Efficacy and mapping of resistance to *Mycosphaerella graminicola* in wheat

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Dedicated to my beloved family

Contents

Chapter 1

General Introduction

General Introduction

Cultivated wheat is represented by two species, bread wheat (*Triticum aestivum L.*; 2n= 6x = 42; AABBDD) and durum wheat (*Triticum turgidum* L., 2n= 4x= 28; AABB), and is globally the most important food and feed commodity, ranking fourth among the 20 most important agricultural crops, with an annual production of over 600 million tons (MT) (FAO 2010a. Fig.1). Together, the cereals maize, rice and wheat contribute directly 47% and indirectly - by including animal feed - 50%, to the global human consumption (Tweeten and Thompson 2009). The global average contribution of wheat to the human dietary energy (2794 kcal/capita/day) is estimated at 19% (529 kcal/capita/day), although this varies over regions with the diversity in nutrition habits (FAO 2010b). The nutritional importance of wheat is increasing in Central-West Asia (35- 47% dietary energy per capita) as well as North Africa and Europe (24%) (Fig. 2). The increased demand for wheat in Asia and Africa is due to the strong economic growth since the late 1990s as well as to the international attention for biofuel crops. In addition, limited investigations in infrastructure and technology (particularly irrigation) put more pressure on available land and water, which are two main production factors for agricultural staple crops (Rosegrant 2008). Consequently, the price of wheat increased between 2005 to 2007 by 70%, subsequently decreased in November 2008, but is currently still above the 2005 level (Ivanic and Martin 2008).

Since 1961, wheat production increased globally with almost 300% beyond 600 MT in 2008 on a virtually stable cultivation area of 200 million ha., hence the progress was largely achieved by increased average yields rather than expansion of arable land (FAO 2010c). The global average wheat yield increased from one to three tons per ha., with a parallel expansion of consumption from 400 to 530 kcal/capita/day during the last four decades (Fig. 3), due to human population growth that doubled since 1961 and is projected to triple to nine billion people in 2050 (FAO, 2010e). However, the annual growth rate of global wheat production is below one percent, which eventually cannot meet the global market requirements during the four decades ahead (Fischer et al. 2009; Fischer and Edmeades 2010). Hence, in order to maintain the current global food security, the average yield of all major cereals (wheat, rice and maize) should be higher than five tons per ha. in 2050 (Gilland

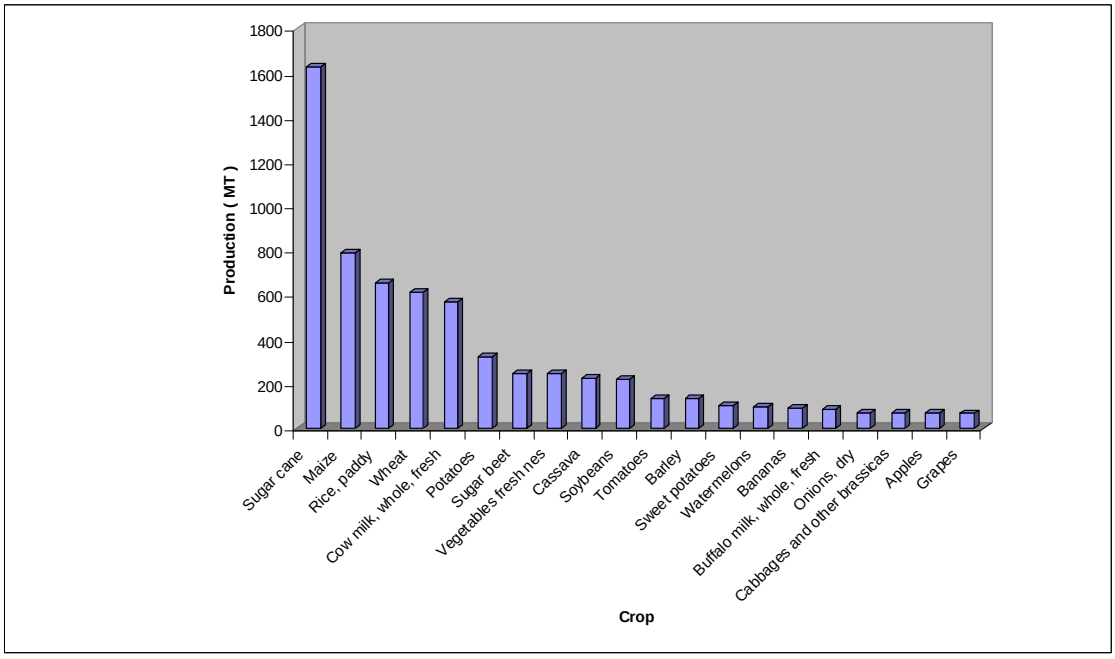

Figure 1. Global production of leading agricultural food and feed crops in 2010 (FAO 2010a).

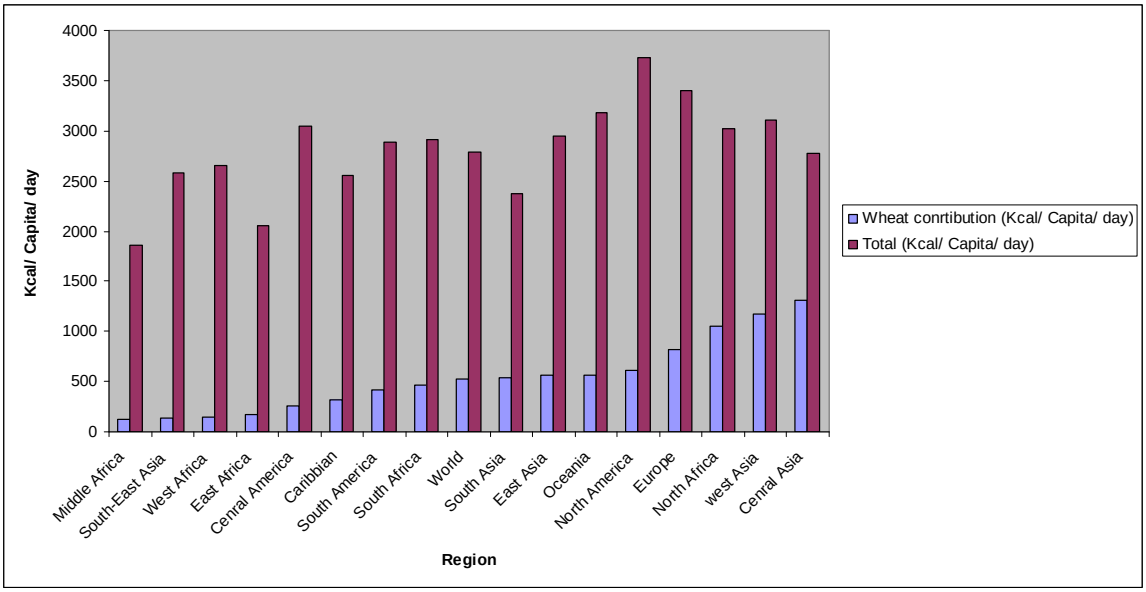

Figure 2. The contribution of wheat to regional human daily dietary demands (FAO 2010b).

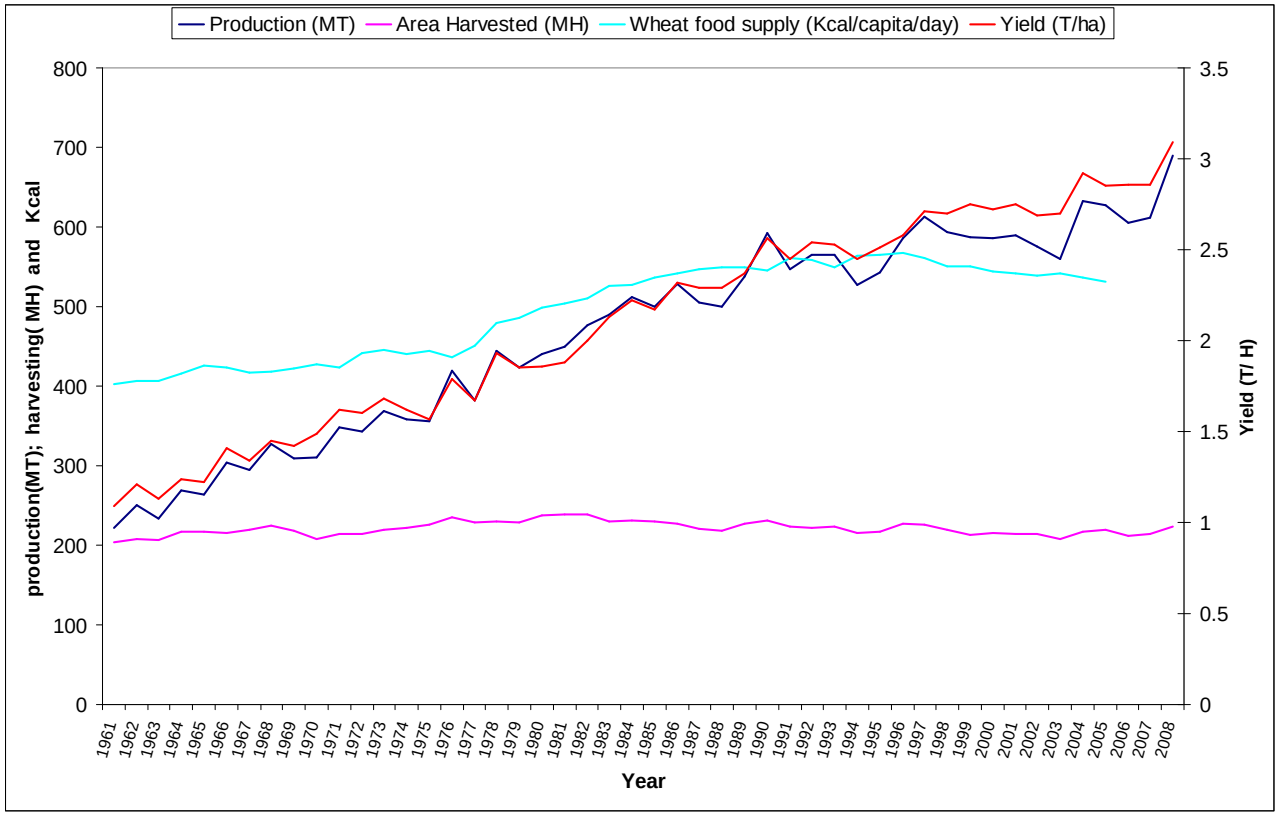

Figure 3. Temporal trend of the harvested wheat area, overall production, yield per hectare and its contribution to global food demands (FAO 2010b and FAO 2010c).

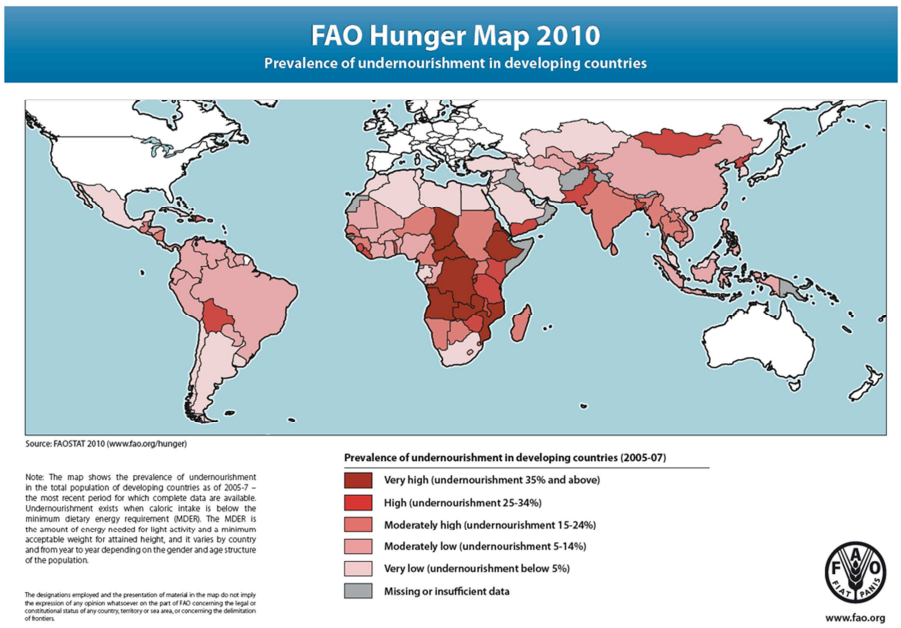

Figure 4. The latest map of global undernourishment (FAO 2010d).

2006). Nevertheless, hunger is presently still a major challenge and FAO estimates that more than 800 million people suffer from malnutrition all over the world (Fig. 4), which is due to variable production potentials, poor distribution and varying dietary energy demands. Therefore, the gap between farmer's yield and attainable yield should be urgently bridged to increase global food production. The generation of cultivars with enhanced resistance to biotic and abiotic stress along with optimized management practices is currently considered to be the best strategy to achieve this goal (Fischer and Edmeades 2010).

Septoria tritici blotch (STB) is one of the most destructive wheat diseases and was first described in Europe by Desmazières (1842) and later by Sprague (1938). The causal agent is the ascomycete *Mycosphaerella graminicola* (Fuckel) J. Schröt, which was observed since 1894, but the connection between this fungus and STB was only discovered almost 80 years later by Sanderson in New Zealand (1972, 1976). The importance of STB increasingly surfaced since the early 1970s, possibly due to a combination of improved genetic control of wheat rusts and the promotion of conservation tillage that supports the over summering of many pathogens, including *M. graminicola* (Forrer and Zadoks 1983; Mergoum et al. 2007; Saari and Wilcoxson 1974; Shipton et al. 1971). Moreover, industrial activities and global climate change also influenced the incidence of *M. graminicola* and *Stagonospora nodorum* (Bearchell et al. 2005; Shaw et al. 2008). Current forecasts project a geographically variable but steady importance of STB (Roos et al. 2010).

M. graminicola has an asexual (Quaedvlieg et al. 2011) as well as a sexual life cycle that is driven by its heterothallic bipolar mating system resulting in splash-dispersed pycnidiospores and airborne ascospore, respectively (Fig. 5). Ascospores are an important source of primary inoculum that is released from wheat debris, whereas disease progress during the growing season is largely driven by the splash-borne pycnidiospores, although ascospores can be formed year round (Eyal 1987; Eyal 1999; Hunter et al. 1999; Kema et al. 1996b; McDonald and Linde 2002; Ponamorenko et al., 2011; Shaw and Royle 1989; Zhan et al. 2007).

Temperature and relative humidity (RH %) have long been considered as the two most critical success factors for *M. graminicola* establishment. A range of temperatures (12-25^0 C) was tested and 22^{0}C was determined as the optimal

temperature for disease development. During incubation, a leaf wetness period of at least 48 hours post inoculation is required for penetration and the initialization of colonization (Chungu et al. 2001; Eyal 1987; Hess and Shaner 1987; Holmes and Colhoun 1974; Kema et al. 1996a; Magboul et al. 1992; Weber 1922). After incubation, the relative humidity should be ≥85% for optimal disease development. In the field, pycnidia exude cyrrhi containing the conidia at a range of different relative humidities, but it is maximized at 100 % and reduced by 50% at 98% (Gough and Lee 1985; Pachinburavan 1981). Daamen and Stol (1992) described a positive correlation between post-harvest (August) sunshine hours and STB incidence in the next year. Shaw et al. (2008) considered that this relationship might be due to reduced reproduction of saprotrophic organisms that leaves more nutrition in the wheat straw for *M. graminicola* pseudothecia development. Currently, greenhouse experiments as well as host-pathogen relationships of related wheat pathogens increasingly indicate that light is a crucial environmental factor for disease development (Carretero et al. 2010; Friesen et al. 2007; Kema et al. 1996c; Manning and Ciuffetti 2005).

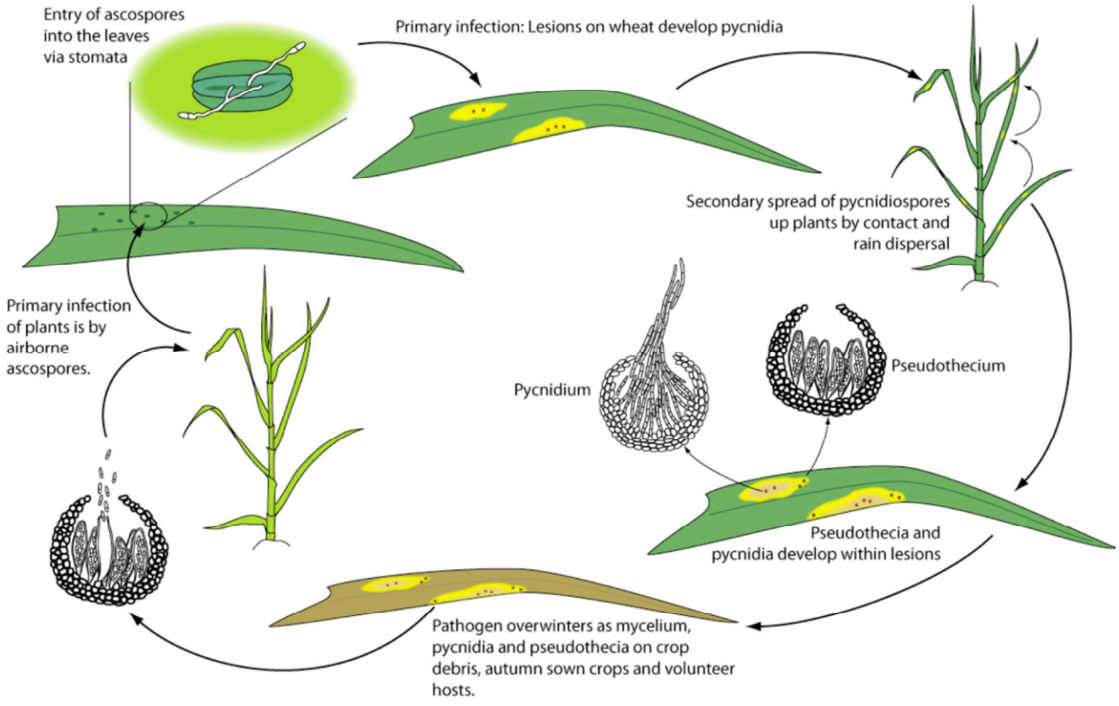

Figure 5. The life cycle of *Mycosphaerella graminicola* on wheat (Ponomarenko et al., 2011).

Suboptimal field conditions do significantly prolong the latency period of *M. graminicola* and hence delay the appearance of disease symptoms, but rarely reduce the damage on susceptible cultivars (Henze et al. 2007; Lovell et al. 2004; Shaw and Royle 1993; Viljanen-Rollinson et al. 2005). The mega-environment classification of the International Maize and Wheat Improvement Center (CIMMYT) (Braun et al. 1996) has identified STB as a main breeding target in at least one third of the total spring wheat growing area of developing countries in Central and Western Asia, North Africa and Latin America (Braun et al. 1996; Duveiller et al. 2007). The incidence of STB on winter wheat is particularly high in moderately to severely cold climates with high rainfall at higher altitudes as well as in Europe, Russia, Australia and New Zealand (Braun et al. 1996; Byerlee and Moya 1993; Heisey et al. 2002; Lantican et al. 2005; Abramova et al. 2008; Daamen and Stol 1992; Eriksen and Munk 2003; Halama 1996; Murray et al. 1990; Pastircak 2005; Polley and Thomas 1991; Royle et al. 1986; Sanderson 1972, 1976; Schnieder et al. 2001; Scott et al. 1988; Shipton et al. 1971).

As mentioned above, both spring and winter cultivars suffer variable yield losses depending on seasonal and regional conditions, cultivar susceptibility, crop history and management (Hardwick et al. 2001; Murray et al. 1990). Linear and exponential regression analysis models showed that yield loss was highly correlated with the STB percentage on the first and second leaf layers at GS 75 in winter wheat (King et al. 1983b). The combined yield penalty of *M . graminicola* and *S. nodorum* was reported to be 35% per year (Jenkins and Morgan 1969). Comparative fungicide experiments under field conditions showed that STB damage alone ranged from 8-18 % in spring wheat and from 10-25 % in winter wheat and can easily increase to 50% during epidemics (Forrer and Zadoks 1983; King et al. 1983a). Total yield losses in England and Wales were estimated at 329 Mt/year during 1985-1989 worth >40 M€ per year (Cook et al. 1991). This was confirmed for the entire UK in 1998, a year with a unique and dramatic disease incidence primarily due to STB (Hardwick et al. 2001). Until now disease management has strongly focused on chemical control, but presently host resistance is also considered a crucial control strategy to minimize STB yield penalties (Loughman and Thomas 1992).

Fungicides have been used for over 200 years to protect small grain cereals, but the demand has significantly increased since the Second World War, due to a

greater variety of crops and fungicide availability (Brent and Hollomon 1995; Morton and Staub 2008). The contemporary annual fungicide trade values over six billion € with a market share of 36% for Europe (Knight and Turner 2009). Initially, the largest market share was on horticultural and vegetable crops, but this changed to barley and later wheat since the 1960s (Russell 2005). The early copper and sulfur-based fungicide formulations had controlled plant disease from the 1940s to the 1980s. These were replaced by sterol demethylation-inhibiting (DMIs) fungicides until the early 1990s (Brent and Hollomon 1995; Fraaije et al. 2003). STB and glume blotch control commenced in 1964 in Western Europe. Over time, STB increased in importance and is currently the main target of the agrochemical and breeding industry (Daamen and Stol 1992; Goodwin et al., 2011; McDougall 2006; Russell 2005). In 1997 Quinine Outside Inhibitors (QoI) were introduced and largely replaced DMIs for STB management. However, contrary to the expectations, resistance rapidly developed and disseminated over Europe (Fraaije et al. 2003; Heaney et al. 2000; Torriani et al. 2009; Ware et al., unpublished). Therefore, STB management is currently virtually entirely azole based (imidazoles and triazoles; DMIs), with imminent risks on resistance development and consequently reduced efficacy of STB control (Cools and Fraaije 2008; Gisi et al. 2005). Integrated pest management programs enabled the development of decision support systems that optimized fungicide applications, thus responding to increasing economic and environmental demands (Bahat et al. 1980; Burke and Dunne 2008; Paveley et al. 1997; Paveley et al. 2001; te Beest et al. 2009; Wiik and Rosenqvist 2010). Currently, national pesticide reduction programs and European legislation further delimit fungicide applications (Epstein and Bassein 2003; Freier and Boller 2009; Gullino and Kuijpers 1994; Ragsdale and Sisler 1994; Sande et al. 2010). This contributed to priority setting for the cereal market with increasing emphasis on the identification and deployment of host resistance to control STB (Angus et al. 2010; Jorgensen et al. 2008; Verreet et al. 2000).

The first genetic study of resistance to STB in wheat was published by Narvaez and Caldwell (Narvaez and Caldwell 1957). Subsequently, resistance genes *Stb1-Stb4* were identified and later mapped (Rillo and Caldwell 1966; Somasco et al. 1996; Wilson 1979, 1985; Adhikari et al. 2004a; Adhikari et al. 2004b; Adhikari et al. 2004c). Arraiano et al. (2001) characterized *Stb5* in a synthetic hexaploid line that

provided broad resistance to at least 12 *M. graminicola* isolates. The discovery of the mating system in *M. graminicola* (Kema et al. 1996b; Waalwijk et al. 2002) resulted in the formal genetic proof of an operational gene-for-gene interaction in the wheat-*M. graminicola* pathosystem. This further enabled the identification of a range of additional *Stb* genes, including *Stb6* (Brading et al. 2002) that is predominant among European wheat cultivars (Arraiano and Brown, 2006). Since 2003 nine additional resistance genes (*Stb7*-*Stb15*) have been characterized and mapped in spring and winter wheat cultivars (Table 1).

Resistance gene *Stb1* originates from the winter wheat cv. Bulgaria 88 and is the first resistance gene that was commercially deployed in cvs. Oasis and Sullivan, providing long- lasting resistance to STB in the Midwest of the United States (Goodwin 2007; Patterson et al. 1975; Patterson et al. 1979). The Brazilian cv. Veranopolis that carries *Stb2* was released in 1950 and was deployed as a progenitor of other wheat cultivars such as cvs. Cotipora, Lagoa-Vermelha, Nova Prata and Vacaria (Kohli and Skovmand 1997; McIntosh R.A. 1991; Prestes and Hendrix 1975; Wilson 1979). The breeding line Israel 493 carries *Stb3*(Wilson 1979), but there is no official report on its commercial deployment (Adhikari et al. 2004a; Goodwin 2007). *Stb4* originates from cv. Tadinia, which is a derivative of a cross between the Dutch cv. Tadorna and Inia 66 and was introduced as a commercial cultivar in 1985 in California with adequate resistance to STB that lasted almost 15 years (Jackson et al. 2000; Somasco et al. 1996). *Stb5* was described in the Chinese Spring/Synthetic hexaploid substitution line of chromosome 7D that presented resistance to 12 of the 13 tested *M. graminicola* isolates (Arraiano et al. 2001), providing a relatively broad resistance that is however, not yet commercially applied. *Stb6* was described in the cvs. Shafir and Flame and was later identified in a range of cultivars suggesting that it is among the most widespread *Stb* genes in contemporary wheat breeding programs (Arraiano and Brown 2006; Brown et al. 2001; Chartrain et al. 2005b; Kema et al. 2000; Kema and van Silfhout 1997). Another predominant gene is *Stb7* that was first identified in the Uruguayan line ST6 that was selected from cv. Estanzuela Federal (McCartney et al. 2003), which is derived from the cross EHRO/CNT8 (GRIPI). *Stb7* is also reported in cvs. KK4500 and TE9111 (Chartrain et al. 2005a; Chartrain et al. 2005c). The International Triticeae Mapping Initiative (ITMI) population is developed from a cross between cv. Opata85 and the synthetic hexaploid derived line W7984,

which carries *Stb8* (Adhikari et al. 2003; Röder et al. 1998). Hence, W7984 has been deployed in the development of marker assisted selection (MAS) programs (Francki et al. 2009; Song et al. 2005; Varshney et al. 2007), but thus far not in commercial wheat breeding for resistance to STB. *Stb9* was discovered in the French winter wheat cv. Courtot as well as the British spring wheat cv. Tonic (Chartrain et al. 2009). The breeding line Kavkaz-K4500 L.6.A.4 (KK4500) was developed at CIMMYT and was derived from winter wheat cvs. Kavkaz and Frontana that originate from Russia and Brazil, respectively (Eyal 1999). It is an important international source of resistance to STB and genetic analysis indicated that it carries *Stb6, Stb7, Stb10* and *Stb12* (Chartrain et al. 2005a), suggesting that gene pyramiding is an effective strategy for STB resistance breeding. Brown et al. (2001) studied STB resistance in the Portuguese line TE9111 and concluded that it carries resistance genes *Stb11, Stb7* and *Stb6* (Chartrain et al. 2005c). *Stb13* and *Stb14* are described in cv. Salamouni (USDA-Annual wheat newsletter volume 53) and *Stb15* was reported in the Swiss cv. Arina and could also be present in the British cv. Riband (Arraiano et al. 2007).

Unfortunately, the efficacy of the above mentioned *Stb* genes (Table 1) is generally narrow (This thesis, Chapter two). Compared to the number of resistance genes that has been identified to yellow rust (88), leaf rust (96), stem rust (64), hessian fly(33) and powdery mildew (104) (Komugi, 2011) this is a very limited arsenal for ongoing breeding programs. It is therefore prudent to explore more wheat germplasm in order to identify new genes for resistance to STB and to provide breeders with up to date tools for the incorporation of these genes in commercial breeding programs.

