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Identification and mapping of QTL for resistance against *Zymoseptoria tritici* in the winter wheat accession HTRI1410 (*Triticum aestivum* L. subsp. *spelta*)

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“Identification and mapping of QTL for resistance against

***Zymoseptoria tritici* in the winter wheat accession**

HTRI1410 (*Triticum aestivum* L. subsp. *spelta*)”

Dissertation

zur Erlangung des
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SUMMARY

Zymoseptoria tritici, the causal agent of Septoria tritici blotch (STB), causes yield losses of up to 50 % in wheat, globally. Growing of resistant cultivars is the most cost effective and environmental friendly way to avoid these losses. *Zymoseptoria tritici* is present worldwide and has gained evident importance due to changes in wheat growing practices. Fungicides such as strobilurins and azoles lost their efficiency in controlling STB. Therefore, there is a need to conducted screening of gene bank accessions for resistance, get information on the genetics of resistance and develop molecular markers for the efficient deployment of new resistances in wheat breeding. In extensive screening programs for resistance, the spelt wheat gene bank accession HTRI1410 turned out to be resistant in field trials and to be a valuable source for improvement of resistance to *Zymoseptoria tritici* in wheat, therefore. In order to get information on the genetics of the STB resistance in HTRI1410, a DH population consisting of 135 lines derived from crosses of HTRI1410 to three susceptible cultivars, i.e. 'Alcedo', 'Jenga' and 'Solitär', was generated. Based on two years and three locations, the heritability for STB resistance was calculated at h^2= 0.55. In addition to the extensive field trials, artificial inoculation in detached leaf assays was conducted using three isolates (IPO323, IPO98022, IPO98050) and the necrotic mean leaf area was determined. A quantitative variation for the reaction to a *Zymoseptoria* infection was observed and a significant genotypic effect detected. In parallel the DH population was genotyped by the wheat 90k iSelect SNP chip. The genotypic data were used for map construction. About 6,000 SNPs turned out to be polymorphic between HTRI1410 and the three susceptible cultivars. Out of these, 1,118 SNPs were mapped to the A genome, 1,326 SNPs mapped to the B genome and 267 SNPs to the D genome. QTL analyses based on field trials revealed QTL on chromosomes 5A, 4B and 7B. In addition, based on the detached leaf assay, 17 QTL were detected on chromosomes 1A, 2A, 3A, 4A, 6A and 1B, 2B, 5B. Furthermore, KASP markers for respective QTL were developed facilitating efficient marker based selection for resistance to STB.

ZUSAMMENFASSUNG

Zymoseptoria tritici, der Erreger der Septoria-Blattdürre (STB), verursacht weltweit Ertragsverluste von bis zu 50 % und hat an Bedeutung durch Veränderungen im Weizenanbau gewonnen. Der Anbau resistenter Sorten ist der kostengünstigste und umweltfreundlichste Weg, diese Verluste zu reduzieren. Typische Symptome dieses Schaderregers sind nekrotische Blattflecken. Häufig genutzte Fungizide, wie Strobilurine und Azole verlieren ihre Wirksamkeit bei der Bekämpfung von STB. Folglich besteht die Notwendigkeit, Genbank-Akzessionen auf Resistenzen zu untersuchen, Informationen über die Genetik der Resistenz zu gewinnen und molekulare Marker für den effizienten Einsatz neuer Resistenzen in der Weizenzüchtung zu entwickeln. Die Spelzweizen Genbankakzession HTRI1410 erwies sich in Feldversuchen als resistent und damit wertvolle Quelle für die Verbesserung der Resistenz gegen *Z. tritici* in Brotweizen. Um die Genetik der STB-Resistenz in HTRI1410 zu untersuchen, wurde eine DH-Population, bestehend aus 135 Linien, die aus Kreuzungen von HTRI1410 mit den drei anfälligen Sorten 'Alcedo', 'Jenga' und 'Solitär' stammen, erzeugt. Basierend auf zweijährigen und dreiortigen Feldversuchsergebnissen ergab sich eine Heritabilität h^2= 0,55 für die STB-Resistenz. Zusätzlich zu diesen umfangreichen Versuchen wurde eine künstliche Inokulation in einem Blattsegementtest mit drei ausgewählten Isolaten (IPO323, IPO98022, IPO98050) durchgeführt und die mittlere, nekrotisierte Blattfläche bestimmt. Eine quantitative Variation für die Reaktion hinsichtlich einer *Zymoseptoria*-Infektion wurde beobachtet und ein signifikanter genotypischer Effekt festgestellt. Parallel dazu wurde die Population mit dem 90k iSelect SNP Chip genotypisiert. Die genotypischen Daten wurden für die Erstellung einer genetischen Karte verwendet. Etwa 6.000 SNPs erwiesen sich als polymorph zwischen der resistenten Akzession und den drei anfälligen Eltern. Von diesen wurden 1.118 SNPs auf dem A-Genom kartiert, 1.326 SNPs auf dem B-Genom und 267 SNPs auf dem D-Genom. In QTL-Analysen basierend auf den Feldversuchsergebnissen, wurden QTL auf den Chromosomen 5A, 4B und 7B lokalisiert. Basierend auf dem Blattsegmenttest wurden 17 QTL auf den Chromosomen 1A, 2A, 3A, 4A, 6A und 1B, 2B, 5B nachgewiesen. Weiterhin wurden KASP-Marker für entsprechende QTL entwickelt, die eine markergestützte Selektion auf STB-Resistenz erlauben.

1 GENERAL INTRODUCTION

WHEAT (*TRITICUM AESTIVUM* L.)

Bread wheat (*Triticum aestivum* L.) belongs to the *Poaceae*, one of the largest family in the monocotyledons (Strasburger et al. 2008). The genus *Triticum* contains diploid, tetraploid as well as hexaploid species (Sharma 2012) such as the allohexaploid bread wheat which consists of 2n = 6x = 42 (AABBDD) chromosomes comprising three homologous genomes, i.e. A, B, and D. Thus, bread wheat has a very large and complex genome of about 17 Gb (Bennett and Leitch 2010) and compared to the sequenced rice genome (489 Mb), it is about 35 times larger and consists of up to 80 % repetitive deoxyribonucleic acid (DNA) (Bennett and Leitch 2010; Goff et al. 2002; Smith and Flavell 1975; Yu et al. 2002). Meanwhile, the complete sequence of the wheat genome is known (Appels et al. 2018).

The origin and center of diversity of wheat is the region of the Fertile Crescent (Brown et al. 2009). About 300.000 - 500.000 years ago, via allopolyploidization of the A genome´s donor, *Triticum urartu* (AA) and the donor of the B genome, which due to sequence similarities most likely originates from the Sitopis section of *Aegilops* (BB) (Jiang et al. 2012), the tetraploid wheat *T. turgidum* ssp. *dicoccoides* (AABB) arose. Thus, based on their ploidy level, *Triticum* species can be categorized into three groups (Figure 1) (Borrill et al. 2019). By cultivation, wild Emmer (AABB) (*T. turgidum* ssp. *dicoccoides*) got domesticated and emmer wheat (*T. turgidum* ssp. *dicoccum*) arose (Ozkan et al. 2002; Peng et al. 2011) and resulted in durum wheat (*Triticum turgidum* L. ssp. *durum*) (Maccaferri et al. 2019).

The hexaploid bread wheat (AABBDD) (*Triticum aestivum*) developed by allopolyploidization of the tetraploid domesticated Emmer (AABB) with *Aegilops tauschii* (DD), the donor of the D genome (Kilian et al. 2010). In the course of the domestication of wheat, selection processes took place on morphologically characteristic and important traits i.e. non brittle rachis or the reduction of glumes (Faris et al. 2003; Peng et al. 2003).

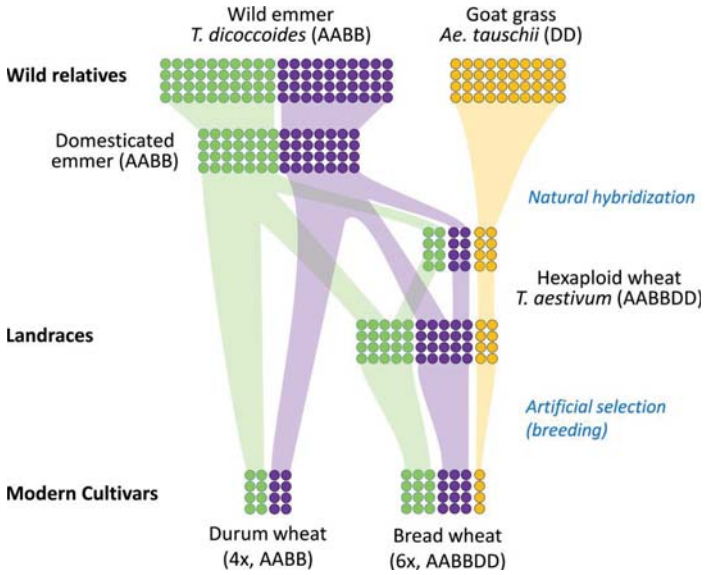

FIGURE 1 Taxonomy of cultivated *Triticum* species (Borrill et al. 2019).

Based on the ploidy level, the products for global consumption for human nutrition may be divided in those derived from hexaploid and tetraploid wheat. The largest part of approx. 95 % is bread wheat consumed mostly as bakery products and biscuits. The smaller part is built on the tetraploid durum wheat with 5 % used for pasta, bulgur and and couscous (Borrill et al. 2019; Peng et al. 2011). Depending on its quality level, wheat is used in different ways. Most important for human nutrition is the production of bread and bakery products. Because of its importance for human nutrition, wheat is an essential commodity and economic factor. In 2017, 771 million tons of wheat were produced and exported with a total value of 39 billion US$, globally (FAO 2017). Wheat also plays an important role in animal feeding (BMEL 2018). Furthermore, wheat is used for biogas and fuel production (Christen 2009). Thus, together with rice (*Oryza sativa* L.) and maize (*Zea mays* L.), hexaploid bread wheat (*Triticum aestivum*) is one of the most important crop species for feeding the earth´s growing population (http://www.fao.org/faostat/en/#data/QC). Worldwide, wheat was grown on 218 million hetares and 771 million tons were produced in 2017. In Germany, winter wheat is the most important

crop grown on 3.2 million hectares and a production of 24 million tons per year in 2017 (FAO 2017). On the worldwide level, the average yield is 3.5 t/ ha whereas in Germany 7.6 t/ ha and in Europe 4.3 t/ ha were produced in 2017 (FAO 2017).

Due to climatic changes and its consequences, new challenges arose for instance heat and drought stress which lead to changing growing conditions and a different situation with respect to pests and diseases, especially in intensive cropping systems like in Germany (Freyer 2003). In future, varieties are needed that are better adapted to the changing climate and to a changing occurrence of pathogens. Up to now, about 30 % of yield improvements in wheat are achieved by breeding and 70 % by changes in cropping systems (Ahlemeyer and Friedt 2012). Current varieties are shorter, harvested earlier, less susceptible to lodging and are more resistant to important wheat diseases like Powdery mildew (*Blumeria graminis*), leaf rust (*Puccinia tritici*) and Septoria tritici blotch (*Zymoseptoria tritici*, STB) than older cultivars (Ahlemeyer and Friedt 2012).

WHEAT DISEASES

A broad range of fungal pathogens is able to infect winter wheat cultivars causing high yield losses worldwide (Oerke and Dehne 2004). The most frequent wheat diseases in northern Europe are Powdery mildew (*Blumeria graminis*), rust diseases (*Puccinia tritici*, *Puccinia striiformis*), Septoria tritici blotch (*Zymoseptoria tritici*) and tan spot (*Pyrenophora tritici-repentis*) damaging leaves; Fusarium head blight (*Fusarium* spp.) and Septoria nodorum blotch (*Phaeosphaeria nodorum*) damaging ears and eyespot (*Pseudocercosporella herpotrichoides*) and so called take-all (*Gaeumannomyces graminis* var. *tritici*) disturbing the roots (Kosellek 2013). In total, in Germany about 310 million euro are spent for fungicide applications (Fones and Gurr 2015; Torriani et al. 2015).

In Germany and northern Europe, i.e. in regions with periods of cool and wet weather, Septoria tritici blotch has become one of the most important wheat diseases causing yield losses up to 30 % in susceptible cultivars (Eyal et al. 1987; Fones and Gurr 2015; Ponomarenko et al. 2011).

In addition to controlling diseases by fungicides, another option is to improve the resistance to fungal diseases by breeding resistant cultivars (Christen 2009), although breeding of resistant varieties and the development of new, effective fungicides is time consuming (Orton et al. 2011).

SEPTORIA TRITICI BLOTCH

In northern Europe, Septoria tritici blotch turns out to be the most important foliar disease in wheat which led to yield losses up to 30 % in susceptible cultivars (Eyal et al. 1987; Jahn et al. 2012). To control STB, around 70 % of the estimated fungicides used in cereals in Europe is applied to wheat (Ponomarenko et al. 2011). The efficiency of triazoles and quinine outside inhibitors (QoIs), the two main groups of fungicides against STB has decreased as fungal populations have developed high degree of insensitivity (Fraaije et al. 2005; Fraaije et al. 2007). The current varieties released in Germany include merely moderately STB resistant cultivars. Regarding wheat cultivars with the largest acreage of propagation in Germany, susceptible cultivars with STB scores larger than four are predominant (Figure 2).

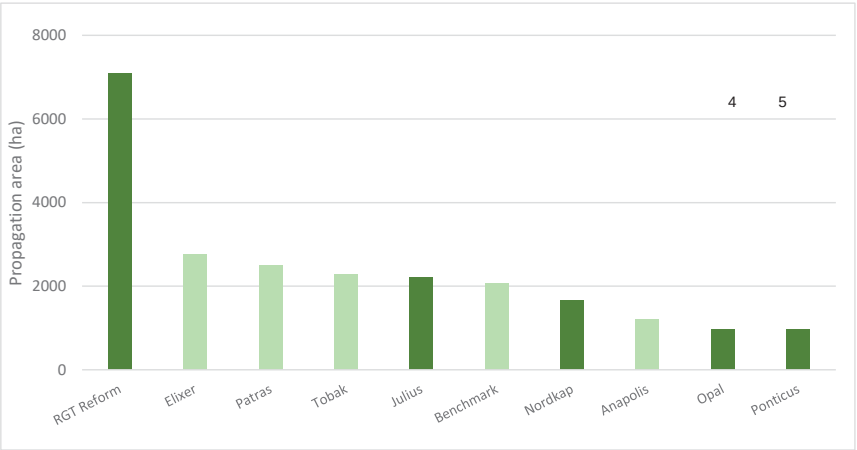

FIGURE 2 Acreage (ha) of seed multiplication of the ten most important wheat cultivars in Germany and their corresponding Septoria tritici blotch (STB) score (1: minimum susceptibilty; 9: maximum susceptibility (Kosellek 2013; Strotmann 2017).

The ascomycete fungus *Zymoseptoria tritici* (Desm.) (Quaedvlieg et al. 2011) is the causal agent of Septoria tritici blotch. Besides bread wheat and durum wheat, also Einkorn (*T. monococcum*), Emmer (*T. turgidum* var. *dicoccum*) and *Aegilops* ssp. are host species of *Z. tritici* (Jing et al. 2008; McKendry and Henke 1994). A new genus for Septoria-like fungi was generated in 2011 and from that time, the teleomorphic form *Mycosphaerella graminicola* describes the sexual stage and the anamorph *Septoria tritici* was renamed into *Zymoseptoria tritici* (Quaedvlieg et al. 2011). Stukenbrock et al. (2007) reported about an already existing co-evolution of *Mycosphaerella graminicola* with the known domestication of wheat in the Fertile Crescent. Septoria tritici blotch is now a worldwide occurring disease spreading by wind-borne ascospores. As a result of gene flow the pathogen is highly variable (Orton et al. 2011; Zhan et al. 2003). Characteristic for *Zymoseptoria tritici* is the hemibiotrophic lifestyle. The disease cycle of *Z. tritici* starts in autumn with the primary inoculum, i.e. air-borne ascospores are transferred by wind-dispersal from neighboring or more distant infected wheat debris to the leaves (Figure 3) (Shaw and Royle 1989; Suffert and Sache 2011).

Beginning with the biotrophic growth stage living cells are needed and sexual ascospores and asexual pycnidiospores germinate on wheat leaves and the fungus penetrates the leaf surface through stomata after 24 to 48 h (Palmer and Skinner 2002). After penetrating the stomata, *Z. tritici* grows very slowly intracellularly in the apoplast. No haustoria-like structures are observed for *Z. tritici,* the fungus feeds by the germinating spore and nutrients in the apoplast during the biotrophic phase (Brunner et al. 2013; Goodwin and Thompson 2011; Rudd et al. 2015).

Then, a necrotrophic phase follows in which host cells are killed, necrotic lesions appear on the leaves on the surface and black fruiting bodies develop inside the lesions (Perfect and Green 2001). The shift from the biotrophic to the necrotrophic stage is not well understood up to now. It is difficult to categorize *Zymoseptoria tritici* as a hemibiotrophic fungus since *Z. tritici* already nourishes on collapsed mesophyll cells although it is actually still in the biotrophic phase. Consequently, *Z. tritici* is defined as a ‘latent necrotrophic’ fungus (Sánchez-Vallet et

al. 2015). The distribution in wheat fields is mainly due to the pycnidiospores which are produced and located in the pycnidia, the fruit bodies of *Z. tritici,* whereas the spores of the sexual stage *M. graminicola*, the ascospores, are produced inside the sexual fruiting bodies, i.e. pseudothecia (Cunfer and Ueng 1999; Sanderson 1972, 1976). After an incubation period of 21 to 28 days after infection, Septoria tritici blotch water-soaked, chlorotic, ambiguous spots on leaves are visible which develop into irregular necrotic lesions. After a certain period of time combined with high humidity, the sexual and asexual spores are released from the tendril-like spore masses. During winter, the fungus survives on plant debris as pseudothecia and in pycnidia (Ponomarenko et al. 2011).

