1.1	R	4	–	S	MS
43	PI 348093	Germany	–	4	S	–	1	R	3.4	–	MS	S
44	PI 348115	Germany	–	3.6	MS	–	1.6	R	4	–	S	S
45	PI 348132	Germany	–	4.1	S	–	1.1	R	1.9	–	R	S
46	PI 348143	Germany	–	3	MS	–	1	R	1.3	–	R	S
47	PI 348213	Germany	–	3.5	MS	–	1.3	R	1.9	–	R	S
48	PI 348390	Belgium	–	0		–	1	R	2	–	MR	MR
49	PI 348432	Spain	–	1	R	–	1	R	1.3	–	R	S
50	PI 348443	Spain	–	2.6	MR	–	3.9	MS	2.7	–	MR	S
51	PI 348457	Spain	–	1.9	R	–	3.3	MS	1.4	–	R	S
52	PI 348465	Spain	–	1	R	–	3.4	MS	1.4	–	R	S
53	PI 348478	Spain	–	1.6	R	–	3.1	MS	2.3	–	MR	S

S. No.	Accession #	Country	Ptr Tox A	Lesion Type	Race 1	Ptr Tox B	Lesion Type	Race 5	Spot blotch	BS ToxA	Spot blotch lesion type	Leaf rust
54	PI 348485	Spain	–	1.1	R	–	3.7	MS	1.6	–	R	S
55	PI 348495	Spain	–	1.8	R	–	1.6	R	4	–	S	S
56	PI 348502	Spain	–	1.1	R	–	3.3	MS	2.3	–	MR	S
57	PI 348526	Spain	–	3.9	MS	–	1	R	2.1	–	MR	S
58	PI 348568	Spain	–	4	S	–	2.3	MR	2	–	MR	S
59	PI 348584	Spain	–	3.3	MS	–	1.5	R	1.9	–	R	S
60	PI 348604	Spain	–	2.7	MR	–	1.3	R	1.2	–	R	S
61	PI 348619	Spain	–	3.5	MS	–	1	R	3	–	MS	S
62	PI 348631	Spain	–	4	S	–	2.1	MR	2.3	–	MR	S
63	PI 348642	Spain	–	2	MR	–	1	R	3	–	MS	S
64	PI 348654	Spain	–	2.1	MR	–	1	R	3.8	–	MS	S
65	PI 348679	Spain	–	1.9	R	–	3.1	MS	3.3	–	MS	S
66	PI 348708	Spain	–	1.6	R	–	1	R	3.1	–	MS	S
67	PI 348736	Spain	–	3.2	MS	–	1	R	2.8	–	MR	S
68	PI 355551	Germany	–	3.3	MS	–	4	S	4	–	S	MS
69	PI 355553	Germany	–	4	S	+	4	S	3.6	–	MS	R
70	PI 355558	Germany	–	1.4	R	–	1.3	R	2	–	MR	S
71	PI 355561	Switzerland	–	3.6	MS	–	1.4	R	3.6	–	MS	MS-S
72	PI 355596	Switzerland	–	2.1	MR	–	1	R	3	–	MS	S

S. No.	Accession #	Country	Ptr Tox A	Lesion Type	Race 1	Ptr Tox B	Lesion Type	Race 5	Spot blotch	BS ToxA	Spot blotch lesion type	Leaf rust
73	PI 355597	Switzerland	–	3.4	MS	–	3.9	MS	2	–	MR	S
74	PI 355599	Switzerland	–	1.9	R	–	1	R	1.5	–	R	S
75	PI 355600	Switzerland	–	1.9	R	–	1	R	1.3	–	R	S
76	PI 355602	Switzerland	–	2.9	MR	–	1.4	R	4	–	S	S
77	PI 355603	Switzerland	–	3.1	MS	–	1	R	3.1	–	MS	S
78	PI 355607	Switzerland	–	2.1	MR	–	1	R	1.3	–	R	MS
79	PI 355610	Switzerland	–	3.9	MS	–	1.7	R	3.8	–	MS	R
80	PI 355615	Germany	–	3.9	MS	–	1	R	2.3	–	MR	MR
81	PI 355616	Switzerland	–	1.1	R	–	1	R	1.3	–	R	R
82	PI 355617	Switzerland	–	1.6	R	–	1.1	R	3	–	MS	S
83	PI 355619	Belgium	–	3.7	MS	–	1	R	3.8	–	MS	S
84	PI 355622	Germany	–	2	MR	–	1.3	R	3	–	MS	S
85	PI 355625	Belgium	–	2.4	MR	–	1	R	3.8	–	MS	MS
86	PI 355626	Germany	–	2.8	MR	–	1.4	R	3	–	MS	S
87	PI 355627	Switzerland	–	3.3	MS	–	2.1	MR	4	–	S	MS

