Inheritance of resistance to *Septoria tritici* blotch in the winter wheat doubled haploid population Solitär x Mazurka

Dissertation (kumulativ)
zur Erlangung des
Doktorgrades der Agrarwissenschaften (Dr. agr.)

der

Naturwissenschaftlichen Fakultät III
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Verteidigt am: 18.11.2013

Halle (Saale) 2013

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Summary

In areas of intensive wheat cultivation *Septoria tritici* blotch (STB), caused by the ascomycete fungus *Mycosphaerella graminicola* (anamorph: *Septoria tritici*), is one of the most important foliar diseases. Severe yield losses are incurred by reduction of the photosynthetic active area due to necrotization of the leaves. *M. graminicola* is a hemibiotrophic pathogen that spreads in the field via splash-borne dispersal of conidia (pycnidiospores) from asexual fruiting bodies. Field populations of *M. graminicola* are genetically diverse, with additional variation occurring during the growing season. Fungicides such as strobilurins and triazoles lost their effectiveness in managing STB own to the development of resistances in the fugal population. Thus, breeding is an essential component in this disease control.

The objective of this study was the detection of qualitative and quantitative STB resistance sources in the progeny of the doubled haploid population Solitär x Mazurka. Solitär has shown the highest STB field resistance among registered German cultivars with high stability of resistance over time. Its resistance has a quantitative character at which disease is reduced but not completely absent. For the genetic analysis of STB resistance the doubled haploid population derived from a cross between Solitär - late heading, tall, outstanding field resistance to several diseases - and the STB susceptible Hungarian cultivar Mazurka – early flowering, short, highly tolerant to drought and frost - was established. The population consists of 137 lines which were covered with 779 molecular markers (162 SSR, 512 DArT and 105 AFLP) and one phenotypic marker for awnedness. For QTL mapping a reduced map of 252 molecular markers and one phenotypic marker was used. The entire map covered all 21 wheat chromosomes and altogether 2,887 cM of the wheat genome.

To identify qualitative resistance sources in the Solitär x Mazurka doubled haploid population, first the parents were screened with a worldwide set of 30 different *M. graminicola* isolates in the seedling stage. Both wheat genotypes clearly differentiated in their response to STB for the majority of isolates. On average Solitär showed lower percentage of disease severity. Due to the distinct response observed in the parental genotypes, isolates IPO323, IPO99015, IPO92034, Hu1 and Hu2 (resistance donor = Solitär) as well as isolates IPO90015 and BBA22 (resistance donor = Mazurka) were chosen to analyze the doubled haploid lines for STB seedling

resistance. Solitär conferred resistance to isolate IPO323, governed by *Stb6* on chromosome 3AS, as well as to IPO99015, IPO92034, Hu1 and Hu2 controlled by a QTL on chromosome 1BS, possibly corresponding to *Stb11* or *Stb2*. Quantitative disease resistance loci, each with small effect, were detected on chromosomes 3B, 6B, 3D and 4B. QTL confer either specific resistance against pycnidia formation (3B), necrotization of leaf area (4B) or both disease parameters (3D, 6B). Resistance of Mazurka to IPO90015 and BBA22 was caused by a QTL located in a region on 4AL which harbors *Stb7* or *Stb12*. Furthermore, Mazurka conferred quantitative resistance against IPO92034 with specificity to necrotic leaf area located on chromosome 1A. Pairwise epistatic interactions were reliably detected with five isolates. Although their contributions to the total variance are generally low, the genotypic effect of the QTL x QTL interaction of 4AL (*Stb7* or *Stb12*) and 3AS (*Stb6*) made up almost 15 % of disease expression. On the one hand inheritance of STB resistance is conferred by isolate-specific genes. Resistance response of *Stb* genes to single fungal genotypes is nearly complete and shows a gene-for-gene interaction. On the other hand STB resistance is inherited by the joint action of genes with minor effects. The results suggest a complex inheritance of resistance to STB in the seedling stage in terms of isolate-specificity and resistance mechanisms, which have implications for marker-assisted breeding in an attempt to pyramid STB resistance genes.

In a QTL mapping approach of six environments and four locations, STB field resistance was studied in previously developed population. The doubled haploid lines differed in diseased leaf area as well as time to heading and plant height. QTL for time to heading have been identified on chromosomes 1D and 7B, both positions coincide with that of known QTL for earliness *per se*. Reduced height genes *Rht-B1* and *Rht8*, both conferred by Mazurka, were responsible for differences in plant height. Apart from time to heading only plant height correlated positive with STB resistance (r = 0.47) and was henceforward considered as covariate in the genetic analyses. To draw a holistic picture of STB resistance in the Solitär x Mazurka doubled haploid population, efficacy of detected *Stb* genes and QTL identified in the seedling stage has to be validated under natural infection conditions. Surprisingly, none of the isolate-specific genes *Stb6*, *Stb11/Stb2* identified in Solitär, and *Stb7/Stb12* detected in Mazurka effected STB field resistance. Also none of the isolate-specific quantitative disease resistance sources were involved in the control

of field resistance. Adaptations of the fungal *M. graminicola* population to the identified *Stb* genes and QTL as well as seedling specificity are assumed.

With a multi-environmental QTL mapping approach five loci for quantitative disease resistance were identified on chromosomes 5A, 6D and 7D. Together with QTL x QTL interactions 20 % of the genotypic variance in STB field resistance is explained by the detected loci. QTL x environment interactions were minor. None of the previously identified isolate-specific resistance sources in the population contributed to the superior field resistance of Solitär. However, correspondences with sources for STB field resistance in other genetic backgrounds were found. Further studies with locally adapted isolates might confirm adaptations to single *Stb* genes in the fungal population and characterize the identified QTL for STB field resistance in more detail. This study sheds light on the genetic control of STB seedling resistance against single isolates and under field conditions. To infer from seedling to field resistance was not possible in the case of the Solitär x Mazurka doubled haploid population. In conclusion, the efficacy of isolate-specific seedling resistance sources has to be confirmed in field studies under the pressure of the complex fungal populations before they are successful utilized in breeding programs.

Zusammenfassung

In Gebieten mit intensivem Weizenanbau hat sich die *Septoria*-Blattdürre, verursacht durch den ascomyceten Pilz *Mycosphaerella graminicola* (anamorph: *Septoria tritici*), zu einer der bedeutendsten Blattkrankheiten entwickelt. Durch Nekrotisierung des Blattgewebes wird die Photosyntheseleistung der Weizenpflanzen herab gesetzt, was zu schweren Ertragsverlusten führen kann. *M. graminicola* zeichnet sich durch eine hemibiotrophe Lebensweise aus. Die Verbreitung der Krankheit im Pflanzenbestand erfolgt durch die in asexuellen Fruchtkörpern des Pilzes (Pyknidien) gebildeten Kondiden (Pyknidiosporen). Durch sexuelle Rekombination, welche auch im Laufe der Vegetationsperiode stattfindet, ist die im Feld natürlich auftretende *M. graminicola* Population genetisch divers. Die Effektivität von fungiziden Wirkstoffen wie Strobilurine und Triazole ist durch Resistenzbildung auf Seiten der Pilzpopulation zunehmend eingeschränkt. Somit stellt die Züchtung von resistenten Winterweizensorten eine essentielle Komponente zur Kontrolle der *Septoria*-Blattdürre dar.

Ziel der vorliegenden Arbeit war die Lokalisierung von qualitativen und quantitativen Resistenzquellen gegenüber der *Septoria*-Blattdürre in der Solitär x Mazurka doppelhaploid Population. Unter den registrierten deutschen Sorten zeigte Solitär, seit ihrer Einführung im Jahr 2004, die besten Resistenzeigenschaften gegen die *Septoria*-Blattdürre im Feld. Zur genetischen Analyse der *Septoria*-Blattdürre Resistenz wurde die doppelhaploid Population aus Solitär – spät blühend, hoch gewachsen - und der anfälligen ungarischen Sorte Mazurka – frühzeitig, niedrig gewachsen - erstellt. Die Population besteht aus 137 Genotpyen, deren Genom mit insgesamt 779 molekularen Markern (162 SSR, 512 DArT und 105 AFLP) und einem phänotypischen Marker für Begrannung abgedeckt wurde. Zur Kartierung von quantitativen Loci zur Resistenzkontrolle wurde eine reduzierte genetische Karte, bestehend aus 252 molekularen Markern und dem phänotypischen Marker verwendet. Mit der erstellten Karte der Solitär x Mazurka doppelhaploid Population konnten auf 2,887 cM alle 21 Chromosomen des Weizengenoms abgedeckt werden.
Zur Identifizierung von qualitativen Resistenzquellen wurden zunächst mit 30 verschiedenen *M. graminicola* Isolaten beide Eltern einem Pathogenitätstest im Keimlingsstadium unterzogen. Auf einen Großteil der Isolate reagierten die Sorten

mit einer klaren Differenzierung in “resistent” und “anfällig”. Im Mittel über alle Isolate wurde Solitär allerdings weniger befallen als Mazurka. Aufgrund einer deutlichen Differenzierung der beiden Eltern wurden die Isolate IPO323, IPO99015, IPO92034, Hu1 und Hu2 (Resistenzträger = Solitär) sowie die Isolate IPO90015 und BBA22 (Resistenzträger = Mazurka) für die Analyse der doppelhaploid Linien im Keimlingsstadium ausgewählt.

Solitär zeigte Resistenz gege das Isolat IPO323, die von dem bereits charakterisierten Resistenzgen *Stb6* auf Chromosom 3AS gesteuert wird. Außerdem vererbte Solitär eine Resistenz gegen IPO99015, IPO92034, Hu1 und Hu2, welche durch ein QTL auf Chromosom 1BS kontrolliert wird und möglicherweise mit *Stb11* oder *Stb2* korrespondiert. Loci für quantitative Krankheitsresistenzquellen wurden auf den Chromosomen 3B, 6B, 3D und 4B lokalisiert. Diese erklärten jeweils nur einen Teil der phänotypischen Varianz und zeigen geringe Effekte auf die Resistenzausprägung. Entweder war die Resistenzantwort spezifisch und reduzierte die Bildung von Pyknidien (3B) beziehungsweise verringerte die nekrotisierte Blattfläche (4B) oder beeinflusste beide Krankheitssymptome (3D, 6B). Die durch Mazurka vererbte, nahezu vollständige Resistenz gegen die Isolate IPO90015 und BBA22, wurde durch ein QTL auf Chromosom 4AL hervorgerufen und korrespondiert mit *Stb7* oder *Stb12*. Zudem vermittelte Mazurka eine, auf Chromosom 1A lokalisierte, quantitative Resistenz gegen Isolat IPO92034, welche spezifisch mit einer Reduktion der nekrotisierten Blattfläche verbunden war. Epistatische Wechselwirkungen zwischen QTL wurden mit fünf Isolaten identifiziert. Ihr Beitrag an der gesamten, phänotypisch erklärbaren Varianz war generell gering. Die Interaktion zwischen dem QTL auf Chromosom 3AS (*Stb6*), welches epistatisch mit dem auf 4AL (*Stb7/Stb12*) identifizierten QTL interagierte, verringerte beide untersuchten Krankheitssymptome um nahezu 15 %. Einerseits wurde die Keimlingsresistenz gegen die *Septoria*-Blattdürre durch isolat-spezifische Gene kontrolliert - hierbei war die Resistenzantwort nahezu vollständig und zeigte den Charakter einer Gen-für-Gen Wechselwirkung. Andererseits wurde die Krankheit durch das Zusammenwirken mehrerer Gene mit geringfügigen Effekten gesteuert. Die Ergebnisse deuten auf eine komplexe Vererbung der Keimlingsresistenz gegen einzelne *Septoria*-Blattdürre Isolate in der untersuchten Population hin. Die Isolatspezifität der identifizierten QTL, das Auftreten von QTL x QTL Interaktionen, sowie die Beteiligung verschiedener Resistenzmechanismen haben Auswirkungen auf die Marker gestützte Selektion. Für eine erfolgreiche Pyramidisierung von Resistenzgenen bzw. QTL müssen die

genannten Einflussfaktoren beachtet werden.
Die Feldresistenz gegen *Septoria*-Blattdürre wurde mit Hilfe einer QTL Studie in sechs Umwelten und vier Standorten untersucht. In der Solitär x Mazurka doppelhaploid Population wurden neben der befallenen Blattfläche auch der Zeitpunkt des Ährenschiebens sowie die Pflanzenhöhe erfasst. Je ein QTL auf den Chromosomen 1D und 7B beeinflusste den Zeitpunkt des Ährenschiebens, wobei sich beide Positionen mit bekannten QTL für das Merkaml „earliness per se“ deckten. Die Variation in der Pflanzenhöhe wurde durch die beiden Gene für Kurzstrohigkeit *Rht-B1* und *Rht8*, vererbt durch Mazurka, gesteuert. Hierbei korrelierte nur die Pflanzenhöhe positiv mit der Resistenz (r = 0,47) und wurde fortwährend als Kovariable in der gegentischen Resistenzanalyse betrachtet. Insgesamt konnten fünf QTL auf den Chromosomen 5A, 6D und 7D für die Variation in der Feldresistenz verantwortlich gemacht werden. Diese erklärten zusammen mit den QTL x QTL Wechselwirkungen 20 % der genotypischen Varianz. QTL x Umwelt Interaktionen zeigten einen vernachlässigbaren Einfluss. Keine der vorher in der Solitär x Mazurka doppelt haploiden Population identifizierten, isolat-spezifischen Quellen für *Septoria*-Blattdürre Resistenz, hatte einen Einfluss auf die Feldresistenz. Anpassungen der Erregerpopulation an die im Keimlingsstadium identifizierten *Stb* gene und QTL, sowie sämlingsspezifische Resistenz werden als warscheinlich angenommen.

Zusammenfassend wird die Feldresistenz gegen die *Septoria*-Blattdürre weniger durch die Wirkung einzelner Resistenzgene, als vielmehr durch das Zusammenspiel mehrerer Gene mit jeweils geringem Effekt hervorgerufen. Pathogenitätstest mit lokal angepassten Pilzisolaten können die identifizierten QTL für Feldresistenz genauer charakterisieren. Anpassungen der Erregerpopulation an isolatspezifische Resistenzgene könnten zudem mit solchen Untersuchungen validiert werden. Die hier voliegenden Studie konnte erfolgreich Licht auf die Steuerung der Septoria-Blattdürre Resistenz auf Basis von Einzelisolaten sowie unter Feldbedingungen werfen. Die isolatspezifischen Resistenzquellen waren im Fall der Solitär x Mazurka doppelhaploid Population gegen die lokal angepassen Erregerpopulationen nicht wirksam. Bevor isolatspezifische Keimlingsresistenzen erfolgreich in der Pflanzenzüchtung Verwendung finden, muss zuvor deren Wirksamkeit in Feldstudien nachgewiesen werden.

1 General introduction

1.1 Wheat (*Triticum aestivum* L.) – The pressure of fungal diseases

The hexaploid bread wheat *Triticum aestivum* L. belongs together with corn and rice to the three fundamentally important major crops for human nutrition worldwide (FAO 2012). In consideration of the ongoing worldwide climatic change assurance for safe food production against biotic stress like diseases caused by pathogens challenges plant breeders worldwide. Especially in intensive crop growing areas like Germany with narrow crop rotations the buildup of pathogens and pests is encouraged (Freyer 2003). Additionally, the costs for plant protection in those systems are high; the most efforts have to be done by fungicides (Anonymous 2012). Under the pressure of European agricultural policy pesticide application rates have to be decreased (Directive 2009/128/EC of the European Parliament and of the Council) and common used fungicides like azoles might be forbidden in near future (Regulation No 1107/2009 of the European Parliament and of the Council). Consequently, farmers will have to face strong cuts in wheat productivity caused by fungal diseases (Schmitz et al. 2010).

In Germany winter wheat is the major crop grown on 3.2 Mio hectares (ha) of arable land with a production of 25 Mio tons (t) per year which primary flow in the milling and baking industry. In comparison to the world average of 3.0 tons per hectare (t/ha) German farmers harvest in average 7.2 t/ha (FAO 2012). From 1966 to 2007 - a period of forty years - German winter wheat yields have been enhanced on average by 31 kilogram per ha and year (kg/ha/a) attributed by cultivar improvement alone (Ahlemeyer and Friedt 2012a; b). For comparison, over the same period in Germany the increase in grain yield on the farm level achieved by the combined action of high yielding cultivars and application of improved agrochemicals (fertilization, plant protection) has been estimated at 100 kg/ha/a (FAO 2012). Modern cultivars exhibit a shorter plant stature, are earlier ripen, show less lodging and are more resistant to important wheat diseases like powdery mildew, leaf rust and *Septoria tritici* blotch (STB) than older cultivars (Ahlemeyer and Friedt 2012b). These breeding inventions made the intensive production systems with high inputs of nitrogen fertilizer only possible (FAO 2012). Parallel the usage of agrochemicals increased (Anonymous 2012) and facilitated yield improvement. Accordingly, 30 % of the yield improvement was contributed by breeding and 70 % by production intensification (Ahlemeyer and Friedt 2012b). But intensive production systems are also vulnerable. A single

mutation in the fungal genome of *Mycosphaerella graminicola* the causal agent of STB provoked a complete resistance against strobilurins – until that day a very effective fungicide to manage STB and other diseases like powdery mildew and leaf rust. This development increased the use of tirazoles and enhanced the selection pressure towards tolerant *M. graminicola* genotypes (Cools and Fraaije 2008).