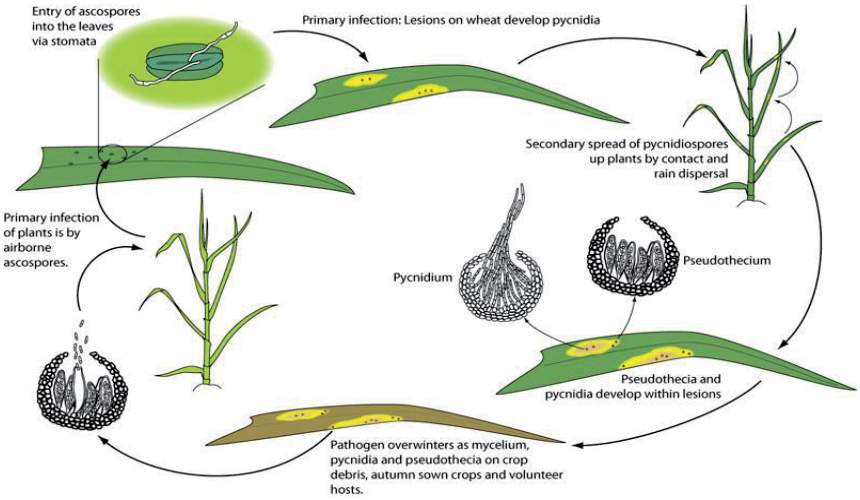

FIGURE 3 Life cycle of *Zymoseptoria tritici* (Ponomarenko et al. 2011).

Disease severity depends on cultivar, temperature and humidity. For a successful infection, the fungus needs periods of wet weather and moderate temperature (20 °C to 25 °C) and high humidity (≥ 85 %) (Ponomarenko et al. 2011; Raman and Milgate 2012). Sunlight and high temperatures decrease the number of airborne spores derived from pycnidia and ascomycetes (Duvivier et al. 2013; Suffert and Sache 2011). Infection of neighbored plants by *Z. tritici* pycnidiospores takes place by physical contact and rain splash. The sexual cycle takes place only

a few times between growing seasons on plant debris whereas several cycles of asexual reproduction take place (Goodwin 2007; Ponomarenko et al. 2011). For most infections pycnidiospores are responsible since pycnidia encompass a significantly higher number of spores compared to the ascospores contained in pseudothecia (Eriksen and Munk 2003).

In the latent period, *Z. tritici* is already present in the plants but no symptoms are visible, yet. *Z. tritici* grows within the leaf for a period of 12 to 28 days until first symptoms are visible (Ponomarenko et al. 2011). Until the first symptoms appear, fungicides only have a limited effect (Fones and Gurr 2015). Several factors influence the latent period. At optimum temperatures of 15 °C to 20 °C and relative humidity ≥ 85 % the latent period is 14 to 20 days whereas colder temperatures of 5 °C prolong the latent period up to 40 days (Eyal et al. 1987; Shaw 1990). Tolerant or resistant wheat varieties have a longer latent period compared to susceptible varieties (Mojerlou et al. 2009; Viljanen-Rollinson et al. 2005) but also the developmental stage and the age of the leaf have an impact on the latent period (Suffert and Thompson 2017). Visible, necrotic leaf areas contain small black pycnidia scattered within the entire lesion. Pycnidia are often arranged in rows since they are formed in sub-stomatal cavities (Figure 4) (Palmer and Skinner 2002).

FIGURE 4 Symptoms of *Zymoseptoria tritici*.

Severity of STB in wheat crops decreases with later sowing (Gladders et al. 2001a) since the primary infection takes place in atumn (Suffert and Sache 2011). The use of fungicides is the most common way of preventing losses due to STB. Due to the sexual reproduction of *Z. tritici*

and the intensive use of fungicides many *Z. tritici* populations have rapidly developed resistance to demethylation inhibitor *(*DMIs) fungicides (Torriani et al. 2009). The most common fungicides currently being applied against STB are azoles (Ponomarenko et al. 2011). Furthermore, the use of fungicides in general is regarded as a hazard to the environment. Therefore, there has been a major focus on resistance breeding in recent years.

GENETICS OF RESISTANCE TO *ZYMOSEPTORIA TRITICI*

When breeding resistant wheat varities, European wheat breeders have to take different genetic meachanisms for STB resistance into account (Arraiano and Brown 2006), i.e. quantitative and qualitative resistance (Brown et al. 2015; Dreisigacker et al. 2015). Quantitative resistance is isolate non-specific, horizontal, and inherited oligo- or polygenically (Keller et al. 2000). By increasing the latency period, the quantiative resistance slows down the disease development (Chartrain et al. 2004b). The second type of resistance to *Z. tritici* is characterized as a qualitative, isolate-specific, vertical resistance based on major genes (Brown et al. 2015) and based on the effective interaction of the resistance gene and the avirulence gene (Flor 1971). The quantitative, non-isolate-specific resistance of wheat to STB is more durable but less effective than the qualitative resistance. Consequently, a combination of quantitative resistances with major genes may extend the effectiveness of major genes.

To date, 21 *Stb* genes and numerous quantitative trait loci (QTL) have been identified in different mapping populations (Table 1) (Brown et al. 2015).

Stb1 is located on the long arm of chromosome 5B and was identified in the winter wheat cultivar ‘Bulgaria88’ (Adhikari et al. 2004a). Together with *Stb1*, resistance gene *Stb2* is mapped on chromosome 1B while *Stb3* is located on chromosome 7A (Brown et al. 2015). Both were originally detected in the wheat cultivars ‘Veranopolis’ and ‘Israel 493’.

Stb4 on chromosome 7D was identified in the winter wheat cultivar ‘Tadinia’ (Adhikari et al. 2004b) which was released in 1985. *Stb4* was effective for almost 15 years but has meanwhile been overcome (Jackson et al. 2000; Somasco et al. 1996). On the short arm of chromosome 7D, the resistance gene *Stb5* is located (Brown et al. 2001). *Stb6* was detected in the cultivar

'Flame' and mapped on the short arm of chromosome 3A using *Z. tritici* isolate IPO323 (Brading et al. 2002). In QTL studies additional STB QTL have been located close to *Stb6* (Eriksen et al. 2003; Ghaffary et al. 2011; Goudemand et al. 2013; Kelm et al. 2012; Zwart et al. 2010). Furthermore, *Stb6* was already cloned and it turned out that *Stb6* encodes a kinase identifying pathogen effector proteins and gives a resistance response without a hypersensitive reaction. Resistance is due to a single nucleotide polymorphism (Saintenac et al. 2018). *Stb7* is located on the long arm of chromosome 4A and was detected in line 'St6' and later on in 'TE9111' (Chartrain et al. 2005a; McCartney et al. 2003). *Stb8* was mapped on the long arm of chromosome 7B (Adhikari et al. 2003). *Stb9* provides resistance against IPO89011 and was located on chromosome 2B in the wheat cultivar 'Courtot' (Chartrain et al. 2009). Even though *Stb10* is efficient against the same isolate as *Stb5*, these genes are located on different chromosomes (Chartrain et al. 2005c). *Stb11* is located on the short arm of chromosome 1B (Arraiano et al. 2007), same as *Stb2* but up to now no allelism tests have been conducted. *Stb12* is mapped on the long arm of chromosome 4A and therefore is located in the same region as *Stb7* but provides resistance to another isolate than *Stb7*. Resistance genes *Stb13* and *Stb14* were mapped on chromosomes 7B and 3B, respectively, (McIntosh 2007) encoding resistance against the same isolate as *Stb7* on chromosome 4A, but all three genes are located on different chromosomes. *Stb15* is located on the short arm of chromosome 6A and was identified in the cultivar 'Arina' (Arraiano et al. 2007). *Stb16* was identified in the synthetic hexaploid wheat line 'M3' on the long arm of chromosome 3D (Ghaffary et al. 2012). It confers seedling resistance to all tested isolates of *Z. tritici* (Brown et al. 2015; Raman and Milgate 2012). *Stb17* was identified in a synthetic hexaploid wheat line and it is the first qualitative gene specifically expressing resistance in the adult plant stage (Ghaffary et al. 2012). *Stb18* has been located in the French winter wheat variety 'Balance' on the short arm of chromosome 6D and confers resistance to five different isolates of *Z. tritici* (Ghaffary et al. 2012). *StbWW* and *TmStb1* confer resistance in the seedling stage and were mapped on chromosomes 1B and 7A, respectively (Jing et al. 2008; Raman et al. 2009).

In addition to the *Stb* genes, up to now 89 QTL have already been identified (Brown et al. 2015) conferring resistance in the seedling and adult plant stage. Some of these QTL are co-located with qualitative *Stb* resistance genes.

TABLE 1 Overview of Septoria tritici blotch resistance genes in wheat.

Gene	Chromo-some	Closest marker	Stage	Variety	Reference
Stb1	5BL	Xgwm335	S, A	Bulgaria 88	Adhikari et al. (2004a)
Stb2	1BS	Xwmc230	A	Veranopolis	Liu et al. (2013)
Stb3	7AS	Xwmc83	A	Israel 493	Goodwin and Thompson (2011)
Stb4	7DS	Xgwm111	S, A	Tadinia	Adhikari et al. (2004c)
Stb5	7DS	Xgwm44	S, A	Synthetic 6x	Arraiano et al. (2001b)
Stb6	3AS	Xgwm369	S, A	Flame, Hereward	Brading et al. (2002)
Stb7	4AL	Xwmc313	S	ST6	McCartney et al. (2003)
Stb8	7BL	Xgwm146	A	Synthetic W7984	Adhikari et al. (2003)
Stb9	2BL	Xfbb226	S	Courtot, Tonic	Chartrain et al. (2009)
Stb10	1Dc	Xgwm848	S	Kavkaz-K4500	Chartrain et al. (2005c)
Stb11	1BS	Xbarc008	S	TE9111	Chartrain et al. (2005a)
Stb12	4AL	Xwmc219	S	Kavkaz-K4500	Chartrain et al. (2005c)
Stb13	7BL	Xwmc396	S	Salamouni	Cowling (2006)
Stb14	3BS	Xwmc500	S	Salamouni	Cowling (2006)
Stb15	6AS	Xpsr904	S	Arina, Riband	Arraiano et al. (2007b)
StbSm3	3AS	barc321	S	Salamouni	Cuthbert (2011)
Stb16q	3DL	Xgwm494	S, A	SH M3	Tabib Ghaffary et al. (2012)
Stb17	5AL	Xhbg247	A	SH M3	Tabib Ghaffary et al. (2012)
Stb18	6DS	Xgpw5176	S, A	Balance	Tabib Ghaffary et al. (2011)
StbWW	1BS	Xbarc119b	S	WW1842, WW2449, WW2451	Raman et al. (2009)
TmStb1	$7A^{m}S$	Xbarc174	S	MDR043	Jing et al. (2008)

It has been demonstrated that already known wheat varieties with STB resistance as 'Kavkaz-K4500 L.6.A.4 (KK)' (Chartrain et al. 2004b; Chartrain et al. 2005c), 'Salamouni' (Cowling 2006; Cuthbert 2011) and 'TE9111' (Chartrain et al. 2005a) contain multiple qualitative resistances (Brown et al. 2015; Chartrain et al. 2004b). This leads to the fact that 'pyramiding' of *Stb* resistance genes increases the effectiveness of resistance, an approach which already has been proven to be effective in controlling serveral diseases (Mundt 2014), e.g. stem and leaf rust (Green and Campbell 1979; McIntosh and Brown 1997; Mundt 2014; Roelfs and Bushnell W. R. 1985; Samborski 1985; Schafer and Roelfs 1985).

MOLECULAR BREEDING FOR STB RESISTANCE

Scoring symptoms of Septoria tritici blotch as a quantitative trait is complicated since phenotyping highly depends on environmental factors and the interaction of the QTL in different environments (Zhu et al. 2008). Many of the released cultivars in Europe carry known *Stb* genes. Partially these genes are still effective and confer resistance but in many studies it is reported that these *Stb* genes have only small effects (Arraiano et al. 2007; Arraiano and Brown 2006; Chartrain et al. 2009; Ghaffary et al. 2011).

Up to now, 21 *Stb* genes and dozens of QTL have been identified in various populations showing high and complex diversity (Brown et al. 2015). The majority of these were identified by using simple sequence repeat (SSR) markers, restriction fragment length polymorphism (RFLP) markers or amplified fragment length polymorphism (AFLP) markers (Table 1) (Brown et al. 2015).

The major gene *Stb6* being located on chromosome 3A (Brading et al. 2002) which has been isolated recently (Saintenac et al. 2018) has shown to also confer quantitative resistances in field trials (Arraiano et al. 2009). Moreover, Brown et al. (2015) have detected QTL for STB resistance close to the resistance gene *Stb6* in several populations.

To use STB resistances such as resistance gene *Stb6* and transfer them to wheat genotypes, several markers are available. Today, array technologies e.g. the 90K single nucleotide polymorphism (SNP) array (Wang et al. 2014) was already successfully applied to detect new genomic regions harbouring STB resistance (Karlstedt et al. 2019). Standard QTL mapping for STB resistance is based on bi-parental populations (Brown et al. 2015; Würschum 2012) allowing the detection of rare alleles also in populations with low genetic diversity (Vagndorf 2018). Latest QTL studies for STB resistance have detected new QTL conferring resistance to STB by using SNPs (Kidane et al. 2017; Vagndorf et al. 2017; Würschum et al. 2017) which have been used for numerous applications e.g. genome-wide association studies (GWAS), characterization of genetic resources, marker-assisted-selection (MAS) and genomic selection (Ganal et al. 2009; Ganal et al. 2012). Today, association genetics studies to identify QTL are

applied frequently to identify markers which are tightly linked to traits of interest such as STB resistance. It is a powerful tool to determine the genetics of complex traits based on linkage disequilibrium (LD), which is defined as "the non-random association of alleles at different loci within a population" (Ingvarsson and Street 2011; Korte and Farlow 2013; Rafalski 2010).

Opposite to QTL studies, GWAS are applied in diverse populations providing higher level of genetic diversity (Vagndorf et al. 2017). Several studies report about detection of loci conferring resistance to STB by using GWAS (Arraiano and Brown 2017; Gurung et al. 2014; Kollers et al. 2013).

SNP markers detected by GWAS or QTL analyses can be used to produce efficient tools for MAS in breeding, such as competitive allele specific (KASP) PCR markers. The detected QTL regions are flanked by KASP markers developed from SNP sequences, facilitating MAS for several traits (Cabral et al. 2014; Chhetri et al. 2017; Dreisigacker et al. 2015). The use of KASP markers allows a precise bi-allelic scoring of SNPs, insertions and deletions at specific loci (LGC protocol 2014) and thereby an easy implementation in breeding programs (Vagndorf 2018). Breeding methods such as MAS and pyramiding of resistances become more effective by the availability of the genome reference sequence (Appels et al. 2018) which recently has been published (Appels et al. 2018; Barabaschi et al. 2015; Perovic et al. 2018).

AIMS OF THE THESIS

The aim of the present thesis was mapping of the quantitative resistance against Septoria tritici blotch derived from the spelt gene bank accession HTRI1410 in field and greenhouse trials. To achive this, three doubled-haploid (DH) populations derived from crosses of HTRI1410 to the susceptible parental lines ‘Alcedo’, ‘Jenga’ and ‘Solitär’ were analysed.

In detail, the aims were (1) to map QTL involved in STB field resistance in adult plants based on a multi-environmental field trial, (2) to characterize the resistance of the DH populations to a worldwide *Zymoseptoria tritici* isolate collection in a detached leaf assay, (3) to identify genes and/or QTL conferring resistance to these STB isolates and (4) to develop molecular markers which facilitate the use of MAS in wheat breeding for STB resistance.

2 CHAPTER 1)

MAPPING OF QUANTITATIVE TRAIT LOCI (QTL) FOR RESISTANCE AGAINST *ZYMOSEPTORIA TRITICI* IN THE WINTER SPELT WHEAT ACCESSION HTRI1410

ABSTRACT

Zymoseptoria tritici, the causal agent of Septoria tritici blotch (STB) causes yield losses in wheat of up to 40 %, globally. Growing of resistant cultivars is the most cost effective and environmentally friendly way to avoid these losses. Therefore, there is a need to identify new resistances in gene bank accessions and to get information on the genetics of resistance followed by the development of molecular markers for the efficient deployment of these resistances in wheat breeding. In extensive screening programs for resistance, the spelt wheat gene bank accession 'HTRI1410' turned out to be resistant to *Zymoseptoria tritici* in field conditions. In order to get information on the genetics of the STB resistance in 'HTRI1410', a DH population consisting of 135 lines derived from crosses of 'HTRI1410' to three susceptible cultivars was developed. Significant genotypic differences and a quantitative variation for the reaction to *Zymoseptoria tritici* were observed. Based on these phenotypic data and a genetic map comprising 714 90K iSelect derived SNP markers four quantitative trait loci on chromosomes 5A, 4B and 7B, explaining 8.5 % to 17.5 % of the phenotypic variance were identified.

INTRODUCTION

Bread wheat is the staple food for about 35 % of the world's population. But wheat production suffers from many diseases resulting in considerable yield losses (Serfling et al. 2017). Therefore, with respect to feeding the earth´s growing population, protecting wheat against fungal diseases is of prime importance for food security (Curtis and Halford 2014; Eyal et al. 1987; Wheat Initiative 2018). As resistance is the most efficient and cost-effective way of plant protection, it is a permanent task for wheat breeding to improve resistance. Spelt wheat (*Triticum aestivum* subsp. *spelta)* is gaining increasing interest due to its adaptability to diverse growing conditions (high rainfall, heavy soils, cool temperatures) and its high quality with respect to human nutrition and animal feed (Lacko-Bartošová and Korczyk-Szabó 2011; Skrabanja et al. 2001). Besides this, spelt is a valuable source for improving resistance in bread wheat (*Triticum aestivum* L.) (Kema 1992). Resistances to stem rust (McVey 1990), stripe rust (Kema 1992), Septoria tritici blotch and Fusarium head blight (Wiwart 2004) were detected in spelt wheat, e.g. major genes and QTL for resistance to stripe rust (Sun et al. 2002) and leaf rust (Mohler et al. 2012) were located on chromosomes 2B and 2A, respectively.

