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IDENTIFICATION OF RESISTANCE GENES TO FOLIAR DISEASES IN SYNTHETICS HEXAPLOID WHEATS

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IDENTIFICATION OF RESISTANCE GENES TO FOLIAR DISEASES IN SYNTHETICS HEXAPLOID WHEATS

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ABSTRACT

In recent years, foliar diseases have become relevant in wheat production, leading to significant limitation to grain yield and grain quality. Synthetic hexaploid wheat (SHW) has shown effective resistance to a diversity of diseases and insects. Tan spot (TS) caused by *Pyrenophora tritici-repentis* (Died.) Drechs is an important foliar disease that attack all types of wheat and several grasses. Spot blotch (SB) caused by *Bipolaris sorokiniana* (Sacc.) Shoem syn. *Drechslera sorokiniana* (Sacc.) is a destructive fungal disease in humid and high temperature regions affecting wheat and many other crops. In this research, a diverse panel of 443 SHW lines were evaluated for their resistance/susceptibilityto TS and SB under controlled environmental conditions. Additionally, a genome-wide association mapping (GWAS) study was conducted by genotyping all entries with the DArTSeq technology to identify marker-trait associations for TS and SB. In TS of the 443 SHW plants, 233 showed resistant and 183 moderately resistant reactions, and only 27 were moderately susceptible or susceptible to TS. In the case of SB, 250 SHW lines showed resistant and 161 moderately resistant reactions, and only 30 were moderately susceptible or susceptible to SB. Durum wheat parents of the SHW showed moderately susceptible to susceptible reactions. In the GWAS for TS a total of 30 significant marker-trait associations were found on chromosomes 1B (4 markers), 1D (1 marker), 2A (1 marker), 2D (2 markers), 3A (4 markers), 3D (3 markers) 4B (1 marker), 5A (4 markers), 6A (6 markers), 6B (1 marker) and 7D (3 markers). A total of 41 significant markers related to resistances to SB were identified, 5 markers were found in chromosome 1B and 3 in chromosome 1D. Chromosomes 2A and 2D had 3 QTLs each, whereas 2B had two QTLs. Two markers near each were detected in chromosome 3A, also 3 markers were identified in chromosome 3B, and 2 in chromosome 3D. While 4 significant markers are in chromosome 4A and 2 in 4D, chromosomes 5A and 5D contained two pairs of significant markers. Chromosome 6D had 1 significant QTL, and chromosomes 7A and 7D had 3 markers significant markers each and 7B only 1 QTL. Increased resistance in the SHW in comparison to the DW parents, along with the significant association of resistance with the A and B genome, supported the concept of activating epistasis interaction across the three wheat genomes. Candidate genes for TS and SB that play significant roles in biotic stress resistance were identified for the significant markers. The identified resistant SHW lines can be deployed in wheat breeding for both foliar diseases.

Keywords: Synthetic hexaploid wheat; Tan spot; Spot blotch, Genome-wide association study, Foliar disease

IDENTIFICACIÓN DE GENES DE RESISTENCIA A ENFERMEDADES FOLIARES EN TRIGOS SINTÉTICOS HEXAPLOIDES

Nérida Lozano Ramírez, D.C.
Colegio de Postgraduados, 2022

RESUMEN

En los últimos años, las enfermedades foliares se han vuelto cada vez más relevantes en la producción de trigo, lo que ha llevado a una limitación significativa en el rendimiento y la calidad del grano. El trigo hexaploide sintético (SHW por sus siglas en ingles) ha demostrado una resistencia efectiva a una diversidad de enfermedades e insectos. La mancha bronceada (TS por sus siglas en ingles), causada por *Pyrenophora tritici-repentis* (Died.) Drechs, es una importante enfermedad foliar que puede atacar todo tipo de trigo y varias gramíneas. Por otro lado, Spot blotch (SB por sus siglas en inglés) es causado por *Bipolaris sorokiniana* (Sacc.) Shoem syn. *Drechslera sorokiniana* (Sacc.) es una enfermedad fúngica destructiva en regiones húmedas y de altas temperaturas que afecta al trigo y muchos otros cultivos. En este estudio, se evaluó la resistencia/susceptibilidad a TS y SB en un panel diverso de 443 líneas SHW en condiciones ambientales controladas. Además, se realizó un estudio de mapeo de asociación de todo el genoma (GWAS por sus siglas en inglés) mediante el genotipado de todas las entradas con la tecnología DArTSeq para identificar asociaciones de rasgos de marcador para TS y SB. En TS de las 443 plantas SHW, 233 mostraron reacciones resistentes y 183 moderadamente resistentes, y solo 27 fueron moderadamente susceptibles o susceptibles a TS. En el caso de SB, 250 líneas SHW mostraron reacciones resistentes y 161 moderadamente resistentes, y solo 30 fueron moderadamente susceptibles o susceptibles a SB. Los progenitores de trigo duro (DW por sus siglas en inglés) de los SHW se mostraron moderadamente susceptibles a reacciones susceptibles. En el GWAS para TS se encontraron un total de 30 asociaciones significativas marcador-rasgo en los cromosomas 1B (4 marcadores), 1D (1 marcador), 2A (1 marcador), 2D (2 marcadores), 3A (4 marcadores), 3D (3 marcadores) 4B (1 marcador), 5A (4 marcadores), 6A (6 marcadores), 6B (1 marcador) y 7D (3 marcadores). Se identificaron un total de 41 marcadores significativos relacionados con las resistencias a SB, 5 marcadores se encontraron en el cromosoma 1B y 3 en el cromosoma 1D. Los cromosomas 2A y 2D tenían 3 QTL cada uno, mientras que el 2B tenía dos QTL. Se detectaron dos marcadores cerca de cada uno en el cromosoma 3A, también se identificaron 3 marcadores en el cromosoma 3B y 2 en el cromosoma 3D. Mientras que 4 marcadores significativos están en el cromosoma 4A y 2 en el 4D, los cromosomas 5A y 5D contenían dos pares de marcadores significativos. El cromosoma 6D tenía 1 QTL significativo, y los cromosomas 7A y 7D tenían 3 marcadores significativos cada uno y el 7B solo 1 QTL. El aumento de la resistencia en SHW en comparación con los progenitores DW, junto con la asociación significativa de resistencia con el genoma A y B, apoyó el concepto de activación de la interacción de la epistasis en los tres genomas de trigo. Los genes candidatos para TS y SB que juegan un papel importante en la resistencia al estrés biótico se identificaron para los marcadores significativos. Las líneas SHW resistentes identificadas se pueden implementar en el mejoramiento de trigo para ambas enfermedades foliares.

Palabras clave: Trigo hexaploide sintético; mancha bronceada; Mancha manchada, estudio de asociación del genoma completo, enfermedad foliar

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GENERAL INTRODUCTION

Problem Statement

Diseases and pests of wheat (*Triticum aestivum* L.) cause devastating losses to growers when susceptible varieties are grown under favorable environmental conditions. An economical solution for disease and pest losses is host resistance

Pyrenophora tritici-repentis (PTR) (Died.) Drechs. [*Drechslera tritici-repentis* (Died.) Shoemaker (anamorph)] is a homothallic (self-fertile) ascomycete that causes the tan spot (TS) of wheat (also called yellow leaf spot). In some regions, the disease has the potential to cause up to 50% yield loss with an average of 12% loss per year (Hosford, 1982; Riede *et al*., 1996. PTR survives from one season to the next as pseudothecia on wheat residue (Hosford, 1971). During the spring, pseudothecia release ascospores that serve as the primary inoculum (Hosford, 1972). Later in the season conidia produced on conidiophores serve as secondary inoculum (Hosford and Morrall, 1975). Tan spot symptoms are described as initially appearing as tan to brown flecks that expand into elliptical lesions associated with varying degrees of necrosis and/or chlorosis (Wiese, 1987; Ciuffetti and Tuori, 1999). The necrosis and chlorosis associated with tan spot result from toxins produced by the pathogen as initially demonstrated by Tomás and Bockus (1987) and Lamari and Bernier (1989). Tan spot incidence and severity is greater in conservation-tillage farming where survival of the causal fungus is favored by the high amounts of wheat residue retained on the soil surface (Bailey, 1996). Tan spot is controlled with fungicides, but deployment of resistant cultivars is a more cost effective and an environmentally preferred alternative (Ciuffetti and Tuori, 1999).

Spot blotch (SB) caused by *Bipolaris sorokiniana* (Sacc.) Shoem syn. *Drechslera sorokiniana* (Sacc.) Subrm and Jain (syn. *Helminthosporium sativum*, teleomorph *Cochliobolus sativus*) is one of the most destructive fungal diseases that affects wheat and several other small grains worldwide (Dubin and Rajaram, 1996; Duveiller and Dubin, 2002; Joshi and Chand, 2002; Sharma *et al*., 2007; Singh and Singh, 2007; Gurung *et al*., 2009; Chowdhury *et al*., 2013). It has a wide range of hosts within wild and cultivated Poaceae species (Kumar *et al*., 2002; Pandey *et al*., 2005; O'Boyle *et al*., 2014). In susceptible lines, SB symptoms are characterized by small, dark brown lesions that extend 1–2 mm long without chlorotic margins during initial infection (Chand *et al*., 2003). Later, the leaves are killed when the light brown to dark brown colored oval to elongated

blotches extend and merge very quickly. The fungus also causes common root rot (Wildermuth *et al*., 1997), seedling blight and seed rot or black point on the embryo (Kumar *et al*., 2002; Hudec and Muchova, 2008). Average yield loss of 15–20% due to SB has been reported from several countries, but under suitable climatic conditions the losses in yield can reach up to 70% in susceptible genotypes, in addition to the reduction in seed quality (Mehta *et al*., 1992; Lemerle *et al*., 1996; Fernandez *et al*., 1998, 2014; Wang *et al*., 2002; Fernandez and Jefferson, 2004; Sharma *et al*., 2007; Siddique *et al*., 2006; Sharma and Duveiller, 2007; Acharya *et al*., 2011).

Identification of novel sources of genetic resistance to tan spot and spot blotch is critical. To identify novel, more effective sources of genetic resistance, breeding programs have focused on synthetic hexaploid germplasm that harbors effective resistance to diseases (Trethowan and van Ginkel, 2009). Synthetic hexaploid wheat provides convenient access to desirable genes from *Aegilops tauschii* Coss. and *T. turgidum* L. for genetic improvement of common bread wheat.

Synthetic hexaploid wheat (SHW; 2n=6x=42, AABBDD, *Triticum aestivum* L.) is produced from an interspecific cross between durum wheat (2n=4x=28, AABB, *T. turgidum* L.) and goat grass (2n=2x=14, DD, *Aegilops tauschii* Coss.). It is reported to have a considerable amount of genetic diversity and is a potential source of novel alleles controlling abiotic and biotic stresses resistance and improving wheat quality. This dissertation was focused on understanding the genetic diversity of unique sets of SHW germplasm and unlocking their genomic regions for controlling the diseases foliar resistance such as tan spot caused by PTR and SB caused by *Bipolaris sorokiniana*.

Use of genome-wide association studies (GWAS) is a robust strategy for identifying genomic regions associated with resistance that can facilitate introgression of novel resistance genes via marker-assisted selection; thus, the process of resistant cultivar development can be accelerated relative to field-based phenotypic selection. The choices of candidate markers and genes to be deployed in breeding rely on the confirmatory as well as unique genomic resources available in a given set of germplasm. The donor genotypes are identified with the presence of a significant portion of heritable variance explained by the target genomic regions. Many GWAS studies have been done on diseases in bread wheat, but few studies have been done for PTR and SB in SHW.

A genome-wide association study (GWAS) is useful to identify genomic regions associated with tan spot and spot blotch resistance. The goals of this research were the followings:

General objective

Identify molecular markers linked to resistance to tan spot and spot blotch by means of genome-wide association study (GWAS).

Specific objectives of this study were as follows:

1. Evaluate 443 lines of SHW for their resistance to tan spot and spot blotch under controlled environmental conditions
2. Perform the GWAS analysis with the phenotypic and genotypic SHW data.
3. Identify marker trait association and new genomic regions conferring tan spot and spot blotch resistance.
4. Compare results of this study with many others of the literature

Hypothesis

The tan spot (TS) disease caused by *Pyrenophora tritici-repentis* and spot blotch (SB) caused by *Bipolaris sorokiniana* are among the main diseases of wheat and can cause great losses in the yield of its world production. Synthetic hexaploid wheats (2n=6x=42; AABBDD) derived from crosses between durum wheat (2n=4x=28; AABB) and *Aegilops tauschii* syn. *squarossa* (2n=2x=14; DD) are important source of useful traits in wheat breeding for resistance to multiple fungal pathogens.

Background

Fungal diseases are one of the main biotic limitations that reduce the expression of the potentiality of wheat crop yields. Wheat foliar diseases have increased in recent years, due to cultural factors such as the increase in direct seeding, as well as the susceptibility of cultivars and the high genetic variability of the causal pathogens. However, changing climate conditions and the onset of severe plant disease epidemics significantly limit wheat grain yield and quality (Gurung et al., 2014). About 5–14% of global wheat yield is lost each year due to diseases (Oerke, 2006). The causal agents parasitize the tissues of the root, stems, leaves, spikes and grains. Due to the spread, frequency of appearance and levels of epidemic development that they reach, the diseases of greater relative importance are those that affect the leaf tissues and the spike (and their grains).

Two of the major foliar diseases causing damage to wheat are tan spot and spot blotch (Tomás and Bockus 1987, Lamari and Bernier 1989, Mehta *et al*, 1992; Sharma and Duveiller, 2007; Acharya *et al*., 2011).

Tan spot (synonymous with yellow spot or yellow leaf spot) is caused by the necrotrophic fungal pathogen *Pyrenophora tritici-repentis* (PTR) (Died.) Drechs. (anamorph: *Drechselera tritici-repentis* Died.), which belongs to the order of dothideomycete in ascomycete (Manning et al. 2013) and is a foliar disease of wheat found worldwide. The pathogen attack both durum and common wheat, as well as numerous other grass species. Symptoms of the disease, which mainly include necrosis and chlorosis on leaf tissue, can cause severe yield losses by reducing the photosynthetic area of leaves resulting in reduced grain fill, kernel shriveling and reduced numbers of kernel per head (Shabeer and Bockus 1988). Disease symptoms on susceptible hosts appear as tan-colored oval shaped necrotic and/or chlorotic spots with a black pinhead spot in the center. In highly susceptible genotypes, these lesions may coalesce and cover the larger/whole leaf surface area; these symptoms are associated with the fungal-produced necrotrophic effectors (NEs), previously known as host-selective toxins (HSTs) (Strelkov and Lamari 2003). Yield losses of up to 49% have been attributed to tan spot during favorable disease conditions.

Losses attributed to tan spot cause low thousand-kernel weight, reduced the number of kernels per head, or if the infection occurs early, then a smaller number of tillers, low biomass, and low leaf area index. Additionally, the disease can lead to reductions in grain quality by forming red or pink smudge. The necrosis and chlorosis associated with tan spot result from toxins produced by the pathogen as initially demonstrated by Tomás and Bockus (1987) and Lamari and Bernier (1989). Currently, eight races of PTR have been identified based on necrosis and chlorosis symptoms induced by host-selective toxins (HST) on a set of differential wheat varieties (Lamari *et al*., 2003).

Although fungus can be controlled using cultural and chemical methods, host resistance against tan spot is the most cost-effective and environmentally friendly way to limit yield losses. Due to its overwintering habit on crop residues or stubble, tan spot is a major concern in sustainable no-tillage agricultural systems, as the inoculum of primary infection is always there in the field. The disease cycle consists of the fungus surviving in wheat stubble as pseudothecia, a primary infection of plants caused by fungal ascospores at the beginning of the growing season, and

numerous subsequent infections by fungal conidia throughout the growing season. Due to its polycyclic nature, the fungus can cause great damage to wheat plants. Shifts from conventional tillage and stubble burning to reduced or no-till practices, intensified wheat production and shorter or no crop rotations are some examples of cultural practices that favor tan spot disease (Bockus & Shroyer, 1998). Although several control strategies exist, including crop-rotation and burning the infested stubble and foliar fungicides, the most cost-effective and environmentally friendly method is the use of resistant cultivars (De Wolf *et al*., 1998; Bockus & Claasen, 1992).

Bipolaris sorokiniana (teleomorph *Cochliobolus sativus*) is of prime economic importance. It has a wide host range in the Poaceae family and causes seedling blight, foliar blight, common root rot, black point disease (Acharya *et al.*, 2011), and spot blotch (SB), also called helminthosporium leaf blight or foliar blight, of wheat (Zhu *et al*., 2014). The disease is typically characterized by small dark brown lesions of 1–2 mm length, that extend to form elongated light to dark brown blotches of several centimeters before coalescing and causing leaf necrosis (Mercado Vergnes *et al*., 2006; Duveiller and Sharma, 2009). This pathogen induced foliar necrosis reduces the photosynthetic area of the leaf and results in premature senescence (Sharma *et al*., 1997). During favorable conditions, the pathogen can also infect the spikes, resulting in shriveling of the grain, black point of the kernels and deterioration of grain quality (Sharma *et al*., 1997; Kumar *et al*., 2002).

Spot blotch (SB) is one of the most devastating foliar diseases of wheat particularly under warm and humid conditions, for example, in South Asia (Singh *et al*., 2016; Friesen *et al*., 2018). Globally, 25 million hectares of wheat-growing areas are affected by SB (Sharma *et al*., 2007). It is prevalent in wheat-growing regions of Bangladesh, Nepal, southeast Asia, Latin America, eastern India, south-east China, south-east Australia, sub-Saharan Africa, northern Kazakhstan and the Great Plains of the USA and Canada causing significant losses in yield of up to 70% under suitable climatic conditions with susceptible cultivars, and deteriorating the grain quality (Duveiller *et al*., 2005; Sharma *et al*., 2007; Zhu *et al*., 2014; Singh *et al*., 2015; Ayana *et al*., 2018). In the wake of global climate change, SB is also becoming a serious concern in new areas with irrigated and low-rainfall (Gupta *et al*., 2018). The widely adopted rice–wheat cropping system of South Asia provides a favorable environment for the survival and multiplication of the

SB pathogen, as rice is an alternative host with the rice crop debris a substrate for the fungus (Saari, 1998).

SB management using several agronomic and cultural approaches have been proposed including the use of disease-free seed, optimized sowing time based on the cropping system, timely irrigation, adequate fertilization, crop rotation, removal of infected plant debris, etc., but none of them have been completely effective (Duveiller *et al*., 2005; Pandey *et al*., 2005; Sharma and Duveiller, 2007; Sharma *et al*., 2006). While chemical control approaches including seed treatment and foliar fungicide spray have provided acceptable SB control, their non-affordability by resource poor farmers, the environment and health hazards associated with their use and the possibility of pathogen populations developing resistance to classes of fungicides have limited their usage (Duvellier and Gilchrist, 1994; Duveiller and Sharma, 2009). Hence, the deployment of resistant varieties is the most economical and sustainable SB management strategy, and an integrated approach that combines host-plant resistance as the key component with good agronomic and cultural practices and reasonable chemical control has been recommended (Joshi *et al*., 2004; Duveiller and Sharma, 2009).

To identify novel and more effective sources of resistance, breeding programs have explored synthetic hexaploid wheat (SHW) that harbors a broad spectrum of resistance to diseases and insects (Trethowan and van Ginkel, 2009). SHW (2n = 6x = 42, AABBDD) derives from a cross between modern durum wheat (DW) (2n=4x=28, AABB, *T. turgidum* L.) and wild goat grass (2n=2x=14, DD, *Ae. tauschii* Coss.) Fig.1. SHW is considered as an ideal bridging germplasm for the transfer of desirable genes from DW and *Ae. tauschii* to bread wheat (Siedler *et al.*, 1994). Embryo rescue is performed after crossing to save the embryos which have the genomic constitution of ABD genomes. As this form is amphiploid, sterile, and unstable, the chromosomes are doubled using colchicine to form a stable hexaploid wheat, commonly referred to as SHW (Mujeeb-Kazi *et al*., 2008)

SHW is easily crossable with elite bread wheat cultivars because the two have similar floral attributes and the same genomic constitution. It has also carried a plethora of disease resistance genes for different pests, insects and fungi and have been exploited for wheat improvement (Gill *et al*., 1986). It is a bridging germplasm for the transfer of desirable genes from *Ae. tauschii* to common wheat (Siedler *et al*., 1994). It has been reported to have a considerable amount of genetic

diversity and is a potential source of novel alleles controlling abiotic and biotic stresses resistance and improving wheat quality.

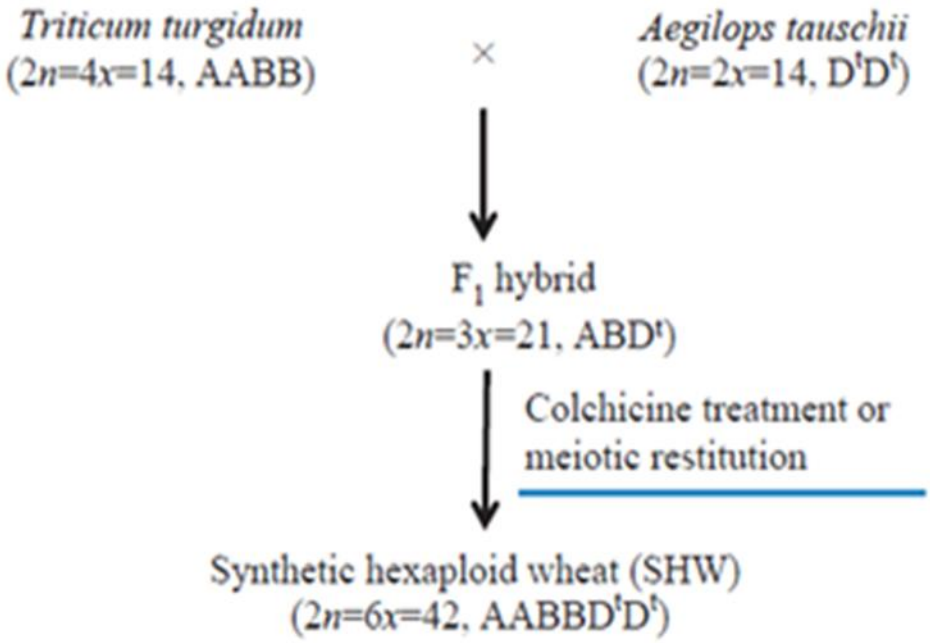

Figure 1. Schematic of a synthetic hexaploid wheat

Production of synthetic hexaploid wheat

Most SHW lines have been developed by crossing durum (pasta) wheat (*T. turgidum* ssp. *durum*, AABB) and wild goat grass (*Ae. tauschii*, DD). In most cases, the diploid species is used as a paternal parent to pollinate durum wheat. The reciprocal cross is possible, albeit with less success due to smaller embryo sizes or embryo defects. In certain cases, embryos derived from the interspecific cross (durum x *Ae. tauschii*) may develop, but endosperms may not. Thus, it is necessary to conduct embryo rescue 2–3 weeks after pollination. During this process, embryos are dissected from immature seeds and transferred to an agar medium with nutrients such as sugar and salt for proper development. The type of the tetraploid parent, such as a different accession of durum wheat, may affect endosperm formation in the cross. For example, Langdon is a well-known durum variety that has a relatively high endosperm development rate. This durum variety is preferred for synthetic wheat production so as to skip the embryo rescue process. The regenerated plants are triploid and are usually treated with colchicine to double their chromosome number

before they are transplanted to soil in pots to produce mature plants and, ultimately, seeds. In addition to the conventional artificial colchicine treatment, chromosome doubling can be achieved by the union of unreduced gametes (2n) derived from spontaneous meiotic restitution in *T. turgidum–Ae. tauschii* hybrids (Figure 2) (Zhang *et al*., 2010 and Hao *et al*., 2014). In fact, spontaneous chromosome doubling has been widely observed during the creation of new SHW lines (Zhang *et al*., 2010 and Luo *et al*., 2012). These SHW lines are useful for producing amphidiploids and double haploids for genetic improvement of existing wheat varieties (Liu *et al*., 2016).

An overview of SHW history.

The first attempt to develop synthetic wheat was made in the middle of the last century with ‘‘synthetic spelta” in a study to determine the progenitors of *T. aestivum* subsp. *spelta* L. Thell (McFadden and Sears., 1946). These earliest allopolyploid hybrid forms of common wheat were named ‘‘synthetic hexaploid wheat.” Since the late 1980s, the International Maize and Wheat Improvement Center (CIMMYT) has developed more than 1000 SHW lines (Das *et al*., 2016). In subsequent studies, SHW has been recognized and confirmed as a valuable genetic source with better performance under biotic and abiotic stresses, as well as with better yield potential such as larger kernels and spikes (Pritchard *et al*., 2002 and Mujeeb-Kazi *et al*., 2008).

However, synthetic wheat itself cannot be used as a cultivar because of the presence of ‘‘wild ,” or “agronomically” undesirable characters such as tenacious glumes that causes non-free threshing grains; it is necessary to remove these characters or transfer desired traits of synthetic wheat into common wheat varieties by developing synthetic derivative lines (SDLs) through crossing with elite common wheat cultivars (Zhang *et al*., 2010). In 2003, Spain pre-registered a CIMMYT synthetic wheat derivative under the name Carmona (Masood *et al*., 2016, 2017). At the same time, China also registered the first synthetic-derived cultivar. Since then, at least 62 SDLs have been registered as cultivars around the world. Synthetic derivatives showed a significant increase in genetic diversity when compared with their parents (Warburton., *et al*., 2006).

Synthetic hexaploid wheat as a potential source of biotic and abiotic stresses resistance

Breeding for drought tolerance is important for wheat improvement. However, bread wheat has limited genetic and phenotypic diversity available for breeding for drought tolerance (Becker *et al*., 2016). The SHWs are potential sources of new genetic variation for drought tolerance in wheat improvement. Several studies on synthetic derived lines (SDLs) indicated that the SDLs provided up to 45% yield increase compared to their wheat parents under drought stressed conditions (Becker *et al*., 2016 and Trethowan, 2008). Similarly, SDLs produced up to 30% yield increase compared with parent lines and local checks under rainfed conditions (Dreccer *et al*., 2007). The synthetic derived cultivar named Chuanmai-42 developed in China was found to have 35% higher grain yield than the commercial check cultivar (Li *et al*., 2014). Therefore, exploiting SHWs for drought tolerance is needed for the global food security.

Biotic stresses such as diseases and insect-pest infestation are a major constraint to wheat production. genetic resistance against biotic stress is a major goal in wheat breeding program. Modern wheat cultivars have a limited genetic variation for diseases and insect-pest resistance (Smith *et al*., 2009) and there is always the possibility of the evolution of new 5 diseases/insect-pest or races to overcome previously identified resistance genes. A wide range of genetic variation is prerequisite for protecting crop productivity and genetic gain. Identification of genetic resistance to multiple diseases and pests is a prerequisite for any breeding programs for the sustainable agricultural productivity and production., it is important to study new genetic resources that have the potential to add genetic variation for several biotic and abiotic stresses resistance. This need may be helped by increasing the genetic variation of wheat through the utilization of SHWs (Smith *et a*l., 2009). The D-genome from wild goat grass used in the SHWs have shown have many desirable genes for wheat improvement including disease and insect pest resistance (Smith *et al*., 2009).

Several studies identified that SHWs are resistance to biotic stresses. For example, SHWs were found to have resistance to leaf rust (incited by *Puccinia triticina*) (Das *et al*., 2016., Ogbonnaya *et al*., 2008 and Jighly *et al.*, 2016), stem rust (incited by *P. graminis*) (Ogbonnaya *et al*., 2008 and Jighly *et al*., 2016), stripe rust (incited by *P. striiformis*) (Ogbonnaya *et al*., 2008., Jighly *et al*., 2016 and Zegeye *et al*., 2014), Fusarium head blight (incited by *Fusarium graminearum*) (Das *et al*., 2016), yellow spot (incited *by Pyrenophora tritici-repentis*)

(Ogbonnaya *et al*., 2008 and Jighly *et al*., 2016), Septoria nodorum (incited by *Parastagonospora nodorum*) (Ogbonnaya *et al*., 2008 and Jighly *et al*., 2016), Septoria tritici blotch (incited by *Mycosphaerella graminicola*) (Ogbonnaya *et a*l., 2008 and Jighly *et al*., 2016), cereal cyst nematode (incited by *Heterodera avenae*) (Ogbonnaya *et al*., 2008), crown rot (incited by *F. pseudograminearum*) (Jighly *et al*., 2016), root-lesion nematode (incited *by Pratylenchus thornei* and *P. neglectus*) (Ogbonnaya *et al*., 2008), and Karnal bunt (*Tilletia indica*) (Villareal *et al*., 1994). Additionally, SHWs had multiple insect-pest resistance (Morgounov *et al*., 2018, Das *et al*., 2016 and Jighly *et al*., 2016). Therefore, exploiting genetic variation of SHWs is needed for the genetic improvement of wheat under biotic stress.

The genome-wide association study

The genome-wide association study (GWAS) is based on the exploitation of linkage disequilibrium found in a collection of varieties or accessions (Zhu *et al*., 2008; Waugh *et al*., 2009; Rafalski 2010; Hamblin *et al*., 2011) and is a powerful tool to identify QTLs in plants. This method uses the recombination which occurred during the history of variety development, resulting in an often-improved genetic resolution compared to bi-parental mapping populations for identifying QTL´s, which have usually only undergone one or a few generations of recombination. In addition, it is possible to monitor a whole spectrum of lines. GWAS, which rely on linkage disequilibrium (LD) between a genetic marker and a locus affecting a trait, were used to identify significant marker–trait correlations in animal and plant genetics (Shirasu and Schulze-Lefert 2003; Neumann *et al*., 2011). In GWAS, a collection of diverse accessions is phenotyped and genotyped to examine marker–trait association and it can be seen as a promising strategy to identify QTL for traits of interest which take advantage of historical recombination. (Shirasu and Schulze-Lefert 2003; Flint-Garcia *et al*., 2003.