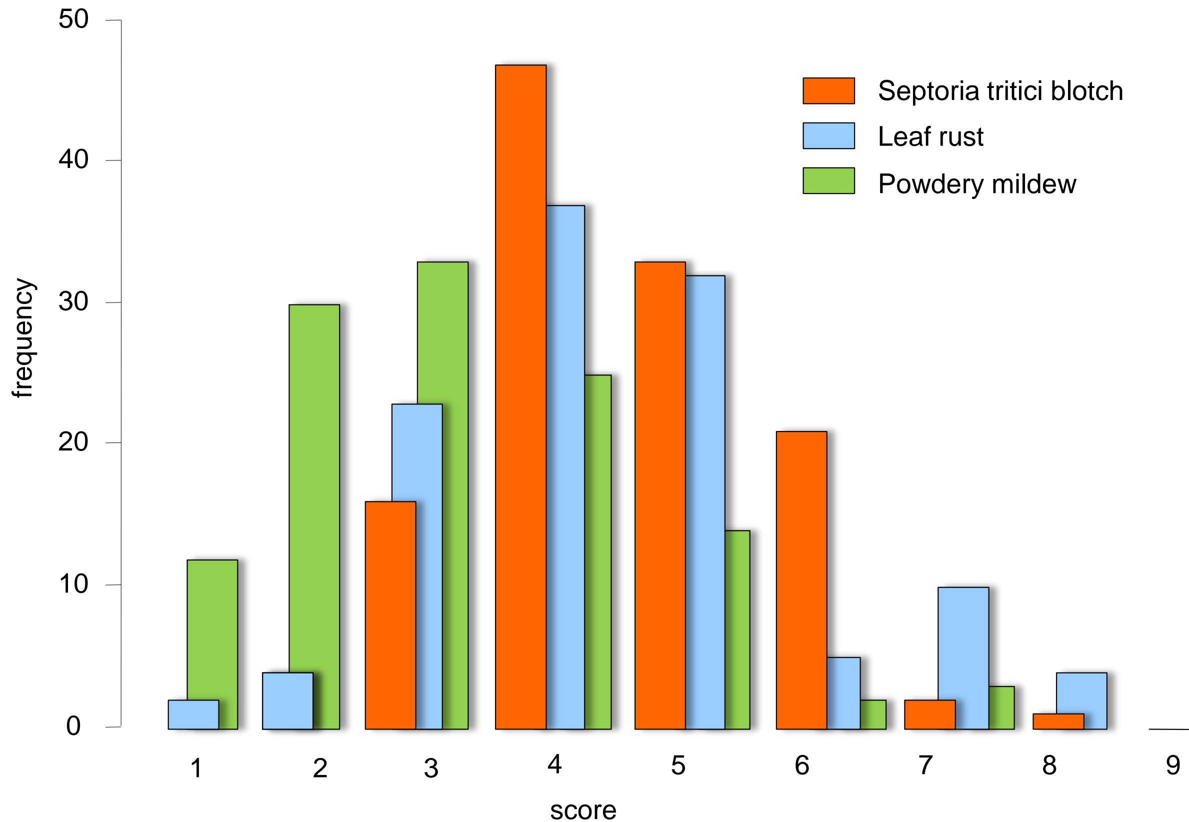

Fig. 1 Distribution of German winter wheat cultivars corresponding to their susceptibility classification by the Bundessortenamt (Anonymous 2010) for the three most frequent leaf diseases *Septoria tritici* blotch (*Mycosphaerella graminicola*; n=120), leaf rust (*Puccinia triticina*, n=117) and powdery mildew (*Blumeria graminis*; n=119); (1: no symptoms; 9: symptoms fully expressed)

Plant breeders create crossing populations of high genetic variability by the use of existing resistance sources in cultivars, accessions or wild ancestors of wheat. Selection processes develop new cultivars which should be high yielding, resistant to the most occurring diseases and of good baking quality. The most important wheat diseases in northern Europe are (i) powdery mildew (*Blumeria graminis*), leaf rust (*Puccinia triticina*), yellow rust (*Puccinia striiformis*), STB (*Mycosphaerella graminicola*) and DTR (*Drechslera tritici repentis*) affecting the leaves; (ii) Fusarium

head blight (*Fusarium* spec.) and *Septoria nodorum* blotch (*Phaeosphaeria nodorum*) affecting the ears as well as (iii) eyespot (*Pseudocercosporella herpotrichoides*), take-all (*Gaeumannomyces graminis* var. *tritici*) affecting the roots. In Germany and whole northern Europe STB turns to be the most frequent wheat disease with yield losses up to 30 % in susceptible cultivars (Eyal et al. 1987, LfL 2004).

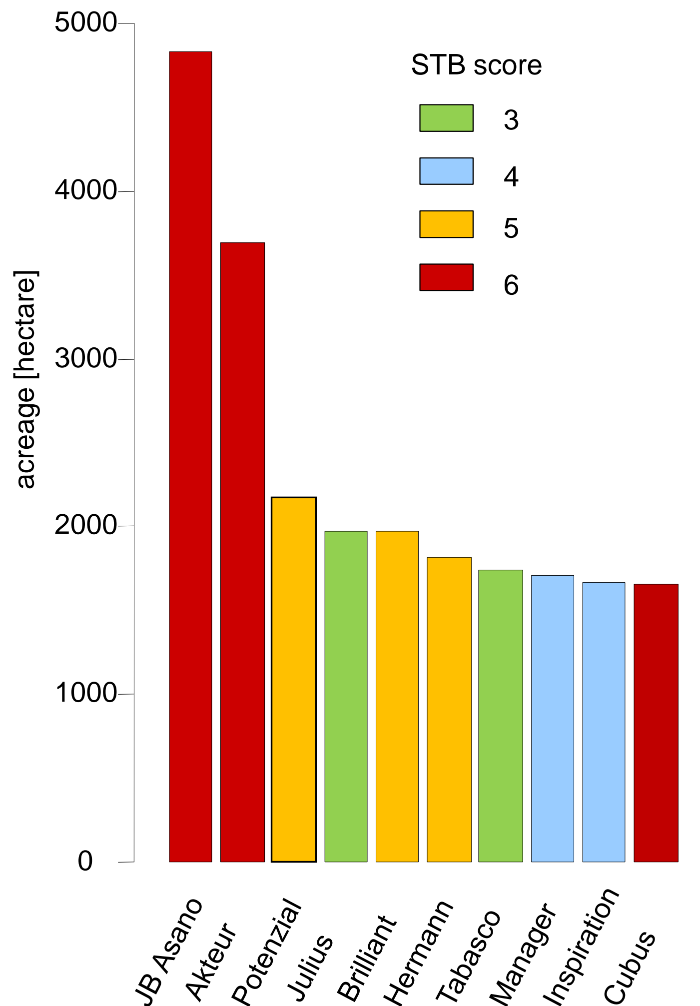

Fig. 2 Acreage of seed multiplication of the ten most important wheat cultivars in Germany and their corresponding *Septoria tritici* blotch (STB) score. Scores 1, 2 and 7 to 9 were absent. (1: no symptoms; 9: symptoms fully expressed) (Anonymous 2010)

Only moderate STB resistant cultivars are available in Germany. The Federal Plant Variety Office in Germany (Bundessortenamt), responsible for cultivar registration, classifies every year the susceptibility of registered cultivars to different wheat diseases on a one to nine scale (1: no symptoms; 9: symptoms fully expressed). Long resistance breeding to powdery mildew and leaf rust resulted in cultivars with resistance classification of one and two (**Fig. 1**). However, regarding STB, resistance classification of registered cultivars starts with a value of three what can be

interpreted as moderate resistant. As long as fungicides for managing STB are available yield potential can be exploited also with a susceptible cultivar. Additionally, highly resistant cultivars often do not exhibit the same yield as susceptible cultivars under intensive field management (Singh et al. 1991). Taking a look at the ten most important cultivars, which account for more than 50 % of the wheat area in Germany, susceptible cultivars with scores greater than four dominate (**Fig. 2**). Reasons for the minor acceptance of STB resistant cultivars on the farm level might be first the trade-off between yield and resistance and second the availability of effective fungicides. However, resistance breeding to STB remains to be the most suitable way to ensure high yields under conditions where (i) a less amount of fungicides is applicable e. g. due to political decisions (ii) fungicides are not effective due to adaptations of the fungal population (iii) the yield losses associated with resistance are less than the costs of a fungicide treatment.

1.2 The *Triticum aestivum* – *Mycosphaerealla graminicola* pathosystem

Triticum species can be classified into three groups based upon their ploidy level (**Fig. 3**; Kilian et al. 2010).

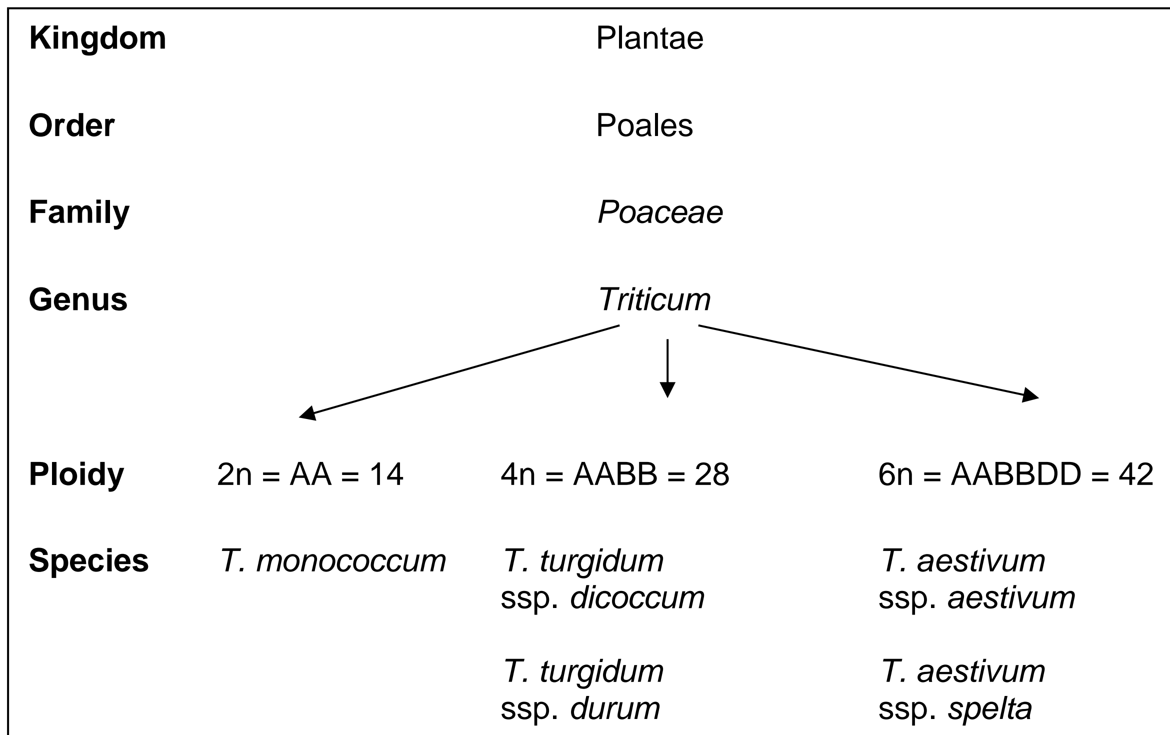

Fig. 3 Taxonomy of the most important *Triticum* species and genome information (van Slageren 1994)

By allopolyploidization of the A genome of Einkorn (*T. monococcum*) and the B

genome, which certainly originates from the Sitopis section of *Aegilops* (Kilian et al. 2007), the tetraploid forms arose from which Emmer (*T. turgidum* ssp. *dicoccum*) got domesticated. The hexaploid wheat developed by allopolyploidization of tetraploid Emmer (*T. turgidum* ssp. *dicoccum*) with the D genome of *Aegilops tauschii* (Kilian et al. 2010).

The ascomycete fungus *Mycosphaerella graminicola* (anamorph: *Septoria tritici*) is the causal agent of *Septoria tritici* blotch (**Fig. 4**). It attacks bread and durum wheat as well as their ancestors Einkorn (*T. monococcum*) (Jing et al. 2008), Emmer (*T. turgidum* var. *dicoccum*) and *Agilops* ssp. (McKendry and Henke 1994). A co-evolution of *M. graminicola* and wheat is supposed to have happened in the Fertile Crescent (Stukenbrock et al. 2007). The fungus developed *formae speciales* on wheat, barley, rye, oat and other grasses including *Lolium* and *Holcus* spp. (Eyal 1999). Grass weeds may act as disease foci out of field boundaries (Brokenshire 1975).

Kingdom	Fungi
Phylum	Ascomycota
Class	Loculoascomycetes
Order	*Dothideales*
Family	*Mycosphaerellaceae*
Genus	*Mycosphaerella*
Species	*M. graminicola* anamorph:*Septoria tritici*

Fig. 4 Taxonomy of *M. graminicola*

M. graminicola has a hemibiotrophic mode of living. An initial period of biotrophic growing – energy is derived from living cells – is followed by a necrotrophic phase – energy is derived from killed cells (Perfect and Green 2001). After incubation time of 48 h at ≥ 85 % relative humidity the fungus penetrates the stomata, passes the sub-stomatal cavity and reaches the mesophyll cells. From here it starts colonization by strictly intercellular growth. No haustoria-like structures for feeding are possessed. Hyphae stay in close contact to mesophyll cells and obtain nutrients from the plant´s apoplast. After 8 to 10 days post inoculation (dpi) the fungus begins to attack

mesophyll cells and cell collapses appear as chlorotic areas on the leaf surface which develop to necrotic lesions. Induced by the release of nutrients, fungal biomass increases massive. This occurs especially in sub-stomatal cavities where “baskets” are formed which develop to pycnidia, the asexual fruiting bodies (Kema et al. 1996c). *In vitro* - on agar dishes or in nutrient solutions - the fungus grows as a yeast-like mass or as a filamentous mycelium (Orton et al. 2011). This makes it easy to multiply and use *M. graminicola* in artificial inoculation experiments.

Besides Northern Europe all wheat growing regions where high humidity and moderate temperature conditions occur are affected by STB, like parts of the United States, Mediterranean countries, Ethiopia and the Fertile Crescent. The word field populations of *M. graminicola* possess a high genetic diversity due to gene flow on a global scale by air borne ascospores, frequent sexual recombination and large effective population sizes with more than 60 genotypes per square meter (Zhan et al 2003). Isolates of *M. graminicola* represent single genotypes in the fungal population usually gained out of the asexual fungal fruiting bodies.

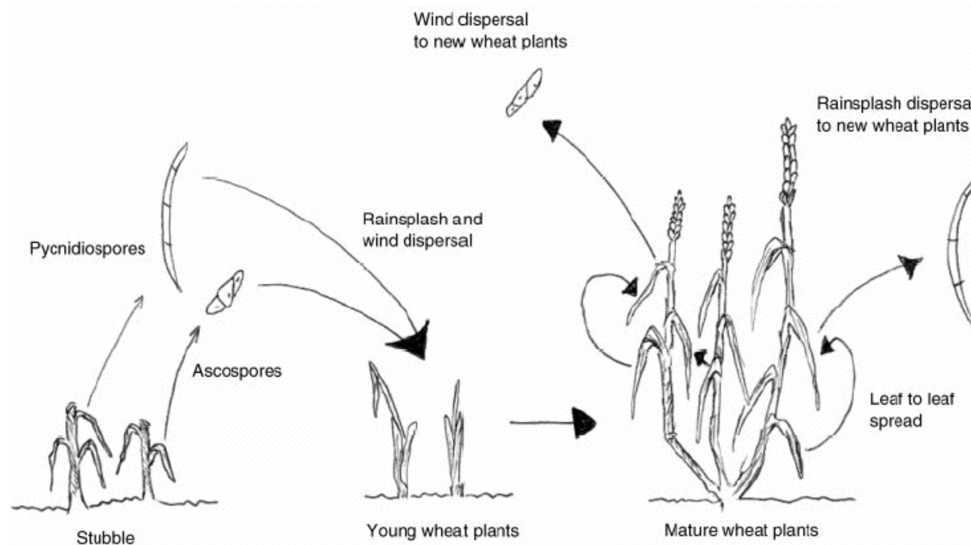

Fig. 5 Life cycle of *M. graminicola* (Palmer and Skinner 2002)

The disease cycle of *M. graminicola* (**Fig. 5**) begins with air-borne ascospores discharged from mature sexual fruiting bodies (pseudothecia) on stubbles remained from the pre-season crop and on plant debris in autumn (Suffert et al. 2011). Ascospores discharge was also observed during the growing season (June/July) in Europe (Kema et al. 1996b; Hunter et al. 1999; Eriksen and Munk 2003).

Dispersion in the canopy mainly occurs via pycnidiospores released by the asexual

fruiting bodies (pycnidia) and dispersed via rain-splash. After a long latent period of 21 to 28 days after infection – depending on cultivar, temperature and humidity - disease initiates with the appearance of water-soaked, chlorotic, ambiguous spots on leaves which develop into irregular necrotic lesions. These necrotic leaf regions bear small black pycnidia scattered within the entire lesion. Pycnidia often appear in rows since they form in sub-stomatal cavities (Palmer and Skinner 2002).