Septoria tritici blotch (STB) caused by the fungal pathogen *Zymoseptoria tritici* (Rob. ex Desm.) Quaedvl. & Crous (teleomorph: *Mycosphaerella graminicola* (Fuckel) J. Schröt) (Quaedvlieg et al. 2011) is one of the economically most important foliar diseases of wheat (*Triticum aestivum* L.) worldwide (Eyal et al. 1987; Ponomarenko et al. 2011), and also in Western Europe (Pillinger et al. 2004).

Because of the increased proportion of cereals in crop rotations and the trend to non-tillage agronomical practices in wheat cultivation, a general increase in disease pressure with respect to fungal pathogens has been observed (Dreisigacker et al. 2015). This holds especially true in case of STB as an early and severe infestation with STB can result in yield losses of 20 % to 40 % in Europe (Klöhn 2011), especially in regions with continuous rainfall and moderate night temperatures during the growing season (Cornish et al. 1990). Since the spread of the asexual pycnidiospores of *Zymoseptoria tritici* in the field depends on moisture on the leaf

surface, conidia spread throughout the crop canopy via rain splash (Gladders et al. 2001b; Pietravalle et al. 2003). An infection with *Zymoseptoria tritici* results in a strong reduction of photosynthesis and a dwarfed growth habit leading to a reduced grain yield (Verreet and Klink 2010).

Several studies show that *Zymoseptoria tritici* has developed resistances against different fungicides such as azoles, strobilurins and triazoles (Cools and Fraaije 2008). Therefore, the development of resistant cultivars is the most environmentally friendly and economical solution for a sustainable wheat production as well as for long-term bio safety of agriculture systems. In order to broaden the genetic base of resistance to STB, extensive screening programs for resistance were conducted (Kosellek et al. 2013; Vagndorf et al. 2017).

Up to now, 21 qualitative resistance genes have been detected for resistance to Septoria tritici blotch (Brown et al. 2015). Additionally, 89 quantitative trait loci (QTL) for *Zymoseptoria tritici* resistance have been identified (Brown et al. 2015). Simón et al. (2005a) identified resistance to *Mycosphaerella graminicola,* the sexual stage of *Zymoseptoria tritici,* in wheat substitution lines on chromosome 7D derived from of *Triticum aestivum* subsp. *spelta*.

In former times, marker development was time consuming and laborious since e.g. simple sequence repeat (SSR) markers require a large investment of time and money to be develop and are not suited for high-density mapping due to their limited number (Sanchez et al. 2000; Shan et al. 1999; Shariflou et al. 2001; Sharp et al. 2001). Due to rapid advances in sequencing and genotyping technologies, single nucleotide polymorphism markers (SNPs) are increasingly applied in genetics and genomics (Elshire et al. 2011; Steemers et al. 2006). SNPs as the most abundant type of polymorphism are used extensively for marker development detected e.g. by genotyping by sequencing (GBS) (Elshire et al. 2011) or chip technology (Chen et al. 2013), e.g. the 90K iSelect chip (Cavanagh et al. 2013; Maccaferri et al. 2014; Maccaferri et al. 2015; van Poecke et al. 2013; Wang et al. 2014). Due to its enormous amount of repetitive DNA (> 80 %), the huge genome size (~ 17 Gbp) as well as the complexity of wheat´s evolution (Paux et al. 2006), molecular analyses in this crop are difficult.

A lot of sequence information of wheat sorted chromosome arms (Brenchley et al. 2012;

IWGSC 2014; Raats et al. 2013), *T. urartu* (Ling et al. 2013) and *A. tauschii* (Jia et al. 2013) has been created in the last decade and made available in public databases like the CerealsDB web page (University of Bristol. 2012), URGI (URGI 2018a), IWGSC (URGI 2018b). To date, the current version of the 'Chinese Spring' IWGSC RefSeq v1.0 (URGI 2018a) opens new avenues for marker development, fine mapping and map-based cloning of agronomical relevant traits (Holušová et al. 2017).

The aims of the current study were (1) to map quantitative trait loci involved in STB field resistance by analyzing doubled haploid populations derived from crosses of 'HTRI1410 × (Alcedo, Jenga, Solitär)' and (2) to develop molecular markers for STB resistance facilitating efficient marker-based selection procedures.

MATERIAL AND METHODS

PLANT MATERIAL

The gene bank accession (IPK Gatersleben, Germany) 'HTRI1410' (*Triticum aestivum* L. subsp. *spelta* var. *album*, Zeiners Schlegeldinkel, Germany before 1945) conferring resistance to Septoria tritici blotch (D. Kopahnke, personal communication) was crossed to three susceptible wheat cultivars. A DH population consisting of 135 DH lines, i.e. 33 DH lines derived from a cross to cv. 'Alcedo', 20 DH lines from a cross to cv. 'Jenga' and 82 DH lines from a cross to cv. 'Solitär', was generated by Saaten-Union Biotec GmbH (Gatersleben, Germany) by using anther culture technique, which is still difficult in wheat and explains the low number of DH lines per cross. This population was originally constructed to map resistance to tan spot (*Pyrenophora tritic repentis*) to which 'HTRI1410' is resistant and the other parental lines are susceptible (Engelmann 2014). However, in subsequent field trials it turned out that 'HTRI141'0 is also resistant to STB (Engelmann 2014) while the other parental lines expressed a higher level of susceptibility. Consequently, this population was used to map resistance to STB in addition.

PATHOGEN ISOLATES AND INOCULATION

For artificial inoculation, inoculum was prepared from sporulating isolates of *Zymoseptoria tritici* which were provided by Dr. Gert Kema from Plant Research International Wageningen and maintained at the Julius Kuehn-Institute (JKI) in Quedlinburg. The isolates were first grown as pre-cultures on potato dextrose agar (PDA) for 5 to 7 days (depending on the isolate) under ultraviolet (UV) light for 16 h per day at 18 °C. In order to harvest conidia, plates were flooded with sterile distilled water and conidia were scraped off with a soft loop (VWR, Germany) into an autoclaved 500 ml Erlenmeyer flask containing 250 ml yeast-glucose (YG) liquid medium (30 g glucose, 10 g yeast per liter demineralized water). The flasks were placed on a rotating shaker at 120 rpm at 18 °C for 6 to 7 days. This method was used to obtain a sufficient amount of inoculum for the adult plant inoculation in the field. The concentration of the conidia suspension was set to 1 x 10^7 conidia/ ml for the three isolates used which were mixed in equal amounts for inoculation (Table 2). The isolates were chosen based on results of preliminary tests, i.e. clearly differentiating between parental lines concerning resistance.

TABLE 2 Name, virulence and origin of the single conidial isolates of *Zymoseptoria tritici* used for the field trials (Dr. Gert Kema, Plant Research International, Wageningen).

Code	Corresponding *Stb* genes	Origin	Source
IPO98028	*Stb2*	France	Bread wheat
IPO323	*Stb3, Stb6, Stb10, Stb18*	The Netherlands	Bread wheat
IPO98001	*Stb4*	France	Bread wheat

FIELD TRIALS AND PHENOTYPING

Field trials were conducted at three locations in Germany, i.e. at the Julius Kuehn-Institute in Quedlinburg in Saxony-Anhalt (lat. 51.7 °, long 11.1 °), in Söllingen in Lower Saxony (lat. 52.0 °, long. 10.9 °) and in Mallersdorf in Bavaria (lat. 48.7 °, long. 12.2 °). Trials were conducted during the growing seasons 2014/2015, 2015/2016 and 2016/2017 in two replications in 1 m^2 plots in a randomized block design. DH lines were sown in early October in four rows, 25 kernels per row, separated by two rows of the highly susceptible wheat cultivar ‘Drifter’ as disease spreader.

Starting at the booting stage, spray inoculation was performed with the help of battery back sprayers twice after three days during growth stage BBCH 39 to 55 in the mid of May.

For each inoculation, an amount of 100 liters of spore suspension per 100 m² was applied. Before, during and after the inoculation, plants were artificially irrigated. Furthermore, inoculation was carried out in the late evening when dew formation and low temperature in the night gave optimal infection conditions.

Temperature, rainfall and leaf wetness varied highly during the three years´ field trials at Quedlinburg and Söllingen. The temperature during the vegetation period ranged from 7.1 °C (min) to 28.8 °C (max). Compared to the rainfall in 2016 in Quedlinburg and Söllingen (Ø 185 ml/ m²) and in 2017 (Ø 315 ml/ m²), the rainfall in 2015 (Ø 135 ml/ m²) was very low, while in Mallersdorf constantly high rainfall was recorded (Ø 236 ml/ m²). Due to the unfavorable weather and the persistent drought after the artificial infection in 2015, followed by a low infection with *Zymoseptoria tritici*, an additional watering was applied in 2016 and 2017, in order to increase the infection rate.

Due to the massive infestation with stripe rust (*Puccinia striiformis* f. sp. *tritici*, PST) in 2015, no reliable scoring of STB was possible. In order to avoid infection with PST and thus to allow a reliable screening for *Zymoseptoria tritici* resistance, trials were treated with the fungicide 'Corbel' (BASF, Germany, 750 g/l *Fenpropimorph*, emulsion concentrate) at BBCH 31 in 2016 and 2017.

When first symptoms were detected (mostly at the end of May) the *Zymoseptoria tritici* development was monitored at least three times at an interval of seven days between individual scorings. The symptoms were scored by estimating the percentage of leaf area with chlorosis and necrosis according to Moll and Flath (2000).

STATISTICAL ANALYSES

The area under the disease progress curve (AUDPC) was calculated using the software RESI (Moll and Flath 2000) followed by the calculation of the average ordinate (AO) by using the formula:

$$AO = \frac{\sum_{i=1}^{N_{i-1}} \frac{(y_i + y_{i+1})}{2} * (t_{i+1} - t_i)}{tp}$$

where (N) is the total number of observations, (yi) the disease level at the *i*th observation, (ti) the time at the *i*th observation and (tp) the trial period in days. The calculation of the adjusted means as well as all further statistical analyses were conducted using the software package Rx64 3.3.1 (R Core Team). Based on the average ordinate, the adjusted means were calculated by using the formula:

$$AEM = \mu + Gi + Lj + Yk + R + G * L + G * Y + G * L * Y + \varepsilon ijk$$

whereas μ is the general mean, Gi is the fixed genotype effect of the ith environment, Lj is the effect of the jth location, Yk is the effect of the kth year, R is the effect for the replication and *εijk* are the residuals with $E(\varepsilon_{ijk}) = 0$ and $(Var(\varepsilon_{ijk}) = \sigma^2)$.

The testing of the normal distribution of the residuals of the adjusted means was calculated by using the Kolmogorov–Smirnov test ($p< 0.05$). Analysis of variance was conducted using Rx64 3.3.1 (R Core Team). Based on the analysis of variance (ANOVA) using a mixed linear model, the heritability was calculated based on the results obtained in 2015/ 2016 and 2016/ 2017 by the formula:

$$h^2 = (VG/(VG + (VGL/L) + (VGY/Y) + (VGLY/(L * Y)) + (VE/(L * Y * R))),$$

(VG) is the genotypic variance, (VGL) the variance associated with the genotype by location interaction, (VGY) the genotype by year interaction, $(VGLY)$ the genotype by location by year interaction and (VE) the error variance, R is the replication.

The principal component analysis (PCA) was calculated according to Abdi and Williams (2010).

MAP CONSTRUCTION AND QTL ANALYSES

Genomic DNA was extracted from the first leaf of a single plant of each DH line and the parental genotypes of the DH population using the genomic DNA Miniprep method according to Stein et al. (2001).

The DNA was adjusted to a concentration of 50 ng/ ml by using the NanoDrop ND-100 spectrophotometer (PeQLab, Erlangen, Germany) and quality was checked by gel electrophoresis. Genotyping of the 135 DH lines and 4 parental lines was carried out with the 90K iSelect chip at TraitGenetics (Gatersleben, Germany). Due to the low population size of each cross, the three subpopulations were combined, and a genetic map constructed according to Perovic et al. (2009), as a prerequisite to have a larger population for QTL analyses. Out of the 81587 SNPs, those with < 10 % missing data and < 10 % heterozygosity were used for map construction. 12 DH lines turned out to be highly heterozygous and were eliminated from further analyses. In the population, between 'HTRI1410 × Alcedo' 11.011 markers were polymorphic, in the population 'HTRI1410 × Jenga' 11422 markers and in the population 'HTRI1410 × Solitär' 11952 markers. Out of these, 5989 were monomorphic between the susceptible parental lines and showed a polymorphism to 'HTRI1410'. Based on these SNP markers, a genetic map was constructed. For map construction the software JoinMap 4.0 (van Ooijen 2006) was used, applying the Kosambi function (Kosambi 1944) in combination with MapManager (Manly et al. 2001) as described by Comadran et al. (2012). Only markers with a logarithm of odds (LOD) higher than 3.0 were used for map construction. QTL analyses based on phenotypic field data of 2015/ 2016 and 2016/ 2017 were conducted by MapQTL 5.0 (Van Ooijen 2004) applying MQM mapping. A permutation test was conducted to calculate the significant LOD scores, resulting in a threshold of LOD= 3.0 for the whole genome. Chromosomes were visualized using the MapChart software (Voorrips 2002).

The adjusted R^2 of the phenotypic variance was calculated by a multiple regression analysis using software Rx64 3.3.1 (R Core Team) for multiple QTL markers as previously described by von Korff et al. (2005). To compare the marker position of the genetic map with the reference genome (URGI 2018a), SNP markers were blasted as fasta file with their marker sequences

(NCBI) against the IWGSC RefSeq v1.0 based on the wheat variety 'Chinese Spring' (*Triticum aestivum* L.) (URGI 2018a). The best uniquely assigned hit was chosen (expected threshold= 0.0001) to determine the start position and end position of QTL on the reference genome. Furthermore, a local downloadable integration tool developed by the Julius Kühn-Institute was used to determine the exact SNP position on the reference genome (Afgan et al. 2018).

In addition, peak markers and flanking markers were converted into KASP markers to facilitate an easy and reliable use of the QTL detected in applied wheat breeding. KASP primers were designed on the LGC Genomics database (LGC Hoddesdon, UK, http://www.lgcgenomics.com) and PCR analyses were performed as described by Yi et al. (2017). Respective KASP markers were re-analysed on the whole DH population.

RESULTS

PHENOTYPIC ANALYSES

The principal component analysis (PCA), representing the relationship of the DH population tested during 3 years (2015 - 2017) at three locations (Quedlinburg; Söllingen; Mallersdorf) in two replications for the mean leaf area diseased with *Zymoseptoria tritici,* clearly reveals that due to persistent drought after inoculation and massive infection with *Puccinia striiformis* at all locations during 2015, the infection pressure and infestation with *Z. tritici* was very low (Figure 5). The first and second principal component axes account for 24.92 % and 11.18 % of the variation in the DH line × mean leaf area diseased matrix. A high correlation for 2016 and 2017 over all locations ($r= 0.59$, $p< 0.0001$***) was observed while a low correlation was obtained between 2015 and 2016 ($r= 0.25$) and 2017 ($r= 0.33$). Therefore, the results of 2015 were excluded from the following analyses and only the phenotypic data of the years 2016 and 2017 were taken into account.

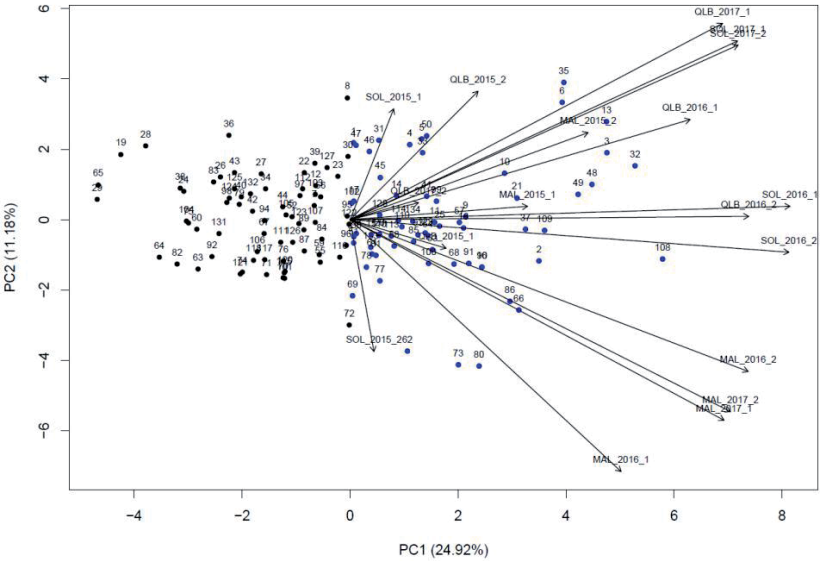

FIGURE 5 Biplot of the Principal Component Analysis (PCA) showing the relationship of the DH lines during three years, at three locations based on the adjusted means calculated for *Zymoseptoria tritici* infection. Arrows indicate the locations (QLB=- Quedlinburg; SOL = Söllingen; MAL = Mallersdorf), years and replications, while DH lines are indicated as dots (black=below 0 concerning PC1, blue=higher 0 concerning PC1).

A quantitative variation for the reaction to a *Zymoseptoria tritici* infection was observed, which fits to a normal distribution (Kolmogorov Smirnov test, ($p < 0.77$)). Besides a clear differentiation between 'HTRI1410' and the crossing parents, also differences in the reaction to *Z. tritici* between the susceptible parental lines were observed (Figure 6).