Scope of the thesis

The aim of the research presented in this thesis was to identify and characterize new genes for resistance to STB and to identify linked molecular markers that will facilitate the introgression of the associated *Stb* genes.

In **Chapter 2** the genetic diversity in *Mycosphaerella graminicola* isolates from a wide and diverse origin is described based on phenotyping assays as well as SSR genotyping. Screening of these isolates on a wide range of wheat cultivars

Table 1. Genes for resistance to septoria tritici blotch (*Stb*) of wheat that have been reported in winter and spring wheat cultivars along with their chromosomal positions and associated molecular markers.

Stb genes	Cultivars source	Chromosomal position	Closest(Flanking) marker	Reference
Stb1	Bulgarai 88[1]	5BL	*Xgwm335*	(Adhikari et al. 2004d)
Stb2	Veranopolis[1]	3Bs	*Xgwm389*	(Adhikari et al. 2004c)
Stb3	Israel 493[1]	7As	Not published yet	(Goodwin 2007)
Stb4	Tadinia[1]	7Ds	*Xgwm111*	(Adhikari et al. 2004b)
Stb5	Cs Synthetic 6X (7D)[1]	7Ds	*Xgwm44*	(Arraiano et al. 2001)
Stb6	Shafir	3As	*Xgwm369*	(Brading et al. 2002)
Stb7	Estanzuela Federal	4AL	*Xwmc313; Xwmc219*	(McCartney et al. 2003)
Stb8	W7984	7BL	*Xgwm146; Xgwm577*	(Adhikari et al. 2003)
Stb9	Courtot	2B	*XksuF1; Xfbb226*	(Chartrain et al. 2009)
Stb10	KK4500 [2]	1D	*Xgwm603; Xgwm458*	(Chartrain et al. 2005a)
Stb11	TE9111 [2]	1Bs	*Xbarc008*	(Chartrain et al. 2005c)
Stb12	KK4500 [2]	4AL	*Xwmc313; Xwmc219*	(Chartrain et al. 2005a)
Stb13	Salamouni	7BL	*Xwmc396*	USDA-Annual wheat newsletter volume 53
Stb14	Salamouni	3Bs	*Xwmc500*	USDA-Annual wheat newsletter volume 53
Stb15	Arina [1]	6As	*Xpsr904*	(Arraiano et al. 2007)

[1]These lines also carry *Stb6*

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

enabled the identification of specific *M. graminicola* isolates that are particularly useful in preliminary *Stb* gene postulations in breeders' germplasm, both in the seedling as well as adult plant stage. These analyses also showed that many of the described *Stb* genes have a limited efficacy in Europe, which underscores the necessity to extend the number of genes for practical breeding in both bread and durum wheat. The confirmation of the phenotypic dichotomy of STB on bread and durum wheat necessitates the application of separate *M. graminicola* isolate panels for these wheat species for detailed characterization of resistance.

Subsequently, genetic analyses – using the well characterized *M. graminicola* strains described in **Chapter 2** - of several recombinant inbreed lines (RILs) and double haploid (DH) populations are described in **Chapters 3, 4, and 5**.

The focus of **Chapter 3** is on synthetic hexaploid wheats (SHs), which are a rich source of new *Stb* resistance genes with an unusual wide efficacy towards broad panels of *M. graminicola* isolates. Analyses of a RIL population derived from the cross between the SH M3 and the highly susceptible bread wheat cv. Kulm revealed two novel resistance loci on chromosomes 3DL and 5AL that explain over 63 of the observed phenotypic variation at 28 days post inoculation in adult plant stage. The 3DL resistance was designated as *Stb16* and is expressed in the seedling and adult plant stages. The resistance locus on chromosome 5AL, designated as *Stb17*, was specifically expressed at the adult plant stage.

Chapter 4 described the genetic analysis of STB resistance in the French commercial wheat cvs. Apache and Balance. Five *M. graminicola* isolates were used to detect four QTLs on chromosomes 3AS, 1BS, 6DS and 7D (7DS/7DL switch) in seedlings and one QTL on 2DS in the adult plant stage. The QTL on chromosome 6DS is a novel QTL that was designated *Stb18.* Since known and new *Stb* genes segregated in the Apache/Balance DH population, the interaction between these genes could be studied with the applied *M. graminicola* isolates. Epistatic and additive effects were prominent and resulted in various levels of explained variation that significantly varied over *M. graminicola* isolates. Nevertheless, pyramiding of *Stb* genes generally contributes to a wider efficacy towards a broader range of isolates. The 2DS QTL that was discovered in adult plant field experiments is most likely a

major genetic component in the regulation of earliness and tallness and therefore indirectly contributes to STB resistance.

Chapter 5 describes the genetic analysis of resistance to STB in the German cvs. Solitär and cv. Mazurka. Seven *M. graminicola* isolates were used and enabled the identification major effect QTLs on chromosomes 3AS, 1BS and 4AL and minor effect QTLs on chromosomes 1B, 3D, 6B and 7D that were contributed by both parental cultivars. The major QTLs on chromosomes 3AS, 1BS and 4AL were tightly linked to the positions where *Stb6*, *Stb11* and *Stb7+12* have been reported. Two specific QTLs controlling necrosis were detected on chromosomes 1A and 3B. Epistatic effects have reliably been detected, but contributed less to the total variance. Altogether, seedling analyses showed a complex inheritance of resistance to STB with regard to isolate-specificity and resistance mechanisms, which complicates marker assisted deployment of these genes.

Chapter 6 eventually puts the results of chapters 2-5 in a broader context and provides a critical review of past methodologies and the current alternatives that provide a higher resolution and better characterization of STB resistance. Furthermore, the chapter anticipates on improved phenotyping protocols to stabilize data generation that will contribute to enhanced genotyping and mapping analyses and hence to successful commercial deployment of *Stb* genes.

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Chapter 2

Challenges of phenotyping and gene postulations in the wheat-*Mycosphaerella graminicola* pathosystem

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Abstract

The ascomycete *Mycosphaerella graminicola* causes the foliar disease septoria tritici blotch (STB), which is currently the most important wheat disease in Europe with potential yield losses of up to 50% under conducive conditions. Fungicide application and host resistance are the two major disease management strategies to control STB. The occurrence of fungicide resistance and the implementation of pesticide reduction programs have resulted in an increased focus on host resistance. To date 15 major resistance genes have been identified and mapped using different phenotyping methods. In this research we screened a set of 94 cultivars, landraces and breeding lines including a differential set of cultivars carrying the mapped *Stb* genes, with a wide range of 50 European and global *M. graminicola* isolates in three seedling experiments and used a subset in a comparative field trial. This delivered pathogenicity characteristics – both necrosis development and the success of asexual fructification - of the *M. graminicola* isolates that can be further deployed in forthcoming host and fungal genetic studies. Furthermore, it showed the wide diversity of host resistance in the tested germplasm. The data enabled *Stb* gene postulations – with a prevalence of *Stb6, Stb8, Stb4* and *Stb2* in French breeding lines - and identified new sources of resistance to STB that can be readily applied in commercial breeding programs. Resistance gene *Stb5*, present in the wheat line Cs/ Synthetic 7D, was the most effective against the European *M. graminicola* isolates and provided a substantial level of resistance to the global set of isolates. Cultivar Arina that carries *Stb6+15* was the most resistant line to the global set of isolates. Conversely, *Stb9*, present in the French cv. Courtot, and *Stb6*, were susceptible to the majority of isolates. Comparative seedling and adult plant experiments showed that resistance genes expression depends on the physiological stage of the wheat plant. Many resistances were specific to the seedling stage and fewer were specific to the adult plant stage. All *M. graminicola* isolates were genotyped with SSR markers and represented unique genotypes, except for two isolates from a field in Northern France. Accompanying phenotypic data from hierarchically sampled isolates from five French wheat fields confirmed a distribution of pathogenicity at a fine spatial scale with multiple significantly different strains among and within wheat field and even within the same spot in such wheat field.

Introduction

Septoria tritici blotch (STB) is caused by *Mycosphaerella graminicola* (Fuckel) J.Schröt (Sanderson, 1976), and is one of the most devastating foliar wheat diseases since its discovery in France (Desmazieres, 1842, Sprague, 1938). The ascomycete *M. graminicola* was already observed in 1894, but was not recognized as the *Septoria tritici* (Crous et al., 2001) teleomorph until the 1970s in New Zealand (Sanderson, 1972, Sanderson, 1976). Intensive worldwide wheat cropping using susceptible cultivars, lack of rotation and minimum tillage practices as well as global climate change increased the incidence and severity of STB epidemics (Bearchell et al., 2005, Eyal, 1999, Fraaije et al., 2005, Mergoum et al., 2007). Generally, STB driven yield losses range between 8-25%, but easily can reach 50% under conducive conditions (Forrer & Zadoks, 1983, King et al., 1983). STB control is traditionally accomplished by fungicide applications that cost between 12-58 € ha^{-1}, depending on cultivar susceptibility and STB severity (Jorgensen, 2008, Wiik & Rosenqvist, 2010, Te Beest et al., 2009). However, fungicide resistance development in *M. graminicola* populations is a great concern (Fraaije et al., 2005, Mavroeidi & Shaw, 2005, Stergiopoulos et al., 2003, Torriani et al., 2009, Jorgensen et al., 2010, EPPO, 2010).

M. graminicola has a heterothallic bipolar mating system that is characterized by two *mat* alleles at a single locus (Kema et al., 1996c). Sexual reproduction results from cellular interactions between two pathogen strains with opposite mating types leading to a transient diploid phase enabling genetic recombination that is presented in the progeny (Coppin et al., 1997). *M. graminicola* continually completes sexual cycles, depending on weather conditions, that each take five to seven weeks and results in complex natural populations with extensive genetic variation (Kema et al., 1996c, McDonald et al., 1996). However, genotypes are short-lived in *M. graminicola* populations due to the concatenation of sexual cycles (Wittenberg et al., 2009; Goodwin et al., 2011), enabling the fungus to adapt to adverse conditions as exemplified by the rapid development of fungicide resistance (Gisi et al., 2000, Gisi et al., 2002, Torriani et al., 2009, Ware, 2006). Linde et al. (2002), therefore designate *M. graminicola* as a pathogen that poses a significant threat on crop production due to its lifestyle. Nevertheless, fungicide applications and breeding for resistance are still, rather than cultural methods, the major STB disease management strategies

(McDonald & Linde, 2002, Loughman & Thomas, 1992; Mergoum et al., 2007, Jing et al., 2008).

In recent years, 18 major resistance genes and QTLs, *Stb1-Stb18*, were identified (Arraiano et al., 2007, Chartrain et al., 2009, Goodwin, 2007, Tabib Ghaffary et al., 2011a, 2011b). However, the majority has been poorly deployed in breeding programs, partly due to their low efficacy. In addition, the number of available *Stb* genes for practical breeding programs is low compared to other wheat diseases and pests such as the rusts, powdery mildew and Hessian fly (Komugi, 2011). It is therefore necessary to invest in gene discovery by screening programs using state of the art phenotyping protocols that exploit existing genetic variation in *M. graminicola.*

In this study we summarize several extensive studies where we genotyped and phenotyped 50 *M. graminicola* isolates from 14 different countries in four continents on 94 wheat cultivars in three seedling experiments and one adult plant field experiment. This contributed to new *Stb* gene discovery and resulted in new tools for an improved understanding of the wheat – *M. graminicola* pathosystem.

Material and Methods

Wheat cultivars and M. graminicola isolates

In total 94 cultivars, breeding lines and landraces including 13 differential wheat cultivars/lines, carrying 15 *Stb* genes (Table 1 and 2), were tested in three independent seedling experiments over the period 1999-2008. The first experiment (EXP1) comprised a set of 50 breeding lines and cultivars, including cvs. Bulgaria, Veranopolis, Shafir and Tadinia that at the time had reported resistance genes, and which were studied with 30 isolates (Table 3). The French isolates were obtained from hierarchically sampled leaves from five individual wheat fields in five geographically different regions (Appendices, Fig. S1). The entire French panel of isolates was later used to test a suite of cultivars in which *Stb* genes were mapped using well-characterized isolates after a gene-for-gene relationship between *M. graminicola* and wheat was described (Brading et al., 2002, Arraiano et al., 2007, Chartrain et al., 2009, Goodwin, 2007) (EXP2). A subset of eight isolates was used to verify seedling

Table 1. Differential set of cultivars carrying mapped genes for resistance to septoria tritici blotch (*Stb*) of wheat that have been reported in winter and spring wheat cultivars.

	Stb1	*Stb2*	*Stb3*	*Stb4*	*Stb5*	*Stb6*	*Stb7*	*Stb8*	*Stb9*	*Stb10*	*Stb11*	*Stb12*	*Stb13*	*Stb14*	*Stb15*	*Stb16*	*Stb17*	*Stb18*	Reference
Bulgaria 88[1]	x					x													Adhikari et al., 2004c
Veranopolis[1]		x				x													Adhikari et al., 2004b
Israel 493[1]			x			x													Adhikari et al., 2004b
Tadinia[1]				x		x													Somasco et al., 1996; Adhikari et al., 2004a
CS/synthetic(6x) 7D					x														Arraiano et al., 2001
Shafir						x													Brading et al., 2002
Estanzuela Federal							x												McCartney et al., 2003
M6 Synth(w7984)								x											Adhikari et al., 2003
Courtot									x										Chartrain et al., not published
Kavkaz - K4500[2]						x	x			x		x							Chartrain et al., 2005a
TE9111[2]						x	x				x								Chartrain et al., 2005c
Salamouni													x	x					USDA-Annual wheat newsletter volume 53
Arina[1]						x									x				Arraiano et al., 2007;
M3 (Synthetic)																x	x		Tabib Ghaffary et al., 2011a
Balance						x												x	Tabib Ghaffary et al., 2011b

[1] These lines also carry *Stb6* (Chartrain et al.,2005b)

[2] These lines also carry *Stb6* and *Stb7* (Chartrain et al., 2005a; Chartrain et al., 2005c)

Table 2. List of used wheat germplasm, with their origin and characteristics, in seedling and adult plant experiments.

						Seedling Trials[4]			Adult Trial[4]
Wheat line	Code label	Ploidy[1]	Growing type[2]	Breeding type[3]	Origin	EXP1	EXP2	EXP3	EXP4
Bulgaria 88	Bulgaria	H	W	BL	Bulgaria	+	+	+	
Veranopolis	Veranopolis	H	S	CV	Brazil	+	+	+	
Israel 493	ISR493	H	S	BL	Israel		+	+	
Tadinia	Tadinia	H	S	CV	USA	+	+	+	
CS/synthetic(6x) 7D	CS/Syn 7D	H	S	BL	USA		+	+	
Shafir	Shafir	H	S	CV	Israel	+	+	+	
Estanzuela Federal	E. Federal	H	S	CV	Uruguay		+	+	
M6 Synth(w7984)	W7984	H	w	BL	USA		+	+	
Courtot	Courtot	H	W	CV	France		+	+	
Kavkaz - K4500	KK4500	H	W	BL	CIMMYT		+	+	
TE9111	TE9111	H	S	BL	Portugal		+	+	
Salamouni	Salamouni	H	S	CV	Lebanon			+	
Arina	Arina	H	W	CV	Switzerland			+	
Taichung 29[5]	T29	H	S	LR	Japan	+	+	+	
00/st/01	SE1	H	W	BL	France	+			+
00/st/02	SE2	H	W	BL	France	+			+
00/st/03	SE3	H	W	BL	France	+		+	+
00/st/04	SE4	H	W	BL	France	+			+
00/st/05	SE5	H	W	BL	France	+			+

Table 2. List of used wheat germplasm, with their origin and characteristics, in seedling and adult plant experiments.

						Seedling Trials[4]			Adult Trial[4]
Wheat line	Code label	Ploidy[1]	Growing type[2]	Breeding type[3]	Origin	EXP1	EXP2	EXP3	EXP4
00/st/06	SE6	H	W	BL	France	+			
00/st/07	SE7	H	W	BL	France	+			+
00/st/08	SE8	H	W	BL	France	+			
00/st/09	SE9	H	W	BL	France	+			
00/st/10	SE10	H	W	BL	France	+			
00/st/11	SE11	H	W	BL	France	+		+	
00/st/12	SE12	H	W	BL	France	+			
00/st/13	SE13	H	W	BL	France	+			+
00/st/14	SE14	H	W	BL	France	+			+
00/st/15	SE15	H	W	BL	France	+			+
00/st/16	SE16	H	W	BL	France	+			
00/st/17	SE17	H	W	BL	France	+			
00/st/18	SE18	H	W	BL	France	+			+
00/st/19	SE19	H	W	BL	France	+			+
00/st/20	SE20	H	W	BL	France	+			+
FD NL 01	FD1	H	W	BL	France	+			
FD NL 02	FD2	H	W	BL	France	+			+
FD NL 03	FD3	H	W	BL	France	+		+	+
FD NL 04	FD4	H	W	BL	France	+			

Table 2. List of used wheat germplasm, with their origin and characteristics, in seedling and adult plant experiments.

						Seedling Trials[4]			Adult Trial[4]
Wheat line	Code label	Ploidy[1]	Growing type[2]	Breeding type[3]	Origin	EXP1	EXP2	EXP3	EXP4
FD NL 05	FD5	H	W	BL	France	+			+
FD NL 06	FD6	H	W	BL	France	+			+
FD NL 07	FD7	H	W	BL	France	+			+
FD NL 08	FD8	H	W	BL	France	+			
FD NL 09	FD9	H	W	BL	France	+			
FD NL 10	FD10	H	W	BL	France	+			+
FD NL 11	FD11	H	W	BL	France	+			+
FD NL 12	FD12	H	W	BL	France	+		+	
FD NL 13	FD13	H	W	BL	France	+			
FD NL 14	FD14	H	W	BL	France	+			+
FD NL 15	FD15	H	W	BL	France	+			
FD NL 16	FD16	H	W	BL	France	+			
FD NL 17	FD17	H	W	BL	France	+			
FD NL 18	FD18	H	W	BL	France	+			+
FD NL 19	FD19	H	W	BL	France	+			+
FD NL 20	FD20	H	W	BL	France	+			+
Triticum polonicum	*T. polonicum*	T	W	WT		+			
Iassul20	Iassul20	H	S	BL	Italy	+			
Olaf	Olaf	H	S	CV	USA	+			

Table 2. List of used wheat germplasm, with their origin and characteristics, in seedling and adult plant experiments.

						Seedling Trials[4]			Adult Trial[4]
Wheat line	Code label	Ploidy[1]	Growing type[2]	Breeding type[3]	Origin	EXP1	EXP2	EXP3	EXP4
Kavkaz	Kavkaz	H	W	CV	Russia	+			
Erik	Erik	H	S	CV	USA			+	
Kulm	Kulm	H	S	CV	USA			+	
M3	M3	H	S	BL	CIMMYT			+	
Chinese Spring	CS	H	S	LR	China			+	
Largo	Largo	H	S	BL	USA			+	
ND495	ND495	H	S	BL	USA			+	
TA 4152-37	TA4152-37	H	S	BL	CIMMYT			+	
TA 4152-19	TA4152-19	H	S	BL	CIMMYT			+	
TA 4152-60	TA4152-60	H	S	BL	CIMMYT			+	
BR34	BR34	H	S	CV	Brazil			+	
Grandin	Grandin	H	S	CV	USA			+	
Katepwa	Katepwa	H	S	CV	Canada			+	
Altar84	Altar 84	T	S	CV	CIMMYT			+	
Ben	Ben	T	S	CV	USA			+	
T. dicoccoïdes (TA106)	*T. dic.* TA106	T	S	WT	Middle East			+	
T. dicoccoïdes IsraelA	*T. dic.* ISR A	T	S	WT	Middle East			+	
T. dicoccoïdes (PI 478742)	*T. dic.* (PI 478742)	T	S	WT	Middle East			+	
T. dicoccoïdes (PI 481521)	*T. dic.* (PI 481521)	T	S	WT	Middle East			+	

Table 2. List of used wheat germplasm, with their origin and characteristics, in seedling and adult plant experiments.

						Seedling Trials[4]			Adult Trial[4]
Wheat line	Code label	Ploidy[1]	Growing type[2]	Breeding type[3]	Origin	EXP1	EXP2	EXP3	EXP4
T. dicoccoïdes (PI 41025)	*T. dic.* (PI 41025)	T	S	WI	Middle East			+	
Solitär[6]	Solitär	H	W	CV	Germany			+	
Mazurka	Mazurka	H	W	CV	Hungry			+	
Wangshuibai	Wangshuibai	H	S	LR	China			+	
Falat (Seri82)	Falat	H	S	CV	CIMMYT			+	
Frontana	Frontana	H	S	CV	Brazil	+		+	
Sumai-3	Sumai-3	H	S	CV	China			+	
Florett	Florett	H	W	CV	Germany			+	
Tuareg	Tuareg	H	W	CV	Germany			+	
Biscay	Biscay	H	W	CV	Germany			+	
Nogal	FD02112	H	W	BL	France			+	
02CY 399	02CY 399	H	W	BL	CIMMYT			+	
FHD 2054.3	FHD 2054.3	H	W	BL	France			+	
Bio2000	Bio2000	H	W	BL	France			+	
Sankara	Sankara	H	W	CV	France			+	
Apache	Apache	H	W	CV	France			+	
Balance	Balance	H	W	CV	France			+	

[1]H for hexaploid; T for tetraploids; [2]S for spring wheat, W for winter wheat; [3]cv. for cultivar; BL for breeding line; LR for landrace and WT for wild type; [4]used in that particular experiment; [5]Susceptible check; [6]not identical with British cv. Solitaire

data in field trials (EXP4, Tables 1-2). Finally, we tested a broad selection of *M. graminicola* isolates on eight durum wheat and 46 bread wheat cultivars, breeding lines, landraces as well as 13 cultivars carrying *Stb1-Stb15* (EXP3).

Phenotyping - experimental design, pre- and post-inoculation growth conditions, data collection and analysis

Essentially all experiments were performed according to a split plot design with two or three replicates. Main plots are rows of plots and subplots are the plots within rows. Main plot treatments are isolates and cultivars are subplot treatments. For a first impression of the incidence of P in EXP1-4, two-way tables of isolate by cultivar means sorted to ascending marginal means have been calculated. Percentage data Y were logistically transformed (i.e. Z=ln(Y/(100-Y)) (and 0.5 and 99.5 were taken to accommodate for Y=0 and Y=100 respectively) prior to analysis. The logistic transformed data Z were analyzed with a mixed model analysis of variance model

Z= systematic part +random part

Where the *systematic part* refers to fixed effects of isolate and cultivar and their interaction, whereas the *random part* refers to random effects of replicate, main plots within replicate, plots within main plots. In the 2001 adult plant and seedling experiment the interaction replicate x cultivar was found not to be significant and analysis was done using the model without replication cultivar interaction The mixed models were analyzed by restricted maximum likelihood (REML) (Searle et al., 1992). Approximate F-tests according to Kenward & Rogers (1997) were used to test for main effects of isolate and cultivar and the interaction of isolate and cultivar. In case the denominator of the F-distribution could not be calculated, fixed effects were tested by computing Wald statistics and comparing these with chi-square distributions, ignoring variability in the estimated variance components. In case of significant cultivar by isolate interaction the agglomerative hierarchical clustering procedure (Corsten & Denis, 1990) and implemented in the GenStat procedure CINTERACTION was used for identifying simultaneously groups of isolates and groups of cultivars in the two way table of isolate by cultivar predicted means on the logistic scale, such that interaction is due to interaction between those groups. The clustering procedure assumes independently distributed means with constant variance.

Table 3. List of *Mycosphaerella graminicola* isolates, their origin and in which seedling and adult plant experiments they were used.

	Origin					Seedling Trials			Adult Trials
Isolate[2]	Country	Sampling field location	Spot[1]	Leaf[1]	Code label	EXP1	EXP2	EXP3	EXP4
IPO323	Netherlands	W.Brabant			IPO323-NLD	+	+		+
IPO94269	Netherlands	Kraggenburg			IPO94269-NLD	+	+		
IPO98031	France	Aire D'Havrincourt	1	1	98031-ADH	+	+		
IPO98047	France	Aire D'Havrincourt	1	1	98047-ADH	+	+		
IPO98094	France	Aire D'Havrincourt	2	1	98094-ADH	+	+		
IPO98097	France	Aire D'Havrincourt	2	1	98097-ADH	+	+		
IPO98099	France	Aire D'Havrincourt	3	1	98099-ADH	+	+		
IPO98113	France	Aire D'Havrincourt	4	5	98113-ADH	+	+		+
IPO99018	France	Beauce			99018-BEA	+	+		
IPO99031	France	Beauce			99031-BEA	+	+		
IPO99032	France	Beauce			99032-BEA	+	+		
IPO99038	France	Beauce			99038-BEA	+	+		+
IPO99042	France	Beauce			99042-F	+			+
IPO99048	France	Beauce			99048-BEA	+	+		
IPO98032	France	Capelle-en-Pévèlle	1	1	98032-CEP	+	+		
IPO98033	France	Capelle-en-Pévèlle	1	1	98033-CEP	+	+		
IPO98034	France	Capelle-en-Pévèlle	1	4	98034-CEP	+	+		
IPO98035	France	Capelle-en-Pévèlle	1	3	98035-CEP	+	+		

Table 3. List of *Mycosphaerella graminicola* isolates, their origin and in which seedling and adult plant experiments they were used.

	Origin					Seedling Trials			Adult Trials
Isolate[2]	Country	Sampling field location	Spot[1]	Leaf[1]	Code label	EXP1	EXP2	EXP3	EXP4
IPO98028	France	St. Pol de Léon	1	1	98028-SPL	+	+		
IPO98038	France	St. Pol de Léon	2	1	98038-SPL	+	+		
IPO98046	France	St. Pol de Léon	2	2	98046-SPL	+	+		
IPO98050	France	St. Pol de Léon	3	1	98050-SPL	+	+		
IPO98075	France	St. Pol de Léon	1	2	98075-SPL	+	+		+
IPO98078	France	St. Pol de Léon	4	1	98078-SPL	+	+		
IPO98001	France	Villaines la Gonais	1	1	98001-VLG	+	+		+
IPO98021	France	Villaines la Gonais	1	1	98021-VLG	+	+		+
IPO98022	France	Villaines la Gonais	3	2	98022-VLG	+	+		
IPO98051	France	Villaines la Gonais	2	1	98051-VLG	+	+		
IPO98057	France	Villaines la Gonais	2	2	98057-VLG	+	+		
IPO98072	France	Villaines la Gonais	4	1	98072-VLG	+	+		
IPO95054	Algeria	Berrahal			95054-ALG			+	
IPO92034	Algeria	Guelma			92034-ALG			+	
IPO86068	Argentina	Balcarce			86068-ARG			+	
IPO99015	Argentina	*Unknown*			99015-ARG			+	
IPO94218	Canada	Saskatoon			94218-CAN			+	
IPO88018	Ethiopia	Holetta			88018-ETH			+	

Table 3. List of *Mycosphaerella graminicola* isolates, their origin and in which seedling and adult plant experiments they were used.