Fig. 6 STB symptoms on a wheat flag leaf

In the field STB can be confused with *Phaeosphaeria* (*Stagnospora*) *nodorum*, which also produces necrotic lesions and pycnidia on leaves. Both fungi co-exist on the same plant. However, necrotic lesions caused by *Stagnospora nodorum*, the causal agent of Glum blotch, are more common on sheaths and glumes. A clear distinction can be made by morphological analysis of pycnidiospores, which are more than ten times as long as wide in the case of *M. graminicola* (Eyal 1999). Epidemics of STB are favored by prolonged periods of wet weather and moderate temperatures. Optimal conditions of 20 to 25 °C and relative humidity ≥ 85 % promote penetration. However, in susceptible cultivars infection conditions do not seem to be limiting factors. This is consistent with the ability of the fungus to tolerate interruptions of humid periods (Shaw 1991; Gough and Lee 1985). A reduction of the photosynthetic capacity leads to severe yield losses in the field up to 30-50 % (*M. graminicola* and *Stagnospora nodorum*) (Eyal 1981). Conspicuously yield losses were observed when STB becomes severe on the flag leaf and second leaf (Lovell et al. 1997) due to a reduction of photosynthetic capacity in the grain filling period.

Fungicides are widely used to control STB but loss of efficacy due to resistance development, high costs, growing awareness of health and environmental impact will minimize their application in future. With the introduction of QoI fungicides or quinone outside inhibitors (called strobilurins) this new class raised to the most applied fungicide in cereals (Bartlett et al. 2002). But a single mutation in the fungus of a glycine to alanine at base 143 in the cytochrome b protein sequence (G143A) led to

a loss of strobilurin efficacy only a few years after its release. Torriani et al. (2009) demonstrated that this mutation took place in genetically and geographically independent backgrounds in Europe, increased by fungicide selection and wind dispersed ascospores. Since that date sterol demethylation-inhibitors (DMI) are the backbone of STB control. But a shift to triazols (fungicide component that belongs to the DMIs) resistant fungal isolates have been observed in European monitoring programs (Cools and Fraaije 2013). Regulation 1107/2009 of the European Union concerning placing of plant protection products on the market will impact chemical control of STB by azoles which are at a risk of regulatory exclusion due to their association with endocrine activity (Kjærstad et al. 2010). In high-input management systems the risk of STB epidemics are reducible by (i) primarily reduction of infected debris, volunteer plants and weeds (ii) chemical control with fungicides and (iii) genetic control with STB resistant cultivars.

1.3 Inheritance of *Septoria tritici* blotch resistance

Inheritance of STB resistance is controlled by either single genes with a near-complete resistance response (**Tab. 1**) following gene for gene interactions (Brading et al. 2002) or quantitatively with incomplete resistance response and involvement of multiple genes each having minor effects (Jlibene et al. 1994; Simon and Cordo 1998, **Tab. 2**). *Stb1*, identified in the winter wheat cultivar Bulgaria 88 (Rillo and Caldwell, 1966), was the first STB resistance gene analyzed (Wilson 1985), and deployed in commercial cultivars in the 1970s. *Stb2* and *Stb3* originally found in cultivars Veranopolis and Israel 493 were used as resistance sources in worldwide breeding programs. However, only *Stb2* was introduced in commercial cultivars (Goodwin 2007) and is the first gene for which a recessive inheritance was identified (Goodwin and Thompson 2011). Cultivar Tadinia, released 1985 was grown in California with low infestation of STB, controlled by *Stb4*, but lost its resistance 15 years later (Jackson et al. 2000; Somasco et al. 1996). Parallel to the identification of *Stb* genes one to four, progress has been made in understanding host-pathogen interactions (Eyal et al. 1987; Kema et al. 1996a; Brown et al. 2001).

Tab. 1 *Septoria tritici* blotch resistance genes (*Stb*) in the respective hexaploid spring and winter wheat cultivar used for identification and mapping along with their chromosomal positions and flanking marker(s) in the wheat genome

Stb gene	Cultivar source	Chromosomal position	Flanking markers(s)	Reference
Stb1	Bulgaria 88[a]	5BL	*Xgwm335*	Adhikari et al. 2004c
Stb2	Veranopolis [a]	1BS	*Xwmc406; Xbarc008*	Liu et al. 2013
Stb3	Israel 493 [a]	7AS	*Xwmc83*	Goodwin et al. 2008
Stb4	Tadinia [a]	7DS	*Xgwm111*	Adhikari et al. 2004b
Stb5	CS Synthetic 7D [a]	7DS	*Xgwm44*	Arraiano et al. 2001b
Stb6	Flame	3AS	*Xgwm369*	Brading et al. 2002
Stb7	Estanzuela Federal	4AL	*Xwmc313; Xwmc219*	McCartney et al. 2003
Stb8	W7984 (Synthetic)	7BL	*Xgwm146; Xgwm577*	Adhikari et al. 2003
Stb9	Courtot	2B	*XksuF1; Xfbb226*	Chartrain et al. 2009
Stb10	KK4500 [b]	1D	*Xgwm603; Xgwm458*	Chartrain et al. 2005a
Stb11	TE9111 [b]	1BS	*Xbarc008*	Chartrain et al. 2005b
Stb12	KK4500 [b]	4AL	*Xwmc313; Xwmc219*	Chartrain et al. 2005a
Stb13	Salamouni	7BL	*Xwmc369*	McIntosh et al. 2007
Stb14	Salamouni	3BS	*Xwmc500*	McIntosh et al. 2007
Stb15	Arina [a]	6AS	*Xpsr904*	Arraiano et al. 2007a
Stb16q[c]	M3 (Synthetic)	3DL	*Xbarc125; Xbarc128*	Ghaffary et al. 2012
Stb17[d]	M3 (Synthetic)	5AL	*Xgwm617; Xhbg247*	Ghaffary et al. 2012
Stb18	Balance[a]	6DS	*Xgpw3087; Xgpw5176*	Ghaffary et al. 2011

[a] These lines also carry *Stb6*

[b] These lines also carry *Stb6* and *Stb7*

[c] Quantitative resistance response

[d] specifically expressed in the adult plant stage

Tab. 2 QTL for *Septoria tritici* blotch resistance mapped in hexaploid wheat and *T. monoccocum* (Einkorn), developmental stage at which disease was scored (A= adults, S= seedlings), disease parameter scored (P = pycnidial covered leaf area; N = leaf area covered with necrosis, Px = pycnidial density), chromosomal location, closest linked marker(s), phenotypic variance explained, resistance carrier, fungal material used for identification and putative resistance gene assumed by the reference authors or according to the corresponding *Stb* gene position out of the literature

QTL name	A / S	Disease para-meter	Chromo -some position	Closest marker(s)	Phenotypic variance explained (%)	Resistance source	Fungal material	*Stb* candi-date gene	Reference
QStb.lsa_fb-1A	A[b]	P	1A	*Xwmc0312*	<10	Florett	mixture		Risser et al. 2011
StbWW2449, 1842,2451	S, A	P,Px	1BS	*Xbarc008, Xwmc230*	S: >60 A: 30-40	WW2449, 1842, 2451	79.2.1A	*Stb11, Stb2*	Raman et al. 2009
not named	S	P	1BS	*wPt-2019, Xbarc008*	>60	Apache	IPO87016	*Stb11, Stb2*	Ghaffary et al. 2011
QStb.lsa_fb-1B	A[b]	P	1B	*Xwmc0419*	<10	Biscay	mixture	*Stb11, Stb2*	Risser et al. 2011
QStb.ipk.-1D	S	P	1DS	*Xksud14d*	20-30	W7984 (Synthetic)	IPO92067; IPO93014	*Stb10*	Simón et al. 2004a
QStb.risø-2B	A[b]	P	2BL	*Xwmc175b*	20-30	Senat	mixture	*Stb9*	Eriksen et al. 2003
QStb.lsa_af-2B	A[b]	P	2B	*Xcfd276*	10-20	Arina	mixture		Miedaner et al. 2012
QStb.lsa_fb-2B	A[b]	P	2B	*XP2553-222*	10-20	Biscay	mixture		Risser et al. 2011
QStb.ipk-2D	S	P	2DS	*Xcdo405a*	20-30	Opata 85	IPO92067; IPO93014		Simón et al. 2004a
QStb.risø-3A.1	S	P	3AS	*Xgwm369*	>60	Senat	IPO323; Risø97-86	*Stb6*	Eriksen et al. 2003
not named	A	P,N	3AS	*wPt-0836*	>60	Balance	IPO323	*Stb6*	Ghaffary et al. 2011
QStb.risø-3A.2	S, A[c]	N	3AS	*Xwmc505, Xwmc388a*	20-30	Senat	IPO323; Risø97-86; mixture		Eriksen et al. 2003
QStb.risø-3B	S	N	3BS	*M62/P38-373*	10-20	Senat	Risø97-86		Eriksen et al. 2003
QStb.lsa_af-3B	A[b]	P	3B	*Xgwm131*	10-20	Arina	mixture		Miedaner et al. 2012
QStb.lsa_fb-3B	A[b]	P	3B	*Xstb10*	<10	Florett	mixture		Risser et al. 2011
QStb.ipk-3D	A	P	3DL	*Xbcd515*	10-20	W7984 (Synthetic)	IPO92067	*Stb16q*	Simón et al. 2004a
QStb.wai-3D	S	N+P[d]	3DL	*Xwmc169b*	20-30	CPI133872 (Synthetic)	79.2.1A	*Stb16q*	Zwart et al. 2010
QStb.lsa_tb-4A	A[b]	P	4AL	*XwPt-5434*	<10	Tuareg	mixture	*Stb12*	Risser et al. 2011
QStb.lsa_tb-4B	A[b]	P	4B	*Xwmc0238*	<10	Tuareg	mixture		Risser et al. 2011
QStb.lsa_fb-4B	A[b]	P	4B	*XwPt-8092*	<10	Florett	mixture		Risser et al. 2011
QStb.lsa_tb-4D	A[b]	P	4D	*Xgwm0129*	<10	Tuareg	mixture		Risser et al. 2011
QStb.lsa_fb-5B	A[b]	P	5B	*XwPt-4577*	<10	Biscay	mixture		Risser et al. 2011
QStb.lsa_af-5B	A[b]	P	5B	*Xpsr574*	<10	Arina	mixture		Miedaner et al. 2012

Tab. 2 continued

QTL name	A / S	Disease para-meter	Chromo-some position	Closest marker(s)	Phenotypic variance explained (%)	Resistance source	Fungal material	*Stb* candi-date gene	Reference
QStb.risø-6B.1	S	N	6BS	*M48/P32-112*	10-20	Senat	IPO323		Eriksen et al. 2003
QStb.risø-6B.2	S,A	N, P	6BC	*Xwmc397, Xwmc341*	>60	Senat	Risø97-86; mixture		Eriksen et al. 2003
QStb.ipk-6B	S	P	6BS	*Xksuh4b*	20-30	W7984 (Synthetic)	IPO92067; IPO93014		Simón et al. 2004a
QStb.psr-6B-1	A	P	6B	*StaMct.200A*	20-30	Riband	IPO94269 ; IPO98011; IPO290		Chartrain et al.2004b
QStb.lsa_tb-6B	A[b]	P	6B	*XwPt-6286*	10-20	Tuareg	mixture		Risser et al. 2011
QStb.lsa_fb-6D	A[b]	P	6D	*Xcfd0013*	10-20	Florett	mixture	*Stb18*	Risser et al. 2011
QStb.lsa_af-6D	A[b]	P	6D	*Xgdm14*	<10	Arina	mixture		Miedaner et al. 2012
TmStb1	S	P	$7A^m$	*Xbarc174*	>60	MDR043 (Einkorn)	IPO323	*Stb3*	Jing et al. 2008
QStb.lsa_fb-7A	A[b]	P	7A	*Xbarc0108*	<10	Florett	mixture	*Stb3*	Risser et al. 2011
QStb.risø-7B	A[b]	P	7B	*M49/P11-229*	10-20	Senat	mixture	*Stb8*	Eriksen et al. 2003
QStb.ipk-7B	A[b]	P	7BL	*Xksud2a*	10-20	W7984 (Synthetic)	IPO93014	*Stb8*	Simón et al. 2004a
QStb.lsa_tb-7B	A[b]	P	7B	*Xwmc0517*	10-20	Tuareg	mixture		Risser et al. 2011
QStb.ipk-7D1[a]	S	N,P	7DS	*Xgwm0111*	NA	'CS' (*T.a.s.*7D)	FALP0103	*Stb4, Stb5*	Simón et al. 2010
QStb.ipk-7D2[a]	A	N,P	7DS	*Xgwm1220*	NA	'CS' (*T.a.s.*7D)	FALP0103		Simón et al. 2010
QStb.psr-7D-1	S	P	7DS	*Xcdo475b, Xswm5*	10-20	Arina	IPO92006		Arraiano et al. 2007a
QStb.lsa_fb-7D	A[b]	P	7D	*XwPt-7842*	<10	Biscay	mixture		Risser et al. 2011
not named	S	P	7DS	*Xgwm111*	20-30	Apache	IPO98046; IPO98022	*Stb4*	Ghaffary et al. 2011
not named	S	P	7DL	*wPt-1859*	<10	Apache	IPO89011		Ghaffary et al. 2011

[a] phenotypic variance explained not indicated by the authors

[b] infection with an isolate mixture in the field solely in the adult plant stage

[c] seedling stage: IPO323 and Risø97-86; adult plant stage: isolate mixture

[d] discrete scale; taking lesion color, size and pycnidia density under consideration

The first gene-for-gene relationship has been identified between the cultivar Flame and the Dutch *M. graminicola* isolate IPO323 (Brading et al. 2002). *Stb5* was the first gene mapped with a single isolate in a synthetic hexaploid line on chromosome 7DS providing resistance to a broad-spectrum of *M. graminicola* isolates (Arraiano et al. 2001a; b) on the global scale (Ghaffary 2011) but was not introduced into a

commercial cultivar. Since now mapping of isolate-specific *Stb* genes progressed and molecular markers linked to *Stb* genes were identified. In 2004 the previously identified genes *Stb1* to *Stb4* were mapped by Adhikari et al. (2004a-c). Map location of *Stb3* was revised from chromosome 6D to 7A after validation in different wheat populations (Goodwin et al. 2008). Recently, *Stb2* was relocated from chromosome 3B to 1B, where also *Stb11* has been mapped. It is not clear yet if *Stb2*, *Stb11* and QTL mapped on chromosome 1B (see **Tab. 2**) are different but closely linked, allelic or the same gene (Liu et al. 2013). *Stb6* first described by Brading et al. (2002) was identified in a wide range of cultivars and is one of the most widespread *Stb* genes in Europe (Arraiano and Brown 2006; Brown et al. 2001). Subsequently *Stb7* to *Stb15* were mapped in segregating populations of commercial cultivars, synthetic wheats and breeding lines with advanced STB resistance like Kavkaz-K4500 L.6.A.4 (KK4500) and TE9111. More recently *Stb16q*, the first *Stb* gene exhibiting a quantitative resistance response, and *Stb17*, the first *Stb* gene specifically expressed in the adult plant stage, were identified both in a synthetic hexaploid wheat line with single isolates (Ghaffary et al. 2012). *Stb18* was mapped in the French winter cultivar Balance conferring resistance to five *M. graminicola* isolates (Ghaffary et al. 2011).

Parallel to the identification of isolate-specific *Stb* genes sources for quantitative disease resistance were detected with quantitative trait loci (QTL) mapping approaches (**Tab. 2**). In comparison to complete resistance contributed by most isolate-specific *Stb* genes, quantitative resistance is incomplete and conditioned usually by multiple genes with small effects that can interact with the environment and each other (Doerge 2002). In terms of STB, quantitative resistance is characterized by a reduction of disease development and fungal dispersion in the plants. To find loci for quantitative disease resistance in a segregating population, associations between molecular marker genotypes and trait phenotypes are calculated statistically (Doerge 2002). Phenotypic variance (V_P) within a population is the result of genetic variance (V_G) and environmental variance (V_E). The relationship can be summarized as follows (Falconer and Mackay 1996; Lynch und Walsh 1998):

$$V_P = V_G + V_E$$

Due to polygenetic inheritance of quantitative traits a QTL explains only a part of the genetic variance, even when no environmental interaction exists. Phenotypic variance of a QTL usually ranges between a few percent and more than 60 %. A so called major QTL generally accounts for 20-25 % or more of the phenotypic variance explained (Mackay et al. 2009). Separation of QTL and *Stb* genes is often not

unambiguous. Detected QTL are often found in regions where *Stb* genes have been identified (**Tab. 2**). Recent changes in the nomenclature of *Stb* genes approved the addition of a “q” as a suffix where phenotypic data do not unequivocally show that a single gene underlies resistance (Ghaffary et al. 2012).