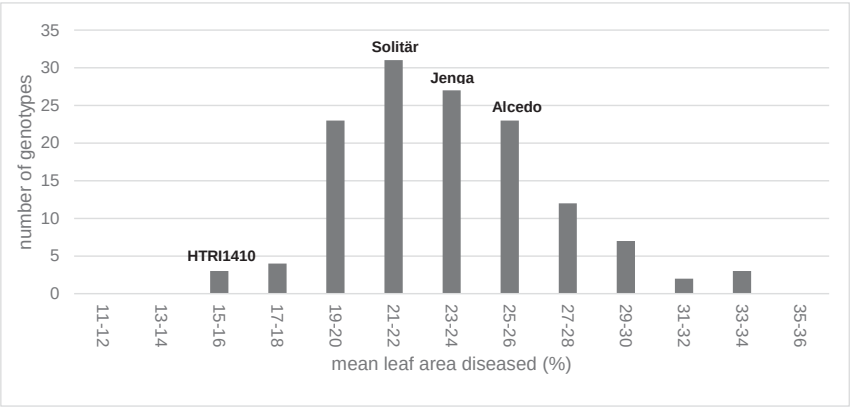

FIGURE 6 Distribution of DH lines for mean leaf area diseased of the population 'HTRI1410 × three susceptible parents' (Alcedo, Jenga and Solitär) calculated using the adjusted means from three locations during two years.

The mean value for the susceptible standard 'Drifter' over the three experimental years was around 50 % leaf area diseased. Mean leaf area diseased in the DH lines ranged from 15.9 % to 34.1 % with a mean value of 23.2 %. For the 135 DH lines tested over two years and three locations the heritability of resistance to *Zymoseptoria tritici* was calculated at h^2= 0.55. ANOVA revealed a significant effect of genotype, year and location and significant interactions for genotype and location and for year and location (Table 3).

TABLE 3 Analysis of variance (ANOVA) for the calculated adjusted means for the mean leaf area diseased with *Zymoseptoria tritici*.

Trait	Degrees of freedom	Mean Square	F value	p value
Genotype	122	154	4.9628	<2.20e-16
Year	1	34634	1115.4304	<2.2e-16
Location	2	2696	86.8325	<2.2e-16
Genotype * Year	121	37	1.1768	0.10961
Genotype * Location	244	46	1.4701	6.750e-05
Year * Location	2	4624	148.9153	<2.2E-16
Genotype * Year * Location	242	36	1.1651	0.06761

GENETIC MAP CONSTRUCTION

Based on the 90K iSelect wheat chip, 5989 marker turned out to be polymorphic between the resistant gene bank accession 'HTRI1410' and the three susceptible parental lines. Out of these, 2711 marker were mapped to 714 loci resulting in a total map length of 2388.3 cM. The size of the chromosomal maps varied from 0.8 cM for chromosome 4D to 209.1 cM for chromosome 2B. The average marker distance over all chromosomes is 3.3 cM (Table 4). In general, the marker saturation on the A and B genome was similar, whereas the marker density on the D genome was much lower.

TABLE 4 Distribution of SNP marker over the three genomes A, B and D and the seven chromosomes per genome.

	Chromosome									
Genome	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Total number**	**Number of loci**	**Map length (cM)**
A	68	200	193	46	223	255	133	1118	313	1072.4
B	302	230	110	102	291	103	188	1326	354	990.4
D	95	107	22	4	8	6	25	267	47	325.5

QTL MAPPING

Based on the map constructed and the two years´ phenotypic data four QTL for resistance to *Zymoseptoria tritici* were detected on chromosomes 5A, 4B and 7B (Figure 7).

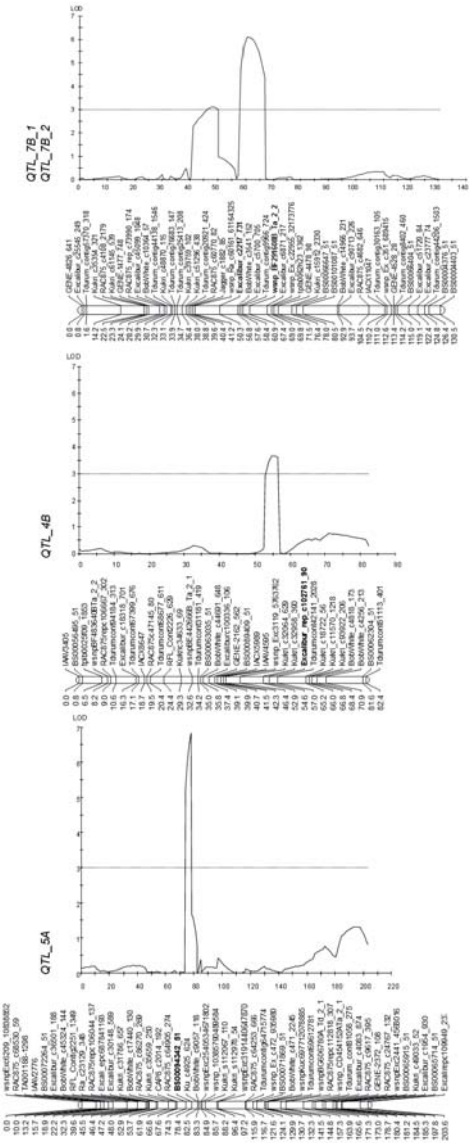

FIGURE 7 Results of QTL analyses for *Zymoseptoria tritici* resistance in the DH population 'HTRI1410 × susceptible parental lines'.

The positive allele of the *QTL_5A* located on chromosome 5A derived from the resistant gene bank accession 'HTRI1410' whereas the positive allele of the *QTL_4B* on chromosome 4B derived from the susceptible parental lines. The positive alleles of the QTL mapped on chromosome 7B originate from both, the resistant and susceptible lines. *QTL_7B_1* derived from the resistant accession 'HTRI1410' and *QTL_7B_2* derived from susceptible parental lines. The QTL on chromosome 5A is located at position 78.3 cM in a marker interval ranging from 74.2 cM to 82.4 cM on the long arm of this chromosome, with the peak marker *BS00094342_51* (LOD 6.83). This QTL accounts for 17.5 % of the phenotypic variance. The QTL on chromosome 4B comprises a marker interval from 52.9 cM to 56.9 cM with *Excalibur_rep_c102761_90* as peak marker at position 54.5 cM (LOD 3.25). The positive allele for the reduction of the leaf area diseased with *Zymoseptoria tritici* originates from the susceptible parental lines and explains 8.8 % of the phenotypic variance for this QTL.

The interval of *QTL_7B_1* spans 15.6 cM with *Excalibur_c2217_731* as the peak marker (LOD 3.12) and explains 8.5 % of phenotypic variance. The positive allele is derived from 'HTRI1410'. The interval for *QTL_7B_2* ranges from 58.4 to 67.4 cM with *wsnp_BF291608B_Ta_2_2* as the peak marker and explains 15.3 % of the phenotypic variance (LOD 6.11) (Table 5). The adjusted R^2 for all QTL is calculated at R^2= 26.9 %.

TABLE 5 Overview on QTL for resistance to *Zymoseptoria tritici* identified showing position of QTL, LOD score, explained phenotypic variance and peak markers.

QTL	Chr.	Position (cM)	Interval	Closest Marker	LOD	Phenotypic Variance explained (%)	Additive effect	Origin
QTL_5A	5A	78.3	74.2-82.4	*BS00094342_51*	6.83	17.5	1.49	HTRI1410
QTL_4B	4B	54.5	52.9-56.9	*Excalibur_rep_c102761_90*	3.25	8.8	0.103	[a]SP
QTL_7B_1	7B	50.2	41.2-56.8	*Excalibur_c2217_731*	3.12	8.5	1.5	HTRI1410
QTL_7B_2	7B	60.8	58.4-67.4	*wsnp_BF291608B_Ta_2_2*	6.11	15.3	2.2	[a]SP

[a]SP (Susceptible parent)

In addition to the genetic map, the positions of the QTL were anchored to the IWGSC RefSeq (Table 6). The marker order of the genetic map in the QTL interval on chromosome 5A is consistent with the IWGSC RefSeq and the 8.2 cM interval is equivalent to 106.7 Mbp.

The *QTL_4B* on chromosome 4B spans a 78.1 Mbp. While the marker order in our map corresponds to that of Wang et al. (2014) marker order on the physical map is inverted. The QTL on chromosome 7B comprise 9.7 Mbp and 37.3 Mbp, respectively.

TABLE 6 Overview of the QTL detected on chromosomes 5A, 4B and 7B with peak and flanking marker and comparison to the IWGSC RefSeq (MBp).

QTL	Marker	Genetic map (cM)	Physical map (Mbp)
	RAC875_c64905_274	74.25	388.1
QTL_5A	*BS00094342_51*	78.39	494.8
	Ku_c4925_624	82.46	503.1
	Kukri_c32958_390	52.92	636.7
QTL_4B	*Excalibur_rep_c102761_90*	54.55	641.5
	Tdurum_contig42141_2028	56.99	558.5
	wsnp_Ra_c60161_61164325	41.22	583.4
QTL_7B_1	*Excalibur_c2217_731*	50.26	593.1
	BobWhite_c3541_152	56.80	605.9
	Tdurum_contig9966_724	58.42	608.7
QTL_7B_2	*wsnp_BF291608B_Ta_2_2*	60.86	614.2
	Excalibur_c6871_217	67.40	646.0

In order to facilitate the use of respective QTL in wheat breeding, KASP markers were developed for the peak and flanking SNP markers. Based on these KASP markers the allelic composition of DH lines for respective QTL was determined (Table 7). As can be seen, the line which combines all positive alleles is the most resistant one (19.10 %) while those carrying only one positive QTL allele and especially those without any positive allele (29.84 %) are more susceptible.

TABLE 7 Analysis of the four quantitative trait loci (QTL) found in QTL studies. Positive alleles are indicated by grey cells. Disease severity shows the mean percentage of leaf area diseased of lines with the respective QTL combination.

QTL_5A	*QTL_4B*	*QTL_7B_1*	*QTL_7B_2*	Disease (%)	No. lines
T	T	A	T	19.10	1
T	T	A	C	20.73	10
T	T	G	T	20.73	9
T	T	G	C	NA	NA
T	C	A	T	NA	NA
T	C	A	C	22.97	16
T	C	G	T	21.06	11
T	C	G	C	24.74	2
G	T	A	T	18.98	2
G	T	A	C	22.45	14
G	T	G	T	21.36	13
G	T	G	C	23.13	2
G	C	A	T	NA	NA
G	C	A	C	24.47	19
G	C	G	T	25.11	22
G	C	G	C	29.84	2

Positive alleles are marked in grey

DISCUSSION

Spelt wheat (*Triticum aestivum* L. subsp. *spelta*) serves as a valuable source for resistance in bread wheat breeding as resistance to several foliar diseases e.g. stripe rust, Fusarium head blight and Septoria tritici blotch has been detected in spelt wheat (Simon et al. 2010).

However, to get detailed information on STB resistance reliable field trials are a prerequisite. In this respect, disadvantageous weather conditions for pathogen development, such as persistent drought, can result in decreased infection pressure (Simón et al. 2005b). By using a combination of irrigation and artificial infection we were able to obtain good *Z. tritici* infections also under low rainfall conditions and in contrast to other studies (Kollers et al. 2013; Naz et al. 2015; Risser et al. 2011) no significant genotype × environment interaction was observed in our study. The importance of artificial inoculation has already been pointed out by Chartrain et al. (2004a), Eriksen et al. (2003) and Schilly et al. (2011). As an isolate mixture was used in

our studies, no analysis of the genotype × isolate interaction was possible. In this respect, it is interesting to note, that based on results on studies on cultivar-by-isolate interactions, response to *Zymoseptoria tritici* in the adult plant stage may differ from the response at the seedling stage (Chartrain et al. 2004a; Kema and van Silfhout 1997), i.e. the expression of partial resistance to *Zymoseptoria tritici* may depend on the growth stage (Cavanagh et al. 2013; Chartrain et al. 2004a) whereas qualitative resistances are independent from the growth stage (Arraiano et al. 2001; Brown et al. 2001; Grieger et al. 2005; Kema and van Silfhout 1997).

The 90K iSelect chip has shown to be an efficient tool for mapping genes and QTL in wheat (Wang et al. 2014; Wen et al. 2017). In the case of this study, 11952 markers turned out to be polymorphic between 'HTRI1410' and 'Solitär', 11011 markers between 'HTRI1410' and 'Alcedo' and 11422 markers between 'HTRI1410' and 'Jenga' corresponding to a level of polymorphism of 14.6 %, 13.5 % and 14.0 %, respectively. However, due to the special DH population used in this study consisting of four parental lines out of these only 5989 markers could be used for mapping and after eliminating cosegregating markers a map consisting of 714 markers was constructed. The distribution of SNP markers showed that the B genome was covered best (1326 markers), followed by the A genome (1118) and the D genome (267) (Table 3). However, due to the special mapping population genome coverage was lower in comparison to Vagndorf et al. (2017) and Gutierrez-Gonzalez et al. (2019) but higher compared to Zeng et al. (2019). The huge discrepancy between the genomes can be explained by to evolutionary development of hexaploid wheat (Chao et al. 2009; Nielsen et al. 2014) as the cross of *T. dicocoides* (AABB) with *Aegilops tauschii* (DD) occurred only recently and very rarely (Kilian et al. 2010; Wicker et al. 2009).

The marker order of the genetic map constructed is in agreement with the dense genetic map of wheat (Wang et al. 2014). No consensus map was constructed in order to increase the marker density because of the quite low number of individuals in the DH populations and the problems related to consensus mapping (Wu et al. 2008).

With respect to breeding, the phenotypic variance explained by a QTL is of prime importance (Collard et al. 2005). *QTL_5A* and *QTL_7B_2* account for 17.5 % of the phenotypic variance

explained and 15.3 % of the phenotypic variance explained, respectively. Therefore, these QTL may be recognized as major QTL whereas *QTL_4B* and *QTL_7B_1* explain 8.8 % and 8.5 % of the phenotypic variance and are minor QTL, therefore. The four QTL detected in our studies were blasted against the physical map based on 'Chinese Spring'. Comparisons of marker intervals of *QTL_5A*, *QTL_4B*, *QTL_7B_1* and *QTL_7B_2* indicated that the SNP marker order is in accordance with the physical map, but the marker order within the intervals of *QTL_4B* is inverted.

QTL_5A is located on the long arm of chromosome 5A in an interval from 74.2 cM to 82.4 cM. In previous studies, a major gene and QTL were already located on chromosome 5A (Ghaffary et al. 2012; Kosellek et al. 2013). The race-specific gene *Stb17* (Ghaffary et al. 2012) was located on the long arm of chromosome 5A. This resistance gene has a quantitative effect on the disease, which can be found in the adult growth stage but is not expressed in the seedling stage (Ghaffary et al. 2012). However, a possible co-localization of *QTL_5A* with *Stb17* cannot be determined because the sequence of the SSR markers used for mapping *Stb17* is not available. Furthermore, the QTL detected by Goudemand et al. (2013) are not in the same genomic region as our QTL and they do not confer resistance in the adult plant stage. As *QTL8* is located on the short arm of chromosome 5A and *QTL9* only reveals resistance in the seedling stage (Goudemand et al. 2013) we can assume that we found a new QTL for adult plant resistance against *Zymoseptoria tritici* on chromosome 5A.

A second QTL, named *QTL_4B*, has been located on chromosome 4B in the marker interval of 52.9 cM to 56.9 cM. Several QTL for STB resistance have already been detected on chromosome 4B, i.e. *QSTB.lsa_fb-4B* and *QSTB.lsa_tb-4B* (Risser et al. 2011) and a QTL detected in 'Solitär' (Kelm et al. 2012). Kelm et al. (2012) analysed the doubled haploid population 'Mazurka × Solitär' and found one QTL on chromosome 4B. As the positive allele detected in our study derived from the susceptible parental lines including the cultivar 'Solitär', which shows a better resistance than 'Alcedo' and 'Jenga', this may be a hint that these QTL are the same. However, this cannot be proven because only markers monomorphic between the susceptible cultivars were used for mapping. Single maps of 'HTRI1410 × Solitär' may be used to get

detailed information whether respective QTL are the same. In addition, Risser (2010) also mapped a QTL on chromosome 4B in the teleomeric region, whereas our QTL is located on the long arm of chromosome 4B but not in the telomeric region.

By assigning the SSR markers used for mapping *Stb8* and *Stb13* to the IWGSC RefSeq it turned out that these genes are located at 711 Mbp and 546 Mbp while *QTL_7B_1* and *QTL_7B_2* are located at 593.1 Mbp and 614.2 Mbp. The already known *QStb.ipk-7B* (Simón et al. 2005b) is in the same genomic region as *QTL_7B_2* but located at 683 Mbp in a more telomeric region. The *QTL_5A* and *QTL_7B_1* derived from 'HTRI1410' are most likely new and may be used in applied wheat breeding, therefore. It may be useful to combine these QTL with other already known QTL. Pyramiding of QTL has proven to enhance the level of resistance in wheat already against leaf rust, yellow rust, stem rust (Tyagi et al. 2014) and Fusarium head blight (Badea et al. 2008; Shi et al. 2008; Tamburic-Ilincic et al. 2011). For this purpose, flanking SNP markers where converted into KASP markers. In this respect, it turned out that combining all positive alleles of the four QTL results in a higher level of resistance. In addition to this, qualitative resistance genes as well as quantitative resistance genes should be combined (Brown et al. 2015; Palloix et al. 2009) as earlier studies have demonstrated, that single qualitative *Stb* genes are quickly overcome by the rapidly evolving *Z. tritici* populations (Cowger et al. 2000; Vagndorf et al. 2017). Due to the quantitative nature, another option to improve STB resistance may be the use of genomic selection as this has been shown e.g. for resistance to Fusarium head blight (Dong et al. 2018).

3 Chapter 2)

IDENTIFICATION AND MAPPING OF QTL FOR SEEDLING RESISTANCE AGAINST *ZYMOSEPTORIA TRITICI* IN THE GENE BANK WINTER WHEAT ACCESSION HTRI1410

ABSTRACT

A set of 16 isolates of *Zymoseptoria tritici* was tested on four German winter wheat cultivars to detect differential reactions. Based on these results three selected isolates were tested on a doubled-haploid (DH) population derived from crosses between the resistant spelt wheat gene bank accession HTRI1410 and the susceptible bread wheat cultivars 'Jenga', 'Solitär' and 'Alcedo'.