	Origin					Seedling Trials			Adult Trials
Isolate[2]	Country	Sampling field location	Spot[1]	Leaf[1]	Code label	EXP1	EXP2	EXP3	EXP4
IPO88004	Ethiopia	Kulumsa			88004-ETH			+	
IPO2166	Iran	Dezful, Safi Abad			02166-IRN			+	
IPO2159	Iran	Gorgan, Aq Qaleh			02159-IRN			+	
IPO90006	Mexico	Toluca			90006-MEX			+	
IPO89011	Netherlands	Barendrecht			89011-NLD			+	
IPO90015	Peru	*Unknown*			90015-PRU			+	
IPO92004	Portugal	Casas Velhas			92004-PRT			+	
IPO95036	Syria	Minbeg			95036-SYR			+	
IPO86013	Turkey	Adana			86013-TUR			+	
IPO87016	Uruguay	Dolores			87016-URY			+	
IPO00003	USA	Colusa	-	3	00003-USA			+	
IPO00005	USA	Colusa	-	3	00005-USA			+	
IPO95052[3]	Algeria	Berrahal			95052-ALG			+	
IPO86022[3]	Turkey	Altinova			86022-TUR			+	

[1]Information on hierarchical sampling

[2]All isolates are available at the KNAW-Fungal Diversity Center, http://www.cbs.knaw.nl/

[3]These isolates are durum wheat adapted isolates, all others are bread wheat adapted isolates

Significant isolate by cultivar interaction on the logit scale was also explored by assessing means for cultivars within isolates on the logit scale using pairwise t-tests. LSD-values were determined and applied to the table of means of the transformed data that were subsequently back transformed percentage for presentation in Tables and Figures. For EXP1-3 disease severities were evaluated 21 days after inoculation as percentages of the total first leaf area bearing necrosis (N) and pycnidia (P). Field experiments were evaluated between 21 and 28 days after inoculation as total STB symptoms on the flag leaves for N and P hardly deviated from each other under these conditions.

We considered that N and P levels as resistant once they did not significantly differ from minimal N and P levels. Similarly, susceptibility was considered once N and P values did not significantly differ from maximal N and P values. Values that differed significantly from both minimal and maximal N and P levels were considered as intermediate. This enabled a statistically sound *Stb* gene postulation and also provides isolate characteristics that can be widely applied in forthcoming genetic studies. All calculations were performed with the statistical programming language Genstat (Payne et al., 2009). Comparative seedling – adult plant analyses were individually performed per isolate using a Spearman rank correlation test.

For seedling experiments ten seeds per pot were linearly sown in VQB 7x7x8 cm TEKU® plastic potswith a steamed sterilized peat/sand mixture. Seedling experiments were temporally replicated twice (EXP1-4) or thrice (EXP2-3) with pots as experimental units. Plants were grown in controlled greenhouse compartments with light conditions of 16 hour/day, pre- and post-inoculation temperature and relative humidity (RH) settings of $18/16^0$ C vs. 22 ^{O}C (day/night rhythm) and RH values of 70% vs. ≥85%, respectively.

All strains were isolated from individual pycnidia from collected leaf material (Table 3). Each isolate was pre-cultured in an autoclaved 100 ml Erlenmeyer flask containing 50 ml yeast-glucose (YG) liquid medium (30gr Glucose, 10 gr yeast per liter demineralised water). The flasks were inoculated using a small piece of isolate mycelium maintained at -80^0 C and were incubated in an orbital incubated shaker (Innova 4430, New Brunswick Scientific, USA) at125 rpm and 18^0 C for 5-6 days. Each pre-culture was subsequently used to inoculate three 250 ml Erlenmeyer flasks

containing 100 ml YG media that were incubated under the abovementioned conditions to produce inoculum - 10^7 spores.ml^{-1}, supplemented with two drops of Tween 20 (MERCK®, Nottingham, UK), total volume of 40 ml for a set of 18 plastic pots - for plant infections at growth stage (GS) 11(Wiik & Rosenqvist, 2010). Adult plant experiments were performed in 2001 at Cappelle-en-Pévèle in Northern France at the breeding station of breeding company Florimond Desprez. Each field plot contained two 0.3m spaced rows of 1.5m length. Inoculations were carried out using a backpack air-pumped sprayer, calibrated at a rate of 10 L/100 m^2 at flag leaf appearance stage (GS 47-49), using a concentration of 10^6 spores/ml supplemented with 36 ml of four times diluted Tween 20 surfactant. One hour before inoculation a sprinkler irrigation system was turned on for a few minutes to provide enough humidity in the plant canopy. Inoculations started when the flag leaves of the earliest DH lines had developed and were subsequently repeated twice at 3-5 day intervals to compensate for earliness differences.

Genotyping -DNA extractions, microsatellite markers analysis

The 50 *M. graminicola* isolates used in the EXP1-3 (20 Global, 28 French and two Dutch reference isolates) were cultured on yeast-peptone-dextrose (YPD) agar plates (10 g of yeast extract, 20 g of peptone, 20 g of dextrose, 20 g of agar per liter). Mycelium samples were collected from the plates after 2-3 days growth at 17°C, lyophilized and stored at -80°C prior to DNA extraction. Total DNA was extracted from about 10 mg lyophilized mycelium using the QIAGEN® Biorobot 3000 and DNeasy® 96 Plant Kit, according to the manufacturer's instructions.

The genotypes of 48 isolates were determined with seven polymorphic microsatellite markers previously identified in EST sequences: ac-0001, ac-0002, ag-0003, ag-0009, caa-0003, caa-0005, tcc-0009 (Goodwin et al., 2007). The forward primers were 5'-labeled (WellRED-Sigma-Aldrich®) with one of three fluorochromes (D2, D3 and D4, respectively black, green and blue). Each microsatellite marker was amplified by polymerase chain reaction (PCR) with the EUROGENTEC® HotGoldStar Mix® adding about 20 ng template DNA and 0,5 µM of each primer in a final volume of 10 µl. Reactions were performed in an Applied Biosystems® 96-Well GeneAmp® PCR System 9700 or a MJ Research PTC-200 thermal cycler at 95°C for 10 min, followed by 35 cycles of 94°C for 10 sec, 55°C for 20 sec, 72°C for 30 sec,

and a final extension step of 72°C for 5 min before cooling to 10°C. PCR products were diluted 2-3 times to prevent signal saturation and analyzed using a CEQ8000 DNA sequencer (Beckman Coulter, Inc.), with allele sizing and grouping manually performed by visual inspection of chromatograms.

Results

All *M. graminicola* isolates grew well under laboratory conditions and were successfully used for plant inoculation in seedling and field experiments.

The microsatellite marker data showed that all the isolates used in these experiments were genetically distinct except for IPO98034 and IPO98035 (Appendices, Table S5). IPO98034 and IPO98035 had identical alleles for the seven microsatellite markers (Appendices, Table S5) and for an additional 15 microsatellite markers not reported in this study (unpublished data). Both isolates originate from the same wheat field in Cappelle-en-Pévèle in the North of France and with a few exceptional cases the phenotypic data also supported the similarity of the interactions on the evaluated wheat germplasm and, hence, we consider them to be clones (Appendices, Table S5, Fig.S1). All 2,899 seedling interactions (EXP1: 1,500; EXP2: 319 and EXP3: 1,080), showed that N and P-values correlated well (r=0,68) but that the former were always higher than the latter. In a few exceptionally susceptible responses (e.g. Chinese Spring *vs.* IPO02166) pycnidia appeared ahead of full necrosis development. Individual analyses of both parameters showed highly significant cultivar-isolate interactions in all three experiments (P=0.001), as well as the adult plant experiments, but the highest Wald/d.f. ratio of EXP3 indicated a relatively high genetic variation in that experiment compared to EXP1 and EXP2 (Table 4).

EXP1 and EXP2: French isolates and cultivars.

The N data showed a significantly lower resolution in describing genetic variation among the French isolates and cultivars (Appendices, Fig. S2). N data from EXP1 distributed cultivars and isolates in each three significantly different groups, whereas P clustered them in 13 and 10 significantly different groups, respectively (Appendices, Fig S3).

Table 4. Results of REML analyses of necrosis (N) and pycnidia (P) data obtained in seedling (EXP1-3) and adult plant field (EXP4) experiments with wheat and *Mycosphaerella graminicola* isolates.

		N				P			
	Fixed term	Wald statistic	d.f.[1]	Wald/d.f.	Chi-square probability[2]	Wald statistic	d.f.	Wald/d.f.	Chi-square probability
Experiment 1	Isolate	118.9	29	4.1	***	152.78	29	5.27	***
	Cultivar	2511.01	49	51.25	***	2543.38	49	51.91	***
	Isolate.Cultivar	2739.83	1421	1.93	***	3015.7	1421	2.12	***
Experiment 2	Isolate	226.67	28	8.1	***	129.92	28	4.64	***
	Cultivar	170.71	10	17.07	***	233.38	10	23.34	***
	Isolate.Cultivar	621.58	280	2.22	***	700.87	280	2.5	***
Experiment 3	Isolate	52.83	19	2.78	***	178.31	19	9.38	***
	Cultivar	5052.24	53	95.33	***	4273.29	53	80.63	***
	Isolate.Cultivar	2506.01	1007	2.49	***	3880.03	1007	3.85	***
Experiment 4 Seedling	Isolate	-	-	-	-	148.70	6	24.78	***
	Cultivar	-	-	-	-	352.67	22	16.03	***
	Isolate.Cultivar	-	-	-	-	361.98	132	2.74	***
Experiment 4 Adult plant	Isolate	-	-	-	-	7.47	6	1.24	ns
	Cultivar	-	-	-	-	152.19	22	6.92	***
	Isolate.Cultivar	-	-	-	-	220.81	132	1.67	***

[1]d.f.= degrees of freedom

[2]Significant at P=0.001, ns=not significant

A similar trend was observed when the French isolates were tested on the differential cultivars although the number of significantly different groups was larger for N and smaller for P (Appendices, Fig. S4-5). Apart from the reference isolates IPO323 and IPO94269 (Wittenberg et al., 2009) that were consequently placed in significantly different clusters, both experiments clearly showed that isolates from the same field were in all cases distributed over significantly different groups (Table 3; Appendices, Fig. S2-5).

More specifically, the range of data in EXP1 varied between 0-98N and 0-80P. Isolate IPO98001 was the least aggressive isolate with means of 15N and 1.7P, whereas IPO99042 was the most aggressive isolate with means of 48N and 36P. The most susceptible cultivar was cv. Taichung 29 with means of 90N and 41P, whereas line SE11 with means of 17N and 0P and the tetraploid *Triticum polonicum* with mean values of 23N and 0.6P were the most resistant lines. The cvs. Bulgaria 88, Veranopolis, Tadinia and Shafir clustered in four different groups for P, confirming that these cultivars carry different *Stb* genes. (Table 1; Appendices, Fig. S3).

In EXP2 the French isolates were tested on *Stb* differentials, which resulted in a range of 1-100N and 0-91P. Isolate IPO98047 was the most aggressive strain (87N and 43P) and IPO98001was the least aggressive isolate (28N and 8P). None of the differentials was resistant to all isolates. Cs/Synthetic 7D, carrying *Stb5* was the most resistant (28N and 4P) and cv. Courtot (98N and 56P) was the most susceptible cultivar. Analysis of the cultivar clustering suggested that *Stb6* was a major factor for cluster assembly (Appendices , Fig. S3 and 5). This resulted in two distinct major clusters among the French breeding lines (Appendices , Fig. S3) and also showed such clusters among the *Stb* differentials, where the *Stb6* cluster could be further divided in cultivars with two or more *Stb* genes (Appendices , Fig. S5; see for further details the phenotyping section).

Overall, the pathogenicity patterns of the French isolates on the set of differentials as well as on the French germplasm varied significantly among and within fields, within individual spots in a field and even on the same spot (Appendices, Tables S1-2).

EXP3: Global panels

In the final seedling experiment we tested 54 cultivars with 20 *M. graminicola* isolates that were distributed in 12/13 clusters for N and in 14/19 clusters for P

(Appendices , Figs. S6-7). The latter parameter clearly separated isolates that originated from durum wheat and bread wheat. The *M. graminicola* isolates did not cluster according to geographical origin and in the wheat clusters, tetraploid and synthetic hexaploid (derived) wheat lines were lumped together as they expressed a surprisingly broad resistance spectrum to the 18 applied bread wheat adapted *M. graminicola* strains. The overall range of 0-100N and 0-79P in this experiment enabled a thorough analysis of the data. Isolate IPO00005 had the lowest aggressiveness on the 54 cultivars (means 30N and 5P) and was only pathogenic on the differential cvs. Shafir and W7984 whereas the most pathogenic isolate on the total set of cultivars was IPO95036 with an average of 46N and 18P. Isolate IPO89011was the most aggressive strain on the differential sub-set and compromised the resistance of 11 out of 13 cultivars. Interestingly, this isolate was avirulent on cv. Courtot and 'CS/ synthetic 7D'. The former was among the most susceptible cultivars in EXP2. Among the bread wheat cultivars 'Shafir' and 'W7984' were overcome by 17 bread wheat strains, whereas cvs. Arina and TE9111 were only showing significant P values for three and four strains, respectively (Table 7).

Among the 16 durum wheat - *M. graminicola* interactions we observed varying levels of disease development (43-98N and 0-48P), but both isolates produced very similar phenotypes (Table 7; Appendices Fig S6-7). In accordance with earlier reports (Kema et al., 1996a, Kema et al., 1996b), the durum isolates induced substantial overall N levels in many cultivars (IPO86022, 18N and IPO95052,10N) that peaked surprisingly high in some specific cultivars such as 'Chinese Spring' (90N with IPO95052). Also, bread wheat isolates provoked substantial N levels in the durum wheat cultivars ranging from 31 to 74N with isolates IPO86068 and IPO88004, respectively. In contrast to earlier reports (Kema et al., 1996a), we also observed durum strains producing substantial P levels on cvs. Veranopolis and Chinese Spring that ranged between 5P and 15P. Conversely, bread wheat isolates, such as IPO95036, produced up to 30P on durum wheat cultivars in the *T. dicoccoïdes* accession PI 41025 (Table7). Nevertheless, in general terms the two wheat species expressed a non-compatible relationship with sympatric strains that originated from the other species.

Finally, we compared the cluster assemblies in EXP2 vs. EXP3 that clearly showed their incongruence (Appendices , Fig. S8).

Table 5. Results of inoculation experiments with 29 *Mycosphaerella graminicola* isolates on 11 wheat cultivars that carry 12 *Stb* genes (EXP1). Figures represent P data. Colors indicate resistant (not significantly different from 0P, green boxes), intermediate significantly different from 0P as well as maxP, yellow boxes) and susceptible (not significantly different from maxP, red boxes).

	IPO323-NLD	98021-VLG	IPO94269-NLD	99048-BEA	98050-SPL	98032-CEP	98034-CEP	98001-VLG	98031-ADH	98038-SPL	98028-SPL	98078-SPL	99018-BEA	98033-CEP	98035-CEP	98099-ADH	99031-BEA	99032-BEA	99038-BEA	98046-SPL	98072-VLG	98075-SPL	98097-ADH	98113-ADH	98094-ADH	98022-VLG	98051-VLG	98057-VLG	98047-ADH
	Stb6 detectors			Stb8 detectors			Stb4 detector				Stb2 detectors																		
Bulgaria	1	3	7	1	1	10	11	6	4	8	10	8	17	5	6	1	2	4	11	2	22	17	35	6	6	37	29	14	43
Veranopolis	1	0	17	1	1	4	8	0	5	10	3	1	1	2	2	2	2	3	7	2	16	4	17	25	12	26	9	1	16
ISR493	1	0	11	1	3	4	7	0	4	5	8	5	3	14	1	5	1	3	10	6	9	9	15	13	14	3	6	6	41
Tadinia	1	0	20	1	1	5	4	0	1	5	1	17	21	7	5	3	15	15	12	5	13	25	39	9	14	16	8	12	36
Cs/synthetic 7D	1	1	8	1	1	1	8	0	3	3	2	3	1	3	1	2	2	4	2	17	2	4	4	4	6	6	3	3	9
Shafir	1	2	44	1	16	6	23	1	15	26	16	17	28	15	20	12	14	24	26	36	22	18	34	38	43	55	3	3	61
E. Federal	64	31	56	24	14	38	13	13	21	30	26	21	8	26	9	8	33	43	44	47	30	60	56	40	60	52	56	36	50
W7984	21	41	54	3	1	3	17	8	10	24	17	15	32	5	6	5	34	40	37	42	14	24	48	36	71	24	11	15	58
Courtot	83	66	80	62	39	49	48	61	17	58	35	64	74	44	12	23	54	49	81	47	33	73	91	45	57	71	64	72	60
KK4500	1	2	6	1	1	15	14	0	2	3	1	1	11	4	19	1	1	3	17	27	1	6	10	23	1	2	2	1	36
TE9111	1	0	10	1	1	28	42	1	6	13	4	2	10	21	20	27	41	13	38	41	24	28	34	21	62	5	14	10	60

Table 6. Results of inoculation experiments with 18 *Mycosphaerella graminicola* isolates on 50 wheat cultivars and breeding lines for gene postulation (EXP2). Figures represent P data. Colors indicate resistant (not significantly different from 0P, green boxes), intermediate significantly different from 0P as well as maxP, yellow boxes) and susceptible (not significantly different from maxP, red boxes).

Cultivar	IPO323-NLD	98021-VLG	IPO94269-NLD	***Stb6***	99048-BEA	98050-SPL	98032-CEP	***Stb8***	98034-CEP	98001-VLG	98031-ADH	98038-SPL	***Stb4***	98028-SPL	98078-SPL	99018-BEA	98033-CEP	98035-CEP	98099-ADH	99031-BEA	99032-BEA	***Stb2***
FD1	0	0	25	x	0	3	10		14	1	40	7		5	14	21	14	2	20	21	21	
FD10	0	0	25	x	0	40	40		17	1	14	14		16	33	18	29	3	20	40	10	
FD11	0	0	45	x	0	50	30		21	1	30	10		14	44	8	18	30	40	25	33	
FD12	30	21	14		16	35	18		7	3	20	1		16	38	5	21	5	35	25	21	
FD13	45	25	40		33	55	21		13	2	18	2		10	50	18	35	7	25	19	35	
FD14	5	14	33		5	14	20		10	1	29	3		7	35	13	14	10	21	25	18	
FD15	0	0	33	x	1	25	7		2	1	29	3		1	40	13	14	1	25	5	2	
FD16	25	7	19		5	14	3		5	1	3	1	x	3	22	3	14	1	29	14	6	
FD17	55	35	45		21	44	35		18	25	29	29		33	60	40	50	20	40	35	29	
FD18	2	0	67	x	1	40	29		2	1	40	17		7	44	30	14	6	5	13	10	
FD19	2	0	50	x	0	2	18		14	1	29	10		9	20	26	10	14	25	25	3	
FD2	50	18	50		25	60	5		10	2	25	10		19	45	6	25	10	55	29	25	
FD20	0	0	25	x	0	1	3		2	1	14	3		3	21	6	10	1	25	3	2	
FD3	50	21	45		7	67	29		20	4	50	7		9	45	8	9	7	40	34	33	
FD4	50	33	43		25	50	14		18	13	35	14		5	45	29	6	10	40	20	30	
FD5	56	10	40		3	45	2		14	2	16	16		5	33	6	34	10	35	26	5	
FD6	0	0	14	x	0	10	14		7	1	3	1	x	18	30	2	5	14	40	14	18	
FD7	75	38	40		29	56	20		5	7	20	10		3	45	6	20	7	45	25	10	
FD8	0	0	30	x	0	33	25		21	1	35	25		40	56	18	43	14	44	25	29	

Table 6. Results of inoculation experiments with 18 *Mycosphaerella graminicola* isolates on 50 wheat cultivars and breeding lines for gene postulation (EXP2). Figures represent P data. Colors indicate resistant (not significantly different from 0P, green boxes), intermediate significantly different from 0P as well as maxP, yellow boxes) and susceptible (not significantly different from maxP, red boxes).

Cultivar	IPO323-NLD	98021-VLG	IPO94269-NLD	***Stb6***	99048-BEA	98050-SPL	98032-CEP	***Stb8***	98034-CEP	98001-VLG	98031-ADH	98038-SPL	***Stb4***	98028-SPL	98078-SPL	99018-BEA	98033-CEP	98035-CEP	98099-ADH	99031-BEA	99032-BEA	***Stb2***
FD9	67	29	25		33	71	40		25	1	9	14		22	55	11	33	21	45	29	29	
Frontana	50	25	10		2	5	1	x	0	1	0	2	x	2	10	1	3	1	14	3	0	
Iassul20	30	33	7		18	18	5		7	1	20	2		2	7	5	14	1	10	4	10	
Kavkaz	0	0	33	x	1	25	9		21	1	60	21		7	16	2	43	25	5	29	19	
Olaf	10	0	25		0	40	5		13	1	30	16		45	29	20	20	10	45	45	35	
SE1	35	3	25		5	18	5		14	1	5	7		18	14	8	30	5	18	30	35	
SE10	0	0	1	x	0	16	2		14	1	3	6		4	7	5	21	2	20	8	18	
SE11	0	0	0	x	0	0	2		7	1	0	0	x	0	0	7	0	1	1	8	25	
SE12	20	5	0		13	21	10		10	1	1	14		10	29	5	2	10	25	25	18	
SE13	0	0	2	x	0	14	3		1	1	20	10		3	25	2	6	3	13	25	13	
SE14	0	0	2	x	0	20	2		10	1	10	10		3	18	1	9	1	14	16	21	
SE15	1	0	3	x	1	40	5		7	1	14	10		3	29	5	33	3	10	10	6	
SE16	0	0	0	x	0	3	3		7	1	3	1	x	3	14	13	14	2	14	25	2	
SE17	2	10	1		18	21	5		5	1	40	29		2	9	25	18	5	25	29	7	
SE18	0	0	2	x	0	29	5		14	1	25	20		35	33	13	21	2	29	25	10	
SE19	10	4	0		2	20	7		3	1	10	2		4	50	3	7	3	25	3	3	
SE2	0	0	25	x	0	21	14		5	1	2	5	x	14	20	7	3	3	33	7	13	
SE20	0	0	21	x	0	7	10		3	1	14	1		3	3	21	2	1	20	22	3	
SE3	0	0	0	x	0	7	2		2	1	7	0	x	2	7	1	0	2	5	2	2	x

Table 6. Results of inoculation experiments with 18 *Mycosphaerella graminicola* isolates on 50 wheat cultivars and breeding lines for gene postulation (EXP2). Figures represent P data. Colors indicate resistant (not significantly different from 0P, green boxes), intermediate significantly different from 0P as well as maxP, yellow boxes) and susceptible (not significantly different from maxP, red boxes).

Cultivar	IPO323-NLD	98021-VLG	IPO94269-NLD	***Stb6***	99048-BEA	98050-SPL	98032-CEP	***Stb8***	98034-CEP	98001-VLG	98031-ADH	98038-SPL	***Stb4***	98028-SPL	98078-SPL	99018-BEA	98033-CEP	98035-CEP	98099-ADH	99031-BEA	99032-BEA	***Stb2***
SE4	3	4	14	x	5	14	10		7	2	6	2	x	3	9	18	18	3	14	18	5	
SE5	0	0	3	x	0	21	5		1	1	14	10		6	40	4	25	1	35	40	9	
SE6	10	10	1		2	29	14		2	2	10	0		3	10	8	4	1	20	21	2	
SE7	50	29	25		19	50	10		1	2	25	20		14	50	21	29	7	14	44	10	
SE8	29	3	6		2	20	7		20	1	1	14		25	25	25	2	3	45	40	5	
SE9	0	0	45	x	0	50	10		20	1	50	13		25	55	13	50	3	40	60	21	
Bulgaria	2	0	5	x	0	1	3		3	1	4	2	x	38	2	40	3	7	10	4	2	
Veranopolis	0	0	38	x	0	0	2		2	1	2	10		0	0	0	0	1	2	1	0	x
Tadinia	0	0	5	x	0	5	14		1	1	0	1	x	4	13	14	2	1	18	21	9	
Shafir	0	0	50	x	0	43	7		10	1	40	10		2	45	10	5	1	18	20	40	
T29	80	55	7		45	80	26		13	2	56	21		18	57	35	25	40	25	25	25	
T._polonicum	0	0	2		0	0	0		0	0	0	0		1	0	0	0	0	2	0	0	
Total				27				1					10									2

Table 7. Results of inoculation experiments with 20 *Mycosphaerella graminicola* isolates on 54 wheat cultivars including 13 differential that carry 15*Stb* genes (EXP3). Figures represent P data. Colors indicate resistant (not significantly different from 0P, green boxes), intermediate significantly different from 0P as well as maxP, yellow boxes) and susceptible (not significantly different from maxP, red boxes).

Cultivar	90006-MEX	88004-ETH	86013-TUR	92004-PRT	00003-USA	89011-NLD	94218-CAN	87016-URY	95054-ALG	00005-USA	90015-PRU	99015-ARG	86068-ARG	92034-ALG	02159-IRN	88018-ETH	95036-SYR	02166-IRN	86022-TUR	95052-ALG
Bulgaria	0	3	13	0	6	4	0	10	1	0	0	0	8	1	4	6	24	13	0	0
Veranopolis	0	5	35	13	1	38	22	4	0	2	38	1	1	0	0	3	33	31	5	1
ISR493	0	0	17	3	1	5	1	3	1	1	0	0	1	24	1	1	6	4	0	0
Tadinia	0	11	17	10	1	10	0	35	6	1	1	10	2	1	6	3	15	13	0	0
CS/Syn 7D	0	4	12	1	3	0	25	2	2	1	1	1	1	6	1	1	12	16	2	2
Shafir	45	24	24	15	21	9	24	60	15	13	41	22	4	45	1	22	23	12	0	0
E. Federal	42	45	26	16	31	30	4	1	1	3	3	32	10	21	16	33	6	43	0	0
W7984	8	17	35	11	20	6	26	12	28	7	6	19	8	35	7	3	58	26	0	0
Courtot	30	4	1	18	52	1	1	5	13	1	45	55	3	46	0	1	1	18	0	0
KK4500	23	19	0	8	11	6	1	0	0	0	2	21	2	2	0	2	1	0	0	0
TE9111	0	1	2	4	10	13	0	0	0	0	0	0	1	4	1	1	0	0	0	0
Salamouni	1	21	31	1	1	7	0	30	2	1	4	7	8	20	10	1	5	24	0	0
Arina	0	0	0	3	6	6	0	2	0	0	5	0	2	1	0	1	1	1	0	0
CS	55	45	41	46	53	48	50	43	34	37	30	33	26	48	34	40	57	52	12	15
Katepwa	32	3	57	53	33	49	47	79	31	30	35	48	15	4	30	11	63	5	0	0
Erik	42	1	25	17	34	36	57	55	31	13	8	35	17	53	4	24	61	3	0	0
FHD 2054.3	0	1	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
M3	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Table 7. Results of inoculation experiments with 20 *Mycosphaerella graminicola* isolates on 54 wheat cultivars including 13 differential that carry 15*Stb* genes (EXP3). Figures represent P data. Colors indicate resistant (not significantly different from 0P, green boxes), intermediate significantly different from 0P as well as maxP, yellow boxes) and susceptible (not significantly different from maxP, red boxes).