Until now 41 QTL conferring resistance to STB are described in the literature (**Tab. 2**). Phenotypic variance explained by each QTL was below 10 % (13 QTL), between 10 and 20 % (12 QTL), between 20 and 30 % (8 QTL), more than 60 % for 6 QTL and for 2 QTL no details were available. With the inoculation of locally adapted isolate mixtures in the adult plant stage, a high number of QTL were detected, each explaining only a small part of the phenotypic variance (Eriksen et al. 2003; Miedaner et al. 2012; Risser et al. 2011). But also isolate-specific resistance is controlled by quantitative loci explaining 30 % or less of the phenotypic variance (Eriksen et al. 2003; Simón et al. 2004a; Chartrain et al. 2004b; Arraiano et al. 2007a; Zwart et al. 2010). Also in Einkorn (*T. monococcum*), an ancestor of hexaploid wheat, a source for STB resistance located on chromosome $7A^m$ has been identified (Jing et al. 2008). Its chromosomal location is in accordance to *Stb3* in hexaploid wheat (Goodwin et al. 2008). In studies on synthetic hexaploid wheat *Stb8*, *Stb16q*, *Stb17*, *Stb5* and five QTL were identified (**Tab. 1 and 2**). Substitution lines with synthetic wheat also implement further sources for isolate-specific resistance on chromosomes 5A and 5D in Synthetic 6x which have not been further analyzed (Simón et al. 2012). Since *Stb* genes and QTL have been located on each of the three wheat genomes and on near all of the seven homologous chromosomes, the three ancestors of wheat seem to have their own set of *Stb* genes (Goodwin and Thompson 2011).

1.4 Efficacy of *Septoria tritici* blotch resistance

Efficacy of a *Stb* gene has been considered as the capacity to confer resistance to a wide range of *M. graminicola* isolates under artificial inoculation conditions (Ghaffary 2011) and/or under natural inoculation in the field (= field resistance) (Arraiano et al. 2009). Apart from the adult plant specific gene *Stb17*, all *Stb* genes were shown to assign resistance in the seedling and adult plant stage. Among 94 cultivars those carrying *Stb5* were most effective against European *M. graminicola* isolates and those carrying *Stb15* against a global set of isolates. Cultivars carrying *Stb9* and *Stb6* were not effective to the majority of tested French isolates (Ghaffary 2011). In the adult plant stage under field conditions among *Stb9, Stb6* and *Stb15* only *Stb6* has been associated with a reduction in diseased leaf area in a set of 226 wheat lines

and cultivars (Arraiano et al. 2009). *Stb6* was in focus of several studies and is after *Stb15* the most widespread *Stb* gene in present breeding programs (Brown et al. 2001; Chartain et al. 2005c; Arraiano and Brown 2006; Ghaffary 2011). Since some cultivars carrying *Stb6* have been found to be susceptible under field conditions it is assumed that *Stb6* may also confer partial resistance or is linked to another gene conferring partial resistance (Arraiano et al. 2009; Arraiano and Brown 2006; Chartrain et al. 2005a). Another way to detect single *Stb* gene efficacy would be the development of near-isogenic lines for each *Stb* gene in a susceptible background. Goodwin and Thompson (2011) began to backcross *Stb1*-*Stb8* in susceptible wheat cultivars. However, the presence of additional QTL with minor effects in the *Stb* gene donors induced an erosion of resistance during backcrossing and enhanced the needed number of backcrosses.

In comparison to most *Stb* genes, a dependency of resistance expression on plant development exists for STB resistance QTL (Eriksen et al. 2003). In most cases efficacy of an isolate-specific QTL identified in the seedling stage was neither validated in the adult plant stage nor under natural inoculation in the field. With one exception, Raman et al. (2009) validated the isolate-specific QTL on chromosome 1BS in the seedling stage in three mapping populations by artificial inoculation in field. In contrast, Simón et al. (2004a) could not validate isolate-specific seedling resistance in the adult plant stage. Due to the results of a pathogenicity assay, Ghaffary (2011) describes a differential response pattern in the seedling and adult plant stage depending on the isolate tested. Out of 161 genotype x isolate interactions 42 were seedling specific and only 10 adult plant specific. In 109 interactions no specificity to the plant developmental stage was observed; 28 interactions showed resistance and 81 susceptibility.

Most *Stb* genes confer near-complete resistance; such genes are also termed as R-genes, only *Stb16q* shows a quantitative resistance response. When the product of a host plant R-gene has recognition specificity for a compound produced by a pathogen avirulence (*Avr*) gene, disease resistance is observed. But *Avr*-genes tend to mutate, especially when sexual recombination takes place during the vegetation period like in *M. graminicola* populations. Host plants are disabled to recognize the pathogen and can be infected. This effect is described as R-gene lost of efficacy (St. Clair 2010).

Such lost of efficacy has been taken place with *Stb4* in the year 2000 in parts of the USA (Jackson et al. 2000). STB resistance of cultivar Gene, carrying *Stb6* (Chartrain

et al. 2004a) and *Stb4* (Cowger et al. 2000), was nearly complete at its release in the early 1990s but broke down rapidly. A clear adaptation of *M. graminicola* isolates to two commercial cultivars of which both probably carry *Stb4* and one cultivar an additional quantitative STB resistance was observed. Resistance of both cultivars eroded over years of growing in wide areas. However, at least some of the resistance maintained. Quantitative resistance was much more durable than the complete resistance of cultivar Gene (Krenz et al. 2008).

Durable resistance is not certainly accompanied with quantitative genetic inheritance. Resistance that remains effective, even when the cultivar possessing it is widely grown, was described as durable by Johnson in 1981. Some examples for mono-genetically inherited durable fungal resistance genes are *Mlo* against powdery mildew in barley; *rym4/rym5* conferring resistance to Barley mild mosaic virus and Barley yellow mosaic virus type 1; *Lr34* against the three biotrophic fungi leaf rust, stripe rust and powdery mildew; *Yr36* conferring resistance against stripe rust and *Pch1*; the resistance gene against eyespot on wheat. *Lr34* and *Yr36* express incomplete adult plant resistance and have been approved to be single genes due to cloning and sequencing approaches (Miedaner and Korzun 2012). In summary, quantitative disease resistance can also be inherited by single genes. *Stb16q* conferred resistance to two *M. graminicola* isolates explaining 60 % and 30 % of the phenotypic variance in the seedling and in the adult plant stage, respectively (Ghaffary et al. 2012).

Until now only *Stb1* can be regarded as durable because it provided resistance to cultivars grown over a wide range in area and time. None of the other *Stb* genes have been proven to be durable because either they haven’t been used successfully in breeding programs or commercial cultivars were not grown over a longer time period or finally broke down (*Stb4*). The highly resistant breeding lines KK4500 and TE9111 carry at least four (*Stb6*, *Stb7*, *Stb10*, *Stb12*) (Chartrain et al. 2005a) and three (*Stb6*, *Stb7*, *Stb11*) (Chartrain et al. 2005b) *Stb* genes, respectively and also unknown isolate-specific resistance. However, these breeding lines have not been introduced in the commercial market to show whether pyramiding genes is also successful in the term of durability. Quantitative isolate non-specific resistance seems to be worthwhile to select on and was successful for resistance breeding against powdery mildew in wheat (Miedaner and Korzun 2012).

For all 18 *Stb* genes closely linked molecular markers are available (**Tab. 1**) and might be used for marker assisted breeding purposes. But in most instances these

markers are not feasible in elite breeding material (Miedaner and Korzun 2012) and their use is restricted due to adaptation processes of the fungal populations (Cowger et al. 2000).

1.5 Phenotyping of *Septoria tritici* botch resistance

The availability of molecular marker data and beyond considering the next generation sequencing techniques, the disentanglement of the genome proceeds continuously (Berkman et al. 2012). Admittedly, adequate phenotyping techniques are missing especially when traits are difficult to determine like disease infestation (Tester and Langridge 2010). Studying STB resistance has been undertaken on different levels considering plant age (seedling, adult plant), plant structure (single leaf, single plant, and canopy) and plant environment (petri dish, growth chamber, greenhouse, and field). For artificial inoculation blastospores of single isolates or mixtures of isolates with known virulence structure were sprayed on the plants (Kema et al. 1996a). Under field conditions the natural occurring fungal population was utilized for STB infection (Arraiano et al. 2009). More often mixtures of diverse *M. graminicola* isolates, more or less adapted to a specific environment, were sprayed in field trials to test STB resistance (Jackson et al. 2000). The advantages of seedling tests in the greenhouse in comparison to adult plant tests or field trials are obvious. More genotypes can be scored on a smaller area in a shorter time period, in subsequent experiments, as no vernalization is required yielding in quick results. To ensure a sufficient infection process seedlings have to be kept in the dark at near 100 % humidity for 48 h after inoculation. Afterwards a day/night rhythm with a moderate light intensity for 16 hours, a temperature regime of 18/22 °C and a humidity of 70 % is utilized. Usually the first leaves are inoculated and STB symptoms are scored 21 dpi (Kema et al 1996a). Also detached seedling leaf assays exist (Arraiano et al. 2001a) but are difficult to handle and need a good touch of the experimenter.

The long symptomless phase is a bottleneck in STB resistance analysis. In comparison, leaf rust (*Puccinia triticina*) takes only two weeks for symptom development (Singh 1992). To overcome this effect Adhikari et al. (2004d) developed a fluorescence real-time PCR assay to quantify fungal biomass in the leaves. Already 12 – 15 dpi fungal biomass quantity differed between resistant and susceptible cultivars. But this method has not become a standard for STB phenotyping because it is too expensive and laborious (Goodwin and Thompson 2011).

Symptoms of STB are necrotic blotches on which the typical black pycnidia are

formed (Kema et al. 1996a). Pycnidia formation is conditioned on cell collapse but not necessarily on necrotic tissue (Kema and van Silfout 1997). As necrosis reduces the leaf area for assimilation and the pycnidia are responsible for disease dispersion both parameters are scored as leaf area covered with necrosis and necrotic leaf area bearing pycnidia on a quantitative scale. Additionally, disease development can be assessed by scoring necrotic leaf area and pycnidial coverage several times starting with 18 dpi to calculate of the area under the disease progress curve (AUDPC) (Chartrain et al. 2004b, 2005c). Both disease parameters are suggested to be under different genetic control (Kema et al. 1996a; b), as proven by Ghaffary et al. (2011) who found isolate-specific QTL which control either necrotic leaf area or pycnidial coverage. However, in some studies only pycnidial coverage (Chartrain et al. 2005a-c; Arraiano et al. 2007a; Arraiano and Brown 2006; Ghaffary et al. 2012) or necrotic leaf area (Simón et al. 2001) was scored. To describe the level of sporulation as quantitative character of STB infection the amount of pycnidia in necrotic lesions was scored as pycnidia density (Cowger et al. 2000; Adhikari et al. 2003; Raman et al. 2009) which correlated strongly with pycnidial coverage (Adhikari et al. 2003). Adhikari et al. (2003; 2004a-c) calculated a disease index by multiplying scores of pycnidial coverage and density. But no genetic basis for pycnida density has been identified yet (Adhikari et al. 2003; 2004a). Likewise pycnidial coverage and necrotic leaf area are interrelated more (Brown et al. 2001; Simón et al. 2010) or less (Chartrain et al. 2005b) with an underlying isolate-specificity (Simón et al. 2010; Ghaffary et al. 2012). As the dimension of necrotic leaf area is also influenced by environmental factors during the experiment, pycnidial coverage provided the most efficient data for mapping STB resistance (Ghaffary et al. 2011).

Under epidemiological aspects the period between inoculation and symptom appearance (latency period) seems to be crucial. Ghaffary et al. (2011b) determined additionally to the quantitative scoring of necrotic leaf area and pycnidial coverage in the seedling stage the latency period of both parameters. QTL with minor effects on the observed traits were detected on chromosomes 5A and 2B.

To understand the *T. aestivum - M. graminicola* pathosystem holistically, adult plant tests are needed because specificity of resistance to plant developmental stages has been observed (Kema et al. 1996c, Ghaffary et al. 2012). Adult plant resistance was studied either on single plants in the greenhouse (Adhikari et al. 2004a; Simón et al. 2001), in the field (Simón et al. 2004b) or in outside polytunnels (Chartrain et al. 2004b; Arraiano et al. 2001b). STB resistance on the canopy level was scored in field

plots (Brown et al. 2001; Somasco et al. 1996; Miedaner et al. 2012). Field inoculations were conducted after the flag leaves had been fully unrolled with additional sprinkler irrigation (Ghaffary 2011) or at a cloudy day with high humidity (Risser et al. 2011) to ensure the infection.

In field experiments pycnidial coverage and necrotic leaf area are more difficult to distinguish than in the seedling tests. With the presence of other diseases (Arraiano et al 2009) or early senescence (Brown et al. 2001) necrotic leaf area cannot be determined confidently. Most field experiments assessed only disease severity of STB symptoms on the flag leaf (STB lesions) (Ghaffary et al. 2012; Eriksen et al. 2003). Only Brown et al. (2001) distinguished between pycnidial coverage and necrotic leaf area. In studies of isolate-specific adult plant resistance also natural inoculation should be accounted for in order to separate it from the isolate-specific reaction.

Disease escape occurs when a susceptible host does not become infected even under favorable environmental conditions because of separation from the present virulent pathogen by space or time (Agrios 2005). The advantage of artificial field inoculation is that disease escape like plant height and heading date can be avoided albeit its right accomplishment is crucial for confidential results. Especially in populations segregating for disease escape artificial inoculation has to be timed according to the particular maturity of each genotype (Adhikari et al. 2004a; Ghaffary et al. 2011). More difficult is the separation of disease escape and STB resistance in field experiments with natural inoculation of the locally occurring *M. graminicola* population. Assuming that asexual pycnidiospores are mainly dispersed via rain-splash in the canopy (Ponomarenko et al. 2011), taller plants tend to be more resistant because higher leaf levels have to be climbed up by the fungus. Earlier cultivars tend to be more diseased because the flag leaf which amounts for the main assimilate production is affected by a longer time period than later heading cultivars (Shaw and Royle 1993). Both, plant height and heading date, may influence STB resistance but follow independent segregation patterns (Somasco et al. 1996; Arama et al. 1999; Simon et al. 2005) and must therefore considered as disease escape. The possibility of artificial inoculation in the field should equal effects of plant height and heading date when the flag leaves are treated. But even in studies with repeated artificial inoculations, correlations to either plant height or heading date or both parameters occurred (Eriksen et al. 2003; Arraiano et al. 2006; Schilly et al. 2011). Such relationships to morpho-physiological traits might be due to environmental

factors like humidity at inoculation and subsequent weather conditions. Epidemiological factors like development stage at inoculation and effects of spore accumulations on early genotypes through repeated inoculation events are known to be problematic at *Fusarium* head blight resistance studies (Mesterhazy et al. 1978). Furthermore, the natural infection may establish better on semi-dwarf genotypes and falsify the results of an artificial inoculation. Arraiano et al. (2009) observed the contribution of disease escape traits plant height, leaf spacing, leaf morphology and heading date to STB resistance on more than 200 wheat lines under natural infection in the field. The authors supported a positive correlation between plant height and STB resistance previously identified in several studies (van Beunigen and Kohli 1990; Simon et al. 2004b; 2005). The presence of different *Rht* genes influences STB resistance. Wheat lines carrying *Rht-D1b* (former *Rht2*) were found to be more resistant than lines carrying *Rht-B1b* (former *Rht1*) (Baltazar et al. 1990). In conclusion correlations of STB resistance and plant height dependent on the absolute maximum and the range between maximum and minimum value for plant height as well as the presence of *Rht* genes but without any genetic linkage or pleiotropy to STB resistance (Simón et al. 2012).

Another difficulty in identifying sources for STB resistance is to classify a phenotype as susceptible or resistant. Due to the quantitative character of STB symptom development, the presence of several possible symptom characteristics (necrotic leaf area, pycnidial coverage, pycnidial density) and numbers of experimental methods (seedlings, detached leaves, single adult plants, field trials) it is not easy to keep comparability of phenotypic classes as long as no consistent methodology has been stated (Arraiano and Brown 2006; Ghaffary 2011). Phenotypic classes were often chosen arbitrary (Adhikari et al. 2004c; Brading et al. 2002) or according to different rating scales (Rosielle 1972; Zwart et al. 2010). The chosen phenotypic classification did not match consequently the allelic segregation of the associated markers (Adhikari et al. 2003; 2004a-c) what is also known as phenotyping versus genotyping problem (Dowell et al. 2010). In QTL mapping approaches no need for phenotype classification exists. In STB resistance studies the assumption of normal distributed phenotypes is often violated. For example when a spike in the phenotype distribution occurs or the normal-distribution is skewed (Zwart et al. 2010; Eriksen et al. 2003) and data cannot be transformed (Risser et al. 2011). However, statistical models exist to overcome these difficulties (Broman 2003).

1.6 Aims and key aspects

The scope of this thesis was the identification of qualitative and quantitative resistance sources in the winter wheat doubled haploid population Solitär x Mazurka by a QTL mapping approach.

Solitär has shown the highest STB field resistance among registered German cultivars (Anonymous 2009) with high stability of resistance over time (Schilly et al. 2011). Its resistance has a quantitative character at which disease is reduced but not completely absent. For the genetic analysis of STB resistance a doubled haploid population derived from a cross between Solitär - late heading, tall, outstanding field resistance to several diseases - and the STB susceptible Hungarian cultivar Mazurka – early flowering, short, highly tolerant to drought and frost - was established.