'HTRI1410' turned out to partially resistant to isolates IPO323 virulent to *Stb6*, IPO98022 virulent to *Stb18* and IPO98050 virulent to *Stb8*. In genetic analyses of resistance of 'HTRI1410' QTL for resistance against these isolates were detected on several chromosomes. Resistance to IPO323 is controlled by a major QTL on chromosome 2A and minor QTL on chromosomes 2A, 3A and 6A. QTL against isolate IPO98022 were detected on chromosomes 2A, 4A, 1B and 2B. Furthermore, six major QTL on chromosomes 1A 3A and 4A and three minor QTL on chromosomes 4A and 5B were identified for resistance against *Zymoseptoria tritici* isolate IPO98050.

In summary, our results point to a complex inheritance of resistance to STB in the seedling stage.

INTRODUCTION

Septoria tritici leaf blotch (STB) is one of the most important diseases of bread wheat worldwide (Chartrain et al. 2004b). It is caused by the ascomycete *Zymoseptoria tritici* (teleomorph: *Mycosphaerella graminicola*) and leads to severe yield losses up to 30 % to 50 % mainly by reducing the photosynthetically active leaf area (Eyal et al. 1987; Ponomarenko et al. 2011). Even with a moderate fungicide use and the cultivation of resistant varieties yield losses of 5 % - 10 % are observed (Fones and Gurr 2015).

For improving disease resistance in *Triticum aestivum*, spelt wheat (*Triticum spelta* L. subsp. *spelta*) may serve as a source since resistances against stripe rust (Kema 1992) and common bunt (Dumalasová et al. 2017) have been identified in spelt wheat, already. For identifying specific cultivar x isolate interactions in wheat for Septoria tritici blotch, a detached seedling leaf technique was developed by Arraiano et al. (2001) and successfully applied in several studies for detecting resistance against *Zymoseptoria tritici* (Arraiano and Brown 2006; Brading et al. 2002; Chartrain et al. 2005b; Radecka-Janusik and Czembor 2014; Stukenbrock et al. 2012). In contrast to extensive field trials, the advantage of detached seedling leaf assay is the possibility to test numerous isolates simultaneously all year long in controlled conditions (Arraiano et al. 2001). Detached leaf assays were also applied for resistance in wheat against leaf rust (*Puccinia triticina*) (Boydom et al. 2015).

Septoria tritici blotch causes necrotic lesions that lead to a massive cell collapse (Kema 1996). After a latency period of ten to fourteen days, chlorotic spots appear developing to necrotic blotches. The ascospores of the teleomorphic form are causing the disease in late autumn and winter (Royle 1994; Shaw and Royle 1989). Out of the pycnidia, mucous membranes swell. In the tendrils, the pycnospores are embedded in a gel and after a period of persistent high humidity, the pycnidiospores are spread throughout the plant canopy by rain splash (Ponomarenko et al. 2011).

Controlling Septoria tritici blotch is mostly conducted by crop rotation, delayed sowing of winter wheat, use of fungicides and growing of resistant cultivars (Ponomarenko et al. 2011). Breeding of STB resistant wheat varieties is the most efficient way to prevent fungicide resistances

due to the intensive use of azoles, strobilurins and triazoles (Cools and Fraaije 2008; Ponomarenko et al. 2011).

The *Zymoseptoria tritici* - wheat pathosystem is characterized by interactions of quantitative and qualitative responses to an STB infection (Kema and van Silfhout 1997) and consequently studies on STB resistance dealt with qualitative, isolate-specific vertical resistances as well as quantitative, isolate non-specific, horizontal resistances (Arraiano and Brown 2006).

SNP markers flanking putative QTL can be efficiently used for MAS in breeding, such as competitive allele specific (KASP) PCR markers developed from SNP sequences (Cabral et al. 2014; Chhetri et al. 2017; Dreisigacker et al. 2015). KASP markers allow a precise bi-allelic scoring of SNPs, insertions and deletions at specific loci (LGC protocol 2014) and thereby an easy implementation in breeding programs (Vagndorf 2018).

Up to now, 21 major *Stb* genes and 89 quantitative trait loci (QTL) conferring partial resistance to Septoria tritici blotch have been detected (Brown et al. 2015). Recently, Sainetac et al. (2018) were are able to clone *Stb6*. It turned out that *Stb6* encodes a kinase, which identifies a pathogen effector protein resulting in resistance without leading to a hypersensitive reaction (Saintenac et al. 2018).

Pyramiding QTL has shown to be an efficient tool to produce stable resistance as it was already shown in wheat e.g. for *Fusarium pseudograminearum* (Bovill et al. 2010) and powdery mildew (Koller et al. 2018) while the use of single major resistance genes often leads to a quick break down resulting in the need to identify new *R* genes and transfer these to adapted cultivars. *Zymoseptoria tritici* has a high evolutionary potential (Croll and Karisto 2018) which explains the rapid development of fungicide resistance and the rapid failure of major STB resistance genes like *Stb6* (Brunner et al. 2008; Estep et al. 2013; Torriani et al. 2009).

The aim of this study was therefore (1) to characterize the resistance of a DH population derived from crosses of the gene bank accession ‘HTRI1410’ to three susceptible cultivars to a worldwide *Zymoseptoria tritici* isolate collection in a detached leaf assay and (2) to identify new QTL conferring resistance to STB and (3) to develop molecular markers which facilitate the use of MAS in wheat breeding for STB resistance.

MATERIAL AND METHODS

PLANT AND FUNGAL MATERIAL

Three doubled-haploid (DH) wheat populations were used to analyze the genetics of resistance to *Zymoseptoria tritici* isolates. The German winter spelt wheat accession 'HTRI1410' (*Triticum aestivum* L. subsp. *spelta* var. *album*, Zeiners Schlegeldinkel, Germany before 1945), resistant to STB in the field, was crossed to the susceptible German winter wheat cultivars 'Alcedo' (Porsche Züchtergemeinschaft, Germany) and 'Jenga' (Ackermann Saatzucht, Germany) as well as the cultivar 'Solitär' (Schweiger Saatzucht, Germany) being partially resistant to STB. The DH populations consisting of 135 lines were generated at Saaten-Union BioTec GmbH (Gatersleben, Germany) by anther culture. DH lines of these three populations were used for the resistance screening under controlled conditions at the Julius Kühn-Institute (JKI, Quedlinburg, Germany) as well as for map construction.

The screening for STB resistance on the parental lines was conducted using a set of 16 different isolates of *Zymoseptoria tritici*, originating from 7 different countries (Table 8). In addition, wheat leaves showing *Z. tritici* symptoms, were collected from naturally infected wheat fields in Quedlinburg, so that besides the worldwide sampled isolates derived from Plant Research International Wageningen (PRI) which were provided by Dr. Gert H.J. Kema, locally collected isolates (JKI) were included in these studies.

PATHOGENICITY ASSAY

For conducting the detached leaf assay four plants per DH-line were grown in plastics pots in three replications. The substrate used was "Einheitserde Torf mit Sand" (Gebr. Patzer GmbH & Co. KG, Sinntal-Altengronau). The DH lines and the four parental lines were grown for three weeks at 22 °C with a lightning regime of 12/ 12 h (light/dark) until the third leaf was fully visible. The STB isolates were grown as pre-cultures on potato dextrose agar (PDA) for 5 to 7 days (depending on the isolate) under ultraviolet (UV) light for 16 h per day at 18 °C. Pycnidiospores were harvested by adding 5 ml of sterile water and rubbing them off with a soft loop (VWR,

Germany) followed by filtering through a gauze piece. The concentration of the spore suspension was determined by using a haemocytometer (Fuchs-Rosenthal cell counter (www.carlroth.de)) and adjusted to $1x10^7$ spores/ ml.

For each isolate, the detached leaf assay was conducted in plastic trays using ten leaf segments for each DH line, derived from ten different plants following the protocol of Arraiano et al. (2001). Each segment was inoculated with 0.5 ml conidial suspension by spraying. Leaves were incubated in plastic trays with a relative humidity of 85 % for 48 h at 18 °C in the dark followed by 18 days at 20 °C and a lightning regime of 16/ 8 h (light/ dark).

TABLE 8 List of *Zymoseptoria tritici* isolates used for seedling tests in a detached leaf assay of the parental lines, i.e. 'HTRI1410', Alecdo, Jenga, Solitär.

Isolate	Corresponding *Stb* genes	Origin	Source
IPO98028	*Stb2*	France	PRI
IPO98078	*Stb2*	France	PRI
IPO99018	*Stb2*	France	PRI
IPO323	*Stb3, Stb6, Stb10, Stb18*	Netherlands	PRI
IPO98034	*Stb4*	France	PRI
IPO92067	*Stb5*	Argentina	PRI
IPO93014	*Stb5*	Argentina	PRI
IPO98021	*Stb6*	France	PRI
IPO99015	*Stb7, Stb11*	Argentina	PRI
IPO98050	*Stb8*	France	PRI
IPO90015	*Stb12*	Peru	PRI
IPO88004	*Stb15*	Ethiopia	PRI
IPO92006	*Stb15*	Portugal	PRI
IPO98022	*Stb18*	France	PRI
IPO98046	*Stb18*	France	PRI
Pop 1	*unknown*	Germany	JKI

Isolates underlined were selected to analyze the STB resistance in all three DH populations
[a] *PRI* Plant Research International, Wageningen, The Netherlands; *JKI* Julius Kühn-Institute, Quedlinburg, Germany

SCORING OF THE LEAF AREA DISEASED

Symptoms of *Zymoseptoria tritici* were scored using the scheme of Moll et al. (2000) 15 to 18 days after inoculation, depending on the isolate.

STATISTICAL ANALYSES

All statistical analyses were conducted with the software packages R x64 3.3.1 (R Core Team). The adjusted means were calculated by using the formula:

$$AEM = \mu + Gi + Lj + R + G * L + \varepsilon ij,$$

whereas μ is the general mean, Gi is the fixed genotype effect of the ith environment, Lj is the effect of the jth location, R is the effect for the repetition and εij are the residuals with $E(\varepsilon_{ij}) = 0$ and $(Var(\varepsilon_{ij}) = \sigma^2)$.

The calculation of best linear unbiased estimators (BLUES) for the parental lines based on the adjusted means was conducted according to Piepho et al. (2008).

The test for a Gaussian distribution of the residuals of the adjusted means was carried out by using the Kolmogorov–Smirnov test ($p < 0.05$). Based on the detached leaf assay repeatability was calculated according to the formula:

$$rep = (VG/(VG + (VGL/L) + (VE/(L * R))),$$

whereas the variance components for the genotypes are coded by (VG), the variance associated with the genotype by location interaction (VGL) and error variance (VE), R is coded as replication.

GENETIC MAPPING

Genomic DNA extraction was done using the genomic DNA Miniprep method according to Stein et al. (2001) using 14 days old seedlings.

The DNA was adjusted to a concentration of 50 ng/ ml by using the NanoDrop ND-100 spectrophotometer (PeQLab, Erlangen, Germany) and quality was checked by gel electrophoresis. Genotyping of the 135 DH lines and 4 parental lines was carried out with the 90K iSelect chip at TraitGenetics (Gatersleben, Germany). Due to the low population size of each cross, the three subpopulations were combined and a genetic map constructed according to Perovic et al. (2009), as a prerequisite to have a larger population for QTL analyses. Out of the 81587 SNPs, those with < 10 % missing data and < 10 % heterozygosity were used for map construction. 12 DH lines turned out to be highly heterozygous and were eliminated from further

analyses. In the population'HTRI1410 × Alcedo' 11.011 markers were polymorphic, in the population 'HTRI1410 × Jenga' 11422 markers and in the population 'HTRI1410 × Solitär' 11952 markers. Out of these, 5989 were monomorphic between the susceptible parental lines and showed a polymorphism to 'HTRI1410'. Based on these SNP markers, a genetic map was constructed. For map construction the software JoinMap 4.0 (van Ooijen 2006) was used, applying the Kosambi function (Kosambi 1944) in combination with MapManager (Manly et al. 2001) as described by Comadran et al. (2012). Only markers with a logarithm of odds (LOD) higher than 3.0 were used for map construction.

By combining the phenotypic data from the detached leaf assay with the genotypic data, QTL analyses were performed with MapQTL 5.0 using MQM mapping to map the resistance to Septoria tritici blotch (Van Ooijen 2004). Using the permutation test in MapQTL 5.0, the significant LOD threshold was calculated for the combined population for each isolate resulting in LOD= 3.1 for isolates IPO323 and IPO98050 and LOD= 3.5 for isolate IPO908022. Chromosomes were visualized using the MapChart software (Voorrips 2002).

To compare the marker position of the genetic map with the reference genome (Appels et al. 2018), SNP markers were blasted as fasta file with their marker sequences (NCBI) against the IWGSC RefSeq v1.0 based on the wheat variety 'Chinese Spring' (*Triticum aestivum* L.) (Appels et al. 2018). The best uniquely assigned hit was chosen (expected threshold= 0.0001) to determine the start position and end position of QTL on the reference genome. Furthermore, a local downloadable integration tool developed by the Julius Kühn-Institute was used to determine the exact SNP position on the reference genome (Afgan et al. 2018).

RESULTS

RESPONSE OF PARENTAL LINES TO *ZYMOSEPTORIA TRITICI*

A set of 16 worldwide sampled single conidia isolates of *Zymoseptoria tritici* was tested on the four parental lines in a detached leaf assay (Figure 8). A clear differentiation between all four parental lines was observed for STB reaction for the majority of isolates tested. 'HTRI1410' showed the highest resistance level to almost all isolates and had the lowest mean leaf area

diseased, whereas ‘Jenga’ and ‘Alcedo’ were quite similar in susceptibility and showed higher levels of infestation. For isolates IPO323, IPO98050 and IPO98022 the highest level of resistance was observed for ‘HTRI1410’, while the other parental lines turned out to be quite susceptible. Therefore, the isolates IPO323, IPO88050, and IPO98022 were selected for analyzing the DH population.

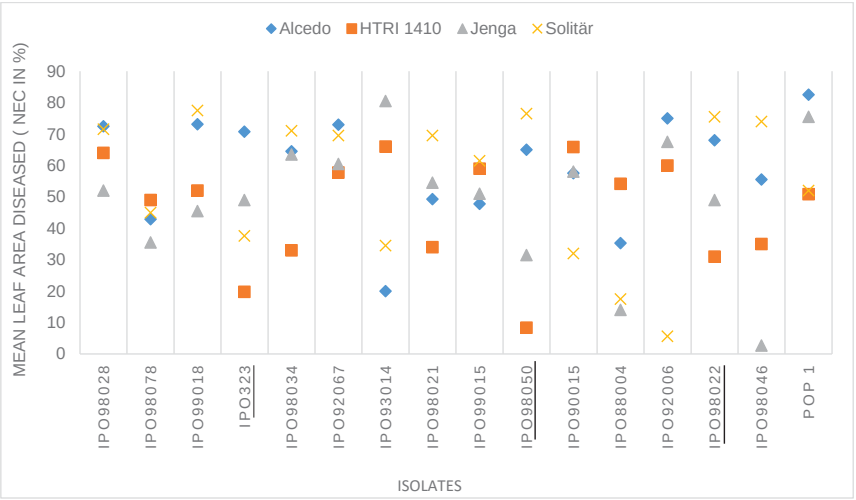

FIGURE 8 Distribution of BLUES for the mean leaf area diseased with necrosis (NEC %) in the parental lines Alcedo, Jenga, HTRI1410 and Solitär in the detached leaf assay with 16 *Zymoseptoria tritici* isolates. Underlined isolates were chosen for analyzing the DH-population.

DISTRIBUTION OF STB RESISTANCE IN THE DH POPULATION

The DH population was tested for STB resistance to three defined single conidial isolates (see above) in a detached leaf assay. The distribution of the mean leaf area diseased (NEC %) in the DH populations for respective isolates is shown in Figure 9.

Calculating the Kolmogorov–Smirnov test for each isolate, the distributions observed on the DH populations do not fit to a normal distribution (IPO323: $p > 0.05$; IPO98022: $p > 0.05$; IPO98022: $p > 0.05$). Based on the results obtained, the repeatability for STB resistance was calculated at rep= 0.96 for isolate IPO323, at rep= 0.95 for isolate IPO98055 and at rep= 0.95 for isolate IPO98022.

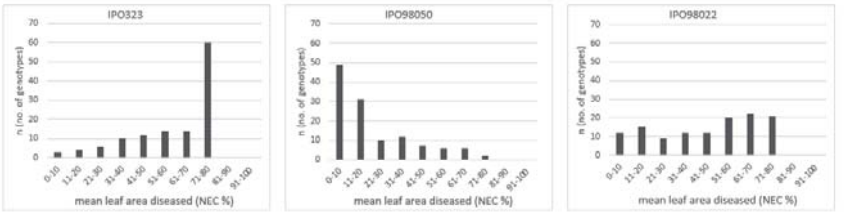

FIGURE 9 Frequency distribution observed for the reaction to single conidial lines of *Zymoseptoria tritici* in the DH populations HTRI1410 x Jenga, HTRI1410 x Solitär and HTRI1410 x Alcedo.

Based on the analysis of variance (ANOVA), significant effects were detected between the lines of the DH population and isolates of *Z. tritici* for the mean leaf area diseased (NEC %). Furthermore, significant differences were detected between the isolates used in this study and a genotype x isolate interaction with regards to IPO323, IPO98050 and IPO98022 hints to an isolate-specific reaction to Septoria tritici blotch (Table 9).

TABLE 9 Analysis of variance (ANOVA) for isolate specific (IPO98050, IPO98022 and IPO323) and genotype effects.