Use of GWAS is a robust strategy for identifying genomic regions associated with resistance that can facilitate introgression of novel resistance genes via marker-assisted selection; thus, the process of resistant cultivar development can be accelerated relative to field-based phenotypic selection. The choices of candidate markers and genes to be deployed in breeding rely on the confirmatory as well as unique genomic resources available in each set of germplasm. The donor genotypes are identified with the presence of a significant portion of heritable variance

explained by the target genomic regions. Many studies of GWAS have been carried out on diseases in bread wheat but few studies have been carried out on diseases in SHW.

CHAPTER 1. GENOME-WIDE ASSOCIATION STUDY FOR RESISTANCE TO TAN SPOT IN SYNTHETIC HAXAPLOID WHEAT.

1.1. ABSTRACT

Synthetic hexaploid wheat (SHW) has shown effective resistance to a diversity of diseases and insects. Tan spot, caused by *Pyrenophora tritici-repentis* (Died.) Drechs, is an important foliar disease that can attack all types of wheat and several grasses. In this study, 443 SHW plants were evaluated for their resistance to tan spot under controlled environmental conditions. Additionally, a genome-wide association study was conducted by genotyping all entries with the DArTSeq technology to identify marker-trait associations for tan spot resistance. Of the 443 SHW plants, 233 showed resistant and 183 moderately resistant reactions, and only 27 were moderately susceptible or susceptible to tan spot. DW parents of the SHW showed moderately susceptible to susceptible reactions. A total of 30 significant marker-trait associations were found on chromosomes 1B (4 markers), 1D (1 marker), 2A (1 marker), 2D (2 markers), 3A (4 markers), 3D (3 markers) 4B (1 marker), 5A (4 markers), 6A (6 markers), 6B (1 marker) and 7D (3 markers). Increased resistance in the SHW in comparison to the DW parents, along with the significant association of resistance with the A and B genome, supported the concept of activating epistasis interaction across the three wheat genomes. Candidate genes coding for F-box and cytochrome P450 proteins that play significant roles in biotic stress resistance were identified for the significant markers. The identified resistant SHW lines can be deployed in wheat breeding for tan spot resistance.

Key words: *Aegilops tauschii*; Durum wheat; Synthetic hexaploid wheat; Tan spot; Genome-wide association study.

1.2. INTRODUCTION

Diseases are major threats that significantly reduce yield when crops are grown under conducive conditions. Wheat foliar diseases have gained increased importance in recent years due to various factors such as the adoption of conservation agriculture practices, commercial cultivation of susceptible varieties, and high-evolution dynamics of the causal pathogens [1]. Furthermore, climate change often results in severe disease epidemics that significantly limit grain yield and quality in wheat [2]. About 12-14% of the global wheat production is lost each year due to diseases

[3]. The causative agents of these diseases, mainly fungal pathogens, infect multiple wheat tissues such as root, stem, leaf, spike, and grain. Based on the frequency and severity levels of disease epidemics, the diseases that infect leaf and spike/grain are considered of greater importance. In this sense, many researchers agree that "leaf rust", caused by *Puccinia triticina* Eriks; "tan spot", by *Pyrenophora tritici-repentis* (Died.) Drechs. (Anamorph *Drechslera tritici-repentis* (Deceased) Shoem.); “Septoria nodorum blotch (SNB)”, by *Parastagonospora nodorum* [syn. ana. *Stagonospora*; teleo. *Phaeosphaeria*] (Berk.) Quaedvlieg, Verkley & Crous, and “Septoria leaf spot”, by *Mycosphaerella graminicola* (Fuckel) Schroeter, in Cohn (anamorph *Zymoseptoria tritici* Rob ex Desm.) are some of the most important foliar diseases [4].

Tan spot (synonymous with yellow spot) pathogen *P. tritici-repentis* belongs to the order of dothideomycete in ascomycete [5] and can attack both durum and bread wheat, as well as many other grass species. This foliar wheat disease is found globally, with symptoms mainly including necrosis and chlorosis on leaf tissues, reducing the photosynthetic area, and resulting in poor grain filling, kernel shriveling, a reduced number of kernels per head, and severe yield losses [6]. Yield losses of up to 49% have been attributed to tan spot under favorable disease conditions. Additionally, the disease can lead to reductions in grain quality by forming red or pink smudge. The pathogen-induced lesions may coalesce and cover most, or the entirety of, the leaf surface; these symptoms are associated with the fungal-produced necrotrophic effectors (NEs), previously known as host-selective toxins (HSTs) [7]. The necrosis and chlorosis associated with tan spot result from toxins produced by the pathogen as initially proven by [8,9]. Currently, eight races of *P. tritici-repentis* have been identified based on symptoms of necrosis and chlorosis on a set of differential wheat varieties/lines [10].

Due to the overwintering habit of *P. tritici-repentis* on crop residues or stubbles, tan spot is a major concern in sustainable zero-tillage agricultural systems. The disease cycle consists of a primary infection caused by fungal ascospores at the beginning of the growing season, and numerous subsequent infections by fungal conidia throughout the growing season. Although the disease can be controlled using cultural and/or chemical methods, host resistance against tan spot is the most cost-effective and environmentally friendly way to limit yield losses.

To identify novel and more effective sources of resistance, breeding programs have explored synthetic hexaploid wheat (SHW) that harbors a broad spectrum of resistance to diseases

and insects [11]. SHW (2n = 6x = 42, AABBDD) derives from a cross between modern durum wheat (DW) (2n=4x=28, AABB, *T. turgidum* L.) and wild goat grass (2n=2x=14, DD, *Ae. tauschii* Coss.). SHW is considered as an ideal bridging germplasm for the transfer of desirable genes from DW and *Ae. tauschii* to bread wheat [12].

The genome-wide association study (GWAS) explores linkage disequilibrium (LD) in a collection of varieties or accessions [13-16] and is a powerful tool to identify quantitative trait loci (QTL). It uses recombination events that occurred during the history of variety development, resulting in an often-improved genetic resolution for identifying QTL compared to bi-parental mapping populations, which have usually undergone only one or a few generations of recombination. In addition, GWAS allows for the screening of a large number of lines for a whole spectrum of traits. GWAS has been applied to identify genomic regions associated with tan spot resistance in common wheat. [17] conducted the first GWAS for tan spot resistance in a spring common wheat landrace collection and found QTL on chromosomes 1D, 2A, 2B, 2D, 4A, 5B and 7D for race 1 and on chromosomes 1D, 2B, 2D and 7D for race 5. Furthermore, GWAS has been performed with different races of *P. tritici-repentis* in panels of spring wheat landraces [18] and with unknown races on a European winter wheat collection [19]. Multiple races were used for a GWAS in a collection of North American winter wheat cultivars and breeding lines [20], and race 1 isolates in the Vavilov wheat collection at both seedling and adult stages [21]. The QTL identified in those studies corresponded partly to the NE sensitivity loci and previously reported loci, whereas others were novel.

Very few GWAS studies have also been performed to identify significant markers related to tan spot resistance in CIMMYT wheat germplasm [22-24]. Singh et al. [22] indicated the association of tan spot resistance with markers on multiple A- and B-genome chromosomes. Similarly, Juliana et al. [23] identified 14 markers on A- and B-genome chromosomes. [24] performed GWAS on a panel of South Asian and CIMMYT spring bread wheat genotypes and found significant markers on chromosomes 1B, 2A, 2B, 3B, 4A, 5A, 5B, 6A, and 7D. However, none of these studies included SHW.

The current GWAS study was conducted on a diverse panel of 443 SHW plants to (1) evaluate their resistance to tan spot under controlled environmental conditions and (2) identify possible new genomic regions for tan spot resistance.

1.3. RESULTS

1.3.1. Resistance to tan spot at the seedling stage

Uniform and consistent tan spot development was observed during seedling evaluation in the greenhouse. Analyses of variance (ANOVA) showed significant differences among SHW plants ($p < 0.001$) for reaction to tan spot. The checks Erik, Glenlea, 6B-662, and 6B-365 displayed scores of 1.0, 4.8, 2.5 and 3.4, respectively (Table 1), verifying the identity of *P. tritici-repentis* and successful inoculation.

Most SHW plants displayed resistant and moderately resistant reactions. Out of the 443 SHW plants, 233 (52.6%) showed resistance (R) and 183 (41.4%) moderate resistance (MR) with disease scores of 1.0 to 2.5 that were comparable to the resistant check Erik and the moderately resistant check 6B-662. Only 27 SHW plants (6.0%) were moderately susceptible (MS) or susceptible (S) with disease scores of 3.0 to 3.5 that were still better than the susceptible check Glenlea and 6B-365 (Table 1, Supplementary Table S1, and Figure 1).

Of the 40 DW parents, 6 (15%) had reaction scores of 1.0-1.5 (R) and 12 (30%) had reactions scores of 1.6-2.5 (MR), developing mostly small dark to maroon lesions on the leaves. Twenty-two entries (55%) were observed to have a mean reaction score between 2.6 and 4.3, being considered moderately susceptible (MS) to susceptible (S), wherein large necrotic lesions with or without chlorosis was observed. (Table 1, Supplementary Table S1, and Figures 2A-4A (Manhattan plots for consensus, Chinese spring, and Durum-*Ae. tauchii*, respectively) and Figures 2B-4B (QQ-plots for consensus, Chinese spring, and Durum- *Ae. tauchii*, respectively).

1.3.2. Genome-wide association mapping under different references maps

Using the markers mapped on the 100K consensus map, the first two principal components (PCs) separated two clear groups of entries of similar sizes and some entries in between, explaining around 34% of the total variability (Supplementary Figure S1). As described in the Material and Method section, possible population structure was controlled by fitting the first five PCs from the correlation matrix as a fixed variate. Also, the coefficient of parentage used as random variable for fitting the GWAS mixed linear model (MLM) effectively controlled the remnant population structure after fitting the first three PCs.

Significant marker-trait associations detected using the consensus map are shown in Table 2 and Figure 2A (Figure 2B). The 16 significant markers were located on chromosomes 1B (3), 2A (1), 4A (1), 5A (2), 5B (1), 6A (5), 6B (1) and 7D (2). The markers with the highest allele substitution effects were located on chromosomes 4A (-0.55), 6B (-0.44), and 7D (0.59).

Significant marker-trait associations when markers were aligned to the whole genome sequence of Chinese Spring (CS, IWGSC RefSeq v1.0) are shown in Table 3 and Figure 3A (Figure 3B). The 18 significant markers were located on chromosomes 1B (1), 1D (1), 2A (1), 3A (2), 3D (3), 4D (1), 5A (2), 6A (3), 6B (2) and 7D (2). Ten of the markers overlapped with those presented in Table 2, out of which six exhibited the same chromosome assignments on the genetic and physical maps, whereas four showed different chromosome assignments (yet mainly homologous chromosomes) on the two maps. The markers with the highest allele substitution effects were located on chromosomes 3A (-0.44), 4D (-0.56), and 7D (0.61).

Thirteen markers were significantly related to tan spot resistance, aligned to the durum wheat cultivar Svevo and the *Ae. tauschii* reference genomes. These markers were located on chromosomes 1B (4), 2D (2), 3A (2), 4A (1), 5A (1), 6A (2) and 7D (1) (Table 4 and Figure 4A) (Figure 4B). Only three markers from Table 4 coincided with the significant markers found in Tables 2 and 3. Marker 3026113 on chromosome 1B in Svevo was found to be significant on chromosome 1D aligned to the physical map of CS. Similarly, marker 1125862 on chromosome 3A in Svevo aligned to chromosome 3D in the physical map of CS (Table 3). Marker 16793126 aligned to chromosome7D in the *Ae. tauschii* and CS physical maps (Table 3)**.** The markers with the highest allele substitution effects ranged from -0.20 to -0.27 and were located on chromosomes 1B, 3A, 5A, and 6A.

1.3.3. Comparison of the significant markers across the different maps

Table 5 summarizes the 30 genomic regions identified with different maps. A re-alignment of the sequences to the ABD, AB and D genomes, could verify the physical position of several of the significant SNPs. Furthermore, 16 SNPs were found within annotated high-confidence gene sequences. Eight of these 16 possible candidate genes were annotated in the CS reference genome, four in Svevo and the residual four in *Ae. tauschii* reference genome (Supplementary Table S2).

1.3.4. Marker-trait associations and QTL for tan spot resistance

The allele frequency correlations (R^2) among the markers were used to estimate LD. Based on the physical positions of observed marker-trait association in the CS reference genome, three potential QTL were identified on each of the chromosomes 3A, 5A and 6A. Out of the four significant markers on chromosome 3A, with marker IDs 1125872, 1668224, 1019955, and 1065211, the latter two markers were positioned at 474,447,292 Mb and 474,447,226 Mb, respectively, only 66 bp apart with a R^2 of 0.89 and a significant LD *p*-value of 8.62E-16. The third marker (ID 1668224), despite being located 5.9 Mb apart from the previous two, still had R^2 values of 0.87 and 0.89 and significant LD *p*-values of 6.54E-16 and 2.30E-16, with the two SNPs, respectively. Therefore, these three markers can be considered for a single QTL for resistance to tan spot. Marker 112872, however, was located far from the markers mentioned above and must represent an independent QTL.

Likewise, two markers on chromosome 5A (100034112 and 3064590) and four markers on chromosome 6A (1254459, 2266481, 100027398, and 1862737) were located in LD and thus represented one same QTL, whereas all the remaining SNPs identified in our study represented independent QTL, due to their mutually unlinked physical positions.

1.4. DISCUSSION

The development of genetically resistant wheat cultivars is an effective and environmentally friendly mechanism for the control of diseases such as tan spot. In the following subsections, we discuss the findings of this GWAS in relations to previous studies performed.

1.4.1. Tan spot resistance in SHW

Modern bread wheat cultivars are only a few broad-spectrum sources of resistance to the major foliar spotting diseases, such as tan spot [25], and great efforts have been made in recent decades to identify and introduce new sources of resistance. Despite the number of studies performed and published for wheat diseases, only a few included SHW. For example, [26] studied 125 SHW plants for their resistance to diseases and pests like rust, crown rot, cereal cyst nematodes, and Hessian fly. To the best of our knowledge, so far, no GWAS was performed to evaluate SHW for tan spot resistance.

Our study indicates that SHW plants present considerable resistance to tan spot due to the diverse genetic backgrounds of these lines. The DW parents were mostly of reaction types of MS and S, suggesting that the resistance in the SHW was either derived from *Ae. tauschii* or through possible favorable epistatic interaction (activation) between A/B- and D- genomes.

1.4.2. Comparisons with previous studies

1.4.2.1.Significant markers found in the D- genome (1D, 2D, 3D, 4D, and 7D)

Our study found significant marker-trait associations for tan spot resistance on chromosome 1D (marker ID 3026113, 2D (marker IDs 1217275, 1046621), 3D (marker IDs 987556, 1125862, 1217411), 4D (marker ID 4993454) and 7D (marker IDs 16793126, 991140, 993425). Thus, this is the first study to detect several significant genomic regions to tan spot resistance in the D-genome, in addition to the few loci reported previously. [24] found a significant marker on chromosome 7D located at 550,216,751 Mb in CS. The closest significant marker on chromosome 7D in this study (marker ID 993425) was positioned at 620,252,508 Mb, physically distant and suggesting that at least two of the three marker-trait associations on chromosome 7D in this study are novel. The physical position of the third marker 991140 in CS could not be determined.

[27] studied resistance to tan spot in segregating $F_{2:3}$ derived populations of SHW using simple sequence repeat (SSR) markers. The authors found that loci *tsn*3*a*, *tsn*3*b* and *tsn*3*c* are all located in the vicinity of the marker *Xgwm*2a located on chromosome 3D. The physical distance of this SSR marker to the SNP markers in our study was difficult to determine. [17] performed GWAS in spring wheat landraces and using DArT markers to identify chromosome regions associated to tan spot race 1 and 5 resistances. The authors found significant markers, among others, on chromosomes 1D and 7D associated to tan spot race 1 and in regions of chromosomes 2D and 7D for tan spot race 5. Similar to the study by [27], genomic regions could not be compared, as different genotyping platforms were used.

1.4.2.2.Significant markers found at the A and B genome (1B, 2A, 3A, 4B, 5A, 6A, and 6B)

The present study found significant marker-trait associations on the A-genome chromosomes 2A (marker ID 10770935), 3A (marker IDs, 1125872, 1668224, 1019955, 1065211) and those forming a QTL on chromosome 6A (marker IDs, 1862737, 100027398, 1254459,

2266481, 4993056, 5331622). None of the marker-trait associations coincided with those reported by [23], except on chromosome 3A. Marker 1125872 was located at 135,590,641 Mb in our study and the marker in [23], at 182,028,651 Mb. In the B-genome chromosomes, we found significant marker-trait associations on chromosomes 1B (markers IDs, 1106306, 6045377, 1089962, and 4909460), 4B (marker ID, 4993454), 5A (marker IDs, 4393896, 1200982, 100034112, and 3064590), 6B (marker ID, 1112961); none of them were reported by [23].

[24] also found several marker-trait associations in the A- and B-genomes. The authors found a significant marker on chromosome 2A but in a different position than the one found in this study. A significant locus on chromosome 1B mapped to a physical position in 465,584,555 Mb and was also distant from markers in chromosome 1B of this study located in 340,462,174 Mb and 558,561,647 Mb. Significant markers on chromosome 6A were located in 596,903,177 Mb and coincided with the physical position of the QTL found in this study in physical positions 599,622,814 Mb, 601,233,092 Mb, 602,989,232 Mb, and 602,745,555 Mb, thus representing the same QTL. The marker located on chromosome 5A in [24] mapped to the physical position of 597,291,565Mb, whereas the markers identified in this study forming a QTL are located a distance apart, in 454,770,615 Mb, 471,723,681 Mb, and 470,186,523 Mb, thus likely presenting a novel QTL.

The study by [28] detected three significant loci on chromosome 1B within the range of 86.7-92.2 cM, not distant from marker ID 1089962 located at 83.6 cM in this study using the same 100K consensus map. Furthermore, the QTL on chromosome 6A were in proximity to the markers found by [28] in the same chromosome.

[29] performed bi-parental QTL mapping for resistance to tan spot race 1 in a population with a SHW parent. QTL identified were located only on the A-genome, on chromosomes 1A, 6A, and 7A**.** Because DArT markers were used in this study, the physical positions of the QTL were, once again, difficult to compare**.** Similarly, [30] identified QTL on chromosomes in the A- and B-genome (2A, 5A and 5B) in a bi-parental mapping study using a SHW parent. The authors hypothesized that the expression of tan spot resistance genes in DW is suppressed (or diluted) but are activated when DW is crossed with *Ae. tauschii*, which could be due to inter-locus interaction (epistasis effects) between loci on A/B- and D-genomes. In the current study, increased resistance in SHW in comparison to their direct DW parents supports this hypothesis.

1.4.3. Underlying candidate genes based on protein

Two markers, one on chromosome 5A (marker ID 3064590) positioned at 470,186,523 Mb and the other one located on chromosome 6A (marker ID 1862737) in position 599,622,814 Mb were of particular interest in this study as they were positioned within genes that code for disease resistance related proteins, i.e., TraesCS5A02G254500/TRITD5Av1G155700 (F-box protein) and TraesCS6A02G378800/TRITD6Av1G217060 (cytochrome P450).

Candidate genes TraesCS5A02G254500 / TRITD5Av1G155700 code for F-box proteins that play a role in protein regulation and degradation, plant photoperiodic and hormone signaling transduction. A total of 1796 F-box proteins have been identified and classified in wheat [31], many of which have been related to biotic stresses, particularly to fungal pathogens. In addition, F-box proteins have been observed to affect the plant metabolism and the regulation of plant enzymes involved in several diverse cellular processes [31]. It has been found that the F-box proteins can act in different development stages in a wheat cultivar. The identification of underling genes being related to specific disease resistance should offer an opportunity to further elucidate the biological functions of F-box genes and proteins in wheat.

The cytochrome P450 (CYP) enzyme in plants is involved in the biosynthetic pathway of phytoalexins that are synthetized by plants to deter hostile organisms [32]. This CYP enzyme plays an important role in in the metabolism of herbicides as a key factor in providing tolerance to some species and thus selectively between crops and weeds. Plants encounter various biotic and abiotic factors at different stages of their growth and development and the group of CYP enzymes are important in the synthesis of certain metabolites which play a fundamental part in the response to biotic stresses. The CYT enzymatic protein participates in the formation of numerous secondary synthetized metabolites that protect plants from biotic and abiotic stresses [33]. The mycotoxin deoxynivalenol (DON) is a virulent factor for the development of Fusarium head blight in wheat. A wheat cytochrome P450 subfamily was found in chromosome 3B and 3D of the wheat genome that was activated in the wheat spikelet as a response to the mycotoxin DON [34].

1.5. MATERIAL AND METHODS

1.5.1. Plant Material

A total of 443 SHW plants generated by the CIMMYT Wheat Wide Crosses Program throughout several years were evaluated (Supplementary Table 1). These SHW plants were selected from a group of 1,524 SHW plants for resistance to diseases such as Fusarium head blight, Septoria tritici blotch, and rusts and phenological traits such as plant height and days to heading. The SWH plants were derived from crosses involving 40 DW parents and 277 *Ae. tauschii* accessions, where the DW parents were used in 1 to 54 crosses and the *Ae. tauschii,* accessions were used in 1 to 7 crosses (Supplementary Table 1).

1.5.2. Phenotypic evaluations for tan spot

The disease screening for tan spot was carried out in a greenhouse in CIMMYT, El Batán Mexico (19°31'N, 98°50'W, elevation 2249 m above sea level) in 2018-2019. In addition to the 443 SHWplants, the 40 DW parents were also evaluated, while the *Ae. tauschii* parents could not be screened due to their challenging phenology as a wild species. The SHW seeds were vernalized to break dormancy and to obtain an even germination. Experiments were arranged in a randomized complete block design with 12 replicates for the SHW and eight replicates for the DW parents, with four plants per entry and four checks—Erik (resistant), Glenlea (susceptible), 6B-365 (moderately susceptible), and 6B-662 (moderately resistant)— grown in plastic trays as experimental units to derive mean values for subsequent analysis. The seedlings were grown under controlled conditions in a temperature of 22–25/16–18°C (day/night) and with a 16 h photoperiod.

For the induction of disease, the Mexican *tan spot* isolate CIMFU 531-Ptr1 (race 1), well characterized by the CIMMYT Wheat Pathology Laboratory, was used. This isolate produces ToxA, based on inoculation experiments with differential genotypes, infiltration experiments, and PCR with the ToxA specific marker (data not shown). The isolate was grown on V8-PDA media [9], and the conidia concentration for inoculation was adjusted to 4×10^3 spores mL−1 using a Fuchs-Rosenthal counting chamber, with one drop of Tween 20 (a surfactant reagent) per 100 ml added to the spore suspension.

In the two-leaf stage, when the second leaf was fully expanded or two weeks after sowing, the seedlings were inoculated with a conidial suspension of the CIMFU 531-Ptr1 isolate until runoff. Subsequently, the trays were moved to a mist chamber (RH 100%, 21-22°C) to facilitate infection. After 24 h, the plants were transferred back to the greenhouse bench. Seedling response was evaluated seven days post inoculation following the 1–5 lesion rating scale developed by [9]. The readings from 12 and 8 inoculation experiments of the SHW plants and DW parents, respectively, were used to calculate the average seedling response, which was used for subsequent statistical analysis. The scale used for the tan spot reaction was based on continuous data given by the mean of the replicates: 1.0-1.5=Resistant (R); 1.6-2.5=Moderately Resistant (MR); 2.6-3.5=Moderately Susceptible (MS); 3.6-5.0=Susceptible (S).

1.5.3. Plant genotyping

The genomic DNA was extracted from 10-day-old seedlings of each SHW line using the modified cetyltrimethyl ammonium bromide (CTAB) method described in the CIMMYT laboratory protocols [35]. The DArTseq^{TM} technology [36] was applied to all samples at the Genetic Analysis Service for Agriculture (SAGA) in CIMMYT, Mexico. DArTseq uses a complexity reduction method including two enzymes (PstI and HpaII) to create a genome representation of the samples. A PstI-RE site-specific adapter is then tagged with 96 different barcodes enabling the multiplexing of a 96-well microtiter plate with equimolar amounts of amplification products to run in an Illumina sequencer Novaseq6000 (Illumina Inc., San Diego, CA). The successfully amplified fragments were sequenced up to 83 bases.

A proprietary analytical pipeline developed by DArT P/L was used to generate allele calls for SNP and SilicoDArT (presence/absence variation markers) [36]. A 100K consensus map [37] was used to obtain genetic positions of the SNPs. To obtain the physical positions, sequence reads were aligned to the reference genome of Chinese Spring (CS) IWGSC RefSeq v1.0 [38], the reference genome of DW cv. Svevo Ref Seq Rel. 1.0 [39] and the reference genome of *Ae. tauschii* (v.4, 2017) [40].

A total of 67,436 DArTSeq SNP markers were originally scored, out of which 50% (34,790) were aligned to the reference genomes. Filtering was carried out excluding SNP with <0.05 allele frequency and >20% missing data points. Finally, 5,800 DArTSeq markers were

retained and used for GWAS analysis. The allele substitution effects for the significant marker-trait association were estimated by the mean phenotypic differences of alleles assuming that one genotype has effects equal to zero. Marker sequences were re-aligned (BLASTn) to the diverse reference sequences using the Emsembl plant public website (https://plants.ensembl.org/) to verify the position of the SNPs.

1.5.4. Statistical analysis and Genome-Wide Association Analysis

For the disease data, statistical analyses were performed using the Statistical Analysis System version 9.1 [41]. Analyses of variance (ANOVA) were conducted on the average reactions of the SHW, the DW parents and checks for tan spot. The Best Linear Unbiased Estimates (BLUE) were computed for each of the 443 SHW genotypes.

The Best Linear Unbiased Estimates (BLUE) for disease severity was used as an input to conduct GWAS using the TASSEL (Trait Analysis by Association Evolution and Linkage) software ver. 5 [42]. We used the mixed linear model (MLM) of [43] to simultaneously include the level of relatedness based on marker data and identical by descent (IBD) computed from the coefficient of parentage, which controls population structure. Additionally, population structure was controlled by fitting the first three principal components (PC) from the kinship matrix taken as the fixed variate and the coefficient of parentage (COP) as the random variable. The false-discovery rate (FDR) was used to assess the significance of the *p* value (<0.05). The allelic effects of the significant marker-trait associations were estimated as the difference between the mean value of lines, with and without the favorable alleles, and was presented as box plots.

The results of the GWAS from MLM are presented in the Manhattan plots and the corresponding QQ-plots are displayed to compare the quantiles of the empirical distribution of the results obtained in this study with those of the distribution that we would expect theoretically if the null hypothesis is true.

1.6. CONCLUSIONS

Our research identified new sources of resistance to tan spot in CIMMYT's SHW that can be used in wheat breeding via crosses and backcrosses with elite bread wheat lines. A total of 30 significant marker-trait associations were found on chromosomes 1B, 1D, 2A, 2D, 3A, 3D, 4B, 4D, 5A, 6A,

6B, and 7D, of which some SNP markers clustered and likely represent single QTL. Several the MTA found in this study can contribute to the genetic diversity of resistance, specifically those on D genome contributed by *Ae. tauschii*, which were almost all novel, but also several on the A- and B-genomes. Furthermore, our study supports the previous concept of possible inter-locus effects caused by the activation of resistance genes in the DW genomes by interaction with the D genome of *Ae. tauschii* after hybridization.

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Table 1. Reaction to tan spot in 40 durum wheat (DW) parents and their respective synthetic hexaploid wheat (SHW) progeny groups. Reactions are defined as Resistant (R, 1.0-1.5), Moderately Resistant (MR, 1.6-2.5), Moderately Susceptible (MS, 2.6-3.5), and Susceptible (S, 3.6-5.0).