In **Chapter 2.1** the parents of the Solitär x Mazurka doubled haploid population were screened for STB resistance in the seedling stage with a worldwide set of 30 different *M. graminicola* isolates to identify such which differentiate the parental genotypes. In assessment of disease severity, pycnidial coverage and necrotic leaf area were distinguished to detect differences in the inheritance of both traits. The objectives were (i) to analyze resistance response of the doubled haploid lines to the selected fungal isolates (ii) to map QTL for isolate-specific STB resistance and (iii) to calculate QTL x QTL interactions to reveal a complete view on the genetic architecture of seedling resistance. Furthermore, molecular markers utilizable for marker assistant selection should be identified.

In **Chapter 2.2** inheritance of STB resistance under natural infection conditions was observed in a multi-environmental field trial. The influence of plant height and heading date, which are known disease escapes, should be determined. The objectives were (i) to analyze field resistance of the doubled haploid lines in a multi-environmental field trial (ii) to map QTL for STB field resistance and QTL x environment interactions under consideration of disease escape and (iii) to investigate the effectiveness of the in **Chapter 2.1** identified isolate-specific QTL in the field.

2 Original Papers

2.1 The genetic architecture of seedling resistance to *Septoria tritici* blotch in the winter wheat doubled-haploid population Solitär x Mazurka

Published in:
Molecular Breeding 29:813-830 in 2012

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Abstract

Breeding for resistance to Septoria tritici blotch (STB), caused by *Mycosphaerella graminicola* (anamorph: *Septoria tritici*), is an essential component in controlling this important foliar disease of wheat. Inheritance of seedling resistance to seven worldwide pathogen isolates has been studied in a doubled haploid (DH) population derived from a cross between the field resistant cv. Solitär and the susceptible cv. Mazurka. Multiple quantitative trait locus (QTL) mapping revealed major and minor genetic effects on resistance as well as several epistatic relationships in the seedling stage. Solitär conferred resistance to isolate IPO323, governed by *Stb6* on chromosome 3A, as well as to IPO99015, IPO92034, Hu1 and Hu2 controlled by a QTL on chromosome arm 1BS, possibly corresponding to *Stb11* and minor QTL on chromosomes 1B, 3D, 6B and 7D. Resistance of Mazurka to IPO90015 and BBA22 was caused by a QTL located in a region on 4AL which harbours *Stb7* or *Stb12*. QTL specific to pycnidial coverage on 3B and specific to necrosis on 1A could be discovered for isolate IPO92034. Pairwise epistatic interactions were reliably detected with five isolates. Although their contributions to the total variance are generally low, the genotypic effect of the QTL by QTL interaction of 4AL (*Stb7 or Stb12*) and 3AS (*Stb6*) made up almost 15% of disease expression. Altogether, the results suggest a complex inheritance of resistance to STB in the seedling stage in terms of isolate-specificity and resistance mechanisms, which bear implications for marker-assisted breeding in an attempt to pyramid STB resistance genes.

Keywords

Triticum aestivum; Mycosphaerella graminicola; Septoria tritici blotch; resistance; QTL; epistasis

2.2 Inheritance of field resistance to Septoria tritici blotch in the wheat doubled-haploid population Solitär x Mazurka

Published in:

Euphytica 194:161-176 in 2013

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Abstract

Breeding for field resistance to Septoria tritici blotch (STB), caused by *Mycosphaerella graminicola* (anamorph: *Septoria tritici*) is the most suitable strategy for controlling this important disease of wheat. Although many *Stb* genes for resistance to single pathogen isolates have been identified in wheat, knowledge of their efficiency against natural fungal populations is lacking. In a quantitative-trait-locus (QTL) mapping approach in six environments and four locations, field resistance to STB was studied in a doubled-haploid population derived from a cross between the field-resistant cultivar Solitär and the susceptible cultivar Mazurka. After plant height as a disease escape trait was accounted for, five QTL with effects on STB resistance on chromosomes 5A, 6D and 7D explained 20% of the genotypic variance, while QTL x environment interactions were minor. Field resistance was conferred exclusively by alleles from Solitär, which was previously shown to carry the isolate-specific genes *Stb6* and *Stb11* as well as minor QTL detected with seven fungal isolates. Surprisingly, neither the *Stb6* nor *Stb11* isolate-specific genes nor minor QTL previously detected in Solitär were found to be involved in its field resistance. The study suggests that resistance breeding for STB should not rest solely on the deployment of *Stb* genes. Field tests are indispensable to show their efficacy and durability and to identify genes of partial field resistance to STB.

Keywords: *Triticum aestivum*, Septoria tritici blotch, *Mycosphaerella graminicola*, field resistance, QTL

3 General discussion and future prospects

3.1 The genetic architecture of *Septoria tritici* blotch resistance in the Solitär x Mazurka doubled haploid population

Resistance that remains effective, even when the cultivar possessing it is widely grown, was described as durable by Johnson in 1981. Solitär is, since its release in 2004, the most resistant winter wheat cultivar in Europe with a stable STB resistance response over years and locations (Schilly et al. 2011). Solitär and the STB susceptible cultivar Mazurka showed isolate-specific STB resistance in the seedling stage. To verify identified isolate-specific resistances the Solitär x Mazurka doubled haploid population was developed, and at first covered with 178 SSR and AFLP as well as one phenotypic marker (**Chapter 2.1**). To increase genome coverage the genetic map was augmented with 512 DArT markers, altogether the entire map comprised 779 molecular markers (162 SSR, 512 DArT and 105 AFLP)) and one phenotypic marker for awnedness (**Chapter 5**; **Fig. A1**). A high number of DArT markers tended to cluster along chromosomes. These markers could not be ordered accurately, given the single recombination event taking place in a doubled haploid line and the limited number of lines. We accordingly used a reduced map for QTL analyses; this comprised 252 molecular markers (120 SSR, 95 DArT, 37 AFLP loci) and one phenotypic marker. In the course of the augmentation with DArT markers the previously identified isolate-specific QTL for STB resistance (**Chapter 2.1**) were re-evaluated (**Chapter 5**, **Tab. A1**). To determine the efficacy of identified isolate-specific resistance against the natural *M. graminicola* population the doubled haploid population was analyzed in a multi-environmental trial. Under consideration of plant height as disease escape, only Solitär was identified to carry STB field resistance loci (**Chapter 2.2, Tab. 3**). Genetic regions with effects on isolate-specific resistance in Solitär did not fit field resistance QTL (**Fig. 7**). Until now this is the first report of genetic inheritance of isolate-specific resistance and field resistance against STB in Solitär.

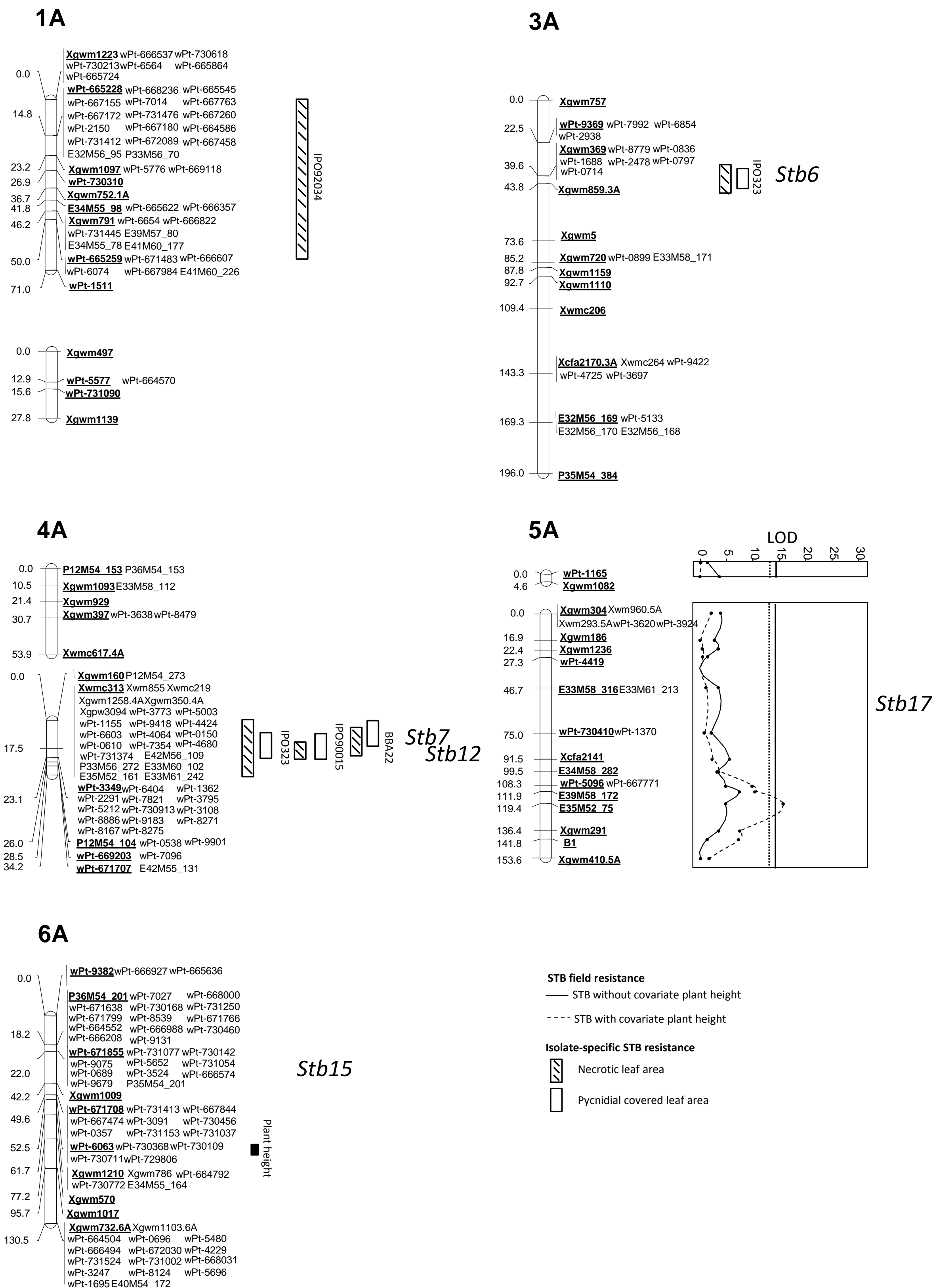

Fig. 7 Location of *Septori tritici* blotch field and seedling resistance QTL in the genetic map of the Solitär x Mazurka doubled haploid population and known *Stb* genes on the respective chromosomes. Isolate-specific QTL for pycnidial covered leaf area (unfilled bars) and necrotic leaf area (textured bars) were detected with seven single isolates in the seedling stage. LOD profiles of QTL detected in six locations in the field with and without plant height as a covariate. Horizontal lines indicate significance thresholds of P = 0.05 and P = 0.1 (dotted line). Locations of QTL for plant height and heading are drawn as filled bars. Bar size indicates 1.5-LOD support interval.

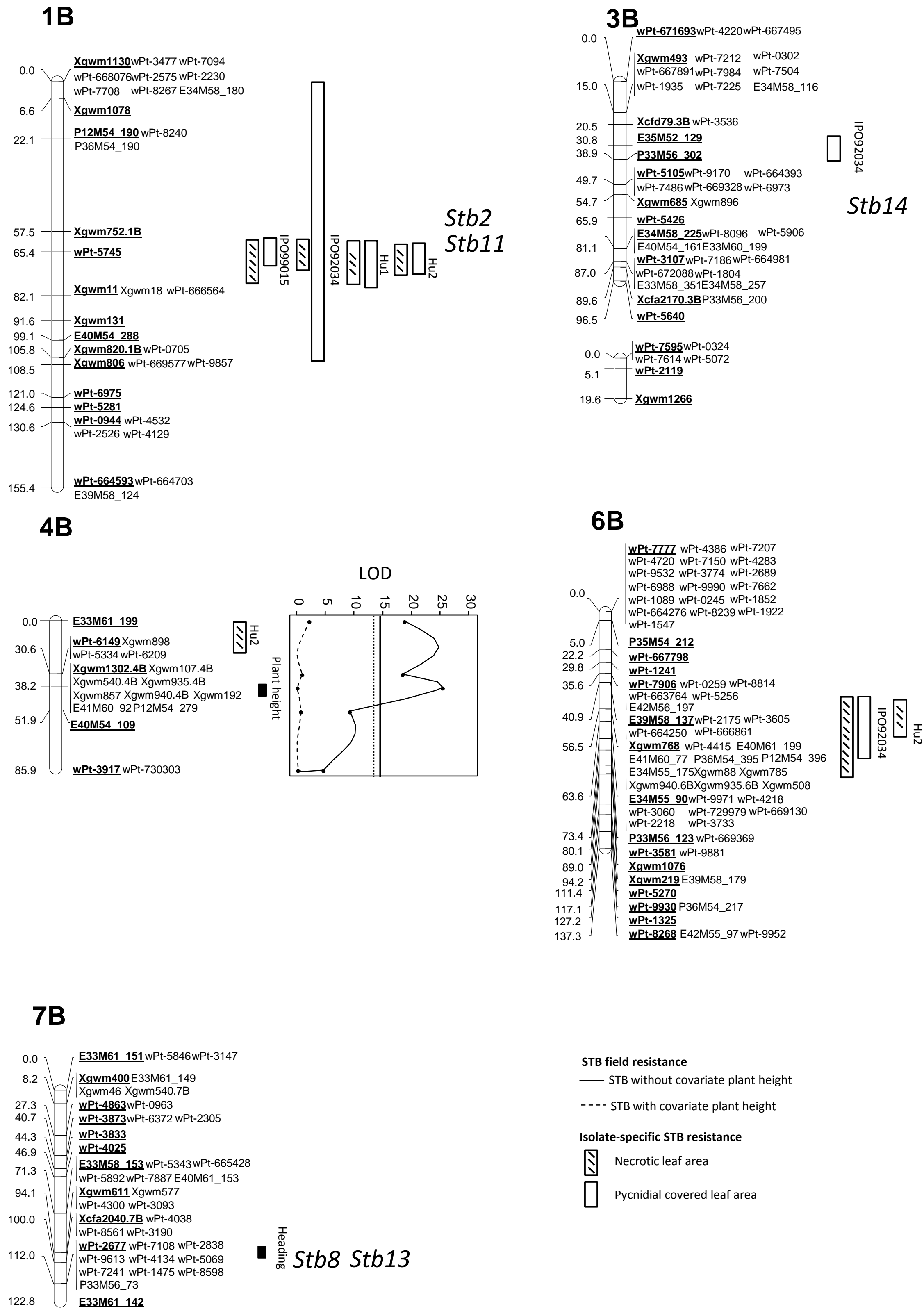

Fig. 7 continued

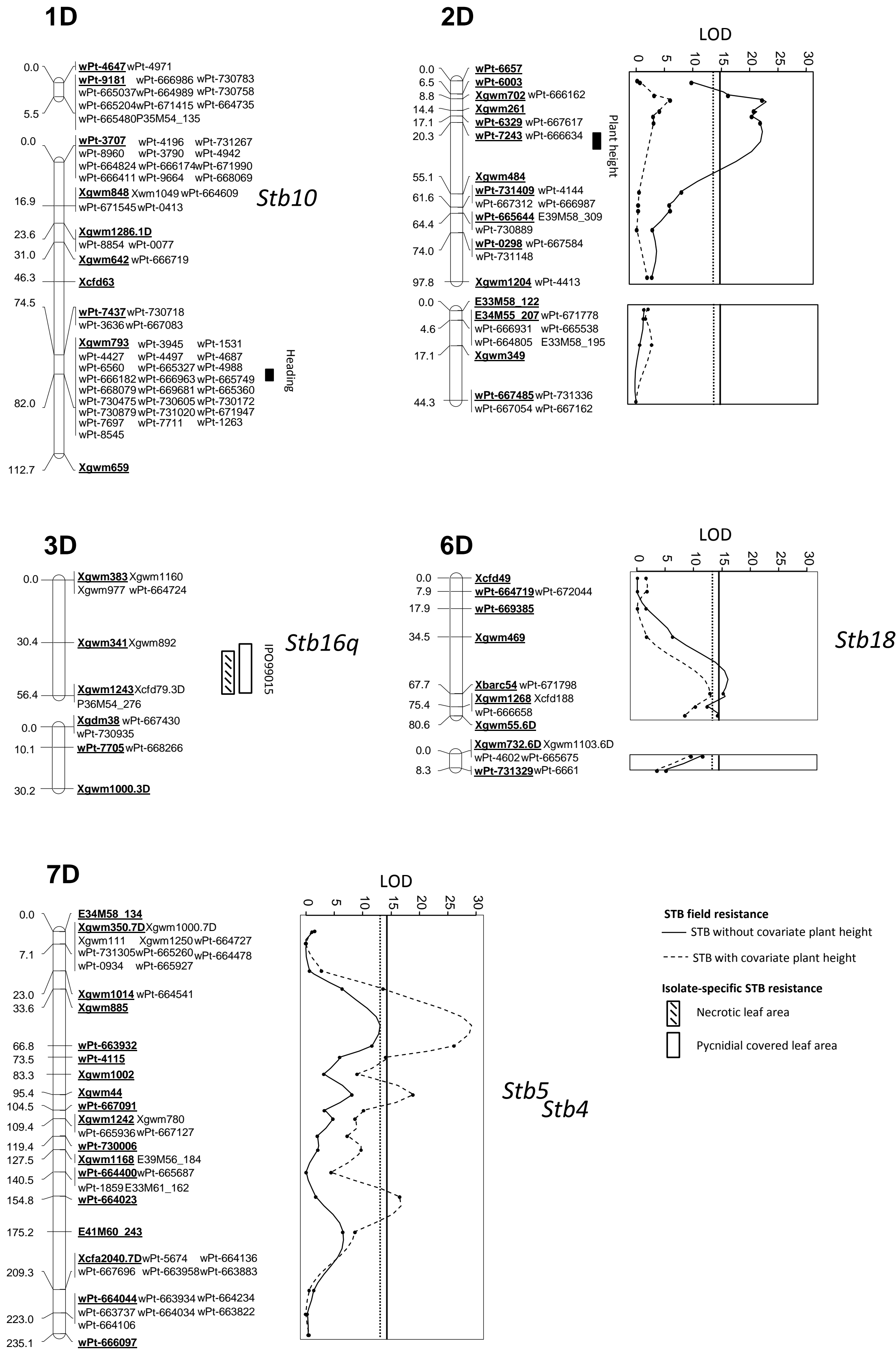

Fig. 7 continued

Solitär carries two isolate-specific QTL on chromosomes 3A and 1B which can be attributed to *Stb* genes; and quantitative disease resistance on chromosomes 3B, 6B, 3D and 4B. QTL confer either specific resistance against pycnidia formation (3B), necrotization of leaf area (4B) or both disease parameters (3A, 1B, 3D, 6B) (**Chapter 5, Tab. A1**). QTL with specificity to plant development were identified previously (Eriksen et al. 2003; Ghaffary et al. 2012) and indicate different operation modes of resistance.

