MARKER ASSISTED BACKCROSS INTROGRESSION OF A RACE NON-SPECIFIC QTL TO DEVELOP DURUM WHEAT LINES WITH IMPROVED TAN SPOT RESISTANCE

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

By

Namrata Acharya

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department
Plant Sciences

March 2024

Fargo, North Dakota

North Dakota State University
Graduate School

Title

MARKER ASSISTED BACKCROSS INTROGRESSION OF A RACE NON-SPECIFIC QTL TO DEVELOP DURUM WHEAT LINES WITH IMPROVED TAN SPOT RESISTANCE

By

Namrata Acharya

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

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Xuehui Li
Chair

Dr. Zhaohui Liu

Dr. Wun Chao

Dr. Md Mukhlesur Rahman

Approved:

April 30, 2024
Date

Dr. Richard D. Horsley
Department Chair

ABSTRACT

Tan spot is a foliar disease caused by the fungus *Pyreniphora tritici-repentis* affecting durum wheat cultivation and production worldwide. Developing tan spot resistant varieties is an environmentally friendly way to control the disease. In this study, I introgressed the favorable allele of a race non-specific QTL mapped on chromosome 3B (TS-3B-QTLrn) into two durum wheat cultivars "Grano" and "Riveland" via marker-assisted backcross selection. I found that the introgression lines with the favorable allele had significantly lower disease severity than the recurrent parents "Grano" and "Riveland" for all four isolates tested. Phenotyping of the BC_4F_2 progenies indicated that the TS-3B-QTLrn showed partially dominant resistance. Further research will be conducted to test the effectiveness of the TS-3B-QTLrn on disease severity of adult plants under field conditions, as well as to test if the introgression of the TS-3B-QTLrn has effects on grain yield and quality related traits.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my major advisor Dr. Xuehui Li for guiding me throughout my master's study. His thoughtfulness and professionalism have provided me with invaluable support in conducting research despite several challenges. His knowledge and enthusiasm to delve into wheat breeding and genetics has not only enhanced my academic experience but also built invaluable pillars for my future research career.

I would also like to extend my gratitude to my supervisory committee member Dr. Zhaohui Liu (Plant Pathology, NDSU) for imparting knowledge on inoculation procedure and aiding in scoring disease severity. Additionally, I wish to express my appreciation to Dr. Wun Chao (USDA-ARS, Fargo) for providing me with the opportunity to work in his lab as well as Dr. Md Mukhlesur Rahman (Plant Science, NDSU) for his invaluable support in both my research endeavors and career development.

I am grateful towards our research specialist Justin Hegstad as well as to past lab members Runhao Wang and Ramesh Pilli. I am also thankful towards current lab members Suraj Basyal, Hayat Khan, Harika Pothula and Evan Salsman.

I would also like to extend my gratefulness to my family members for always encouraging me to move forward and strive for excellence in my academic career.

This project is supported by the North Dakota Agricultural Experiment Station and North Dakota Wheat commission.

DEDICATION

I dedicate this thesis to my grandparents, Dharma Raj Acharya and Narbada Devi Acharya, my parents, Atma Ram Acharya and Ramila Acharya, as well as my brother, Bijay Acharya. Their belief and unwavering support in every step of my academic journey have inspired me to strive for excellence.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

CHAPTER 1: LITERATURE REVIEW

Distribution and importance of wheat

Wheat is a major cereal crop and one of the main sources of carbohydrate worldwide (Curtis, 2002). Global wheat production in 2021/22 was over 778 million metric tons (Shahbandeh, 2023a). In 2021/22, European Union emerged as the foremost contributor to the global output with the production of 138 million metric tons followed by China with 136 million metric tons and India with 109 million metric tons (Shahbandeh, 2023a). The United States ranked in fifth on wheat production with about 44 million metric tons. China led the world on wheat consumption (148 million metric tons) followed by India (109 million metric tons) and European Union (108 million metric tons) in 2022 (Shahbandeh, 2023b). The United States ranks in fifth on wheat consumption (30 million metric tons) (Shahbandeh, 2023b).

In the United States, North Dakota is the leading producer with the total production of 8 million metric tons followed by Kansas, Washington, and Montana (Shahbandeh, 2023c). In the United States, wheat ranks in third among field crops after corn and soyabean in terms of planted acreage, production, and gross farm receipts (Sowell and Swearingen, 2023). There are five major classes of wheat cultivated in the United States: hard red spring wheat, hard red winter wheat, soft red winter wheat, white wheat, and durum wheat. The cultivation of different classes of wheat tends to be region specific. Hard red winter wheat is mainly grown for making bread flour and grown primarily in the Great Plains ranging from northern Texas through Montana (Statista, 2023). Hard red spring wheat is suitable for specialty breads and is mainly grown in the Northern Plains where winter is harsh including North Dakota, South Dakota, Minnesota, and Montana. Flour obtained from soft red winter wheat is suitable for cookies, cakes, and crackers and soft red winter wheat is primarily grown along the Mississippi river and eastern states. White

wheat is famous for the preparation of white bread. Durum wheat contributes 1.6 million metric tons, known for its qualities suitable for making pasta and couscous, and is primarily grown in North Dakota and Montana (USDA, 2023). Durum wheat is hardest amongst all wheat classes with high gluten content and amber color.

Domestication and origin of durum wheat

Durum wheat (*Triticum turgidum* ssp. *durum*, 2n=4x=28, AABB genome) is tetraploid. The tetraploidization event involved a cross between *Triticum urartu* (2n=2x=14, genome AA) and its close relative of *Aegilops speltoides* (2n=2x=14, genome BB), which occurred less than 0.8 million years ago (Ozkan et al., 2005). Durum wheat direct ancestor was domesticated emmer wheat (*Triticum turgidum* ssp. *dicoccum*, 2n=4x=28) (Ozkan et al., 2005). Domesticated emmer wheat was domesticated from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*, 2n=4x=28) in the Fertile Crescent (Dubcovsky and Dvorak, 2007). Many desirable genes have been identified in wild emmer and domesticated emmer wheat such as strip rust resistance genes, powdery mildew resistance genes, and high protein content genes, providing good source for genetic improvement of durum wheat (Reviewed by Li et al., 2020).

Tan spot of wheat

Tan spot, stagonospora nodorum blotch (SNB) and septoria tritici blotch (STB) are three major fungal leaf spot diseases that commonly come into sight in North Dakota (McMullen and Adhikari, 2009). Among the three foliar diseases, tan spot is the most common and destructive one in all wheat classes throughout North Dakota (McMullen and Adhikari, 2009). Tan spot, also called yellow spot, is caused by the fungus *Pyrenophora tritici-repentis*. The incidence of the disease was first detected in New York, U.S. in 1940 (Barrus, 1942). The fungus causes foliar disease in both bread wheat and durum wheat (Ali and Francl, 2003).

The fungus infects wheat and causes small, oval to diamond shaped spots on leaves that will later develop into larger, tan-colored lesions often surrounded by chlorotic halos in susceptible genotypes. The development of lesions and chlorotic regions leading to the reduction of photosynthetic area that ultimately causes yield losses (McMullen and Adhikari, 2009, Faris et al., 2013). On average, grain yield loss due to tan spot was about 10% to 15%, but up to 49% loss has been reported (Rees et al., 1982; Shabeer and Bockus, 1988). Therefore, it is important to control the disease using various measures.

Symptoms and disease cycle of tan spot

The fungus *Pyrenophora tritici-repentis* was first identified in 1823 (Hosford, 1982) and it has been found in most of the world's wheat growing regions. Tan spots initially appear as small, tan to brown lens or diamond shaped dots on wheat leaves. Characteristics of symptoms include elliptical to elongated, tan colored patches with dark brown area close to the center and yellow border. A characteristic "eye-spot" appearance is created by the pattern of tiny black spot within a tan spot lesion and yellow border around it. In susceptible varieties, the lesions grow and tend to group together to form larger, asymmetrical patches of dead tissues. As the disease worsens, the pathogen travels to the top leaves from the lower developed leaves. The dots often stay small when the leaves are young and actively growing. The leaves may become yellow in the areas with many spots giving yellow cast to the field (Figure 1). Although leaf infection is the most common form of infection, kernel infections can also occur, characterized by a reddish discoloration on the seed coat known as red smudge (Figure 2). As the plant matures it invades straw producing tiny, elevated, black, fruiting structures called pseudothecia. Pseudothecia are indicative of tan spot and noticeable on the stubble that is still present after harvest (Wegulo et al., 2012).

Figure 1. Wheat leaves with tan spot symptoms (NDSU Photos).

Figure 2. Durum wheat showing "red or pink smudge" (left and middle) and healthy seeds (right) (NDSU Photos).

The tan spot fungus can survive and reproduce in bales of infected straw, on standing wheat stubble, or even on wheat stubble and straw that is on soil surface or partially buried. During spring and summer, spores are grown and spread by wind or rain (Figure 3). The spores are called ascospores and they are born in pseudothecia. They are the sources of primary infection in field. After the primary infection, different types of spores called conidia are produced at the site of infection and spread throughout the field by wind or rain (Figure 3) (Wegulo et al., 2012).

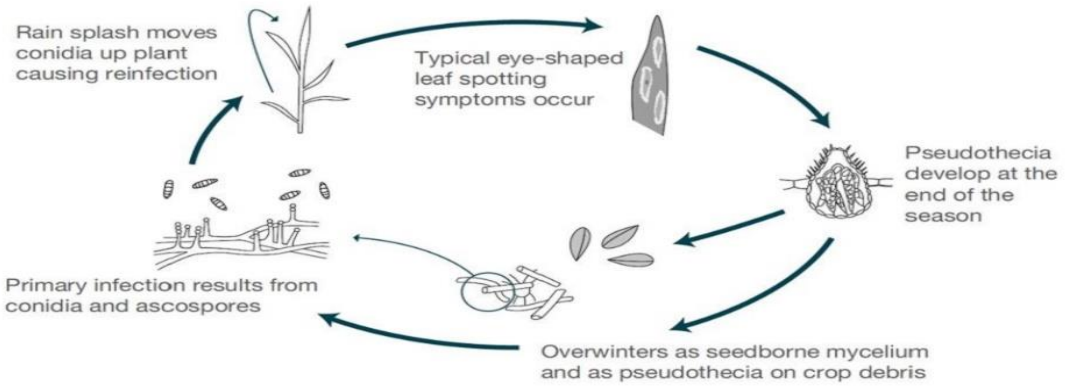

Figure 3. Life cycle of tan spot pathogen *(*Agriculture and Horticulture Development board, 2023).

Host selective toxins produced by the tan spot pathogen

The fungus *Pyrenophora tritici-repentis* releases host selective toxin (HST), called necrotrophic effectors (NEs) and the recognition of NEs by specific host sensitivity genes results in susceptibility, whereas if the host doesn't recognizes NEs it results in the incompatible reaction by showing resistance to the disease (reviewed by Faris et al., 2013). This type of host-pathogen interaction model is known as inverse gene-for-gene model (Fenton et al., 2009).

There are three NEs produced by *Pyrenophpra tritici-repentis* and are designated as Ptr ToxA, Ptr ToxB and Ptr ToxC (Ciuffetti et al., 1998). According to the standard nomenclature, the respective encoding genes in *Pyrenophora tritici-repentis* are designated as *ToxA*, *ToxB and ToxC*, respectively (Ciuffetti et al., 1998).

Ptr ToxA is encoded by a single copy gene *ToxA* in *Pyrenophora tritici-repentis* (Ciuffetti et al., 1997). Ballace et al. (1996) and Ciuffetti et al. (1997) identified *ToxA* gene in *Pyrenophora tritici-repentis* which was known to transferred from *Parastagonospora nodorum* through horizontal gene transfer (Friesen et al., 2006). Recently, *ToxA* gene had been identified in another fungal pathogen *Bipolaris sorokiniana* which causes spot blotch on wheat (Friesen and Holmes, 2018). Ptr ToxA is a 13.2 kDa secreted protein with a distinctive structure, and its

arginyl-glycyl-aspartic acid (RGD) motif, along with other amino acids in the loop is essential for its activity to enter the cells of susceptible hosts (Ciuffetti et al., 2010). Internalization of Ptr ToxA requires recognition of RGD motif and binding of this motif to a high-affinity receptor. Ptr ToxA is then internalized into endosome, enters the cytosol and targets chloroplast. It was reported that Ptr ToxA is able to cause cell death when it is accumulated internally (Manning & Ciuffetti, 2005; Tai & Bragg, 2007; Manning et al., 2010). The cell death is caused by the accumulation of reactive oxygen species(ROS) in chloroplast (Manning et al., 2010). Hence, Ptr ToxA distrupt chloroplast function by reducing the photosynthetic area in wheat leaf.

Ptr ToxB is a small secreted protein (about 6.5 kDa) that causes chlorosis (yellowing) of wheat leaves encoded by *ToxB* gene. Unlike the *ToxA* gene, the *ToxB* gene has multiple copies and copy number can vary between different isolates of the fungus. The ability of Ptr ToxB to induce chlorosis is directly related to the number of copies of the *ToxB* gene (Amaike et al., 2008). Interestingly, the plant's response to Ptr ToxB is slower compared to its response to Ptr ToxA (Ciuffetti et al., 2010). ToxB-induced yellowing in wheat leaves depends on light conditions (Strelkov et al., 1998). It is speculated that the fungus's ability to cause disease may be linked to disruptions in chloroplast function and changes proteome in wheat leaves even before the yellowing becomes visible (Ciuffetti et al., 2010; Kim et al., 2010). Ptr ToxB was reported to be heat stable and have toxicity with a minimum concentration of 40nM (Strelkov et al., 1999). Ptr ToxB has been identified as an apoplastic effector, meaning that it acts outside plant cells unlike Ptr ToxA (Figueroe et al., 2015).