S. No.	Accession #	Country	Ptr Tox A	Lesion Type	Race 1	Ptr Tox B	Lesion Type	Race 5	Spot blotch	BS ToxA	Spot blotch lesion type	Leaf rust
88	PI 355632	Switzerland	–	3.4	MS	–	3	MS	3.4	–	MS	MS
89	PI 355633	Germany	–	3.7	MS	–	1	R	3.9	–	MS	R
90	PI 355638	Switzerland	–	1.1	R	–	1	R	2.1	–	MR	MR
91	PI 355640	Germany	–	1.3	R	–	1	R	4	–	S	R
92	PI 355641	Germany	–	1	R	–	1	R	1.5	–	R	R
93	PI 355642	Italy	–	1.1	R	–	1	R	3	–	MS	R
94	PI 355650	Belgium	–	1.1	R	–	1	R	3.8	–	MS	MS
95	PI 355652	Switzerland	–	1	R	–	1	R	1.3	–	R	R
96	PI 355664	Germany	–	1	R	–	3	MS	3	–	MS	S
97	PI 355665	Germany	–	1.5	R	–	3.6	MS	3.3	–	MS	MS
98	PI 355667	Germany	–	1.5	R	–	1	R	3.7	–	MS	R
99	PI 355668	Switzerland	–	1	R	–	1.8	R	1.9	–	R	MR
100	PI 355681	Belgium	–	1	R	–	3.3	MS	4	–	S	S
101	PI 355685	Switzerland	–	3.3	MS	–	1	R	2.4	–	MR	R
102	PI 355687	Germany	–	1	R	–	1	R	3.4	–	MS	MS
103	PI 355692	Germany	–	1.6	R	–	3.1	MS	4	–	S	S
104	PI 355694	Germany	–	1	R	–	1.7	R	4	–	S	S
105	PI 355704	United Kingdom	–	1.4	R	–	1	R	3.5	–	MS	MS

S. No.	Accession #	Country	Ptr Tox A	Lesion Type	Race 1	Ptr Tox B	Lesion Type	Race 5	Spot blotch	BS ToxA	Spot blotch lesion type	Leaf rust
106	PI 362062	Romania	–	1	R	–	1	R	4	–	S	MS
107	PI 362063	Romania	–	1.5	R	–	1	R	4	–	S	S
108	PI 378469	North Macedonia	–	1	R	–	1	R	4	–	S	MS
109	PI 469022	Spain	–	1.2	R	–	1	R	4	–	S	MS
110	PI 469027	Spain	–	3.5	MS	–	1.5	R	3.9	–	MS	S
111	PI 469035	Spain	–	3.4	MS	–	1	R	4	–	S	MS
112	PI 469042	Spain	–	1	R	–	1	R	4	–	S	MS
113	PI 469054	Spain	–	1	R	–	1	R	4	–	S	MS
114	PI 572914	Tajikistan	+	3.9	MS	–	1.4	R	4	+	S	MS
115	PI 572915	Tajikistan	+	4	S	–	1.1	R	4	+	S	MS
116	PI 591890	Austria	–	1	R	–	1	R	1.9	–	R	MS
117	PI 591891	Spain	–	2.3	MR	–	4.4	S	4.1	–	S	MS
118	PI 591900	Spain	–	1.3	R	–	3	MS	2.3	–	MR	MS
119	PI 591901	Germany	–	1.9	R	–	1	R	4	–	S	MR
120	PI 615238	Belgium	–	1.3	R	–	1.3	R	2.3	–	MR	MR
121	PI 615284	Belgium	–	3.4	MS	–	1	R	3.7	–	MS	MS
122	PI 615314	Belgium	–	2.4	MR	–	1	R	3.1	–	MS	MS
123	PI 668171	Czechoslovakia	–	3.9	MS	–	2.4	MR	4	–	S	MR

S. No.	Accession #	Country	Ptr Tox A	Lesion Type	Race 1	Ptr Tox B	Lesion Type	Race 5	Spot blotch	BS ToxA	Spot blotch lesion type	Leaf rust
124	PI 668174	Czechoslovakia	–	1	R	–	1	R		–		S
125	PI 674998	Georgia	+	3.9	MS	–	1.1	R	4.3	+	S	MS
126	225271 TR04ID	Iran	–	3.6	MS	–	2	MR	4.4	–	S	MS
127	221419 TR16ID	Serbia	–	3.6	MS	+	1	R	4.7	–	S	R
128	591899 TR13ID	United Kingdom	–	1	R	–	1	R	4.3	–	S	MS
129	591900 TR97ID	Spain	–	3.7	MS	–	3	MS	4.9	–	S	S
130	591901 TR13ID	Germany	–	3.3	MS	–	1	R	4.1	–	S	MS
131	591904 TR13ID	Bulgaria	–	1.3	R	–	1	R	4.9	–	S	MS
132	225295 TR04ID	Iran	–	1.4	R	–	1	R	4.6	–	S	S
133	272529 TR17ID	Hungary	–	4	S	–	4.1	S	5	–	S	
134	266848 TR17ID	United Kingdom	–	4	S	–	1.9	R	4.5	–	S	MS
135	191392 TR15ID	Ethiopia	–	3.3	MS	+	3.4	MS	4.3	–	S	MS
136	191393 TR05ID	Ethiopia	–	1.6	R	–	1.6	R	4.2	–	S	MS