Cultivar	90006-MEX	88004-ETH	86013-TUR	92004-PRT	00003-USA	89011-NLD	94218-CAN	87016-URY	95054-ALG	00005-USA	90015-PRU	99015-ARG	86068-ARG	92034-ALG	02159-IRN	88018-ETH	95036-SYR	02166-IRN	86022-TUR	95052-ALG
Nogal	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
TA4152-19	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
TA4152-37	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
SE11	1	1	1	0	1	1	1	6	0	1	0	1	1	1	0	1	8	1	0	0
SE3	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0
FD12	0	8	23	6	18	15	0	2	1	0	1	1	1	4	9	1	17	15	0	0
FD3	1	0	8	2	28	8	0	4	0	0	1	2	2	3	3	2	22	7	0	0
02CY 399	1	27	9	7	1	1	1	16	8	1	2	1	1	13	4	11	4	1	1	1
Apache	0	18	23	0	7	23	0	0	0	0	4	0	1	0	0	1	8	2	0	0
Balance	2	14	25	16	5	1	4	51	4	1	7	23	2	27	20	14	31	11	0	0
Bio2000	0	0	16	1	3	1	0	4	1	0	2	1	1	2	0	0	16	9	0	0
Biscay	1	16	15	3	6	6	2	2	0	0	2	1	1	23	1	1	10	25	0	0
Florett	5	0	4	3	15	10	3	0	1	0	3	0	1	0	1	8	1	0	0	0
Frontana	16	21	2	9	8	4	1	20	3	13	8	10	8	18	1	18	4	15	0	0
Grandin	18	4	19	4	40	27	42	61	17	10	28	19	14	26	1	4	46	27	1	0
Kulm	7	1	33	22	23	22	38	13	5	16	21	19	31	26	1	16	31	0	0	0
Largo	1	5	10	6	9	2	5	5	0	1	6	8	2	2	4	10	16	32	2	2
Mazurka	38	40	19	20	41	15	18	5	4	3	2	50	31	34	11	22	10	32	0	0

Table 7. Results of inoculation experiments with 20 *Mycosphaerella graminicola* isolates on 54 wheat cultivars including 13 differential that carry 15*Stb* genes (EXP3). Figures represent P data. Colors indicate resistant (not significantly different from 0P, green boxes), intermediate significantly different from 0P as well as maxP, yellow boxes) and susceptible (not significantly different from maxP, red boxes).

Cultivar	90006-MEX	88004-ETH	86013-TUR	92004-PRT	00003-USA	89011-NLD	94218-CAN	87016-URY	95054-ALG	00005-USA	90015-PRU	99015-ARG	86068-ARG	92034-ALG	02159-IRN	88018-ETH	95036-SYR	02166-IRN	86022-TUR	95052-ALG
ND495	35	1	25	28	52	40	37	51	37	20	51	24	5	47	10	21	40	0	0	0
Sankara	1	3	10	1	10	2	0	19	0	0	1	0	2	0	0	1	8	5	0	0
Solitär	2	16	25	13	16	31	2	1	1	9	20	0	1	1	6	1	6	6	0	0
Sumai-3	36	35	53	35	17	10	10	48	30	13	32	22	8	52	19	32	16	21	0	0
T29	45	3	32	24	63	48	42	55	31	15	39	41	15	45	11	24	66	43	1	0
Tuareg	1	0	16	6	3	3	1	11	1	0	11	1	1	10	0	0	4	0	0	0
Wangshobai	31	26	7	39	32	13	7	23	17	4	41	6	3	43	8	18	42	30	1	0
TA4152-60	13	19	39	3	9	1	15	24	17	5	6	17	3	6	5	11	6	32	0	0
BR34	15	32	33	21	31	28	39	52	36	7	14	55	34	27	16	22	36	66	0	0
Falat	62	39	26	15	27	50	16	8	9	3	12	37	19	58	10	13	21	63	0	0
Altar 84	0	6	1	3	1	0	0	0	0	2	6	0	1	6	0	3	5	1	20	20
Ben	2	28	6	6	8	4	5	11	1	1	12	9	3	8	2	12	9	8	26	36
Langdon16	3	8	10	11	1	8	3	0	4	0	9	1	1	4	0	10	20	10	48	31
T. dic. (PI 41025)	1	22	2	1	9	21	0	1	0	0	1	0	4	2	0	9	30	0	6	29
T. dic. (PI 481521)	0	15	8	0	1	2	6	0	0	1	12	0	3	1	0	1	14	2	19	17
T. dic. ISR A	6	9	1	1	1	2	0	0	0	1	17	0	2	0	0	0	4	1	12	0
T. dic. TA106	1	3	1	0	0	0	0	1	0	0	0	1	7	17	0	1	1	1	2	1
T. dic. (PI 478742)	2	14	0	1	1	0	3	0	0	0	0	5	4	28	2	0	1	3	10	2

EXP4: Adult plant field experiments

Disease symptoms developed well under conducive conditions in the field experiment (Table 8; Appendices, Fig S9). The comparative seedling-adult plant response analysis resulted in significant rank correlations for only two (IPO323 and IPO98021) out of the seven used *M. graminicola* strains, indicating significant differences in the expression of resistance under these different physiological stages of wheat plants.

Phenotyping – compatibility thresholds and gene postulation

Due to the higher resolution of P data we focused gene postulations primarily on this parameter and used a conservative but flexible approach by determining the thresholds for resistance and susceptibility through statistical analyses. Each figure that was not significantly different from 0P was considered as a resistant response. Likewise, each figure not significantly different from the highest score in the entire experiment was considered as susceptible. Values being significantly different from these two threshold values were considered as intermediate (all at P=1% level). This translates to varying thresholds for each experiment but clearly indicates what interactions are incompatible (EXP1≤9P, EXP2≤6P and EXP3≤4P) and compatible (EXP1≥17P, EXP2≥ 49P and EXP3≥26P), which in turn provides a basis for gene postulations (Tables 5-7).

A starting point for these analyses was the data set on the differential *Stb* cultivars (EXP2). These data were used to characterize (in)compatibility and provided a matrix that was superimposed over the EXP1 and EXP3 data. Despite the limitations of this approach, due to the lack of near isogenic lines with individual *Stb* genes, it provided a first insight in genetic diversity in these large phenotypic data sets. Cultivar Shafir carries *Stb6*, which is surprisingly prevalent in the *Stb* differential cultivars (Table 1). The reference strain IPO323 as well as isolate IPO98021 are avirulent on cv. Shafir and have parallel responses on all other cultivars, including compatible interactions with cvs. Estanzuela Federal, W7984 and Courtot that lack *Stb6* (Table 1). The other reference strain, IPO94269, circumvents *Stb6* in all differentials except in cv. KK4500 that carries four *Stb* genes (Chartrain et al., 2005a) (Table 1, Table 5). Hence, we used these three isolates for *Stb6* detection and

Table 8. Relationship between seedling and adult plant stage resistance in a subset of 23 French breeding lines that were inoculated with seven *Mycosphaerella graminicola* isolates. Resistant in seedling (P< 9%) and adult plant stages (P<10%), green boxes; resistant in seedling but susceptible in adult plant stage, yellow boxes; susceptible in seedling but resistant in adult plant stage, brown boxes; and susceptible at both seedling (P>9%) and adult plant stages (P>10%), red boxes

Isolate	IPO323-NLD		IPO98021-VLG		IPO98113-ADH		IPO98001-VLG		IPO99042-BEA		IPO99038-BEA		IPO98075-SPL	
Cultivars	S[1]	A	S	A	S	A	S	A	S	A	S	A	S	A
FD2	50	65	20	40	11	25	3	40	35	25	25	45	30	80
FD3	50	18	25	18	25	20	15	23	45	20	20	18	65	13
FD5	55	50	13	50	15	13	3	45	30	35	5	55	30	40
FD6	0	15	0	10	8	20	0	20	33	13	13	10	25	43
FD7	75	50	40	28	35	23	8	11	55	30	20	20	45	18
FD10	0	10	0	15	25	25	0	15	35	13	10	15	35	30
FD11	0	13	0	13	25	30	0	18	40	8	30	40	55	45
FD14	11	38	15	20	40	10	1	10	45	25	5	30	20	25
FD18	5	15	0	5	25	20	0	3	50	18	13	35	25	65
FD19	5	8	0	8	10	10	0	5	35	33	3	15	40	40
FD20	0	8	0	8	3	13	0	23	30	8	1	13	20	10
SE1	35	10	6	40	10	10	0	30	35	13	15	20	50	40
SE2	0	8	0	13	1	8	0	10	55	33	20	30	55	45
SE3	0	5	0	8	3	5	0	1	8	13	6	13	3	40
SE4	6	40	15	40	15	13	3	55	40	28	8	15	15	30
SE5	0	8	0	3	5	3	0	28	45	13	20	11	30	18
SE7	50	28	30	33	30	18	3	13	60	18	30	20	45	18
SE13	0	5	0	8	13	18	0	13	20	23	15	18	26	18
SE14	0	13	0	35	5	13	0	40	15	40	10	50	11	60
SE15	1	5	0	6	8	20	0	11	45	33	8	40	33	5
SE18	0	10	0	10	25	18	0	8	25	18	11	40	55	35
SE19	13	75	15	13	8	30	0	30	40	25	6	23	26	50
SE20	0	8	0	23	15	8	0	31	35	15	10	13	20	15
Correlation[2]	0.72***		0.66***		0.41ns		0.25ns		0.14ns		0.13ns		-0.07ns	

[1]S=seedling data, A=adult plant data

[2]Significant at P=0.001, ns=no correlation

postulate it in 16 breeding lines and cultivars including cv. Shafir (Table 6). In addition, another set comprising cvs. Tadinia, Bulgaria and breeding lines SE3, SE5, SE10, SE11, SE13,SE14, SE15, SE16 and SE18 were resistant to these three isolates and 26 out of these 27 entries clustered in one major clade (Appendices, Fig. S3) suggesting a common *Stb6* presence among these accessions. Exceptions include line SE4 that shows identical responses to IPO323, IPO98021 and IPO94269 as cv. Shafir; as well as cv. Olaf and line SE17 that are clustered as *Stb6* carriers despite their opposite compatibility with isolates IPO323 and IPO98021 (Table 6). Likewise we have determined *M. graminicola* isolates that are indicative for the presence of *Stb8, Stb4 and Stb2* in wheat germplasm (Table 5). These 18 isolates were subsequently used to postulate genes in the French breeding lines, which showed that *Stb6* and *Stb4* are the most prevalent genes, whereas *Stb2* and *Stb8* were detected at a lower frequency (Table 6).

To further validate EXP1 and EXP2 we tried to postulate *Stb* genes in 46 commonly used cultivars out of 54 that were tested in EXP3, which also included the differential wheat cultivars carrying the 15 reported *Stb* genes. Statistical analyses confirmed the great diversity in pathogenic and resistance profiles of the applied *M. graminicola* isolates and wheat cultivars, clearly contrasting with the same analyses using the French panel (Appendices, Fig S8). This enabled the identification of valuable additional isolates, compared to the European strains (Table 5) that can be used for *Stb* postulations. For example, the Mexican isolate IPO90006 is an additional master differentiator as it is virulent for *Stb6, Stb7* and *Stb9*. The former gene is very prevalent in wheat germplasm and frequently co-occurs with other *Stb* genes in wheat germplasm (Table 1). Hence, germplasm resistant to this strain may carry at least *Stb1, Stb2, Stb3, Stb4, Stb5, Stb11* or *Stb15*, which then can be further analyzed using the determined pathogenicity patterns of other strains. Others include strains with specific virulences for individual genes such as *Stb7* (IPO88018), *Stb8* (IPO95054) as well as several combinations of *Stb* genes including *Stb2+Stb6+Stb8* (IPO95036), *Stb2+Stb6+Stb9* (IPO90015), *Stb2+Stb7+Stb8* (IPO02166), *Stb6+Stb8+Stb9* (IPO92034), *Stb4+Stb6+Stb13+Stb14* (IPO87016) and a variety of other combinations. This project enabled the validation of these differential strains on a number of cultivars with multiple mapped *Stb* genes (Table 7). 'Arina' carries *Stb6* and *Stb15* (Table 1) and is specifically resistant to IPO323 (Tabib Ghaffary and

Kema, *unpublished data*, Chartrain et al., 2005b) and IPO90006 as well as to 14 more bread wheat adapted isolates from four continents. All other differentials were circumvented by at least one of these isolates, indicating that a subset of these isolates can differentiate *Stb15* from the other *Stb* genes. This set comprises IPO323, IPO90006, IPO88004, IPO86013 and IPO92004 in which IPO323/IPO90006 differentiate *Stb6* from *Stb15*, and the others differentiate the latter from all other *Stb* genes. Differential TE9111 carries *Stb6+Stb7+Stb11* (Chartrain et al. 2005c) and indeed is resistant to IPO90006 that carries virulence for *Stb6+Stb7*, but is avirulent for *Stb11*, which is effective across all other isolates in this test. Surprisingly few other cultivar susceptibility patters matched the patters of the *Stb* differentials. An exception might be 'Bio2000' that matched the *Stb5* (CS/Syn7D) pattern closely.

Geographical differences represented in the global vs. the French panel clearly demonstrated that cvs. like Estanzuele Federal (*Stb7*) and Courtot (*Stb9*), being among the most susceptible to the French panel (Table 6), can still be used as a source of resistance elsewhere (Table 7).

Finally, by using a wide panel of well-characterized *M. graminicola* isolates we were able to identify potential new sources of resistance. None of the 15 described *Stb* genes was completely effective to this panel. Hence, germplasm such as M3, Nogal, FHD 2054.3, TA4152-19, TA4152-37, which are derived from synthetic hexaploids, and showed a broad resistance spectrum to the global panel, potentially carries new genes as these responses can hardly be explained by combinations of the available *Stb* gene arsenal (Table 7). Similarly, more adapted germplasm such as breeding lines SE3 and SE11 as well as cv. Apache have reasonably good levels of resistance towards this panel (Table 6-7).

Discussion

This project has resulted in a large database of wheat-*M. graminicola* interactions that will enable new studies into the genetic background of host resistance. The availability of well-characterized *M. graminicola* isolates has shown to be indispensable for such studies (Adhikari et al., 2003, Adhikari et al., 2004a, Adhikari et al., 2004b, Adhikari et al., 2004c, Arraiano et al., 2007, Arraiano et al.,

2001b, Brading et al., 2002, Chartrain et al., 2005a, Chartrain et al., 2005c, Chartrain et al., 2009, McCartney et al., 2003, Somasco et al., 1996). However, reliable phenotyping in the *M. graminicola*-wheat pathosystem remains an area for intensive consideration as it truly impacts the trustfulness of *Stb* gene discovery. Since STB emerged as an important wheat disease different phenotyping methods have been developed and applied. Various qualitative and quantitative phenotyping scales were used over the years. In some reports both N and P were quantitatively scored (Kema et al., 1996a), while others only scored P (Arraiano et al., 2001a, Brown et al., 2001, Chartrain et al., 2009). A combined qualitative/quantitative assessment method evaluated disease severity as the leaf area with pycnidia bearing necrosis along with the level of sporulation (a variation on the earliest qualitative 0-5 scale for STB phenotyping) (Adhikari et al., 2003, McCartney et al., 2003, Rosielle, 1972). In fact, all the reported *Stb* genes were identified by different scoring methods in either attached or detached leaf assays (Arraiano et al., 2001a, Kema et al., 1996a). A combination of the attached/detached leaf technique was also applied to induce sporulation in overall symptomless responses of the diploid *T. monococcum* (Jing et al., 2008). This, evidently is far from ideal and hampers effective introgression of *Stb* genes into breeding programs, particularly as these program most often rely on field studies using specific isolates and accompanying marker assisted approaches (Goodwin, 2007). We, therefore, chose to evaluate a vast array of interactions in conjunction with tests on *Stb* differentials to validate *Stb* efficacy and to provide a new starting point for *Stb* gene discovery. Ten out of the 18 currently mapped *Stb* genes were identified and mapped with well-characterized strains from our laboratory (IPO strains). Recently, Czembor et al. (2010) used IPO isolates to a subset of *Stb* differentials, but only evaluated necrosis development. With our analysis we extend previous studies (Arraiano & Brown, 2006, Chartrain et al., 2005b, Czembor et al., 2010, Kema et al., 1996a, Kema et al., 1996b, Kema & vanSilfhout, 1997) by testing all differentials in an attached leaf assay for both N and P using one scale. Recently, we identified three new *Stb* genes in 'M3' and the French wheat cv. Balance by exploiting some of these isolates in detailed mapping studies (Tabib Ghaffary et al., 2011a,b), illustrating the value of deep screening studies to identify new sources or resistance.

In one of these studies we identified *Stb17* which is specifically expressed in adult plants. We, therefore, also screened germplasm under field conditions in the adult plant stage with a subset of the described *M. graminicola* isolates. Evidently, single isolate inoculations under field conditions are challenging in terms of experimental management (Brown et al., 2001, Eriksen et al., 2003, Kema & vanSilfhout, 1997, Simon et al., 2004), but provide a wealth of information that cannot be achieved by natural infections. The application of individual isolates under field conditions is required to test gene efficacy and is the only way for reliable phenotyping that helps breeders to select premium germplasm. We confirmed earlier data (Arraiano & Brown, 2006, Brown et al., 2001, Kema & vanSilfhout, 1997) that specific seedling and adult plant responses are commonly observed in the wheat – *M. graminicola* pathosystem. Specific adult plant resistance is commonly observed for other cereal diseases (Lin & Chen, 2007, Liu et al., 2001) due to genes that are exclusively expressed in plant in a different physiological stage such as *Stb17* (Tabib Ghaffary et al., 2011b). Such differential responses, however, depend strongly on the used isolates. The results with IPO323 do not significantly differ at the two stages, but the seedling resistance to isolate IPO98001 was hardly expressed in the adult plant stage, whereas specific adult plant resistance was only observed in 10 out of the 141 interactions (Table 8).

Western Europe totally produces 69 million tons and contributes more than 10% to the global wheat production and France is major wheat producer with a 6% global and a 55% regional share (FAO 2010). STB is considered the most important constraint of the French wheat production, which has triggered substantial interest from the government (Freier & Boller, 2009), breeding companies and commodity boards (Jorgensen et al., 2010). Surfacing fungicide resistance issues recently also underscored the importance of STB for French wheat growers (Halama, 1996, Leroux et al., 2005, Loyce et al., 2008). We, therefore, have included a panel of French isolates in our studies to address genetic diversity for pathogenicity at the field level. Diversity for anonymous markers has been known for a long time (Abrinbana et al., 2010, El Chartouni et al., 2011, Jürgens et al., 2006), but associations within field variation for pathogenicity have not been addressed. Our studies, confirmed genetic diversity at a fine spatial scale as all strain represented individual genotypes (Linde et al., 2002) in accord with the expectations for a heterothallic pathogen (Kema et al.,

1996c, Wittenberg et al., 2009). Additionally, we demonstrate extensive genetic variation within and between fields for pathogenicity. The SSR data showed that the 25 French isolates of EXP1-2 represented 24 different genotypes and screening on the French breeding lines and the differential panel of cultivars distributed them into 22 and 20 significantly different phenotypes, respectively. For instance, the six isolates originating from St. Pol de Léon were placed in five significantly different clades (Appendices, Fig. S3 and 5) and arranging all French isolates by location shows the extensive pathological variation within wheat fields, both on the differentials as well as on the tested breeding lines. This result calls for an extensive study into the population dynamics of genes that control pathogenicity. With the ongoing sequencing initiatives and parallel genetic studies (Goodwin et al., 2011; Wittenberg et al., 2009) we are close to elucidating effector genes and determining the distribution of such genes will further contribute to STB management.

Resistance genes *Stb6* and *Stb4* were most frequently postulated, which confirmed their prevalence in a wide diversity of European germplasm (Arraiano et al., 2009, Arraiano & Brown, 2006, Brown et al., 2001, Chartrain et al., 2005b, Eriksen et al., 2003). Brading et al., (2002) suggested that this inevitably relates to hitherto applied breeding strategies where natural inoculum, that is by definition a mix of many pathogenic variants, rather than well characterized *M. graminicola* isolates, is being used for selection purposes. *Stb4* originates from the Dutch wheat cv. Tadorna that was abundantly used in breeding programs in the 1960s (Somasco et al., 1996, Gervais et al., 2003, Huang et al., 2007, Johnson, 1978, Zeven, 1972), hence, its prevalence in European wheat cultivars is not surprising.

Any genetic analysis suffers from restrictions, and compromises have to be accepted with respect to the number of populations, their size, or the number of isolates that can be managed. The differential Cs/Synthetic 7D is a substitution line of chromosome 7D from a synthetic hexaploid into cv. Chinese Spring (Nicholson et al., 1993). Chartrain et al. (2005b) showed that cv. Chinese Spring carries an allele of *Stb6* on chromosome 3AS. Therefore, we suggest that Cs/Synthetic 7D not only carries *Stb5* on chromosome 7D, but also *Stb6* as it is resistant to IPO323. Arraiano et al. (2001b, 2007) also reported that 'Cs/Synthetic' is resistant to IPO323, IPO89011 and IPO88004, which we confirmed in the current study suggesting that the broad efficacy of Cs/Synthetic 7D is actually due to a combination of several genes that at

least include *Stb5* and *Stb6*. Another possibility is that the 7D chromosome apparently carries more genes than *Stb5*, but this can only be addressed by using a wide set of isolates with different specificities in genetic analyses. However, Arraiano et al. (2007) and Chartrain et al. (2009) tested their segregating populations with two isolates in the seedling stage. We, therefore recommend that future genetic studies should include more precisely characterized *M. graminicola* isolates, such as the ones reported in this study, to ensure sound conclusions on the genetic basis of STB resistance in wheat (Tabib Ghaffary et al., 2011b). In addition, individual lines from segregating population should be exposed to a wide variety of *M. graminicola* strains to ascertain that mapped QTLs do not represent clusters of genes with different specificities (This thesis, general discussion).

Our data provide an overview of wide *Stb* efficacy, but also demonstrate the incongruence of different data sets. Gene postulations were possible by testing French breeding materials with French *M. graminicola* isolates, but were hardly possible by testing a wide panel of global isolates on a wide variety of unrelated wheat germplasm. Still, genes with good efficacy towards European strains, such as *Stb5*, showed also an acceptable efficacy to a much wider range of *M. graminicola* isolates. Moreover, cultivars with apparent low value in terms of resistance in a European context, such as cv. Courtot, showed high levels of resistance to strains from other geographical regions. Additionally, the value of using different panels was clearly demonstrated by the identification of new sources of resistance that were recently confirmed (Tabib Ghaffary et al., 2011a). Thus, for new *Stb* gene discovery, application of wide and diverse genetic screens are required as narrower panels may discover new genes, but their efficacy is usually of limited commercial interest as was recently shown by the genetic analysis of the French cv. Balance (Tabib Ghaffary et al., 2011b) and the German wheat cv. Solitär (Kelm et al., 2011).

The current panel of isolates might therefore be an ideal suite of strains for association genetics approaches that have recently worked well for other wheat diseases (Crossa et al., 2007, Tommasini et al., 2007, Zhu et al., 2008). However, such studies require excellent and repeatable phenotyping protocols. Despite the value of the current study and its contribution to *Stb* gene discovery and a better understanding of gene efficacy, it also addresses an undesirable variety of protocols over the years and by different research groups. Kema et al. (1996a) also addressed

this when gene-for-gene interactions in the wheat – *M. graminicola* pathosystem were still considered suspicious. Over time, these interactions have been proven and confirmed over and over again. However, the effect of different geographical locations on instability of data sets needs to be elucidated and, additionally, we now need to capitalize on insight from related species such as the wheat – *Stagonospora nodorum* and wheat – *Pyrenophora tritici-repentis* pathosystems that are characterized by inverse gene-for-gene interactions driven by host sensitivity loci and small pathogen derived proteins with toxic effects (Friesen et al., 2007). With the current excellent genome information of *M. graminicola* (Goodwin et al., 2011), we have a new window of opportunities for enhanced understanding of the wheat – *M. graminicola* pathosystem that will benefit breeders and growers around the world.

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Chapter 3

New broad-spectrum resistance to septoria tritici blotch derived from synthetic hexaploid wheat

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Abstract

Septoria tritici blotch (STB), caused by the ascomycete *Mycosphaerella graminicola*, is one of the most devastating foliar diseases of wheat. We screened five synthetic hexaploid wheats (SHs), 13 wheat varieties that represent the differential set of cultivars and two susceptible checks with a global set of 20 isolates and discovered exceptionally broad STB resistance in SHs. Subsequent development and analyses of recombinant inbred lines (RILs) from a cross between the SH M3 and the highly susceptible bread wheat cv. Kulm revealed two novel resistance loci on chromosomes 3D and 5A. The 3D resistance was expressed in the seedling and adult plant stages, and it controlled necrosis (*N*) and pycnidia (*P*) development as well as the latency periods of these parameters. This gene, which is closely linked to the microsatellite marker *Xgwm494*, was designated *Stb16* and explained from 41 to 71% of the phenotypic variation at seedling stage and 28 to 31% in mature plants. The resistance locus on chromosome 5A was specifically expressed in the adult plant stage, associated with SSR marker *Xhbg247*, and explained 12 to 32% of the variation in disease. This quantitative trait locus (QTL) was designated *Stb17q*, and is the first QTL for adult plant resistance to *M. graminicola* to be identified. Our results confirm that common wheat progenitors might be a rich source of new *Stb* resistance genes/QTLs that can be deployed in commercial breeding programs.

Introduction

Since early history, wheat (*Triticum aestivum* L., 2n = 6× = 42, AABBDD) was a main source of food and feed. It is the oldest and has been the most widely grown crop since 10,000-8,000 B.C (Heun et al. 1997; Luo et al. 2007; Nesbitt and Samuel 1998). Due to its importance and increasing demand, it is a key commodity to eradicate global hunger not only by ensuring sufficient production to feed a world population that will grow by 50 percent and reach 9 billion by 2050, but also by guaranteeing access to food (FAO 2010). Still, in 2010 annual bread wheat production is projected to decline and diseases play a significant role in such reductions (USDA 2010). In Western Europe, which is among the largest wheat production areas, Septoria tritici blotch (STB) caused by the ascomycete *Mycosphaerella graminicola* (Fuckel) J. Schröt is the most recurrent and important wheat disease. STB is also a

major disease in the Americas, Central and West Asia, and particularly on durum wheat in North Africa. STB can cause yield losses that typically range from 10-15%, but under conducive weather conditions, losses can easily exceed 50%, particularly in low-input agriculture where disease management is frequently suboptimal (Duveiller et al. 2007; Eyal 1999; King et al. 1983).

In intensive wheat production areas, disease management is often accomplished by fungicide applications and the deployment of resistant wheat cultivars (Goodwin 2007; Lehoczki-Krsjak et al. 2010). STB is the major target of the agrochemical industry that has Western Europe as its prime market (Jorgensen 2008), but fungicide applications are not always timely, environmentally sound or economically viable (Paveley et al. 1997). Under conditions favorable for disease, two to 12 fungicide applications are required to control STB (Burke and Dunne 2008), and the costs easily reach approximately 150 Euro per hectare (Beest et al. 2009). Most importantly, fungicide efficacy towards STB is hampered by the development of fungicide resistant strains of the pathogen (Fraaije et al. 2005; Mavroeidi and Shaw 2005; Stergiopoulos et al. 2003). Therefore, host resistance is an important component of effective disease management strategies for commercial wheat production.