Ptr ToxC has been characterized as non-ionic, polar, low molecular mass molecule (Effertz et al., 2002). Althogh Effertz (2002) partially purified Ptr ToxC using gel infilteration, ion exchange, and reverse-phase chromatography, this molecule has not yet been fully purified

and characterized. The genetic locus *ToxC* was known to encode for PtrToxC. In a recent study it was shown that gene *ToxC1* is required but not sufficient for the production of Ptr ToxC, suggesting that other genes are involved in the biosynthesis of Ptr ToxC (Shi et al., 2022).

Classification of the tan spot pathogen

Initially, scientists group strains of the fungus *Pyrenophora tritici-repentis* by looking at lesion size, the percentage of leaf necrosis, and the percentage of infection (Misra and Singh, 1972; Schilder and Bergstrom, 1990; Da Luz and Hosford, 1980). However, these methods weren't very effective in understanding how the fungus interacts with its host plant. Later, Lamari and Bernier introduced a classification system based on the symptoms caused by the fungus (Lamari and Bernier, 1989). They divided the strains into four groups or "pathotypes" based on whether they induced necrosis, chlorosis, or both on the host plant, including pathotype 1 (both chlorosis and necrosis), pathotype 2 (only necrosis, no chlorosis), pathotype 3 (only chlorosis, no necrosis), and pathotype 4 (neither chlorosis nor necrosis). However, it was found that strains caused the same symptoms on different host genotypes. To address the limitations of previous classification system, new race-based classification system was introduced, in which isolates of the *Pyrenophora tritici-repentis* are classified into races based on the HST produced (Lamari et al., 1995). Race 1 produces Ptr ToxA and Ptr ToxC; race 2 produces Ptr ToxA; race 3 produces Ptr ToxC; race 4 does not produce any necrotrophic effectors; race 5 produces Ptr ToxB; race 6 produces Ptr ToxB and Ptr ToxC; race 7 produces Ptr ToxA and Ptr ToxB (Lamari and Strelkov, 2010).

Some strains from Arkansas caused necrosis but did not produce the Ptr ToxA, which challenges the current race classification. It was also suggested that additional unknown toxins may be produced by some Arkansas isolates (Ali et al., 2010; Gamba et al., 1998; Strelkov and

Lamari, 2003).

Genes confering NE sensitivity in wheat-*Pyrenophora tritici-repentis* system

Fungi secret NEs that are recognized by corresponding sensitivity or susceptibity genes in the host leading to the compatible interaction, and this is necrotrophic effector triggered susceptibility (NETS) (Liu et al., 2009). The corresponding genes conferring sensitivity to Ptr ToxA, Ptr ToxB, and Ptr ToxC in wheat are *Tsn1, Tsc2 and Tsc1*, respectively (reviewed by Ciuffetti et al., 2010; Faris et al., 2013).

Faris et al. (1996) found that *Tsn1* is located on long arm of chromosome 5B in common wheat. *Tsn1* was mapped on chromosome 5B in durum wheat population as well (Anderson et al., 1999). Faris et al. (2010) found that *Tsn1* gene is a single gene and contains S/TPK-NBS-LRR domain, which is a similar feature for disease resistance gene. It was demonstrated that the protein encoded by *Tsn1* doesn't directly interact with Ptr ToxA, instead it monitors ToxA receptor leading to the cell import of ToxA. The low expression of *Tsn1* gene in dark implies that the expression of *Tsn1* gene is light dependent and their interaction was thought to be linked to pathways related to photosynthesis. The studies conducted on durum wheat popualtions shows that Ptr ToxA and *Tsn1* gene encoded protein interaction does not play any role in the development of disease symptoms in wheat-*Pyrenophora tritici-repentis* pathosystem (Faris et al., 2020; Galagedara et al., 2020; Virdi et al., 2016).

The gene in wheat response to Ptr ToxB effector is called *Tsc2*. Genetic mapping studies in both tetraploid and hexaploid wheat populations found that the gene is on the distal end of the short arm of chromosome 2B (Abeysekara et al., 2010; Friesen and Faris, 2004). *Tsc2* was delimited to a 3.3 cM genetic interval (Abeysekara et al., 2010). A recent mapping study in common wheat found that the gene *Tsc2* is located in a 192-kb interval containing 104 candidate

genes (Corsi et al., 2020).

The gene responsible for sensitivity to Ptr ToxC, known as *Tsc1*, was mapped in a 1.4 cM genetic interval spanning 184 kb on the short arm of chromosome 1A (Effertz et al., 2002; Kariyawasam et al., 2016; Running et al., 2022). Nine candidate genes were identified in Chinese Spring reference v2.1 genome and seven of them are found to have domains common to disease resistance genes (Running et al., 2022).

Other dominant susceptibility genes have also been identified on chromosomes 3A, 3B and 3D which might interact with other unknown NEs (Singh et al., 2006; Singh et al., 2008; Tadesse et al., 2006; Tadessee et al., 2008). Identification of new NEs and their corresponding sensitivity genes would help in developing resistant varieties by removal of the susceptibity genes from wheat.

QTLs associated with tan spot resistance

In addition to *Tsn1*, *Tsc1*, and *Tsc2*, many QTLs for tan spot resistance have been identified in hexaploid and tetraploid wheat. For example, a major QTL was mapped on chromosome 3B showing resistance to race 3 in a tetraploid wheat population derived from a cross between PI352519 and Coulter (Singh et al., 2006). A single dominant QTL on chromosome 2B was found to control chlorosis caused by race 5 in a hexaploid common wheat population (Singh et al., 2008). Tadesse et al. (2006) found a single recessive gene that confers resistance to race 1 in common wheat cultivar Salamouni located on chromosome 3A. Faris et al. (1997) found a major QTL in short arm of chromosome 1A, a QTL with minor effect on long arm of chromosome 4A, and an interaction between the QTL on chromosome 1AS and a QTL on chromosome 2DL explaining 49% phenotypic variation in a RIL population derived from the cross between synthetic hexaploid wheat variety W-7984 and a hard red spring wheat variety

Opata 85 when inoculated with isolate Pti2. A genome wide association study using a synthetic hexaploid wheat population identified markers on chromosme 1B, 1D, 2A, 2D, 3A, 3D, 4B, 5A, 6A, 6B, and 7D associated with tan spot resistance (Lozano-Ramirez et al., 2022). Dominant resistant QTLs and race non-specific QTLs were reported, suggesting resistance genes are not limited to the recessive insensitivity.

First race non-specific QTL was reported on chromosome 3B in a population derived from a cross between the hard red spring wheat varieties Grandin and BR34, from which the QTL was associated with resistance to races 1, 3, and 5 (Faris and Friesen, 2005). Another study using a wheat population derived from a cross between Louise and Penawawa identified four QTLs on chromosomes 1A, 2D, 3B, and 5A, and were designated as QTs.zhl-1A, QTs.zhl-2D, QTs.zhl-3B, and QTs.zhl-5A, respectively (Kariyawasam et al., 2016). Of them, QTs.zhl-3B and QTs.zhl-5A were highly effective against all isolates tested including Pti2, 86-124, 331-9, DW5, and AR CrossB10 (Kariyawasam et al., 2016). The race non-specific QTL on chromosome 3B was also found in durum wheat. Faris et al. (2020) evaluated a set of chromosome substitution lines of durum wheat cultivar Langdon against tan spot isolates Pti2, 86-124, 331-9, and DW5 and found that the chromosome 3B from a wild emmer wheat accession IsraelA conferred high level of resistance to all races tested. The QTL on chromosome 3B was designated as *Tsr7* (Faris et al., 2020). QTL mapping in a durum wheat double haploid population derived from a cross between Lebsock and PI94749 identified five QTLs and one QTL on chromosome 3B was significantly associated with tan spot resistance caused by isolates Pti2 and 86-124 (Chu et al., 2010). A meta-QTL analysis was conducted for tan spot resistance using 21 mapping populations, of which 10 were tetraploid wheat populations and 11 were hexaploid wheat populations (Liu et al., 2020). A consensus map with a total length of 4080.5 cM was developed

and 19 meta-QTL were identified (Liu et al., 2020). The meta-QTL analysis confirmed that the QTLs identified on chromosome 3B from the common wheat and durum wheat populations mentioned above were the same QTL. Furthermore, the meta-QTL analysis located the meta-QTL on chromosome 3B (named as MQTL-3B) in the genomic region of 466.6-474.3 Mbp (Liu et al., 2020).

A genome wide association study using ~400 durum wheat worldwide collections found a resistant QTL on chromosome 3B is race non-specific and works against isolates Pti2, 86-124, 331-9 and DW5 (Galagedara et al., 2020). Significant markers were located within the genomic region of the MQTL-3B (Galagedara et al., 2020; Liu et al., 2020). Another association mapping study of tan spot resistance using global durum wheat panel found that markers within the region of the MQTL-3B or *Tsr7* is significantly associated with resistance to races 2 and 3, but not with races 1, 4, and 5 (Szabo-Hever et al., 2023). The genome wide association studies further supported that there is a race non-specific gene for tan spot resistance on chromosome 3B. After genotyping the significant markers for over 1,800 elite durum breeding lines and cultivars from the North Dakota State University (NDSU) durum wheat program, it was found that none of them has the desirable allele of the MQTL-3B (Li et al., unpublished data). Therefore, introgression of the favorable allele of the MQTL-3B to the NDSU durum wheat breeding pool is promising to develop new breeding germplasm with improved tan spot resistance adapted to Northern Great Plains.

Marker-assisted backcross selection

Introgression of superior genes/QTLs from wild relatives to elite breeding lines is a common way to develop pre-breeding germplasm or new cultivar for disease resistance in wheat. However, the traditional phenotypic backcross selection method can be slow and ineffective

because it relies solely on selecting plants based on their visible traits. Sometimes, we can't easily see if the desired resistance has been passed on. Also, traditional phenotypic backcross selection might unintentionally bring along other unwanted genes that may have negative impacts on other important traits (Li et al., 2017). Marker-assisted backcross selection (MABC) allows effective introgression of a gene while retaining the background of recurrent parent by using markers linked to the gene and/or genome wide markers (Collard and Mackill, 2008). MABC has been widely used for introgression of disease resistance genes or QTLs in wheat (Mallick et al., 2022; Sunilkumar et al., 2022; Vida et al., 2009). In MABC, the F_1 plants are backcrossed to the recurrent parent; selection of the progenies with the favorable allele and backcrossing to the recurrent parent is continued until the background of recurrent parent is obtained; at final stage, those desirable progenies are self-pollinated to obtain inbred lines with homozygous favorable allele. In addition to selection for the target gene/QTL, background selection can accelerate the process by reducing the number of backcrosses required to recover the recurrent parent phenotype (Collard, 2005). Recombinant selection can reduce linkage drag but can be only employed for those finely mapped genes (Hasan et al., 2015).

Kompetitive Allele Specific PCR (KASP) marker

Effective marker-assisted selection (MAS) hinges on choosing informative markers. Single nucleotide polymorphisms (SNPs), discovered in 1974, are ideal due to their abundance throughout genomes, high variation between individuals (polymorphism), and ability to show both parental versions (co-dominant). While recent advancements allow easier SNP identification, converting them into user-friendly tools for breeders remains a challenge.

Kompetitive Allele Specific PCR (KASP) markers offer a simple, fast, and cost-effective method for SNP validation (Allen et al., 2013). This technique relies on fluorescence resonance

energy transfer (FRET) to detect specific SNPs (Suo et al., 2020). In KASP, two sets of fluorescent molecules compete to bind the target SNP region after DNA replication (PCR) (He er al., 2014). If a specific SNP is present, only the matching fluorescent molecule binds, emitting a signal upon separation during PCR completion. This unique signal allows researchers to identify specific genetic variations, making KASP valuable for genotyping applications.