	DW parents		SHW		
Pedigree	Tan spot scores	Reaction type	Number of progeny (*Ae. tauschii)*	Mean tan spot scores	Mean reaction type
BOTNO	4.3	S	1	2.2	MR
SCAUP	3.9	S	3	2.2	MR
CROC_1	3.7	S	30	1.7	MR
D67.2/PARANA 66.270	3.7	S	13	1.7	MR
YAR	3.7	S	4	1.4	R
68.111/RGB-U//WARD RESEL/3/STIL	3.6	S	31	1.5	R
DECOY 1	3.5	MS	30	2.1	MR
SORA	3.4	MS	14	1.6	MR
6973/WARD.7463//74110	3.3	MS	3	1.6	MR
CPI8/GEDIZ/3/GOO//ALB/CRA	3.3	MS	31	1.9	MR
LCK59.61	3.2	MS	2	2.3	MR
68.111/RGB-U//WARD	3.1	MS	7	1.6	MR
CHEN_7	3.0	MS	1	1.2	R
ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE	2.9	MS	3	2	MR
YAV_2/TEZ	2.9	MS	12	1.6	MR
LOCAL RED	2.9	MS	7	2.2	MR
TK SN1081	2.9	MS	3	1.2	R
YARMUK	2.8	MS	4	1.7	MR
ROK/KML	2.7	MS	4	2.2	MR
STY,DR/CELTA//PALS/3/SRN_5	2.7	MS	2	1.5	R
ALTAR 84	2.6	MS	20	1.6	MR
ACONCHI 89	2.6	MS	4	1.5	R

DVERD_2	2.5	MR	13	1.5	R
FGO/USA2111	2.5	MR	1	1.1	R
ARLIN_1	2.4	MR	13	1.5	R
68.111/RGB-U//WARD/3/FGO/4/RABI	2.4	MR	31	1.5	R
SCOT/MEXI_1	2.4	MR	1	1.8	MR
GARZA/BOY	2.3	MR	7	1.8	MR
68112/WARD	2.3	MR	4	1.2	R
LARU	2.3	MR	4	1.1	R
RASCON_37	2.2	MR	2	1.3	R
KAPUDE_1	2.1	MR	1	1.9	MR
CERCETA	1.9	MR	54	1.6	MR
RABI//GS/CRA	1.6	MR	4	1.5	R
SNIPE/YAV79//DACK/TEAL	1.5	R	7	1.1	R
FALCIN_1	1.5	R	5	1.9	MR
SHAG_22	1.5	R	6	1.5	R
GREEN_3	1.2	R	1	1	R
GAN	1.1	R	39	1.4	R
SCOOP_1	1.1	R	3	1	R
Erik (Resistant check)	1.0	R	---	1	R
Glenlea (Susceptible check)	4.8	S	---	4.8	S
6B-662 (Moderately resistant check)	2.0	MR	---	2.50	MR
6B-365 (Moderately susceptible check)	3.1	MS	---	3.30	MS

Table 2. Significant markers associated with seedling resistance to tan spot detected with the consensus genetic maps**.** Allele ID, genetic position in cM, F statistics, Probability (Prob), Marker R^2, -log10Pvalue and the effect of allele substitution are given for each marker

Chr	Marker ID	Allele ID	Genetic position on consensus map (cM)	F statistics	Prob.	Marker R^2	-log10 Pvalue	Effect of allele substitution (genotype effect)
1B	987556	987556|F|0-61:G>A-61:G>A	60.43	8.36	2.78E-04	0.042	3.56	-0.22
1B	6045377	6045377|F|0-16:T>C-16:T>C	51.29	8.06	3.71E-04	0.040	3.43	-0.10
1B	1089962	1089962|F|0-56:C>T-56:C>T	83.57	7.21	8.40E-04	0.036	3.08	-0.19
2A	1070935	1070935|F|0-45:G>A-45:G>A	68.84	7.48	6.46E-04	0.038	3.19	-0.28
4A	4993454	4993454|F|0-12:T>C-12:T>C	10.72	8.20	3.24E-04	0.041	3.49	-0.55
5A	1200982	1200982|F|0-30:C>G-30:C>G	47.79	7.68	5.36E-04	0.038	3.27	0.05
5A	4393896	4393896|F|0-34:T>C-34:T>C	48.67	7.21	8.43E-04	0.036	3.07	-0.20
5B	100034112	100034112|F|0-10:C>T-10:C>T	39.26	7.80	4.77E-04	0.039	3.32	-0.14
6A	1862737	1862737|F|0-44:C>G-44:C>G	90.36	9.15	1.30E-04	0.046	3.89	-0.20
6A	100027398	100027398|F|0-42:A>G-42:A>G	77.32	8.21	3.20E-04	0.041	3.49	-0.15
6A	5331622	5331622|F|0-5:A>G-5:A>G	98.51	8.05	3.72E-04	0.040	3.43	-0.12
6A	1254459	1254459|F|0-8:A>C-8:A>C	94.09	7.35	7.36E-04	0.037	3.13	-0.22
6A	4993056	4993056|F|0-26:A>T-26:A>T	91.17	7.18	8.68E-04	0.036	3.06	-0.23
6B	1019955	1019955|F|0-55:A>G-55:A>G	46.69	8.82	1.79E-04	0.044	3.75	-0.44
7D	991140	991140|F|0-11:G>C-11:G>C	153.02	10.19	4.84E-05	0.051	4.31	-0.15
7D	993425	993425|F|0-28:A>G-28:A>G	168.74	8.35	2.81E-04	0.041	3.55	0.59

Table 3. Significant markers for seedling resistance to tan spot detected with the physical map based on the Chinese spring reference genome (RefSeqV.1.0). Allele ID, physical position in CS, F statistics, Probability (Prob), Marker R^2, -log10Pvalue and the effect of allele substitution

Chr	Marker	Allele ID	Pos	F statistic	Prob.	Marker R^2	-log10 Pvalue	Effect of allele substitution (genotype effect)
1B	1089962	1089962|F|0-56:C>T-56:C>T	340462174	7.37	7.23E-04	0.037	3.14	-0.19
1D	3026113	3026113|F|0-19:G>T-19:G>T	375647840	7.92	4.22E-04	0.040	3.37	0.16
2A	1070935	1070935|F|0-45:G>A-45:G>A	525822786	7.99	3.97E-04	0.040	3.40	-0.29
3A	1019955	1019955|F|0-55:A>G-55:A>G	474447292	9.28	1.16E-04	0.046	3.94	-0.44
3A	1668224	1668224|F|0-18:T>C-18:T>C	468520788	7.03	1.00E-03	0.035	3.00	-0.24
3D	1125862	1125862|F|0-8:C>A-8:C>A	603632716	8.86	1.72E-04	0.044	3.76	-0.13
3D	1217411	1217411|F|0-6:C>T-6:C>T	610566593	8.06	3.71E-04	0.040	3.43	-0.21
3D	987556	987556|F|0-61:G>A-61:G>A	288544777	7.88	4.41E-04	0.039	3.36	-0.21
4D	4993454	4993454|F|0-12:T>C-12:T>C	449396486	8.57	2.26E-04	0.043	3.64	-0.56
5A	100034112	100034112|F|0-10:C>T-10:C>T	471723681	8.21	3.20E-04	0.041	3.50	-0.15
5A	1200982	1200982|F|0-30:C>G-30:C>G	454770585	7.28	7.83E-04	0.036	3.11	-0.05
6A	100027398	100027398|F|0-42:A>G-42:A>G	601233092	8.92	1.62E-04	0.045	3.79	-0.15
6A	1254459	1254459|F|0-8:A>C-8:A>C	602989232	8.23	3.15E-04	0.041	3.50	-0.23
6A	2266481	2266481|F|0-54:C>T-54:C>T	602745555	7.19	8.56E-04	0.036	3.07	-0.21
6B	1862737	1862737|F|0-44:C>G-44:C>G	689032602	9.46	9.65E-05	0.047	4.02	-0.20
6B	1112961	1112961|F|0-43:G>A-43:G>A	62173247	7.44	6.75E-04	0.037	3.17	-0.13
7D	16793126	16793126|F|0-15:G>T-15:G>T	161842641	9.59	8.59E-05	0.048	4.07	0.05
7D	993425	993425|F|0-28:A>G-28:A>G	620252466	8.28	3.00E-04	0.041	3.52	0.61

Table 4. Significant markers associated with seedling resistance to tan spot based on durum wheat (cv. Svevo) and *Ae. tauchii* reference genomes. Allele ID, physical positions, F-statistics, Probability (Prob), Marker R^2, log10 p-value and the effect of allele substitution are given for each marker

Chr	Marker	Allele ID	Position	F- statistic	Prob.	Marker R^2	-log10 *p*-value	Effect of allele substitution (genotype effect)
1B	1106306	1106306|F|0-31:A>G-31:A>G	18733634	9.04	1.45E-04	0.045	3.84	-0.24
1B	1089962	1089962|F|0-56:C>T-56:C>T	333205076	8.01	3.89E-04	0.040	3.41	-0.20
1B	3026113	3026113|F|0-19:G>T-19:G>T	493514948	7.86	4.47E-04	0.039	3.35	0.16
1B	4909460	4909460|F|0-15:T>C-15:T>C	551136407	7.33	7.45E-04	0.037	3.13	-0.17
2D	1046601	1046601|F|0-37:C>G-37:C>G	543349511	7.33	7.47E-04	0.037	3.13	-0.01
2D	1217245	1217245|F|0-50:G>A-50:G>A	49063764	7.27	7.90E-04	0.036	3.10	-0.15
3A	1065211	1065211|F|0-46:G>A-46:G>A	477078596	7.49	6.43E-04	0.037	3.19	-0.26
3A	1125872	1125872|F|0-29:C>T-29:C>T	141341740	7.14	9.00E-04	0.036	3.05	-0.27
4A	1125862	1125862|F|0-8:C>A-8:C>A	558758715	8.35	2.80E-04	0.042	3.55	-0.14
5A	3064590	3064590|F|0-39:T>A-39:T>A	433029624	7.17	8.76E-04	0.036	3.06	-0.22
6A	100027398	100027398|F|0-42:A>G-42:A>G	597038442	11.53	1.36E-05	0.058	4.87	-0.17
6A	1254459	1254459|F|0-8:A>C-8:A>C	598610204	8.69	2.01E-04	0.043	3.70	-0.23
7D	16793126	16793126|F|0-15:G>T-15:G>T	162738314	9.30	1.13E-04	0.047	3.95	0.05

Table 5. List of potential candidate genes found in regions identified by marker-trait associations for seedling resistance to tan spot based on Consensus Map, Physical Map (Chinese spring Ref Seq_v1.0) and Durum Wheat (cv. Svevo) aligned to *Ae. tauchii*. Information on chromosome (Ch.) marker, genetic position on the consensus map (cM), position on the Chinese Spring RefV.10gene ID (CS), GWAS, *p* value, marker R2 and -log10 *p*-value. Underlined marker ID, Consensus map, and Position (CS) indicate candidate genes.

Ch.	Marker ID	Consensus map (cM)	Position (CS)	Position (Svevo)	Pos (Ae.t.)	Gene (s)	GWAS	*p*-value	Marker R^2	-log10 *p*-value
1B	1106306			1B-18733634		-	Durum-*tauschi*i (phy. pos)	1.45E-04	0.045	3.84
1B	6045377	1B-51.3					Bread wheat (genetic map)	3.71E-04	0.040	3.43
1B	1089962	1B-83.6	1B-340462174	1B-333205076		-	*Aestivum* (genetic map)	8.40E-04	0.036	3.08
						-	*Aestivum* (phy. pos.)	7.23E-04	0.037	3.14
						-	Durum-*tauschii* (phy. pos)	3.89E-04	0.040	3.41
1B	4909460		1B-558561647	1B-551136407		-	Durum-*tauschii* (phy. pos)	7.45E-04	0.037	3.13
1D	3026113		1D-375647840		1D-381593800	-	*Aestivum* (phy. pos.)	4.22E-04	0.040	3.37
						AET1Gv20669700	Durum-*tauschii* (phy. pos)	4.47E-04	0.039	3.35
2A	1070935	2A-68.8					*Aestivum* (genetic map)	6.46E-04	0.038	3.19
			2A-525822786	2A-519747584		-	Aestivum (phy. pos.)	3.97E-04	0.040	3.40
2D	1217245		2D-48123061		2D-49063764	-	Durum-*tauschii* (phy. pos)	7.90E-04	0.036	3.10
2D	1046601		2D-544685083		2D-543349511	TraesCS2D02G432700	Durum-*tauschi*i (phy. pos)	7.47E-04	0.037	3.13
3A	1125872		3A-135590641	3A-141341769		-	Durum-*tauschii* (phy. pos)	9.00E-04	0.036	3.05
3A	1668224		3A-468520788	3A-471432162		-	*Aestivum* (phy. pos.)	1.00E-03	0.035	3.00
3A or 6B	1019955	6B-46.7*	3A-474447292, 6B-665557108	3A-477078694			*Aestivum* (genetic map)	1.79E-04	0.044	3.75
						-	*Aestivum* (phy. pos.)	1.16E-04	0.046	3.94
3A	1065211		3A-474447226	3A-477078596		-	Durum-*tauschi*i (phy. pos)	6.43E-04	0.037	3.19
3D	987556	1B-60.4*					*Aestivum* (genetic map)	2.78E-04	0.042	3.56
			3D-288544838		3D-295969303	-	*Aestivum* (phy. pos.)	4.41E-04	0.039	3.36
3D	1125862		3D-603632716		3D-614682837	-	*Aestivum* (phy. pos.)	1.72E-04	0.044	3.76
						-	Durum-*tauschii* (phy. pos)	2.80E-04	0.042	3.55
3D	1217411		3D-610566592		3D-622597928	-	*Aestivum* (phy. pos.)	3.71E-04	0.040	3.43
4B or	4993454	4A-10.7*	4B-561892901,	4B-566325530	4D-455660733		*Aestivum* (genetic map)	3.24E-04	0.041	3.49

4D			4D-449396542			-	*Aestivum* (phy. pos.)	2.26E-04	0.043	3.64
5A	4393896	5A-48.7					*Aestivum* (genetic map)	8.43E-04	0.036	3.07
5A	1200982	5A-47.8	5A-454770615	5A-416482338		TraesCS5A02G238600 TRITD5Av1G148960	*Aestivum* (genetic map) *Aestivum* (phy. pos.)	5.36E-04 7.83E-04	0.038 0.036	3.27 3.11
5A	100034112	5B-39.3*	5A-471723681	5A-433814227		- -	*Aestivum* (genetic map) *Aestivum* (phy. pos.)	4.77E-04 3.20E-04	0.039 0.041	3.32 3.50
5A	3064590		5A-470186523	5A:433029663		TraesCS5A02G254500 TRITD5Av1G155700	Durum-*tauschi*i (phy. pos)	8.76E-04	0.036	3.06
6A	1862737	6A-90.4	6A-599622814	6A-595687891		TraesCS6A02G378800, TRITD6Av1G217060	*Aestivum* (genetic map) *Aestivum* (phy. pos.)	1.30E-04 9.65E-05	0.046 0.047	3.89 4.02
6A	100027398	6A-77.3	6A-601233092	6A-597038469		TraesCS6A02G381900 TRITD6Av1G217800	*Aestivum* (genetic map) *Aestivum* (phy. pos.) Durum-*tauschi*i (phy. pos)	3.20E-04 1.62E-04 1.36E-05	0.041 0.045 0.058	3.49 3.79 4.87
6A	1254459	6A-94.1	6A-602989232	6A-598610265		- -	*Aestivum* (genetic map) *Aestivum* (phy. pos.) Durum-*tauschii* (phy. pos)	7.36E-04 3.15E-04 2.01E-04	0.037 0.041 0.043	3.13 3.50 3.70
6A	2266481		6A-602745555	6A-598380242		TraesCS6A02G384200	*Aestivum* (phy. pos.)	8.56E-04	0.036	3.07
6A	4993056	6A-91.2					*Aestivum* (genetic map)	8.68E-04	0.036	3.06
6A	5331622	6A-98.6					*Aestivum* (genetic map)	3.72E-04	0.040	3.43
6B	1112961		6B-62173280	6B-59030547		-	*Aestivum* (phy. pos.)	6.75E-04	0.037	3.17
7D	16793126		7D-161842695		7D-162738368	TraesCS7D02G203900 AET7Gv20511100 AET7Gv20511200	*Aestivum* (phy. pos.) Durum-*tauschii* (phy. pos)	8.59E-05 1.13E-04	0.048 0.047	4.07 3.95
7D	991140	7D-153.0					*Aestivum* (genetic map)	4.84E-05	0.051	4.31
7D	993425	7D-168.7	7D-620252508		7D-625050620	TraesCS7D02G524200 AET7Gv21298500	*Aestivum* (genetic map) *Aestivum* (phy. pos.)	2.81E-04 3.00E-04	0.042 0.041	3.55 3.52

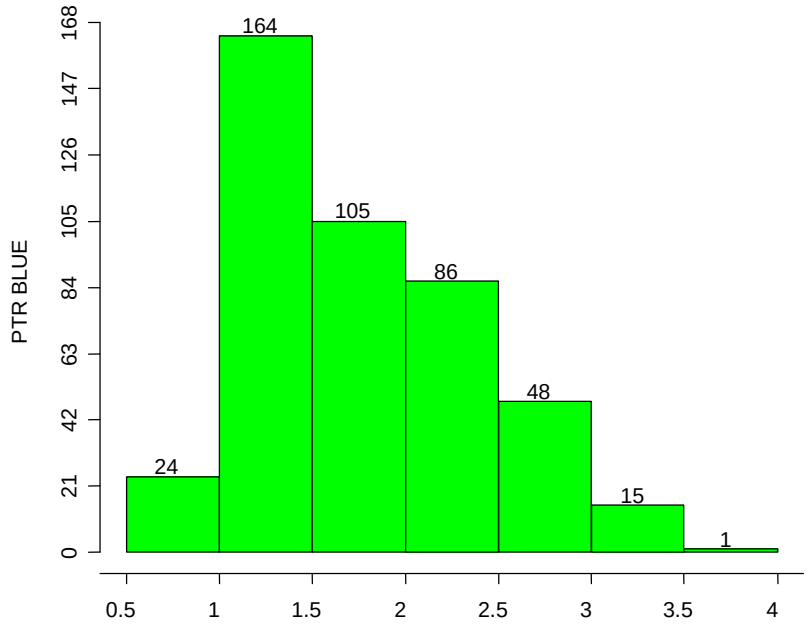


Figure 1. Histograms of tan spot disease scores for different intervals of the diseases are 0.5-1.0, 1.0-1.5, 1.5-2.0, 2.0-2.5, 2.5-3.0, 3.0-3.5, 3.5-4.0.

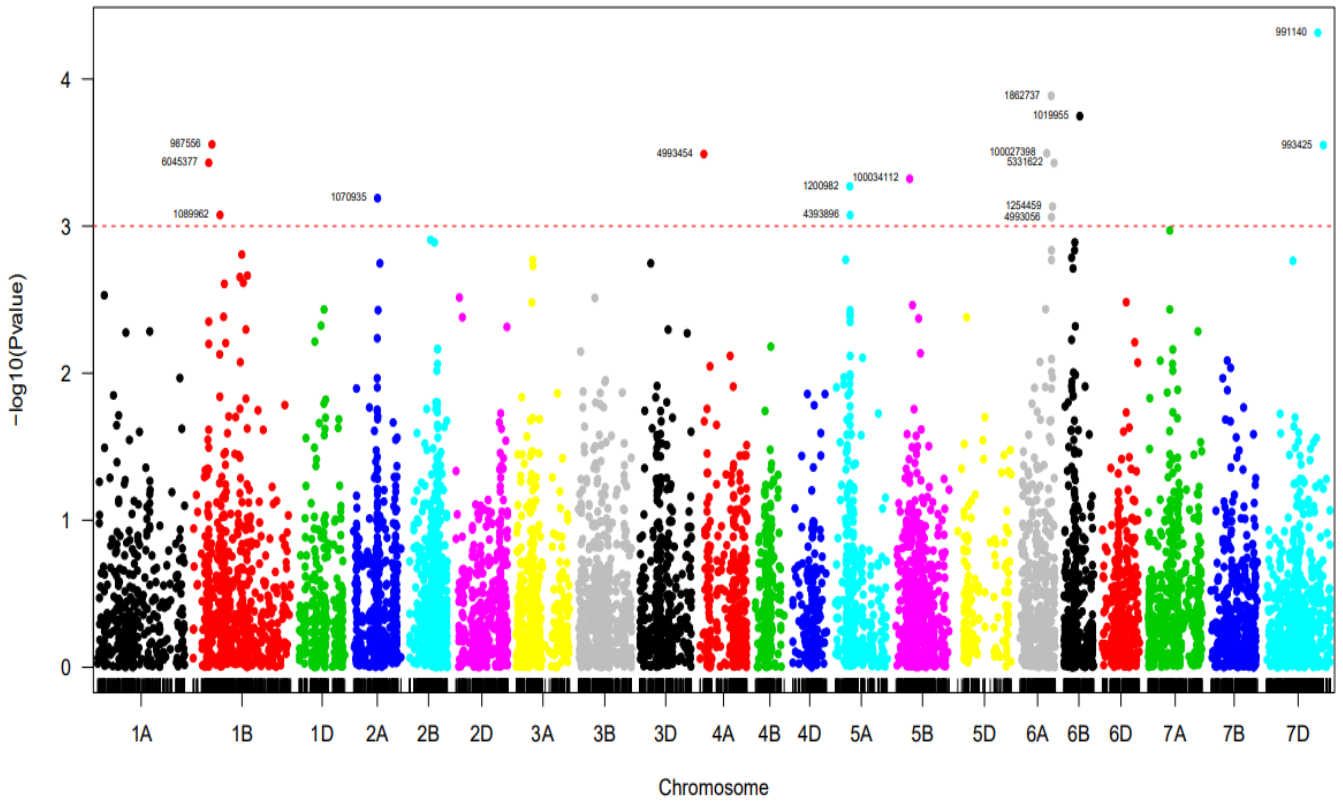


Figure 2A. Manhattan plots for tan spot disease corresponding to the Consensus Map. The *p* values are shown on a $\log_{10}$ scale. The marker is considered significant if $\log_{10}$ scale is 3 or higher.

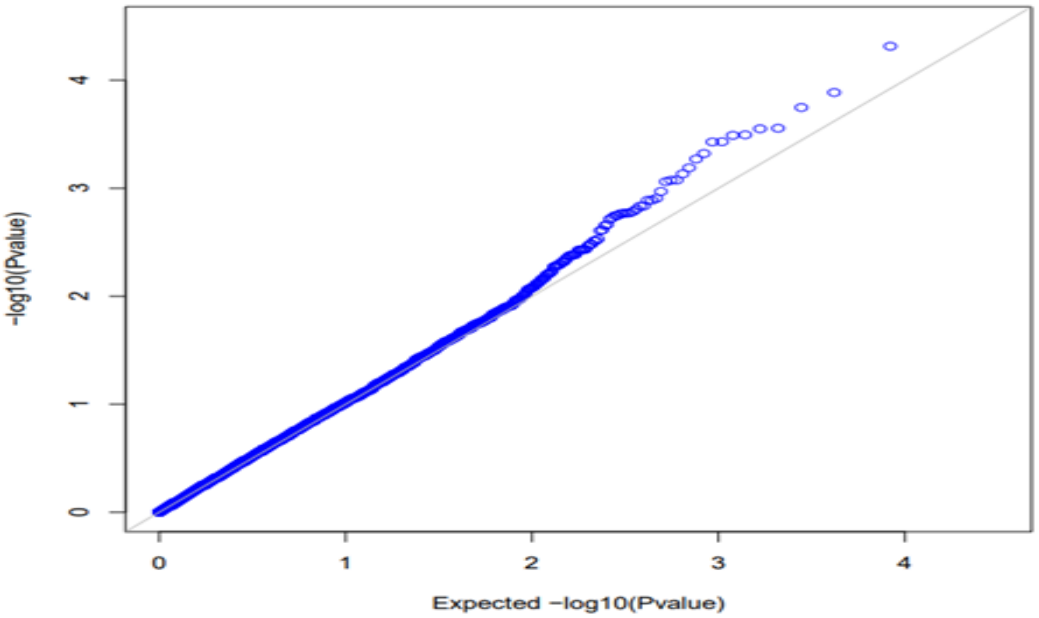


Figure 3B. Consensus Map QQplot.

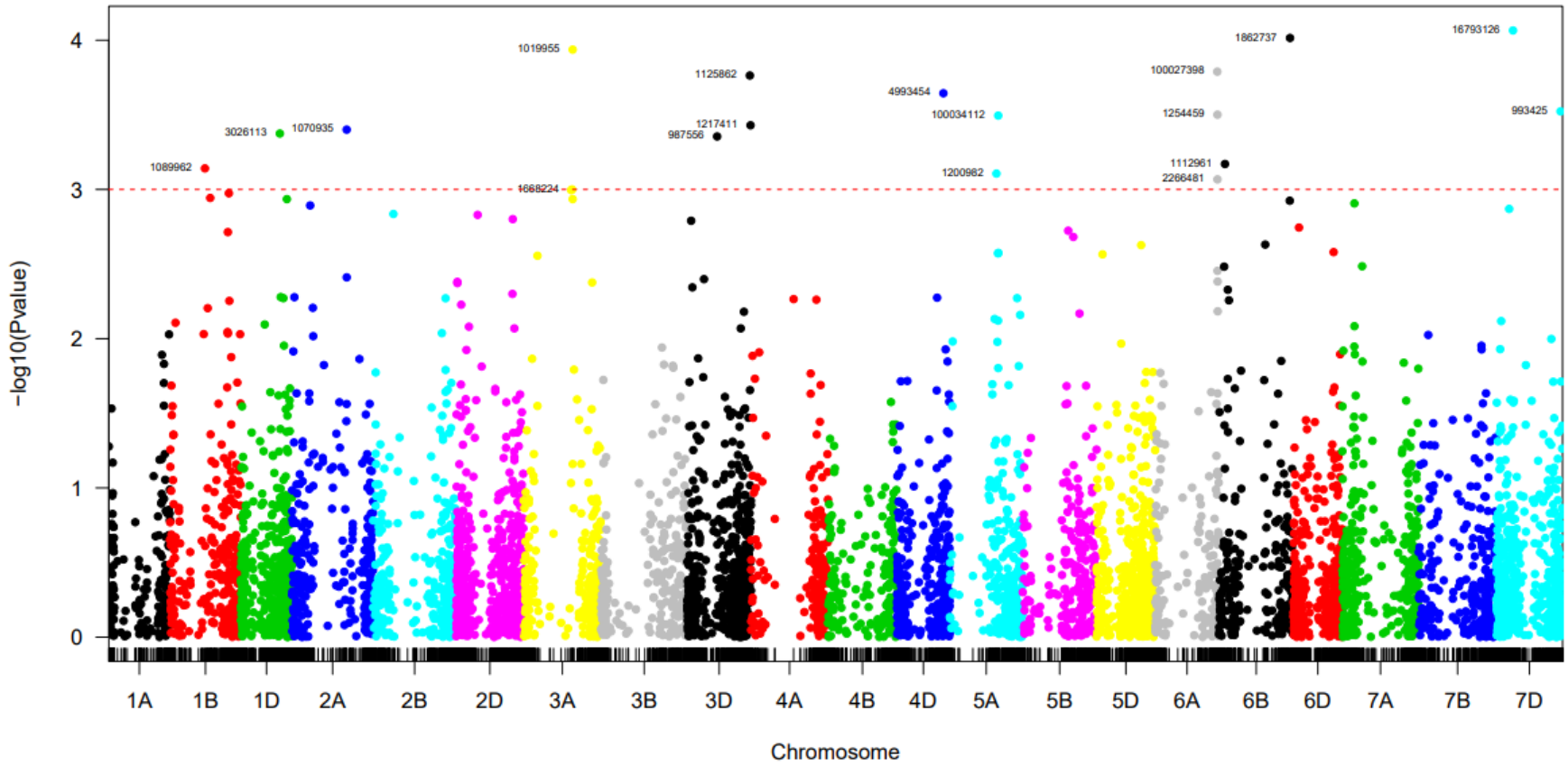


Figure 4A. Manhattan plots for tan spot disease corresponding to the Physical position (Chinese spring Ref Seq ver.1.0). The *p* values are shown on a $\log_{10}$ scale. The marker is considered significant if $\log_{10}$ scale is 3 or higher. Physical position (Chinese spring)

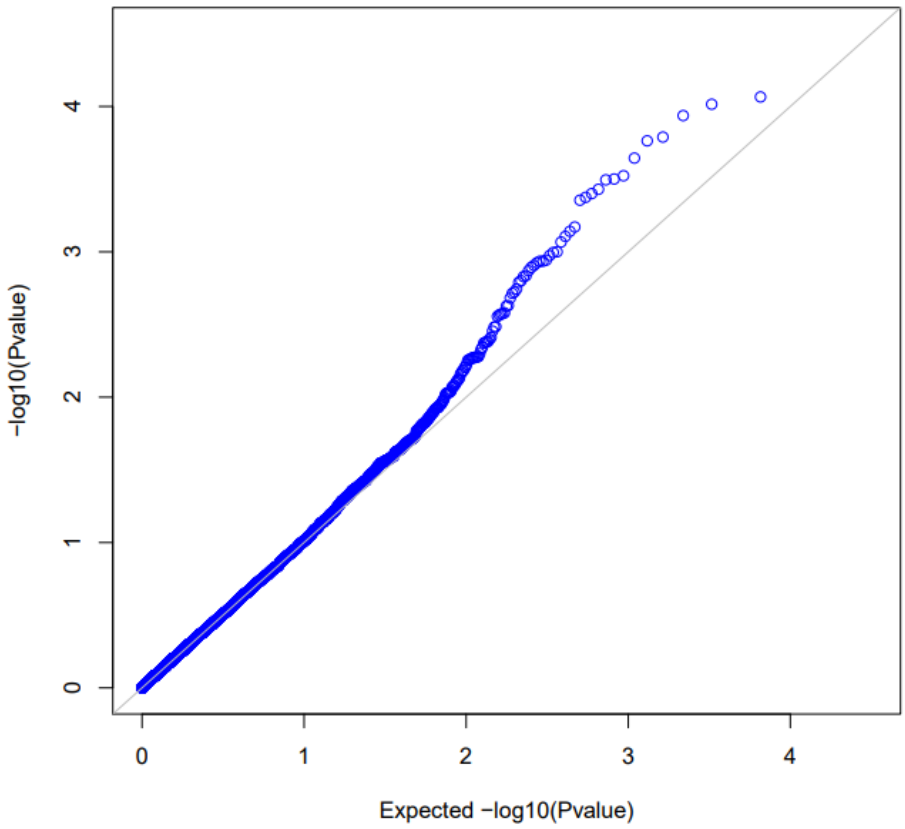


Figure 5B. Physical position (Chinese spring). QQplot

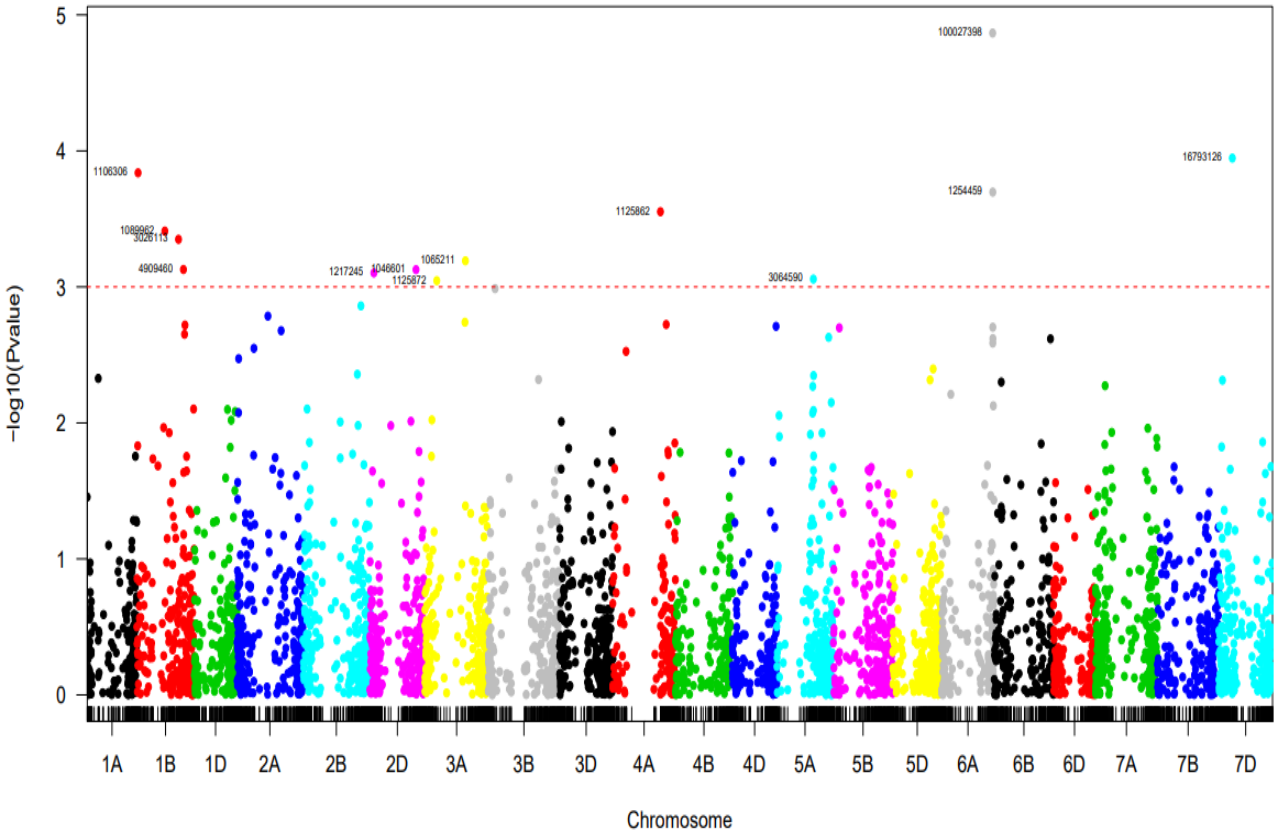


Figure 6A. Manhattan plots for tan spot disease corresponding to the Durum Wheat (cv. Svevo) and *Ae. tauchii* reference genomes (Ref Seq Rel. 1.0). The *p* values are shown on a $\log_{10}$ scale. The marker is considered significant if $\log_{10}$ scale is 3 or higher.