To date, 15 major resistance genes, *Stb1-Stb15*, have been identified and characterized, but compared to yellow rust, leaf rust, stem rust, hessian fly and powdery mildew – with 88, 96, 64, 33 and 104 mapped resistance genes, respectively - this number is limited. Moreover, the majority of these genes have narrow spectra of specificity towards *M. graminicola* isolates that represent current field populations in major wheat producing areas, and this limits their use (Arraiano and Brown 2006; Chartrain et al. 2005b). Furthermore, *M. graminicola* is a heterothallic filamentous fungus with multiple sexual cycles during the growing season that defines its complex genetic population structure and influences disease management (Chen and McDonald 1996; Kema et al. 1996c; McDonald et al. 1996). The wheat- *M. graminicola* pathosystem complies with the gene-for-gene hypothesis where a pathogen effector interacts with a host target (Brading et al. 2002).Hence, the selection pressure that new *Stb* genes may exert on natural *M. graminicola* populations calls for responsible deployment strategies and a continuous effort to unveil key genes that control this disease (Cowger et al. 2000; Linde et al. 2002; Zhang et al. 2001).

Modern wheat improvement programs and wheat domestication processes resulted in narrow diversity of wheat germplasm (Christiansen et al. 2002; Raman et al. 2010). For this reason, wild wheat progenitors have been considered potential sources for the recovery of genetic diversity (Dreisigacker et al. 2008; Ortiz et al. 2008; Warburton et al. 2006; Zhang et al. 2006). The production of synthetic hexaploid (SH) wheats goes back to the 1940s but is recently considered a strategic approach to exploit germplasm of wild wheat progenitors in commercial breeding programs (Mizuno et al. 2010; van Ginkel and Ogbonnaya 2007; Warburton et al. 2006; Xie and Nevo 2008; Yang et al. 2009). SHs are produced by crossing tetraploid wheat (*T. turgidum* L., 2n = 4× = 28, A and B genomes) with diploid goatgrass (*Aegilops tauschii* Coss., 2n = 2× = 14, DD genomes) followed by chromosome doubling of the F_1 hybrid. The resulting synthesized hexaploids provide a rich source of genetic variation and can be readily hybridized with elite bread wheat cultivars and germplasm. Breeders have exploited these sources for resistance to a wide range of biotic and abiotic stresses (Adhikari et al. 2003; Arraiano et al. 2001; Assefa and Fehrmann 1998, 2000, 2004; Berzonsky et al. 2004; Cakmak et al. 1999; Genc and McDonald 2004; Gororo et al. 2001; Konik-Rose et al. 2009; Lage et al. 2003, 2004; Lage and Trethowan 2008; Mujeeb-Kazi et al. 2001a; Mujeeb-Kazi et al. 2001b; Sotelo et al. 2009; Xu et al. 2006; Xu et al. 2004). Here, we further investigate the potential of SHs and derived breeding lines as sources of resistance to *M. graminicola* in commercial resistance breeding programs.

Material and methods

Mycosphaerella graminicola isolates, plant materials and experimental design

A global panel of 20 *M. graminicola* isolates (Table 1) was assembled and used to characterize the response of mapped *Stb* genes and compare their resistance spectrum with uncharacterized resistance to STB in SHs and derived breeding lines. A set of 20 wheat accessions comprising 13 *M. graminicola* differential cultivars, five SHs, and the susceptible checks cv. Taichung 29 and the hard red spring wheat cv. Kulm (Table 2), was tested in a triplicate seedling experiment using the set of isolate.

F_1 and F_2 plants, and an $F_{6:7}$ population of recombinant inbred lines (RILs) developed by single-seed descent were produced from a cross between the SH M3 and cv. Kulm. M3 (W-7976) was developed at CIMMYT by A. Mujeeb-Kazi and has the pedigree Cando/R143//Mexi'S'/3/*Ae. tauschii* (C122), whereas cv. Kulm was developed at North Dakota State University, Fargo, ND. The hexaploid wheat cv. Chinese Spring (CS) and CS chromosome 5A deletion lines 5AS-1, 5AS-3, 5AL-10, 5AL-12, and 5AL-17 (Endo and Gill 1996) were used to locate chromosome 5A markers to deletion bins.

The various wheat accessions were grown in VQB 7x7x8 cm TEKU® plastic pots with 10 linearly sown seeds per pot. RILs were planted in 5.5 × 5 cm round Jiffy® pots with three seeds per pot using a steam-sterilized peat/sand mixture. All plants were grown in a controlled greenhouse compartment with a 16/8 hour light/dark cycle supplemented with son-T Agro 400W lamps (Hortilux, Boca Raton, Florida, USA). Pre-inoculation temperature and relative humidity (RH) were 18/16° C (day/night rhythm) and 70% RH, while post-inoculation temperature and RH were 22/21° C and ≥ 85% RH, respectively. Plants were grown in an alpha lattice experimental design with pots as experimental units that were randomly arranged for each isolate-replication combination on separate parallel tables in the greenhouse compartment.

Inoculation procedures

Pre-cultures of each isolate (Table 1) were prepared in an autoclaved 100 ml Erlenmeyer flask containing 50 ml yeast-glucose (YG) liquid medium (30 g glucose, 10 g yeast per liter demineralized water). The flasks were inoculated using a small piece of frozen isolate mycelium maintained at -80°C and were placed in an incubated rotary shaker (Innova 4430, New Brunswick Scientific, USA) set at 125 rpm and 18°C for 5-6 days. These pre-cultures were then used to inoculate three 250 ml Erlenmeyer flasks containing 100 ml YG media per isolate that were incubated under the aforementioned conditions to provide enough inoculum for the seedling inoculation assays at growth stage (GS) 11 (Zadoks et al. 1974). The inoculum concentration was adjusted to 10^7 spores/ml in a total volume of 40 ml for a set of 18

Table 1. The original hosts and origin of the global panel of *Mycosphaerella graminicola* isolates used in the present study.

	Origin		
Isolate nr	Country	Location	Year of collection
IPO94218	Canada	Saskatoon	1994
IPO00003	USA	Colusa	2000
IPO00005	USA	Colusa	2000
IPO90006	Mexico	Toluca	1990
IPO90015	Peru	Unknown	1990
IPO87016	Uruguay	Dolores	1987
IPO86068	Argentina	Balcarce	1986
IPO99015	Argentina	Unknown	1999
IPO89011	Netherlands	Barendrecht	1989
IPO92004	Portugal	Casa Valhas	1992
IPO95054	Algeria	Berrahal	1995
IPO92034	Algeria	Guelma	1992
IPO88018	Ethiopia	Holetta	1988
IPO88004	Ethiopia	Kulumsa	1988
IPO95036	Syria	Minbeg	1995
IPO86013	Turkey	Adana	1986
IPO02166	Iran	Dezful, Safi Abad	2002
IPO02159	Iran	Gorgan, AqQaleh	2002
IPO95052[1]	Algeria	Berrahal	1995
IPO86022[1]	Turkey	Altinova	1986

[1]All isolates are bread wheat isolates except IPO95052 and IPO86022, which are durum adapted isolates.

plastic pots or 24 Jiffy® pots and was supplemented with two drops of Tween 20 (MERCK®, Nottingham, UK). The screening of the 20 wheat accessions as seedlings was conducted using the collection of 20 isolates (Table 1). Seedlings of the entire RIL population were initially tested with *M. graminicola* isolates IPO92004, IPO92034, IPO94218 and IPO88018, and the results of these pre-screening experiments were used to select the most appropriate isolates (IPO94218 and IPO88018) for the second and third replications that were also used to screen F_1 and F_2 seedlings.

Adult plant screening of the RILs and parents was carried out in a greenhouse experiment with three replications using *M. graminicola* isolate IPO88018 (0.6×10^6 spores/ml) at GS 47- 58.

Data collection and analysis

Wheat germplasm. Disease severity was evaluated 21 days after inoculation by estimating the percentage necrosis (*N*) and pycnidia (*P*) on the inoculated first leaves (GS 11-12) (Zadoks et al. 1974) in the seedling assays. Data were transformed to the logit scale for statistical analysis using Residual Maximum Likelihood (REML) variance component analysis (Genstat 13th edition, VSN International Ltd, Hemel Hempstead, UK.) Significant differences were determined using the Least Significant Difference (LSD) of back-transformed *N* and *P* values. Logit transformed data analysis resulted in minor changes between observed and processed data to cope with zero scores of *N* or *P*.

RILs. A total of 96 RILs were evaluated in the pre-screening (first replication) and 103 RILs in the second and third replications. Disease severity on the seedlings was evaluated 23 days post inoculation (dpi) by scoring *N* and *P* on the primary leaves. Latency periods (*NLP* and *PLP*: days between inoculation and first *N* and *P* appearance) were also determined in the second and third replications of the seedling assays. Adult plant responses - total leaf area covered with STB lesions - were scored on the flag leaves (F) or the second leaf layer (F-1) at 21 and 28 dpi. Bartlett's χ^2 test was employed to evaluate the homogeneity of replication error variances and calculated using the Excel formula option. Data homogeneous across replications

were subsequently averaged and used for QTL analysis (Chu et al. 2010; Friesen et al. 2009).

Molecular mapping in the RIL population. DNA was extracted from M3, cv. Kulm and the RILs as described in Faris et al. (2000). A total of 609 microsatellite (simple sequence repeat; SSR) primer pairs were tested on M3 and cv. Kulm to reveal polymorphisms. The microsatellite primers were derived from the following sets: GWM (Roder et al. 1998), WMC (Somers et al. 2004), HBG, HBD, HBE (Torada et al. 2006), CFA, CFD (Sourdille et al. 2004), BARC (Song et al. 2005), and FCP (Faris et al. 2010; Reddy et al. 2008; Zhang et al. 2009). Methods for PCR, polyacrylamide gel electrophoresis, and fragment visualization were as described in Lu et al. (2006). Primer pairs revealing polymorphism between M3 and cv. Kulm were subsequently used to genotype the 103 RILs.

A total of 284 of the 609 (47%) primer sets revealed polymorphisms and detected 349 marker loci (1.2 loci per primer set). Linkage analysis of the 349 loci was conducted using Mapmaker (Lander et al. 1987) for Macintosh and the Kosambi mapping function (Kosambi 1944) as described in Liu et al. (2005).

QTL analysis. Linkage maps consisting of 296 markers giving the most complete genome coverage were used to detect genomic regions associated with phenotypic means. Composite interval mapping (CIM) was performed using the computer program QGene (Joehanes and Nelson 2008). A permutation test with 1,000 permutations was conducted to determine that a critical LOD threshold of 4.7 in this population yields an experiment-wise significance level of 0.05.

Genotype to phenotype discrepancy. Analysis of the allelic marker segregation and concurrent phenotypic data of the RILs enabled us to study genotype to phenotype discrepancies with respect to STB resistance. We used all observed disease parameters (*N*, *P*, *NLP* and *PLP*) and distributed the RILs in statistically significant different ($\chi^2_{1:1}$) groups. Subsequently, marker segregation was superimposed on these

data to determine sliding windows of lower to upper limits of the aforementioned disease parameters for each isolate to determine the threshold values for segregation analyses.

Results

Wheat germplasm screen. All control inoculations resulted in excellent disease development enabling precise phenotyping of wheat germplasm and the Kulm/M3 RIL population. None of the differential cultivars was completely resistant to the global *M. graminicola* panel, whereas all SHs, including M3, were widely resistant to the entire set of isolates (Table 3). The number of identified *Stb* genes in each differential cultivar (Table 2) positively correlated with broader efficacy ($r = 0.75$, $P<0.01$; $N=13$, $df=11$) indicating that accumulation of *Stb* genes is a valid resistance breeding strategy. In contrast, the SHs showed a significantly different pattern for they were resistant to all *M. graminicola* isolates (Fig. 1, Table 3). We therefore focused further analyses on the cv. Kulm/M3 RIL population. The parental lines differed significantly for *N* (values for cv. Kulm and M3 ranging from 2.2-91.8 and 1.1-6.8, respectively) and *P* (values for cv. Kulm and M3 ranging from 0-37.5 and 0, respectively) over the 20 isolates (Fig. 1). This enabled the selection of isolates IPO94218, IPO92004, IPO88018 and IPO92034 for further analysis.

RIL screening. We produced 103 cv. Kulm/M3 RILs and 96 were inoculated with *M. graminicola* isolates IPO94218, IPO92004, IPO88018 and IPO92034 in the first replication (Fig. 2). The results of this experiment indicated that segregation ratios of *P* fit 1:1 ratios for *M. graminicola* isolates IPO92004, IPO88018 and IPO92034, suggesting segregation of a single genetic factor. The result with IPO94218, however, indicated that more genes could be involved. We, therefore, continued analyses for the second and third replications with *M. graminicola* isolates IPO88018 and IPO94218. Ranking of the RILs for *N* and *P* showed highly significant correlations for *N* and *P*, indicating that the same genetic factor(s) could control resistance to these isolates (Table 4).

Table 2. Hexaploid wheat germplasm that was tested with a global panel of 20 *Mycosphaerella graminicola* isolates to determine potentially new genes for resistance to septoria tritici blotch.

Line	Growth Habit[1]	Origin	*Stb* gene	Reference
Bulgaria 88	W	Bulgaria	*Stb1* (5BL) +*Stb6*	(Adhikari et al. 2004c; Chartrain et al. 2005b)
Veranopolis	S	Brazil	*Stb2* (3BS) +*Stb6*	(Adhikari et al. 2004b; Chartrain et al. 2005b)
Israel493	S	Israel	*Stb3* (7AS) +*Stb6*	(Adhikari et al. 2004b; Chartrain et al. 2005b)
Tadinia	S	USA	*Stb4* (7DS) +*Stb6*	(Adhikari et al. 2004a; Chartrain et al. 2005b; Somasco et al. 1996)
Cs Synthetic (6x)7D	S	China/USA	*Stb5* (7DS) +*Stb6*	(Arraiano et al. 2001b)
Shafir	S	Israel	*Stb6* (3AS)	(Brading et al. 2002)
Estanzuela Federal	S	Uruguay	*Stb7* (4AL)	(McCartney et al. 2003)
M6 synthetic (W-7984)	W	USA	*Stb8* (7BL)	(Adhikari et al. 2003)
Courtot	W	France	*Stb9* (2BL)	(Chartrain et al. 2009)
Kavkaz-K4500	F	CIMMYT	*Stb10* (1D) + *Stb12* (4AL) +*Stb6* +*Stb7*	(Chartrain et al. 2005a)
TE9111	S	Portugal	*Stb11* (1BS) +*Stb6* +*Stb7*	(Chartrain et al. 2005c)
Salamouni	S	Canada	*Stb13* (7BL) + *Stb14* (3BS)	http://wheat.pw.usda.gov/ggpages/awn/53/Textfile/WGC.html
Arina	W	Switzerland	*Stb15* (6AS) +*Stb6*	(Arraiano et al. 2007; Chartrain et al. 2005b)
Kulm	S	USA	*Susceptible parent*	
M3 synthetic (W-7976)	S	USA	*Stb16* (3DL) + *Stb17* (5AL)	This study
Nogal synthetic	W	France	*Unknown*	
FD 2054.3 synthetic	W	France	*Unknown*	
TA4152-19 synthetic	S	USA	*Unknown*	
TA4152-37 synthetic	S	USA	*Unknown*	
Taichung 29	S	Japan	*Susceptible check*	

[1]: S=Spring type; W= winter type; F=Facultative

Table 3. Phenotypic responses of wheat cultivars and synthetic hexaploids or derivatives to a global panel of 20 *Mycosphaerella graminicola* isolates. Significant differences are based on Least Significant Differences of back transformed logit values of *P*.

	Bread wheat isolates																		Durum wheat isolates	
	North and Latin America								Europe		North Africa				West Asia					
Cultivar	**IPO 94218**	**IPO 00003**	**IPO 00005**	**IPO 90006**	**IPO 90015**	**IPO 87016**	**IPO 86068**	**IPO 99015**	**IPO 89011**	**IPO 92004**	**IPO 92034**	**IPO 95054**	**IPO 88004**	**IPO 88018**	**IPO 95036**	**IPO 86013**	**IPO 02166**	**IPO 02159**	**IPO 86022**	**IPO 95052**
Bulgaria	0	6	0	0	0	10	8	0	4	0	1	1	3	6	24	13	13	4	0	0
Veranopolis	22	1	2.3	0	38	4	1	1	37.6	13	0	0	5	3	33	35	31	0	5	1
Israel 493	1	1	1	0	0	3	1	0	5	3	24	1	0	1	6	17	4	1	0	0
Tadinia	0	1	1	0	1	35	2	10	10	10	1	6	11	3	15	17	13	6	0	0
Cs/synthetic (6x)7D	25	3	1	0	1	2	1	1	0	1	6	2	4	1	12	12	16	1	2	2
Shafir	24	21	13	45	41	60	4	22	9	15	45	15	24	22	23	24	12	1	0	0
Estanzuela Federal	4	31	3	42	3	1	10	32	30	16	21	1	45	33	6	26	43	16	0	0
W-7984	26	20	7	8	6	12	8	19	6	11	35	28	17	3	58	35	26	7	0	0
Courtot	1	52	1	30	45	5	3	55	1	18	46	13	4	1	1	1	18	0	0	0
Kavkaz-K4500	1	11	0	23	2	0	2	21	6	8	2	0	19	2	1	0	0	0	0	0
TE9111	0	10	0	0	0	0	1	0	13	4	4	0	1	1	0	2	0	1	0	0
Salamouni	0	1	1	1	4	30	8	7	7	1	20	2	21	1	5	31	24	10	0	0
Arina	0	6	0	0	5	2	2	0	6	3	1	0	0	1	1	0	1	0	0	0
Kulm	37.5	23	16	7	21	13	31	19	22	22	26	5	1	16	31	33	0	1	0	0
M3	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Nogal	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
FHD 2054.3	0	1	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0
TA4152-19	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
TA4152-37	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Taichung 29	42	63	15	45	39	55	15	41	48	24	45	31	3	24	66	32	43	11	1	0

not significantly different from *P*=0% (P<0.05).

not significantly different from maximal *P* value (P<5%).

2.3<*P*<37.6 Significantly different from either *P*=0% or maximal *P* value (P<5%).

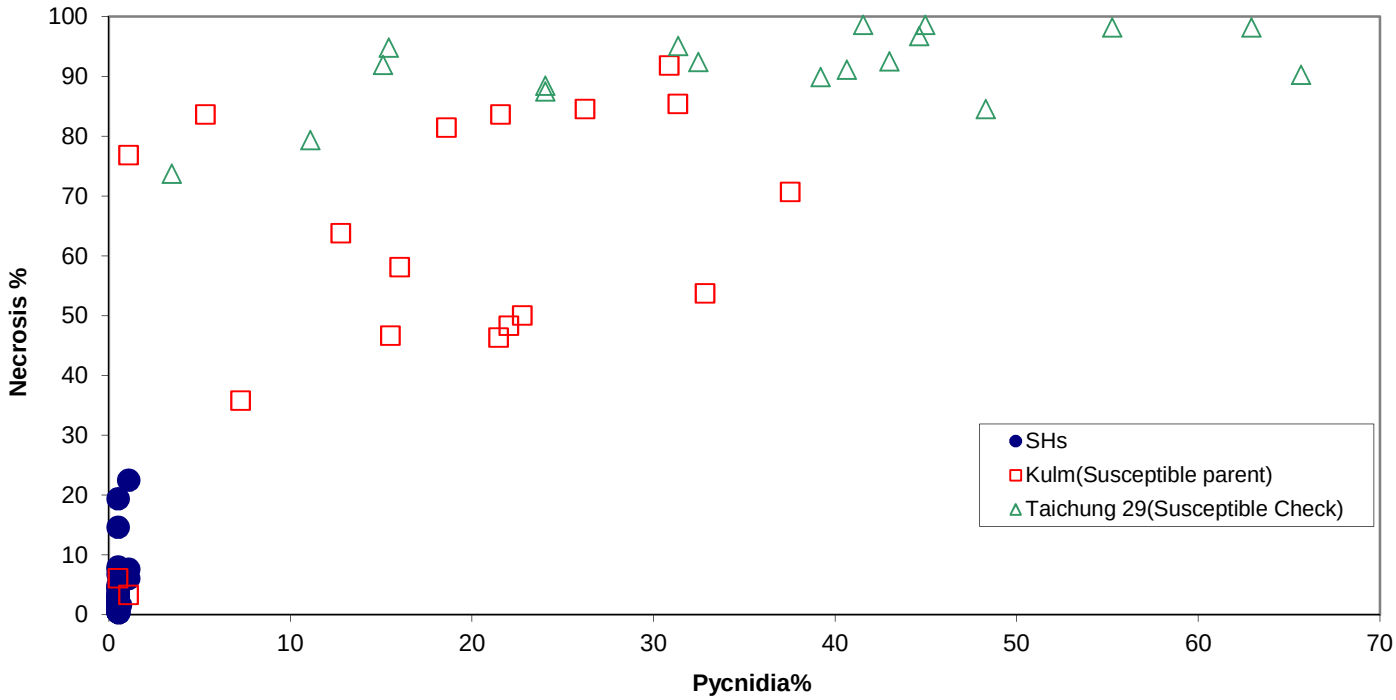

Figure 1. Scatter plot of *N* and *P* values of SHs and the cvs. Kulm and Taichung 29 after inoculation with 18 bread wheat *Mycosphaerella graminicola* isolates.

F_1 and F_2 screening. Four F_1 plants were inoculated with *M. graminicola* isolate IPO88018 and showed only minor tip leaf necrosis and no pycnidia formation at 21 dpi (data not shown). Thirty-two and 28 F_2 plants were then inoculated with *M. graminicola* isolates IPO88018 and IPO94218, respectively. Segregation ratios (resistant:susceptible) for *N* and *P* did not significantly differ from the expected 3:1 (Table 5), suggesting the inheritance of a single dominant gene.

Table 4. Correlation coefficients between ranked *P* values of 86 (96 – 10 missing values for some isolates) cv. Kulm/M3 RILs after inoculations with four *Mycosphaerella graminicola* isolates.

		N			
		IPO88018	IPO92004	IPO94218	IPO92034
IPO88018			0.77^{***}	$0.62^{***}(0.83^{***})^{1}$	0.68^{***}
IPO92004	P	0.68^{***}		0.59^{***}	0.61^{***}
IPO94218		$0.58^{***}(0.83^{***})^{1}$	0.56^{***}		0.53^{***}
IPO92034		0.70^{***}	0.61^{***}	0.62^{***}	

*** Significant at P=0.001

1 Correlation coefficient of the second and third replication between IPO88018 and IPO94218

Mapping and QTL analysis

Mapping. The 349 microsatellite markers were assembled into linkage groups representing the 21 hexaploid wheat chromosomes and spanned a genetic distance of 2,465 cM. Only chromosomes 3D and 5A were associated with STB resistance and these will be shown here, details of map construction and analysis will be published elsewhere. The genetic map of chromosome 3D in the cv. Kulm/M3 RIL population consisted of 27 markers spanning a genetic distance of 67.9 cM and included a cluster

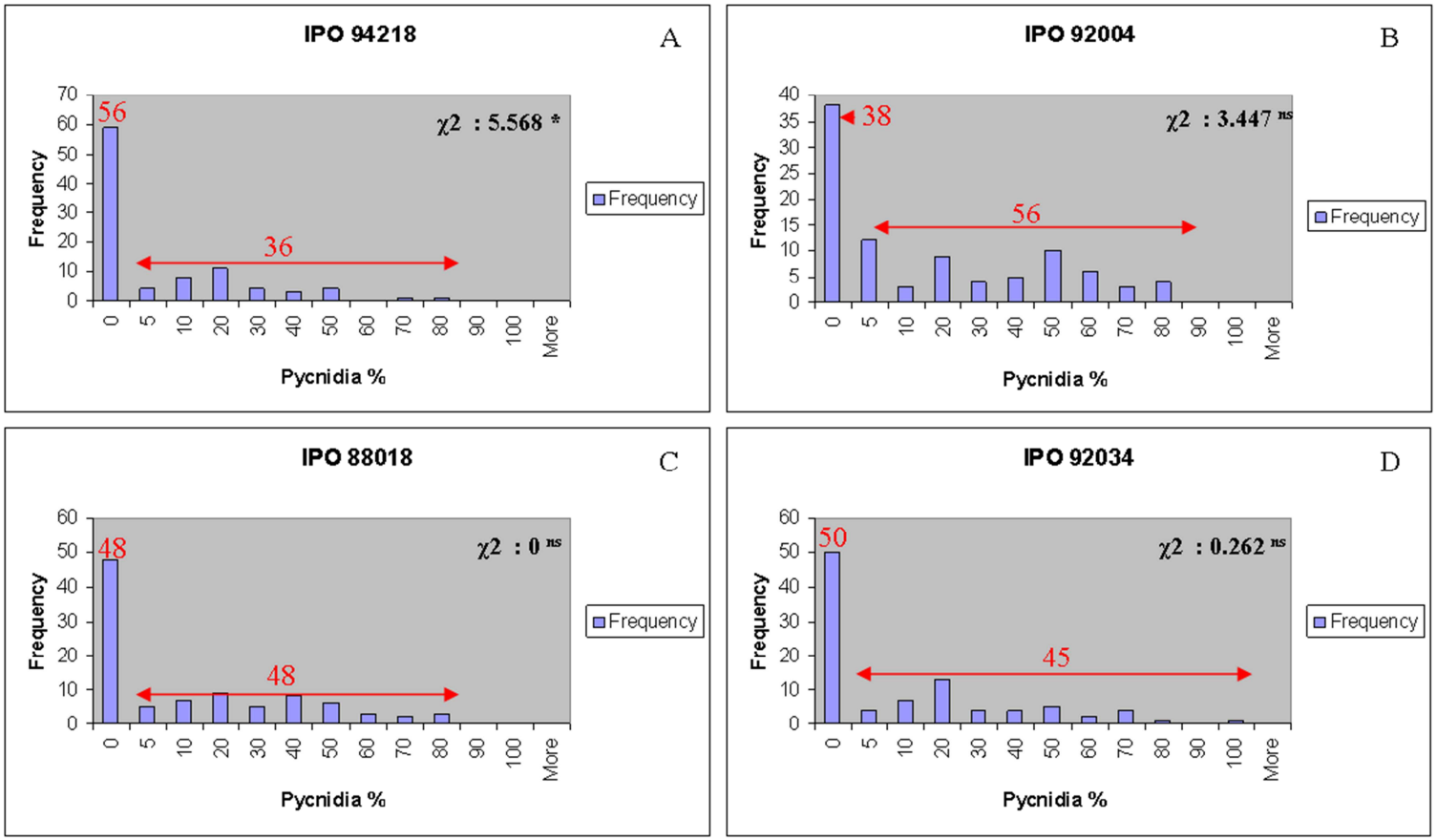

Figure 2. Pre-screening results (*P*) of the cv. Kulm/M3 RIL population with four *Mycosphaerella graminicola* isolates. Box A significantly deviates from a 1:1 ratio, whereas boxes B-D have segregation ratios that are not significantly different from 1:1 (based on χ^2test; P=0.05).

of 18 co-segregating markers near the distal end of the long arm (Fig. 3). Comparison with the 3D deletion-based physical map indicated that this suppressed recombination occurred across much of the long arm of chromosome 3D (Fig. 4). Closer evaluation of the 3D marker profiles indicated that most were codominant, and hence, there was no indication of a large deletion on chromosome 3D in either M3 or cv. Kulm. The linkage map of chromosome 5A consisted of 13 markers spanning 125.4 cM (Fig. 3). Of the markers mapped to 5A in the cv. Kulm/M3 population, only *Xbarc180, Xcfa2250, Xbarc141, Xgwm617, Xgwm595,* and *Xgwm291* were previously located on the deletion-based physical map (Sourdille et al. 2004). Therefore, we tested markers *Xhbd160, Xhbg247, Xhbg219, Xbarc232, Xhbd150,* and *Xwmc524* on the 5A deletion lines to determine their locations on the physical map. Comparison of the cv. Kulm/M3 5A genetic map with the 5A physical map indicated that the genetic linkage map of 5A developed in the cv. Kulm/M3 population accounted for most of the chromosome (Fig. 4).