The phenotypic distribution pattern in resistance to IPO323 for both pycnidial coverage and necrotic leaf area showed a clear peak of resistant genotypes what implies the action of a single gene (**Chapter 5, Fig. A2**). The QTL on the short arm of chromosome 3A linked to *Xgwm369* can be assigned to *Stb6,* one of the most frequent *Stb* genes in European germplasm (Brown et al. 2001). *Stb6* confers seedling and adult plant resistance to IPO323 as well as resistance in the field against the natural occurring *M. graminicola* population (Arraiano et al. 2009). The QTL identified on the short arm of chromosome 1B can either be *Stb11*, allelic to *Stb11* or a different unknown *Stb* gene. Recently *Stb2*, first identified in cultivar Veranopolis and mapped on chromosome 3B (Adhikari et al. 2004a), has been relocated to the short arm of chromosome 1B (Liu et al. 2013). Whether *Stb2*, *Stb11* and further QTL identified in the same genomic region on 1BS (Raman et al. 2009; Ghaffary et al. 2011) refer to the same gene is not clear yet. Each *Stb* gene or QTL has been detected in different mapping populations with different *M. graminicola* isolates and due to a paucity of shared molecular markers it is impossible to draw a clear conclusion. Resistance response of *Stb2* seems to be recessively inherited (Goodwin and Thompson 2011). *Stb11* and *Stb2* both have been identified in DH populations where dominance has been assumed (Chartrain et al. 2005b; Adhikari et al. 2004b). A simple replication of the original cross which segregated for *Stb11* would shade light on the respective inheritance pattern. A recessive inheritance of *Stb11* will be expected when the F_1 generation would be susceptible and the F_2 generation would segregate into a 1 (resistant lines) : 3 (susceptible lines) phenotypic ratio. Liu et al. (2013) stated that the “quantitative character” of *Stb11* explaining 50 % of the phenotypic variance in the seedling stage (Chartrain et al. 2005a) could be used to discriminate *Stb2* from *Stb11* and further QTL on 1BS. Resistance response of *Stb2* differentiated clearly in resistant and susceptible phenotypes in the adult plant stage (Liu et al. 2013). QTL on 1BS explained 90 % of the phenotypic variance in the seedling but only 38 % in the adult plant stage in three mapping populations with a single *M. graminicola* isolate (Raman et al. 2009). The QTL identified by Ghaffary et al. (2011) wth isolate IPO87016 in the seedling stage explained > 60 % of the phenotypic variance (**Chapter 1**, **Tab. 2**). The QTL on chromosome

1B identified in **Chapter 2.1** was only detected in the seedling stage but with four different *M. graminicola* isolates (IPO99015, IPO92034, Hu1 and Hu2). In the Solitär x Mazurka doubled haploid population the phenotypic effect varied with the isolate from 14 % (Hu1) to 69 % (IPO99015) (**Chapter 5, Tab. A1**). The right-skewed phenotypic distribution of resistance response against IPO92034 indicates the activity of multiple loci. In the presence of multiple loci, phenotypic variance explained by each single QTL is lower in comparison to the activity of a unique QTL. However, continuously distributed phenotypes are not necessarily the consequence of the action of multiple loci. Unfavorable environmental conditions during disease development might influence resistance response of the doubled haploid lines differentially. This was suspected for resistance against the Hungarian isolate Hu1 where a continuous phenotypic distribution was detected. Common pathogenicity analyses of the single isolates on the segregating populations previously utilized to identify resistance on chromosome 1B are required to differentiate *Stb11*, *Stb2* and QTL on 1BS.

Environmental conditions were shown to influence resistance expression in the seedling stage and thereby decrease the proportion of phenotypic variance explained (St. Clair 2010). Besides Solitär also the susceptible parent Mazurka, showed isolate-specific resistance to single *M. graminicola* isolates in the seedling stage. Mazurka carries one isolate-specific QTL on chromosomes 4A which can either be attributed to *Stb7* or *Stb12* and quantitative disease resistance on chromosomes 1A, conferring specific resistance against necrotization of leaf area (**Chapter 5, Tab. A1**). Resistance response of *Stb7/Stb12* against IPO90015 explained 50 % of the phenotypic variance. At the same chromosomal location on 4AL resistance against the German isolate BBA22 and the Dutch isolate IPO323 has been identified. Like resistance responses of the doubled haploid lines to Hu1 and Hu2, unfavorable conditions during disease development of BBA22 might increase environmental influence and decrease phenotypic variance explained by *Stb7/Stb12*. In response to IPO323 the QTL on chromosome 4A was hypostatic to *Stb6* on chromosome 3A. The effect of *Stb7/Stb12* was masked by *Stb6;* only in genotypes where *Stb6* was not present, activity of *Stb7/Stb12* has been recognized. In comparison to genotypes which carried the Solitär allele on 4A and the Mazurka allele on 3A, genotypes which carried on both loci the Mazurka allele showed a decrease in mean pycnidial coverage. Hereby, the single effect of *Stb7/Stb12* was not unambiguously distinguishable from the interaction effect of *Stb7/12* and *Stb6*. The effect of *Stb7/Stb12* against IPO323 is rather low whereas resistance response against IPO90015 shows a qualitative character (**Chapter 2.1**). An epistatic effect of *Stb6* was also recognized in the Apache x Balance wheat mapping population in the seedling stage (Ghaffary et al.

2011). *Stb18*, located on chromosome 6D and conferring resistance to four different *M .graminicola* isolates, was found to be hypostatic to *Stb6* when inoculated with IPO323.

Environmental effects during disease development, isolate-specific resistance response and the presence of multiple loci which additionally can interact with each other influence the phenotypic variance explained by isolate-specific STB resistance QTL. Thus, differentiation in minor and major QTL or in QTL and *Stb* gene is not reasonable when comparing single QTL effects.

In the attempt to explain quantitative disease inheritance one hypothesis indicates, that quantitative resistance loci are weak forms of R-genes. The co-localization of R-genes and loci for quantitative disease resistance gives evidence that allelic variants of R-genes might account for a proportion of quantitative resistance. R-genes are defined as single genes conferring qualitative complete resistance (Poland et al. 2008). But exceptions of this definition exists, like *Lr34* and *Yr36* which express quantitative resistance and have been approved to be single genes due to cloning and sequencing approaches (Miedaner and Korzun 2012). Furthermore, mutations in the pathogen might erode effectiveness of R-genes and convert a complete resistance response to a quantitative one (Poland et al. 2008). In the Solitär x Mazurka doubled haploid population *Stb7/Stb12* exhibits a different resistance response in dependency of the tested isolate. Likewise, QTL for STB resistance were found in the same chromosomal region as known *Stb* genes (**Chapter 5, Tab A1**).

For a holistic few on STB resistance inheritance the Solitär x Mazurka doubled haploid population was tested under natural inoculation pressure in six environments (**Chapter 2.2**). One aim was to verify efficacy of previously identified *Stb* genes and resistance QTL under the pressure of locally adapted *M. graminicola* populations. No *Stb* gene (*Stb6*; *Stb11*/*Stb2; Stb7/Stb12*) or QTL conferring seedling resistance in Solitär and Mazurka were confirmed in the field trials. Two reasons which might be responsible for this discrepancy are described. First, the *Stb* genes/ QTL identified in **Chapter 2.1** are seedling specific with no effects in the adult plant stage. Second, adaptation of the local *M. graminicola* population induced a lost of efficacy of the previously identified isolate-specific *Stb* genes and QTL in the Solitär x Mazurka doubled haploid population.

Stb6 (Arraiano and Brown 2006, and *Stb11/Stb2* (Raman et al. 2009; Liu et al. 2013) were effective in both the seedling and adult plant stage. Genotypes carrying *Stb6* were shown to confer also field resistance (Arraiano et al. 2009). However, effects of *Stb6* were not consistent and depend strongly on the cultivar. Also *Stb2* showed effects on field resistance

and was introduced in commercial cultivars (Goodwin 2007). Single isolate efficacy of *Stb11* against the fungal *M. graminicola* population is not known. To test the Solitär x Mazurka DH lines with the selected seven single fungal isolates also in the adult plant stage would give a clear answer to the questioned seedling or adult plant specificity of the defected QTL.

Approaches which try to develop isogenic lines for certain *Stb* genes in the same genetic background are underway (Goodwin and Thompson 2011). These lines will allow testing single *Stb* gene efficacy against local adapted field populations of *M. graminicola* or against a wide range of single isolates. Fungal adaptation can also be confirmed by testing single isolates of the local fungal population on a set of wheat cultivars which differ in *Stb* genes (differential set) (Ghaffary et al. 2012). The lost of *Stb* gene efficacy due to adaptations of *M. graminicola* isolates has been detected for *Stb4* (Jackson et al. 2000; Krenz et al. 2008). *Stb7/Stb12* carried by Mazurka is already not efficient because Mazurka is susceptible to the fungal population in field trials in Germany. Solitär, which carries STB field resistance, was susceptible to six out of seven single isolates sampled in Germany (**Chapter 2.1**). This implies that also STB resistance of Solitär can get inefficient after a certain period of growing.

Field resistance of Solitär is quantitative and inherited by five QTL on chromosomes 5A, 6D and 7D which, together with the QTL x QTL interactions, explain 20 % of the phenotypic variance. Quantitative STB resistance inheritance was also detected in four winter wheat populations infected with a mixture of adapted *M. graminicola* isolates (Miedaner et al. 2012; Risser et al 2011). Five QTL explained between 3 and 24 % of the phenotypic variance (Miedaner et al. 2012).

One of the three QTL detected on chromosome 7D had the largest effects on STB resistance and were detected in four out of six environments. QTL on chromosomes 5A and 6D were only identified in single environments (**Chapter 2.2, ESM 2 Fig. 3**). Differences in virulence spectra of the local occurring *M. graminicola* populations are expected. However, a relative low genetic diversity of the fungal population among fields of the same region is described (Linde et al. 2002). Furthermore, the infestations with other diseases like leaf rust or powdery mildew could also have altered STB symptom expression in the field trials (Orton 2012; Brokenshire 1974).

The QTL on chromosome 7D is located close to a cluster of *Stb* genes (Adhikari et al. 2004b; Arraiano et al. 2001b) and isolate-specific QTL (Arraiano et al. 2007a; Simón et al. 2010; Ghaffary et al. 2011). QTL on chromosome 5A is located close to *Stb17* (Ghaffary et al. 2012), the first named *Stb* gene expressed specifically at the adult plant stage and the only

source for STB resistance on chromosome 5A. Except for *Stb18*, which is mapped ~ 60 cM apart from the QTL for STB field resistance no QTL has been mapped on 6D (Goodwin 2007). QTL for STB field resistance in the Solitär x Mazurka doubled haploid population were identified while accounting for plant height as a covariate. The influence of heading date was excluded as covariate due to the low relationship between STB symptoms and plant development. Disease escape is a common phenomenon in STB field trials (Eriksen et al. 2003; Arraiano et al. 2009). The best procedure is to exclude disease escape e.g. by choosing a similar plant height of both crossing partners what let expect only a low variation of this trait in the offspring. Solitär and also other sources for STB resistance like the Swiss cultivar Arina (Risser 2010) is taller than most of the high yielding but STB susceptible wheat cultivars with which a cross is most suitable. The second best option is what has been done in this study; to use plant height as source of disease escape as a covariate in the genetic analyses of STB resistance.

A complex genetic architecture of STB resistance inheritance has been identified in the Solitär x Mazurka doubled haploid population. Isolate-specific qualitative and quantitative resistance sources accounted primarily for seedling resistance in both parents. But isolate-specific resistance sources detected in the seedling stage were not effective in field resistance. While accounting for plant height, superior STB field resistance of Solitär is inherited by quantitative resistance sources. Those were only detected in Solitär. Interactions between detected *Stb* genes (solely in the seedling stage) as well as QTL x QTL interactions were identified in the seedling tests as well as in the field trials. However, interactions had only minor effects on resistance response. Without knowledge of the local *M. graminicola* population no final conclusion of fungal adaptations to *Stb* genes presented in Solitär are possible. A greater number of doubled haploid lines and molecular markers might enhance the resolution in mapping quantitative resistance in Solitär.

3.1 Future prospects in *Septoria tritici* blotch resistance breeding

Beyond identification of STB resistance genes/QTL also possible gene functions and interactions with other fungal resistance sources should be considered. This knowledge could enhance the breeding success. Evidence for isolate-specific gene action in dependence of necrosis/pycnidia formation and under epistasis is feasible in STB resistance inheritance (Kema et al. 1996a; Ghaffary et al. 2011; 2012). *M. graminicola* is able to penetrate susceptible and resistant hosts, resistance response starts after the fungus has entered the plant. *M. graminicola* seems to overcome the first line plant defense which recognizes basal

fungal components like chitin via so called effectors. These effectors again are recognized by products of R-genes and trigger an immunity response. In contrast to resistance against biotrophic fungi, host-specific toxins function as effectors in necrotrophic fungi. *M. graminicola* is classified as a hemibiotrophic fungus which shows an endophytic-like and a necrotrophic growing phase. It is not clear whether *M. graminicola* produces toxins to introduce cell collapse like *Stagnospora nodorum* (Liu et al. 2004). Resistance to *M. graminicola* concentrates on a deceleration of fungal growth and prevention of pycnidia formation (Kema 1996c). Possibly, *Stb6* and *Stb7/Stb12* are active short after penetration of *M. graminicola* through the stomata, thereby preventing programmed cell death (PCD) and slow down fungal growth. The pycnidia-specific QTL on chromosome 3B could interfere at a later stage of pycnidia formation and maturation by inhibiting fungal synthesis of reactive oxygen species (Shetty et al. 2003).

Common clusters of resistance genes against STB and biotrophic fungi like yellow rust, leaf rust, and powdery mildew are described on chromosomes 1BS (Liu et al. 2013; Bariana et al. 2002; Schnurbusch et al. 2004) and 7DS (Krattinger et al 2009). IPO323 has been shown to hijack plant resistance signaling against biotrophic fungi of a STB susceptible host by accelerating PCD (Hammond-Kosack and Rudd 2008; Keon et al. 2007). Biotrophic and necrotrophic fungi react differently to PCD; usually biotrophs are inhibited by the hypersensitive reaction (HR), whereas necrotrophic pathogens are able to utilize it (Keon et al. 2007). To induce susceptibility, host receptors are required which recognize fungal toxins and start the immunity response usually applied against biotrophic fungi (Hok et al. 2010). It has been shown, that a STB susceptible host infected with dead and unviable *M. graminicola* spores and additionally inoculated with living *Blumeria graminis f.sp. tritici* spores, reduced the colonization with powdery mildew (Orton 2012). Mutation might induce a loss of function in the host receptor or toxin sensitivity gene which prevents the recognition process and so PCD (Hok et al. 2010). Loss of function of genes for susceptibility will lead to a resistance response that inherits recessively like assumed for *Stb2* (Goodwin and Thompson 2011). In barley, a mutation of the recessively inherited susceptible locus *Mlo* conferred a durable resistance to all races of *Blumeria graminis f.sp. tritici* (Büschges et al. 1997). In comparison to the *mlo*-derived resistance, *Stb2* does not confer resistance to all *M. graminicola* isolates tested but at least to a broad-spectrum of them (Ghaffary 2011). Chromosome 5BS, which carries resistance genes against yellow rust and powdery mildew, seems to possess also genes, which either promote susceptibility or suppress resistance to STB (Arraiano et al. 2007b). Genes that response on one hand with resistance to biotrophic fungi and on the

other hand with susceptibility against necrotrophic fungi seem to encode similar protein pattern as shown for a susceptibility gene in *Arabidopsis* (Hok et al. 2010; Sweat et al. 2008). A mutation in a R-gene against a biotrophic fungus might result in a resistance locus for a necrotrophic fungus or in the case of STB, against the necrotrophic growth step of *M. graminicola*. To prove this hypothesis for the *Triticum aestivum* L. – *M. graminicola* pathosystem the genetic sequences of *Stb* genes and genes against biotrophic fungi have to be compared. Until now no *Stb* gene has been cloned and sequenced yet. Also not all *Stb* genes are clustered with R-genes for biotrophic fungi. This may be an indication for different types of resistance genes against STB. More attention in identification and analysis of susceptible genes will certainly improve STB breeding for durable, broad-spectrum resistance. But this approach would require a cloning of promising susceptible genes and a gene silencing procedure afterwards what is not yet feasible today for STB (Hok et al. 2010).