Source of variation	Degrees of freedom	NEC		
		Mean square	*F* value	*p* value
Genotype	122	58489	305.897	< 2.2e-16 ***
Isolate	2	500619	2618.254	< 2.2e-16 ***
Genotype x Isolate	242	4270	22.332	< 2.2e-16 ***

GENETIC MAP CONSTRUCTION

Based on the 90K iSelect wheat chip, 5989 marker turned out to be polymorphic between the resistant gene bank accession 'HTRI1410' and the three susceptible parental lines. Out of these, 2711 marker were mapped to 714 loci resulting in a total map length of 2388.3 cM. The size of the chromosomal maps varied from 0.8 cM for chromosome 4D to 209.1 cM for chromosome 2B. The average marker distance over all chromosomes is 3.3 cM.

QTL MAPPING OF RESISTANCE AGAINST DIFFERENT ZYMOSEPTORIA TRITICI ISOLATES

Based on the map constructed and present data resulting from the detached leaf assay, QTL mapping for resistance to Septoria tritici blotch with single defined isolates was conducted (Figure 10, 11, 12).

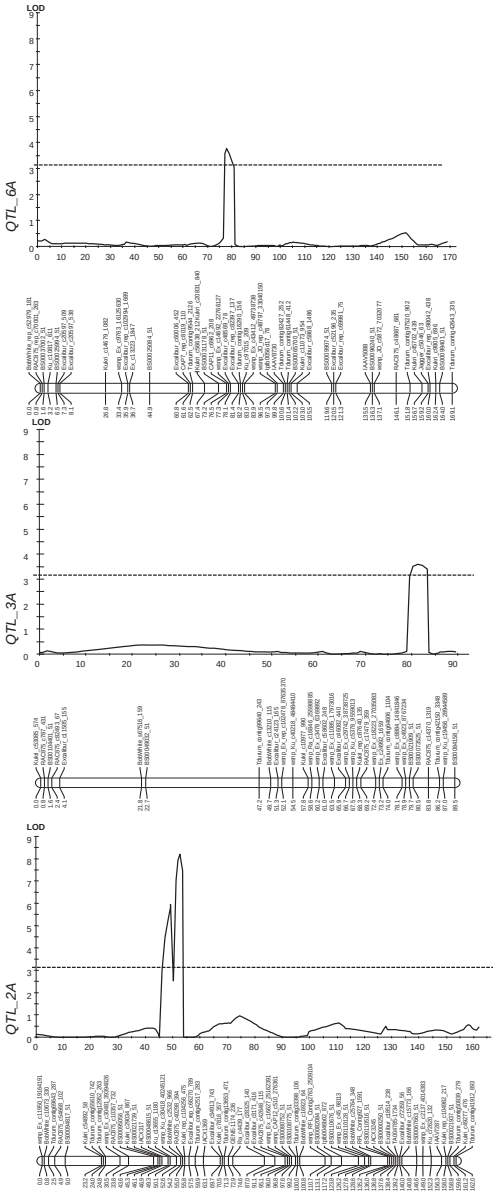

FIGURE 10 Results of QTL analyses for *Zymoseptoria tritici* resistance in the DH population 'HTRI1410 x susceptible parental lines' for isolate IPO323.

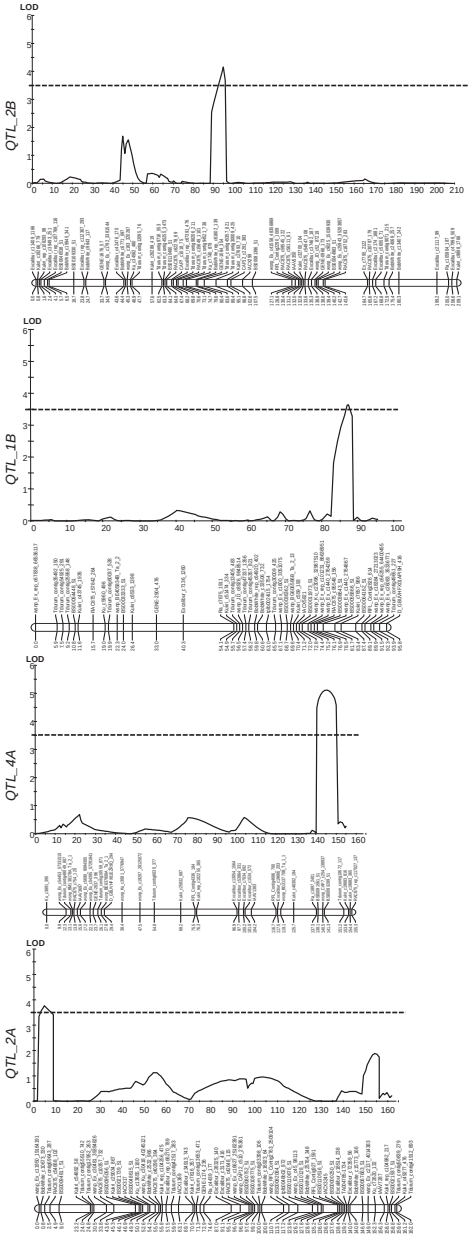

FIGURE 11 Results of QTL analyses for *Zymoseptoria tritici* resistance in the DH population 'HTRI1410 x susceptible parental lines' for isolate IPO98022.

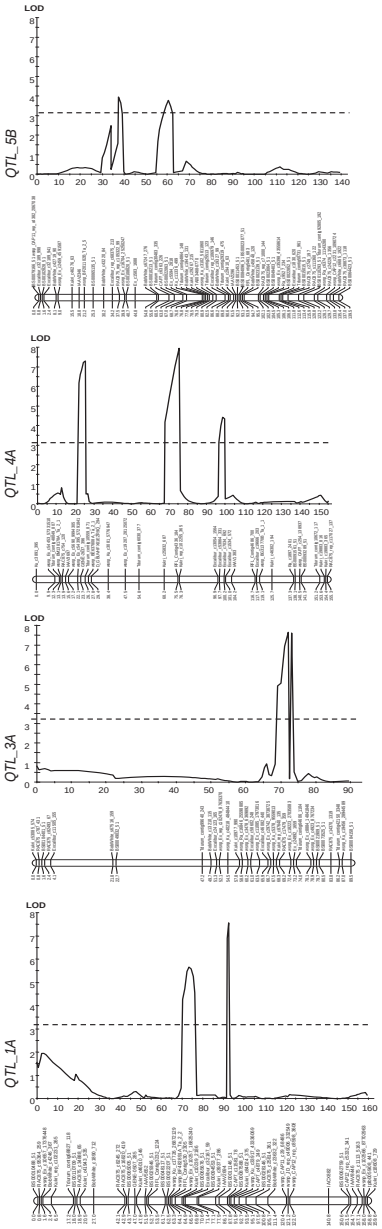

FIGURE 12 Results of QTL analyses for *Zymoseptoria tritici* resistance in the DH population 'HTRI1410 x susceptible parental lines' for isolate IPO98050.

For isolate IPO323, four QTL in the population 'HTRI1410 x SP' effective against Septoria tritici blotch were detected (Table 10). Two of them (*QTL_2A_1; QTL_2A_2*) were identified on chromosome 2A located in an interval of 8 cM between 46.89 cM and 54.20 cM. The closest markers are *BS00048615_51* and *wsnp_Ku_c30418_40245121*. The positive allele for *QTL_2A_1* derived from the susceptible parental lines, the positive allele for *QTL_2A_2* derived from the resistant cultivar 'HTRI1410' explaining 13.5 % and 19.4 % of the phenotypic variance, respectively. The QTL on chromosome 3A deriving from the susceptible parental lines is located at 80.53 cM. The closest marker is *BS00073525_51* explaning 8.0 % of the phenotypic variance. The QTL on 6A, closest to marker *Excalibur_c48569_78* is located at 78.12 cM, explaning 8.2 % of the phenotypic variance.

With regard to isolate IPO98022, four QTL were detected (Table 10). On chromosomes 2A, 4A and 1B, the positive allele derived from the susceptible parental lines. The phenotypic variance explained ranged from 7.7 % to 10.2 %. An additional QTL was located on the long arm of chromosome 2B in an interval ranging from 89.35 cM to 96.79 cM, closest to marker *Kukri_c27693_710*. The explained phenotypic variance is 8.8 % and the positive allele derived from 'HTRI1410'.

Nine QTL were detected for isolate IPO98050 (Table 10). One QTL (*QTL_1A_1*) was mapped on the long arm on chromosome 1A, closest to *Excalibur_c32167_59*, explaining 10.6 % of the phenotypic variance. The positive allele derived from the resistant parent 'HTRI1410'. A second QTL deriving from 'HTRI1410' was detected on chromosome 3A at 73.22 cM, explaining 13 % of the phenotypic variance. Two QTL were located on chromosomes 4A and 5B, both derived from the resistant cultivar 'HTRI1410'. Furthermore, five QTL were detected on chromosomes 1A, 3A, 4A, and 5B, all positive alleles derived from the susceptible parental lines.

TABLE 10 Overview on single QTL detected for resistance to *Zymoseptoria tritici* in a detached leaf assay (NEC %).

Isolate	QTL	Chr.	Position (cM)	Closest Marker	Intervall	LOD	Phenotypic variance explained (%)	Origin
IPO323	*QTL_2A_1*	2A	49.33	*BS00048615_51*	46.89-50.14	5.95	13.5	[a]SP
	QTL_2A_2	2A	52.58	*wsnp_Ku_c30418_40245121*	50.14-54.20	8.21	19.4	HTRI1410
	QTL_3A_1	3A	80.53	*BS00073525_51*	79.72-83.79	3.60	8.0	[a]SP
	QTL_6A	6A	78.12	*Excalibur_c48569_78*	77.30-81.40	3.78	8.2	HTRI1410
IPO98022	*QTL_2A_3*	2A	4.91	*RAC875_c54668_102*	2.45-8.98	3.77	7.9	[a]SP
	QTL_4A_1	4A	141.31	*BS00093289_51*	140.5-151.19	4.75	10.2	[a]SP
	QTL_1B	1B	87.43	*BS00064162_51*	83.36-89.07	3.65	7.7	[a]SP
	QTL_2B	2B	95.11	*Kukri_c27693_710*	89.35-96.79	4.16	8.8	HTRI1410
IPO98050	*QTL_1A_1*	1A	71.42	*Excalibur_c32167_59*	70.61-77.13	5.66	10.6	HTRI1410
	QTL_1A_2	1A	92.65	*BS00087588_51*	91.84-93.46	7.56	11.4	[a]SP
	QTL_3A_2	3A	72.41	*wsnp_Ex_c18223_27035083*	69.15-73.22	7.62	13.4	[a]SP
	QTL_3A_3	3A	73.22	*Ex_c24992_1659*	72.41-74.03	7.59	13.0	HTRI1410
	QTL_4A_2	4A	24.70	*Kukri_c46057_646*	23.70-26.14	7.22	12.0	[a]SP
	QTL_4A_3	4A	75.51	*RFL_Contig4336_184*	68.15-76.33	7.95	13.0	HTRI1410
	QTL_4A_4	4A	98.74	*Excalibur_c53864_331*	96.93-100.18	4.42	7.1	[a]SP
	QTL_5B_1	5B	37.48	*RAC875_rep_c105322_99*	34.22-39.92	3.95	6.1	[a]SP
	QTL_5B_2	5B	60.51	*Tdurum_contig30483_335*	55.58-62.95	3.78	5.3	HTRI1410

[a]SP (Susceptible parent)

In addition to the genetic map, the positions of the QTL were anchored to the IWGSC RefSeq (Table 11). For isolate IPO323, the marker order of the genetic map in the QTL interval on chromosomes 2A, 3A and 6A are consistent with the IWGSC RefSeq and the 3.25 cM, 4.07 cM and 4.10 cM marker intervals are equivalent to 309.5 Mbp, 155.1 Mbp and 2.3 Mbp, respectively. The *QTL_2A_2* on chromosome 2A spans a 4.06 cM interval but the marker order on the physical map is inverted.

For isolate IPO98022, the marker order of the genetic map in the QTL interval on chromosomes 2A, 4A and 1B are consistent with the IWGSC RefSeq and the 6.53 cM, 10.69 cM and 5.71 cM marker intervals are equivalent to 13.0 Mbp, 5.1 Mbp and 17.9 Mbp. The *QTL_2B* on chromosome 2B spans a 7.44 cM interval but the marker order on the physical map is inverted. The SNP information for marker *RFL_Contig2826_614* is not available yet.

For isolate IPO98050, the marker order of the genetic map in the QTL interval on chromosomes 1A, 3A, 4A and 5B is consistent with the IWGSC RefSeq and the 6.52 cM, 1.62 cM, 4.07 cM, 1.62 cM, 8.18 cM and 5.70 cM marker intervals are equivalent to 23.2 Mbp, 0.7 Mbp, 31.3 Mbp, 9 Mbp, 6.1 Mbp and 100.9 Mbp.

The *QTL_4A_2, QTL_4A_4 and QTL_5B_2* on chromosomes 4A and 5B span a 2.44 cM, 3:25 cM and 7.37 cM interval but the marker order on the physical map is inverted. The SNP information for marker *RFL_Contig4336_184* is not available yet.

TABLE 11 Overview of the QTL detected on chromosomes 5A, 4B and 7B with peak and flanking marker and comparison to the IWGSC RefSeq (Mbp).

Isolate	QTL	Marker	Genetic map (cM)	Physical map (Mbp)
IPO323		*IACX317*	46.89	101.3
	QTL_2A_1	*BS00048615_51*	49.33	200.9
		Ku_c13655_1180	50.14	410.8
		Ku_c13655_1180	50.14	410.8
	QTL_2A_2	*wsnp_Ku_c30418_40245121*	52.58	361.3
		BobWhite_c2532_966	54.20	154.4
		BS00021909_51	79.72	490.8
	QTL_3A_1	*BS00073525_51*	*80.53*	636.5
		Tdurum_contig42150_3348	83.79	645.9
		wsnp_Ex_c14692_22766127	77.30	58.1
	QTL_6A	*Excalibur_c48569_78*	*78.12*	60.0
		Excalibur_rep_c82397_137	81.40	60.4
IPO98022		*Tdurum_contig69643_287*	2.45	18.1
	QTL_2A_3	*RAC875_c54668_102*	4.91	27.8
		BS00094817_51	8.98	31.1
		wsnp_CAP7_c254_138937	140.50	712.8
	QTL_4A_1	*BS00093289_51*	141.31	713.1
		Tdurum_contig10672_117	151.19	717.9
		Kukri_c7657_986	83.36	583.1
	QTL_1B	*BS00064162_51*	87.43	601.0
		RFL_Contig2826_614	89.07	[a]NA
		Tdurum_contig30009_425	89.35	512.6
	QTL_2B	*Kukri_c27693_710*	95.11	494.4
		CAP12_c2291_383	96.79	551.0
IPO98050		*BS00065676_51*	70.61	477.7
	QTL_1A_1	*Excalibur_c32167_59*	71.42	485.3
		BS00094553_51	77.13	500.9
		CAP7_c11581_78	91.84	532.0
	QTL_1A_2	*BS00087588_51*	92.65	532.2
		Kukri_c84024_375	93.46	532.7
		RAC875_c17479_359	69.15	574.5
	QTL_3A_2	*wsnp_Ex_c18223_27035083*	72.41	602.9
		Ex_c24992_1659	73.22	605.8
		wsnp_Ex_c18223_27035083	72.41	602.9
	QTL_3A_3	*Ex_c24992_1659*	73.22	605.8
		Tdurum_contig64606_1104	74.03	611.9
		GENE-2637_396	23.70	387.5
	QTL_4A_2	*Kukri_c46057_646*	24.70	348.0
		Tdurum_contig10559_871	26.14	258.8
		Kukri_c25832_687	68.15	623.3
	QTL_4A_3	*RFL_Contig4336_184*	75.51	[a]NA
		Kukri_rep_c102255_365	76.33	629.4
		Excalibur_c13054_1564	96.93	675.9
	QTL_4A_4	*Excalibur_c53864_331*	97.74	673.4
		Excalibur_c7034_692	100.18	660.9
		Excalibur_rep_c68375_213	34.22	330.1
	QTL_5B_1	*RAC875_rep_c105322_99*	37.48	403.7
		wsnp_Ex_c16704_25250247	39.92	431.0
		BS00010213_51	55.58	422.3
	QTL_5B_2	*Tdurum_contig30483_335*	60.51	418.0
		CAP7_c6363_226	62.95	421.4

[a]NA (not available)

DISCUSSION

The purpose of the current study was to get information on the resistance to different isolates of Zymoseptoria *tritici.* Therefore, the resistant gene bank spelt wheat accession 'HTRI1410' and wheat cultivars 'Alcedo', 'Jenga' and 'Solitär', parents of the DH populations, were analysed for their reaction to a larger set of defined isolates of *Zymoseptoria tritici* representing 13 mapped *Stb* resistance genes (Brown et al. 2015).

The isolate specificity of the *Triticum aestivum* - *Zymoseptoria tritici* host pathogen system has been discovered in numerous experiments using single spore isolates (Kelm et al. 2012; Kema 1996; Kema and van Silfhout 1997). Respective segregation ratios giving hint that resistance is due to major qualitative resistance genes could not be detected in our study. Similar results were also obtained by Kelm et al. (2012). The same holds true with respect to Pop1, an isolate derived from a locally adapted *Z. tritici* population. Mundt et al. (1999) reported that the adaption of Z. *tritici* isolates to quantitative resistance of wheat cultivars strongly depends on the host and the environmental conditions (Asher and Thomas 1984; Newton 1989). By combining the effective qualitative resistance with a high number of quantitative trait loci in new wheat varieties is supposed to be the most efficient way (Risser et al 2011). Strains with increased aggressiveness occur more often under weather conditions favorable for pathogen development (Mundt et al. 1999). The four parental lines were all susceptible towards the local isolate Pop1 but 'HTRI1410' showed in comparison the lowest level of infestation. Adaption of *Z. tritici* to resistant and partly resistant wheat cultivars has already been reported in several studies (Jackson et al. 2000; Krenz et al. 2008).