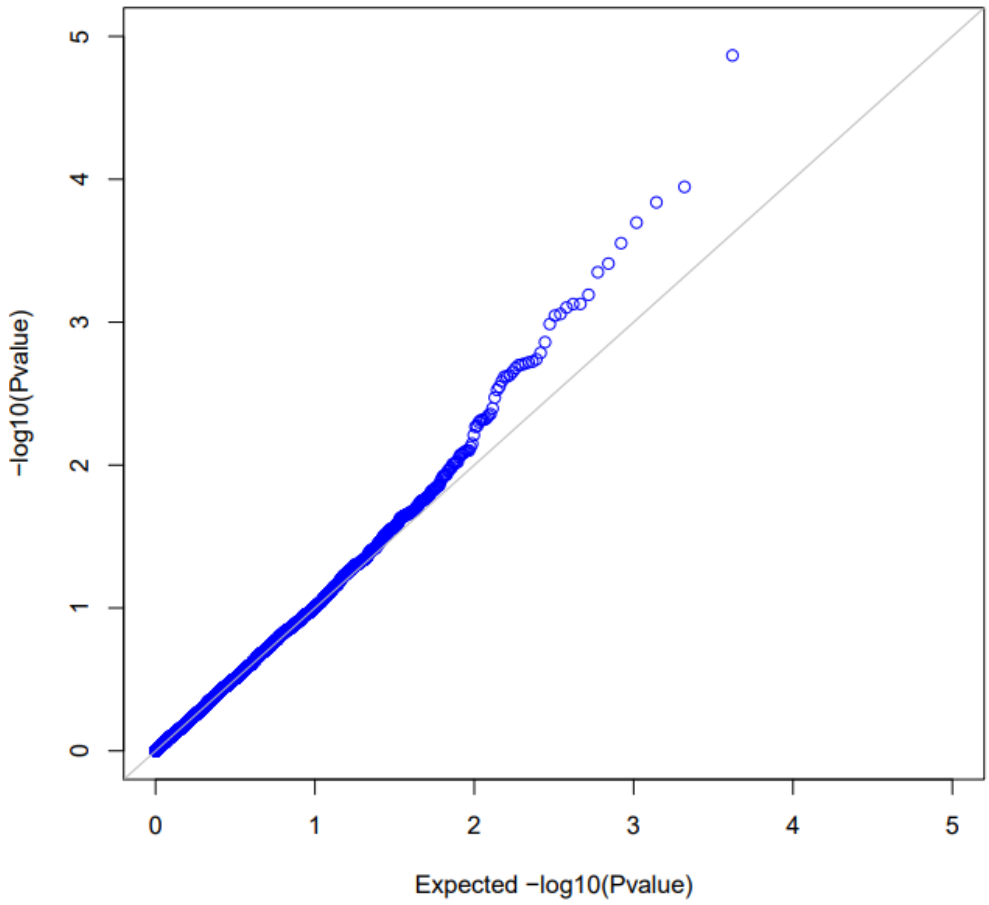


Figure 7B. Durum Wheat (cv. Svevo) and *Ae. tauchii* reference genomes (Ref Seq Rel. 1.0). QQplot

Table S1. Seedling tan spot reaction scores of synthetic hexaploid wheat (SHW) lines and their durum wheat (DW) parents

		Reaction to PTR		
Entry No.	Pedigree	AVG**	Score	Number of progeny
1	**BOTNO***	4.31	S	1
2	BOTNO/AE.SQUARROSA (617)	2.2	MR	--
3	**68.111/RGB-U//WARD RESEL/3/STIL***	3.56	S	31
4	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (332)	1.00	R	--
5	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (1030)	1.08	R	--
6	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (389)	1.14	R	--
7	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (630)	1.16	R	--
8	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (628)	1.17	R	--
9	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (392)	1.19	R	--
10	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (684)	1.21	R	--
11	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (631)	1.22	R	--
12	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (631)	1.23	R	--
13	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (627)	1.23	R	--
14	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (659)	1.24	R	--
15	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (390)	1.25	R	--
16	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (623)	1.28	R	--
17	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (1038)	1.30	R	--
18	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (781)	1.40	R	--

19	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (1029)	1.41	R	--
20	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (675)	1.41	R	--
21	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (385)	1.45	R	--
22	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (386)	1.50	R	--
23	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (625)	1.51	R	--
24	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (768)	1.54	R	--
25	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (783)	1.64	MR	--
26	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (164)	1.75	MR	--
27	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (672)	1.83	MR	--
28	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (681)	1.84	MR	--
29	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (685)	1.85	MR	--
30	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (700)	1.88	MR	--
31	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (662)	1.92	MR	--
32	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (1090)	2.05	MR	--
33	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (188)	2.29	MR	--
34	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (1010)	2.68	MS	--
35	**68.111/RGB-U//WARD***	3.15	MS	7
36	68.111/RGB-U//WARD/3/AE.SQUARROSA (202)	1.75	MR	--
37	68.111/RGB-U//WARD/3/AE.SQUARROSA (426)	1.00	R	--
38	68.111/RGB-U//WARD/3/AE.SQUARROSA (316)	1.39	R	--
39	68.111/RGB-U//WARD/3/AE.SQUARROSA (329)	1.43	R	--
40	68.111/RGB-U//WARD/3/AE.SQUARROSA (322)	1.50	R	--
41	68.111/RGB-U//WARD/3/AE.SQUARROSA (321)	1.73	MR	--
42	68.111/RGB-U//WARD/3/AE.SQUARROSA (511)	2.09	MR	--
43	**68.111/RGB-U//WARD/3/FGO/4/RABI***	2.41	MR	31
44	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (882)	1.03	R	--
45	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	1.08	R	--
46	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (701)	1.08	R	--

47	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (1050)	1.08	R	--
48	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (675)	1.08	R	--
49	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (778)	1.08	R	--
50	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (768)	1.10	R	--
51	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (720)	1.13	R	--
52	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (809)	1.20	R	--
53	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (710)	1.21	R	--
54	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (661)	1.27	R	--
55	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (191)	1.28	R	--
56	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	1.29	R	--
57	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (719)	1.30	R	--
58	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (974)	1.30	R	--
59	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (905)	1.30	R	--
60	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (1093)	1.33	R	--
61	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (788)	1.35	R	--
62	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	1.42	R	--
63	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (504)	1.43	R	--
64	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (809)	1.50	R	--
65	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	1.58	MR	--
66	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (784)	1.59	MR	--
67	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	1.60	MR	--
68	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	1.67	MR	--
69	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (709)	1.84	MR	--
70	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	1.91	MR	--
71	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (809)	1.92	MR	--
72	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (1010)	1.99	MR	--
73	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	2.42	MR	--
74	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	2.61	MS	--

75	**68112/WARD***	2.34	MR	4
76	68112/WARD//AE.SQUARROSA (369)	1.05	R	
77	68112/WARD//AE.SQUARROSA (369)	1.15	R	
78	68112/WARD//AE.SQUARROSA (369)	1.21	R	
79	68112/WARD//AE.SQUARROSA (369)	1.22	R	
80	**6973/WARD.7463//74110***	3.34	MS	3
81	6973/WARD.7463//74110/3/AE.SQUARROSA (665)	1.00	R	
82	6973/WARD.7463//74110/3/AE.SQUARROSA (438)	1.11	R	
83	6973/WARD.7463//74110/3/AE.SQUARROSA (35A)	2.63	MS	
84	**ACONCHI 89***	2.55	MR	4
85	ACO89/AE.SQUARROSA (178)	1.50	R	
86	ACO89/AE.SQUARROSA (309)	1.08	R	
87	ACO89/AE.SQUARROSA (290)	1.48	R	
88	ACO89/AE.SQUARROSA (282)	2.11	MR	
89	**ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE***	2.94	MS	3
90	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/AE.SQUARROSA (389)	2.08	MR	
91	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/AE.SQUARROSA (451)	2.31	MR	
92	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/AE.SQUARROSA (723)	1.73	MR	
93	**ALTAR 84***	2.59	MS	20

94	ALTAR 84/AE.SQUARROSA (1012)	2.06	MR
95	ALTAR 84/AE.SQUARROSA (174)	2.05	MR
96	ALTAR 84/AE.SQUARROSA (188)	1.12	R
97	ALTAR 84/AE.SQUARROSA (191)	1.06	R
98	ALTAR 84/AE.SQUARROSA (198)	1.89	MR
99	ALTAR 84/AE.SQUARROSA (220)	1.44	R
100	ALTAR 84/AE.SQUARROSA (221)	1.11	R
101	ALTAR 84/AE.SQUARROSA (223)	1.39	R
102	ALTAR 84/AE.SQUARROSA (224)	1.37	R
103	ALTAR 84/AE.SQUARROSA (224)	1.22	R
104	ALTAR 84/AE.SQUARROSA (224)	1.04	R
105	ALTAR 84/AE.SQUARROSA (244)	1.69	MR
106	ALTAR 84/AE.SQUARROSA (291)	1.34	R
107	ALTAR 84/AE.SQUARROSA (319)	1.45	R
108	ALTAR 84/AE.SQUARROSA (333)	1.94	MR
109	ALTAR 84/AE.SQUARROSA (507)	2.19	MR
110	ALTAR 84/AE.SQUARROSA (531)	1.52	R
111	ALTAR 84/AE.SQUARROSA (539)	2.52	MR

112	ALTAR 84/AE.SQUARROSA (793)	1.46	R	
113	ALTAR 84/AE.SQUARROSA(Y86-87 S401)	1.28	R	
114	**ARLIN_1***	2.44	MR	13
115	AE.SQUARROSA (1031)/ARLIN_1	1.02	R	
116	ARLIN/AE.SQUARROSA (283)	2.29	MR	
117	ARLIN/AE.SQUARROSA (317)	1.16	R	
118	ARLIN/AE.SQUARROSA (410)	2.65	MS	
119	ARLIN_1/AE.SQUARROSA (1018)	1.07	R	
120	ARLIN_1/AE.SQUARROSA (310)	1.00	R	
121	ARLIN_1/AE.SQUARROSA (320)	1.00	R	
122	ARLIN_1/AE.SQUARROSA (333)	1.53	R	
123	ARLIN_1/AE.SQUARROSA (335)	1.03	R	
124	ARLIN_1/AE.SQUARROSA (368)	1.23	R	
125	ARLIN_1/AE.SQUARROSA (430)	1.06	R	
126	ARLIN_1/AE.SQUARROSA (536)	2.40	MR	
127	ARLIN_1/AE.SQUARROSA (802)	1.59	MR	
128	**CERCETA***	1.85	MR	54
129	CETA/AE.SQUAROOSA (263)	1.65	MR	--
130	CETA/AE.SQUARROSA (1016)	1.66	MR	--
131	CETA/AE.SQUARROSA (1018)	1.22	R	--

132	CETA/AE.SQUARROSA (1026)	1.20	R	--
133	CETA/AE.SQUARROSA (1027)	1.09	R	--
134	CETA/AE.SQUARROSA (1030)	1.24	R	--
135	CETA/AE.SQUARROSA (1031)	1.28	R	--
136	CETA/AE.SQUARROSA (1036)	1.73	MR	--
137	CETA/AE.SQUARROSA (1038)	1.00	R	--
138	CETA/AE.SQUARROSA (1043)	1.88	MR	--
139	CETA/AE.SQUARROSA (1047)	1.70	MR	--
140	CETA/AE.SQUARROSA (1053)	1.87	MR	--
141	CETA/AE.SQUARROSA (1073)	1.69	MR	--
142	CETA/AE.SQUARROSA (1090)	1.72	MR	--
143	CETA/AE.SQUARROSA (166)	1.13	R	--
144	CETA/AE.SQUARROSA (174)	1.47	R	--
145	CETA/AE.SQUARROSA (187)	1.22	R	--
146	CETA/AE.SQUARROSA (230)	1.05	R	--
147	CETA/AE.SQUARROSA (231)	1.79	MR	--
148	CETA/AE.SQUARROSA (244)	2.25	MR	--
149	CETA/AE.SQUARROSA (246)	2.30	MR	--
150	CETA/AE.SQUARROSA (248)	2.88	MS	--
151	CETA/AE.SQUARROSA (262)	1.33	R	--
152	CETA/AE.SQUARROSA (310)	1.03	R	--
153	CETA/AE.SQUARROSA (335)	1.09	R	--
154	CETA/AE.SQUARROSA (356)	1.86	MR	--
155	CETA/AE.SQUARROSA (371)	1.17	R	--
156	CETA/AE.SQUARROSA (391)	1.16	R	--
157	CETA/AE.SQUARROSA (418)	1.08	R	--
158	CETA/AE.SQUARROSA (442)	1.35	R	--
159	CETA/AE.SQUARROSA (445)	2.30	MR	--
160	CETA/AE.SQUARROSA (450)	1.70	MR	

161	CETA/AE.SQUARROSA (485)	1.78	MR
162	CETA/AE.SQUARROSA (496)	1.08	R
163	CETA/AE.SQUARROSA (499)	1.52	R
164	CETA/AE.SQUARROSA (506)	2.42	MR
165	CETA/AE.SQUARROSA (525)	1.86	MR
166	CETA/AE.SQUARROSA (530)	2.43	MR
167	CETA/AE.SQUARROSA (533)	2.66	MS
168	CETA/AE.SQUARROSA (539)	2.46	MR
169	CETA/AE.SQUARROSA (540)	2.48	MR
170	CETA/AE.SQUARROSA (541)	2.72	MS
171	CETA/AE.SQUARROSA (615)	1.52	R
172	CETA/AE.SQUARROSA (629)	1.74	MR
173	CETA/AE.SQUARROSA (681)	1.35	R
174	CETA/AE.SQUARROSA (682)	1.52	R
175	CETA/AE.SQUARROSA (683)	1.51	R
176	CETA/AE.SQUARROSA (684)	1.27	R
177	CETA/AE.SQUARROSA (750)	1.68	MR
178	CETA/AE.SQUARROSA (783)	1.23	R

179	CETA/AE.SQUARROSA (796)	1.53	R	
180	CETA/AE.SQUARROSA (895)	1.35	R	
181	CETA/AE.SQUARROSA (895)	1.11	R	
182	CETA/T.URARTU (557)	1.05	R	
183	**CHEN_7***	3.01	MS	1
184	CHEN_7/AE.SQUARROSA (429)	1.19	R	
185	**CPI8/GEDIZ/3/GOO//ALB/CRA***	3.25	MS	31
186	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (1017)	1.95	MR	
187	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (1018)	1.69	MR	
188	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (1021)	1.78	MR	
189	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (1026)	1.86	MR	
190	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (1029)	1.69	MR	
191	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (1031)	1.83	MR	
192	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (184)	1.69	MR	
193	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (188)	2.49	MR	
194	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (193)	2.60	MS	
195	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (196)	1.81	MR	
196	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (205)	2.14	MR	

197	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)	2.17	MR
198	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (215)	1.64	MR
199	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (227)	2.03	MR
200	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (244)	2.33	MR
201	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (273)	2.42	MR
202	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (296)	2.18	MR
203	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (305)	1.64	R
204	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (334)	2.09	MR
205	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (358)	1.35	R
206	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (409)	1.90	MR
207	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (439)	2.15	MR
208	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (461)	1.56	MR
209	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (533)	2.67	MS
210	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (629)	1.75	MR
211	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (633)	1.70	MR
212	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (637)	2.06	MR
213	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (659)	1.52	R
214	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (684)	1.02	R

215	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (698)	1.98	MR	
216	AE.SQUARROSA (1043)/4/CPI8/GEDIZ/3/GOO//ALB/CRA	1.08	R	
217	**CROC_1***	3.74	S	30
218	CROC_1/AE.SQUARROSA (168)	2.33	MR	
219	CROC_1/AE.SQUARROSA (170)	2.11	MR	
220	CROC_1/AE.SQUARROSA (176)	1.63	MR	
221	CROC_1/AE.SQUARROSA (177)	2.86	MS	
222	CROC_1/AE.SQUARROSA (205)	1.60	MR	
223	CROC_1/AE.SQUARROSA (210)	1.74	MR	
224	CROC_1/AE.SQUARROSA (210)	1.67	MR	
225	CROC_1/AE.SQUARROSA (210)	1.65	MR	
226	CROC_1/AE.SQUARROSA (213)	1.46	R	
227	CROC_1/AE.SQUARROSA (215)	1.92	MR	
228	CROC_1/AE.SQUARROSA (224)	1.75	MR	
229	CROC_1/AE.SQUARROSA (224)	1.25	R	
230	CROC_1/AE.SQUARROSA (224)	1.15	R	
231	CROC_1/AE.SQUARROSA (224)	1.08	R	
232	CROC_1/AE.SQUARROSA (229)	2.39	MR	

233	CROC_1/AE.SQUARROSA (239)	1.10	R	
234	CROC_1/AE.SQUARROSA (256)	2.98	MS	
235	CROC_1/AE.SQUARROSA (275)	1.77	MR	
236	CROC_1/AE.SQUARROSA (298)	2.63	MS	
237	CROC_1/AE.SQUARROSA (310)	1.33	R	
238	CROC_1/AE.SQUARROSA (333)	1.06	R	
239	CROC_1/AE.SQUARROSA (397)	1.38	R	
240	CROC_1/AE.SQUARROSA (493)	1.34	R	
241	CROC_1/AE.SQUARROSA (516)	1.08	R	
242	CROC_1/AE.SQUARROSA (517)	1.71	MR	
243	CROC_1/AE.SQUARROSA (518)	2.05	MR	
244	CROC_1/AE.SQUARROSA (662)	1.33	R	
245	CROC_1/AE.SQUARROSA (725)	1.62	MR	
246	CROC_1/AE.SQUARROSA (826)	1.44	R	
247	CROC_1/AE.SQUARROSA (886)	1.00	R	
248	**D67.2/PARANA 66.270***	3.72	S	13
249	D67.2/PARANA 66.270//AE.SQUARROSA (1148)	1.36	R	
250	D67.2/PARANA 66.270//AE.SQUARROSA (211)	1.67	MR	

251	D67.2/PARANA 66.270//AE.SQUARROSA (213)	2.34	MR	
252	D67.2/PARANA 66.270//AE.SQUARROSA (218)	1.12	R	
253	D67.2/PARANA 66.270//AE.SQUARROSA (220)	1.58	MR	
254	D67.2/PARANA 66.270//AE.SQUARROSA (221)	2.00	MR	
255	D67.2/PARANA 66.270//AE.SQUARROSA (222)	2.16	MR	
256	D67.2/PARANA 66.270//AE.SQUARROSA (223)	1.55	R	
257	D67.2/PARANA 66.270//AE.SQUARROSA (246)	2.05	MR	
258	D67.2/PARANA 66.270//AE.SQUARROSA (633)	1.91	MR	
259	D67.2/PARANA 66.270//AE.SQUARROSA (634)	1.73	MR	
260	D67.2/PARANA 66.270//AE.SQUARROSA (657)	1.91	MR	
261	D67.2/PARANA 66.270//AE.SQUARROSA (668)	1.03	R	
262	**DECOY 1***	3.50	MS	30
263	DOY1/AE.SQUARROSA (1016)	2.14	MR	
264	DOY1/AE.SQUARROSA (1018)	2.04	MR	
265	DOY1/AE.SQUARROSA (1024)	2.59	MS	
266	DOY1/AE.SQUARROSA (1026)	1.87	MR	
267	DOY1/AE.SQUARROSA (1029)	2.29	MR	
268	DOY1/AE.SQUARROSA (177)	2.56	MS	

269	DOY1/AE.SQUARROSA (188)	2.35	MR
270	DOY1/AE.SQUARROSA (216)	1.56	MR
271	DOY1/AE.SQUARROSA (255)	3.43	MS
272	DOY1/AE.SQUARROSA (258)	2.29	MR
273	DOY1/AE.SQUARROSA (267)	1.28	R
274	DOY1/AE.SQUARROSA (295)	1.30	R
275	DOY1/AE.SQUARROSA (322)	2.24	MR
276	DOY1/AE.SQUARROSA (334)	1.68	MR
277	DOY1/AE.SQUARROSA (360)	1.59	MR
278	DOY1/AE.SQUARROSA (415)	2.41	MR
279	DOY1/AE.SQUARROSA (428)	2.11	MR
280	DOY1/AE.SQUARROSA (446)	1.63	MR
281	DOY1/AE.SQUARROSA (447)	1.75	MR
282	DOY1/AE.SQUARROSA (488)	2.06	MR
283	DOY1/AE.SQUARROSA (507)	1.83	MR
284	DOY1/AE.SQUARROSA (510)	1.87	MR
285	DOY1/AE.SQUARROSA (515)	2.35	MR
286	DOY1/AE.SQUARROSA (516)	1.43	R

287	DOY1/AE.SQUARROSA (517)	2.78	MS	
288	DOY1/AE.SQUARROSA (532)	2.73	MS	
289	DOY1/AE.SQUARROSA (540)	2.58	MS	
290	DOY1/AE.SQUARROSA (632)	1.48	R	
291	AE.SQUARROSA (1026)/DOY1	2.51	MR	
292	AE.SQUARROSA (1043)/DOY1	1.90	MR	
293	**DVERD_2***	2.54	MR	13
294	DVERD_2/AE.SQUARROSA (1022)	1.32	R	
295	DVERD_2/AE.SQUARROSA (1026)	1.17	R	
296	DVERD_2/AE.SQUARROSA (1029)	1.22	R	
297	DVERD_2/AE.SQUARROSA (1031)	1.27	R	
298	DVERD_2/AE.SQUARROSA (214)	1.52	R	
299	DVERD_2/AE.SQUARROSA (221)	1.28	R	
300	DVERD_2/AE.SQUARROSA (247)	1.43	R	
301	DVERD_2/AE.SQUARROSA (247)	1.20	R	
302	DVERD_2/AE.SQUARROSA (333)	1.20	R	
303	DVERD_2/AE.SQUARROSA (507)	1.76	MR	
304	DVERD_2/T.URARTU (545)	2.92	MS	

305	AE.SQUARROSA (1031)/DVERD_2	1.13	R	
306	AE.SQUARROSA (1029)/DVERD_2	1.77	MR	
307	**FALCIN_1***	1.50	R	5
308	FALCIN/AE.SQUARROSA (312)	1.35	R	
309	FALCIN/AE.SQUARROSA (389)	2.67	MS	
310	FALCIN_1/AE.SQUARROSA (1073)	2.06	MR	
311	FALCIN_1/AE.SQUARROSA (176)	1.78	MR	
312	FALCIN_1/AE.SQUARROSA (197)	1.63	MR	
313	**FGO/USA2111***	2.47	MR	1
314	FGO/USA2111//AE.SQUARROSA (658)	1.08	R	
315	**GAN***	1.06	R	39
316	GAN/AE.SQUARROSA (1080)	1.23	R	
317	GAN/AE.SQUARROSA (163)	1.20	R	
318	GAN/AE.SQUARROSA (180)	1.27	R	
319	GAN/AE.SQUARROSA (182)	1.62	MR	
320	GAN/AE.SQUARROSA (201)	1.92	MR	
321	GAN/AE.SQUARROSA (206)	2.13	MR	
322	GAN/AE.SQUARROSA (231)	2.10	MR	
323	GAN/AE.SQUARROSA (233)	1.14	R	

324	GAN/AE.SQUARROSA (257)	1.02	R
325	GAN/AE.SQUARROSA (264)	1.21	R
326	GAN/AE.SQUARROSA (267)	2.15	MR
327	GAN/AE.SQUARROSA (268)	2.26	MR
328	GAN/AE.SQUARROSA (285)	1.29	R
329	GAN/AE.SQUARROSA (296)	1.24	R
330	GAN/AE.SQUARROSA (300)	1.58	MR
331	GAN/AE.SQUARROSA (335)	1.00	R
332	GAN/AE.SQUARROSA (408)	1.02	R
333	GAN/AE.SQUARROSA (413)	1.11	R
334	GAN/AE.SQUARROSA (446)	2.27	MR
335	GAN/AE.SQUARROSA (459)	1.54	R
336	GAN/AE.SQUARROSA (479)	1.32	R
337	GAN/AE.SQUARROSA (522)	1.86	MR
338	GAN/AE.SQUARROSA (536)	1.87	MR
339	GAN/AE.SQUARROSA (620)	1.28	R
340	GAN/AE.SQUARROSA (621)	1.48	R
341	GAN/AE.SQUARROSA (623)	1.04	R

342	GAN/AE.SQUARROSA (624)	1.06	R	
343	GAN/AE.SQUARROSA (633)	1.00	R	
344	GAN/AE.SQUARROSA (638)	1.02	R	
345	GAN/AE.SQUARROSA (643)	1.38	R	
346	GAN/AE.SQUARROSA (658)	1.08	R	
347	GAN/AE.SQUARROSA (668)	1.23	R	
348	GAN/AE.SQUARROSA (680)	1.35	R	
349	GAN/AE.SQUARROSA (721)	2.29	MR	
350	GAN/AE.SQUARROSA (735)	1.36	R	
351	GAN/AE.SQUARROSA (741)	1.00	R	
352	GAN/AE.SQUARROSA (768)	1.13	R	
353	GAN/AE.SQUARROSA (779)	1.78	MR	
354	GAN/AE.SQUARROSA (890)	1.15	R	
355	**GARZA/BOY***	2.34	MR	7
356	GARZA/BOY//AE.SQUARROSA (271)	1.33	R	
357	GARZA/BOY//AE.SQUARROSA (286)	2.39	MR	
358	GARZA/BOY//AE.SQUARROSA (307)	1.21	R	
359	GARZA/BOY//AE.SQUARROSA (311)	2.26	MR	

360	GARZA/BOY//AE.SQUARROSA (350)	1.26	R	
361	GARZA/BOY//AE.SQUARROSA (439)	2.27	MR	
362	GARZA/BOY//AE.SQUARROSA (764)	1.98	MR	
363	**GREEN_3***	1.19	R	1
364	GREEN/AE.SQUARROSA (458)	1.00	R	
365	**KAPUDE_1***	2.13	MR	1
366	KAPUDE/AE.SQUARROSA (175)	1.88	MR	
367	**LARU***	2.31	MR	4
368	LARU/AE.SQUARROSA (309)	1.00	R	
369	LARU/AE.SQUARROSA (309)	1.00	R	
370	LARU/AE.SQUARROSA (333)	1.18	R	
371	LARU/AE.SQUARROSA (TA2459)	1.41	R	
372	**LCK59.61***	3.18	MS	2
373	LCK59.61/AE.SQUARROSA (308)	1.23	R	
374	LCK59.61/AE.SQUARROSA (783)	3.47	MS	
375	**LOCAL RED***	2.90	MS	7
376	LOCAL RED/AE.SQUARROSA (189)	2.20	MR	
377	LOCAL RED/AE.SQUARROSA (219)	2.64	MS	
378	LOCAL RED/AE.SQUARROSA (220)	1.80	MR	
379	LOCAL RED/AE.SQUARROSA (221)	2.40	MR	

380	LOCAL RED/AE.SQUARROSA (222)	3.04	MS	
381	LOCAL RED/AE.SQUARROSA (223)	2.19	MR	
382	LOCAL RED/AE.SQUARROSA (449)	1.42	R	
383	**RABI//GS/CRA***	1.63	MR	4
384	RABI//GS/CRA/3/AE.SQUARROSA (190)	2.01	MR	
385	RABI//GS/CRA/3/AE.SQUARROSA (457)	1.54	R	
386	RABI//GS/CRA/3/AE.SQUARROSA (891)	1.08	R	
387	RABI//GS/CRA/3/AE.SQUARROSA (904)	1.57	MR	
388	**RASCON_37***	2.18	MR	2
389	RASCON/AE.SQUARROSA (312)	1.08	R	
390	RASCON/AE.SQUARROSA (367)	1.44	R	
391	**ROK/KML***	2.72	MS	4
392	ROK/KML//AE.SQUARROSA (214)	1.65	MR	
393	ROK/KML//AE.SQUARROSA (295)	2.03	MR	
394	ROK/KML//AE.SQUARROSA (333)	2.27	MR	
395	ROK/KML//AE.SQUARROSA (507)	2.70	MS	
396	**SCAUP***	3.85	S	3
397	SCA/AE.SQUARROSA (248)	2.90	MS	
398	SCA/AE.SQUARROSA (409)	1.58	MR	