Marker-assisted selection (MAS) has been effective for the implementation of large effect alleles with known association to a molecular marker in breeding lines (Zhong et al. 2006; Miedaner and Korzun 2012). Certainly, for STB resistance and *Stb* gene pyramidization MAS have until now not been feasible. One reason is that available molecular markers linked to *Stb* genes are not useful in different genetic backgrounds (Miedaner and Korzun 2012). Knowledge of efficacy of single *Stb* genes against the natural *M. graminicola* population seems to be crucial before starting MAS and pyramidization approaches (Goodwin and Thompson 2011). Furthermore, occurrence of non-additive interactions like they are proven for *Stb6* and *Stb7/Stb12* in this study as well as for *Stb6* and *Stb18* by Ghaffary et al. (2011) have to be considered when combining *Stb* genes and QTL for STB resistance. Under the rapid development of sequencing techniques (Poland et al. 2012) the identification of molecular markers linked to *Stb* genes or QTL seems the less challenging mission in STB resistance breeding. Thus, reliable phenotyping methods are getting more and more important. Versatile phenotyping methods for STB resistance are available (**Chapter 1.5**). The declaration of a standard method, adjusted for the seedling and adult plant stage, would considerably facilitate the identification of new sources for STB resistance.

STB field resistance of Solitär has shown to be quantitatively inherited by QTL each having small effects on resistance response. Using biparental populations to identify such QTL may have some disadvantages like missing level of allelic diversity, high costs of population generation, small population sizes, need of validation of discovered QTL and usage of stringent significance thresholds (Jannink et al. 2010). A promising alternative to use small effective QTL in breeding is genomic selection (GS; Meuwissen et al. 2001). Genome-wide

marker coverage is utilized to predict genotypic values for quantitative traits. One advantage of GS is that there is no need for previous identification and mapping of QTL. Statistical methods enable the simultaneous estimation of all marker effects. In comparison MAS utilizes only a small number of markers for selection. The frequency of phenotyping events can be reduced because selection is based rather on genotypic data than on phenotypic data. Corresponding to the development of GS models for adult plant resistance to stem rust (Rutkoski et al. 2011), GS models for STB field resistance could be developed. At first a population of individuals with variation in STB field resistance would be used for GS model training. Isolate-specific seedling resistance of the training population should be known to ensure that field resistance can be evaluated without the confounding effects of *Stb* genes. Phenotyping of the training population should be done under high disease pressure and the presence of isolates which lead to efficacy lost of existing *Stb* genes.

With phenotypic data resistant candidate individuals will be selected and together with the genotypic data marker effects will be estimated for the whole training population. By combination of marker effect estimates and the marker data of the selection candidates, genomic estimated breeding values (GEBVs) will be calculated. A GEBV can be defined as the sum of all marker effects included in the model for an individual and used as selection criterion. Thus total additive genetic variance is captured with genome-wide marker coverage and effect estimates. Once the GS model has been built it can be utilized in the breeding material for selection. Using GEBVs for selection of quantitative traits has been proved a high accuracy in simulation studies (Meuwissen et al. 2001) as well as in empirical studies of corn (Zhao et al. 2012) and wheat (Heffner et al. 2011, Poland et al. 2012).

4 References General introduction and Discussion

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Tab. A1 QTL and QTL-by QTL-interactions for *Septoria tritici* blotch (means of three experiments) to seven *M. graminicola* isolates in the seedling stage identified in the Solitär x Mazurka doubled-haploid population by multiple QTL mapping

Isolate	Disease parameter[a]	QTL / QTL pair[b]	Resistance Donor[c]	Position(s) (cM)[d]	Nearest marker / marker pair	QTL Heritability[e] (%)	Genotypic Effect[f] (%)	F-value [g]	Putative gene
IPO90015	PYC	*QStb.4AL*	M	14	*Xwmc313*	49.4	12.5	166.1 ***	*Stb7/Stb12*
		QStb.1B.b:QStb.2AL	S:M; M:S	102:12	Xgwm820.1B:Xgwm1151	3.2	6.1	10.5 **	
	NEC	*QStb.4AL*	M	14	*Xwmc313*	74.2	25.9	NA	*Stb7/Stb12*
IPO323	PYC	*QStb.3AS*	S	42	*Xgwm369*	67.8	19.5	139.3 [h] ***	*Stb6*
		QStb.4AL	M	18	*Xwmc313*	14.4	6.4	29.9 [h] ***	*Stb7/Stb12*
		QStb.3AS:QStb.4AL	S:M	42:18	*Xgwm369:Xwmc313*	4.8	13.0	20.0 [h] ***	
	NEC	*QStb.3AS*	S	42	*Xgwm369*	81.7	43.2	276.6 ***	*Stb6*
		QStb.4AL	M	18	Xwmc313	5.2	7.5	17.4 ***	*Stb7/Stb12*
		QStb.3AS:QStb.4AL	S:M	42:18	*Xgwm369:Xwmc313*	1.5	13.9	10.5 **	
IPO99015	PYC	*QStb.1B.a*	S	66	*wPt-5745*	68.9	12.7	354.5 ***	*Stb2/Stb11*
		QStb.3DS	S	54	*Xgwm1243*	4.5	3.5	22.8 ***	
	NEC	*QStb.1B.a*	S	66	*wPt-5745*	66.4	22.0	381.5 ***	*Stb2/Stb11*
		QStb.3DS	S	54	*Xgwm1243*	8.6	8.7	49.5 ***	

Tab. A1 continued

Isolate	Disease parameter[a]	QTL / QTL pair[b]	Resistance Donor[c]	Position(s) (cM)[d]	Nearest marker / marker pair	QTL Heritability[e] (%)	Genotypic Effect[f] (%)	F-value [g]	Putative gene
IPO92034	PYC	*QStb.3B*	S	30	*E35M52_129*	37.0	8.9	33.0 [h] ***	*Stb14*
		QStb.1B.a	S	68	*wPt-5745*	14.4	5.5	19.2 [h] ***	*Stb2/Stb11*
		QStb.6B.a	S	76	*wPt-3581*	10.3	4.1	13.7 [h] ***	
		QStb.3B:QStb.6B.a	S:S	30:76	*E35M52_129:wPt-3581*	4.0	6.6	10.6 [h] **	
		QStb.3B:QStb.1B.a	S:S	30:68	*E35M52_129:wPt-5745*	3.2	6.1	8.6 [h] **	
	NEC	*QStb.1B.a*	S	66	*wPt-5745*	24.7	11.0	52.0 ***	*Stb2/Stb11*
		QStb.6B.a	S	80	*wPt-3581*	6.0	6.0	14.0 ***	
		QStb.1A	M	10	*wPt-665228*	9.2	7.1	19.3 ***	
Hu1	PYC	*QStb.1B.a*	S	65	*wPt-5745*	14.3	3.2	NA	*Stb2/Stb11*
	NEC	*QStb.1B.a*	S	65	*wPt-5745*	18.0	4.2	NA	*Stb2/Stb11*
Hu2	PYC	*QStb.1B.a*	S	65	*wPt-5745*	50.1	7.5	148.4 ***	*Stb2/Stb11*
		QStb.2B:QStb.7D	S:M; M:S	18:127	*Xgwm374:Xgwm1168*	3.0	3.9	8.8 **	
	NEC	*QStb.1B.a*	S	65	*wPt-5745*	35.5	7.5	105.1 ***	*Stb2/Stb11*
		QStb.6B.b	S	60	*E34M55_90*	2.8	2.3	8.3 **	
		QStb.4B	S	0	*E33M61_199*	4.0	2.7	12.0 ***	
		QStb.2B:QStb.7D	S:M; M:S	18:127	*Xgwm374:Xgwm1168*	4.5	5.6	13.3 ***	
BBA22	PYC	*QStb.4AL*	M	14	*Xwmc313*	13.7	3.3	NA	*Stb7/Stb12*
	NEC	*QStb.4AL*	M	14	*Xwmc313*	10.8	3.8	NA	*Stb7/Stb12*

[a] PYC = pycnidial coverage, NEC = necrotic leaf area
[b] QTL name described by chromosome or chromosome arm; a lower-case character indicates different QTL on the same chromosome
[c] single QTL allele, QTL-by-QTL interaction allele combination(s) conferring resistance; S = cv. Solitär; M = cv. Mazurka
[d] QTL position(s) determined by refined MQM analysis
[e] QTL heritability defined as phenotypic variance explained by the QTL or QTL-by-QTL interaction
[f] QTL effect was estimated as the difference in the mean between the two homozygous QTL genotypes
[g] ** P = 0.01; *** P = 0.001 ; [h] estimated single QTL effect and QTL-by-QTL interaction effect not unambiguously distinguishable

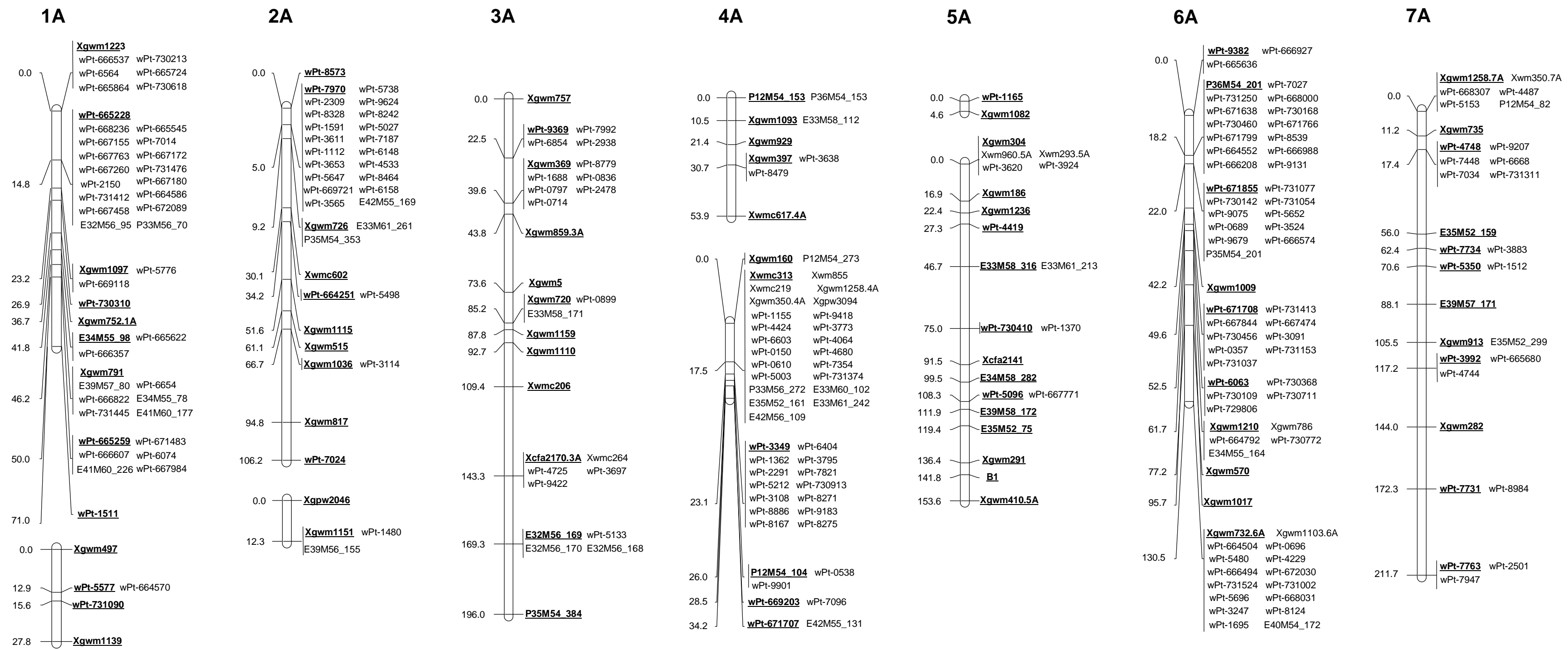

Fig. A1 Genetic map of the Solitär x Mazurka doubled haploid population covered with 779 molecular markers. Bold and underlined markers were used for QTL mapping (Chapter 2.2)