References

Abeysekara, N. S., Friesen, T. L., Liu, Z., McClean, P. E., & Faris, J. D. (2010). Marker development and saturation mapping of the tan spot Ptr ToxB sensitivity locus *Tsc2* in hexaploid wheat. *The Plant Genome*, *3*(3), 179-189. https://doi.org/10.3835/plantgenome2010.07.0017

Agriculture and Horticulture Development Board. (2023). *Management of tan spot disease in Wheat, Barley and Rye*. Retrieved from https://ahdb.org.uk/knowledge-library/management-of-tan-spot-disease-in-wheat-barley-and-rye

Ali, S., & Francl, L. J. (2003). Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and noncereal grasses in the Great Plains. *Plant Disease*, *87*(4), 418-422. https://doi.org/10.1094/PDIS.2003.87.4.418

Ali, S., Gurung, S., & Adhikari, T. B. (2010). Identification and characterization of novel isolates of *Pyrenophora tritici-repentis* from Arkansas. *Plant Disease*, *94*(2), 229-235. https://doi.org/10.1094/PDIS-94-2-0229

Allen, A. M., Barker, G. L., Wilkinson, P., Burridge, A., Winfield, M., Coghill, J., Uauy, C., Griffiths, S., Jack, P., Berry, S., Werner, P., Melicher, J.P.E., McDougall, J., Gwilliam, R., Robinson, P. & Edwards, K. J. (2013). Discovery and development of exome-based, co-dominant single nucleotide polymorphism markers in hexaploid wheat (*Triticum*

aestivum L.). *Plant biotechnology journal*, *11*(3), 279-295. https://doi.org/10.1111/pbi.12009

Amaike, S., Ozga, J. A., Basu, U., & Strelkov, S. E. (2008). Quantification of ToxB gene expression and formation of appressoria by isolates of *Pyrenophora tritici-repentis* differing in pathogenicity. *Plant Pathology, 57*(4), 623-633. https://doi.org/10.1111/j.1365-3059.2007.01821.x

Anderson, J. A., Effertz, R. J., Faris, J. D., Francl, L. J., Meinhardt, S. W., & Gill, B. S. (1999). Genetic analysis of sensitivity to a *Pyrenophora tritici-repentis* necrosis-inducing toxin in durum and common wheat. *Phytopathology, 89*(4), 293-297. https://doi.org/10.1094/PHYTO.1999.89.4.293

Ballance, G. M., Lamari, L., Kowatsch, R., & Bernier, C. C. (1996). Cloning, expression and occurrence of the gene encoding the Ptr necrosis toxin from *Pyrenophora tritici-repentis*. *Molecular Plant Pathology On-Line*. Retrieved from https://www.cabidigitallibrary.org/doi/full/10.5555/19971002889

Barrus, M. F. (1942). Yellow-spot disease of wheat in New York state. *Plant Disease Reporter, 26*(11), 246-247. Retrieved from https://www.cabidigitallibrary.org/doi/full/10.5555/19431101158

Chu, C. G., Chao, S., Friesen, T. L., Faris, J. D., Zhong, S., & Xu, S. S. (2010). Identification of novel tan spot resistance QTLs using an SSR-based linkage map of tetraploid wheat. *Molecular Breeding*, *25*, 327-338. https://doi.org/10.1007/s11032-009-9335-2

Ciuffetti, L. M., Francl, L. J., Ballance, G. M., Bockus, W. W., Lamari, L., Meinhardt, S. W., & Rasmussen, J. B. (1998). Standardization of toxin nomenclature in the *Pyrenophora*

tritici-repentis/wheat interaction. *Canadian Journal of Plant Pathology*, *20*(4), 421-424. https://doi.org/10.1080/07060669809500415

Ciuffetti, L. M., Manning, V. A., Pandelova, I., Betts, M. F., & 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*(4), 911-919. https://doi.org/10.1111/j.1469-8137.2010.03362.x

Ciuffetti, L. M., Tuori, R. P., & Gaventa, J. M. (1997). A single gene encodes a selective toxin causal to the development of tan spot of wheat. *The Plant Cell*, *9*(2), 135-144. https://doi.org/10.1105/tpc.9.2.135

Collard, B. C., & Mackill, D. J. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences, 363*(1491), 557-572. https://doi.org/10.1098/rstb.2007.2170

Collard, B. C., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica, 142*, 169-196. https://doi.org/10.1007/s10681-005-1681-5

Corsi, B., Percival-Alwyn, L., Downie, R. C., Venturini, L., Iagallo, E. M., Campos Mantello, C., McCormick-Barnes, C., See, P.T., Oliver, R.P., Moffat, C.S. & Cockram, J. (2020). Genetic analysis of wheat sensitivity to the ToxB fungal effector from *Pyrenophora tritici-repentis*, the causal agent of tan spot. *Theoretical and Applied Genetics*, 133, 935-950. https://doi.org/10.1007/s00122-019-03517-8

Curtis, B. C., Rajaram, S., & Gomez Macpherson, H. (2002). *Bread wheat: improvement and production*. Food and Agriculture Organization of the United Nations (FAO). Retrieved from https://www.cabidigitallibrary.org/doi/full/10.5555/20033015139

Da Luz, W. C., & Hosford Jr, R. M. (1980). Twelve *Pyrenophora trichostoma* races for virulence to wheat in the Central Plains of North America. *Phytopathology, 70*(1), 193-1196. Retrieved from https://www.apsnet.org/publications/phytopathology/backissues/Documents/1980Articles/Phyto70n12_1193.PDF

Dubcovsky, J., & Dvorak, J. (2007). Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science, 316*(5833), 1862-1866. https://doi.org/10.1126/science.1143986

Effertz, R. J., Meinhardt, S. W., Anderson, J. A., Jordahl, J. G., & Francl, L. J. (2002). Identification of a chlorosis-inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitivity locus in wheat. *Phytopathology, 92*(5), 527-533. https://doi.org/10.1094/PHYTO.2002.92.5.527

Faris, J. D., & Friesen, T. L. (2005). Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. *Theoretical and Applied Genetics, 111*(2), 386-392. https://doi.org/10.1007/s00122-005-2033-5

Faris, J. D., Abeysekara, N. S., McClean, P. E., Xu, S. S., & Friesen, T. L. (2012). Tan spot susceptibility governed by the *Tsn1* locus and race-nonspecific resistance quantitative trait loci in a population derived from the wheat lines Salamouni and Katepwa. *Molecular breeding, 30*, 1669-1678. https://doi.org/10.1007/s11032-012-9750-7

Faris, J. D., Anderson, J. A., Francl, L. J., & Jordahl, J. G. (1996). Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis*. *Phytopathology*, *86*(5), 459-463. Retrieved from https://www.apsnet.org/publications/phytopathology/backissues/Documents/1996Articles/Phyto86n05_459.pdf

Faris, J. D., Anderson, J. A., Francl, L. J., & Jordahl, J. G. (1997). RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. *Theoretical and Applied Genetics*, *94*, 98-103. Retrieved from https://link.springer.com/article/10.1007/s001220050387

Faris, J. D., Liu, Z., & Xu, S. S. (2013). Genetics of tan spot resistance in wheat. *Theoretical and Applied Genetics*, 126, 2197-2217. https://doi.org/10.1007/s00122-013-2157-y.

Faris, J. D., Overlander, M. E., Kariyawasam, G. K., Carter, A., Xu, S. S., & Liu, Z. (2020). Identification of a major dominant gene for race-nonspecific tan spot resistance in wild emmer wheat. *Theoretical and Applied Genetics*, *133*, 829-841. https://doi.org/10.1007/s00122-019-03509-8

Faris, J. D., Zhang, Z., Lu, H., Lu, S., Reddy, L., Cloutier, S., Fellers, J.P., Meinhardt, S.W., Rasmussen, J.B., Xu, S.S., Oliver, R.P., Simons, K.J. & Friesen, T. L. (2010). A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proceedings of the National Academy of Sciences*, *107*(30), 13544-13549. https://doi.org/10.1073/pnas.1004090107.

Fenton, A., Antonovics, J., & Brockhurst, M. A. (2009). Inverse-gene-for-gene infection genetics and coevolutionary dynamics. *The American Naturalist*, *174*(6), E230-E242.

Figueroa, M., Manning, V. A., Pandelova, I., & Ciuffetti, L. M. (2015). Persistence of the host-selective toxin Ptr ToxB in the apoplast. *Molecular Plant-Microbe Interactions*, *28*(10), 1082-1090. https://doi.org/10.1094/MPMI-05-15-0097-R

Friesen, T. L., & Faris, J. (2004). Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. *Theoretical and applied genetics*, *109*(3), 464-471. https://doi.org/10.1007/s00122-004-1678-9

Friesen, T. L., Holmes, D. J., Bowden, R. L., & Faris, J. D. (2018). *ToxA* is present in the US *Bipolaris sorokiniana* population and is a significant virulence factor on wheat harboring *Tsn1*. *Plant disease*, *102*(12), 2446-2452. https://doi.org/10.1094/PDIS-03-18-0521-RE

Galagedara, N., Liu, Y., Fiedler, J., Shi, G., Chiao, S., Xu, S. S., Faris, J.D., Li, X. & Liu, Z. (2020). Genome-wide association mapping of tan spot resistance in a worldwide collection of durum wheat. *Theoretical and Applied Genetics*, *133*, 2227-2237. https://doi.org/10.1007/s00122-020-03593-1

Gamba, F. M., Lamari, L., & Brülé-Babel, A. L. (1998). Inheritance of race-specific necrotic and chlorotic reactions induced by *Pyrenophora tritici-repentis* in hexaploid wheats. *Canadian Journal of Plant Pathology*, *20*(4), 401-407. https://doi.org/10.1080/07060669809500411

Hasan, M. M., Rafii, M. Y., Ismail, M. R., Mahmood, M., Rahim, H. A., Alam, M. A., Ashkani, S., Malek, M.A. & Latif, M. A. (2015). Marker-assisted backcrossing: a useful method for rice improvement. *Biotechnology & Biotechnological Equipment*, *29*(2), 237-254. https://doi.org/10.1080/13102818.2014.995920

He, C., Holme, J., & Anthony, J. (2014). SNP genotyping: the KASP assay. *Crop breeding: methods and protocols* (Vol. 1145, pp.1894-1903). New York, NY: Humana Press. https://doi.org/10.1007/978-1-4939-0446-4_7

Hosford Jr, R. M. (1982*). Tan spot of wheat and related diseases workshop, July 14-15, 1981. North Dakota State University, Fargo, ND*. North Dakota Agricultural Experiment Station, North Dakota State University, 1-24.

Kariyawasam, G. K., Carter, A. H., Rasmussen, J. B., Faris, J., Xu, S. S., Mergoum, M., & Liu, Z. (2016). Genetic relationships between race-nonspecific and race-specific interactions in the wheat–*Pyrenophora tritici-repentis* pathosystem. *Theoretical and Applied Genetics, 129*, 897-908. https://doi.org/10.1007/s00122-016-2670-x

Kim, Y. M., Bouras, N., Kav, N. N., & Strelkov, S. E. (2010). Inhibition of photosynthesis and modification of the wheat leaf proteome by Ptr ToxB: a host-specific toxin from the fungal pathogen *Pyrenophora tritici-repentis*. *Proteomics*, *10*(16), 2911-2926. https://doi.org/10.1002/pmic.200900670

Lamari, L., & Bernier, C. C. (1989). Toxin of *Pyrenophora tritici-repentis*: Host-specificity, significance in disease. *Pytopathology, 79*, 740-744. Retrieved from https://www.apsnet.org/publications/phytopathology/backissues/Documents/1989Articles/Phyto79n07_740.pdf

Lamari, L., & 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*(1), 4-10. https://doi.org/10.1080/07060661003594117.

Lamari, L., Sayoud, R., Boulif, M., & Bernier, C. C. (1995). Identification of a new race in *Pyrenophora tritici-repentis*: implications for the current pathotype classification

system. *Canadian Journal of Plant Pathology*, *17*(4), 312-318. https://doi.org/10.1080/07060669509500668

Li, H., Jiang, B., Wang, J., Lu, Y., Zhang, J., Pan, C., Yang, X., Li, X., Liu, W. & Li, L. (2017). Mapping of novel powdery mildew resistance gene (s) from Agropyron cristatum chromosome 2P. *Theoretical and Applied Genetics, 130*, 109-121. https://doi.org/10.1007/s00122-016-2797-9

Li, S. Y., Xi, L., Liu, H. J., Zhu, W., Xu, L. L., Wang, Y., Zeng, J., Fan, X., Sha, L.N., Zhang, H.Q., Qi, W.L., Chen, G.Y., Zhou, Y.H. & Kang, H. Y. (2020). The classification of tetraploid wheat by phylogenetic and cytogenetic analyses. *Biologia Plantarum*, *64*(1). https://doi.org/10.32615/bp.2020.013

Liu, Y., Salsman, E., Wang, R., Galagedara, N., Zhang, Q., Fiedler, J. D., Liu, Z., Xu, S., Faris, J.D. & Li, X. (2020). Meta-QTL analysis of tan spot resistance in wheat. *Theoretical and Applied Genetics*, *133*, 2363-2375. https://doi.org/10.1007/s00122-020-03604-1

Liu, Z., Faris, J. D., Oliver, R. P., Tan, K. C., Solomon, P. S., McDonald, M. C., McDonald, B.A., Nunez, A., Lu, S., Rasmussen, J.B. & Friesen, T. L. (2009). SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the Snn3 gene. *PLoS Pathogens*, *5*(9), e1000581. https://doi.org/10.1371/journal.ppat.1000581

Lozano-Ramirez, N., Dreisigacker, S., Sansaloni, C. P., He, X., Sandoval-Islas, J. S., Perez-Rodriguez, P., Carballo, A.C., Diaz, C.N., Kishii, M. & Singh, P. K. (2022). Genome-wide association study for spot blotch resistance in synthetic hexaploid wheat. *Genes*, *13*(8), 1387. https://doi.org/10.3390/genes13081387

Mallick, N., Jha, S. K., Agarwal, P., Mall, A., Kumar, S., Choudhary, M. K., Bansal, S., Saharan, M.S., Sharma, J.B. & Vinod. (2022). Marker-assisted improvement of bread wheat

variety HD2967 for leaf and stripe rust resistance. *Plants, 11*(9), 1152. https://doi.org/10.3390/plants11091152

Manning, V. A., & Ciuffetti, L. M. (2005). Localization of Ptr ToxA produced by *Pyrenophora tritici-repentis reveals* protein import into wheat mesophyll cells. *The Plant Cell, 17*(11), 3203-3212. https://doi.org/10.1105/tpc.105.035063

Manning, V. A., Chu, A. L., Scofield, S. R., & Ciuffetti, L. M. (2010). Intracellular expression of a host-selective toxin, ToxA, in diverse plants phenocopies silencing of a ToxA-interacting protein, ToxABP1. *New Phytologist, 187*(4), 1034-1047. https://doi.org/10.1111/j.1469-8137.2010.03363.x

McMullen, M., & Adhikari, T. (2009). Fungal Leaf Spot Diseases of Wheat: tan spot, stagonospora nodorum blotch and septoria tritici blotch (PP-1249). *Plant Disease Management (NDSU Extension Service)*.