399	SCA/AE.SQUARROSA (493)	1.98	MR	
400	**SCOOP_1***	1.06	R	3
401	SCOOP_1/AE.SQUARROSA (358)	1.03	R	
402	SCOOP_1/AE.SQUARROSA (407)	1.00	R	
403	SCOOP_1/AE.SQUARROSA (659)	1.00	R	
404	**SCOT/MEXI_1***	2.35	MR	1
405	SCOT/MEXI_1//AE.SQUARROSA (186)	1.84	MR	
406	**SHAG_22***	1.50	R	6
407	SHAG_22/AE.SQUARROSA (1101)	1.20	R	
408	SHAG_22/AE.SQUARROSA (227)	1.59	MR	
409	SHAG_22/AE.SQUARROSA (319)	1.67	MR	
410	SHAG_22/AE.SQUARROSA (530)	1.33	R	
411	SHAG_22/AE.SQUARROSA (537)	1.55	MR	
412	SHAG_22/AE.SQUARROSA (539)	1.47	R	
413	**SNIPE/YAV79//DACK/TEAL***	1.53	R	7
414	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (411)	1.20	R	
415	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (528)	1.02	R	
416	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (628)	1.00	R	
417	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (629)	1.25	R	

418	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (633)	1.03	R	
419	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (700)	1.08	R	
420	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (904)	1.13	R	
421	**SORA***	3.38	MS	14
422	SORA/AE.SQUARROSA (191)	2.68	MS	
423	SORA/AE.SQUARROSA (192)	2.10	MR	
424	SORA/AE.SQUARROSA (192)	1.68	MR	
425	SORA/AE.SQUARROSA (207)	1.72	MR	
426	SORA/AE.SQUARROSA (208)	2.49	MR	
427	SORA/AE.SQUARROSA (211)	1.28	R	
428	SORA/AE.SQUARROSA (215)	1.25	R	
429	SORA/AE.SQUARROSA (323)	1.04	R	
430	SORA/AE.SQUARROSA (442)	1.13	R	
431	SORA/AE.SQUARROSA (469)	1.30	R	
432	SORA/AE.SQUARROSA (617)	1.15	R	
433	SORA/AE.SQUARROSA (625)	1.18	R	
434	SORA/AE.SQUARROSA (684)	1.39	R	
435	SORA/AE.SQUARROSA (939)	2.42	MR	

436	**STY,DR/CELTA//PALS/3/SRN_5***	2.68	MS	2
437	STY,DR/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (277)	1.72	MR	
438	STY,DR/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (502)	1.27	R	
439	**TK SN1081***	2.88	MS	3
440	TK SN1081/AE.SQUARROSA (222)	1.19	R	
441	TK SN1081/AE.SQUARROSA (222)	1.06	R	
442	TK SN1081/AE.SQUARROSA (690)	1.20	R	
443	**YAR***	3.66	S	4
444	YAR/AE.SQUARROSA (493)	1.77	MR	
445	YAR/AE.SQUARROSA (518)	1.33	R	
446	YAR/AE.SQUARROSA (783)	1.48	R	
447	YAR/AE.SQUARROSA (809)	1.04	R	
448	**YAV_2/TEZ***	2.94	MS	12
449	YAV_2/TEZ//AE.SQUARROSA (1093)	1.54	R	
450	YAV_2/TEZ//AE.SQUARROSA (249)	2.55	MR	
451	YAV_2/TEZ//AE.SQUARROSA (249)	2.22	MR	
452	YAV_2/TEZ//AE.SQUARROSA (249)	1.97	MR	
453	YAV_2/TEZ//AE.SQUARROSA (249)	1.82	MR	
454	YAV_2/TEZ//AE.SQUARROSA (249)	1.78	MR	

455	YAV_2/TEZ//AE.SQUARROSA (249)	1.10	R	
456	YAV_2/TEZ//AE.SQUARROSA (435)	1.11	R	
457	YAV_2/TEZ//AE.SQUARROSA (437)	1.13	R	
458	YAV_2/TEZ//AE.SQUARROSA (721)	2.02	MR	
459	YAV_2/TEZ//AE.SQUARROSA (746)	1.03	R	
460	YAV_2/TEZ//AE.SQUARROSA (882)	1.00	R	
461	**YARMUK***	2.79	MS	4
462	YUK/AE.SQUARROSA (217)	1.61	MR	
463	YUK/AE.SQUARROSA (434)	1.10	R	
464	YUK/AE.SQUARROSA (784)	2.08	MR	
465	YUK/AE.SQUARROSA (864)	1.97	MR	
	Lines without durum wheat parents in this study			
466	KUCUK/AE.SQUARROSA (1080)	1.36	R	
467	KUCUK/AE.SQUARROSA (458)	1.03	R	
468	KUCUK/AE.SQUARROSA (640)	1.28	R	
469	DUKEM_12/2*RASCON_21//AE.SQUARROSA (1090)	1.07	R	
470	DUKEM_12/2*RASCON_21//AE.SQUARROSA (1100)	2.33	MR	
471	SRN/AE.SQUARROSA (358)	1.02	R	

472	CADO/BOOMER_33//AE.SQUARROSA (504)	2.18	MR
473	CADO/BOOMER_33//AE.SQUARROSA (651)	1.00	R
474	CADO/BOOMER_33//AE.SQUARROSA (949)	1.15	R
475	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (381)	1.48	R
476	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (397)	1.18	R
477	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (443)	1.19	R
478	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (460)	1.13	R
479	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (460)	1.05	R
480	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (477)	1.63	MR
481	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (477)	1.48	R
482	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (490)	1.20	R
483	BACANORA T 88	1.83	MR
	Check resistant (Erik)	1.00	R
	Check susceptible (Glenlea)	4.80	S
	Check moderately resistant (6B-662)	2.50	MR
	Check moderately susceptible (6B-365)	3.40	MS

* Durum wheat parents.

**Averaged tan spot reaction of each genotype of SHW (twelve replications) and durum wheat parents (eight replications)

Table S2. Candidate genes for significant marker-trait associations identified from *Triticum aestivum* (IWGSC), *Triticum turgidum* (Svevo.v1), *Aegilops tauschii* (Aet_v4.0), and *Triticum dicoccoides* (WEWSeq_v.1.0). Data was obtained from Emsembl https://plants.ensembl.org/

Chromosome	Marker	Gene	Description
1D	3026113	AET1Gv20669700	-
2D	1046601	TraesCS2D02G432700	-
5A	1200982	TraesCS5A02G23860	-
		TRITD5Av1G148960	Galactoside 2-alpha-L-fucosyltransferase
5A	3064590	TraesCS5A02G254500	-
		TRITD5Av1G155700	F-box family protein
6A	1862737	TraesCS6A02G378800,	-
		TRITD6Av1G217060	Cytochrome P450
6A	100027398	TraesCS6A02G381900	-
		TRITD6Av1G217800	F-box protein PP2
6A	2266481	TraesCS6A02G384200	-
7D	16793126	TraesCS7D02G203900	-
		AET7Gv20511100	-
		AET7Gv20511200	-
7D	993425	TraesCS7D02G524200	-
		AET7Gv21298500	-

CHAPTER 2. GENOME-WIDE ASSOCIATION STUDY FOR SPOT BLOTCH RESISTANCE IN SYNTHETIC HEXAPLOID WHEAT[1]

2.1. ABSTRACT

Spot blotch (SB) caused by *Bipolaris sorokiniana* (Sacc.) Shoem is a destructive fungal disease affecting wheat and many other crops. Synthetic hexaploid wheat (SHW) offers opportunities to explore new resistance genes for SB for introgression into elite bread wheat. The objectives of our study were to evaluate a collection of 441 SHWs for resistance to SB and to identify potential new genomic regions associated with the disease. The panel exhibited high SB resistance, with 250 accessions showing resistance and 161 showing moderate resistance reactions. A genome-wide association study (GWAS) revealed a total of 41 significant marker–trait associations for resistance to SB, being located on chromosomes 1B, 1D, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 4D, 5A, 5D, 6D, 7A, and 7D; yet none of them exhibited a major phenotypic effect. In addition, a partial least squares regression was conducted to validate the marker–trait associations, and 15 markers were found to be most important for SB resistance in the panel. To our knowledge, this is the first GWAS to investigate SB resistance in SHW that identified markers and resistant SHW lines to be utilized in wheat breeding.

Keywords: foliar disease; spot blotch; genome-wide association study; synthetic hexaploid wheat; partial least squares regression.

2.2. INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most widely consumed food grain in the world. Global wheat production must therefore increase to meet the growing demand estimated for the next three decades [1]. It will be paramount to combine climate resilience, yield potential, and disease resistance in single wheat genotypes which could be grown across diverse environments. Known challenges that limit increased production rates are rapid climate change and emergence of new pathogenic variants. Foliar diseases in particular, have become increasingly relevant for wheat in recent years, leading to significant losses in grain yield and quality [2]. Some of the factors driving

[1] Nerida Lozano-Ramirez, Susanne Dreisigacker, Carolina P. Sansaloni, Xinyao He, José Sergio Sandoval-Islas, Paulino Pérez-Rodríguez, Aquiles Carballo Carballo, Cristian Nava Diaz, Masahiro Kishii and Pawan K. Singh. Genes 2022, 13, 1387. https://doi.org/10.3390/genes13081387

foliar diseases are the commercial cultivation of susceptible varieties, the rapid evolution of causal pathogens, climate change, and unfavorable agricultural practices, which often lead to severe disease epidemics. About 21.5% of the global wheat production is lost each year to diseases [2], the majority of the losses attributed to fungal pathogens infecting multiple wheat organs such as root, stem, leaf, spike, and grain.

Spot blotch (SB) is caused by the fungus *Bipolaris sorokiniana* (Sacc.) Shoem syn. *Drechslera sorokiniana* (Sacc.) Subrm and Jain (syn. *Helminthosporium sativum*, teleomorph *Cochliobolus sativus*) and is considered one of the most destructive fungal diseases in humid and high temperature regions; they not only affect wheat, but also several other small grains worldwide such as barley, rye, and triticale [3–9]. The SB pathogen can infect all plant organs, but particularly leaves and grain; thus, reducing plant photosynthetic efficiency and grain quality. SB has a wide range of hosts among wild and cultivated Poaceae species [10–12]. SB symptoms are characterized by light to dark brown lesions on leaves, oval to elongated in shape [13], that extend and merge very quickly, resulting in tissue death. The importance of SB in production losses has been widely documented. On average, yield loss of 15–20% due to SB has been reported in several countries under favorable climate conditions, yet the yield losses can reach up to 70% in susceptible varieties [14–16]. The growing threat of SB due to rising global temperatures and the accelerated evolution of pathogenic races have recently caught the attention of plant breeders and pathologists and created a sense of urgency for the identification of new sources of SB resistance. The commercial cultivation of SB-resistant varieties is the most sustainable and cost effective strategy to manage the losses incurred by SB [17–19]. Cultivar development for resistance to SB is slow due to the quantitative nature of resistance and a limited number of genes are known to have a major effect. Four SB resistance genes with major effects have been named to date, i.e., Sb1 through Sb4 [20–23]. Furthermore, several QTLs with minor effects have been found on almost all wheat chromosomes [24–27]. Most gene discovery studies undertaken to date have used biparental mapping populations, while a genome-wide association study (GWAS) using historical recombination usually provides a better resolution than bi-parental mapping. GWAS for resistance to SB found minor QTLs on chromosomes 2D, 3A, 4A, 4B, 5A, and 7B [28]; 1A, 1B, 1D, 4A, 5A, 5B, 6A, 6B, 6D, 7A, 7B [29]; and 1B, 3B 7B and 7D [30]. Recently, Bainsla *et al*. [31] found 25 marker– trait associations (MTAs) on 13 chromosomes explaining between 2.0 and 17.7% of the phenotypic variance. Tomar *et al*. [32] reported four new QTLs for resistance to SB in spring

wheat on chromosomes 1A, 1D, 2B, and 6D. Most of the studies for resistance to SB concentrated on spring wheat, and only a few focused on winter wheat germplasm. To identify novel and more effective sources, synthetic hexaploid wheat (SHW) (2n = 6x = 42; AABBDD), derived from a cross between *Triticum turgidum* L. (2n = 4x = 28; AABB) and *Aegilops tauschii* syn. *Ae. squarossa* (2n = 2x = 14; DD), could be an alternative source of resistance to SB as envisaged from other studies [33,34]. Previously, considerable levels of genetic variation were already recorded among SHW developed by the Wide Crosses Program of the International Maize and Wheat Improvement Center (CIMMYT) for different agronomic traits, disease resistance, and quality [33,35–37]. SHW was found to be promising in terms of resistance to SB and a few SHW lines showed better resistance than the resistant check variety 'Mayoor' [38]. Spot blotch is a major limiting factor for bread wheat production in hot and humid regions, particularly the Indo-Gangetic plains of South Asia. Despite the extensive breeding efforts, effective resistance to SB has not been observed in released cultivars, and the most promising cultivars have been found to be only partially resistant. Numerous studies have indicated that resistance to SB is polygenic, and multiple QTLs have been reported [24,26]. In CIMMYT, four biparental bread wheat populations were recently tested for SB resistance under Mexican environments, where several QTLs with minor effects were identified [24,25]. The same populations were further evaluated in South Asia with similar results, all QTLs presenting minor effects [26,27]. However, to our knowledge, no large-scale systematic screening and genetic study for SB resistance have been performed yet on SHW. Therefore, the objectives of this study were to (1) evaluate a set of 441 primary SHW lines for SB resistance under controlled environmental conditions and (2) to apply GWAS to identify potential new genomic regions of resistance that are not yet present in elite bread wheat germplasm.

2.3. MATERIALS AND METHODS

2.3.1. Plant Material

A total of 441 SHW lines, generated by the CIMMYT's Wide Crosses Program via hybridizing 40 durum wheat (DW) parents and 277 *Ae. tauschii* accessions, were used in this study. The DW parents were involved in 1–54 crosses and the *Ae. tauschii* accessions were used in 1–7 crosses (Supplementary Table S1). The SHWs were selected from a larger collection of 1524 SHWs for their resistance to diseases such as Fusarium head blight, Septoria tritici blotch, rusts, and have acceptable agronomic traits such as plant height and days to heading [34].

2.3.2. Phenotypic Evaluations of Spot Blotch.

The disease screening was carried out in a greenhouse at CIMMYT, El Batán, Mexico (19◦310 N, 98◦500 W, elevation 2249 m above sea level) during 2018 and 2019. All 441 SHWs, along with the 40 DW parents and four checks including Chirya 3 (resistant), Sonalika and Ciano T79 (susceptible) and Francolin (moderately susceptible) were evaluated for SB resistance at the seedling stage, while the *Ae. tauschii* accessions could not be screened due to their nature and growth as a wild species. The seeds of SHW lines were vernalized to break down seed dormancy and to obtain an even germination. Experiments were planned in a randomized complete block design with six replicates for the SHW and eight replicates for the DW parents, with four plants per entry—grown in plastic containers as experimental units to obtain average values for their subsequent analysis. The size of the containers was 26.5 cm long, 20.5 cm wide, and 5 cm high. The seedlings were grown under controlled conditions with an ambient temperature of 22–25/16–18 ◦C (day/night) and with a 16 h photoperiod. For disease expression, the isolate CIMFU 483 of Mexican *Bipolaris sorokiniana* (BSG40M2), a monosporic strain isolated from wheat collected in Agua Fria, Mexico, was used. This isolate is a ToxA producer, which was confirmed based on inoculation experiments with differential genotypes, infiltration experiments, and PCR with the ToxA1/ToxA2 primers. The isolate was grown in a 30% V8 media [39], and the conidia concentration for inoculation was adjusted to 7500 spores mL−1 using a Neubauer counting chamber. One drop of Tween 20 (a surfactant reagent) was added for every 100 mL of spore suspension. Seedlings were inoculated at the two-leaf stage, when the second leaf was fully expanded, or two weeks after sowing. The seedlings were inoculated with a conidial suspension of the CIMFU 483 isolate until the leaves were at dew point. This inoculum was sprayed four times every 20–30 min using a hand sprayer. After the leaves dried, the trays were moved to a mist chamber (RH 100%, 22–24 ◦C) to promote infection. After 48 h, the plants were transferred back to the greenhouse bench. Seedling response was evaluated seven days post inoculation following the 1–5 ordinal lesion rating scale developed by Lamari and Bernier [40], which is based on the lesion type shown on the second leaf. The genotypes were grouped based on the mean score of replicates following 1.0–1.5 = Resistant (R); 1.6–2.5 = Moderately Resistant (MR); 2.6–3.5 = Moderately Susceptible (MS); and 3.6–5.0 = Susceptible (S).

2.3.3. Genotyping.

Genomic DNA was extracted from the second leaf (0.25 mg per entry) of 10-day-old seedlings of each line of the SHW using the modified cetyl trimethyl ammonium bromide (CTAB) method described in the CIMMYT laboratory protocols [41]. The high-throughput genotyping method DArTseqTM [42] was applied to all samples in the Genetic Analysis Service for Agriculture (SAGA) in CIMMYT, El Batan, Mexico. Briefly, DArTseq is a complexity reduction method that includes two enzymes (PstI and HpaII) to create a genome representation of the set of samples. The PstI-RE site specific adapter is tagged with 96 different barcodes, enabling the multiplexing of a 96-well microtiter plate with equimolar amounts of amplification products to run in an Illumina sequencer Novaseq6000 (Illumina Inc., San Diego, CA, USA). The successfully amplified fragments are sequenced with up to 83 bases, generating approximately 500,000 unique reads per sample. A proprietary analytical pipeline developed by DArT P/L was used to generate allele calls for SNP and presence/absence variation (PAV) markers [42]. A 100K consensus map [43] was used to obtain genetic positions of the SNPs in addition to the alignments to the reference genomes.

From the complete set of 441 SHW lines, 438 were genotyped and used for Genome Wide Association Study (GWAS). A total of 67,436 markers were scored, out of which 50% (34,790) could be aligned to reference genomes. Quality control was carried out based on the minimum lack of alleles, resulting in 5800 markers to be used for GWAS. The reference genomes used in this study were Chinese Spring IWGSC RefSeq v1.0 genome assembly [44] and durum wheat (cv. Svevo) Ref Seq Rel. 1.0 [45], along with the reference genome of *Ae. tauschii* (v.4, 2017) [46].

2.3.4. Statistical

Analysis and Genome-Wide Association Study For the disease data, statistical analyses were performed using the Statistical Analysis System version 9.1 [47]. An analysis of variance (ANOVA) was conducted on the average reactions of the SHW, the DW parents, and SB checks. The Best Linear Unbiased Estimates (BLUE) were computed for each of the 441 SHW genotypes and later used to conduct GWAS using the TASSEL (Trait Analysis by Association Evolution and Linkage) software ver. 5.2.73 [48]. The mixed linear model (MLM) by Yu et al. [49] was used to simultaneously include the level of relatedness based on marker data and identical by descent (IBD)

computed from the coefficient of parentage, which controls population structure. Additionally, population structure was controlled by fitting the first five principal components (PC) from the kinship matrix taken as the fixed variate and the coefficient of parentage (COP) as the random variable. The false-discovery rate (FDR) was used to assess the significance of the p-value (<0.05) [49]. The allelic effects of the significant MTAs were estimated as the difference between the mean value of lines, with and without the favorable alleles, and were presented as box plots.

2.5. Partial Least Squares Regression We used the Partial Least Squares (PLS) method to apply the results of GWAS analyses to practical application to breeding. Extensive studies to assess the importance of environmental and genotypic covariables in multi-environment plant breeding trials were carried out using the PLS method [50–53]. In the context of this study, the PLS relates in a single estimation procedure (1) the two-way table of phenotypic measurements of SB of the SHW lines in 6 replicates in the greenhouse (and on the mean across the six replicated) and (2) the total number of significant markers found in the current GWAS study (41 explanatory variables). PLS regression describes explanatory (markers) as linear combinations of the complete set of measures of SB on SHW cultivars with no limit to the number of marker covariables or to the number of SHW lines that can be used.

2.4. RESULTS

2.4.1. Resistance to Spot Blotch at the Seedling Stage

The SB development observed during seedling evaluation in the greenhouse was even and consistent. ANOVA showed significant differences among SHWs ($p < 0.001$). The checks Chirya 3, Sonalika, Ciano T79, and Francolin displayed scores of 1.4, 4.0, 4.0, and 2.8, respectively (Table 1), verifying the identity of the *B. sorokiniana* isolate used and a successful inoculation. Most of the 441 SHW lines displayed resistant and moderately resistant reactions (Supplementary Table S1), i.e., 250 (56.7%) showed resistance (R) and 161 (36.5%) showed moderate resistance (MR) reactions with disease scores of 1.0–2.5, comparable to the resistant check Chirya 3. Only 30 SHWs (6.8%) were moderately susceptible (MS) or susceptible (S) with disease scores of 3.0–4.1. These scores were still lower than the scores of the susceptible checks, Sonalika, and Ciano T79 (Table 1 and Figure 1)

Table 1. Spot blotch (SB) reactions of 40 durum wheat (DW) parents, their respective synthetic hexaploid wheat (SHW) and four checks. Reactions are defined as Resistant (R, 1.0–1.5), Moderately Resistant (MR, 1.6–2.5), Moderately Susceptible (MS, 2.6–3.5), and Susceptible (S, 3.6–5.0). For 18 SHW lines, their DW parents were not identified.

	DW parents		SHW		
Pedigree	Tan spot scores	Reaction type	Number of progeny (*Ae. tauschii)*	Mean tan spot scores	Mean reaction type
BOTNO	4.3	S	1	2.2	MR
SCAUP	3.9	S	3	2.2	MR
CROC_1	3.7	S	30	1.7	MR
D67.2/PARANA 66.270	3.7	S	13	1.7	MR
YAR	3.7	S	4	1.4	R
68.111/RGB-U//WARD RESEL/3/STIL	3.6	S	31	1.5	R
DECOY 1	3.5	MS	30	2.1	MR
SORA	3.4	MS	14	1.6	MR
6973/WARD.7463//74110	3.3	MS	3	1.6	MR
CPI8/GEDIZ/3/GOO//ALB/CRA	3.3	MS	31	1.9	MR
LCK59.61	3.2	MS	2	2.3	MR
68.111/RGB-U//WARD	3.1	MS	7	1.6	MR
CHEN_7	3.0	MS	1	1.2	R
ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE	2.9	MS	3	2	MR
YAV_2/TEZ	2.9	MS	12	1.6	MR
LOCAL RED	2.9	MS	7	2.2	MR
TK SN1081	2.9	MS	3	1.2	R
YARMUK	2.8	MS	4	1.7	MR
ROK/KML	2.7	MS	4	2.2	MR
STY,DR/CELTA//PALS/3/SRN_5	2.7	MS	2	1.5	R
ALTAR 84	2.6	MS	20	1.6	MR
ACONCHI 89	2.6	MS	4	1.5	R

DVERD_2	2.5	MR	13	1.5	R
FGO/USA2111	2.5	MR	1	1.1	R
ARLIN_1	2.4	MR	13	1.5	R
68.111/RGB-U//WARD/3/FGO/4/RABI	2.4	MR	31	1.5	R
SCOT/MEXI_1	2.4	MR	1	1.8	MR
GARZA/BOY	2.3	MR	7	1.8	MR
68112/WARD	2.3	MR	4	1.2	R
LARU	2.3	MR	4	1.1	R
RASCON_37	2.2	MR	2	1.3	R
KAPUDE_1	2.1	MR	1	1.9	MR
CERCETA	1.9	MR	54	1.6	MR
RABI//GS/CRA	1.6	MR	4	1.5	R
SNIPE/YAV79//DACK/TEAL	1.5	R	7	1.1	R
FALCIN_1	1.5	R	5	1.9	MR
SHAG_22	1.5	R	6	1.5	R
GREEN_3	1.2	R	1	1	R
GAN	1.1	R	39	1.4	R
SCOOP_1	1.1	R	3	1	R
Erik (Resistant check)	1.0	R	---	1	R
Glenlea (Susceptible check)	4.8	S	---	4.8	S
6B-662 (Moderately resistant check)	2.0	MR	---	2.50	MR
6B-365 (Moderately susceptible check)	3.1	MS	---	3.30	MS

Most of the 441 SHW lines displayed resistant and moderately resistant reactions (Supplementary Table S1), i.e., 250 (56.7%) showed resistance (R) and 161 (36.5%) showed moderate resistance (MR) reactions with disease scores of 1.0–2.5, comparable to the re-sistant check Chirya 3. Only 30 SHWs (6.8%) were moderately susceptible (MS) or suscep-tible (S) with disease scores of 3.0–4.1. These scores were still lower than the scores of the susceptible checks, Sonalika, and Ciano T79 (Table 1 and Figure 1).

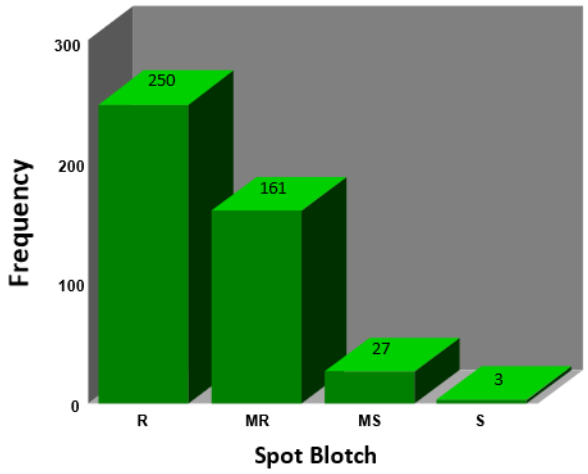


Figure 1. Histogram of spot blotch (SB) scores for different reaction types, which include Resistant (R, 1.0–1.5), Moderately Resistant (MR, 1.6–2.5), Moderately Susceptible (MS, 2.6–3.5), and Susceptible (S, 3.6–5.0).(data extracted from Supplementary Table S1).

The SB reaction of DW parents revealed that 18 (45%) parents had reaction scores of 1.0–1.5 (R) and 14 (35%) reaction scores of 1.6–2.5 (MR), developing mostly small dark to maroon lesions on those that had extended 1–2 mm in length with chlorotic edges during the initial infection. Eight entries (20%) were observed to have a mean reaction score between 2.6 and 3.6, being considered moderately susceptible (MS) to susceptible (S), whereas the leaves were observed to die/senescence when the light brown to dark brown oval to elongated blotches extended and merged very quickly (Tables 1 and S1). The SB reaction scores of the DW parents compared to the scores of the SHW indicated that the SB resistance of SHW was likely inherited from both DW and *Ae. tauschii* parents.

2.4.2. Genome-Wide Association Study Using Different References Genomes.

The first two principal components (PCs) based on the DArTSeq markers separated two clear groups of entries of similar sizes and some entries in between, explaining around 34% of the total variability. This population structure was controlled by fitting the first five PCs derived from the correlation matrix as fixed covariates. Additionally, the coefficient of parentage used as a random variable to fit the GWAS mixed linear model (MLM) effectively controlled the remaining population structure after fitting the first five PCs. From the complete set of 441 SHW lines, 438 were genotyped and used for the Genome Wide Association Study (GWAS). A total of 67,436 markers were scored, out of which 50% (34,790) could be aligned to reference genomes. Quality control was carried out based on the minimum lack of alleles, resulting in 5800 markers to be used for GWAS. Out of the DArTSeq markers that could be aligned to the whole genome sequence of cv. Chinese Spring (CS, IWGSC RefSeq v1.0), 20 significant MTAs were identified as shown in Table S2 and Figure 2, being located on chromosomes 1B (1), 1D (1), 2A (1), 2D (3), 3A (2), 3B (1), 3D (1), 4A (1), 5A (2), 5D (2) 6D (1), 7A (2), and 7D (2). The markers with the highest allele substitution effects were located on chromosomes 7D (1.11), 3A (0.33), and 5D (0.32)

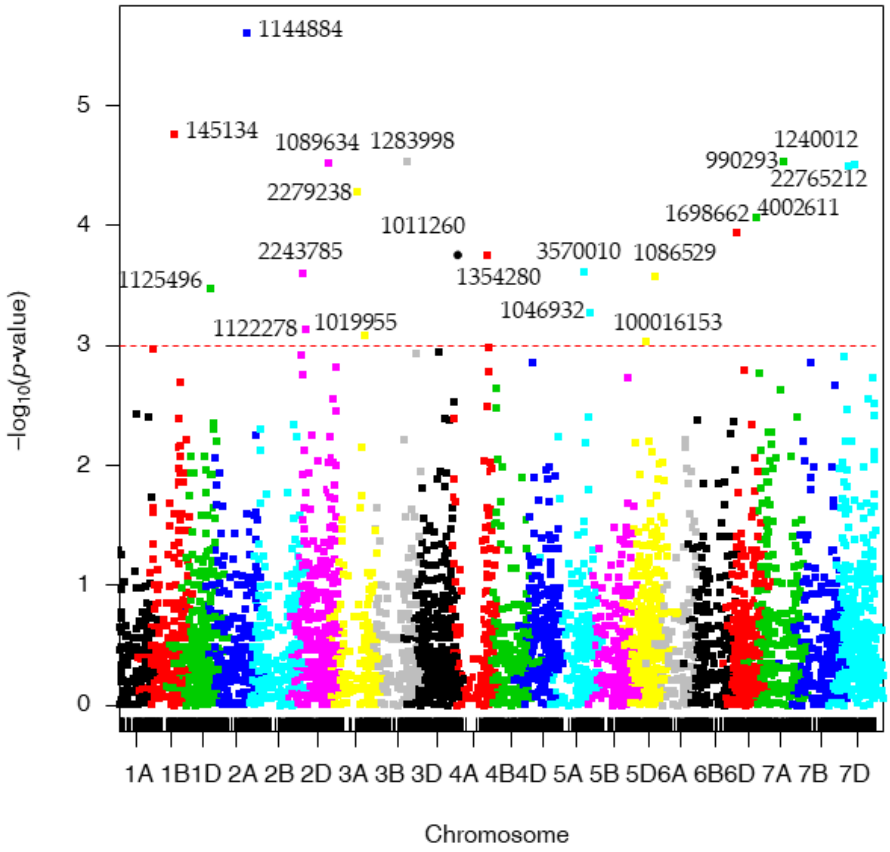


Figure 2. Manhattan plots for spot blotch (SB) disease corresponding to the physical position of Chinese spring Ref Seq ver.1.0. The *p*-values are shown on a $\log_{10}$ scale. The marker is considered significant if $\log_{10}$ scale is 3 or higher

Looking at the markers located on the 100 K consensus map, 32 significant MTAs were detected, as shown in Table S3 and Figure 3, and found to be located on chromosomes 1B (7), 1D (2), 2A (2), 2B (3), 2D (2), 3B (2), 3D (2), 4A (3), 4D (1), 5A (2), 5B (1), 6B (1) 7A (3), and 7B (1). The markers with the highest allele substitution effects were located on chromosomes 5B (1.12), 3B (0.53), and 2B (0.24). Nine MTAs based on the IWGSC Ref Seq v1.0 overlapped with those presented in Table S3. Therefore, three MTAs showed the same chromosome allocation on the genetic and physical maps, while six MTAs showed different chromosome assignments (yet mainly homologous chromosomes) on both maps

When markers aligned to the DW cultivar Svevo and the *Ae. tauschii* reference genomes were considered, 10 MTAs were identified on chromosomes 1B (1), 2A (1), 2B (1), 2D (1), 3A (2), 3B (2), 4D (1), and 7A (1) (Table S4 and Figure 4). However, only three markers in Table S4 coincided with those found in Tables S2 and S3. Marker ID 1240012 on chromosome 2B in Svevo was found to be on chromosome 7D when aligned to the physical map of CS and on chromosome 5B in the 100K consensus map. The markers with the highest allele substitution effects ranged from 1.10 (2B), 0.33 (3A), to 0.16 (3A).