Table 5. Segregation analysis of the cv. Kulm/M3 F_2 population after inoculation with two *Mycosphaerella graminicola* isolates.

Isolates	Criteria	Number of plants		χ^2 (P=0.05)[1]
		No symptom	Symptom	
IPO 88018	*N*	22	10	0.67^{ns}
	P	28	4	2.67^{ns}
IPO 94218	*N*	19	9	0.76^{ns}
	P	23	5	0.76^{ns}

[1] χ^2 for single gene segregation 3R:1S ration when R is for resistance and S is for susceptible

Seedling resistance. QTL analysis using CIM indicated that, for both *M. graminicola* isolates IPO88018 and IPO94218, markers located on the long arm of chromosome 3D were significantly associated with *N*, *P*, *NLP*, and *PLP* in seedlings (Table 6, Fig. 3). The QTLs peaked at position 58.0 cM between SSR markers *Xwmc494* and *Xbarc125* for each trait (Figs. 3 and 4), and resistance effects were derived from M3. LOD values were highly significant ranging from 11.7 to 22.3 for the phenotypes caused by isolate IPO94218 and 19.0 to 27.0 for those caused by isolate IPO88018 (Figure 3, Table 6). The QTL explained from 41 to 64% of the phenotypic variation for the disease caused by isolate IPO94218, and 58 to 71% of the variation for disease caused by isolate IPO88018.

Adult plant resistance. QTL analysis of adult plant reactions to *M. graminicola* isolate IPO88018 indicated that the resistance locus on 3DL identified at the seedling stage, was also significantly associated with resistance at both the 21 and 28 dpi readings (Fig. 3, Table 7). The QTL peaked at the same cM position as for the seedling data for both isolates and had LOD values of 7.2 and 8.4 for the 21 and 28 dpi readings, respectively. The locus explained 28% of the variation in STB at 21 dpi, which increased to 31% at 28 dpi. In addition to the resistance locus on 3DL, an additional QTL associated with adult plant resistance derived from M3 was identified on the long arm of chromosome 5A (Fig. 3). The 5AL QTL had a LOD value of 3 and explained 12% of the variation at 21 dpi, but had stronger effects at 28 dpi with an LOD of 8.9, explaining 32% of the variation (Table 7). The 5AL QTL was flanked by SSR markers *Xgwm617* and *Xhbg247*, and it peaked approximately 3.1 cM proximal to *Xhbg247* (Fig. 3). Comparisons between the genetic and physical maps indicated that this QTL was located in the deletion bin defined by the breakpoints in deletion lines 5AL-10 and 5AL-17, which is in the distal half of 5AL (Fig. 4).

Table 6. LOD and R^2 values for *Stb16* associated with broad-spectrum seedling resistance to *Mycosphaerella graminicola* in the recombinant inbred population derived from the cross between cv. Kulm and M3.

Data set	*Stb16*	
	LOD	R^2
Isolate IPO88018		
% *N* average	27.0	0.71
% *P* average	19.0	0.58
NLP average	20.7	0.61
PLP average	22.8	0.64
Isolate IPO94218		
% *N* average	22.3	0.64
% *P* average	11.7	0.41
NLP average	16.9	0.55
PLP average	18.9	0.59

Discussion

Here we report two new STB resistance genes that were derived from the SH wheat M3. Segregation and QTL analyses as well as genetic and physical mapping suggested that a single locus on chromosome 3D derived from M3 conferred resistance to all STB disease parameters in the seedling stage in the cv. Kulm/M3 RIL population. Since (i) no additional QTLs were significantly associated with any of the seedling phenotypes caused by either isolate in genome-wide scans, (ii) none of the known *Stb* genes were mapped on chromosome 3D and, (iii) the 3D QTL was highly significant and explained a large portion of the phenotypic variation, we propose to designate the underlying gene as *Stb16*. We determined substantial recombination suppression along the long arm of chromosome 3D, which is not due to a large deletion. However, it is possible that a large inversion exists in 3D of one of the parents, which could be the cause of the extreme suppression of recombination on

3DL. Due to the highly suppressed recombination along chromosome arm 3DL, comparison with the physical map of 3D yielded little additional information. Nevertheless, these results indicate that *Stb16* lies on the long arm of chromosome 3D and that it was derived from the *Ae. tauschii* accession C122, which was the donor of the D-genome chromosomes in M3. Zwart et al. (2010) also reported a SH derived QTL with multiple unrelated functions including STB resistance on chromosome 3D, but the LOD scores were relatively low and STB resistance was only tested with a single non-characterized *M. graminicola* isolate. Our study showed that *Stb16* had an unusually broad efficacy in the seedling stage as shown by the resistance to the global panel of isolates, and is also expressed in adult plants.

In addition we determined a QTL on chromosome 5AL that does not confer resistance to STB in seedlings, but specifically in adult plants. None of the previously characterized *Stb* genes was mapped on chromosome 5A (Arraiano et al. 2007; Chartrain et al. 2009; Goodwin 2007). Therefore, we conclude that this QTL likely represents a novel gene for STB resistance that we tentatively designate as *Stb17q*. The addition of 'q' was suggested in a recent community wide discussion on *Stb* nomenclature for cases where presented data do not unequivocally show that a single gene underlies a detected QTL e.g. due to a percentage of explained variation below 50%. *Stb17q* originated from the tetraploid durum wheat line used in the development of M3. Previous experiments conducted to compare seedling and adult plant STB resistance suggested the occurrence of specific seedling resistance loci, but no specific adult plant resistance genes were identified (Kema and van Silfhout 1997). All previously reported *Stb* genes are effective in the seedling stage and sometimes in adult plants (Adhikari et al. 2003;Adhikari et al. 2004a; Adhikari et al. 2004c; Arraiano and Brown 2006; Arraiano et al. 2001b; Chartrain et al. 2005b). Adult plant resistance is very common to other cereal diseases such as the rusts and has been associated with temperature sensitivity and other abiotic environmental factors (McIntosh et al. 1995). The regulation of adult plant resistance in wheat to STB is unknown and the efficacy of *Stb17q* to a wider set of isolates has to be determined. Nevertheless, we can conclude that *Stb17q* is the first authorized adult plant-specific STB QTL to be identified.

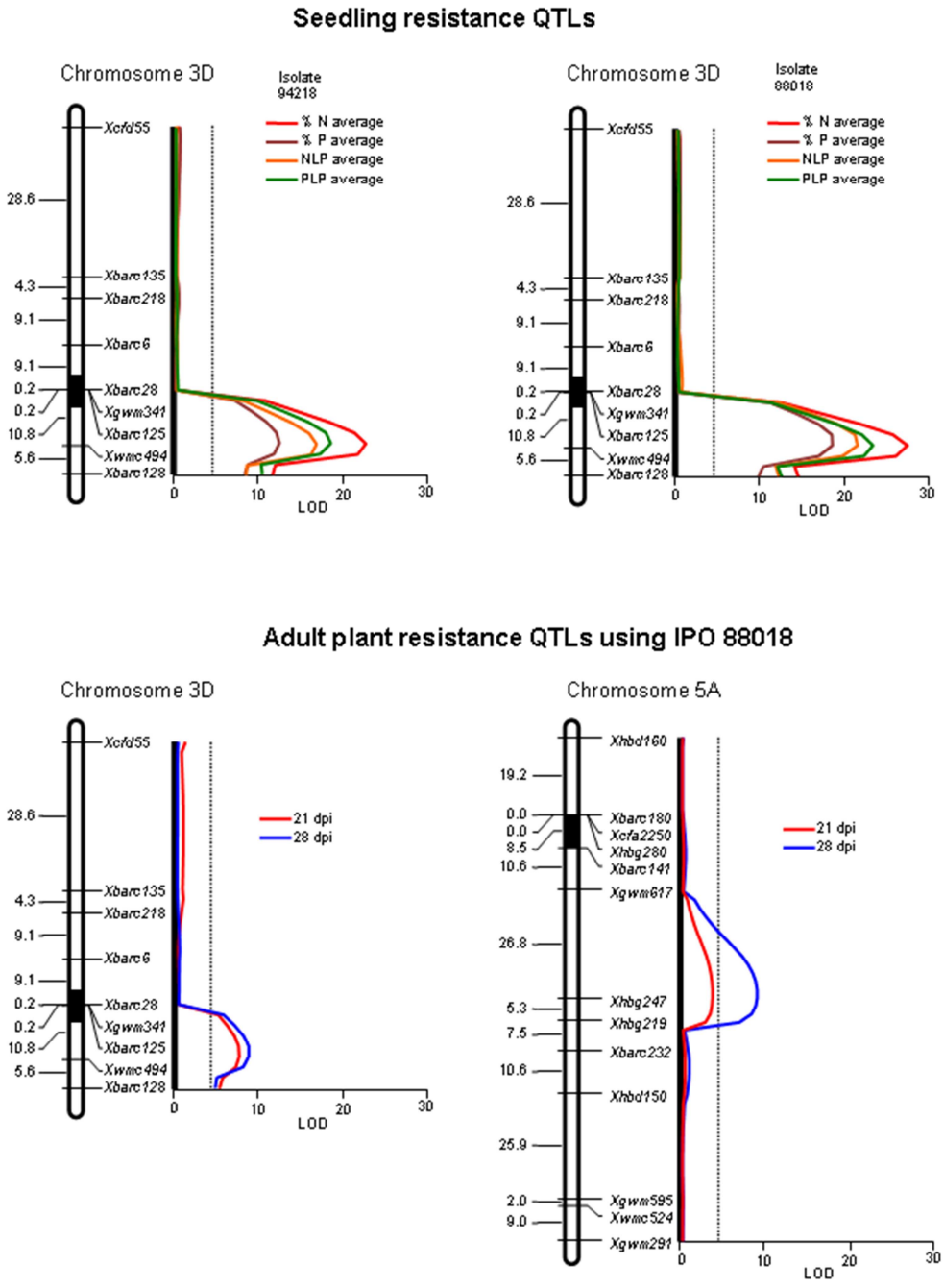

Figure. 3. LOD profiles of detected QTLs associated with resistance to *Mycosphaerella graminicola* isolates IPO94218 and IPO88018 on chromosomes 3DL in the seedling as well as 3DL and 5AL using IPO88018 in the adult plant stage.

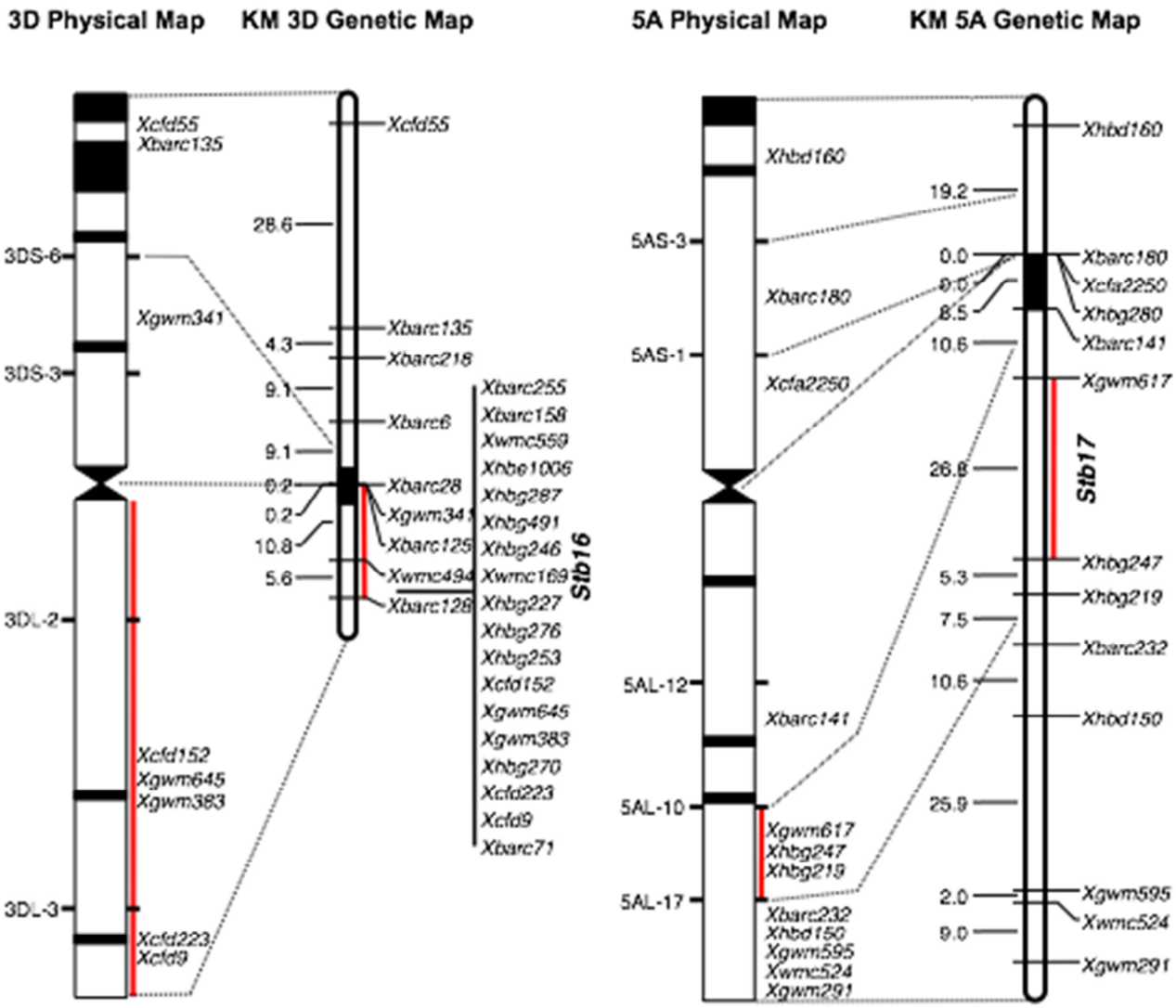

Figure 4. Comparison of the Chinese Spring chromosome 3D and 5A deletion-based physical maps with the 3D and 5A genetic linkage maps developed in the cv. Kulm/M3 population. Deletion breakpoints are indicated to the left of the physical maps and bin-located markers are shown along the right. On the linkage maps, cM distances are shown along the left and markers along the right. The QTL regions associated with STB resistance are indicated by the red lines.

Table 7. Genes for adult plant resistance to *Mycosphaerella graminicola* isolate IPO88018 in the recombinant inbred population derived from the cross between cv. Kulm and M3.

Gene/Chromosome arm	Marker interval	Position (cM)	Resistance source	LOD (21dpi/28dpi)	R^2 (21dpi/28dpi)	Additive effect (21dpi/28dpi)
Stb16/3DL	*Xbarc125-Xbarc128*	58.0	M3	7.2/8.4	0.28/0.31	7.4/11.9
Stb17/5AL	*Xgwm617-Xhbg247*	62.0	M3	3.0/8.9	0.12/0.32	4.5/12.3

Interestingly, the response of M3 to the global panel of *M. graminicola* isolates was very similar to those of the other tested SHs. The broad resistance spectrum of *Stb16* might be due to the apparent dichotomy of host specificity in the wheat-*M. graminicola* pathosystem. Kema et al. (1996a; 1996b) summarized and extended these observations and showed that *M. graminicola* isolates are in general either pathogenic on bread wheat or durum wheat. Recently, Wittenberg et al. (2009) and Ware (2006) showed that genetic recombination during sexual reproduction in *M. graminicola* easily results in progeny with altered cultivar and host specificity. However, tetraploid wheats are in general resistant to *M. graminicola* isolates derived from bread wheat and *vice versa*. This was confirmed in the current experiments because neither of the durum wheat-derived isolates IPO86022 and IPO95052 were virulent on any of the tested bread wheat accessions including the susceptible parent cv. Kulm and the susceptible check cv. Taichung 29. Therefore, a SH is expected to be resistant to such bread wheat derived *M. graminicola* isolates unless the D genome component affects the expression of resistance, which has been shown for rust diseases (Kerber and Green 1980, Kema et al. 1995). Assefa and Fehrmann (1998) also documented broad-spectrum resistance to *M. graminicola* (99% of 194 accessions) in seven *Aegilops* species, while only 8, 11, 16 and 24% of this collection was resistant to stem rust, leaf rust, eyespot and powdery mildew, respectively. Similar broad spectrum resistance was observed in phenotypic screens of the diploid wheat *T. monococcum*, which led to the identification of the resistance locus *TmStb1* and the linked microsatellite locus *Xbarc174* on chromosome $7A^m$ (Jing et al. 2008). Because SHs effectively combine the genomes of tetraploid and diploid wheat progenitors and relatives (Mujeeb-Kazi et al. 1996; Yang et al. 2009), they may carry a reservoir of novel genes for resistance to *M. graminicola*. Despite the value of the genes that we discovered, exposure to *M. graminicola* populations may potentially enable the fungus to adapt and circumvent them (Wittenberg et al., 2009; Ware, 2006; McDonald and Linde 2002a, b; Linde et al. 2002; Zhan et al. 2007). Hence, their commercial deployment should take these observations into consideration to maximize their efficacy under practical conditions.

To date, there has been no report of mapping host QTLs associated with life strategy parameters such as latency period and the lesion development rate of *M. graminicola*. Here, we characterized classical (*N* and *P*) and new parameters (*NLP*,

PLP) to investigate whether a major STB resistance gene also controls underlying pathogenicity factors, which is relevant, as resistance to STB is characterized by the absence of the hypersensitive response (HR) (Kema et al. 1996d). Interestingly, all the analyzed parameters mapped to the *Stb16* locus. In the absence of the HR, resistance is achieved by reducing the development of fungal biomass, which may occur by reducing infection rates. Such partial, or 'horizontal', resistance has been observed in some cereal rust interactions (Aghnoum and Niks 2010; Marcel et al. 2008). One of the best-known 'slow rusting' loci is the *Lr34/Yr18/Pm38* complex (Singh et al. 2007), which confers partial resistance to stripe rust, leaf rust and powdery mildew. Molecular cloning of the *Lr34/Yr18/Pm38* locus indicated that it is a unique functional ABC transporter (Krattinger et al. 2009; Lagudah et al. 2009). On the contrary, genes that confer complete, or 'vertical', resistance to pathogens with biotrophic lifestyles and susceptibility to necrotrophic pathogens usually harbor NBS and LRR domains (Bent and Mackey 2007; Jones and Dangl 2006; McDowell and Simon 2006; Lorang et al. 2007; Nagy and Bennetzen 2008; Faris et al. 2010). *Tsn1*, a gene controlling sensitivity to a host-selective toxin produced by the necrotrophic fungal pathogens *Stagonospora nodorum* and *Pyrenophora tritici-repentis* has resistance gene-like features including protein kinase and NBS-LRR domains (Faris et al. 2010). Interestingly, *S. nodorum*, *P. tritici-repentis* and *M. graminicola* are close relatives and belong to the Dothideomycete class of fungi. However, nothing is currently known about the molecular characteristics of *Stb* resistance genes. Therefore, the wide efficacy of *Stb16* and the abovementioned findings call for the unveiling of the molecular structure and a further understanding of the resistance mechanism exerted by these new genes for resistance to STB.

Because the *M. graminicola*-wheat pathosystem is characterized by the absence of an HR, resistance and susceptibility are currently usually expressed on a quantitative scale. However, symptom expression is strongly affected by environmental fluctuations and hence repeatability of experiments might be low (Arraiano et al. 2001a; Bearchell et al. 2005; Czembor et al. 2010; Kema et al. 1996a). Early reports determined an arbitrary threshold of resistance and susceptibility by using a 0-5 scoring scale (Rosielle 1972) that was more qualitative than quantitative. Later, applications of complex statistics were used to turn qualitative data into qualitative determinants (Eyal and Levy 1987; Eyal et al. 1985; Yechilevich-Auster et

al. 1983). Eventually, Kema et al. (1996a, 1996b) used quantitative data in cluster analyses based on interaction components of analyses of variance to group isolates and cultivars with similar responses and hypothesized that *N* and *P* were controlled by different genetic factors in the fungal genome. This was later corroborated by formal fungal genetics (Kema et al. 2002; Kema et al. 2000; Wittenberg et al., 2009; Ware, 2006). Adhikari et al. (2003, 2004a, 2004b, 2004c) used a modified 0-5 scale, which considered pycnidia percentage and density, for the mapping of several *Stb* genes, but phenotypic classifications were not matched with allelic segregations of the associated markers. A detached leaf assessment method also has been established for the characterization and mapping of some *Stb* genes (Arraiano et al. 2001a; Chartrain et al. 2005a; Chartrain et al. 2005c; Chartrain et al. 2009). Essentially, all these phenotyping assays address the phenotyping *vs*. genotyping problem (Dowell et al. 2010). Here we had the opportunity to study phenotype/genotype variation in more detail using the allelic information of all RILs along with all observed disease assessment parameters. As *Stb16* controls all the observed disease parameters for a global panel of unrelated *M. graminicola* isolates, the phenotypes of RILs with alternative parental alleles at the *Xwmc494* locus are of interest. Our analyses indicated that lines with the *Xwmc494* allele from M3 had *P* values that ranged from 0 to 5 and *N* values from 0 to 30, with averages over both isolates of 1 and 15, respectively. On the contrary, RILs carrying the cv. Kulm allele for *Xwmc494* had values that ranged from 8 -70 *P* and 37-100 *N*, and averaged over both isolates of 30 and 80, respectively. We do not know the origin of such sliding disease parameter windows, but we cannot exclude phenotyping errors due to environmental fluctuations, despite the accordance of all replications. We can exclude genotyping errors and recombination events between the *Xwmc494* marker and *Stb16* as possible sources of error because our results indicate significant recombination suppression in this region evidenced by the fact that 18 SSR markers that co-segregated at a single locus on the genetic map were distributed across 3DL on the deletion-based physical map. However, unknown genetic modifiers could also play an important role in genotype to phenotype variation in wheat. What counts, however, is that despite the presence of *Stb16*, resistant plants may develop up to 5% *P* and 30% *N*, which is close to the lowest values for plants lacking *Stb16*, which had values as low as 8% *P* and 37% *N*. The application is that the distinguishing threshold between resistance and susceptibility in a given population should not be taken arbitrarily (Adhikari et al.

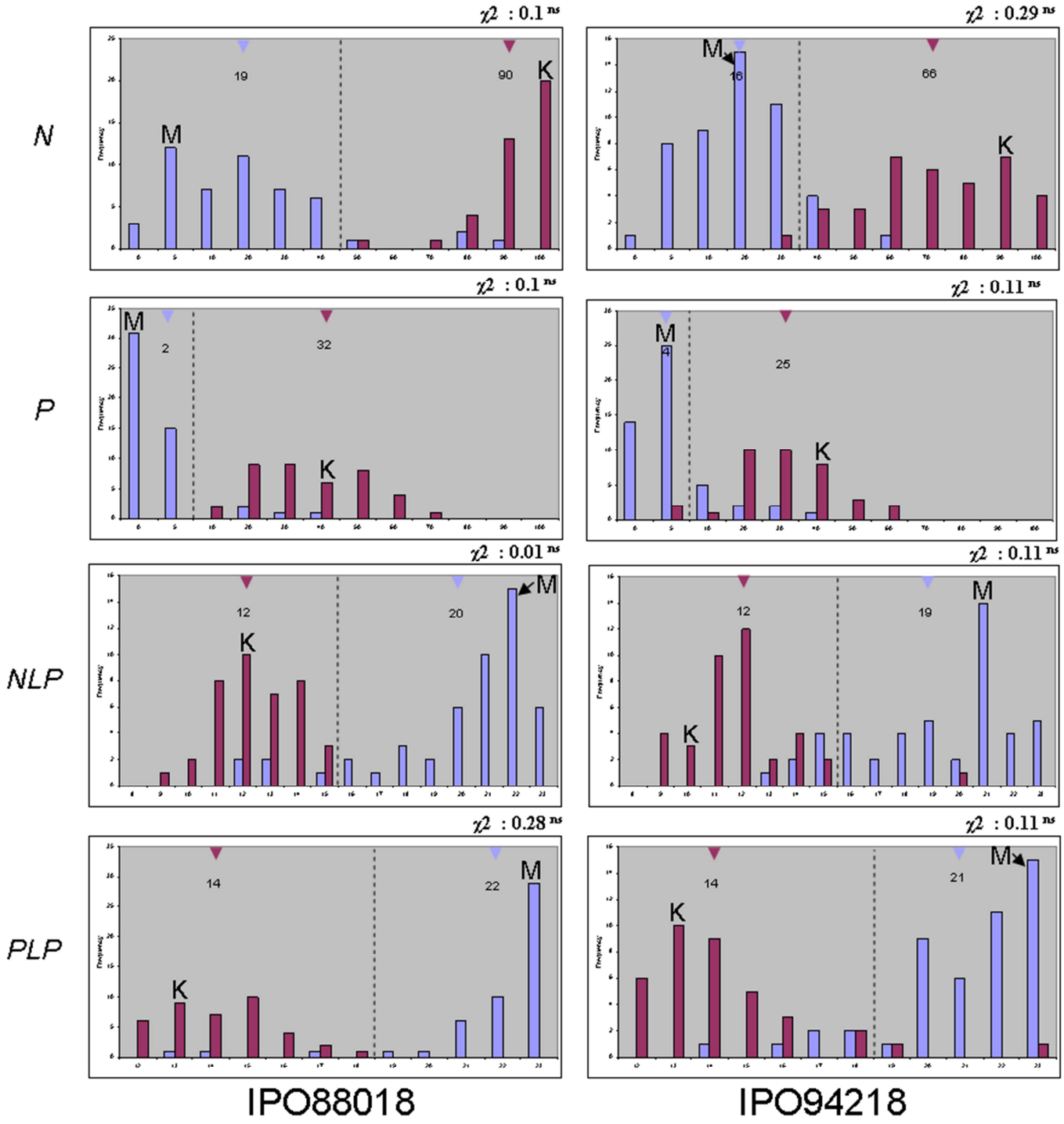

Figure 5. Segregation for *N*, *P*, *NLP* and *PLP* in the cv. Kulm/M3 RIL population inoculated with *M. graminicola* isolates IPO88018 and IPO94218 overlaid with allelic segregation of the *Xwmc494* SSR marker which is linked to *Stb16*. 'M' and 'K' indicate parental bin-values. Blue and purple triangles indicate average values of RILS with 'M' and 'K' alleles, respectively. The vertical dashed line is the $\chi^2_{1:1}$ validated threshold position between resistant and susceptible RILs.

2003; Adhikari et al. 2004b; Chartrain et al. 2005b), but ought to be based on appropriate genotype vs. phenotype analyses (Fig.5).