According to the calculated high repetability of the detached leaf assay, the experimental design is suited for determining differences in the resistance to different *Z. tritici* isolates. The infestation expressed as the mean leaf area diseased with necrosis, is determined by the aggressiveness of the respective isolates and the resistance of the genotype (Chen et al. 2017). The characterization of the *Z. tritici* isolates revealed clear differences in the aggressiveness and in the susceptibility of the genotypes tested. By using 16 different isolates in a detached leaf assay for phenotyping allowed the detection of an isolate-specific resistance reaction in

early developmental stages. The lack of full resistance and partly infestation also of resistant varieties is due to the combined effect of several QTL and environmental effects resulting in quantitative resistance (Eriksen et al. 2003; Simón et al. 2004).

Over all isolates, a good differentiation between parental lines was observed in our studies (Figure 8). This confirms that resistance to *Z. tritici* is encoded by qualitative and quantitative resistances (Kema and van Silfhout 1997).

Regarding QTL mapping, numerous studies reported about QTL on all three genomes of wheat for resistance against *Zymoseptoria tritici* (Brown et al. 2015). In our population, we detected in total 17 QTL using three defined isolates, i.e. IPO323, IPO98022 and IPO98050, all showing a clear differentiation between parental lines.

We compared the localization of QTL detected in our study with known *Stb* genes and QTL described in literature (Brown et al. 2015) by using the reference sequence of wheat (Appels et al. 2018).

We detected two QTL effective against IPO98050 in the population 'HTRI1410 x SP' on chromosome 1A in a marker interval of 477.7 Mbp to 500.9 Mbp and 532.0 Mbp to 532.7 Mbp, respectively. Up to now, no *Stb* gene is mapped on chromosome 1A, but there are several studies reporting about QTL and MetaQTL on chromosome 1A in different populations for resistance against *Z. tritici* (Goudemand et al. 2013; Kelm et al. 2012; Risser et al. 2011). *QTL1 and MQTL1* detected by Goudemand et al. (2013), *QStb.1A* (Kelm et al. 2012) and *QStb.lsa_fb-1A* (Risser et al. 2011) are located in another genomic region, whereas *QTL2* (Goudemand et al. 2013) could be co-located to our QTL defined as *QTL_1A_1* and *QTL_1A_2*.

In our studies, we detected three QTL conferring resistance to STB on chromosome 2A. Up to now, neither a resistance gene nor a QTL is mapped on chromosome 2A. Two MetaQTL are already reported by Goudemand et al. (2013) identified in several bi-parental populations, but located in another genomic region, so it is assumed that *QTL_2A_1* and *QTL_2A_2* conferring resistance to IPO323 are new. Furthermore, our detected QTL explain a much higher phenotypic variance than the previously detected *MetaQTL4* and *MetaQTL5* (Goudemand et al.

2013). We identified two QTL effective against IPO98050 and one QTL effective against IPO323 on chromosome 3A. *QTL_3A_1* for resistance to isolate IPO323 is located in an interval from 636.5 Mbp to 644.8 Mbp and both QTL (*QTL_3A_2; QTL_3A_3*) conferring resistance to IPO98050 are located in intervals of 574.5 Mbp to 602.9 Mbp and 602.9 Mbp to 611.9 Mbp. *Stb6* is located at 25.4 Mbp (Saintenac et al. 2018). In our study *QTL_3A_1* also contributed resistance against IPO323 but *QTL_3A_1* is located in an interval of 636.5 Mbp to 644.8 Mbp, closest to marker *BS00073525_51* and is therefore not in the same genomic region. Resistance gene *Stb6* is effective to isolate IPO323 but not effective to IPO92469 and co-segregates with SSR marker *Xgwm369*, 2 cM from *Stb6* (Brading et al. 2002; Saintenac et al. 2018). Despite the presence of *Stb6* in varieties, these still show a genetic variation in infestation severity (Arraiano and Brown 2006). Furthermore, Kema et al. (2000) have shown that the isolate IPO323 carries another *Avr* virulence gene (i.e. *AvrStb6*) in addition to the one overcomming *Stb6* (Zhong et al. 2017). This was shown in later studies by Chartrain et al. (2005c) who identified in addition to Kema et al. (2000) an additional gene for resistance to IPO323 besides *Stb6* in spring wheat cultivar 'Kavkaz-K4500 L.6.A.4 (KK)'. Furthermore, already known *QStb.risø-3A.1* (25.4 Mbp), *QStb.risø-3A.2* (180 Mbp), *QStb.3AS* (25.4 Mbp) as well as *3AS* and *QStb.wai-3A* are excluded to be similar to our QTL since they are all located on the short arm of chromosome 3A (Eriksen et al. 2003; Ghaffary et al. 2011; Kelm et al. 2012; Zwart et al. 2010).

On chromosome 4A, we detected four QTL on the long arm conferring resistance to IPO98022 and IPO98050. *QTL_4A_1* spans an interval from 712.8 Mbp to 717.9 Mbp. In addition, *Stb12* is mapped on the long arm of chromosome 4A at 732.9 Mbp, so *Stb12* is a candidate for *QTL_4A_1*, conferring resistance to isolate IPO98022. *Stb12* has been differentiated from *Stb7* by the different response of the parental lines to two Israelian isolates and was determined to be more closely linked to *Xwmc219* than to *Xwmc313* (Chartrain et al. 2005b). McCartney et al. (2003) mapped *Stb7* in proximity to *Xwmc313* in crosses with the spring wheat variety 'Estanzuel Federal' and furthermore mapped *Stb7* independently in a population derived from a cross between 'KK' and cultivar 'Shafir' (Chartrain et al. 2005c).

In addition to *Stb* genes, four QTL, i.e. *QTL7, QTL6, QStb.4AL* and *QStb.lsa_tb-4A* conferring resistance in both seedling stage and adult plants are already mapped on this chromosome (Goudemand et al. 2013; Kelm et al. 2012; Risser et al. 2011). *QStb.4AL* is located at 739.4 Mbp in the more distal part of chromosome 4A and therefore not in the region of the QTL detected in our study. The QTL located nearest to this already known QTL, i.e. *QTL_4A_1* spans an interval from 712.8 Mbp to 717.9 Mbp.

On the short arm of chromosome 6A, resistance gene *Stb15* was mapped (Arraiano et al. 2007) and in addition, *MetaQTL20* (Goudemand et al. 2013). *QTL_6A* which covers an interval from 58.1 Mbp to 60.4 Mbp is located in the same genomic region as *Stb15* (Brown et al. 2015). For a more precise localization of already known STB genes and QTL and the comparison to our detected QTL, the sequence information of the STB linked SSR, AFLP and RFLP markers are needed.

Raman et al. (2009) located *Stb11* on chromosome 1BS in an interval between markers *Xwmc230* and *Xbarc119b*. Resistance gene *StbWW* (Goudemand et al. 2013) is also located on the short arm of chromosome 1B. In our study, *QTL_1B* for isolate IPO98022 is located in a marker interval from 585.1 Mbp to 601.0 Mbp on the distal end of the long arm of chromosome 1B and so is not in the same genomic region as *Stb11* and *StbWW.*

QTL_2B for isolate IPO98022 was detected on the distal end of the long arm on chromosome 2B in a marker interval from 512.6 Mbp to 551.0 Mbp, so that there is no match with *Stb9* which covers roughly the same region but is located at 366.5 Mbp (Chartrain et al. 2009).

On chromosome 5B, we detected two QTL conferring resistance to IPO98050 at 403.7 Mbp in a marker interval from 330.1 Mbp to 431.0 Mbp and at 418.0 Mbp in a marker interval of 418.0 Mbp to 422.3 Mbp. Besides four QTL which are already known on chromosome 5B (Goudemand et al. 2013; Mergoum et al. 2013; Miedaner et al. 2012, 2012; Risser et al. 2011), resistance gene *Stb1* is located on the long arm of chromosome 5B at 402.6 Mbp and so is mapped within the same interval as *QTL_5B_1.*

Unfortunately, the lack of common polymorphic markers between different mapping populations often complicates a final comparative QTL study. Furthermore, population size is the

limiting factor when detecting new minor QTL with low heritability (Kelm et al. 2012).

To improve the resistance level in wheat, pyramiding of QTL seems to be an efficient tool as it was already demonstrated for leaf rust, yellow rust, stem rust (Tyagi et al. 2014) and Fusarium head blight (Badea et al. 2008; Shi et al. 2008; Tamburic-Ilincic et al. 2011). For facilitating the use of resistances against Septoria tritici blotch, KASP markers were developed from SNP sequences out of flanking SNP markers. The detected QTL regions, which are saturated by KASP markers differentiating between susceptible and resistant DH lines, allow marker assisted breeding for disease related traits (Dreisigacker et al. 2015).

4 GENERAL DISCUSSION

The identification and mapping of quantitative trait loci for resistance in winter wheat against the hemibiotrophic fungus *Zymoseptoria tritici*, the causal agent of Septoria tritici blotch, was the main aim of this study. Therefore, a DH population consisting of 135 DH lines of crosses between the resistant spelt wheat gene bank accession 'HTRI1410' and three susceptible cultivars (Alcedo, Jenga, Solitär) was analysed in extensive field trials as well as in detached leaf assays under controlled conditions.

In this study, we report about different methods for the detection of genomic regions conferring resistance to STB. For the characterization of new resistances and the analyses of their genetic background, there are different experimental methods, each with advantages and disadvantages. First, the screening in greenhouses guarantees highly controlled conditions for the experiments resulting in a better reproducibility of trials. In contrast, field trials better reflect natural growing conditions, but the results obtained may be subject to environmental influences and their strong variations (Odilbekov et al. 2014) e.g. Septoria tritici blotch infestation degree is highly influenced by biotic and abiotic environmental conditions (Klahr et al. 2007; Schilly et al. 2011). Biotic factors are, on the one hand, the simultaneous occurrence of other leaf pathogens in the field, which must be taken into account and can lead to difficulties in scoring e.g. tan spot (*Pyrenophora tritici-repentis*) and the causal agent of Stagonospora nodorum blotch of wheat (*Parastagonospora nodorum;* previously *Septoria nodorum, Stagonospora nodorum*), occur on wheat plants in field rials at the same time as STB (Ponomarenko et al. 2011). Differentiating between these fungal diseases is difficult but possible by determining their spore shape and size (Salgado and Paul 2016) as well as by the time and place of appearance, e.g. *Parastagonospora nodorum* arises on leaves and glumes (glume blotch), whereas in contrast *Zymoseptoria tritici* occurs on wheat leaves. Additionally, symptoms of *Zymoseptoria tritici* usually can be found on lower leaves in the fall and early spring, whereas *P. nodorum* appears in an interval of two or three weeks to heading mostly on the upper leaves (McMullen and Adhikari 2016).

Tan to brown flecks hint to *Pyrenophora tritici-repentis* which later develop to irregular oval shaped lesions with a yellow or chlorotic circle and a dark spot in the center. When both Septoria pathogens occur at the same time on the same plants, they are referred to as the Septoria blotch complex or Septoria complex (Ponomarenko et al. 2011).

Furthermore, abiotic factors have an impact on the results of field trials. As described in chapter 1, the lower expression of STB symptoms of field trials in 2015 was due to less favorable weather conditions. The spread of *Zymoseptoria tritici* strongly depends on humid and wet weather conditions (Shaw 1990). The lack of rainfall and persistently high temperatures, as they occurred in 2015 (Deutscher Wetterdienst 2015a, 2015b) led to a decreased symptom expression.

Taking into account all biotic and abiotic factors, which influenced the trials of our studies, in the end a combination of both, field and greenhouse experiments is the best way to determine variation of the STB resistance in the DH population. This is also shown by correlation analysis, showing a correlation of r= 0.14 between multi-year field trials and the detached leaf assay for isolate IPO98050. For isolate IPO98022 a correlation of r= 0.06 and for the isolate IPO323 a negative correlation of r= -0.1 was calculated. In summary, there is no significant correlation between the results obtained in field trials and the detached leaf assays for the different isolates used (Figure 13). The adapted controlled conditions in the greenhouse and the following detached leaf assay enabled the detection of isolate-specific interactions (Arraiano et al. 2001), while in the field experiments the environmental influence and the influence of the local *Z. tritici* populations have been considered. Arraiano et al. (2001) were able to demonstrate a close correlation between the results of field trials, whole plant trials and detached leaf assays, which was not the case in our study.

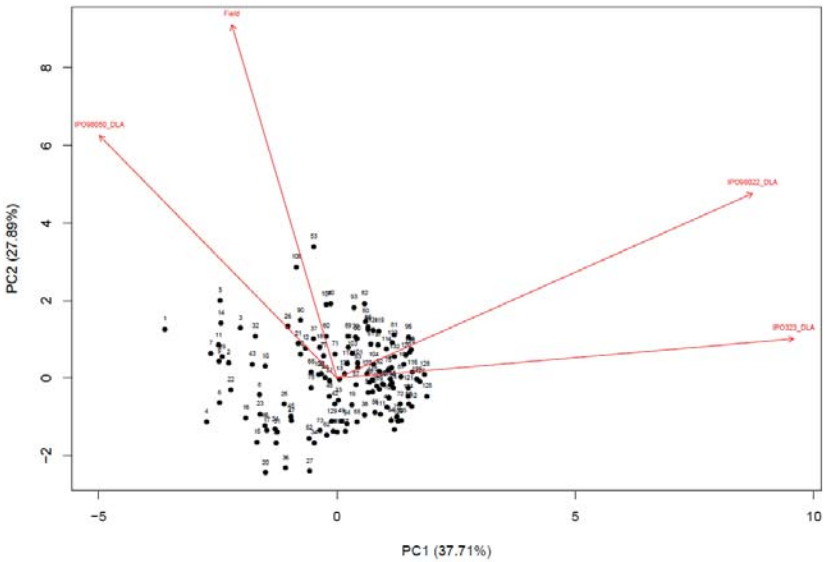

FIGURE 13 Principal Component Analysis (PCA) of the DH lines which are indicated as dots showing the relationship of the three years field trials, at three locations based on the adjusted means calculated for *Zymoseptoria tritici* infection and the detached leaf assay using three different isolates (IPO98050_DLA, IPO98022_DLA, IPO323_DLA).

Wheat varieties classified as resistant or susceptible often show a differentiating response whether single isolates or isolate mixtures are used and whether they are tested under field or controlled conditions (Eyal Z. 1992; Zelikovitch 1991). The effect of individual isolates was clearly observed in various studies while it is more difficult to detect these effects if isolates were used as a mixture (Parlevliet 1983; van der Plank 1982). The isolates used in the field trials showed the highest aggressiveness and disease severity, while the isolates used in the trials under greenhouse conditions showed the highest differentiation between parental lines.

The use of fungicides facilitates a better differentiation between different pathogens in field and greenhouse experiments and enables a precise STB scoring. Crop protection products with strobilurins and azoles as active ingredients show effective control of rust diseases and at the same time a clear ineffectiveness against STB, so that *Z. tritici* as a target pathogen is not eliminated, which allows an accurate evaluation (Vagndorf 2018). In our experiments we applied the fungicide 'Corbel' with the active ingredient *Fenpropimorph*, which has a good effectivity against stripe rust and powdery mildew. The fungicide treatment was done at BBCH 32,

the application of the artificial *Zymoseptoria* inoculum was done at BBCH 39 as the flag leaf became visible. No fungicide application was necessary in the detached leaf assay. Here, the Septoria infection was done at BBCH 12 to 13 to determine the resistance in different developmental stages.

The dependence of resistance analyses on the developmental stage of the plant for the detection of QTL involved in STB resistance has already been demonstrated. Studies of Kema and van Silfhout (1997) have shown a higher susceptibility in adult plants compared to seedlings. However, there are reports about resistance mechanisms at both the adult stage and seedling stage, which are often dependent on the selected isolates of *Zymoseptoria tritici* and the investigated plant material (Kema and van Silfhout 1997). Thus, qualitative, isolate-specific resistance genes may be effective at both stages (Arraiano et al. 2001). Nevertheless, complete resistance up to now has only been detected in the seedling stage and not in adult plant stage (Kema and van Silfhout 1997). Partly in agreement with the results of Kema and van Silfhout (1997), in our studies DH lines seemed to be more resistant in the seedling stage than the adult plant stage in general but there are some DH lines which revealed a higher resistance at the adult plant stage. In this respect, DH lines HxA_DH_1 and HxA_DH_8 derived from the cross of 'HTRI1410 x Alcedo', showed high resistance in the field trials with a mean leaf area diseased of 15.99 % and 16.04 %, respectively, but were highly susceptible in the detached leaf assay showing mean infestation of 37.0 % and 48.0 % for isolate IPO323. On the other hand, there where DH lines being resistant both in the field and under controlled conditions, e.g. AxH_DH_26 derived from the cross 'Alcedo x HTRI1410', with mean leaf area diseased of 22.99 % in the field and showing < 15 % mean leaf area diseased in the detached leaf assay for isolates IPO323 and IPO98050.

In order to detect genomic regions that confer resistance to STB, a quantitative trait locus mapping approach was applied in this study for both, field (Chapter 1) and greenhouse experiments (Chapter 2). Within the frame of this work, a large number of QTL were identified with both screening methods, which underline the efficiency of this method and is in line with pub-

lished studies in which wheat resistance to *Z. tritici* was also investigated (Arraiano et al. 2001). The extent to which breeders can use QTL that confer resistance to a certain trait depends on the percentage of the phenotypic variance explained by the QTL. According to the definition of Miedaner and Korzun (2012), a QTL should explain at least between 10.0 % and 20.0 % of the phenotypic variance in the mapping population in order to allow an application in marker assisted selection (MAS). If, however, the detected QTL only explains a small percentage of the phenotypic variance, the transformation into elite lines and MAS is not appropriate.