Misra, A. P., & Singh, R. A. (1972). Pathogenic differences amongst three isolates of *Helminthosporium tritici-repentis* and the performance of wheat varieties against them. *Indian Phytopathology*, *25*(3), 350-353. Retrieved from https://www.cabidigitallibrary.org/doi/full/10.5555/19731306434

Ozkan, H., Brandolini, A., Pozzi, C., Effgen, S., Wunder, J., & Salamini, F. (2005). A reconsideration of the domestication geography of tetraploid wheats. *Theoretical and Applied Genetics*, *110*, 1052-1060. https://doi.org/10.1007/s00122-005-1925-8

Rees, R. G., Platz, G. J., & Mayer, R. J. (1982). Yield losses in wheat from yellow spot: comparison of estimates derived from single tillers and plots. *Australian Journal of Agricultural Research*, *33*(6), 899-908. https://doi.org/10.1071/AR9820899

Running, K. L., Momotaz, A., Kariyawasam, G. K., Zurn, J. D., Acevedo, M., Carter, A. H., Liu, Z. & Faris, J. D. (2022). Genomic analysis and delineation of the tan spot susceptibility locus Tsc1 in wheat. *Frontiers in Plant Science*, *13*, 793925. https://doi.org/10.3389/fpls.2022.793925

Schilder, A. M. C., & Bergstrom, G. C. (1990). Variation in virulence within the population of *Pyrenophora tritici-repentis* in New York. *Phytopathology, 80*(1), 84-90. Retrieved from https://www.apsnet.org/publications/phytopathology/backissues/Documents/1990Articles/Phyto80n01_84.PDF

Shabeer, A., & Bockus, W. W. (1988). Tan spot effects on yield and yield components relative to growth stage in winter wheat. *Plant Disease*, *72*(7), 599-602. https://doi.org/10.1094/PD-72-0599

Shahbandeh, M. 2023. Leading wheat producers worldwide from 2016/2017 to 2022/2023. Statista. Retrieved from https://www.statista.com/statistics/237908/global-top-wheat-producing-countries/

Shahbandeh, M. 2023. Leading wheat producing U.S. states in 2021 and 2022. Statista. Retrieved from www.statista.com/statistics/190376/top-us-states-in-wheat-production/.

Shahbandeh, M. 2023. Wheat consumption worldwide in 2021/2022 by country. Statista. Retrieved from https://www.statista.com/statistics/1094065/total-global-wheat-consumption-by-country/

Shi, G., Kariyawasam, G., Liu, S., Leng, Y., Zhong, S., Ali, S., Moolhuijzen, P., Moffat, C.S., Rasmussen J.B., Friesen, T.L., Faris, J.D. & Liu, Z. (2022). A conserved hypothetical gene is required but not sufficient for Ptr ToxC production in *Pyrenophora tritici-*

repentis. *Molecular Plant-Microbe Interactions*, *35*(4), 336-348. https://doi.org/10.1094/MPMI-12-21-0299-R

Singh, P. K., Gonzalez-Hernandez, J. L., Mergoum, M., Ali, S., Adhikari, T. B., Kianian, S. F., Elias, E.M. & Hughes, G. R. (2006). Identification and molecular mapping of a gene conferring resistance to *Pyrenophora tritici-repentis* race 3 in tetraploid wheat. *Phytopathology*, 96(8), 885-889. https://doi.org/10.1094/PHYTO-96-0885

Singh, P. K., Mergoum, M., Gonzalez-Hernandez, J. L., Ali, S., Adhikari, T. B., Kianian, S. F., Elias, E.M. & Hughes, G. R. (2008). Genetics and molecular mapping of resistance to necrosis inducing race 5 of *Pyrenophora tritici-repentis* in tetraploid wheat. *Molecular Breeding*, 21, 293-304. https://doi.org/10.1007/s11032-007-9129-3

Sowell A. and Swearingen B. (2023). Wheat Sector at a Glance. Economic Research Service, USDA. Retrieved from https://www.ers.usda.gov/topics/crops/wheat/wheat-sector-at-a-glance/#:~:text=In%202023%2F24%2C%20U.S.%20farmers,from%20last%20year's%20record%20low

Statista. (2023). Wheat consumption worldwide in 2023/2024, by country. Retrieved from https://www.statista.com/statistics/1094065/total-global-wheat-consumption-by-country/.

Strelkov, S. E., & Lamari, L. (2003). Host–parasite interactions in tan spot [*Pyrenophora tritici-repentis*] of wheat. *Canadian Journal of Plant Pathology*, *25*(4), 339-349. https://doi.org/10.1080/07060660309507089

Strelkov, S. E., Lamari, L., & Ballance, G. M. (1998). Induced chlorophyll degradation by a chlorosis toxin from *Pyrenophora tritici-repentis*. *Canadian Journal of Plant Pathology*, *20*(4), 428-435. https://doi.org/10.1080/07060669809500417

Strelkov, S. E., Lamari, L., & Ballance, G. M. (1999). Characterization of a host-specific protein toxin (Ptr ToxB) from *Pyrenophora tritici-repentis*. *Molecular Plant-Microbe Interactions*, 12(8), 728-732. https://doi.org/10.1094/MPMI.1999.12.8.728

Sunilkumar, V. P., Krishna, H., Devate, N. B., Manjunath, K. K., Chauhan, D., Singh, S., Sinha, N., Singh, J.B., Prakasha, T.L., Pal, D., Sivasamy, M., Jain, N., Singh, G.P., Singh, P.K. & Singh, P. K. (2022). Marker assisted improvement for leaf rust and moisture deficit stress tolerance in wheat variety HD3086. *Frontiers in Plant Science, 13*, 1035016. https://doi.org/10.3389/fpls.2022.1035016

Suo, W., Shi, X., Xu, S., Li, X., & Lin, Y. (2020). Towards low cost, multiplex clinical genotyping: 4-fluorescent Kompetitive Allele-Specific PCR and its application on pharmacogenetics. *PLoS One*, *15*(3), e0230445. https://doi.org/10.1371/journal.pone.0230445

Szabo-Hever, A., Singh, G., Haugrud, A. R. P., Running, K. L., Seneviratne, S., Zhang, Z., Shi, G., Bassi, F.M., Maccaferri, M., Cativelli, L., Tuberosa, R., Friesen, T.L., Liu, Z., Xu, S.S. & Faris, J. D. (2023). Association mapping of resistance to tan spot in the Global Durum Panel. *Phytopathology*, *113*(10), 1967-1978. https://doi.org/10.1094/PHYTO-02-23-0043-R

Tadesse, W., Hsam, S. L. K., & Zeller, F. J. (2006). Evaluation of common wheat cultivars for tan spot resistance and chromosomal location of a resistance gene in the cultivar 'Salamouni'. *Plant Breeding*, *125*(4), 318-322. https://doi.org/10.1111/j.1439-0523.2006.01243.x

Tadesse, W., Hsam, S. L. K., Wenzel, G., & Zeller, F. J. (2008). Chromosome location of a gene conferring resistance to *Pyrenophora tritici-repentis* in Ethiopian wheat cultivars. *Euphytica*, *162*, 423-430. https://doi.org/10.1007/s10681-007-9593-1

Tai, Y. S., & Bragg, J. (2007). Dual applications of a virus vector for studies of wheat-fungal interactions. *Biotechnology*, *6*(2), 288-91. Retrieved from http://www.ansinet.org

USDA, Economics, Statistics and Market Information System. September 2023. Small grains 2023 summary. Retrieved from https://usda.library.cornell.edu/concern/publications /5t34sj573/

Vida, G., Gál, M., Uhrin, A., Veisz, O., Syed, N. H., Flavell, A. J., Wang, Z. & Bedő, Z. (2009). Molecular markers for the identification of resistance genes and marker-assisted selection in breeding wheat for leaf rust resistance. *Euphytica*, *170*, 67-76. DOI 10.1007/s10681-009-9945-0

Virdi, S. K., Liu, Z., Overlander, M. E., Zhang, Z., Xu, S. S., Friesen, T. L., & Faris, J. D. (2016). New insights into the roles of host gene-necrotrophic effector interactions in governing susceptibility of durum wheat to tan spot and Septoria nodorum blotch. *G3: Genes, Genomes, Genetics*, *6*(12), 4139-4150. https://doi.org/10.1534/g3.116.036525

Wegulo, S. N., Klein, R. N., & Harveson, R. M. (2012). Tan Spot of Wheat. University of Nebraska–Lincoln Extension, Institute of Agriculture and Natural Resources, Nebraska.

CHAPTER 2: MARKER-ASSISTED BACKCROSS INTROGRESSION OF A RACE NON-SPECIFIC QTL TO DEVLOP DURUM WHEAT LINES WITH IMPROVED TAN SPOT RESISTANCE[1]

Abstract

Durum wheat (*Triticum turgidum* subsp. *durum*) is an important food crop and mainly used for pasta production. Tan spot is a foliar fungal disease affecting durum wheat cultivation and production worldwide. The fungal pathogen infects leaves of wheat causing a reduction in total photosynthetic area and a subsequent decrease in production. Developing wheat tan spot resistant varieties is an environmentally friendly way to control the disease. From previous studies, a race non-specific QTL (named as TS-3B-QTLrn) was mapped on chromosome 3B. In this study, we introgressed the favorable allele of the TS-3B-QTLrn into two durum wheat cultivars "Grano" and "Riveland" via marker-assisted backcross selection. The resulting $BC_4F_{2:3}$, $BC_5F_{2:3}$, and $BC_6F_{2:3}$ lines were evaluated for their reactions to multiple isolates including 331-9, 86-124, DW5, and ND1. It was found that the introgression lines with the favorable allele showed significantly lower disease severity than the recurrent parents "Grano" and "Riveland". Phenotyping of a population of $BC_4F_{1:2}$ progenies indicated that the TS-3B-QTLrn showed partially dominant resistance. Further research will be conducted to test the effectiveness of the

[1] The material in this chapter was co-authored by Namrata Acharya and Xuehui Li. Namrata Acharya had primary responsibility for collecting samples in the field and for interviewing users of the test system. Namrata Acharya was the primary developer of the conclusions that are advanced here. Namrata Acharya also drafted and revised all versions of this chapter. Xuehui Li served as proofreader and checked the math in the statistical analysis conducted by Namrata Acharya.

TS-3B-QTLrn on disease severity of adult plants under field conditions, as well as to test if the introgression of the TS-3B-QTLrn has effects on grain yield and quality related traits.

Keywords: Durum wheat, tan spot, *Pyrenophora tritici-repentis,* race non-specific QTL, marker-assisted backcross selection.

Introduction

Durum wheat (*Triticum turgidum* subsp. *durum*) is an allotetraploid (AABB, 2n=4x=28) and one of the first domesticated food crops. Its direct ancestor is cultivated emmer wheat (*Triticum turgidum* ssp. *dicoccum),* which is domesticated from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) in the Fertile Crescent 6,500-7,500 years ago (Dubcovsky and Dvorak, 2007). Durum wheat is a globally cultivated crop with a total production of 28.1 million metric tons in 2019 (Xynias et al., 2020). In the United States, durum wheat is annually planted on 1.6 million acres with production of 1.6 million metric tons (USDA, 2023). North Dakota and Montana are the major producer of durum wheat in United States with the production of around 0.9 million metric tons and 0.5 million metric tons, respectively (USDA, 2023). The demand of pasta is increasing yearly, and this is estimated to increase every year with a total consumption of 53.7 million metric ton in 2023 to 63.5 million metric ton in 2028 (Statista, 2023). However, multiple diseases threaten durum wheat production. Additionally, the adoption of reduced or no-till farming practice has increased the levels of disease-causing pathogens in crop residues, leading to more frequent outbreaks of diseases (reviewed by Faris et al., 2013).

Tan spot is one of the major foliar diseases threatening wheat production, caused by the fungus *Pyrenophora tritici-repentis* (Ali and Francl, 2003). During winter, the fungus survives on the remains of wheat plants in the form of tiny black structures called pseudothecia. The pseudothecia release spores under wet and warm conditions, which are carried by the wind to

infect wheat seedlings (De Wolf et al., 1998). The infection leads to development of small, oval to diamond shaped tan-colored lesion on leaf surrounded by chlorotic halo in susceptible wheat varieties. The development of lesion tends to coalesce and decreases photosynthesis. A significant decrease in both kernel weight and number of grains per head was observed when inoculated at bolting and flowering stages (Shabeer and Bockus, 1988). A decrease in number of tillers, accumulation of dry matter, leaf area index, and size of grains was reported due to tan spot (Rees and Platz, 1983). Under favorable conditions, the disease can cause up to 50% of grain yield loss (Shabeer and Bockus, 1988). Cultural practices like crop rotation with non-host crops, removal of infested residue and appropriate tillage can help controlling tan spot outbreaks (Bockus and Claassen, 1992; Carigano et al., 2008). When the infection is severe, the common practice is foliar spray of fungicides (Wegulo, 2011). It was reported that some isolates of *Pyrenophora tritici-repentis* had developed resistance over common fungicides over time (Murray and Brennan, 2009). Developing resistant cultivars is the most environmentally and ecologically responsible approach to minimize the loss caused by tan spot.