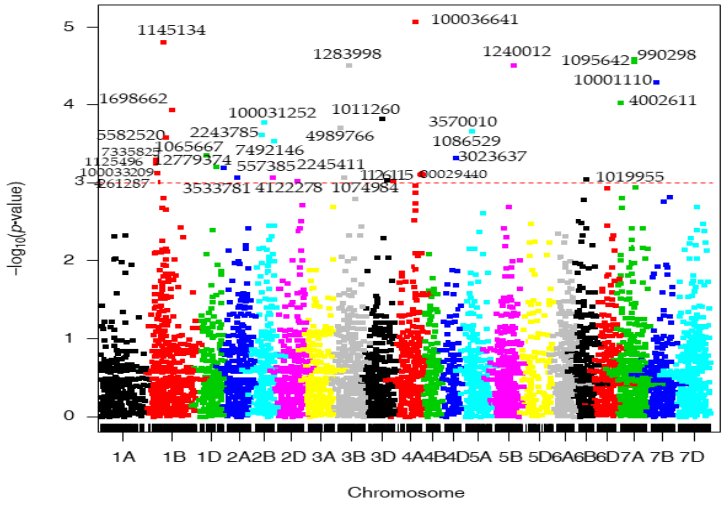


Figure 3. Manhattan plots for spot blotch disease (SB) corresponding to the consensus map. The *p*-values are shown on a $\log_{10}$ scale. The marker is considered significant if $\log_{10}$ scale is 3 or higher.

Overall, a total of 41 genomic regions identified using the different maps are summarized in Table 2. A re-alignment of the marker sequences to the ABD, AB, and D genomes verified the physical position of several of the significant SNPs and could identify their physical positions across species. However, among all, 11 MTAs could not be assigned positions on the physical map. Furthermore, 23 MTAs were found within annotated high-confidence gene sequences, with 10 of these 23 candidate genes annotated in the CS reference genome, 6 in Svevo reference genome, and 7 in the *Ae. tauschii* reference genome (Supplementary Table S5). These significant MTAs were detected on 15 chromosomes with the maximum number of 5 MTAs on chromosome 1B and 1 each on 6D and 7B, and their R^2 values varied from 0.03 to 0.07. Among the five markers detected

on chromosome 1B, the highest R^2 value of 0.06 was found for marker ID 1145134 that is in proximity with marker ID 5582520, with two other markers (IDs 4261287 and 7335825) distal to them and one (ID 100033209) proximal to them. Three MTAs were found on chromosome 2A, with marker ID 1144884 exhibiting the highest R^2 value of 0.07. Two MTAs on chromosome 5A (IDs 3570010 and 1046932) were found with low R^2 values of 0.03 for each one. Allelic effects ranged from 0.01 to 1.11 for the MTAs on 4D (ID 2243087) and 7D (ID 1240012), respectively

Table 2. Significant marker–trait associations for seedling resistance to spot blotch, their position in different reference genomes, associated candidate genes, and GWAS statistics. The table contains the physical position based on Chinese Spring (CS) reference genome, the chromosome and the genetic position based on cM, the BLAST results against the CS, Svevo, and *Ae. tauschii* reference genomes, genes, freq. of resistance markers, *p*-values, Marker R^2. $-\log_{10}$ *p*-values and effect of allele.

Chr.	Marker ID	Physical position (CS) Ref Seq v1.0)	Chr	Genetic Position (cM)	BLASTN to I WGSC Ref Seq V1.0	BLAST to Ref Seq Svevo	BLAST to Ref Seq *Ae. tauschii*	Gene (s)	Frequency of Resistance Marker Allele	*p*-value	Marker R^2	$-\log_{10}$ *p*-value	Effect of Allele
1B	4261287		1B	51.29	1B: 17537160-17537233	no good hit found	no good hit found		0.88	9.83×10^{-4}	0.04	3.01	−0.29
1B	7335825		1B	52.56	no good hit found	no good hit found	no good hit found		0.83	4.96×10^{-4}	0.04	3.30	−0.19
1B	5582520		1B	96.91	no good hit found	no good hit found	no good hit found		0.89	2.70×10^{-4}	0.04	3.57	−0.26
1B	1145134	406039536	1B	98.03	1B: 406039533-406039608	1B: 399260866-399260941			0.63	1.64×10^{-5}	0.06	4.79	−0.05
1B	100033209		1B	139.32	no good hit found	no good hit found	no good hit found		0.83	8.35×10^{-4}	0.04	3.08	−0.66
1D	1065667		1D	12.27	1D: 6248618-6248679		1D: 6917141-6917202		0.94	4.50×10^{-4}	0.04	3.35	0.23
1D	1125496	416590812	1B	51.289	1D: 416590808-416590883		1D: 424102922-424102997	AET1Gv20777500	0.82	3.36×10^{-4}	0.03	3.47	NaN
1D	12779374		1D	130.64	1D: 486387813-486387877	1B: 667753290-667753354	1D: 493826928-493826992	TraesCS1D02G441400 AET1Gv21021400 TRITD1Bv1G224330	0.12	6.25×10^{-4}	0.04	3.20	0.00
2A	5573285		2A	45.45	no good hit found	no good hit found	no good hit found		0.78	5.74×10^{-4}	0.04	3.24	0.17
2A	1144884	583026867			2A: 583026863-583026938	2A: 576091990-576092065			0.77	2.50×10^{-4}	0.07	5.60	0.02
2A	3533784		2A	123.66	aligns only to 2B	2A: 774229337-77422941			0.64	9.75×10^{-4}	0.04	3.01	−0.13
2B	7492146			107.03	no good hit found	no good hit found	no good hit found		0.83	3.01×10^{-4}	0.04	3.52	0.24
2B	100031252			55.48	no good hit found	no good hit found	no good hit found		0.88	1.66×10^{-4}	0.04	3.78	NaN
2D	1122278	21621448	2D	20.85	2D: 21621445-21621520		2D: 22832366-22832441	TraesCS2D02G054200	0.61	8.39×10^{-4}	0.04	3.08	−0.14
2D	2243785	32640660	2B	40.74	2D: 32640657-32640732		2D: 33858967-33859042	TraesCS2D02G076500	0.86	2.46×10^{-4}	0.04	3.61	−0.18
2D	1089634	509231294			2D: 509231291-		2D: 507788059-	AET2Gv20890600	0.05	$3.10\times10^{-}$	0.05	4.51	0.03

					509231366		507788134			4			
3A	1019955	474447292	6B	46.69	3A: 474447288-474447363	3A: 477078635-477078710			0.92	9.28×10^{-4}	0.04	3.03	−0.46
3A	2279238	474554774			3A: 474554770-474554845	3A: 477190300-477190375			0.84	5.27×10^{-5}	0.05	4.28	0.33
3B	4989766		3B	19.56	no good hit found	no good hit found	no good hit found		0.81	1.97×10^{-4}	0.04	3.71	0.53
3B	1283998	593544135.00	3B	68.53	3B: 593544132-593544207	3B: 593903780-593903855		TRITD3Bv1G194800	0.10	3.04×10^{-5}	0.05	4.52	−0.02
3B	4992362	775474348.00			3B: 763236117-763236191	3B: 775474345-775474420		TraesCS3B02G520000 TRITD3Bv1G257410	0.21	9.91×10^{-4}	0.04	3.00	0.02
3D	1074984		3D	61.81	3D: 401883953-401884028		3D: 409258183-409258258	TraesCS3D02G291900 AET3Gv20689000	0.86	9.10×10^{-4}	0.04	3.04	0.17
3D	1011260	520678096	3D	82.16	3D: 520678093-520678168		3D: 529110490-529110565	TraesCS3D02G407000 AET3Gv20921800	0.21	1.83×10^{-4}	0.04	3.74	−0.05
4A	1351280	629433955.00			4A: 629433952-629434027	4A:623641790-623641858		TraesCS4A02G355400	0.84	1.78×10^{-4}	0.04	3.75	−0.06
4A	1162615		4A	96.08	4A: 661535726-661535794	4A:661278198-661278266			0.87	9.57×10^{-4}	0.04	3.02	−0.26
4A	100036641		4A	96.36	no good hit found	no good hit found	no good hit found		0.92	8.42×10^{-6}	0.06	5.07	−0.39
4A	100039440		4A	113.91	aligns to many chromosomes but less than 100%	4A:693427125-693427193 4A:693425785-693425853			0.83	8.99×10^{-4}	0.04	3.05	−0.32
4D	3023637	474561316	4D	66.12	no good hit found	no good hit found	no good hit found		0.05	4.86×10^{-4}	0.04	3.31	−0.02
4D	2243087	54178331			4D: 51304835-51304903		4D:54178332-54178400		0.07	2.61×10^{-5}	0.05	4.58	0.01
5A	3570010	521764788	5A	36.99	5A: 521764784-521764859	5A:484938946-484939014			0.02	2.40×10^{-4}	0.03	3.62	NaN
5A	1046932	622389460			5A: 622389461-622389529 4A:552297214-552297282	5A:583637584-583637652 4A:545007545-545007613			0.85	5.52×10^{-4}	0.03	3.26	NaN
5D	100016153	232599413			5D: 232599413-232599475 5A:322677280-322677342	5A:316073030-316073092	5D:246553454-246553516	AET5Gv20379200, TraesCS5A02G146400 TRITD5Av1G111170	0.72	9.35×10^{-4}	0.04	3.03	0.32

5D	1086529	410253879	5A	36.99	5D: 410253875 - 410253950		5D:418190498-418190566		0.89	2.65×10^{-4}	0.04	3.58	0.21
6D	1698662	42940457.00	1B	148.15	6D: 42940453-42940522		6D: 64808834-64808903		0.76	1.13×10^{-4}	0.05	3.95	−0.27
7A	4002611	7938756.00	7A	7.25	7A:7938757-7938825	7A:6228579-6228647		TraesCS7A02G019400 TRITD7Av1G003410	0.10	8.88×10^{-5}	0.05	4.05	−0.04
7A	1095642		7A	75.85	no good hit found	no good hit found	no good hit found		0.88	2.90×10^{-5}	0.05	4.54	−0.29
7A	990293	621213334.00	7A	88.42	4A:142973443-142973511 7A:621213334-621213402	4A:140470489-140470557 7A:616593441-616593509			0.85	3.11×10^{-5}	0.05	4.51	−0.03
7B	100011110		7B	46.26	no good hit found	no good hit found	no good hit found		0.84	5.27×10^{-5}	0.05	4.28	−0.23
7D	2245411		2D	118.19	7D: 69417014-69417082		7D: 70389436-70389511		0.89	9.54×10^{-4}	0.04	3.02	−0.14
7D	1240012	150762254	5B	98.36	7D: 150762250-150762325	2B: 196456606-196456681	7D: 151389082-151389157	TRITD2Bv1G075350	0.89	3.19×10^{-5}	0.05	4.50	1.11
7D	22765212	268565893			7D: 268565890-268565965		7D: 270502277-270502352	TraesCS7D02G278500 AET7Gv20675900	0.05	3.15×10^{-5}	0.05	4.50	0.02

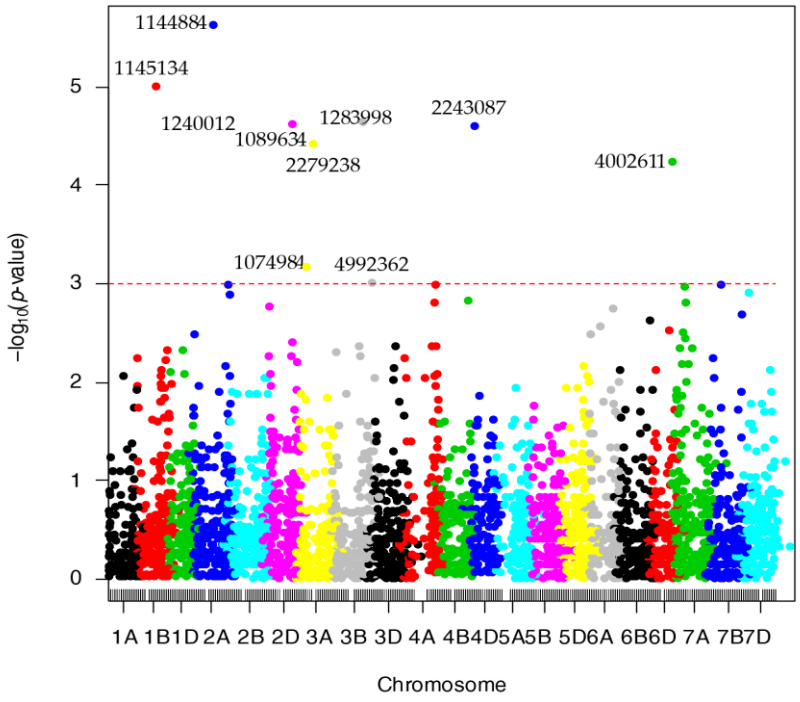


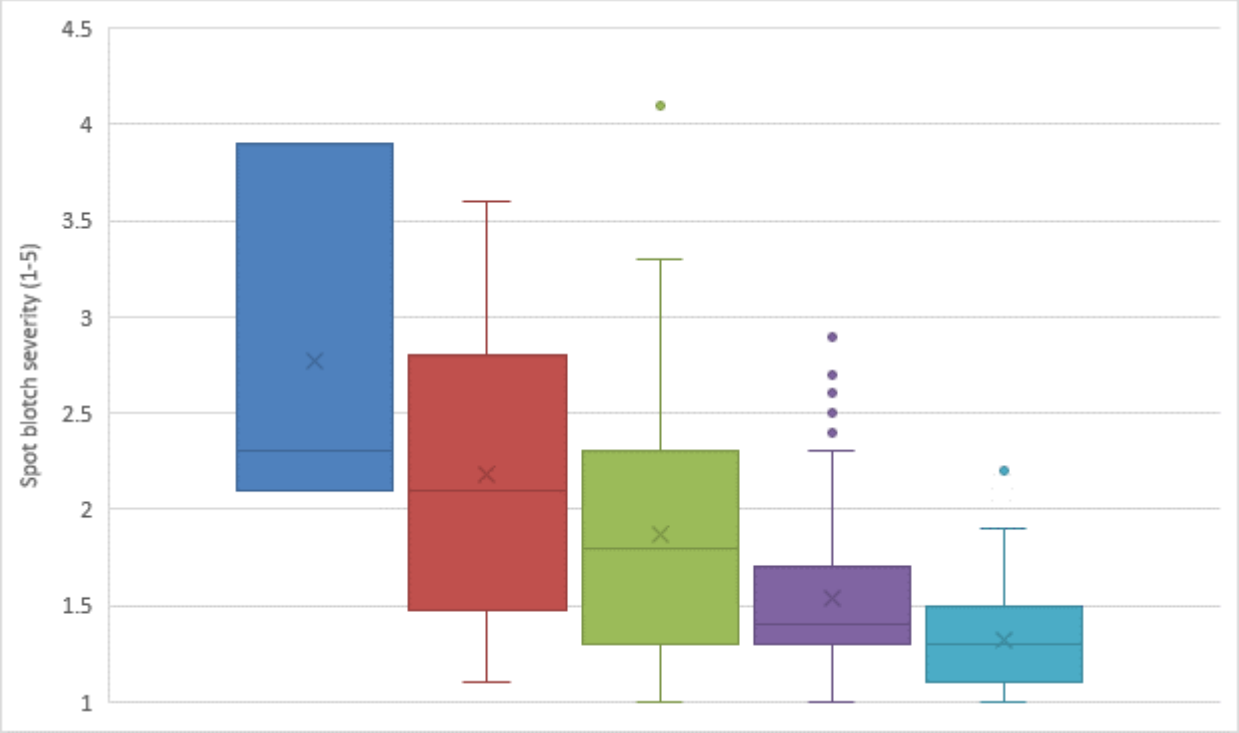
Figure 4. Manhattan plots for spot blotch (SB) disease corresponding to the durum wheat (cv. Svevo) and *Ae. tauschii* reference genomes (Ref Seq Rel. 1.0). The *p* values are shown on a $\log_{10}$ scale.

2.4.3. Identified MTA

On chromosome 1B, the reported positions for five MTAs showed two MTAs (markers 4261287 and 7335825) nearby, at 51.3 and 52.6 cM, and two MTAs (markers 5582520 and 1145134) at 96.9–98.0 cM, respectively, resulting in three different QTLs identified for SB on chromosome 1B. On chromosome 2D, two MTAs (markers 1122278 and 2243785) were positioned 11.02 Mbp apart but with an R^2 of 0.08 and a probability of linkage disequilibrium (LD) of 1.23×10^{-7} forming a third MTA. Additionally, two markers on chromosome 3A with a distance of only 0.11 Mbp (markers ID 1019955 and 474554774) showed a linkage disequilibrium r^2 of 0.8138, with a *p*-value of 1.21×10^{-7}. The two significant markers on 3D were located at a distance of 20 cM; thus, being considered unlinked. On chromosome 4A, markers 1162615 and 100036641 were mapped near each other, at 96.1 and 96.4 cM, respectively, and thus could be considered one single MT

2.4.4. Frecuency of Resistance Alleles within Individual SHSs

The frequency of resistance alleles in the SHWs was examined with the aim of identifying lines with high numbers of resistance alleles to be used for further resistance breeding. A total of 59 SHW lines carried more than 30 of the 41 identified resistance alleles with an average SB score of 1.3 (Figure 5). Although not shown in this figure, there are 32 SHW lines with > 32 resistance alleles and 15 SHW lines with > 34 resistance alleles, which could be the top candidates for further evaluation and breeding. SHW lines with less resistance alleles (<16 R alleles) showed increased susceptibility and demonstrated the additive nature of the resistance alleles.



No. of R alleles	<16	16-20	21-25	26-30	>30
No. of lines	3	26	112	237	59
Mean SB severity	2.8	2.2	1.9	1.5	1.3

Figure 5. Boxplot showing the effects of stacking different number of resistance (R) alleles (QTL) on mean SB severity. The average severity is represented by the 'x' symbol and the median by the horizontal line inside.

2.4.5. Interpretation of results from Partial Least Squares

The results of the PLS are shown in Figure 6, where the first two PLS factors explained around 26% of the total variability, and 15 molecular markers (green color) with a frequency of R alleles greater than 84% and 32 SHW lines (red color) having more than 32 resistance alleles (Figure 6). The arrows from the center to the upper-left quadrant show the six phenotype measurements of SB (SB1-6) and their overall mean (Mean SB). The SHW lines are distributed in a linear manner from the lower-right quadrant (more resistance lines) to the upper-left quadrant (more susceptible lines). The 15 markers were located at the center and on the right-hand side of the biplot (green letter-numeric combination), and the 32 most resistant SHW lines (red numbers) are located towards the lower-right quadrant. From a practical breeding perspective, the 15 markers and the 32 SB resistance lines could be prioritized in crosses between SHW lines and elite bread wheat lines in breeding and pre-breeding programs.

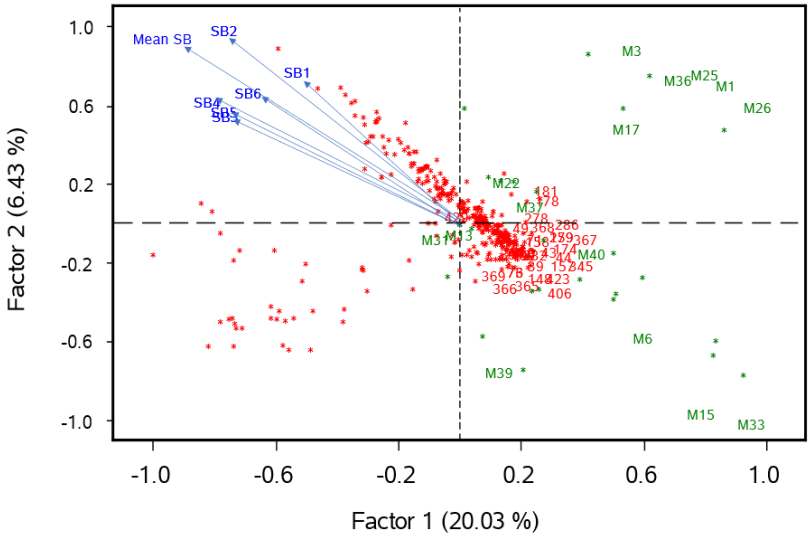


Figure 6. Biplot chart showing the first two PLS factors for 41 significant markers and 438 SHW lines, where SB measured in the greenhouse in six replicates (SB1-6) and overall mean (Mean SB) are shown (lines from the center to the upper right quadrant). The 15 molecular markers with a frequency of resistance alleles greater than 84% were M1 (4261287 chr1B), M3 (5582520 chr1B), M6 (1065667 chr1D), M13 (100031252 chr2B), M15 (2243785 chr2D or chr2B), M17 (1019955 chr3A or chr6B), M22 (1074984 chr3D), M25 (1162615 chr4A), M26 (100036641 chr 4A), M31 (1046932 chr 5A), M33 (1086529 chr5D or chr5A), M36 (1095642 chr7A), M37 (990293 chr7A), M39 (2245411 chr7D or chr2D), and M40 (1240012 chr7D or chr5B) (marker IDs are presented in Table 2). The 32 SHW lines having more than 32 resistance alleles are identified with red numbers. The remaining markers and SHW lines are represented by green and red dots, respectively.

2.5. DISCUSSION

Genome-wide association studies were performed to uncover SNP markers related to SB resistance in bread wheat. One such study was conducted by [54] on 528 spring wheat accessions for seedling resistance against SB, and 11 MTAs were identified. The same panel was analyzed earlier by [30], but only four genomic regions were identified, due to fewer markers being used, emphasizing the importance of high-density marker data. A recent GWAS was reported by [55], who studied a total of 6736 CIMMYT breeding lines for SB resistance in field experiments conducted throughout several years (2014–2019), and up to 214 MTAs were identified in at least one year, 96 were repeatable in at least two years and all had minor effects.

To our knowledge, to date no GWAS has been reported on SB resistance in SHW, although several studies reported good resistance of SHW to SB. In earlier studies, *Ae. tauschii* was used to transfer potential SB-resistant genes through *T. turgidum* × *Ae. tauschii* or *T. aestivum* × *Ae. tauschii* crosses [35]. Diverse *Ae. tauschii* accessions were used to make SHW lines, which exhibited promising SB resistance and often performed better than the resistant check Mayoor [38]. A series of SHW was developed and then screened for several biotic and abiotic stresses, and promising entries were either used for commercial cultivars or as pre-breeding materials to develop new genotypes. The authors of [33] reported eight SHW accessions with SB resistance, along with sources of resistance to other diseases.

Our study revealed that the evaluated SHWs displayed a considerable resistance to SB, with 38% of the SHW lines showing better resistance than the resistant check Chirya 3. According to the pedigree information, SB resistance of the panel might be based on diverse DW and *Ae. tauschii* backgrounds and was thus likely contributed by multiple SB resistance genes that was in agreement with the GWAS results.

2.5.1. Novelties of the Significant Markers Found in the Current Study

Previous genetic studies have identified a range of SB resistance genes/QTL, residing on all wheat chromosomes except 4D and 5D, as summarized recently by [56]. Some of these loci exhibited major effects, such as the nominated *Sb* genes, yet most of them showed minor effects. The same applies to the current study, where a total of 41 significant markers on 15 chromosomes were found to be associated with SB resistance, and none of them showed any major effects. This again

confirmed the polygenic nature of SB resistance described in previous studies [24,26,55]. The significant MTAs were identified on AB genome chromosomes as well as on D genome chromosomes, suggesting that SB resistance in the SHWs was derived from both their DW and *Ae. tauschii* parents.

MTAs were identified on all seven D genome chromosomes, especially chromosomes 4D and 5D, on which no QTL/MTA has been reported so far [56]; thus, confirming their novelty. The two MTAs on chromosome 4D were located on short arm (marker 2243087) and long arm (marker 3023637); on chromosome 5D the physically distant markers must represent two different QTL. MTAs on chromosomes 1B (marker 1145134), 2D (marker 1122278 and 2243785), 3A (marker 1019955 and 2279238), and 6D (marker 1698662) also suggested to be novel since no QTL/MTA has been reported in the vicinity of these markers [56].

However, some MTAs were found within known QTL regions. For example, the two MTAs on chromosome 1BS (markers 4261287 and 7335825) were in close proximity to the MTAs reported by [29]. Likewise, on chromosome 3B, marker 4992362 was closely located to an MTA reported by [31]. Nevertheless, close linkage or coincidence does not necessarily mean that the identified regions represent the same QTL/MTA, especially because our study screened SHW, while those published previously evaluated common wheat. It is noteworthy that some markers did not show any BLAST hit on the three reference genomes, e.g., marker 7335825 on chromosome 1B and marker 7492146 on chromosome 2B. These MTAs represent variants absent in the reference genomes and might be worthy of further investigation.

2.5.2. Candidate Genes for the Identified Marker–Trait Associations

The significant markers identified from the GWAS were further evaluated for their association with disease resistance-related genes. We identified 23 plant defense-related protein families across multiple chromosome regions, of which only 13 have a known protein function. For example, marker 12779374 on chromosome 1D was identified within the gene TRITD1Bv1G224330 (Tables S5 and 2), which is involved in the synthesis of the lectin receptor kinase that has an important function for the general immunity of the plants [57]. Similarly, marker 1240012 on 7D was located within the gene TRITD2Bv1G075350 related to protein U-box domain containing protein 4, associated with the control of grain production [58]. However, it should be

noticed that these candidate genes might not be the underlying genes for the MTAs, due to the usually large linkage disequilibrium blocks in the wheat genome [59].

Furthermore, marker 1283998 on chromosome 3B marked an SNP within gene TRITD3Bv1G194800, which is a protein described as disease resistance protein RPM1 G, again involved in the general resistance of plants to various diseases [60]. Marker 4992362 on chromosome 3B marked the gene TRITD3Bv1G257410, which is identified as protein Serpin that participates in the regulation of proteolytic complex systems [61], whereas marker 1011260 (in chromosome 3D) falls within the gene TraesCS3D02G407000, a peroxidase protein that has the divergence role in different pathogens systems in plants [62]. Furthermore, marker 100016153, aligned on chromosomes 5A and 5D, was located within the genes TraesCS5A02G146400 and TRITD5Av1G111170, in which two proteins, Mannan endo-1,4 -beta-mannosidase 6 and Mannan endo-1,4-beta-mannosidase-like protein, are involved.

Note that marker 4002611 on chromosome 7A did fall within the gene TRITD7Av1G003410, a Pectin lyase-like superfamily protein, which has an important role in the development and maturity process of the plant. This protein also acts on the peptic substances presented as structural polysaccharides in the primary cell walls of the superior plants [63]. Marker 22765212, on chromosome 7D, was included in gene TraesCS7D02G278500, which is found in the ribosomal protein that plays a fundamental integral role in the growth and development of the plant, as well as participating in the general defense mechanism of the plants [64].

2.5.3. Application of GWAS for Use in Practical Breeding

Genome-wide association studies (GWAS) are a powerful option for the genetic characterization of quantitative traits and have been widely used to analyze agronomic and disease traits. With the increasing number of diseases affecting cultivated wheat plants, the option of developing resistance SHW lines has been widely used. This is the first GWAS study to assess significant MTA of SB from a diverse collection of 441 SHW lines, and 41 significant markers and a range of SHW lines with high SB resistance were identified. In the PLS analysis, a subset of markers and SHW lines were identified that are more suitable for future breeding and pre-breeding activities.

Results of this study showed 15 molecular markers with a frequency of R alleles greater than 84% and 32 SHW lines having more than 32 resistance alleles. The PLS plot show the specific locations of the 15 markers and the 32 most resistant SHW lines. From a practical breeding perspective, these markers with R alleles and the SB resistance lines could be used in future breeding crosses.

2.6. . CONCLUSIONS

This is the first GWAS study to investigate MTAs for SB resistance in a diverse collection of 441 SHW lines from CIMMYT. GWAS found a total of 41 significant markers related to SB resistance, being distributed on 15 wheat chromosomes, and many of them are novel. We were able to identify highly resistant SHW lines with most resistance alleles of the significant markers that can be used in wheat breeding programs.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/genes13081387/s1,for Supplementary Tables. Table S1. Seedling spot blotch (SB) reaction scores of synthetic hexaploid wheat (SHW) lines derived from crosses between durum wheat (DW, *T. turgidum* L.) and *Aegilops tauschii* Coss (*Ae. squarrosa*) parents. Table S2. Significant markers for seedling resistance to spot blotch when aligned to the physical map of Chinese spring (IWGSC RefSeqV.1.0). Chromosome (Chr.), marker ID, allele ID, physical position, F statistics, Probability (Prob), Marker R2, −log10 (p-value) and the effect of allele substitution are given for each marker. Table S3. Significant markers associated with seedling resistance to spot blotch when a DArTSeq consensus genetic map was used. Chromosome (Chr), Allele ID, genetic position in cM, F statistics, Probability (Prob), Marker R2, −log10 (p-value) and the effect of allele substitution are given for each marker. Table S4. Significant markers associated with seedling resistance to spot blotch based on durum wheat (cv. Svevo) and *Ae. tauschii* reference genomes. Chromosome (Chr.), Marker ID, allele ID, physical positions, F-statistics, Probability (Prob), Marker R2 , −log10 (p-value) and the effect of allele substitution are given for each marker. Table S5. Candidate genes for significant marker-trait associations identified from *Triticum aestivum* (IWGSC), *Triticum turgidum* (Svevo.v1) and *Aegilops tauschii* (Aet_v4.0) genomes. Data was obtained from Emsembl (https://plants.ensembl.org/ (accessed on 15 March 2022).