Furthermore, based on the R^2 value which defines the proportion of phenotypic variation explained, QTL can be classified into major QTL accounting for a relatively high phenotypic variation explained (e.g. > 10 %) and minor QTL with a phenotypic variation explained of < 10 % (Collard et al. 2005). Several studies report about major QTL being stable across different environments whereas minor QTL are environmentally sensitive, especially QTL linked to disease resistance (Li et al. 2001; Lindhout 2002; Pilet-Nayel et al. 2002). Our four QTL discovered in multi-environmental field trials explained between 8.5 % to 17.5 % of the phenotypic variance and are therfore classified as major or minor QTL, repectively. In other studies on wheat, phenotypic variance explained in field trials was estimated between 7.0 % and 9.0 %, (Vagndorf et al. 2017). The explained phenotypic variance in the greenhouse experiments conducting detached leaf assays was 6.1 % to 19.4 % across all three isolates tested. This is comparable to other studies under controlled environmental conditions, such as Aouini (2018) identifying QTL explaining phenotyping variances between 10.3 % and 32.6 %.

In view of this, the *QTL_5A*, which explains 17.5 % of phenotypic variance in field trials and isolate-specific QTL *QTL_2A_2*, *QTL_1A_1, QTL_3A_3* and *QTL_4A_3* (explained variance ranging from 10.6 % to 19.4 %) all derived from the resistant gene bank accession ‘HTRI1410’ might be of interest to breeders.

The significance level of the QTL identified in this study under field (LOD range 3.12 - 6.83) (chapter 1) and greenhouse conditions (LOD range 3.60 - 8.21, Chapter 2) is in the range of those obtained in other STB resistance studies in wheat, e.g Aouini (2018) (LOD

range 7.83 - 32.57) or Vagndorf et al. (2017) (LOD range 3.0 - 3.75). Pyramiding of QTL may lead to a more durable resistance. The four QTL identified in this study for the field trials explain 29.6 % of phenotypic variation (Table 7, chapter 1). By combining the QTL into a single DH line, the disease score of 19.1 % was significantly lower than the average disease score of 23.2 % of the field data. Our results are consistent with previous studies, which showed that higher resistance can be achieved by pyramiding numerous QTL in individual lines resulting in a decreased disease infestation (Chartrain et al. 2004b; Mundt 2014; Vagndorf et al. 2017).

Pyramiding resistance genes has proven to be an approach for preserving disease resistances durability (Brown et al. 2015; Palloix et al. 2009; Parlevliet 2002), e.g. in wheat stem rust (*Puccinia graminis f. sp. tritici*), where the combination of numerous resistance genes has well controlled the disease for more than half a century till it was overcome by Ug99 race (Mundt 2014; Singh et al. 2011). The QTL detected in our studies may possibly be stacked in a new breeding line by using the KASP markers developed resulting in more stable STB resistance.

In our study it turned out that there is no overlap of the detected QTL in the field trials and the detached leaf assays. In the adult plant stage QTL on wheat chromosomes 5A, 4B and 7B were identified in field trials, whereas in the detached leaf assay QTL were found on 1A, 2A, 3A, 4A, 6A and 1B, 2B and 5B. This fits to the low correlation observed between greenhouse and field tests which may be due to the different conditions and the effect that QTL rely on plant development stages as already reported in previous studies (Eriksen and Munk 2003; Ghaffary et al. 2012; Kelm et al. 2012).

But, despite the use of different isolates and the testing under different environmental conditions, an ecologically stable and non-isolate-specific stability of the QTL detected in this work can be assumed, since overlaps between the QTL detected by us and the QTL already identified in previous studies were found, i.e. *QTL2* on chromosome 1A (Goudemand et al. 2013) may be co-located to *QTL_1A_1* and *QTL_1A_2* identified in our study. This overlap of QTL identified in this work with QTL identified in previous studies associated with resistance to *Z. tritici* may be a proof for the reliability of the detected QTL. The final evidence, however, can

only be provided by additional field trials under a range of environmental conditions and with different isolates (Miedaner and Korzun 2012).

Since many of the detected QTL have only small effects and this is in line with the results of other studies focusing on the detection of resistance QTL (Vagndorf et al. 2017), it may be assumed that this probably due to the complex inheritance of resistances of adult plants.

The resistance of the gene bank accession 'HTRI1410' to Septoria tritici blotch in the field has proved to be a quantitative trait conferred by major and minor QTL with small to moderate effects on the resistance response. In order to increase the number of QTL to be detected and the respective LOD score, additional DH lines had to be tested in order to increase the population size. An alternative to increase additional QTL for STB resistance is the use of genome wide association studies (Jannink et al. 2010). The use of polymorphic SNP markers was the basis for the design of KASP markers that can be used for marker-assisted breeding and on the other hand they are effective tools allowing the detection of marker trait associations (Khalid et al. 2019; Rasheed et al. 2016).

The development of available KASP markers for the detected QTL and the use of these markers in marker assisted selection has already been demonstrated e.g. with respect to wheat strip rust (Wu et al. 2017).

CONCLUSION AND FUTURE PERSPECTIVES

Wheat is the major source for feeding the earth´s growing population, but severe yield losses are each year caused by pathogens. Among these Septoria tritici blotch is of prime importance. The aim of the present thesis was mapping of the quantitative resistance against Septoria tritici blotch derived from the accession 'HTRI1410' in field and greenhouse trials. To achive this three doubled-haploid (DH) populations derived from crosses of 'HTRI1410' to the susceptible parental lines 'Alcedo', 'Jenga' and 'Solitär' were analysed. In detail, the aims were (1) to map QTL involved in STB field resistance in adult plants based on a multi-environmental field trial, (2) to characterize the resistance of the DH populations to a worldwide *Zymoseptoria tritici*

isolate collection in a detached leaf assay, (3) to identify genes and/or QTL conferring resistance to these STB isolates and (4) to develop molecular markers which facilitate the use of MAS in wheat breeding for STB resistance.

In extensive screening programs for resistance, the spelt wheat gene bank accession 'HTRI1410' turned out to be resistant to *Zymoseptoria tritici* in field conditions. In order to get information on the genetics of the STB resistance in 'HTRI1410', a DH population consisting of 135 lines derived from crosses of 'HTRI1410' to three susceptible cultivars was developed. Significant genotypic differences and a quantitative variation for the reaction to *Zymoseptoria tritici* were observed. Based on these phenotypic data and a genetic map comprising 714 90K iSelect derived SNP markers four quantitative trait loci on chromosomes 5A, 4B and 7B, explaining 8.5 % to 17.5 % of the phenotypic variance were identified. In additional studies using IPO323 virulent to *Stb6*, IPO98022 virulent to *Stb18* and IPO98050 virulent to *Stb8* QTL for resistance against these isolates were detected on several chromosomes. Resistance to IPO323 is controlled by a major QTL on chromosome 2A and minor QTL on chromosomes 2A, 3A and 6A. QTL against isolate IPO98022 were detected on chromosomes 2A, 4A, 1B and 2B. Furthermore, six major QTL on chromosomes 1A 3A, 4A and three minor QTL on chromosomes 4A and 5B were identified for resistance against *Zymoseptoria tritici* isolate IPO98050. In summary, our results point to a complex inheritance of resistance to STB in the seedling stage.

In the future due to the availability of the wheat reference genome (Appels et al. 2018) genes conferring resistance to *Z. tritici* may be isolated faster facilitating site directed mutagenesis (Bao et al. 2019) using e.g. CRISPR/Cas9. This may be conducted already today for the isolated resistance gene *Stb6* (Saintenac et al. 2018). Since the resistance of *Zymoseptoria tritici* is highly quantitative, as also shown in our studies, genomic selection may be an efficient tool to accelerate wheat breeding for STB resistance in the future (Bassi et al. 2016; Juliana et al. 2017; Varshney et al. 2005). Genomic selection considers the quantitative loci segregating in breeding germplasm more efficiently than classical marker-assisted selection.

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8 ABBREVIATIONS

AFLP, Amplified fragment length polymorphism

ANOVA, Analysis of variance

AO, Average ordinate

AUDPC, Area under the disease progress curve

cM, Centimorgan

DH, Doubled haploid

DNA, Deoxyribonucleic acid

FAO, Food and Agriculture Organization of the United Nations

GB, Giga base

GBS, Genotyping by sequencing

GWAS, Genom-wide association study

IPM, Integrated pest management

IWGSC, International Wheat Genome Sequencing Consortium

JKI, Julius Kuehn-Institute

KASP, Kompetitive Allele Specific PCR

LOD, Logarithm of odds ratio

LSMEANS, Least squares means

MABC, Marker-assisted backcrossing

MAS, Marker assisted selection

MTA, Marker trait association

PCA, Principal component analysis

PCR, Polymerase chain reaction

PDA, Potato dextrose agar

QoIs, Quinone outside inhibitors

QTL, Quantitative trait locus/loci

RFLP, Restriction fragment length polymorphism

SNP, Single nucleotide polymorphism

SSR, Simple sequence repeats

STB, Septoria tritici blotch

UV, Ultraviolet

YG, Yeast-glucose

9 APPENDIX

KASP marker and sequence of detected QTL for resistance to *Zymoseptoria tritici* (LGC 2018)

Marker	Sequence
BS00094342_51_A	GAAGGTGACCAAGTTCATGCTCacAATgcGtcctAGtcaacatT
BS00094342_51_B	GAAGGTCGGAGTCAACGGATTacAATgcGtcctAGtcaacatC
BS00094342_51_com	atgatgtatcttgccatggattG
Tdurum_contig42141_2028_A	GAAGGTGACCAAGTTCATGCTCacAATccgtgtaactcgctctagcataT
Tdurum_contig42141_2028_B	GAAGGTCGGAGTCAACGGATTacAATccgtgtaactcgctctagcataC
Tdurum_contig42141_2028_com	gccacagacgtgatatgcac
Excalibur_c2217_731_A	GAAGGTGACCAAGTTCATGCTggttcgatccgcagaacgT
Excalibur_c2217_731_B	GAAGGTCGGAGTCAACGGATTggttcgatccgcagaacgC
Excalibur_c2217_731_com	tttcctctcatctggctttctcG
Tdurum_contig9966_724_A	GAAGGTGACCAAGTTCATGCTCggtcgatggTgggatggtT
Tdurum_contig9966_724_B	GAAGGTCGGAGTCAACGGATTCggtcgatggTgggatggtC
Tdurum_contig9966_724_com	ctctcccttgagcccGctT

KASP marker and sequence of detected QTL for resistance to *Zymoseptoria tritici* (LGC 2018)

Marker	Sequence
BS00021739_51_A	GAAGGTGACCAAGTTCATGCTcagtctggacctttgaaataccT
BS00021739_51_B	GAAGGTCGGAGTCAACGGATTcagtctggacctttgaaataccC
BS00021739_51_com	gccaataactgaaaactAGAaaGgA
Ku_c13655_1180_A	GAAGGTGACCAAGTTCATGCTGCAATaccatcatatgcgatgT
Ku_c13655_1180_G	GAAGGTCGGAGTCAACGGATTGCAATaccatcatatgcgatgC
Ku_c13655_1180_com	ctttgcagatttgttttgggattT
BS00073525_51_A	GAAGGTGACCAAGTTCATGCTggccatTgctccgcttgA
BS00073525_51_C	GAAGGTCGGAGTCAACGGATTggccatTgctccgcttgC
BS00073525_51_com	ccTgttgagcaCtgTaaaAgaaaaT
Excalibur_c48569_78_A	GAAGGTGACCAAGTTCATGCTtttatacGgcgctAtacgtAgtT
Excalibur_c48569_78_B	GAAGGTCGGAGTCAACGGATTtttatacGgcgctAtacgtAgtC
Excalibur_c48569_78_com	ggttggagtagatgcatgAacA
Tdurum_contig69643_287_A	GAAGGTGACCAAGTTCATGCTaacagcGcgaggaaataaagcA
Tdurum_contig69643_287_B	GAAGGTCGGAGTCAACGGATTaacagcGcgaggaaataaagcG
Tdurum_contig69643_287_com	catactctgGtcaaactctgcagT
BS00093289_51_T	GAAGGTGACCAAGTTCATGCTtcgatggagctaagttgcactaA
BS00093289_51_C	GAAGGTCGGAGTCAACGGATTtcgatggagctaagttgcactaG
BS00093289_51_com	agatggaggacgtggagagg
Kukri_c7657_986_A	GAAGGTGACCAAGTTCATGCTcggacacCtgttcctccaaT
Kukri_c7657_986_B	GAAGGTCGGAGTCAACGGATTcggacacCtgttcctccaaC
Kukri_c7657_986_com	cgttCgtgGcaattgcaaaT
Tdurum_contig30009_425_A	GAAGGTGACCAAGTTCATGCTtgtAccaagctcttccatttgtA
Tdurum_contig30009_425_B	GAAGGTCGGAGTCAACGGATTtgtAccaagctcttccatttgtG
Tdurum_contig30009_425_com	tcatggagaactttgcAaagaaG
Excalibur_c32167_59_A	GAAGGTGACCAAGTTCATGCTaaggccgatAcgtaggcA
Excalibur_c32167_59_B	GAAGGTCGGAGTCAACGGATTaaggccgatAcgtaggcG
Excalibur_c32167_59_com	ccgatgaaggtgcacatgtT
BS00087588_51_A	GAAGGTGACCAAGTTCATGCTcgcatcgctatatctcgcT
BS00087588_51_B	GAAGGTCGGAGTCAACGGATTcgcatcgctatatctcgcC
BS00087588_51_com	cCtttgaccaatctgcaGcA

wsnp_Ex_c18223_27035083_A	GAAGGTGACCAAGTTCATGCTCggatccagttggtgaagcA
wsnp_Ex_c18223_27035083_B	GAAGGTCGGAGTCAACGGATTCggatccagttggtgaagcG
wsnp_Ex_c18223_27035083_com	ggatgagtaaTcaaggcaacaG
Ex_c24992_1659_A	GAAGGTGACCAAGTTCATGCTcaagtaggacaaattacagcaacaT
Ex_c24992_1659_B	GAAGGTCGGAGTCAACGGATTcaagtaggacaaattacagcaacaG
Ex_c24992_1659_com	ccatttCcGtcatgtaaccctC
wsnp_Ex_c54395_57291841_A	GAAGGTGACCAAGTTCATGCTtgggaggtccatccatgT
wsnp_Ex_c54395_57291841_B	GAAGGTCGGAGTCAACGGATTtgggaggtccatccatgC
wsnp_Ex_c54395_57291841_com	gcctcgtGgtggaagaAatC
Kukri_rep_c102255_365_A	GAAGGTGACCAAGTTCATGCTatctctcgcgacgaacacA
Kukri_rep_c102255_365_B	GAAGGTCGGAGTCAACGGATTatctctcgcgacgaacacG
Kukri_rep_c102255_365_com	cgttgagctgtgccgttG
Excalibur_c7034_692_A	GAAGGTGACCAAGTTCATGCTGtcaaaggactgcggaaaattA
Excalibur_c7034_692_B	GAAGGTCGGAGTCAACGGATTGtcaaaggactgcggaaaattG
Excalibur_c7034_692_com	tctAgtttgCctcttccatatCC
RAC875_rep_c105322_99_A	GAAGGTGACCAAGTTCATGCTcctgacaaatacggcactcT
RAC875_rep_c105322_99_B	GAAGGTCGGAGTCAACGGATTcctgacaaatacggcactcC
RAC875_rep_c105322_99_com	tggttcctgaagaagaaccC
CAP7_c6363_226_T	GAAGGTGACCAAGTTCATGCTaacaacgcgtgggccttT
CAP7_c6363_226_C	GAAGGTCGGAGTCAACGGATTaacaacgcgtgggccttC
CAP7_c6363_226_com	acacatttagcttccggggg

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WORK EXPERIENCE

Since 08/2018 **STATE INSTITUTE OF AGRICULTURE AND HORTICULTURE BERNBURG**
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PUBLICATIONS AND PROCEEDINGS

PUBLICATIONS (PEER- REVIEW)

CHAPTER 1

Karlstedt F, Kopahnke D, Perovic D, Jacobi A, Pillen K, Ordon F (2019) Mapping of quantative trait loci (QTL) for resistance against *Zymoseptoria tritici* in the winter spelt wheat accession HTRI1410 (*Triticum aestivum* L. subsp. *spelta*). Euphytica 215:433. doi: 10.1007/s10681-019-2432-3

CHAPTER 2- PLANED

Karlstedt, F; Kopahnke, D; Perovic, D; Ordon, F (2020) Identification and mapping of QTL for seedling resistance against *Zymoseptoria tritici* Rob. ex Desm. in the winter wheat accession HTRI1410 (*Triticum aestivum* L. subsp. *spelta*)

POSTER PRESENTATIONS

Karlstedt, F; Kopahnke, D; Ordon, F (2016): Mapping of QTL for *Septoria tritici* resistance in the wheat gene bank accession HTRI1410. 9th International Symposium on Septoria Diseases of Cereals, 7-9 April 2016, Paris, France, 123.

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ORAL PRESENTATIONS

Karlstedt, F; Kopahnke, D; Perovic, D; Ordon, F (2015): Identifizierung und Kartierung von QTL für Resistenz gegenüber der Septoria- Blattdürre (*Septoria tritici*) des Weizens in der Akzession HTRI1410 (IKRS). proWeizen-Konferenz, 20.-21.04.2015, Quedlinburg, Deutschland.

Karlstedt, F; Kopahnke, D; Perovic, D; Ordon, F (2016): Identifizierung und Kartierung von QTL für Resistenz gegenüber der Septoria- Blattdürre (*Septoria tritici*) des Weizens in der Akzession HTRI1410 (IKRS). proWeizen-Konferenz, 20.-21.04.2016, Quedlinburg, Deutschland.

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Karlstedt, F; Kopahnke, D; Perovic, D; Ordon, F (2018): Identifizierung und Kartierung von QTL für Resistenz gegenüber der Septoria- Blattdürre (*Septoria tritici*) des Weizens in der Akzession HTRI1410 (IKRS). proWeizen-Konferenz, 18.-19.04.2018, Quedlinburg, Deutschland.

EIDESSTATTLICHE ERKLÄRUNG/ *DECALRATION UNDER OATH*

Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word or content.

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