In conclusion, the present results show that *Stb16* and *Stb17q* are valuable new resistance genes that can be easily deployed in national and international marker-assisted resistance breeding programs. However, *M. graminicola* is classified as a high to moderate risk pathogen due to its multiple asexual and sexual cycles per year and its effective spore dissemination mechanism (McDonald and Linde 2002a, b), which enabled the fungus to circumvent *Stb* genes deployed in commercial wheat (Linde et al. 2002; Wittenberg et al. 2009; Zhan et al. 2007). We, therefore, discourage using *Stb16* or *Stb17q* as single genes, but rather suggest pyramiding strategies with other STB resistance genes in order to maximize their commercial life span.

Acknowledgments

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Chapter 4

Genetic analysis of resistance to septoria tritici blotch in the French winter wheat cultivars Balance and Apache

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Abstract

The ascomycete *Mycosphaerella graminicola* is the causal agent of septoria tritici blotch (STB), one of the most destructive foliar diseases of bread and durum wheat globally, particularly in temperate humid areas. A screening of the French bread wheat cultivars Apache and Balance with 30 *M. graminicola* isolates revealed a pattern of resistant responses that suggested the presence of new genes for STB resistance. Quantitative trait loci (QTL) analysis of a doubled haploid (DH) population with five *M. graminicola* isolates in the seedling stage identified four QTLs on chromosomes 3AS, 1BS, 6DS and 7DS and occasionally on 7DL. The QTL on chromosome 6DS flanked by SSR markers *Xgpw5176* and *Xgpw3087* is a novel QTL that now can be designated as *Stb18.* The QTLs on chromosomes 3AS and 1BS most likely represent *Stb6* and *Stb11*, respectively, and the QTLs on chromosome 7DS are most probably identical with *Stb4* and *Stb5*. However, the QTL identified on chromosome 7DL is expected to be a new *Stb* gene that still needs further characterization. Multiple isolates were used and show that not all isolates identify all QTLs, which clearly demonstrates the specificity in the *M. graminicola*-wheat pathosystem. QTL analyses were performed with various disease parameters. The development of asexual fructifications (pycnidia) in the characteristic necrotic blotches of STB, designated as parameter *P*, identified the maximum number of QTLs. All other parameters identified fewer but not different QTLs. The segregation of multiple QTLs in the Apache/Balance DH population enabled the identification of DH lines with single QTLs and multiple QTL combinations. Analyses of the marker data of these DH lines clearly demonstrated the positive effect of pyramiding QTLs to broaden resistance spectra as well as epistatic and additive interactions between these QTLs. Phenotyping of the Apache/Balance DH population in the field confirmed the presence of the QTLs that were identified in the seedling stage, but *Stb18* was inconsistently expressed and might be particularly effective in young plants. In contrast, an additional QTL for STB resistance was identified on chromosome 2DS that is exclusively and consistently expressed in mature plants over locations and time, but it was also strongly related with earliness, tallness as well as resistance to Fusarium Head Blight. Although to date no *Stb* gene has been reported on chromosome 2D, the data provide evidence that this QTL is only indirectly related to STB resistance. This study shows that detailed genetic analysis of contemporary

commercial bread wheat cultivars can unveil novel *Stb* genes that can be readily applied in marker-assisted breeding programs.

Introduction

Septoria tritici blotch (STB) is a fungal wheat disease that is caused by the ascomycete *Mycosphaerella graminicola* (Fuckel) J.Schröt. The disease was first described in France (Desmazieres 1842; Sprague 1938), but was neglected for a long time due to overwhelming stripe rust and powdery mildew epidemics. Thus STB was long considered as a secondary disease that mostly appeared in years with low levels of other cereal diseases. Nevertheless, it has been present in Europe for over a century, along with *Stagonospora nodorum* (Bearchell et al. 2005) and is currently considered to be one of the most important wheat diseases. Infections result in severe necrosis of the foliage that is filled with the asexual and sexual fructifications (Eyal 1999; Hunter et al. 1999; Kema et al. 1996c; McDonald et al. 1996; Shaw and Royle 1989). In Europe, STB usually establishes through airborne ascospores that are discharged from wheat debris and deposited in young wheat crops in the fall (Suffert et al. 2010). This is followed by rain splash driven spore dispersal during the growing season (Eriksen and Munk 2003; Halama 1996; Pastircak 2005; Scott et al. 1988; Shaw and Royle 1989, 1993). However, *M. graminicola* can reproduce sexually throughout the year, which provides the fungus with a mechanism to overcome adverse biotic or abiotic conditions (Kema et al. 1996c; Zhan et al. 2007; Ware et al. unpublished data).

STB management is largely effectuated by the application of fungicides and breeding for resistance. Due to its increased importance in Europe, STB is a main target as well as a serious concern of the agrochemical and breeding industry due to recent outbreaks of resistance to strobilurins (Cools and Fraaije 2008; Fraaije et al. 2005; Fraaije et al. 2007; McCartney et al. 2007; Stammler et al. 2008; Torriani et al. 2009) and steadily increasing levels of resistance to azole fungicides (Cools and Fraaije 2008; Fraaije et al. 2005; Fraaije et al. 2007; Mavroeidi and Shaw 2005; Stergiopoulos et al. 2003). These problems raised questions about the sustainability as well as the environmental impact of crop protection agents (Verweij et al. 2009). Hence, in several European countries, including France, Spain, Germany, Italy, the Netherlands and UK, pesticide reduction programs have been developed and adopted

by policymakers (Anonymous 2009). Therefore, a new focus on host resistance to increase the commercial lifetime of cultivars is required as part of a strategy to control STB.

In the UK, STB was unheard of as a major wheat disease before the late 1970s, but emerged as a major foliar blight in the early 1980s on susceptible cvs. such as cvs. Norman and Longbow. These cultivars were then replaced by others of similar susceptibility and significant progress in breeding for resistance was not made until the mid-1990s (Paveley 2006). Demands for cultivars with better resistance levels resulted in the release of cv. Claire in 1999 that was replaced by cv. Alchemy (Angus and Fenwick 2008). Currently, other high yield potential cultivars with moderate to high resistance to STB have been recommended, such as cv. Stigg (Anonymous 2010a; Angus et al., 2010). In France, 15 cultivars covered almost 77% of the total wheat acreage in 2003, in which cv. Apache ranked first with 23.7% and cvs. Isengrain, Tremy, Shango, Orvantis, Soissons, Caphorn and Charger together covered 37.3 % (Anonymous 2005). Recent resistance screens indicated that the majority of these cultivars are highly susceptible to a substantial number of isolates in the seedling stage, and hence, their resistances have a narrow efficacy (Tabib Ghaffary et al., unpublished data). Consequently, there is an urgent need for new resistance genes (Tabib Ghaffary et al., 2011 submitted to TAG).

The identification, characterization and processing of resistance to STB in practical wheat breeding programs, however, is not a routine issue and several parameters can be used for disease scoring, such as the percentage induced necrosis (*N*) or the percentage of pycnidia (*P*), the asexual fructifications of *M. graminicola*, in the foliage. Both parameters are strongly interwoven, as necrosis is conditional for pycnidia development, but are suggested to be under different genetic control (Kema et al. 1996d; Shetty et al. 2009; Shetty et al. 2003; Shetty et al. 2007). Currently, most screens involve well-characterized fungal isolates in repeated young plantlet assays and detached leaf assays (Arraiano et al. 2001a; Arraiano and Brown 2006; Kema et al. 1996a; Kema et al. 1996b; Kema and van Silfhout 1997), which have contributed to the data reliability and eventually to the mapping of resistance genes. Seedling screens offer opportunities to identify the efficacy of resistance to a wide panel of isolates, but - due to quarantine limitations - these can only be used to a limited extent under field conditions. Nevertheless, practical resistance breeding is a difficult multi-location, multi-pathogen and multi-pathotype effort responding to actual

epidemiological situations - for instance for cereal rust diseases - and even legislation such as for Fusarium Head Blight (FHB) (Vanloqueren and Baret 2008). This resulted in 88, 96, 64, 33 and 104 identified resistance genes for stripe rust, leaf rust, stem rust, hessian fly and powdery mildew, respectively, but only 17 *Stb* genes have been reported (Tabib Ghaffary et al. 2011, submitted to TAG). The majority of these *Stb* genes has a limited efficacy and hence are only sparsely deployed in breeding programs (Arraiano et al. 2007; Chartrain et al. 2009; Goodwin 2007), whereas the resistance genes to other wheat diseases are widely applied in new commercial wheat cultivars.

The apparent need for additional resistance genes prompted us to screen a wide variety of germplasm that resulted in the identification of new *Stb* genes (Tabib Ghaffary et al. 2011, submitted to TAG; Tabib Ghaffary, unpublished data). Here, we report the characterization of STB resistance in the French winter wheat cvs. Apache and Balance with 30 *M. graminicola* isolates and the identification of new *Stb* genes and associated molecular markers that can be readily applied in marker assisted breeding programs.

Materials and Methods

Plant materials and pathogen isolates

A double-haploid (DH) population of 91 lines derived was developed from a cross between cvs. Apache and Balance. Seedling assays were performed in a greenhouse compartment. The parental cvs. Apache and Balance, were planted in VQB 7x7x8 cm TEKU® plastic pots, 10 linearly sown seeds per pot, while the DH lines were planted in 5.5 x 5 cm round Jiffy® pots, five seeds per pot using a steam-sterilized peat/sand mixture. All plants were grown in a controlled greenhouse compartment with 16 hour/day light supplemented with son-T Agro 400 W lamps (Hortilux, Boca Raton, Florida, USA). Pre-inoculation temperature and relative humidity (RH) were 18/16°C (day/night rhythm) and 70% RH, whereas post-inoculation temperature and RH were 22/21°C and ≥ 85% RH, respectively. Adult plant experiments were carried out in 2007 and 2008 in Cappelle-en-Pévèle and Prémesques in Northern France at the breeding stations of Florimond Desprez and

Serasem, respectively. Each field plot contained two 1.5m length rows with 0.3m spacing.

Seedling evaluations involved deep screening of the parental cultivars with 30 monopycnidial *M. graminicola* isolates in 2007 and 2008 followed by a progeny evaluation in three replications, in which eight isolates were tested in the first replication (pre-screening) and five in subsequent replications (Table 1). In all seedling experiments, an alpha lattice experimental design was adopted that considered each pot as an experimental unit with random arrangement for each isolate-replication combination on separate parallel tables in the above mentioned greenhouse compartment. Field evaluations were performed with isolate IPO323 in a single replicated randomized block experiment in 2007 and a double replicated randomized block design at both locations in 2008.

Inoculation procedures and scoring

Pre-cultures of each isolate (Table 1) were prepared in an autoclaved 100 ml Erlenmeyer flask containing 50 ml yeast-glucose (YG) liquid medium (30 gr Glucose, 10 gr yeast per liter dematerialized water). The flasks were inoculated using a small piece of mycelium maintained at – 80°C and were incubated in a shaker (Innova 4430, New Brunswick Scientific, USA) adjusted at 125 rpm and 18°C for 5-6 days. These pre-cultures were then used to inoculate three 250 ml Erlenmeyer flasks containing 100 ml YG media per isolate that were incubated under the aforementioned conditions to provide enough inoculum for the seedling inoculation assays at growth stage (GS) 11 (Zadoks et al. 1974). The inoculum concentration was adjusted to 10^7 spores/ml in a total volume of 40 ml for a set of 18 plastic pots or 24 Jiffy® pots and was supplemented with two drops of Tween 20 (MERCK®, Nottingham, UK).

Field inoculations were performed with a backpack air-pumped sprayer, which was calibrated at a rate of 10 L/100 m^2 at flag leaf appearance stage (GS 47-49), using a concentration of 10^6 spores/ml supplemented with 36 ml of four times diluted Tween 20 (MERCK®, Nottingham, UK) surfactant. Inoculations started when the foliage of the earliest DH lines developed and were subsequently repeated twice at 3-5 day intervals to compensate for earliness differences.

Table 1. *Mycosphaerella graminicola* isolate panels and their origin that were used for parental cultivars and Apache/Balance doubled haploid progeny screening.

Isolate code	Origin	
	Country	Location
IPO94218 [a]	Canada	Saskatoon
IPO00003 [a]	USA	Colusa
IPO00005 [a]	USA	Colusa
IPO90006 [a]	Mexico	Toluca
IPO90015 [a]	Peru	Unknown
IPO87016 [a,d]	Uruguay	Dolores
IPO86068 [a]	Argentina	Balcarce
IPO99015 [a]	Argentina	Unknown
IPO89011 [a,d]	Netherlands	Barendrecht
IPO92004 [a]	Portugal	Casa Valhas
IPO95054 [a]	Algeria	Berrahal
IPO92034 [a,c]	Algeria	Guelma
IPO88018 [a]	Ethiopia	Holetta
IPO88004 [a]	Ethiopia	Kulumsa
IPO95036 [a,c]	Syria	Minbeg
IPO86013 [a]	Turkey	Adana
IPO02166 [a]	Iran	Dezful,Safi Abad
IPO02159 [a]	Iran	Gorgan, AqQaleh
IPO95052 [a,e]	Algeria	Berrahal
IPO86022 [a,e]	Turkey	Altinova
IPO323 [b,d]	Netherlands	W.Brabant
IPO94269 [b]	Netherlands	Kraggenburg
IPO98022 [b,d]	France	Villaines la Gonais
IPO98046 [b,d]	France	St. Pol de Leon
IPO98047 [b]	France	Aire D'Havrincourt
IPO98094 [b,c]	France	Aire D'Havrincourt
IPO052461 [b*]	France	Unknown (Biogemma)
IPO052462 [b*]	France	Unknown (Biogemma)
IPO052463 [b*]	France	Unknown (Biogemma)
IPO052464 [b*]	France	Unknown (Biogemma)

[a]Used for parental screen in 2008, [b]used for parental screen in 2007, [b*]provided by Biogemma, Clermond-Ferrand, France, [c]used in pre-screening, [d]triplicated on DH lines, [e]durum wheat adapted strains.

Disease severity was evaluated 21 days after inoculation in the seedling and the adult plant stage (with some variation +/- two days depending on weather conditions). In the seedling stage, the percentages of necrosis (*N*) and pycnidia (*P*; asexual fructifications) were scored separately on the first leaves, as well as *NLP* and *PLP* (days between inoculation and first *N* and *P* appearance, respectively). In the adult plant stage the total percentage of STB symptoms on the flag leaf was recorded in 2007 and 2008 as well as earliness and tallness in 2008. Data loggers were installed at the flag leaf level to monitor the actual field conditions (RH and temperature at 10 min. intervals) throughout the experiments.

FHB was established by distributing maize debris among the plants during tillering in the adult plant experiment in 2008. Disease was rated as percentage infected spikelets per ear during STB assessments.

Mapping and QTL analysis

DNA was extracted from first leaf samples of cvs. Apache, Balance and the DH lines using the Promega Wizard® Magnetic DNA Purification System for Food (blc) according to manufacturer's instructions with slight modifications. Genetic polymorphism analyses were performed at Diversity Arrays Technology (DArT) version 2.3 and 3 (Triticarte Pty Ltd, Canberra, Australia) that were supplemented with additional SSR data. Mapping analyses were performed using JoinMap® 4 software with settings LOD≥3 (Log of Odds) for grouping as well as the maximum likelihood mapping option for linkage group generation (Van Ooijen 2006). The DArT markers with low quality parameters (ANOVA based P value <80) were removed form the data set (Akbari et al. 2006) and marker positions were compared and verified using the publicly available data bases at INRA (Anonymous 2010b) , Triticarte (Anonymous 2010c,d) and Grain Genes (Anonymous 2010e,f).

QTL analysis was performed using MapQTL® 5.0 (Van Ooijen 2004) using the interval mapping (IM) option for QTL position detection followed by MQM (Multiple QTL Model) after cofactor selection either by Automatic cofactor selection (ACS) or manual investigation of the marker alignment on the linkage groups where the peaks of IM QTLs were detected. Minimum significant LOD values were calculated by 1000 permutation tests to determine 5% probability thresholds for

seedling and adult plant stage experiments. The Excel formula option was used for Bartlett's χ^2 tests to determine homogeneity of replication error variances enabling QTL analyses with average or individual replicate disease scores (Chu et al. 2010; Friesen et al. 2009). The QTL profiles were drawn with MapChart 2.2 software (Voorrips 2002).

The explained variance (%) of a detected QTL strongly depends on the size of a tested population. For instance, the probability of detecting a QTL that explains 10% of the total variance in a population of 200 individuals is 0,8 (Van Ooijen 2004), but it decreases almost linearly with smaller populations (Charmet 2000; Cornforth and Long 2003; Dupuis and Siegmund 1999; Knapp et al. 1990; Van Ooijen 1992). Here, the size of the Apache/Balance population was limited (N=91). To increase the probability of QTL detection; (i) a wide range of isolates was used to screen the parents and a subset of eight highly distinctive isolates was selected for a pre-screening that was followed by tests with five of these isolates in subsequent replications, (ii) the most recent release of DArT markers was used (DArT marker V.3) that increased the genome coverage from 1497cM to 3431 cM, which strongly contributed to QTL detection and (iii) three replicated data sets were used for final QTL analysis that was preceded by Bartlett's test for homogeneity of these replicates.

Results

Mapping.

A total of 962 polymorphic markers between cvs. Balance and Apache, including 169 SSR and 793 DArT markers (231 and 562 DArT markers of polymorphic chip versions 2.3 and 3, respectively), were used for mapping. A genetic map with 36 linkages group was constructed (Appendix), containing 786 DArT and SSR markers (428 and 205 DArT markers of V3 and V2.3, respectively; as well as 153 SSR markers) covering 3431 cM of the total wheat genome. Hundred seventy-six markers (134 and 26 DArT markers of V3 and V2.3, respectively; plus 16 SSR markers) were excluded from mapping due to marker similarity (109 loci) or significant segregation distortions or unreliable DArT scores (67 loci).

Isolate selection and QTL analyses for seedling resistance to septoria tritici blotch

Disease development in all seedling assays was excellent with maxima of 100% *N* and 83% *P* on the susceptible checks. The field evaluations were prone to strong environmental fluctuations but resulted in adequate STB levels in 2007 and 2008 at both locations. The initial screening of parental cvs. Apache and Balance with 30 *M. graminicola* isolates showed a clear contrast (P=0.05) with 15 isolates (Tables 1 and 2). Nine isolates differentiated the parents for *N* and 12 showed significant differences for *P*. Finally, isolates IPO87016, IPO92034, IPO323, IPO98022, IPO89011and IPO98094 as well as IPO95036 and IPO98046 were selected for a single replicated pre-screening of the DH lines. QTL analysis with *P* phenotypic data resulted in five significant QTLs on chromosomes 3AS, 1BS, 6DS and 7D (7DS/7DL switch) with higher LOD values than the threshold (LOD=3.5) that was determined by permutation test at P=0.05 (Fig. 1, Table 3). The highest LOD values per QTL were obtained with isolates IPO323, IPO98022, IPO98046 and IPO87016 (Table 4), hence these isolates were selected, along with IPO89011 that also detected a major QTL on chromosome 6DS, to complete the data set with two additional replications.

Not all isolates detected all QTLs, which underscores the specificity in the *M. graminicola*-wheat pathosystem. The results clearly show that *P* is the most efficient parameter for QTL detection as nine QTLs were detected using this parameter compared to three for *N* (Table 3). Isolates IPO323 and IPO87016 specifically detected the 3AS and 1BS QTLs, respectively. With the exception of IPO87016, all isolates detected the 6DS QTL. The 7D QTLs were detected by isolates IPO98022, IPO89011 and IPO98046 but the genomic position of the associated marker is not consistent. Moreover, despite the fact that some isolates did not show a significant difference between both parents, DH analyses detected QTLs for *N* and/or *P*. For instance, IPO98046 induced a non-significantly different *P* level in both parents (Table 2), but in the DH analysis it detected the QTLs on 6DS and 7D. The 7D QTL, however, was not consistent in all replications (7DS or 7DL). Isolate IPO323 did not differentiate the parents for *N* but still detected the 3AS QTL in the DH analysis. *NLP* data enabled the detection of more QTLs than *N*, but *PLP* reduced their number compared to *P* (not shown). *NLP* and *PLP* also detected two additional minor QTLs with LODs of 4.8 and 3.9 on chromosomes 5A and 2B, respectively (not shown).

Table 2. Screening with 30 *Mycosphaerella graminicola* isolates from diverse origin resulted in significant differences (Δ) between the parental cvs. Apache and Balance (P=0.05, labeled *). Resistance source cv. Apache, Resistance source cv. Balance.

Isolate	Necrosis %			Pycnidia %		
	Apache	Balance	Δ	Apache	Balance	Δ
IPO00003	90	65	ns	7	5	ns
IPO00005	3	8	ns	0	1	ns
IPO02159	84	89	ns	0	20	*
IPO02166	62	33	ns	2	11	ns
IPO86013	91	77	ns	23	25	ns
IPO86022	6	27	ns	0	0	ns
IPO86068	4	57	*	1	2	ns
IPO87016	10	90	*	0	51	*
IPO88004	98	71	*	18	14	ns
IPO88018	13	97	*	1	14	*
IPO89011	54	15	ns	23	1	*
IPO90006	16	15	ns	0	2	ns
IPO90015	26	58	ns	4	7	ns
IPO92004	18	85	*	0	16	*
IPO92034	30	84	ns	0	27	*
IPO94218	5	22	ns	0	4	*
IPO95036	52	79	ns	8	31	ns
IPO95052	16	2	ns	0	0	ns
IPO95054	16	80	*	0	4	*
IPO99015	3	98	*	0	23	*
IPO323	100	96	ns	25	0	*
IPO94269	100	100	ns	13	19	ns
IPO98022	100	86	*	32	8	*
IPO98046	100	100	ns	24	44	ns
IPO98047	100	100	ns	16	10	ns
IPO98094	96	100	ns	32	10	*
IPO052461	100	98	ns	0	0	ns
IPO052462	100	100	ns	0	0	ns
IPO052463	100	58	*	0	0	ns
IPO052464	100	96	ns	0	0	ns

Table 3. Summary of detected quantitative trait loci for necrosis (*N*) and pycnidia (*P*) in the Apache/Balance mapping population with five *Mycosphaerella graminicola* isolates.

Chromosomal position	IPO 323	IPO 98022	IPO89011	IPO98046	IPO 87016
3AS	*N P*				
6DS	*P*	*N P*	*P*	*P*	
7D*		*P*	*P*	*P*	
1BS					*N P*

* The QTLs detected on 7D vary over isolates. Isolate IPO98022 detected a QTL on 7DS, while IPO89011 detected a QTL on 7DL and isolate IPO98046 detected QTLs on both 7DS and 7DL

The details of the mapped QTLs in seedling experiments are shown in Table 4. The LOD values and explained variances vary substantially with the applied isolates and also with the presence of additional QTLs. For instance the 6DS QTL explains only approximately 10% of the observed variation in the presence of the 3AS QTL in tests with IPO323, but close to 68 % in the presence of the 7DL QTL in tests with IPO89011. In tests with isolate IPO98046, both the 6DS and 7D (7DS/7DL switch) QTLs explain about 20% of the observed variation. Remarkably, in the case of the resistance to isolate IPO98046 the 6DS QTL is expressed in all replications whereas, the QTL on 7DS is identified in two of the replications (Table 4).

Since multiple QTLs in the Apache/Balance DH population (Tables 3 and 4) were detected, additional analyses of the interaction between these QTLs were performed (Figs. 3 and 4). Isolate IPO323 detected the 3AS and 6DS QTLs. Four groups that significantly differed in *P* were generated by averaging the *P* levels for all DH lines with and without the resistant and susceptible alleles of DArT marker *wPt-0836* and flanking SSR markers *Xgpw5176-Xgpw3087*. Marker *wPt-0836* is present in cv. Apache and diagnostic for its susceptibility. The absence of the resistance alleles from both parents resulted in 39% of *P*. The presence of the resistance alleles of the flanking markers linked to the 6DS QTL reduced it to 14% but without the DArT

marker *wPt-0836* (the QTL on 3AS) the average of *P* dropped to just 1%. Accumulation of both resistance alleles associated with the 6DS and 3AS QTLs did not significantly lower *P*. Hence, the 3AS QTL is epistatic over the 6DS QTL in the analysis with isolate IPO323. In tests with isolates IPO98022 and IPO89011, the 6DS QTL had a larger effect than the 7D QTL (7DS/7DL switch), but the presence of both QTLs lowered *P* to 7%. This shows that 6DS and 7D had an additive effect but the additive effect of the former QTL is much stronger as it has a higher LOD value. This was also shown for tests with isolate IPO98046 where the individual QTLs contributed equally to disease reduction, but the combination of both QTLs minimized the disease level. Eventually, the accumulation of four QTLs in the Apache/Balance DH population for average STB levels over all used isolates was tested, which clearly demonstrated that the pyramiding of the associated markers gradually and significantly reduced disease levels (Fig. 4).

Detection of QTLs associated with resistance to STB in the adult plant stage

Due to field size limitations, the Apache/Balance DH population was only tested with isolate IPO323 in both years. The weather conditions for STB development were conducive in both years, resulting in differentiating STB levels (quantified by the average severity of DH lines with/without 3AS associated DArT markers and with/without 6DS flanking SSR markers that were identified in the seedling stage with isolate IPO323). QTL analyses revealed three QTLs on chromosomes 3AS, 2DS and 6DS that were associated with STB resistance (Table 5, Fig. 2). The 3AS and 6DS QTLs were also detected at the seedling stage. The former QTL was consistently expressed at both locations in both years, but the latter QTL was only detected in 2008 at the Serasem location and, similar to the seedling analyses, explained a lower percentage of the observed variation. Interestingly, the 2DS QTL was exclusively and consistently detected throughout all adult plant tests, but was also significantly correlated with earliness (-0.48 and -0.25, P=0.05 at Florimond Desprez and Serasem, respectively), tallness (-0.36, P=0.05 at Serasem) and resistance to FHB (Fig. 2E). Subsequent regression analyses that fitted means of logit transformed STB values on earliness and tallness left no residual STB resistance effect for the 2D locus (p=0.359).