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Conflicts of Interest: The authors declare no conflict of interest

Table S1. Seedling spot blotch (SB) reaction scores of synthetic hexaploid wheat (SHW) from the cross of *T. turgidum* L, durum wheat (DW) parents (in bold phase) by *Aegilops tauschii* Coss (AE.SQUARROSA)

		Reaction to SB		
Entry No.	**Pedigree**	**AVG****	**Score**	**Number of progeny**
1	**68.111/RGB-U//WARD***	3.6	S	7
2	68.111/RGB-U//WARD/3/AE.SQUARROSA (316)	1.3	R	
3	68.111/RGB-U//WARD/3/AE.SQUARROSA (321)	1.1	R	
4	68.111/RGB-U//WARD/3/AE.SQUARROSA (322)	1.6	MR	
5	68.111/RGB-U//WARD/3/AE.SQUARROSA (329)	2.2	MR	
6	68.111/RGB-U//WARD/3/AE.SQUARROSA (511)	1.6	MR	
7	68.111/RGB-U//WARD/3/AE.SQUARROSA (426)	2.2	MR	
8	68.111/RGB-U//WARD/3/AE.SQUARROSA (202)	1.9	MR	
9	**68.111/RGB-U//WARD RESEL/3/STIL***	2.7	MS	31
10	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (164)	1.5	R	
11	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (332)	1.1	R	
12	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (781)	1.4	R	
13	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (783)	1.5	R	

14	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (385)	2.1	MR
15	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (386)	1.7	MR
16	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (389)	1.7	MR
17	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (390)	1.3	R
18	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (392)	1.0	R
19	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (1029)	1.7	MR
20	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (1030)	2.2	MR
21	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (1038)	1.6	MR
22	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (631)	1.7	MR
23	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (662)	2.0	MR
24	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (672)	1.5	R
25	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (685)	2.1	MR
26	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (623)	1.5	R
27	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (625)	1.7	MR
28	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (627)	1.4	R
29	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (628)	1.2	R
30	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (630)	1.2	R
31	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (631)	1.3	R

32	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (1090)	1.3	R	
33	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (188)	1.3	R	
34	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (659)	1.8	MR	
35	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (675)	1.1	R	
36	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (681)	1.8	MR	
37	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (684)	1.2	R	
38	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (700)	1.4	R	
39	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (768)	1.1	R	
40	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (1010)	1.9	MR	
41	**68.111/RGB-U//WARD/3/FGO/4/RABI***	3.2	MS	31
42	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (191)	1.2	R	
43	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	1.3	R	
44	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	1.1	R	
45	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (882)	1.1	R	
46	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (905)	1.3	R	
47	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (809)	1.3	R	
48	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (809)	1.1	R	
49	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (809)	1.3	R	

50	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	1.6	MR
51	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	1.8	MR
52	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	1.5	R
53	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	2.2	MR
54	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	1.2	R
55	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	3.3	MS
56	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	2.5	MR
57	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (1050)	1.2	R
58	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (719)	2.4	MR
59	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (720)	2.0	MR
60	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (768)	1.0	R
61	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (778)	1.4	R
62	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (788)	1.8	MR
63	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (974)	1.7	MR
64	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (661)	1.1	R
65	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (701)	1.0	R
66	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (709)	1.4	R
67	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (710)	.	.

68	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (784)	1.2	R	
69	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (504)	1.2	R	
70	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (675)	1.2	R	
71	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (1010)	1.3	R	
72	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (1093)	1.3	R	
73	**68112/WARD***	2.3	MR	4
74	68112/WARD//AE.SQUARROSA (369)	1.3	R	
75	68112/WARD//AE.SQUARROSA (369)	1.6	MR	
76	68112/WARD//AE.SQUARROSA (369)	1.2	R	
77	68112/WARD//AE.SQUARROSA (369)	2.0	MR	
78	**6973/WARD.7463//74110***	2.3	MR	3
79	6973/WARD.7463//74110/3/AE.SQUARROSA (35A)	2.6	MS	
80	6973/WARD.7463//74110/3/AE.SQUARROSA (665)	1.3	R	
81	6973/WARD.7463//74110/3/AE.SQUARROSA (438)	1.2	R	
82	**ACONCHI 89***	1.7	MR	4
83	ACO89/AE.SQUARROSA (178)	1.5	R	
84	ACO89/AE.SQUARROSA (282)	1.9	MR	
85	ACO89/AE.SQUARROSA (290)	2.5	MR	
86	ACO89/AE.SQUARROSA (309)	2.2	MR	
87	**ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE***	1.8	MR	3

88	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/AE .SQUARROSA (389)	2.4	MR	
89	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/AE .SQUARROSA (451)	3.4	MS	
90	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/AE .SQUARROSA (723)	2.0	MR	
91	**ALTAR 84***	1.3	R	20
92	ALTAR 84/AE.SQUARROSA (188)	1.2	R	
93	ALTAR 84/AE.SQUARROSA (191)	1.1	R	
94	ALTAR 84/AE.SQUARROSA (198)	1.0	R	
95	ALTAR 84/AE.SQUARROSA (220)	2.3	MR	
96	ALTAR 84/AE.SQUARROSA (221)	1.0	R	
97	ALTAR 84/AE.SQUARROSA (223)	1.3	R	
98	ALTAR 84/AE.SQUARROSA (224)	1.0	R	
99	ALTAR 84/AE.SQUARROSA (224)	1.9	MR	
100	ALTAR 84/AE.SQUARROSA (224)	2.2	MR	
101	ALTAR 84/AE.SQUARROSA (291)	1.9	MR	
102	ALTAR 84/AE.SQUARROSA(Y86-87 S401)	1.2	R	
103	ALTAR 84/AE.SQUARROSA (333)	3.9	S	
104	ALTAR 84/AE.SQUARROSA (507)	1.6	MR	
105	ALTAR 84/AE.SQUARROSA (174)	1.5	R	
106	ALTAR 84/AE.SQUARROSA (1012)	1.8	MR	

107	ALTAR 84/AE.SQUARROSA (244)	1.9	MR	
108	ALTAR 84/AE.SQUARROSA (319)	1.3	R	
109	ALTAR 84/AE.SQUARROSA (531)	2.5	MR	
110	ALTAR 84/AE.SQUARROSA (539)	4.1	S	
111	ALTAR 84/AE.SQUARROSA (793)	1.3	R	
112	**ARLIN_1***	2.4	MR	13
113	ARLIN/AE.SQUARROSA (283)	1.6	R	
114	ARLIN/AE.SQUARROSA (317)	2.1	MR	
115	ARLIN_1/AE.SQUARROSA (333)	1.8	MR	
116	ARLIN/AE.SQUARROSA (410)	2.0	MR	
117	ARLIN_1/AE.SQUARROSA (536)	1.5	R	
118	ARLIN_1/AE.SQUARROSA (1018)	2.6	MS	
119	AE.SQUARROSA (1031)/ARLIN_1	1.1	R	
120	ARLIN_1/AE.SQUARROSA (310)	1.1	R	
121	ARLIN_1/AE.SQUARROSA (320)	1.4	R	
122	ARLIN_1/AE.SQUARROSA (368)	1.3	R	
123	ARLIN_1/AE.SQUARROSA (430)	1.3	R	
124	ARLIN_1/AE.SQUARROSA (335)	1.2	R	
125	ARLIN_1/AE.SQUARROSA (802)	1.3	R	

126	**BOTNO***	3.4	MS	1
127	BOTNO/AE.SQUARROSA (617)	1.5	R	
128	**CERCETA***	1.3	R	54
129	CETA/AE.SQUARROSA (230)	1.3	R	
130	CETA/AE.SQUARROSA (783)	1.1	R	
131	CETA/AE.SQUARROSA (895)	1.2	R	
132	CETA/AE.SQUARROSA (895)	1.5	R	
133	CETA/AE.SQUARROSA (796)	1.9	MR	
134	CETA/AE.SQUARROSA (174)	1.3	R	
135	CETA/AE.SQUARROSA (499)	1.3	R	
136	CETA/AE.SQUARROSA (525)	1.1	R	
137	CETA/AE.SQUARROSA (540)	1.9	MR	
138	CETA/AE.SQUARROSA (1016)	1.9	MR	
139	CETA/AE.SQUARROSA (1027)	1.5	R	
140	CETA/AE.SQUARROSA (1030)	2.0	MR	
141	CETA/AE.SQUARROSA (166)	1.9	MR	
142	CETA/AE.SQUARROSA (187)	1.6	MR	
143	CETA/AE.SQUARROSA (244)	3.1	MS	
144	CETA/AE.SQUARROSA (262)	1.2	R	

145	CETA/AE.SQUAROOSA (263)	2.1	MR
146	CETA/AE.SQUARROSA (371)	1.4	R
147	CETA/AE.SQUARROSA (391)	2.8	MS
148	CETA/AE.SQUARROSA (445)	1.5	R
149	CETA/AE.SQUARROSA (450)	2.4	MR
150	CETA/AE.SQUARROSA (485)	2.7	MS
151	CETA/AE.SQUARROSA (530)	2.6	MS
152	CETA/AE.SQUARROSA (533)	2.5	MR
153	CETA/T.URARTU (557)	1.3	R
154	CETA/AE.SQUARROSA (1018)	2.0	MR
155	CETA/AE.SQUARROSA (1026)	1.4	R
156	CETA/AE.SQUARROSA (1031)	1.4	R
157	CETA/AE.SQUARROSA (1036)	1.8	MR
158	CETA/AE.SQUARROSA (1038)	1.9	MR
159	CETA/AE.SQUARROSA (1043)	1.6	MR
160	CETA/AE.SQUARROSA (1053)	1.4	R
161	CETA/AE.SQUARROSA (246)	2.1	MR
162	CETA/AE.SQUARROSA (496)	1.5	R

163	CETA/AE.SQUARROSA (506)	3.3	MS
164	CETA/AE.SQUARROSA (539)	3.6	S
165	CETA/AE.SQUARROSA (541)	3.3	MS
166	CETA/AE.SQUARROSA (231)	1.3	R
167	CETA/AE.SQUARROSA (335)	1.3	R
168	CETA/AE.SQUARROSA (356)	2.0	MR
169	CETA/AE.SQUARROSA (1047)	2.1	MR
170	CETA/AE.SQUARROSA (615)	1.4	R
171	CETA/AE.SQUARROSA (629)	1.3	R
172	CETA/AE.SQUARROSA (750)	1.2	R
173	CETA/AE.SQUARROSA (1090)	1.7	MR
174	CETA/AE.SQUARROSA (248)	1.4	R
175	CETA/AE.SQUARROSA (310)	1.3	R
176	CETA/AE.SQUARROSA (418)	1.2	R
177	CETA/AE.SQUARROSA (442)	2.0	MR
178	CETA/AE.SQUARROSA (681)	1.4	R
179	CETA/AE.SQUARROSA (682)	1.0	R
180	CETA/AE.SQUARROSA (683)	1.4	R

181	CETA/AE.SQUARROSA (684)	1.5	R	
182	CETA/AE.SQUARROSA (1073)	1.6	MR	
183	**CHEN_7***	2.6	MS	1
184	CHEN_7/AE.SQUARROSA (429)	2.3	MR	
185	**CPI8/GEDIZ/3/GOO//ALB/CRA***	2.3	MR	31
186	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (193)	1.4	R	
187	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (196)	1.3	R	
188	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (205)	1.6	MR	
189	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)	1.3	R	
190	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (215)	1.3	R	
191	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (629)	1.5	R	
192	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (633)	2.3	MR	
193	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (637)	1.4	R	
194	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (358)	1.8	MR	
195	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (334)	1.3	R	
196	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (409)	1.6	MR	
197	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (184)	1.8	MR	
198	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (244)	2.5	MR	
199	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (273)	2.6	MS	

200	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (296)	3.0	MS	
201	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (305)	2.9	MS	
202	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (439)	1.9	MR	
203	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (461)	2.2	MR	
204	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (533)	2.2	MR	
205	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (1018)	1.4	R	
206	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (1021)	1.5	R	
207	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (1026)	1.3	R	
208	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (1029)	1.6	MR	
209	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (1031)	2.5	MR	
210	AE.SQUARROSA (1043)/4/CPI8/GEDIZ/3/GOO//ALB/CRA	1.3	R	
211	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (227)	1.4	R	
212	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (1017)	2.1	MR	
213	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (188)	1.5	R	
214	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (659)	1.7	MR	
215	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (684)	1.3	R	
216	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (698)	2.3	MR	
217	**CROC_1***	1.4	R	30
218	CROC_1/AE.SQUARROSA (168)	1.0	R	

219	CROC_1/AE.SQUARROSA (205)	1.3	R
220	CROC_1/AE.SQUARROSA (210)	1.9	MR
221	CROC_1/AE.SQUARROSA (210)	1.8	MR
222	CROC_1/AE.SQUARROSA (210)	1.4	R
223	CROC_1/AE.SQUARROSA (213)	1.3	R
224	CROC_1/AE.SQUARROSA (215)	1.0	R
225	CROC_1/AE.SQUARROSA (224)	1.0	R
226	CROC_1/AE.SQUARROSA (224)	1.0	R
227	CROC_1/AE.SQUARROSA (224)	1.1	R
228	CROC_1/AE.SQUARROSA (224)	1.0	R
229	CROC_1/AE.SQUARROSA (662)	1.3	R
230	CROC_1/AE.SQUARROSA (725)	1.2	R
231	CROC_1/AE.SQUARROSA (826)	1.7	MR
232	CROC_1/AE.SQUARROSA (886)	1.3	R
233	CROC_1/AE.SQUARROSA (518)	1.3	R
234	CROC_1/AE.SQUARROSA (298)	2.4	MR
235	CROC_1/AE.SQUARROSA (333)	1.6	MR
236	CROC_1/AE.SQUARROSA (170)	1.6	MR

237	CROC_1/AE.SQUARROSA (177)	1.4	R	
238	CROC_1/AE.SQUARROSA (256)	2.1	MR	
239	CROC_1/AE.SQUARROSA (275)	1.7	MR	
240	CROC_1/AE.SQUARROSA (516)	1.1	R	
241	CROC_1/AE.SQUARROSA (517)	1.3	R	
242	CROC_1/AE.SQUARROSA (493)	1.9	MR	
243	CROC_1/AE.SQUARROSA (176)	2.0	MR	
244	CROC_1/AE.SQUARROSA (229)	1.9	MR	
245	CROC_1/AE.SQUARROSA (310)	1.5	R	
246	CROC_1/AE.SQUARROSA (239)	2.0	MR	
247	CROC_1/AE.SQUARROSA (397)	1.9	MR	
248	**D67.2/PARANA 66.270***	3.1	MS	13
249	D67.2/PARANA 66.270//AE.SQUARROSA (211)	1.5	R	
250	D67.2/PARANA 66.270//AE.SQUARROSA (213)	1.5	R	
251	D67.2/PARANA 66.270//AE.SQUARROSA (218)	1.4	R	
252	D67.2/PARANA 66.270//AE.SQUARROSA (220)	1.4	R	
253	D67.2/PARANA 66.270//AE.SQUARROSA (221)	1.5	R	
254	D67.2/PARANA 66.270//AE.SQUARROSA (222)	1.1	R	
255	D67.2/PARANA 66.270//AE.SQUARROSA (223)	1.2	R	

256	D67.2/PARANA 66.270//AE.SQUARROSA (633)	1.1	R	
257	D67.2/PARANA 66.270//AE.SQUARROSA (246)	1.3	R	
258	D67.2/PARANA 66.270//AE.SQUARROSA (657)	1.3	R	
259	D67.2/PARANA 66.270//AE.SQUARROSA (634)	1.6	MR	
260	D67.2/PARANA 66.270//AE.SQUARROSA (668)	1.0	R	
261	D67.2/PARANA 66.270//AE.SQUARROSA (1148)	1.3	R	
262	**DECOY 1***	2.5	MR	30
263	DOY1/AE.SQUARROSA (188)	1.1	R	
264	DOY1/AE.SQUARROSA (216)	2.1	MR	
265	DOY1/AE.SQUARROSA (446)	1.8	MR	
266	DOY1/AE.SQUARROSA (447)	2.8	MS	
267	DOY1/AE.SQUARROSA (488)	1.7	MR	
268	DOY1/AE.SQUARROSA (510)	1.5	R	
269	DOY1/AE.SQUARROSA (515)	1.8	MR	
270	DOY1/AE.SQUARROSA (415)	2.7	MS	
271	DOY1/AE.SQUARROSA (428)	1.5	R	
272	DOY1/AE.SQUARROSA (507)	1.2	R	
273	DOY1/AE.SQUARROSA (532)	2.1	MR	
274	DOY1/AE.SQUARROSA (177)	2.1	MR	

275	DOY1/AE.SQUARROSA (255)	1.7	MR	
276	DOY1/AE.SQUARROSA (258)	1.3	R	
277	DOY1/AE.SQUARROSA (267)	1.6	MR	
278	DOY1/AE.SQUARROSA (322)	1.1	R	
279	DOY1/AE.SQUARROSA (334)	1.4	R	
280	DOY1/AE.SQUARROSA (516)	2.0	MR	
281	DOY1/AE.SQUARROSA (517)	1.5	R	
282	DOY1/AE.SQUARROSA (1016)	1.3	R	
283	DOY1/AE.SQUARROSA (1024)	1.7	MR	
284	DOY1/AE.SQUARROSA (1018)	2.6	MS	
285	DOY1/AE.SQUARROSA (1026)	1.3	R	
286	DOY1/AE.SQUARROSA (1029)	1.3	R	
287	AE.SQUARROSA (1043)/DOY1	2.0	MR	
288	AE.SQUARROSA (1026)/DOY1	2.1	MR	
289	DOY1/AE.SQUARROSA (295)	2.4	MR	
290	DOY1/AE.SQUARROSA (360)	2.5	MR	
291	DOY1/AE.SQUARROSA (540)	3.5	MS	
292	DOY1/AE.SQUARROSA (632)	1.3	R	
293	**DVERD_2***	1.5	R	13

294	DVERD_2/AE.SQUARROSA (214)	1.3	R	
295	DVERD_2/AE.SQUARROSA (221)	1.3	R	
296	DVERD_2/AE.SQUARROSA (247)	1.1	R	
297	DVERD_2/AE.SQUARROSA (247)	1.5	R	
298	DVERD_2/AE.SQUARROSA (333)	1.8	MR	
299	DVERD_2/AE.SQUARROSA (507)	2.3	MR	
300	DVERD_2/AE.SQUARROSA (1022)	1.3	R	
301	DVERD_2/T.URARTU (545)	1.8	MR	
302	DVERD_2/AE.SQUARROSA (1026)	1.8	MR	
303	DVERD_2/AE.SQUARROSA (1029)	1.7	MR	
304	DVERD_2/AE.SQUARROSA (1031)	1.8	MR	
305	AE.SQUARROSA (1029)/DVERD_2	1.9	MR	
306	AE.SQUARROSA (1031)/DVERD_2	2.1	MR	
307	**FALCIN_1***	1.0	R	5
308	FALCIN/AE.SQUARROSA (312)	1.6	MR	
309	FALCIN/AE.SQUARROSA (389)	2.2	MR	
310	FALCIN_1/AE.SQUARROSA (176)	2.4	MR	
311	FALCIN_1/AE.SQUARROSA (197)	1.8	MR	
312	FALCIN_1/AE.SQUARROSA (1073)	1.3	R	

313	**FGO/USA2111***	1.3	R	1
314	FGO/USA2111//AE.SQUARROSA (658)	1.0	R	
315	**GAN***	2.0	MR	39
316	GAN/AE.SQUARROSA (201)	1.5	R	
317	GAN/AE.SQUARROSA (446)	3.3	MS	
318	GAN/AE.SQUARROSA (522)	2.1	MR	
319	GAN/AE.SQUARROSA (180)	1.0	R	
320	GAN/AE.SQUARROSA (257)	1.1	R	
321	GAN/AE.SQUARROSA (408)	1.0	R	
322	GAN/AE.SQUARROSA (890)	1.0	R	
323	GAN/AE.SQUARROSA (163)	1.8	MR	
324	GAN/AE.SQUARROSA (182)	1.6	MR	
325	GAN/AE.SQUARROSA (264)	1.4	R	
326	GAN/AE.SQUARROSA (267)	1.5	R	
327	GAN/AE.SQUARROSA (268)	1.3	R	
328	GAN/AE.SQUARROSA (285)	1.0	R	
329	GAN/AE.SQUARROSA (413)	1.1	R	
330	GAN/AE.SQUARROSA (459)	1.3	R	
331	GAN/AE.SQUARROSA (206)	1.4	R	

332	GAN/AE.SQUARROSA (231)	1.3	R
333	GAN/AE.SQUARROSA (233)	1.6	MR
334	GAN/AE.SQUARROSA (296)	1.5	R
335	GAN/AE.SQUARROSA (300)	1.6	MR
336	GAN/AE.SQUARROSA (335)	1.1	R
337	GAN/AE.SQUARROSA (536)	1.7	MR
338	GAN/AE.SQUARROSA (620)	2.4	MR
339	GAN/AE.SQUARROSA (621)	2.7	MS
340	GAN/AE.SQUARROSA (623)	1.0	R
341	GAN/AE.SQUARROSA (624)	1.0	R
342	GAN/AE.SQUARROSA (633)	1.2	R
343	GAN/AE.SQUARROSA (638)	1.3	R
344	GAN/AE.SQUARROSA (658)	1.1	R
345	GAN/AE.SQUARROSA (668)	1.6	MR
346	GAN/AE.SQUARROSA (643)	2.7	MS
347	GAN/AE.SQUARROSA (741)	1.0	R
348	GAN/AE.SQUARROSA (479)	1.0	R
349	GAN/AE.SQUARROSA (680)	1.3	R

350	GAN/AE.SQUARROSA (721)	1.2	R	
351	GAN/AE.SQUARROSA (735)	1.1	R	
352	GAN/AE.SQUARROSA (768)	1.4	R	
353	GAN/AE.SQUARROSA (779)	1.5	R	
354	GAN/AE.SQUARROSA (1080)	1.5	R	
355	**GARZA/BOY***	1.3	R	7
356	GARZA/BOY//AE.SQUARROSA (271)	1.5	R	
357	GARZA/BOY//AE.SQUARROSA (286)	1.3	R	
358	GARZA/BOY//AE.SQUARROSA (307)	2.9	MS	
359	GARZA/BOY//AE.SQUARROSA (311)	2.2	MR	
360	GARZA/BOY//AE.SQUARROSA (350)	1.7	MR	
361	GARZA/BOY//AE.SQUARROSA (439)	1.8	MR	
362	GARZA/BOY//AE.SQUARROSA (764)	2.0	MR	
363	**GREEN***	1.1	R	1
364	GREEN/AE.SQUARROSA (458)	1.4	R	
365	**KAPUDE_1***	2.2	MR	1
366	KAPUDE/AE.SQUARROSA (175)	1.8	MR	
367	**LARU***	1.3	R	4
368	LARU/AE.SQUARROSA (309)	1.5	R	
369	LARU/AE.SQUARROSA (309)	1.4	R	

370	LARU/AE.SQUARROSA (TA2459)	1.4	R	
371	LARU/AE.SQUARROSA (333)	1.6	MR	
372	**LCK59.61***	3.0	MS	2
373	LCK59.61/AE.SQUARROSA (308)	1.4	R	
374	LCK59.61/AE.SQUARROSA (783)	2.1	MR	
375	**LOCAL RED***	1.9	MR	7
376	LOCAL RED/AE.SQUARROSA (219)	2.4	MR	
377	LOCAL RED/AE.SQUARROSA (220)	2.3	MR	
378	LOCAL RED/AE.SQUARROSA (221)	2.0	MR	
379	LOCAL RED/AE.SQUARROSA (222)	2.1	MR	
380	LOCAL RED/AE.SQUARROSA (223)	2.3	MR	
381	LOCAL RED/AE.SQUARROSA (449)	1.3	R	
382	LOCAL RED/AE.SQUARROSA (189)	2.8	MS	
383	**RABI//GS/CRA***	1.1	R	4
384	RABI//GS/CRA/3/AE.SQUARROSA (190)	1.0	R	
385	RABI//GS/CRA/3/AE.SQUARROSA (891)	1.3	R	
386	RABI//GS/CRA/3/AE.SQUARROSA (904)	1.8	MR	
387	RABI//GS/CRA/3/AE.SQUARROSA (457)	1.2	R	
388	**RASCON***	1.1	R	2
389	RASCON/AE.SQUARROSA (312)	1.5	R	

390	RASCON/AE.SQUARROSA (367)	2.3	MR	
391	**ROK/KML***	1.0	R	4
392	ROK/KML//AE.SQUARROSA (214)	1.3	R	
393	ROK/KML//AE.SQUARROSA (295)	2.5	MR	
394	ROK/KML//AE.SQUARROSA (333)	1.5	R	
395	ROK/KML//AE.SQUARROSA (507)	2.3	MR	
396	**SCAUP***	2.0	MR	3
397	SCA/AE.SQUARROSA (493)	2.6	MS	
398	SCA/AE.SQUARROSA (248)	2.1	MR	
399	SCA/AE.SQUARROSA (409)	1.0	R	
400	**SCOOP_1***	1.2	R	3
401	SCOOP_1/AE.SQUARROSA (358)	1.3	R	
402	SCOOP_1/AE.SQUARROSA (407)	1.1	R	
403	SCOOP_1/AE.SQUARROSA (659)	1.1	R	
404	**SCOT/MEXI_1***	1.0	R	1
405	SCOT/MEXI_1//AE.SQUARROSA (186)	1.3	R	
406	**SHAG_22***	1.3	R	6
407	SHAG_22/AE.SQUARROSA (227)	1.3	R	
408	SHAG_22/AE.SQUARROSA (319)	1.6	MR	
409	SHAG_22/AE.SQUARROSA (530)	1.8	MR	

410	SHAG_22/AE.SQUARROSA (537)	2.8	MS	
411	SHAG_22/AE.SQUARROSA (539)	2.5	MR	
412	SHAG_22/AE.SQUARROSA (1101)	1.2	R	
413	**SNIPE/YAV79//DACK/TEAL***	1.0	R	7
414	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (411)	1.6	MR	
415	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (904)	1.0	R	
416	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (528)	1.3	R	
417	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (628)	1.4	R	
418	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (629)	1.3	R	
419	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (633)	1.3	R	
420	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (700)	1.6	MR	
421	**SORA***	1.1	R	14
422	SORA/AE.SQUARROSA (191)	1.4	R	
423	SORA/AE.SQUARROSA (192)	1.4	R	
424	SORA/AE.SQUARROSA (192)	1.2	R	
425	SORA/AE.SQUARROSA (207)	1.5	R	
426	SORA/AE.SQUARROSA (208)	1.4	R	
427	SORA/AE.SQUARROSA (211)	1.4	R	
428	SORA/AE.SQUARROSA (215)	1.6	MR	

429	SORA/AE.SQUARROSA (323)	1.4	R	
430	SORA/AE.SQUARROSA (939)	2.9	MS	
431	SORA/AE.SQUARROSA (617)	1.4	R	
432	SORA/AE.SQUARROSA (625)	2.0	MR	
433	SORA/AE.SQUARROSA (442)	1.5	R	
434	SORA/AE.SQUARROSA (469)	1.1	R	
435	SORA/AE.SQUARROSA (684)	2.2	MR	
436	**STY,DR/CELTA//PALS/3/SRN_5***	1.2	R	2
437	STY,DR/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (277)	1.3	R	
438	STY,DR/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (502)	1.1	R	
439	**TK SN1081***	3.2	MS	3
440	TK SN1081/AE.SQUARROSA (222)	1.2	R	
441	TK SN1081/AE.SQUARROSA (222)	1.0	R	
442	TK SN1081/AE.SQUARROSA (690)	1.7	MR	
443	**YAR***	2.5	MR	4
444	YAR/AE.SQUARROSA (493)	2.7	MS	
445	YAR/AE.SQUARROSA (783)	1.5	R	
446	YAR/AE.SQUARROSA (809)	1.0	R	
447	YAR/AE.SQUARROSA (518)	1.1	R	
448	**YARMUK***	2.1	MR	4

449	YUK/AE.SQUARROSA (217)	2.1	MR	
450	YUK/AE.SQUARROSA (434)	1.4	R	
451	YUK/AE.SQUARROSA (784)	1.6	MR	
452	YUK/AE.SQUARROSA (864)	1.2	R	
453	**YAV_2/TEZ***	2.3	MR	12
454	YAV_2/TEZ//AE.SQUARROSA (249)	2.0	MR	
455	YAV_2/TEZ//AE.SQUARROSA (249)	1.4	R	
456	YAV_2/TEZ//AE.SQUARROSA (249)	1.3	R	
457	YAV_2/TEZ//AE.SQUARROSA (249)	1.2	R	
458	YAV_2/TEZ//AE.SQUARROSA (249)	1.3	R	
459	YAV_2/TEZ//AE.SQUARROSA (249)	1.7	MR	
460	YAV_2/TEZ//AE.SQUARROSA (435)	1.5	R	
461	YAV_2/TEZ//AE.SQUARROSA (437)	1.5	R	
462	YAV_2/TEZ//AE.SQUARROSA (882)	1.4	R	
463	YAV_2/TEZ//AE.SQUARROSA (746)	1.1	R	
464	YAV_2/TEZ//AE.SQUARROSA (721)	1.3	R	
465	YAV_2/TEZ//AE.SQUARROSA (1093)	1.3	R	
466	Lines without durum wheat parents in this study			
467	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (460)	1.5	R	

468	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (460)	1.1	R
469	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (477)	2.3	MR
470	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (477)	1.5	R
471	SRN/AE.SQUARROSA (358)	1.2	R
472	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (381)	1.1	R
473	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (397)	1.2	R
474	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (443)	1.3	R
475	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (490)	1.3	R
476	BACANORA T 88	2.6	MS
477	CADO/BOOMER_33//AE.SQUARROSA (651)	.	.
478	CADO/BOOMER_33//AE.SQUARROSA (949)	1.6	MR
479	CADO/BOOMER_33//AE.SQUARROSA (504)	2.1	MR
480	DUKEM_12/2*RASCON_21//AE.SQUARROSA (1100)	1.1	R
481	KUCUK/AE.SQUARROSA (458)	1.3	R
482	KUCUK/AE.SQUARROSA (1080)	1.9	MR
483	KUCUK/AE.SQUARROSA (640)	1.3	R
484	DUKEM_12/2*RASCON_21//AE.SQUARROSA (1090)	1.5	R
	Check resist Chirya 3	1.4	R
	Check susceptible Sonalika	4.0	S

Check susceptible Ciano T79	4.0	S
Check moderately susceptible Francolin	2.8	MS

* Durum wheat parents.
**Averaged spot blotch reaction of each genotype of SHW (six replications) and durum wheat parents (eight replications)

Table S2. Significant markers for seedling resistance to spot blotch when aligned to the physical map of Chinese spring (IWGSC RefSeqV.1.0). Chromosome (Chr.), marker ID, allele ID, physical position, F statistics, Probability (Prob), Marker R^2, $-\log_{10}$ (*p*-value) and the effect of allele substitution are given for each marker.