Necrotrophic pathogen produces necrotrophic effectors (NEs), also known as host selective toxins (HSTs), which are specifically recognized by dominant host sensitivity genes (reviewed by Faris et al., 2013). This type of host-pathogen interaction system is known as inverse gene-for-gene system (Fenton et al., 2009). *Pyrenophora tritici-repentis* is known to produce three NEs, which are designated as Ptr ToxA, Ptr ToxB and Ptr ToxC (Ciuffetti et al., 1998). The sensitivity genes for Ptr ToxA, Ptr ToxB and Ptr ToxC in wheat are *Tsn1, Tsc2* and *Tsc1*, which were mapped on chromosomes 5B, 2B and 1A, respectively (Reviewed by Faris et al., 2013). In addition, unknown NEs were reported in some isolates (Meinhardt, 2003; reviewed by Faris et al., 2013).

Over time, researchers have identified a growing number of quantitative trait loci (QTLs) for tan spot resistance in hexaploid common wheat and durum wheat (Friesen and Faris 2004; Singh et al., 2006; Singh et al., 2008; Kariyawasam et al., 2016; Faris et al., 2020; Galagedara et al., 2020; Szabo-Hever et al., 2023). Some of them showed dominant resistance and/or were resistant to multiple isolates, suggesting that the wheat-*Pyrenophora tritici-repentis* interaction is more complex than inverse gene-for-gene system. A meta-QTL analysis was conducted using 21 previously studied mapping populations, and a meta-QTL was identified on chromosome 3B, which confers resistance to multiple races of the pathogen in both hexaploid common wheat and tetraploid wheat (Liu et al., 2020). This meta-QTL was defined in a genetic interval of 59.4-66.1 cM on a consensus linkage map and physical interval of 465.2-583.4 Mbp and comprised 11 initial QTLs identified from three bi-parental mapping populations (Liu et al., 2020). Of the 11 initial QTLs, four were identified from a common wheat BR34×Grandin population (Faris and Friesen, 2005), explaining 13% to 41% of the phenotypic variation when inoculated with isolates Pti2 (race1), 86-124 (race 2), OH99 (race 3) and DW5 (race5); five initial QTLs were identified in a common wheat population of Louise×Penawawa (Kariyawasam et al., 2016) and found effective against isolates Pti2, 86-124, 331-9 (race 3), DW5, and ARcrossB10 (unclassified isolate); and the remaining two initial QTLs were identified in a tetraploid wheat population derived from a cross between Lebsock (*T turgidum subsp. durum*) and PI94749 (*T turgidum subsp. carthlicum*) (Chu et al., 2010), where isolates Pti2 and 86-124 were inoculated. A genome wide association study (GWAS) using durum wheat worldwide collections found that markers in the region of 466.6-474.3 Mbp on chromosome 3B were significantly associated with tan spot resistance against multiple isolates including Pti2, 86-124, 331-9 and DW5 (Galagedara et al., 2020). The meta-QTL and GWAS analysis indicated that there is a race non-specific resistance

gene within the region of chromosome 3B, named as TS-3B-QTLrn hereafter. Four KASP markers within the region were developed and were used to genotype over 1,800 durum wheat breeding lines from the North Dakota State University (NDSU) breeding program; none of them had the favorable alleles of the race no-specific QTL on chromosome 3B (Dr. Li, unpublished data).

The objectives of this study are to (1) introgress the favorable allele of the race non-specific QTL TS-3B-QTLrn into two durum wheat cultivars "Grano" and "Riveland" using marker-assisted backcross selection; (2) evaluate its effects on tan spot resistance at seedling stage; (3) determine function mode of the race non-specific QTL.

Materials and method

Plant materials

"Grano" and "Riveland" were used as recipient parents. The two durum wheat varieties were released by the NDSU durum wheat breeding program (Elias and Manthey, 2019; Elias et al., 2021). PI192640, a breeding line from Australia, was used as a donor parent in this study, which showed high resistance to isolates Pti2, 86-124, 331-9 and DW5 in the previous GWAS analysis (Galagedara et al., 2020).

Backcross selection scheme

"Grano" and "Riveland" were crossed to PI192640. The F_1s were backcrossed to each recipient variety, respectively. Each BC_1F_1 population, 48 progenies were planted and genotyped with four KASP markers closely linked to the TS-3B-QTLrn. According to the marker data, four BC_1F_1 progenies with the favorable allele (heterozygous) were backcrossed to the recipient parent, respectively. The backcrossing and selection based on the KASP markers were repeated. BC_4F_1, BC_5F_1, and BC_6F_1 progenies with the favorable allele were self-pollinated to get BC_4F_2,

BC_5F_2, and BC_6F_2 progenies. The BC_4F_2, BC_5F_2, and BC_6F_2 progenies were genotyped with the four KASP markers and homozygous progenies with the favorable allele (Grano-BB or Riveland-BB) and without favorable allele (Grano-AA or Riveland-AA) were self-pollinated to get $BC_4F_{2:3}$, $BC_5F_{2:3}$, and $BC_6F_{2:3}$ lines for phenotypic evaluation.

All plants were grown in greenhouse under controlled conditions. Plants were grown in plastic pots of 5-inch diameter. The pots were filled with a soil mixture comprising 85% sphagnum peat moss, along with perlite, vermiculite, limestone, and a wetting agent. They were then moistened with a standard nutrient solution (Miracle-Gro® Professional Peat-Lite® Special 20-10-20) following the guidelines provided by the manufacturer (The Scotts Company, 1411 Scottslawn Road, Marysville, OH 43041). Slow-release fertilizer (Multicote-6 14-14-16, Haifa Chemicals Ltd, Israel) was incorporated into the pots.

DNA extraction

Leaf tissues were collected from two-week old seedlings. The samples were then sent to the USDA-ARS genotyping lab at Fargo, ND, where the DNA extraction was carried out based on Wheat and Barley DNA Extraction Protocol (96-well plate format) (Pallotta et al., 2003).

KASP marker design and genotyping

Four KASP markers (Table 1) were designed based on the results of meta-QTL analysis (Liu et al., 2020) and GWAS (Galagedara et al., 2020).

Table 1. Information of the Significant Markers Identified on Chromosome 3B for Tan Spot Resistance in the Previous GWAS Analysis (Galagedara et al., 2020).

Marker	Isolates	R^2	p-value	FDR-value
S3B_472243678	Pti2	5.33%	1.13×10^{-5}	3.11×10^{-2}
	86-124	6.45%	1.60×10^{-6}	1.74×10^{-2}
	331-9	7.83%	1.32×10^{-7}	1.23×10^{-3}
	DW5	6.41%	1.66×10^{-6}	2.17×10^{-2}
S3B_472880346	Pti2	5.61%	7.39×10^{-6}	2.90×10^{-2}
	86-124	7.00%	7.65×10^{-7}	1.61×10^{-2}
	331-9	8.55%	5.46×10^{-8}	5.95×10^{-4}
	DW5	6.86%	9.10×10^{-7}	1.97×10^{-2}
S3B_474031065	86-124	6.58%	1.26×10^{-6}	1.65×10^{-2}
	331-9	9.46%	7.53×10^{-9}	1.02×10^{-4}
S3B_474316625	86-124	6.81%	9.87×10^{-7}	1.61×10^{-2}
	331-9	11.73%	1.90×10^{-10}	6.22×10^{-6}
	DW5	8.85%	2.43×10^{-8}	7.93×10^{-4}

The four markers were S3B_472243678 (NDSU-SG-KASP27), S3B_472880346 (NDSU-SG-KASP28), S3B_474031065 (NDSU-SG-KASP29) and S3B_474316625 (NDSU-SG-KASP30). From the GWAS analysis, S3B_472243678 and S3B_472880346 were associated with resistance to four isolates 331-9, 86-124, DW5 and Pti2; S3B_474316625 was associated with resistance to isolates 331-9, 86-124, and DW5; SNP S3B_474031065 was associated with two isolates 86-124 and 331-9 (Table 1). KASP markers with a common reverse primer and biallelic forward primers were designed to identify specific markers of each line (Table 2). The genotypes of the four KASP markers for the three parents were listed in Table 3.

Table 2. Common Reverse Primer and Biallelic Forward Primer Sequences of The Four KASP Markers Used for Marker-Assisted Backcross Selection in This Study.

Marker Alias	Marker	Common reverse primer	forward primers
NDSU-SG-KASP27	S3B_472243678	ggactgcagaggggactagt	GAAGGTGACCAAGTTCATGCTtggcccttcttgcattttgG GAAGGTCGGAGTCAACGGATTtggcccttcttgcattttgT
NDSU-SG-KASP28	S3B_472880346	tgggcctgtggttaattggg	GAAGGTGACCAAGTTCATGCTggctaaagcgggcttccC GAAGGTCGGAGTCAACGGATTggctaaagcgggcttccA
NDSU-SG-KASP29	S3B_474031065	gtgctaagcaggaaccaaca	GAAGGTGACCAAGTTCATGCTgctacttcactgctgcagatC GAAGGTCGGAGTCAACGGATTgctacttcactgctgcagatT
NDSU-SG-KASP30	S3B_474316625	cagattgaaattcactgatgTGgT	GAAGGTGACCAAGTTCATGCTTgttcacattcAtatttggcacaC GAAGGTCGGAGTCAACGGATTTgttcacattcAtatttggcacaT

Table 3. Genotypes of the Four KASP Markers for the Three Parents “Grano”, “Riveland” and “PI192640”.

Genotype	NDSU-SG-KASP27	NDSU-SG-KASP28	NDSU-SG-KASP29	NDSU-SG-KASP30
Grano	G	C	C	T
Riveland	G	C	C	T
PI192640	T	A	T	C

Disease evaluation of the backcross progenies

To evaluate the effect of the TS-3B-QTLrn, $BC_4F_{2:3}$ lines were evaluated for their reactions to isolates ND1 (race 1) and 331-9, $BC_5F_{2:3}$ lines were evaluated for their reactions to isolate DW5, and $BC_6F_{2:3}$ lines were evaluated for their reactions to isolates 331-9 and DW5. For each generation of each background cultivar, 10-20 lines with the favorable alleles (Grano-BB or Riveland-BB) and 10-20 lines without the favorable allele (Grano-AA or Riveland-AA), along with the parents "Grano", "Riveland", and PI192640 were used for disease evaluation. The experimental design was a randomized complete block design (RCBD) with two replications. The seeds were planted in specialized cone-tainers known as super cell cone-tainers. These cone-tainers were filled with Sunshine SB100 soil from Sun Gro Horticulture in Bellevue, Washington, and were arranged in RL98 racks. For each line, two seeds were planted in one cone-tainer. To minimize border effect, the outer edges of the racks were planted with a susceptible variety "Jerry". After planting the seeds, a small amount of Osmocote Plus 15-19-12 fertilizer from Scotts Sierra Horticultural Product Company in Maysville, Ohio, was added to each cone-tainer. The seedlings were grown in a greenhouse with temperatures ranging from 20 to 25˚C over a container filled with water for two weeks (Figure 4).

Figure 4. $BC_6F_{2:3}$ seedlings in greenhouse planted for screening against isolate 331-9.

To prepare the inoculum of *Pyrenophora tritici-repentis* of race 1 isolate ND1, race 3 isolate 331-9, and race 5 isolate DW5, a standard procedure outlined by Liu et al. (2015) was followed (Figure 5). A brief summary of the process is:

a. A piece of fungal mycelium was placed onto V8 (Potato Dextrose Agar) PDA plates, with one plate prepared for each rack. Each rack represents a single replication in the experiment.
b. The plates were kept in a dark area at room temperature for five days.
c. Afterward, each plate was flooded with 10ml of sterilized water, and all the fungal mycelium on the surface of the medium was gently pressed down manually using the bottom of a flame-sterilized test tube.
d. Excess water was drained off, and the plates were allowed to dry for some time.
e. Following flattening, the plates were exposed to fluorescent light for 24 hours at room temperature.
f. Subsequently, the plates were moved to a dark environment at 15°C to encourage sporulation.
g. Once sporulation was abundant, spore harvesting was carried out by adding 30ml of sterilized distilled water to each petri dish and gently scraping the spores with a sterilized inoculation loop.
h. The inoculum concentration was adjusted to 2,000 conidia/ml before the actual inoculation.
i. To this spore suspension, two drops of tween 20 per 100 ml were added as a surfactant, and the mixture was thoroughly shaken before spraying.