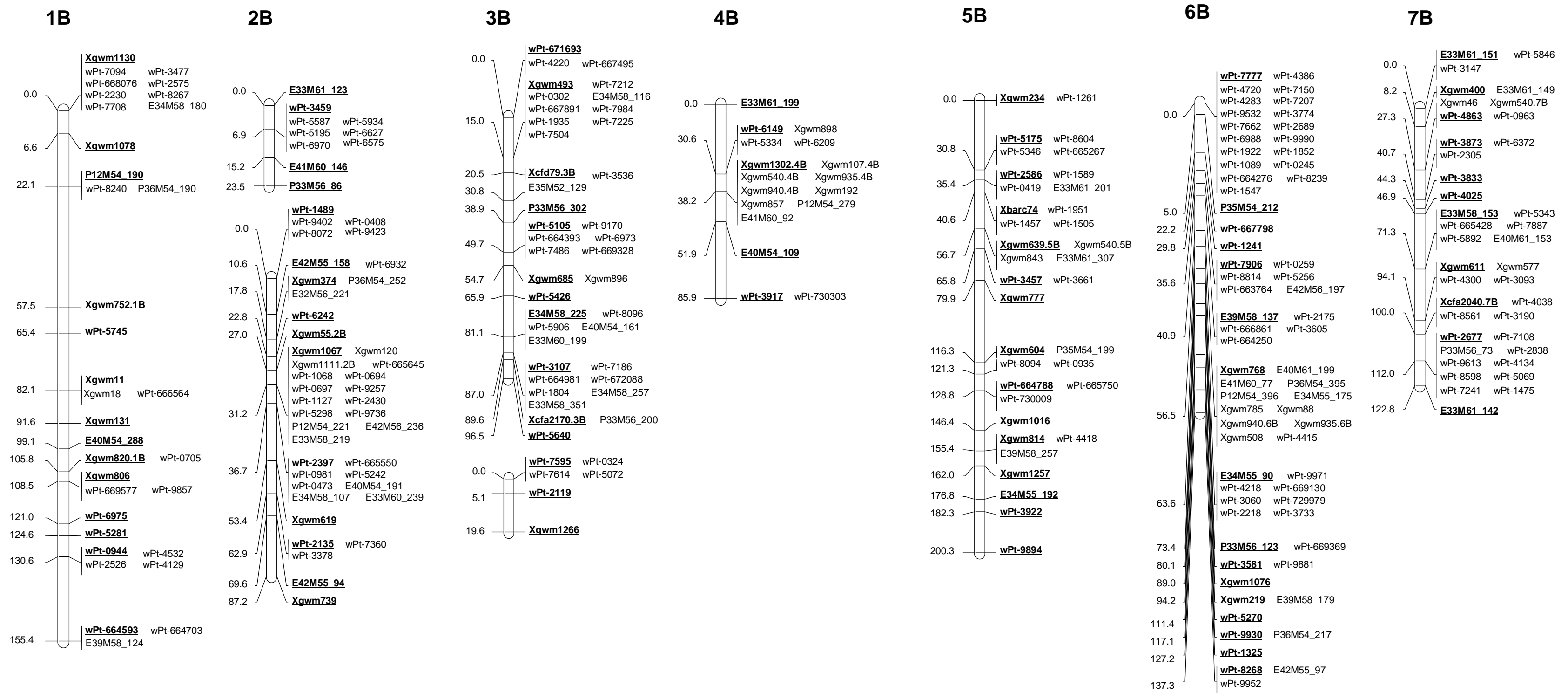

Fig. A1 continued

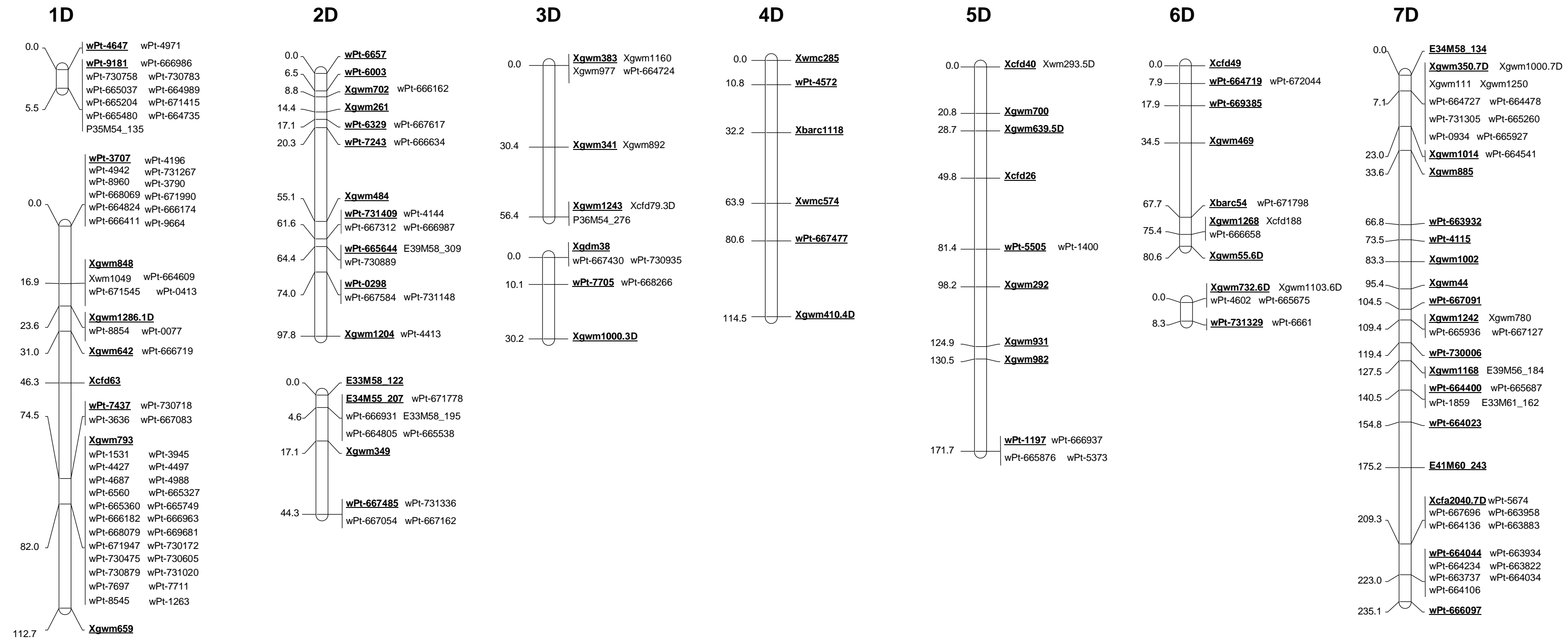

Fig. A1 continued

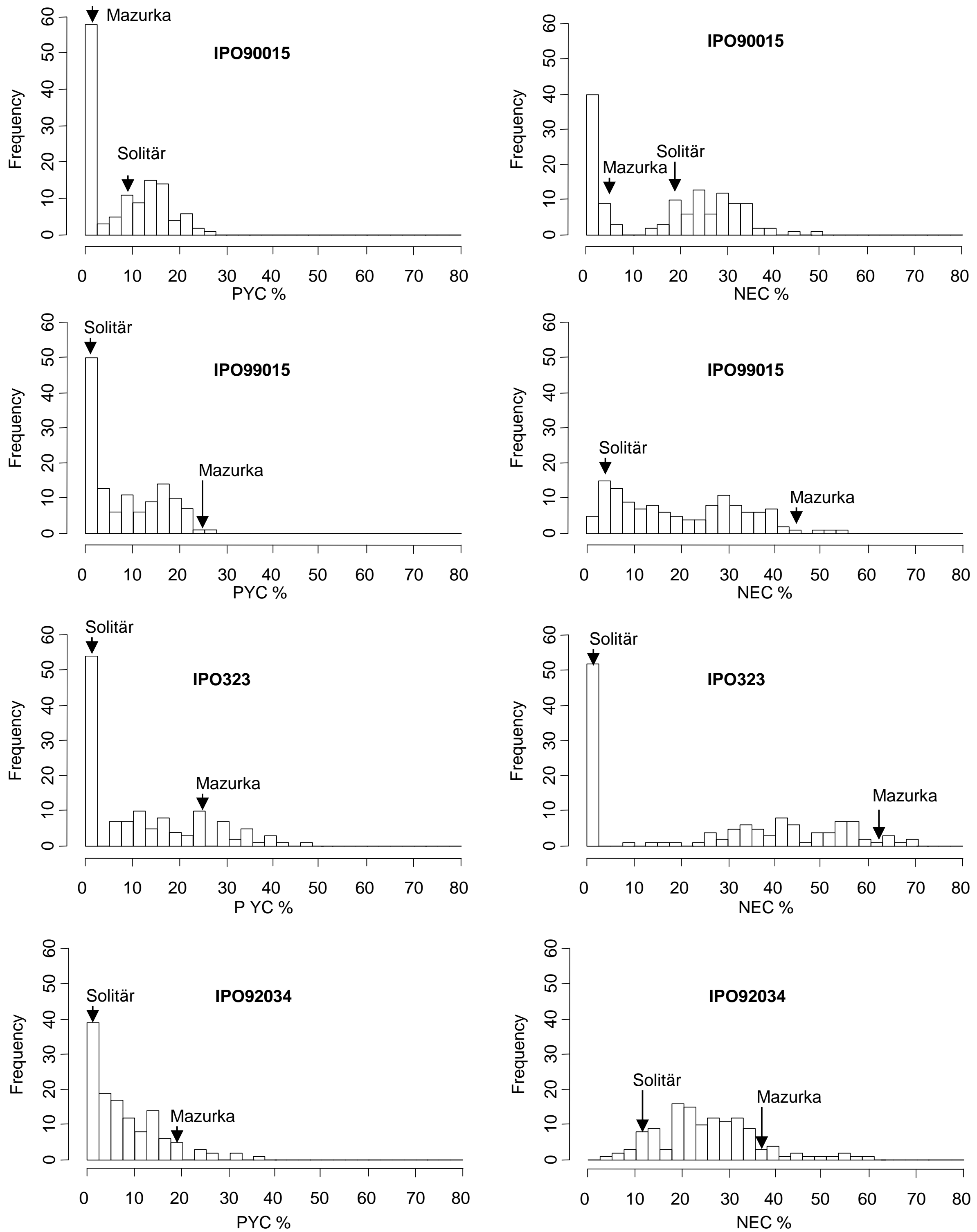

Fig. A2 Phenotypic distributions for mean pycnidial coverage (PYC %) and necrotic leaf area (NEC %) in the seedling stage (mean of 3 replicates) of Solitär, Mazurka and their doubled haploid offspring (IPO isolates: n = 128; Hu1: n=130; Hu2, BBA22: n=131).

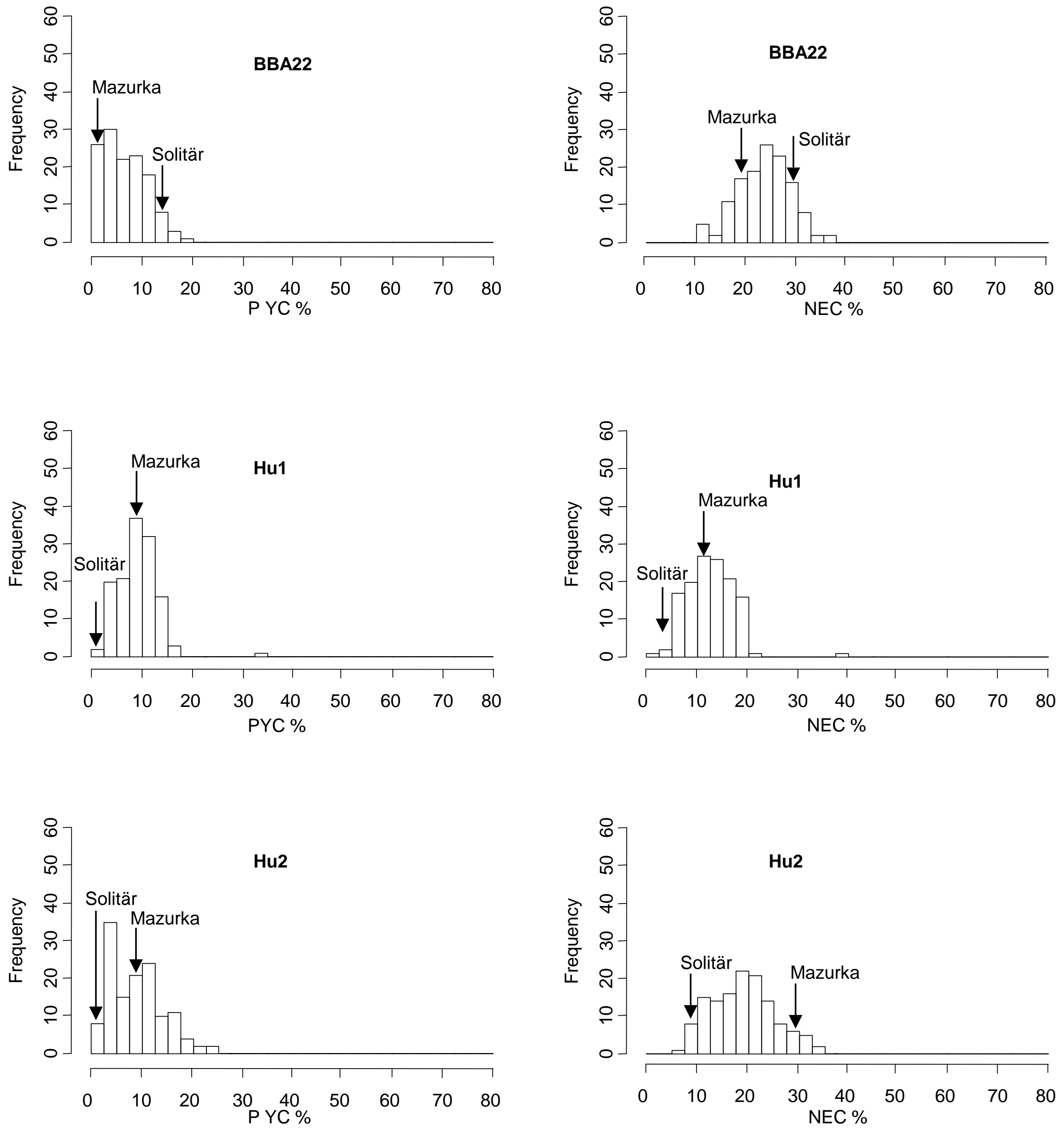

Fig. A2 continued

6 Acknowledgements

I would like to take this opportunity to thank all the persons who have contributed in different aspects to the completion of this thesis. At first I want to thank Prof. W. Eberhard Weber for giving me the chance to start my scientific career. The project he offered me gave me the possibility to develop ideas in different aspects of plant breeding. He guided me in terms of biostatistics and quantitative genetics and taught me to simplify even complex relationships. I am particularly grateful for the assistance of Dr. Bernhard Saal who supervised me in molecular techniques and QTL analysis. With his preciseness in correcting my writing and motivation in biostatistics he helped me to successfully finish my PhD thesis. It was a great luck for me that Dr. Clare Nelson adviced me in the analyses of multienvironmenal QTL during his sabbatical in Halle. I would like to express my appreciation to Dr. Barbara Leithold for her valuable and constructive suggestions during the planning and development of my research work. I would also like to thank the staff of the Chair of Plant breeding in Halle. Mrs. Brigitte Schröder, Mr. Bernd Kollmorgen and Mrs. Helga Sängerlaub supported me with their excellence assistance in my experimental work.

I had a fruitful collaboration with Dr. Gert Kema and Dr. Mahmod Ghaffary of Plant Research International in Wageningen, The Netherlands. Thank you very much for the warm reception in the *Mycosphaerealla* research group and the inspiring scientific discussions. I want to appreciate the cooperation with the IPK in Gatersleben. In the research group “genome and genome mapping” leaded by Mrs. Marion Röder I was able to conduct a great part of my molecular work. I would like to extend my thanks to the staff of all breeding companies I was collaborating with. Namely, Dr. Hilmar Cöster (RAGT2n, Silstedt), Dr. Ebrahim Kazman (SW Seed, Hadmersleben), Dr. Erhard Ebmeyer (KWS-Lochow, Wietze) and Dr. Hubert Kempf (Secobra Saatzucht, Feldkirchen). Only by their sponsorship the field experiments were possible.

Finally I want to thank my parents, my brother and my grandparents for their support and encourage throughout my PhD project. My special thanks go to my husband Sebastian and my lovely daughter Ronja Antonia. With his faith in me Sebastian always kept me grounded and motivated so that I could only finish my PhD thesis with his support.

7 Curriculum Vitae

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Personal Details

Day of birth	06.03.1982
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Professional experience

08/2011 – 07/2013 Research assistant in the Institute for Biological Production Systems at Leibniz University in Hannover

Teaching of B.Sc./ M.Sc. students in yield physiology and international vegetable production (language German/English)

Research focus on: fundraising, association mapping and modeling of supraoptimal temperature stress in barley

Education

10/2009 – 7/2011 Postgraduate scholarship granted by Martin-Luther-University in Halle-Wittenberg with focus on genetic inheritance of yield and quality parameters in winter wheat

10/2005 – 09/2010 Ph.D. student at Martin-Luther-University in Halle-Wittenberg at the chair of plant breeding with focus on quantitative genetics and resistance breeding in winter wheat

Doctoral research study about “Inheritance of resistance to *Septoria tritici* blotch in the winter wheat doubled haploid population Solitär x Mazurka”

Ph. D supervisors: Prof. Dr. W. E. Weber, Dr. B. Saal

10/2000 – 09/2005 Student of Agricultural Science at Martin-Luther-University in Halle-Wittenberg with main focus on plant breeding

Degree dissertation about “Determination of baking quality in biological and conventional winter wheat growing systems”Graduated as agronimist (Dipl. Ing. agr.) with first class

Research periods abroad

4/2008 – 7/2008 DAAD research fellow ship at Plant Research International, Wageningen, The Netherlands, work group of Dr. G. Kema Biointeractions and Plant health; pathogenicity studies in the *Septoria tritici – Triticum aestivum* pathosystem

Skills

Languages English: fluent in writing of scientific papers and contributions to international conferences

Activities and Interests

Assistant coaching of children and adults in traditional Japanese martial arts; gardening

8 List of publications

Kelm C, Ghaffary SMT, Bruelheide H, Röder MS, Miersch S, Weber WE, Kema GHJ, Saal B (2012) The genetic architecture of seedling resistance to *Septoria tritici* blotch in the winter wheat doubled haploid population Solitär x Mazurka. Mol Breeding 29:813-830

Kelm C, Pillen K, Nelson JC, Weber WE, Saal B (2013) Inheritance of field resistance to *Septoria tritici* blotch in the doubled haploid population Solitär x Mazurka. Euphytica 194:161-176

Poster contributions on conferences

Kelm C, Weber WE, Saal B (2010) Multiple QTL mapping of resistance to *Septoria tritici* blotch in a DH population of hexaploid wheat. EUCARPIA Conference 2010, Cambridge, England

Kelm C, Weber WE, Pillen K, Saal B (2010) Relationship between grain size and grain shape in a doubled haploid population of hexaploid wheat. Tagung der Gesellschaft für Pflanzenzüchtung, Giessen, Deutschland

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Kelm C, Saal B, Weber WE (2007) Eignung des Elektrolyt-Leitfähigkeitstests zur indirekten Selektion von Winterweizen auf Frosttoleranz. Vortr. Pflanzenzüchtg.72, 187-190. Tagung der Gesellschaft für Pflanzenbauwissenschaften in Halle, Deutschland

Kelm C, Leithold B, Weber WE (2006) Methoden zur Bestimmung der Backqualität an ausgewählten Winterweizensorten und –stämmen in unterschiedlichen Anbausystemen. Vortr. Pflanzenzüchtg. 68, 4. Tagung der Gesellschaft für Pflanzenzüchtung in Weihenstephan, Deutschland

Oral presentations

Kosellek C (2013) Vererbung der Resistenz gegen *Septoria tritici* in der DH Population Solitär x Mazurka. Tagung der Gesellschaft für Pflanzenzüchtung und der Deutschen Phytomedizinischen Gesellschaft in Fulda, Deutschland, 9.-10.12.2013

Kelm C (2010) Multiple QTL mapping of resistance to *Septoria tritici* blotch in a DH population of hexaploid wheat. Tagung der Gesellschaft für Pflanzenzüchtung, Göttingen, Deutschland

Kelm C (2010) Field resistance to *Septoria tritici* blotch in hexaploid wheat: Mulit-environment QTL mapping using mixed models. Tagung der Gesellschaft für Pflanzenzüchtung, Giessen, Deutschland

Kelm C (2008) Prebreeding program in bread wheat with emphasis on frost tolerance and *Septoria tritici* blotch resistance. Final Meeting, PlantResource II Meeting Szeged, Ungarn *Septoria tritici* blotch resistance. Progress Report, PlantResource II Meeting Szeged, Ungarn

Kelm C (2006) Prebreeding program in bread wheat with emphasis on frost tolerance and *Septoria tritici* blotch resistance. PlantResource II Meeting Gatersleben, Deutschland

Kelm C (2005): Methoden zur Bestimmung der Backqualität an ausgewählten Winterweizensorten und –stämmen in unterschiedlichen Anbausystemen. Mitt. Ges. Pflanzen-bauwiss. 17, 221-222. Tagung der Gesellschaft für Pflanzenbauwissenschaften, Wien, Österreich

9 Eidesstattliche Erklärung / *Declaration under Oath*

Eidesstattliche Erklärung / *Declaration 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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