S. No.	Accession #	Country	Ptr Tox A	Lesion Type	Race 1	Ptr Tox B	Lesion Type	Race 5	Spot blotch	BS ToxA	Spot blotch lesion type	Leaf rust
137	191394 TR09ID	Ethiopia	–	2.5	MR	–	1	R	3.5	–	MS	MR
138	191617 TR16ID	Portugal	–	3	MS	–	3	MS	4.8	–	S	MS
139	348597 TR16ID	Spain	–	1	R	–	1	R	4.3	–	S	S
140	348594 TR16ID	Spain	–	1	R	–	1	R	2	–	MR	S
141	348592 TR17D	Spain	–	1	R	–	1	R	4.2	–	S	S
142	348590 TR13ID	Spain	–	1	R	–	1	R	3.4	–	MS	S
143	348579 TR16ID	Spain	–	1	R	–	1.3	R	3.8	–	MS	S
144	221402 TR13ID	Serbia	+	3.1	MS	–	1	R	4	+	S	S
145	272573 TR16CA	Hungary	–	1	R	+	2.9	MR	3.1	–	MS	R
146	367201 TR16ID	Afghanistan	+	1	R	–	1.3	R	3.4	+	MS	S
147	591898 TR13ID	Switzerland	–	1	R	–	1	R	2.1	–	MR	MR
148	348279 TR16ID	Germany	–	1	R	–	1	R	2.2	–	MR	MR
149	348428 TR16ID	Spain	–	1	R	–	1	R	1.9	–	R	S

S. No.	Accession #	Country	Ptr Tox A	Lesion Type	Race 1	Ptr Tox B	Lesion Type	Race 5	Spot blotch	BS ToxA	Spot blotch lesion type	Leaf rust
150	348429 TR16ID	Spain	–	1.3	R	–	1.4	R	3.9	–	MS	S
151	348430 TR16ID	Spain	–	1.1	R	–	1	R	3.6	–	MS	S
152	348436 TR16ID	Spain	–	1	R	–	1	R	3.4	–	MS	S
153	348437 TR16ID	Spain	–	1.3	R	–	1	R	3.6	–	MS	S
154	348577 TR16ID	Spain	–	3.1	MS	–	1	R	3.3	–	MS	MS
155	348490 TR04ID	Spain	–	1	R	–	1	R	3.3	–	MS	S
156	348475 TR16ID	Spain	–	1	R	–	1	R	4.1	–	S	MS
157	348473 TR16ID	Spain	–	1	R	+	1	R	3.9	–	MS	S
158	348472 TR16ID	Spain	–	1	R	–	4	S	4	–	S	MS
159	347854 TR01ID	Switzerland	–	1	R	–	1	R	1.1	–	R	MS
160	338366 TR05ID	Belgium	–	1	R	+	1.25	R	1.9	–	R	S
161	295064 TR15ID	Bulgaria	–	1.3	R	+	3.75	MS	4.1	–	S	MS
162	295063 TR15ID	Bulgaria	–	2.3	MR	–	3	MS	4	–	S	MS

S. No.	Accession #	Country	Ptr Tox A	Lesion Type	Race 1	Ptr Tox B	Lesion Type	Race 5	Spot blotch	BS ToxA	Spot blotch lesion type	Leaf rust
163	347986 TR16ID	Switzerland	–	1	R	–	1	R	1.5	–	R	MR
164	347918 TR12ID	Switzerland	–	1	R	–	1	R	3	–	MS	MS
165	347911 TR05ID	Switzerland	–	2.9	MR	–	1	R	4	–	S	MR
166	347909 TR16ID	Switzerland	–	3.8	MS	–	1	R	4.1	–	S	MR
167	347875 TR01ID	Switzerland	–	3.3	MS	+	1	R	2	–	MR	MS
168	272574 TR15ID	Hungary	–	4	S	–	1.5	R	4	–	S	S
169	277012 TR15ID	Spain	–	3.9	MS	–	1.5	R	1.9	–	R	MS
170	355657 TR02ID	Germany	–	3.3	MS	–	1	R	3	–	MS	S
171	355661 TR16ID	Italy	–	3.9	MS	+	1.5	R	4	–	S	S
172	355674 TR16ID	Germany	–	3.8	MS	–	3.6	MS	2.5	–	MR	S
173	355676 TR16ID	Germany	–	4	S	–	1.2	R	3.3	–	MS	S
174	348611 TR00ID	Spain	–	1	R	–	1.4	R	4	–	S	S
175	355557 TR16ID	Germany	–	1.4	R	–	1	R	4	–	S	S