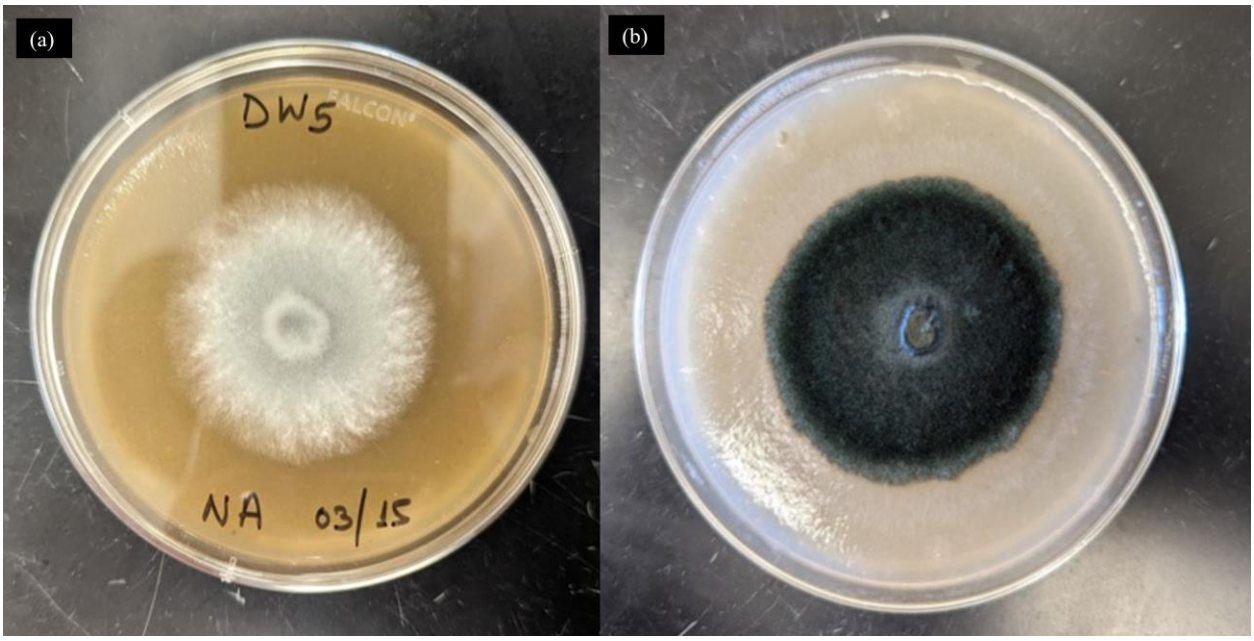

Figure 5. Culture of Pyrenophonra tritici-ripentis on potato dextrose agar showing (a) cottony whitish mycelial growth and (b) black mycelium with abundant sporulation ready for spore collection.

The seedlings at the three-leaf stage were subjected to inoculation within an enclosed room. This inoculation was performed using a paint sprayer connected to an air supply, with the air pressure set at approximately 1.0 bar. The seedlings were sprayed until their leaves were evenly coated with water droplets. After the spraying, the seedlings were transferred to a mist chamber and kept for 24 hours. The mist chamber was configured to release mist for 20 seconds every four minutes, maintaining a constant relative humidity of 100% (Figure 6). Subsequently, the seedlings were moved to a growth chamber where the temperature was maintained at 21°C, and the relative humidity was kept at 75% with 12h photoperiod (Figure 6).

Figure 6: $BC_5F_{2:3}$ seedlings inoculated with isolate DW5 (a) in mist chamber (b) in growth chamber.

Disease severity rating

Seven days after inoculation, the seedlings were assessed for disease severity by assigning a score on a scale of 1 to 5. This rating was determined by examining the type of lesion present on the secondary leaf, following the criteria outlined by Lamari and Bernier in 1989. A score of 1 represents high resistance, while a score of 5 represents high susceptibility.

Statistical analysis

Phenotypic data was analyzed using SAS®9.4 and R package Agricolae (Mendiburu, 2019). The statistical model used for the analysis of each isolate inoculated for each backcross generation of each background cultivar was:

$$Y = G + L(G) + R + \varepsilon \quad (1)$$

Where, Y is disease severity score; G is the fixed effect of the QTL genotype (with favorable allele [BB] vs without favorable allele [AA]); L(G) is the random effect of the line nested within each QTL genotype at each backcross generation of each background cultivar. The best linear unbiased estimators (BLUEs) were estimated.

The statistical model used for the analysis of multiple isolates on different background cultivars across all three backcross generations was

$$Y = C + I + I \times C + G + C \times G + I \times G + C \times I \times G + B + B \times G + L(C \times B \times G) + R(B \times I) + \varepsilon \quad (2)$$

Where, Y is disease severity score; C is the fixed effect of the background cultivar ("Grano" or "Riveland"); I is the fixed effect of the isolate; I × C is the fixed effect of the isolate and background cultivar interaction; G is the fixed effect of the QTL genotype (with favorable allele [BB] vs without favorable allele [AA]); C × G is the fixed effect of the background cultivar and QTL genotype interaction; I × G is the fixed effect of the isolate and QTL genotype interaction; I × C × G is the fixed effect of the isolate, background cultivar and QTL genotype interaction; B is the random effect of the backcross generation ($BC_4F_{2:3}$, $BC_5F_{2:3}$, and $BC_6F_{2:3}$); B×G is the fixed effect of background generation and QTL genotype interaction. L(C × B × G) is the random effect of the line nested within each QTL genotype at each backcross generation of each background cultivar; R(B × I) is the random effect of the replication nested in each backcross generation and each isolate inoculated; ε is the random effect of error. Variance for each component was estimated.

Results

Marker assisted development of improved resistance to tan spot

The durum wheat cultivars "Grano" and "Riveland" were used as recipient parents and crossed with the donor parent "PI192640" to obtain F_1 individual plants. Subsequent backcrossing led to the development of BC_1F_1, BC_2F_1, BC_3F_1, BC_4F_1, BC_5F_1 and BC_6F_1 progenies. In each backcross generation four individual plants with favorable allele at targeted QTL and phenotypes similar to recurrent parent were selected and backcrossed to the recurrent parent.

Table 4. Information on the Number of Individuals with the Favorable Allele and Unfavorable Allele in Respective Backcross Generation.

Generation	with favorable allele	Without favorable allele	Expected frequency with favorable allele	Expected frequency without favorable allele	Chi-square p-value
BC_1F_1	132	181	156.5	156.5	0.005612
BC_2F_1	21	27	24	24	0.386476
BC_3F_1	18	22	20	20	0.527089
BC_4F_1	27	41	34	34	0.089555
BC_5F_1	46	30	38	38	0.066457
BC_6F_1	28	46	37	37	0.036398

In each back cross generation individuals were genotyped for the four KASP markers associated with TS-3B-QTLrn. The deviation from the expected segregation ratio is measured by Chi-square p-value (Table 4).

Disease evaluation of $BC_4F_{2:3}$ lines

The $BC_4F_{2:3}$ lines were evaluated for their reactions to two isolates ND1 and 331-9. A total of 10 lines with the favorable allele (Grano-BB) and 10 lines without the favorable allele (Grano-AA) were evaluated for each isolate. The introgressed lines with the favorable allele (Grano-BB) had significantly lower disease severity than the lines without the favorable allele (Grano-AA) and parent "Grano" for both isolates (Figure 7 and Figure 8). When inoculated with the race 1 isolate ND1, the disease severity scores were 2.82, 2.01, 2.90, 2.87, and 1.83 for

Grano-AA, Grano-BB, “Grano”, “Riveland”, and PI192640. When inoculated with the isolate 331-9, the disease severity scores for Grano-AA, Grano-BB, “Grano”, “Riveland”, and PI192640 were 2.65, 1.25, 2.42, 2.50, and 1.10, respectively.

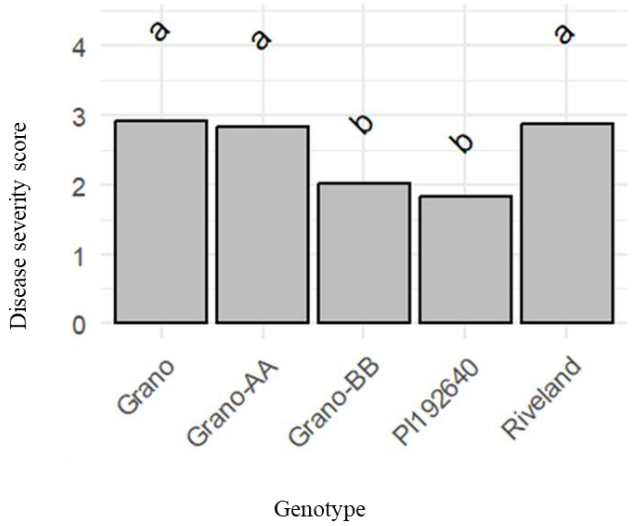

Figure 7. Disease severity scores of $BC_4F_{2:3}$ lines inoculated with the isolate ND1. Different letters represent significant differences at $p<0.05$ probability level.

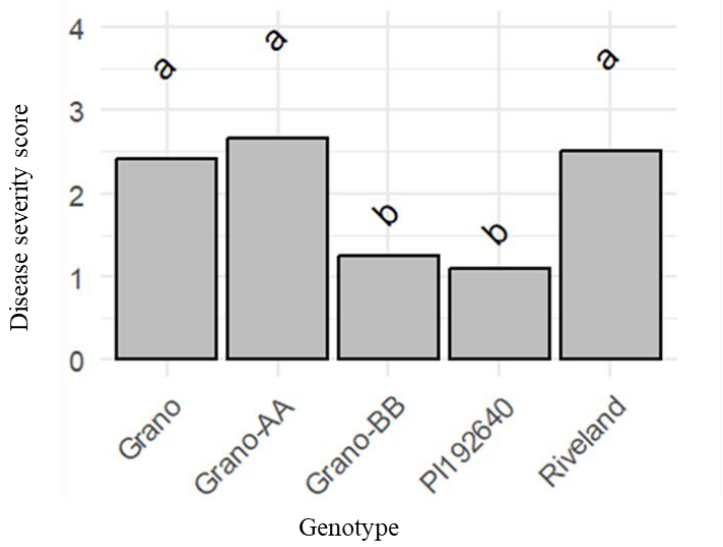

Figure 8. Disease severity scores of $BC_4F_{2:3}$ lines inoculated with the isolate 331-9. Different letters represent significant differences at $p<0.05$ probability level.

Disease evaluation of $BC_5F_{2:3}$ lines

The $BC_5F_{2:3}$ lines were evaluated for their reactions to the race 5 isolate DW5. The introgression lines with the favorable allele had significantly lower disease severity than those lines without the favorable allele for the background cultivar of "Grano" (Figure 9). The disease severity scores for the genotypes Grano-AA, Grano-BB, Riveland-AA, and Riveland-BB were 2.90, 1.70, 2.83, and 2.29, respectively. The disease severity scores for the three parents "Grano", "Riveland", and PI192640 were 2.80, 2.83, and 1.3, respectively.

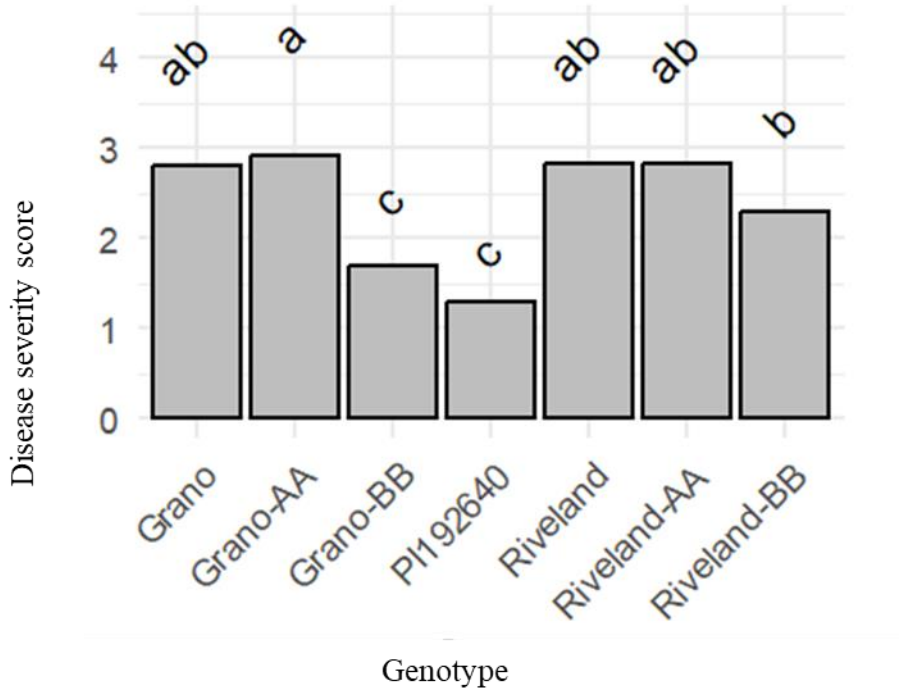

Figure 9. Disease severity scores of $BC_5F_{2:3}$ lines when inoculated with isolate DW5. Different letters represent significant differences at $p<0.05$ probability level.