Chr.	Marker ID	Allele ID	Position	F statistic	Prob	Marker R^2	$-\log_{10}$ *p*-value	Effect of allele substitution (genotype effect)
1B	1145134	1145134|F|0-37:T>C-37:T>C	406039536	11.33	1.64×10^{-5}	0.06	4.79	-0.05
1D	1125496	1125496|F|0-23:T>C-23:T>C	416590812	13.08	3.36×10^{-4}	0.03	3.47	NaN
2A	1144884	1144884|F|0-29:C>T-29:C>T	583026867	13.33	2.50×10^{-6}	0.07	5.60	0.02
2D	1089634	1089634|F|0-38:A>C-38:A>C	509231294	10.66	3.10×10^{-5}	0.05	4.51	0.03
2D	2243785	2243785|F|0-27:T>C-27:T>C	32640660	8.49	2.46×10^{-4}	0.04	3.61	-0.18
2D	1122278	1122278|F|0-8:C>A-8:C>A	21621448	7.21	8.39×10^{-4}	0.04	3.08	-0.14
3A	2279238	2279238|F|0-47:C>T-47:C>T	474554774	10.10	5.27×10^{-5}	0.05	4.28	0.33
3A	1019955	1019955|F|0-55:A>G-55:A>G	474447292	7.11	9.28×10^{-4}	0.03	3.03	-0.46
3B	1283998	1283998|F|0-27:G>A-27:G>A	593544135	10.68	3.04×10^{-5}	0.05	4.52	-0.02
3D	1011260	1011260|F|0-43:A>T-43:A>T	520678096	8.80	1.83×10^{-4}	0.04	3.74	-0.05

4A	1351280	1351280|F|0-50:G>T-50:G>T	629433955	8.83	1.78×10^{-4}	0.04	3.75	-0.06
5A	3570010	3570010|F|0-29:G>A-29:G>A	521764788	13.73	2.40×10^{-4}	0.03	3.62	NaN
5A	1046932	1046932|F|0-42:G>A-42:G>A	622389460	12.13	5.52×10^{-4}	0.03	3.26	NaN
5D	1086529	1086529|F|0-68:G>T-68:G>T	410253879	8.41	2.65×10^{-4}	0.04	3.58	0.21
5D	100016153	100016153|F|0-26:G>A-26:G>A	232599413	7.10	9.35×10^{-4}	0.04	3.03	0.32
6D	1698662	1698662|F|0-37:G>C-37:G>C	42940457	9.30	1.13×10^{-4}	0.05	3.95	-0.27
7A	990293	990293|F|0-7:G>A-7:G>A	621213334	10.66	3.11×10^{-5}	0.05	4.51	-0.03
7A	4002611	4002611|F|0-59:C>G-59:C>G	7938756	9.55	8.88×10^{-5}	0.05	4.05	-0.04
7D	22765212	22765212|F|0-33:C>A-33:C>A	268565893	10.64	3.15×10^{-5}	0.05	4.50	0.02
7D	1240012	1240012|F|0-23:C>T-23:C>T	150762254	10.63	3.19×10^{-5}	0.05	4.50	1.11

Table S3. Significant markers associated with seedling resistance to spot blotch when a DArTSeq consensus genetic map was used**.** Chromosome (Chr), Marker ID**,** Allele ID, genetic position in cM, F statistics, Probability (Prob), Marker R^2, $-\log_{10}$ (*p*-value) and the effect of allele substitution are given for each marker.

Chr.	Marker ID	Allelel ID	Genetic position on consensus map (cM)	F statistic	Prob	Marker R^2	−log10 *p*-value	Effect of allele substitution (genotype effect)
1B	1145134	1145134|F|0-37:T>C-37:T>C	98.03	11.37	1.58×10^{-5}	0.06	4.80	-0.05
1B	1698662	1698662|F|0-37:G>C-37:G>C	148.15	9.28	1.15×10^{-5}	0.05	3.94	-0.26
1B	5582520	5582520|F|0-11:G>A-11:G>A	96.91	8.39	2.70×10^{-4}	0.04	3.57	-0.26
1B	7335825	7335825|F|0-10:C>T-10:C>T	52.56	7.76	4.96×10^{-4}	0.04	3.30	-0.19
1B	1125496	1125496|F|0-23:T>C-23:T>C	51.29	12.12	5.53×10^{-4}	0.03	3.26	NaN
1B	100033209	100033209|F|0-6:A>G-6:A>G	139.32	7.22	8.35×10^{-4}	0.04	3.08	-0.66
1B	4261287	4261287|F|0-17:C>T-17:C>T	51.29	7.05	9.83×10^{-4}	0.04	3.01	-0.29
1D	1065667	1065667|F|0-21:A>T-21:A>T	12.27	7.86	4.50×10^{-4}	0.04	3.35	0.23
1D	12779374	12779374|F|0-30:G>A-30:G>A	130.64	7.52	6.25×10^{-4}	0.04	3.20	0.00

2A	5573285	5573285|F|0-21:A>G-21:A>G	45.45	7.61	5.74×10^{-4}	0.04	3.24	0.17
2A	3533784	3533784|F|0-39:C>T-39:C>T	123.66	7.06	9.75×10^{-4}	0.04	3.01	-0.13
2B	2243785	2243785|F|0-27:T>C-27:T>C	40.74	8.42	2.62×10^{-4}	0.04	3.58	-0.17
2B	7492146	7492146|F|0-17:G>C-17:G>C	107.03	8.28	3.01×10^{-4}	0.04	3.52	0.24
2B	100031252	100031252|F|0-29:T>C-29:T>C	55.48	14.46	1.66×10^{-4}	0.04	3.78	NaN
2D	2245411	2245411|F|0-21:C>A-21:C>A	118.19	7.08	9.54×10^{-4}	0.04	3.02	-0.14
2D	1122278	1122278|F|0-8:C>A-8:C>A	20.85	7.05	9.80×10^{-4}	0.04	3.01	-0.14
3B	1283998	1283998|F|0-27:G>A-27:G>A	68.53	10.66	3.10×10^{-5}	0.05	4.51	-0.02
3B	4989766	4989766|F|0-16:C>T-16:C>T	19.56	8.72	1.97×10^{-4}	0.04	3.71	0.53
3D	1011260	1011260|F|0-43:A>T-43:A>T	82.16	8.82	1.79×10^{-4}	0.04	3.75	-0.05
3D	1074984	1074984|F|0-15:T>G-15:T>G	61.81	7.10	9.36×10^{-4}	0.04	3.03	-0.16
4A	100036641	100036641|F|0-6:C>A-6:C>A	96.36	12.04	8.42×10^{-6}	0.06	5.07	-0.39
4A	100039440	100039440|F|0-27:G>A-27:G>A	113.91	7.14	8.99×10^{-4}	0.04	3.05	-0.32
4A	1162615	1162615|F|0-50:C>T-50:C>T	96.08	7.08	9.57×10^{-4}	0.04	3.02	-0.26
4D	3023637	3023637|F|0-12:C>T-12:C>T	66.12	7.78	4.86×10^{-4}	0.04	3.31	-0.02
5A	3570010	3570010|F|0-29:G>A-29:G>A	36.99	13.96	2.14×10^{-4}	0.04	3.67	NaN

5A	1086529	1086529|F|0-68:G>T-68:G>T	36.99	8.54	2.35×10^{-4}	0.04	3.63	0.20
5B	1240012	1240012|F|0-23:C>T-23:C>T	98.36	10.62	3.20×10^{-5}	0.05	4.49	1.12
6B	1019955	1019955|F|0-55:A>G-55:A>G	46.69	7.17	8.70×10^{-4}	0.04	3.06	-0.46
7A	990293	990293|F|0-7:G>A-7:G>A	88.42	10.76	2.82×10^{-5}	0.05	4.55	-0.05
7A	1095642	1095642|F|0-36:C>T-36:C>T	75.85	10.73	2.90×10^{-5}	0.05	4.54	-0.29
7A	4002611	4002611|F|0-59:C>G-59:C>G	7.25	9.50	9.34×10^{-5}	0.05	4.03	-0.03
7B	100011110	100011110|F|0-15:G>C-15:G>C	46.26	10.10	5.27×10^{-5}	0.05	4.28	-0.23

Table S4. Significant markers associated with seedling resistance to spot blotch based on durum wheat (cv. Svevo) and *Ae. tauschii* reference genomes. Chromosome (Chr.), Marker ID, allele ID, physical positions, F-statistics, Probability (Prob), Marker R^2, $-\log_{10}$ (*p*-value) and the effect of allele substitution are given for each marker

Chr.	Marker ID	Allele ID	Position	F statistic	Prob	Marker R^2	$-\log_{10}$ (*p*-value)	Effect of allele substitution (genotype effect)
1B	1145134	1145134|F|0-37:T>C-37:T>C	399260869	11.85	9.99×10^{-6}	0.06	5.00	-0.06
2A	1144884	1144884|F|0-29:C>T-29:C>T	576091993	13.30	2.56×10^{-6}	0.07	5.59	-0.00
2B	1240012	1240012|F|0-23:C>T-23:C>T	196456610	10.85	2.58×10^{-5}	0.05	4.59	1.10
2D	1089634	1089634|F|0-38:A>C-38:A>C	507788062	10.91	2.43×10^{-5}	0.05	4.61	0.04
3A	1074984	1074984|F|0-15:T>G-15:T>G	524698865	7.13	9.10×10^{-4}	0.04	3.04	0.17
3A	2279238	2279238|F|0-47:C>T-47:C>T	477190304	10.33	4.25×10^{-5}	0.05	4.37	0.34
3B	1283998	1283998|F|0-27:G>A-27:G>A	593903783	10.85	2.59×10^{-5}	0.05	4.58	0.01
3B	4992362	4992362|F|0-58:C>T-58:C>T	775474348	7.04	9.91×10^{-4}	0.04	3.00	0.02
4D	2243087	2243087|F|0-35:G>A-35:G>A	54178331	10.84	2.61×10^{-5}	0.05	4.58	0.01
7A	4002611	4002611|F|0-59:C>G-59:C>G	6228579	9.93	6.22×10^{-5}	0.05	4.21	-0.01

Table S5. Candidate genes for significant marker-trait associations identified from *Triticum aestivum* (IWGSC), *Triticum turgidum* (Svevo.v1) and *Aegilops tauschii* (Aet_v4.0) genomes. Data was obtained from Emsembl https://plants.ensembl.org/ (accessed 15/03/2022)

Chromosome	Marker ID	Gene	Description
1D	1125496	AET1Gv20777500	n/a
1D	12779374	TraesCS1D02G441400	n/a
1D	12779374	AET1Gv21021400	n/a
1D	12779374	TRITD1Bv1G224330	Lectin receptor kinase
2B	1240012	TRITD2Bv1G075350	U-box domain-containing protein 4
2D	1122278	TraesCS2D02G054200	n/a
2D	2243785	TraesCS2D02G076500	n/a
2D	1089634	AET2Gv20890600	n/a
3B	1283998	TRITD3Bv1G194800	Disease resistance protein RPM1 G
3B	4992362	TraesCS3B02G520000	n/a
3B	4992362	TRITD3Bv1G257410	Serpin
3D	1074984	TraesCS3D02G291900	n/a
3D	1074984	AET3Gv20689000	n/a
3D	1011260	TraesCS3D02G407000	Peroxidase
3D	1011260	AET3Gv20921800	n/a
4A	1351280	TraesCS4A02G355400	n/a

5A	100016153	TraesCS5A02G146400	Mannan endo-1,4-beta-mannosidase 6
5A	100016153	TRITD5Av1G111170	Mannan endo-1,4-beta-mannosidase-like protein
5D	100016153	AET5Gv20379200	Mannan endo-1,4-beta-mannosidase 6
7A	4002611	TraesCS7A02G019400	n/a
7A	4002611	TRITD7Av1G003410	Pectin lyase-like superfamily protein
7D	22765212	TraesCS7D02G278500	Ribosomal protein
7D	22765212	AET7Gv20675900	n/a

GENERAL DISCUSSION

The main problem of wheat: foliar diseases

Wheat (*Triticum aestivum* L.) is the most widely consumed food grain in the world. Under a continuous climatic change disease have become a major threat for reducing grain yield specially for crops grown under diseases-favoring conditions. It will be fundamental to combine climate resilience new wheat varieties, with high yield potential, and disease resistance in single wheat cultivar could be grown across diverse environments, demonstrated stability and adaptability to a great number of diverse environments. However, known challenges that limit increased wheat grain production are rapid climate change and emergence of new pathogenic variants. Foliar diseases, have become increasingly important for wheat production in recent years, leading to significant losses in grain yield and quality. Some of the factors influencing increase in foliar diseases are the cultivation of susceptible varieties, the rapid evolution of causal pathogens, climate change, and unfavorable agricultural practices, which often lead to severe disease epidemics. About 21.5% of the global wheat production is lost each year to diseases (Savary, 2019), most of the losses attributed to fungal pathogens infecting multiple wheat organs such as root, stem, leaf, spike, and grain.

The development of genetically resistant wheat cultivars is an effective and environmentally friendly mechanism for the control of diseases such as tan spot and spot blotch. To identify novel and effective sources of resistance many Genome-wide Association studies (GWAS) have been conducted in wheat breeding populations and used as powerful tool to identify marker traits association by exploring linkage disequilibrium in collections of diverse wheat populations.

Modern bread wheat cultivars are only a few broad-spectrum sources of resistance to the major foliar spotting diseases, such as tan spot and spot blotch (Farias, 2005). Although great efforts have been made in recent decades to identify and introduce new sources of resistance to foliar diseases in wheat, only a few included Synthetic Hexaploid Wheat (SHW). For example, (Bhatta, 2019) studied 125 SHW plants for their resistance to diseases and pests like rust, crown rot, cereal cyst nematodes, and Hessian fly. However, to the best of our knowledge, so far, no GWAS was performed to evaluate SHW for tan spot and spot blotch resistance.

In earlier studies, *Ae. tauschii* was used to transfer potential SB-resistant genes through *T. turgidum* × *Ae. tauschii* or *T. aestivum* × *Ae. tauschii* crosses (Mujeeb-Kazi, 1996). Diverse *Ae. tauschii* accessions were used to make SHW lines, which exhibited promising SB resistance and often performed better than the resistant check Mayoor (Mujeeb-Kazi, 2007). A series of SHW was developed and then screened for several biotic and abiotic stresses, and promising entries were either used for commercial cultivars or as pre-breeding materials to develop new genotypes. The authors of (Das, 2016) reported eight SHW accessions with spot blotch (SB) resistance, along with sources of resistance to other diseases.

Resistance to tan spot and Spot Blotch (SB) at the Seedling Stage

Our study used 443 SHW and indicates that SHW plants present considerable resistance to tan spot due to the diverse genetic backgrounds of these SHW lines. Most SHW plants displayed resistant and moderately resistant reactions. Out of the 443 SHW plants, 219 (49.4%) showed resistance (R) and 195 (44.0%) moderate resistance (MR) with disease scores of 1.5 to 2.5 that were comparable to the resistant check Erik and the moderately resistant check 6B-662. Only 29 SHW plants (6.5%) were moderately susceptible (MS) with disease scores of 2.6 to 3.5 that were still better than the susceptible check Glenlea and 6B-365.

For the case of inoculated materials for SB, most of SHW lines displayed resistant and moderately resistant reactions i.e., 250 (56.7%) showed resistance (R) and 161 (36.5%) showed moderate resistance (MR) reactions with disease scores of 1.0–2.5, comparable to the resistant check Chirya 3. Only 30 SHWs (6.8%) were moderately susceptible (MS) or susceptible (S) with disease scores of 3.0–4.1. These scores were still lower than the scores of the susceptible checks, Sonalika, and Ciano T79.

According to the pedigree information, SB and tan spot resistance of the panel might be based on diverse DW and *Ae. tauschii* backgrounds and was thus likely contributed by multiple SB and tan spot resistance genes that was supported by in agreement with the GWAS results.

Significant marker trait association for tan spot

Significant markers found at the D- genome for tan spot

Our study found significant marker-trait associations (MTA) for tan spot resistance on chromosome 1D (marker ID 3026113, 2D (marker IDs 1217275, 1046621), 3D (marker IDs 987556, 1125862, 1217411), 4D (marker ID 4993454) and 7D (marker IDs 16793126, 991140, 993425). Thus, this is the first study to detect several significant genomic regions to tan spot resistance in the D-genome, in addition to the few loci reported previously. (Phuke, 2020) found a significant marker on chromosome 7D located at 550,216,751 Mb in CS. The closest significant marker on chromosome 7D in this study (marker ID 993425) was positioned at 620,252,508 Mb, physically distant and suggesting that at least two of the three marker-trait associations on chromosome 7D in this study are novel. The physical position of the third marker 991140 in CS could not be determined.

(Tadesse, 2007) studied resistance to tan spot in segregating F2:3 derived populations of SHW using simple sequence repeat (SSR) markers. The authors found that loci tsn3a, tsn3b and tsn3c are all located in the vicinity of the marker Xgwm2a located on chromosome 3D. The physical distance of this SSR marker to the SNP markers in our study was difficult to determine. (Gurung, 2011) performed GWAS in spring wheat landraces and using DArT markers to identify chromosome regions associated to tan spot race 1 and 5 resistances. The authors found significant markers, among others, on chromosomes 1D and 7D associated to tan spot race 1 and in regions of chromosomes 2D and 7D for tan spot race 5. Like the study by (Tadesse, 2007), genomic regions could not be compared, as different genotyping platforms were used.

Significant markers found at the A and B genome for tan spot

The present study found significant marker-trait associations on the A-genome chromosomes 2A (marker ID 10770935), 3A (marker IDs, 1125872, 1668224, 1019955, 1065211) and those forming a QTL on chromosome 6A (marker IDs, 1862737, 100027398, 1254459, 2266481, 4993056, 5331622). None of the marker-trait associations coincided with those reported by Juliana (2018), except on chromosome 3A. Marker 1125872 was located at 135,590,641 Mb in our study and the marker in (Juliana, 2018), at 182,028,651 Mb. In the B-genome chromosomes, we found significant marker-trait associations on chromosomes 1B (markers IDs, 1106306, 6045377, 1089962, and 4909460), 4B (marker ID, 4993454), 5A (marker IDs, 4393896, 1200982, 100034112, and 3064590), 6B (marker ID, 1112961); none of them were reported by Juliana, (2018).

Phuke (2020) also found several marker-trait associations in the A- and B-genomes. The authors found a significant marker on chromosome 2A but in a different position than the one found in this study. A significant locus on chromosome 1B mapped to a physical position in 465,584,555 Mb and was also distant from markers in chromosome 1B of this study located in 340,462,174 Mb and 558,561,647 Mb. Significant markers on chromosome 6A were located at 596,903,177 Mb and coincided with the physical position of the QTL found in this study in physical positions 599,622,814 Mb, 601,233,092 Mb, 602,989,232 Mb, and 602,745,555 Mb, thus representing the same QTL. The marker located on chromosome 5A (Phuke, 2020) mapped to the physical position of 597,291,565Mb, whereas the markers identified in this study forming a QTL are located a distance apart, in 454,770,615 Mb, 471,723,681 Mb, and 470,186,523 Mb, thus likely presenting a novel QTL.

The study by Kokhmetova, (2021) detected three significant loci on chromosome 1B within the range of 86.7-92.2 cM, not distant from marker ID 1089962 located at 83.6 cM in this study using the same 100K consensus map. Furthermore, the QTL on chromosome 6A were in proximity to the markers found by Kokhmetova, (2021) in the same chromosome.

Kalia (2018) performed bi-parental QTL mapping for resistance to tan spot race 1 in a population with a SHW parent. QTL identified were located only on the A-genome, on chromosomes 1A, 6A, and 7A. Because DArT markers were used in this study, the physical positions of the QTL were, once again, difficult to compare. Chu (2008) identified QTL on chromosomes in the A- and B-genome (2A, 5A and 5B) in a bi-parental mapping study using a SHW parent. The authors hypothesized that the expression of tan spot resistance genes in DW is suppressed (or diluted) but are activated when DW is crossed with Ae. tauschii, which could be due to inter-locus interaction (epistasis effects) between loci on A/B- and D-genomes. In the current study, increased resistance in SHW in comparison to their direct DW parents supports this hypothesis.

Significant marker trait association for spot blotch

Significant markers found at the A, B and D genome for spot blotch

Previous genetic studies have identified a range of SB resistance genes/QTL, residing on all wheat chromosomes except 4D and 5D (Su, 2021). Some of these loci exhibited major effects, such as the nominated Sb genes, yet most of them showed minor effects. The same applies to the current

study, where a total of 41 significant markers on 15 chromosomes were found to be associated with SB resistance, and none of them showed any major effects. This again confirmed the polygenic nature of SB resistance described in previous studies (Singh, 2018, Roy, 2021 and Juliana, 2022). The significant MTAs were identified on AB genome chromosomes as well as on D genome chromosomes, suggesting that SB resistance in the SHWs was derived from both their DW and *Ae. tauschii* parents. MTAs were identified on all seven D genome chromosomes, especially chromosomes 4D and 5D, on which no QTL/MTA has been reported so far (Su, 2021); thus, confirming their novelty. The two MTAs on chromosome 4D were located on short arm (marker 2243087) and long arm (marker 3023637); on chromosome 5D the physically distant markers must represent two different QTL. MTAs on chromosomes 1B (marker 1145134), 2D (marker 1122278 and 2243785), 3A (marker 1019955 and 2279238), and 6D (marker 1698662) also suggested to be novel since no QTL/MTA has been reported in the vicinity of these markers (Su, 2021).

However, for SB, some MTAs were found within known QTL regions. For example, the two MTAs on chromosome 1BS (markers 4261287 and 7335825) were near the MTAs reported by (Ahirwar, 2018). Likewise, on chromosome 3B, marker 4992362 was closely located to an MTA reported by (Bainsla, 2020). Nevertheless, close linkage or coincidence does not necessarily mean that the identified regions represent the same QTL/MTA, especially because our study screened SHW, while those published previously evaluated common wheat. It is noteworthy that some markers did not show any BLAST hit on the three reference genomes, e.g., marker 7335825 on chromosome 1B and marker 7492146 on chromosome 2B. These MTAs represent variants absent in the reference genomes and might be worthy of further investigation.

Underlying candidate genes based on protein for tan spot

For tan spot, two markers, one on chromosome 5A (marker ID 3064590) positioned at 470,186,523 Mb and the other one located on chromosome 6A (marker ID 1862737) in position 599,622,814 Mb were of particular interest in this study as they were positioned within genes that code for disease resistance related proteins, i.e., TraesCS5A02G254500/TRITD5Av1G155700 (F-box protein) and TraesCS6A02G378800/TRITD6Av1G217060 (cytochrome P450).

Candidate genes TraesCS5A02G254500 / TRITD5Av1G155700 code for F-box proteins that play a role in protein regulation and degradation, plant photoperiodic and hormone signaling transduction. A total of 1796 F-box proteins have been identified and classified in wheat (Zhang, 2019), many of which have been related to biotic stresses, particularly to fungal pathogens. In addition, F-box proteins have been observed to affect the plant metabolism and the regulation of plant enzymes involved in several diverse cellular processes (Zhang, 2091). It has been found that the F-box proteins can act in different development stages in a wheat cultivar. The identification of underling genes being related to specific disease resistance should offer an opportunity to further elucidate the biological functions of F-box genes and proteins in wheat.

The cytochrome P450 (CYP) enzyme in plants is involved in the biosynthetic pathway of phytoalexins that are synthetized by plants to deter hostile organisms (Durst, 1993). This CYP enzyme plays an important role in in the metabolism of herbicides as a key factor in providing tolerance to some species and thus selectively between crops and weeds. Plants encounter various biotic and abiotic factors at different stages of their growth and development and the group of CYP enzymes are important in the synthesis of certain metabolites which play a fundamental part in the response to biotic stresses. The CYT enzymatic protein participates in the formation of numerous secondary synthetized metabolites that protect plants from biotic and abiotic stresses (Jun, 2015). The mycotoxin deoxynivalenol (DON) is a virulent factor for the development of Fusarium head blight in wheat. A wheat cytochrome P450 subfamily was found in chromosome 3B and 3D of the wheat genome that was activated in the wheat spikelets as a response to the mycotoxin DON (Gunupuru, 2018).

Underlying candidate genes based on protein for spot blotch

For the case the spot blotch, the significant markers identified from the GWAS were further evaluated for their association with disease resistance-related genes. We identified 23 plant defense-related protein families across multiple chromosome regions, of which only 13 have a known protein function. For example, marker 12779374 on chromosome 1D was identified within the gene TRITD1Bv1G224330 (Tables S5 and 2), which is involved in the synthesis of the lectin receptor kinase that has an important function for the general immunity of the plants (Wang, 2014). Similarly, marker 1240012 on 7D was located within the gene TRITD2Bv1G075350 related to protein U-box domain containing protein 4, associated with the control of grain production (Song,

2007). However, it should be noticed that these candidate genes might not be the underlying genes for the MTAs, due to the usually large link-age disequilibrium blocks in the wheat genome (Juliana, 2018).

Furthermore, marker 1283998 on chromosome 3B marked an SNP within gene TRITD3Bv1G194800, which is a protein described as disease resistance protein RPM1 G, again involved in the general resistance of plants to various diseases (Gettins, 1996). Marker 4992362 on chromosome 3B marked the gene TRITD3Bv1G257410, which is identified as protein Serpin that participates in the regulation of proteolytic complex systems (Gettins, 1996), whereas marker 1011260 (in chromosome 3D) falls within the gene TraesCS3D02G407000, a peroxidase protein that has the divergence role in different pathogens systems in plants (Dmochowska, 2013). Furthermore, marker 100016153, aligned on chromosomes 5A and 5D, was located within the genes TraesCS5A02G146400 and TRITD5Av1G111170, in which two proteins, Man-nan endo-1,4 -beta-mannosidase 6 and Mannan endo-1,4-beta-mannosidase-like protein, are involved.

Note that marker 4002611 on chromosome 7A did fall within the gene TRITD7Av1G003410, a Pectin lyase-like superfamily protein, which has an important role in the development and maturity process of the plant. This protein also acts on the peptic substances presented as structural polysaccharides in the primary cell walls of the superior plants (Sangeeta, 2009). Marker 22765212, on chromosome 7D, was included in gene TraesCS7D02G278500, which is found in the ribosomal protein that plays a fundamental integral role in the growth and development of the plant, as well as participating in the general defense mechanism of the plants (Vemanna, 2020).

Interpretation of Results from Partial Least Squares (PLS) for SB

In the PLS analysis for SB, a subset of markers and SHW lines were identified that are more suitable for future breeding and pre-breeding activities. In the case of tan spot, the PLS was not carried out because it was not the objective of the research.

Results of this study in SB showed 15 molecular markers with a frequency of R alleles greater than 84% and 32 SHW lines having more than 32 resistance alleles. The PLS plot show the specific locations of the 15 markers and the 32 most resistant SHW lines. From a practical breeding perspective, these markers with R alleles and the SB resistance lines could be used in future breeding crosses.

Application of GWAS for Use in Practical Breeding for tan spot and SB

Genome-wide association studies (GWAS) are a powerful option for the genetic characterization of quantitative traits and have been widely used to analyze agronomic and disease traits. With the increasing number of diseases affecting cultivated wheat plants, the option of developing resistance SHW lines has been widely used. In these GWAS studies we assessed significant MTA of tan spot and SB from a diverse collection of 443 SHW lines, and 41 significant markers for SB and 30 for tan spot as well as a range of SHW lines with high SB and tan spot resistance were identified. These significant markers for SB and tan spot could be used in plant breeding to identify SHW resistance to these foliar diseases that could be introgressed into elite wheat lines.

CONCLUSIONS

This is the first GWAS study to investigate marker trait associations (MTAs) for tan spot and SB resistance in a diverse collection of 443 SHW lines from CIMMYT. GWAS found a total of 41 significant markers related to SB resistance, being distributed on 15 wheat chromosomes, and for tan spot a total of 30 significant marker-trait associations. Several of the MTA found in this study can contribute to the genetic diversity of resistance, specifically those on D genome contributed by *Ae. tauschii*, which were almost all novel, but also several on the A and B genomes. Furthermore, our study supports the previous concept of possible inter-locus effects caused by the activation of resistance genes in the DW genomes by interaction with the D genome of *Ae. tauschii* after hybridization

Our research identified new sources of resistance to tan spot and SB in CIMMYT's SHW that can be used in wheat breeding via crosses and backcrosses with elite bread wheat lines in wheat breeding programs.

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CHAPTER 1. GENOME-WIDE ASSOCIATION STUDY FOR RESISTANCE TO TAN SPOT IN SYNTHETIC HAXAPLOID WHEAT

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General Discussion

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