S. No.	Accession #	Country	Ptr Tox A	Lesion Type	Race 1	Ptr Tox B	Lesion Type	Race 5	Spot blotch	BS ToxA	Spot blotch lesion type	Leaf rust
176	355574 TR16ID	Switzerland	–	2.3	MR	–	1.3	R	4	–	S	S
177	355625 TR16ID	Belgium	–	4	S	–	1.1	R	4	–	S	S
178	286048 TR99ID	Germany	–	1.3	R	–	1	R	4	–	S	MS
179	286060 TR16ID	Poland	–	1.6	R	–	1	R	3.1	–	MS	MR
180	190963 TR01ID	Portugal	–	4	S	–	1.7	R	4	–	S	MR
181	191100 TR17ID	Spain	–	1.3	R	–	1	R	2.4	–	MR	MS
182	348135 TR16ID	Germany	–	3.9	MS	–	1	R	4	–	S	MR
183	190962 TR17ID	Italy	–	3.6	MS	–	1	R	4	–	S	S
184	190960 TR15ID	Belgium	–	1	R	–	1	R	3.3	–	MS	MR
185	168680 TR01CA	United States	–	3.6	MS	+	3.7	MS	3.8	–	MS	S
186	348136 TR16ID	Germany	–	3.6	MS	–	1	R	3.8	–	MS	MS
187	348222 TR01ID	Germany	–	3.1	MS	–	1	R	3.7	–	MS	S
188	348229 TR01ID	Germany	–	1.1	R	–	1	R	4	–	S	S

S. No.	Accession #	Country	Ptr Tox A	Lesion Type	Race 1	Ptr Tox B	Lesion Type	Race 5	Spot blotch	BS ToxA	Spot blotch lesion type	Leaf rust
189	348036 TR16ID	Germany	–	1.8	R	–	1	R	4	–	S	
190	348022 TR16ID	Germany	–	3.9	MS	–	1	R	3.4	–	MS	S
191	348105 TR16ID	Germany	–	3.8	MS	–	1	R	4	–	S	S
192	348007 TR16ID	Switzerland	–	1.1	R	–	1	R	4	–	S	S
193	347990 TR00ID	Switzerland	–	1	R	–	1.3	R	4	–	S	MR
194	348107 TR01ID	Germany	–	1.5	R	–	1	R	3	–	MS	S
195	348109 TR16ID	Germany	–	1	R	–	1	R	2.5	–	MR	MR
196	348111 TR16ID	Germany	–	1.1	R	–	1.4	R	3.4	–	MS	MS
197	348122 TR15ID	Germany	–	1	R	–	1	R	3.4	–	MS	S
198	295056 TR04ID	Bulgaria	–	1	R	–	3.1	MS	4	–	S	S
199	294576 TR16ID	United States	–	3.3	MS	–	1	R	3.5	–	MS	S
200	290514 TR17ID	Hungary	–	3.7	MS	–	3.1	MS	4	–	S	MR
201	348041 TR16ID	Germany	–	2.5	MR	–	1	R	4.1	–	S	S

S. No.	Accession #	Country	Ptr Tox A	Lesion Type	Race 1	Ptr Tox B	Lesion Type	Race 5	Spot blotch	BS ToxA	Spot blotch lesion type	Leaf rust
202	348056 TR16ID	Germany	–	2.2	MR	+	1	R	4	–	S	S
203	348058 TR16ID	Germany	–	3.4	MS	–	1	R	3.5	–	MS	S
204	348060 TR05ID	Germany	+	4	S	+	1	R	4	+	S	S
205	348075 TR16ID	Germany	–	4	S	+	1.8	R	4	–	S	S
206	PI 15865 TR04ID	United States	–	4	S	–	1.3	R	4	–	S	S
207	PI 57394 TR16ID	Ethiopia	–	4	S	–	2.1	MR	1.2	–	R	MS
208	Cltr 15071 TR16ID	United States	–	1.8	R	–	1	R	1.4	–	R	MR
209	Cltr 17764 TR04ID	United States	–	3.6	MS	+	1	R	1.4	–	R	S
210	Cltr 13959 TR11CA	United States	–	1.6	R	–	1	R	3.2	–	MS	MS
	Glenlea		+	4.1	S	+	3.8	MS	4.4	+	S	
	Salamouni		–	1	R	–	1	R	1.1	–	R	
	6B365		–	4.1	S	–	1.3	R				
	6B662		–	2.2	MR	+	4.1	S				
	Bolles											R
	Baart											S