Disease evaluation of $BC_6F_{2:3}$ lines

The $BC_6F_{2:3}$ lines were evaluated for their reactions to isolates 331-9 and DW5. For both isolates, the introgression lines with the favorable alleles showed significantly lower disease severity than those lines without the favorable alleles in both background cultivars "Grano" and "Riveland" (Figure 10 and Figure 11). For the isolate 331-9, disease severity scores for Grano-

AA, Grano-BB, Riveland-AA, Riveland-BB, “Grano”, “Riveland” and PI192640 were 1.79, 1.05, 1.96, 1.37, 1.83, 1.92, and 1.00, respectively. When inoculated with the isolate DW5, the disease severity scores for Grano-AA, Grano-BB, Riveland-AA, Riveland-BB, “Grano”, “Riveland” and PI192640 were 2.41, 1.61, 2.94, 2.33, 2.83, 2.90, and 1.50, respectively.

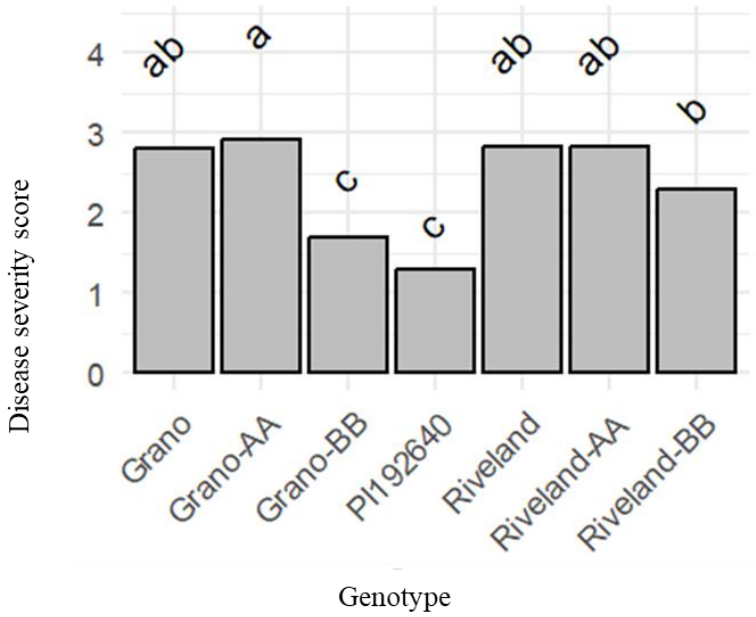

Figure 10. Disease severity scores of $BC_6F_{2:3}$ lines when inoculated with the isolate 331-9. Different letters represent significant differences at $p<0.05$ probability level.

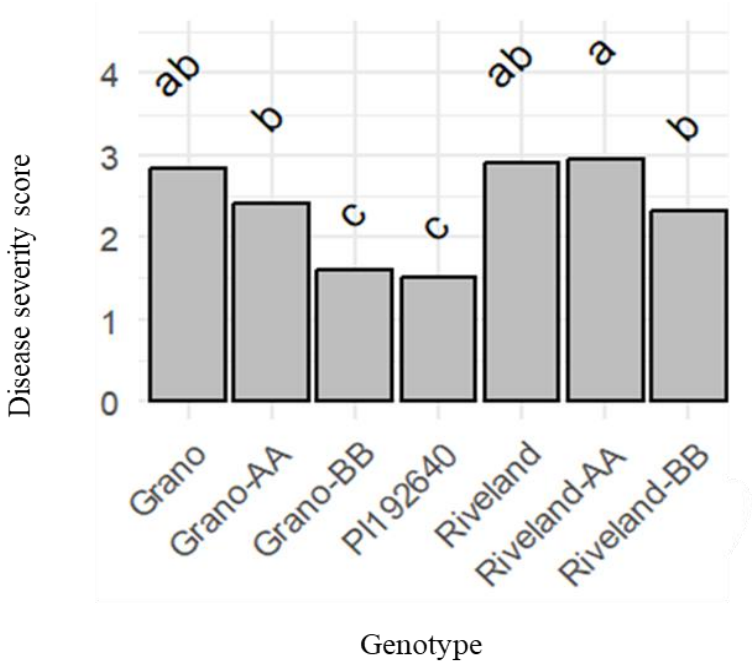

Figure 11. Disease severity scores of $BC_6F_{2:3}$ lines when inoculated with the isolate DW5. Different letters represent significant differences at $p<0.05$ probability level.

Analysis of variance for multiple isolates

To test if the effects of the TS-3B-QTLrn vary against pathogen isolates or in different background cultivars, the phenotypic data collected from the $BC_4F_{2:3}$, $BC_5F_{2:3,}$ and $BC_6F_{2:3}$ lines derived from "Grano" and "Riveland" for their reactions to all isolates tested were combined for analysis of variance. The analysis of variance shows that there is significant effect of background cultivars, isolates, QTL genotype, interaction between QTL genotype and isolates, background generation and the interaction between QTL genotype and backcross generation on disease development (Table 5).

Table 5. Analysis of Variance (ANOVA) Table for Background Cultivar, QTL Genotype, and Isolate Interaction Across all Generations.

Source	DF	Sum of Squares (SS)	Mean Square	F Value
C	1	1.49	1.49	6.82**
I	2	12.19	6.09	27.94****
C × I	1	0.007	0.007	0.03
G	1	28.26	28.26	129.57****
C × G	1	2.33	2.33	10.67**
G × I	2	2.77	1.38	6.35**
C × G × I	1	0.56	0.56	2.59
B	2	5.75	2.87	13.19****
B × G	2	0.45	0.23	1.03
L(B × G × C)	100	34.27	0.34	1.57**
R(B × I)	5	3.07	0.61	2.82*
ε	194	42.31	0.22	

Where, C = background cultivar; I = isolate; I × C = isolate × background cultivar; G = QTL genotype C × G = background cultivar × QTL genotype interaction; I × G = isolate × QTL genotype; I × C × G = isolate × background cultivar × QTL genotype; B = backcross generation (BC4F2:3, BC5F2:3, and BC6F2:3); L(C × B × G) = line (QTL genotype × backcross generation × background cultivar; R(B × I) = replication × backcross generation × isolate inoculated; ε = error.

*$p< .05$ **$p<.01$ ****$p<.0001$

Disease evaluation of BC_4F_2 individuals

BC_4F_1 heterozygous individuals from the cross between "Grano" and PI192640 were self-pollinated and a total of resulting 96 $BC_4F_{1:2}$ individuals were evaluated for their reactions to race 3 isolate 331-9. The 96 individuals were genotyped for the four KASP markers and clustered into three groups, homozygous with the favorable allele (Grano-BB), heterozygous with one copy of the favorable allele (Grano-AB), and homozygous without the favorable allele (Grano-AA). The mean disease severity score for the heterozygous progenies was significantly lower than the homozygous line with the favorable allele but was significantly higher than the homozygous line without the favorable allele (Figure 12). The mean disease severity scores were

2.56, 2.37, 1.51, 1.05 and 1.85 for “Grano”, Grano-AA, Grano-AB, Grano-BB, and PI192640, respectively.

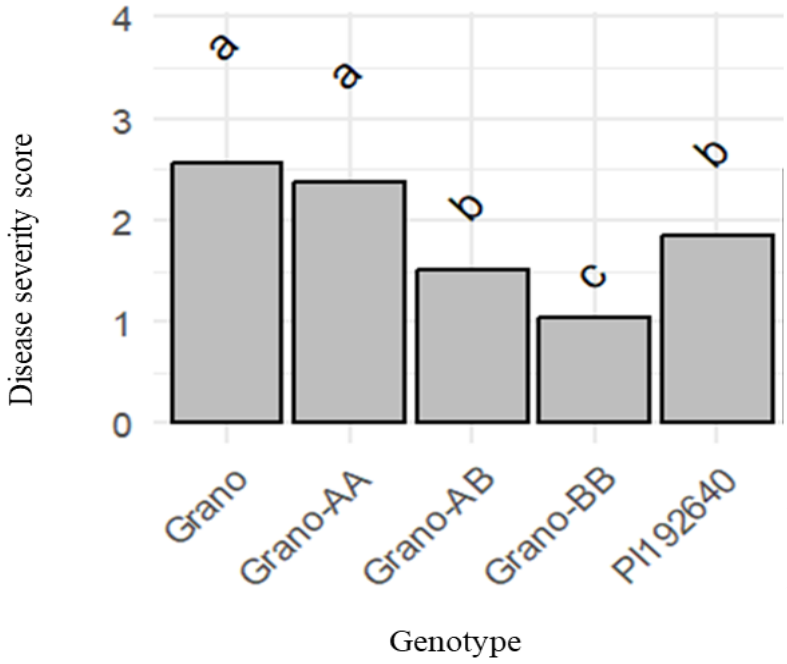

Figure 12. Mean disease severity scores of BC_4F_2 progenies and the two parents “Grano” and PI192640 when inoculated with the isolate 331-9. Different letters represent significant differences at $p<0.05$ probability level.

Discussion

Using resistance cultivars is the most economical and sustainable way to reduce the loss of wheat production due to tan spot. Marker-assisted selection is an efficient way to develop disease resistant germplasm or cultivars. In wheat, marker-assisted backcross selection had been widely used for developing new germplasm or cultivars against stem rust, leaf rust, and stripe rust (Salameh et al., 2011; Mallick et al., 2015), where foreground selection, recombinant selection and background selection were commonly used. Numerous QTLs have been identified for tan spot resistance in wheat (Kariyawasam et al., 2016; Liu et al., 2020; Haugrud et al., 2023). A race non-specific QTL on chromosome 3B (TS-3B-QTLrn) was found to be resistant to multiple isolates but was not presented in the NDSU durum wheat breeding population. No

recombinant selection was applied in this study since the causal gene has not yet been finely located and cloned. Foreground selection for the targeted QTL using four linked KASP markers was applied. In this study, the favorable allele of the race non-specific TS-3B-QTLrn was introgressed into two durum wheat cultivars "Grano" and "Riveland" via marker-assisted backcross selection. The significance of using "Grano" and "Riveland" as recipient parents in our experiment lay in their high yield potential, excellent end use quality and potential for enhancing disease resistance in durum wheat cultivars (Elias and Manthey, 2019; Elias et al., 2021). A total of six generations of backcross was conducted and over 99% of recurrent parent genome is expected to achieve in the resulting introgression lines. The systematic marker assisted backcross selection approach used in our experiment successfully transferred and retained tan spot resistance TS-3B-QTLrn into "Grano" and "Riveland".

The introgression lines including $BC_4F_{2:3}$, $BC_5F_{2:3}$, and $BC_6F_{2:3}$ lines were evaluated for resistance to tan spot by using multiple isolates including race 1 isolate ND1, race 3 isolate 331-9 and race 5 isolate DW5. Previous studies have shown that TS-3B-QTLrn is race non-specific (Galagedara et al., 2020; Liu et al., 2020), indicating that it provides resistance to various isolates. Therefore, it was expected that the introgressed lines would exhibit resistance to any of the isolates used in the study. The introgression lines with the favorable allele of the TS-3B-QTLrn had significantly lower disease severity compared to the recurrent parents "Grano" and "Riveland", suggesting that the causal gene was successfully introgressed into the cultivars. For introgression line of "Riveland" which were evaluated in $BC_5F_{2:3}$ and $BC_6F_{2:3}$ showed some difference in observation even when inoculated with same isolate DW5 in both generations. This might be due to some difference in disease causing factors such as density of spores in inoculation, temperature during disease development. A significantly lower disease severity was

found for all isolates tested, further supporting that the QTL is race non-specific. We also wanted to see if the race non-specific QTL provided equal resistance to all the isolates tested and in both genetic backgrounds for which a combined data analysis across all the generation was carried. Significant interaction between the QTL and isolates was found too, indicating the effects of the gene vary against isolates. Also, the significant interaction between QTL genotype and background cultivars indicates that the effect of QTL varies between background cultivars. No significant interaction between background cultivar and isolates further supports race non-specific nature of the QTL. The absence of significant three-way interaction between QTL genotype, isolate and background cultivar suggests that while the QTL effect varies with the genetic background and the isolate, the effects are independent of each other. The QTL may confer baseline level of resistance that is modulated by the background cultivar and or isolate but not by combination of both. Evaluation of $BC_4F_{1:2}$ individuals suggested that the TS-3B-QTLrn showed partial dominant resistance. It should be noted that one segregating population was tested for one isolate only in this study. Further testing of multiple populations to multiple isolates is needed to determine the functional mode of the race non-specific QTL. It was proposed that the race non-specific QTL on chromosome 3B decreases the toxin activity, which renders the toxin amount unable to cause disease symptoms in host plant (Faris and Friesen, 2005). Further cloning of the gene and function study will help to explain how it provides resistance and different levels of resistance to varied isolates. Currently, we are using BC_6F_2 heterozygous progenies to develop segregating population for fine mapping of the TS-3B-QTLrn. Closely linked markers resulting from the fine mapping will facilitate marker-assisted selection of the causal gene into the elite durum wheat breeding pool without linkage drags. Disease evaluation was conducted for the introgression lines at the seedling stage in greenhouse only in this study.

Conclusion

The race non-specific QTL (TS-3B-QTLrn) from PI192640 was successfully introgressed into two durum wheat cultivars "Grano" and "Riveland" using four KASP markers and showed resistance against multiple isolates at the seedling stage. The race non-specific QTL showed partial dominant resistance with varying resistance against isolates.

Future direction

In this experiment, we only tested for the effect of TS-3B-QTLrn at seedling stage. It will be interesting to see if the race non-specific QTL has an effect on tan spot resistance for adult plants. We will evaluate the $BC_6F_{2:4}$ lines in field to test if TS-3B-QTLrn has effects on tan spot resistance in adult plants. We will also test if the introgression of QTL has any negative impact on grain yield and quality related traits.

References

Ali, S., & Francl, L. J. (2003). Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and noncereal grasses in the Great Plains. *Plant Disease*, *87*(4), 418-422. https://doi.org/10.1094/PDIS.2003.87.4.418

Bockus, W.W. & Claassen, M.M. (1992). Effects of crop rotation and residue management practices on severity of tan spot of winter wheat. *Plant Disease*, *76*(6), 633-636. Retrieved from https://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1992Articles/PlantDisease76n06_633.pdf

Chu, C. G., Chao, S., Friesen, T. L., Faris, J. D., Zhong, S., & Xu, S. S. (2010). Identification of novel tan spot resistance QTLs using an SSR-based linkage map of tetraploid wheat. *Molecular Breeding*, *25*, 327-338. https://doi.org/10.1007/s11032-009-9335-2

Ciuffetti, L. M., Francl, L. J., Ballance, G. M., Bockus, W. W., Lamari, L., Meinhardt, S. W., & Rasmussen, J. B. (1998). Standardization of toxin nomenclature in the *Pyrenophora tritici-repentis*/wheat interaction. *Canadian Journal of Plant Pathology*, *20*(4), 421-424. https://doi.org/10.1080/07060669809500415

De Wolf, E. D., Effertz, R. J., Ali, S., & Francl, L. J. (1998). Vistas of tan spot research. *Canadian Journal of Plant Pathology*, *20*(4), 349-370. Retrieved from https://www.cabidigitallibrary.org/doi/full/10.5555/19991004576

Dubcovsky, J., & Dvorak, J. (2007). Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science*, *316*(5833), 1862-1866. https://doi.org/10.1126/science.1143986

Elias, E. M., & Manthey, F. A. (2019). Registration of 'Riveland' durum wheat. *Journal of Plant Registrations*, 13(3), 387-391. https://doi.org/10.3198/jpr2019.01.0001crc.

Elias, E. M., Manthey, F. A., Kumar, A., & Salsman, E. (2021). Registration of 'Grano' durum wheat. *Journal of Plant Registrations*, *15*(3), 535-541. https://doi.org/10.1002/plr2.20170

Faris, J. D., & Friesen, T. L. (2005). Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. *Theoretical and Applied Genetics*, *111*(2), 386-392. https://doi.org/10.1007/s00122-005-2033-5

Faris, J. D., Liu, Z., & Xu, S. S. (2013). Genetics of tan spot resistance in wheat. *Theoretical and Applied Genetics*, *126*, 2197-2217. https://doi.org/10.1007/s00122-013-2157-y

Faris, J. D., Overlander, M. E., Kariyawasam, G. K., Carter, A., Xu, S. S., & Liu, Z. (2020). Identification of a major dominant gene for race-nonspecific tan spot resistance in wild emmer wheat. *Theoretical and Applied Genetics*, *133*, 829-841. https://doi.org/10.1007/s00122-019-03509-8

Faris, J. D., Zhang, Z., Lu, H., Lu, S., Reddy, L., Cloutier, S., Fellers, J.P., Meinhardt, S. W., Rasmussen, J. B., Xu, S.S., Oliver, R.P., Simons, K.J., & Friesen, T. L. (2010). A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proceedings of the National Academy of Sciences*, *107*(30), 13544-13549. https://doi.org/10.1073/pnas.1004090107

Fenton, A., Antonovics, J., & Brockhurst, M. A. (2009). Inverse-gene-for-gene infection genetics and coevolutionary dynamics. *The American Naturalist, 174*(6), E230-E242. Retrieved from https://www.journals.uchicago.edu/doi/full/10.1086/645087#_i13

Fernandez, M. R., DePauw, R. M., Clarke, J. M., & Fox, S. L. (1998). Discoloration of wheat kernels by *Pyrenophora tritici-repentis*. *Canadian Journal of Plant Pathology*, *20*(4), 380-383. https://doi.org/10.1080/07060669809500407

Friesen, T. L., & Faris, J. (2004). Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. *Theoretical and applied genetics*, *109*(3), 464-471. https://doi.org/10.1007/s00122-004-1678-9

Friskop, A., & Zhaohui, L. (2021). Fungal Leaf Spot Diseases of Wheat: Tan spot, Septoria/Stagonospora nodorum blotch and Septoria tritici blotch (PP-1249). *Plant Disease Management: NDSU Extension Service*.

Galagedara, N., Liu, Y., Fiedler, J., Shi, G., Chiao, S., Xu, S. S., Faris, J.D., Li, X., & Liu, Z. (2020). Genome-wide association mapping of tan spot resistance in a worldwide collection of durum wheat. *Theoretical and Applied Genetics*, *133*, 2227-2237. https://doi.org/10.1007/s00122-020-03593-1

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

in the wheat–*Pyrenophora tritici-repentis* pathosystem. *Theoretical and Applied Genetics, 129*, 897-908. https://doi.org/10.1007/s00122-016-2670-x

Lamari, L., & Bernier, C. C. (1989). Toxin of *Pyrenophora tritici-repentis*: Host-specificity, significance in disease. *Phytopathology, 79*(7), 740-744. Retrieved from https://www.apsnet.org/publications/phytopathology/backissues/Documents/1989Articles/Phyto79n07_740.pdf

Liu, Y., Salsman, E., Wang, R., Galagedara, N., Zhang, Q., Fiedler, J. D., Liu, Z., Xu, S., Faris, J.D. & Li, X. (2020). Meta-QTL analysis of tan spot resistance in wheat. Theoretical and Applied Genetics, *133*, 2363-2375. https://doi.org/10.1007/s00122-020-03604-1

Liu, Z., El-Basyoni, I., Kariyawasam, G., Zhang, G., Fritz, A., Hansen, J., Marais, F., Friskop, A., Chao, S., Akhunov, E., & Baenziger, P. S. (2015). Evaluation and association mapping of resistance to tan spot and Stagonospora nodorum blotch in adapted winter wheat germplasm. *Plant Disease, 99*(10), 1333-1341. https://doi.org/10.1094/PDIS-11-14-1131-RE

Mallick, N., Jha, S. K., Agarwal, P., Mall, A., Kumar, S., Choudhary, M. K., Bansal, S., Saharan, M.S., Sharma, J.B. & Vinod. (2022). Marker-assisted improvement of bread wheat variety HD2967 for leaf and stripe rust resistance. *Plants, 11*(9), 1152. https://doi.org/10.3390/plants11091152

Meinhardt, S., Ali, S., Ling, H., & Francl, L. (2003). A new race of *Pyrenophora tritici-repentis* that produces a putative host-selective toxin. *In Proceedings of Fourth International Wheat Tan Spot and Spot Blotch Workshop, Bemidji, Minnesota, USA, 21-24 July*, 2002 (pp. 117-121). Agricultural Experiment Station, North Dakota State University.

Mendiburu, F. D. (2019). Agricolae: statistical procedures for agricultural research. CRAN. R packages version 4.3.3.

Murray, G. M., & Brennan, J. P. (2009). Estimating disease losses to the Australian wheat industry. *Australasian Plant Pathology*, *38*(6), 558-570. https://doi.org/10.1071/AP09053

Pallotta, M.A., Warner, P., Fox R.L., Kuchel, H., Jefferies S. J., & Langridge, P. (2003, September). Marker assisted wheat breeding in southern region of Australia. *Proceedings of Tenth International Wheat Genetics Symposium* (pp.789-791). Puglia, Italy.

Peters Haugrud, A. R., Shi, G., Seneviratne, S., Running, K. L., Zhang, Z., Singh, G., Szabo-Hever, A., Acharya, K., Friesen., T.L., Liu, Z. & Faris, J. D. (2023). Genome-wide association mapping of resistance to the foliar diseases septoria nodorum blotch and tan spot in a global winter wheat collection. *Molecular Breeding*, *43*(7), 54. https://doi.org/10.1007/s11032-023-01400-5

Rees, R. G., & Platz, G. J. (1983). Effects of yellow spot on wheat: comparison of epidemics at different stages of crop development. *Australian Journal of Agricultural Research*, *34*(1), 39-46. https://doi.org/10.1071/AR9830039

Running, K. L., Momotaz, A., Kariyawasam, G. K., Zurn, J. D., Acevedo, M., Carter, A. H., Liu, Z. & Faris, J. D. (2022). Genomic analysis and delineation of the tan spot susceptibility locus Tsc1 in wheat. *Frontiers in Plant Science*, *13*, 793925. https://doi.org/10.3389/fpls.2022.793925.

Salameh, A., Buerstmayr, M., Steiner, B., Neumayer, A., Lemmens, M., & Buerstmayr, H. (2011). Effects of introgression of two QTL for fusarium head blight resistance from Asian spring wheat by marker-assisted backcrossing into European winter wheat on

fusarium head blight resistance, yield and quality traits. *Molecular Breeding, 28*, 485-494. https://doi.org/10.1007/s11032-010-9498-x.

Shabeer, A., & Bockus, W. W. (1988). Tan spot effects on yield and yield components relative to growth stage in winter wheat. *Plant Disease*, *72*(7), 599-602. https://doi.org/ 10.1094/PD-72-0599

Shi, G., Kariyawasam, G., Liu, S., Leng, Y., Zhong, S., Ali, S., Moolhuijzen, P., Moffat, C.S., Rasmussen J.B., Friesen T. L., Faris, J.D., & Liu, Z. (2022). A conserved hypothetical gene is required but not sufficient for Ptr ToxC production in *Pyrenophora tritici-repentis*. *Molecular Plant-Microbe Interactions*, *35*(4), 336-348. https://doi.org/10.1094/MPMI -12-21-0299-R

Singh, P. K., Gonzalez-Hernandez, J. L., Mergoum, M., Ali, S., Adhikari, T. B., Kianian, S. F., Elias, E.M. & Hughes, G. R. (2006). Identification and molecular mapping of a gene conferring resistance to *Pyrenophora tritici-repentis* race 3 in tetraploid wheat. *Phytopathology*, *96*(8), 885-889. https://doi.org/10.1094/PHYTO-96-0885

Singh, P. K., Mergoum, M., Gonzalez-Hernandez, J. L., Ali, S., Adhikari, T. B., Kianian, S. F., Elias, E.M. & Hughes, G. R. (2008). Genetics and molecular mapping of resistance to necrosis inducing race 5 of *Pyrenophora tritici-repentis* in tetraploid wheat. *Molecular Breeding*, *21*, 293-304. https://doi.org/10.1007/s11032-007-9129-3

Statista. (2023). Wheat consumption worldwide in 2023/2024, by country. Retrieved from https://www.statista.com/statistics/1094065/total-global-wheat-consumption-by-country/.

Szabo-Hever, A., Singh, G., Haugrud, A. R. P., Running, K. L., Seneviratne, S., Zhang, Z., Shi, G., Bassi, F.M., Maccaferri, M., Cattivelli, L., Tuberosa, R., Friesen, T. L., Liu, Z., Xu, S.S. & Faris, J. D. (2023). Association mapping of resistance to tan spot in the Global

Durum Panel. *Phytopathology, 113*(10), 1967-1978. https://doi.org/10.1094/PHYTO-02-23-0043-R

USDA, Economics, Statistics and Market Information System. September 2023. Small grains 2023 summary. Retrieved from https://usda.library.cornell.edu/concern/publications/5t34sj573/

Wegulo, S.N. (2011). Tan spot of cereals. APS. Retrieved from https://www.apsnet.org/edcenter/disandpath/fungalasco/pdlessons/Pages/TanSpot.aspx

Xynias, I. N., Mylonas, I., Korpetis, E. G., Ninou, E., Tsaballa, A., Avdikos, I. D., & Mavromatis, A. G. (2020). Durum wheat breeding in the Mediterranean region: Current status and future prospects. *Agronomy*, *10*(3), 432. https://doi.org/10.3390/agronomy10030432

APPENDIX. ANALAYSIS OF VARIANCE TABLE FOR BC_4 AND BC_5 LINES

Table 1. Analysis of Variance (ANOVA) Table for BC_4F_2 Lines Against Isolate 331-9.

Source of Variation	Degree of freedom	Sum of Squares	F-value
L(G)	141	41.47	0.87
G	5	41.35	24.6*
R	3	2.86	2.83
ε	17	5.71	

*$p<0.5$

Table 2. Analysis of Variance (ANOVA) Table for BC_4F_3 Lines Against Isolate 331-9.

Source of Variation	Degree of freedom	Sum of Squares	F-value
L(G)	38	7.6	1.05
G	4	45.62	60.09*
R	1	0.037	0.2
ε	52	9.87	

*$p<0.5$

Table 3. Analysis of Variance (ANOVA) Table for BC_4F_3 Lines Against Isolate ND1.

Source of Variation	Degree of freedom	Sum of Squares	F-value
L(G)	38	10.37	1.5
G	4	16.77	23.09*
R	1	1.69	9.32
ε	49	8.9	

*$p<0.5$

Table 4. Analysis of Variance (ANOVA) Table of BC5F3 Lines Against Isolate DW5.

Source of Variation	Degree of freedom	Sum of Squares	F-value
L(G)	40	16.62	1.24
G	6	26.68	13.32s
R	1	1.16	3.48
E	39	13.02	

*$p<0.5$