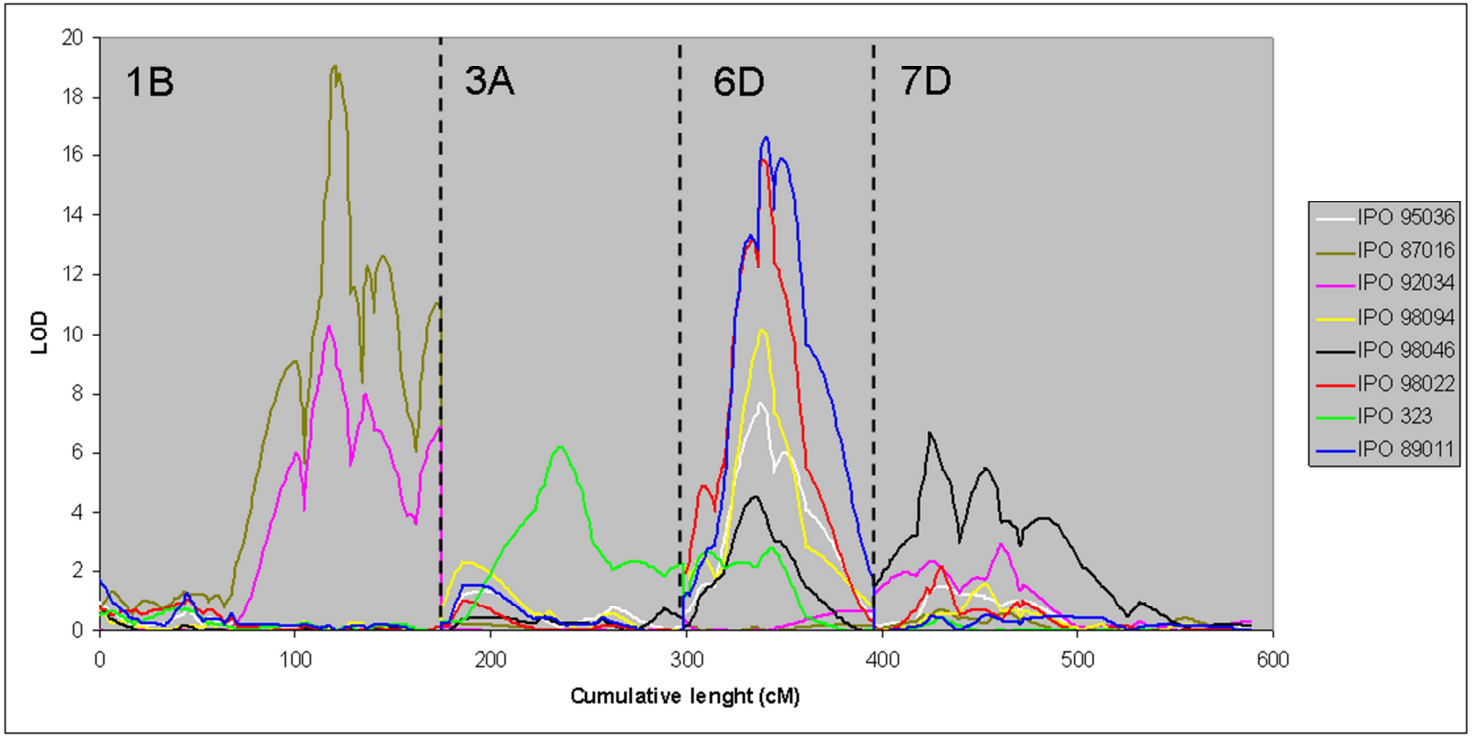


Figure 1. Interval mapping LOD profile of the Apache/Balance DH mapping population using eight *Mycosphaerella graminicola* isolates in a pre-screening test (*P*).

Table 4. Quantitative trait loci (QTLs) associated with necrosis (*N*) and pycnidia development (*P*) in the Apache/Balance DH population after inoculation with five *Mycosphaerella graminicola* isolates in the seedling stage.

Isolate	Closest Marker	Chromosome position	Phenotypic data set[1]	Resistance source	*N*			*P*		
					PD[2] (cM)	LOD	Exp. (%)	PD (cM)	LOD	Exp. (%)
IPO323	*wPt-0836*	3AS	R1	Balance	0	12.2	46.1	1	7.3	27.7
			R2		1	25.5	73.7	1	11.1	39.7
			R3		1	25.6	73.1	1	10.8	38.7
	Xgpw5176-Xgpw3087[3]	6DS	R1	Balance				3.2- 5	3.6	12.7
			R2					0.3- 8	3.1	8.9
			R3					4.3- 4	3.5	11
IPO98022	*Xgpw5176-Xgpw3087*[3]	6DS	R1	Balance	6.3- 2	6.4	30.4	5.3- 3	16.3	47
			R2		8.3- 0	5.4	21.6	5.3- 3	13.1	47.4
			R3		0.3- 8	4.4	18.8	5.3- 3	12.3	48
	Xgwm111	7DS	R1	Apache				1.1	6.2	11.8
			R2					0	5.2	11.2
			R3					0	2.2[4]	5.9
IPO89011	*Xgpw5176-Xgpw3087*[3]	6DS	Ave.	Balance				5.3- 3	23.16	67.5
	wPt-1859	7DL	Ave.	Apache				0	4.5	8
IPO98046	*Xgwm111*	7DS	R1	Apache				0	9.5	27.5
			R2					-[5]	-	-
			R3					0	6.2	20.8
	Xgpw313	7DL	R1	Apache				-	-	-
			R2					5	6.8	20.5
			R3					-	-	-
			Ave.							
	Xgpw5176-Xgpw3087[3]	6DS	R1	Balance				8.3- 0	7	19
			R2					8.3- 0	7.9	24.2
			R3					6.3- 2	7.4	27.1
IPO87016	*wPt-2019*	1BS	R1	Apache				2	19.1	63.3
			R2					1	21.1	68.3
			R3					0	17.8	59.3
			Ave.		1	21.11	67.3			

[1]R1, R2 and R3 represent first, second and third replicate data. QTL analysis was performed on averaged data (Ave) when Bartlett's χ^2 test indicated non significant phenotypic variation over replicates, otherwise replicates were processed individually. [2]PD = QTL peak distance in cM.; [3]Flanking markers.; [4]Not significant but consistent QTL position; [5]- = Non detected QTL in the repetition.

A

B

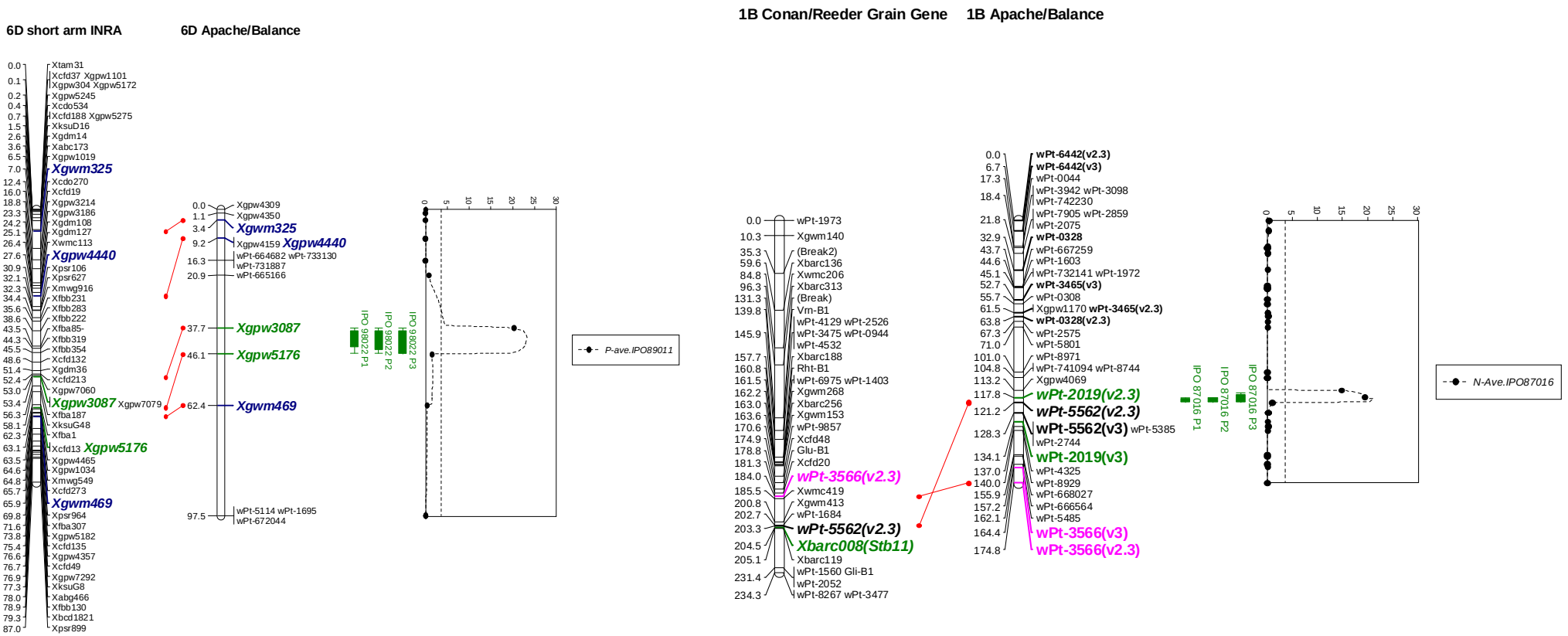
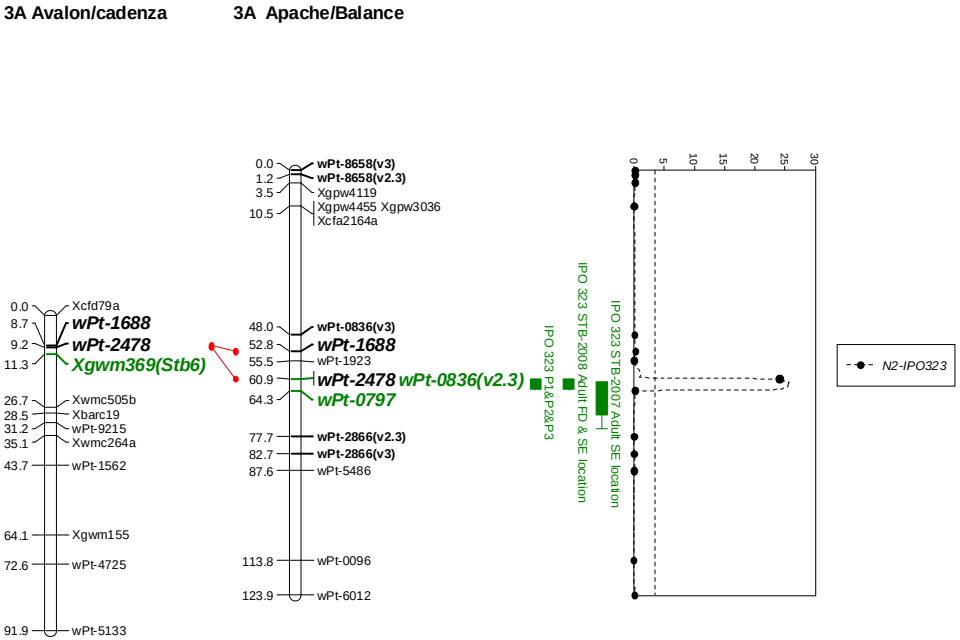
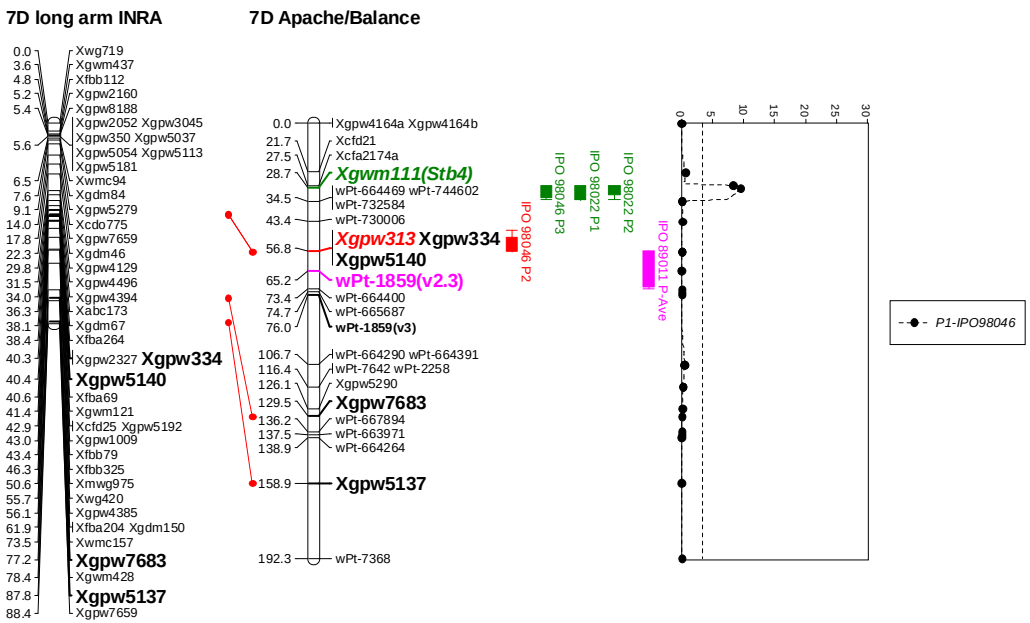


Figure 2. LOD profiles of QTL sections involved in STB resistance in the seedling (A, B, C, D) and adult plant stage (E) after individual inoculations with five *Mycosphaerella graminicola* isolates as well as earliness, tallness and Fusarium Head Blight (FHB) severity in the adult plant stage (E) in the Apache/ Balance DH population. *P* and *N* are disease parameters obtained from replicates 1, 2 and 3 or from the average (Ave) based on Bartlett's test (see Materials and methods).

C

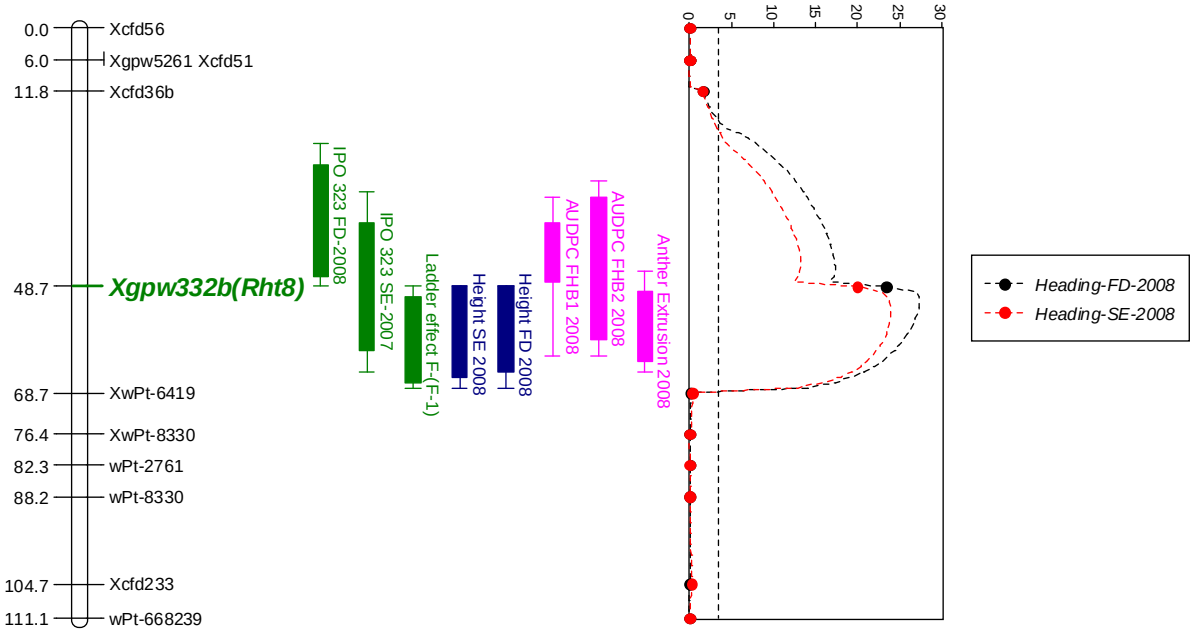

3A Avalon/cadenza
3A Apache/Balance
0.0 Xcfd79a
8.7 wPt-1688
9.2 wPt-2478
11.3 Xgwm369(Stb6)
26.7 Xwmc505b
28.5 Xbarc19
31.2 wPt-9215
35.1 Xwmc264a
43.7 wPt-1562
64.1 Xgwm155
72.6 wPt-4725
91.9 wPt-5133
0.0 wPt-8658(v3)
1.2 wPt-8658(v2.3)
3.5 Xgpw4119
10.5 Xgpw4455 Xgpw3036 Xcfa2164a
48.0 wPt-0836(v3)
52.8 wPt-1688
55.5 wPt-1923
60.9 wPt-2478 wPt-0836(v2.3)
64.3 wPt-0797
77.7 wPt-2866(v2.3)
82.7 wPt-2866(v3)
87.6 wPt-5486
113.8 wPt-0096
123.9 wPt-6012
IPO 323 P1&P2&P3
IPO 323 STB-2008 Adult FD & SE location
IPO 323 STB-2007 Adult SE location
0 5 10 15 20 25 30
N2-IPO323

D


7D long arm INRA
7D Apache/Balance
0.0 Xwg719
3.6 Xgwm437
4.8 Xfbb112
5.2 Xgpw2160
5.4 Xgpw8188
Xgpw2052 Xgpw3045
Xgpw350 Xgpw5037
5.6 Xgpw5054 Xgpw5113
Xgpw5181
6.5 Xwmc94
7.6 Xgdm84
9.1 Xgpw5279
14.0 Xcdo775
17.8 Xgpw7659
22.3 Xgdm46
29.8 Xgpw4129
31.5 Xgpw4496
34.0 Xgpw4394
36.3 Xabc173
38.1 Xgdm67
38.4 Xfba264
40.3 Xgpw2327 Xgpw334
40.4 Xgpw5140
40.6 Xfba69
41.4 Xgwm121
42.9 Xcfd25 Xgpw5192
43.0 Xgpw1009
43.4 Xfbb79
46.3 Xfbb325
50.6 Xmwg975
55.7 Xwg420
56.1 Xgpw4385
61.9 Xfba204 Xgdm150
73.5 Xwmc157
77.2 Xgpw7683
78.4 Xgwm428
87.8 Xgpw5137
88.4 Xgpw7659
0.0 Xgpw4164a Xgpw4164b
21.7 Xcfd21
27.5 Xcfa2174a
28.7 Xgwm111(Stb4)
wPt-664469 wPt-744602
34.5 wPt-732584
43.4 wPt-730006
56.8 Xgpw313 Xgpw334
Xgpw5140
65.2 wPt-1859(v2.3)
73.4 wPt-664400
74.7 wPt-665687
76.0 wPt-1859(v3)
106.7 wPt-664290 wPt-664391
116.4 wPt-7642 wPt-2258
126.1 Xgpw5290
129.5 Xgpw7683
136.2 wPt-667894
137.5 wPt-663971
138.9 wPt-664264
158.9 Xgpw5137
192.3 wPt-7368
IPO 98046 P2
IPO 98046 P3
IPO 98022 P1
IPO 98022 P2
IPO 89011 P Ave
0 5 10 15 20 25 30
P1-IPO98046

E

2D Apache / Balance



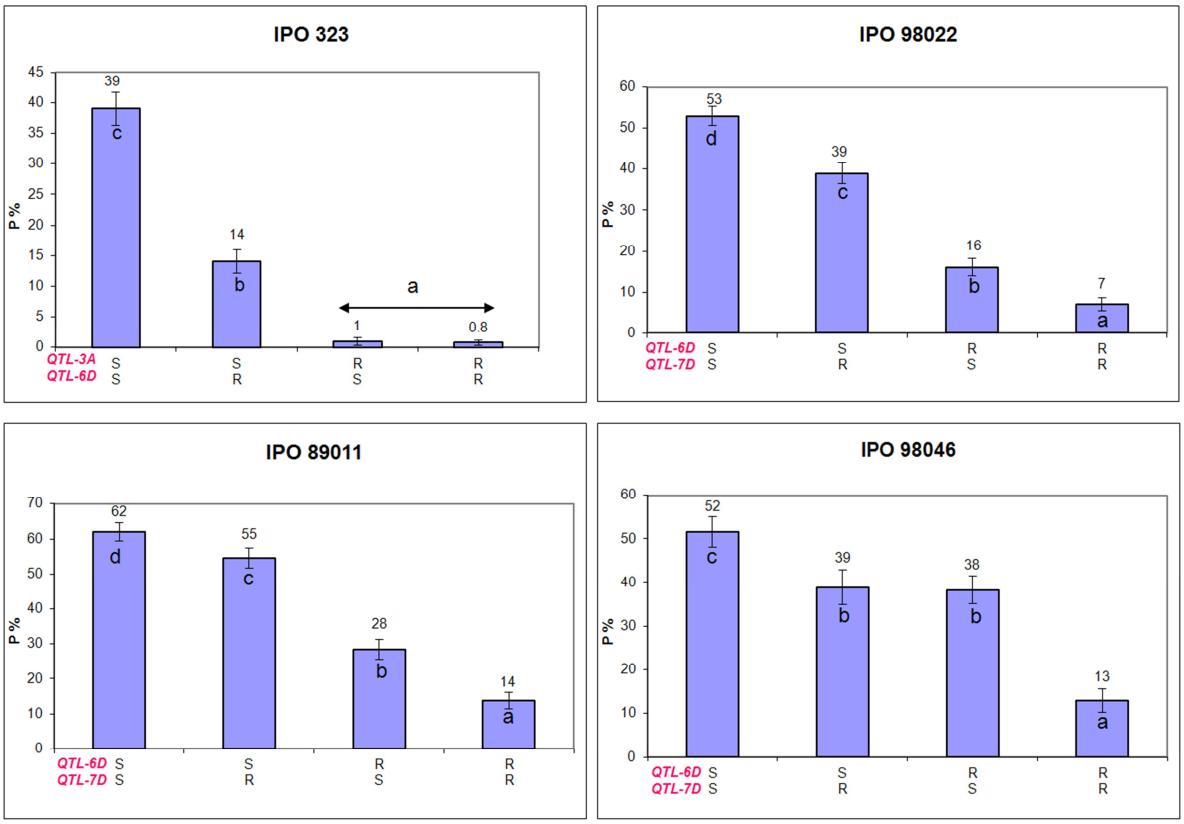


Figure 3. Various interactions between QTLs in the Apache/Balance DH mapping population detected by single isolates controlling *P*. SS, individual DH lines merely carrying susceptibility alleles of two markers associated with QTLs; RS and SR, individual DH lines carrying a resistance allele of a marker linked to one QTL and a susceptibility allele of a marker of another QTL; RR, individual DH lines with both resistance alleles. Same letters in the columns indicate not significantly different *P* values (P=0.05). A. Epistatic effect of the major QTL-3AS over the minor QTL-6DS detected by IPO323; B and C. QTLs detected by IPO98022 and IPO89011, respectively, show a mutual additive effect; D. Additive interaction between two QTLs with almost equal LOD scores.

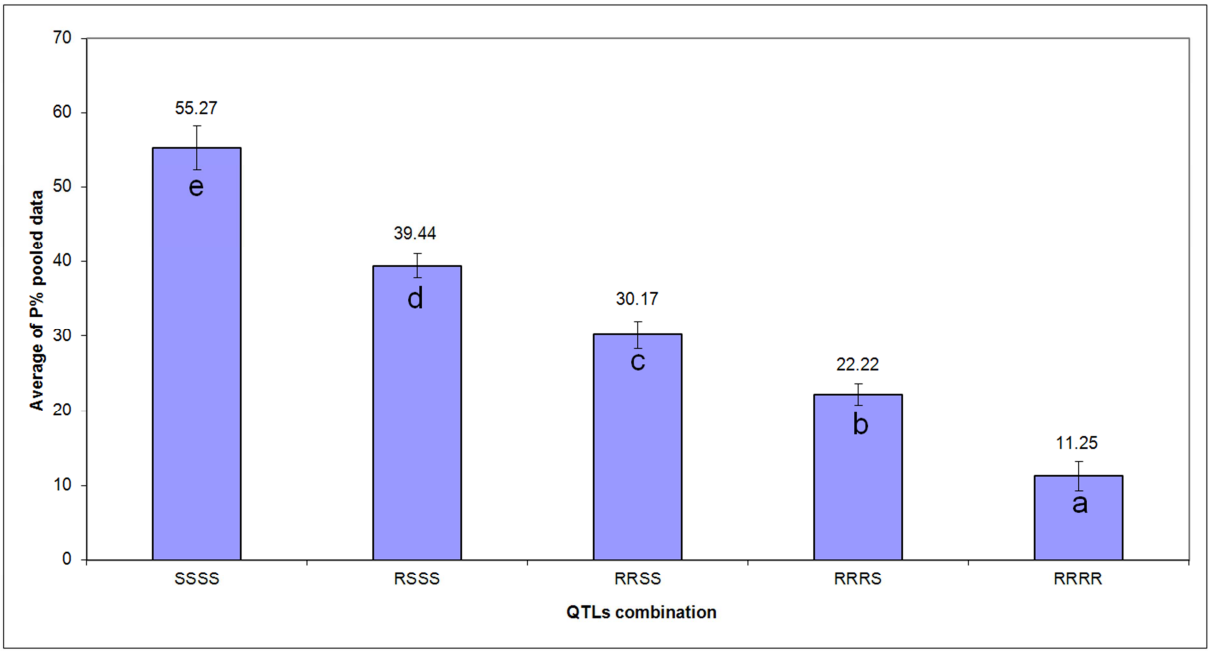


Figure 4. Stacking effect of individual QTLs that were detected in the Apache/Balance DH mapping population. Overall resistance to the five employed *M. graminicola* isolates (*P*) significantly (P=0.01) increased with the number of QTLs in DH lines. SSSS and RRRR; Representative of individual DH lines merely carrying markers of susceptibility or resistance alleles associated with QTLs on chromosomes 3AS, 6DS, 7DS (only the most common marker *Xgwm111*) and 1BS, respectively. RSSS and RRRS: indicate individual DH lines with combinations of one resistance allele linked to a QTL and three susceptibility alleles or vice versa. RRSS: indicates individual DH lines carrying two QTLs associated with resistance and two QTLs associated with susceptibility.

Discussion

The present data show that both cvs. Apache and Balance contributed specific resistance to the DH population. The resistance in both parents could be easily differentiated using the 30 *M. graminicola* isolates panel and enabled the selection of multiple isolates with significant differences that increased the detection of QTLs and helped to understand both the specificity of and interactions between these QTLs. So far, only 17 resistance genes and QTLs have been reported to STB (Arraiano et al.

2007; Chartrain et al. 2009; Goodwin 2007; Tabib Ghaffary et al. 2011, submitted to TAG) and there is a clear need for an extended arsenal of resistance genes to support resistance breeding. The QTL on chromosome 6DS is a new resistance gene as no other *Stb* gene has been mapped to this chromosome, except for the erroneous location of *Stb3* (Adhikari et al. 2004), that was later correctly assigned to chromosome 7AS (Goodwin 2007). Hence, the 6DS QTL that was detected in the French winter wheat cv. Balance with four *M. graminicola* isolates and is flanked by the SSR markers *Xgpw3087* and *Xgpw5176* is associated with a new resistance gene to STB that is designated as *Stb18*.

This is an isolate specific resistance gene that was detected with the French *M. graminicola* isolates IPO98022 and IPO98046 and with the Dutch isolates IPO89011 and IPO323. Isolate IPO89011 detected *Stb18* at the seedling stage, whereas IPO323 identified it in both the seedling and adult plant stage. IPO89011 is also avirulent on *Stb9* (Chartrain et al. 2009) and *Stb5* (Arraiano et al. 2001b), confirming the presence of multiple avirulence factors in *M.graminicola* isolates. Isolate IPO87016 from Uruguay is specifically virulent to *Stb18* as no QTL other than the 1BS QTL was detected with this isolate, which was also confirmed by additional phenotyping assays. In the adult plant stage, *Stb18* was detected only in 2007, but this is most likely due to the epistatic effect of the QTL on chromosome 3AS. All other QTLs also demonstrated gene-for-gene interactions that are operational in the *M. graminicola*-wheat pathosystem (Brading et al. 2002). Earlier findings that *P* rather than *N* is a reliable disease parameter (Kema et al. 1996a) are supported by the current data, as *Stb18* was only detected once for *N* but multiple times for *P*. Previously, Kema et al. (1996a) concluded that *N* and *P* are under different genetic control, which is in accordance with the current MapQTL analyses.

The publicly available map databases show that the flanking markers of *Stb18* on 6DS, *Xgpw3087* and *Xgpw5176*, have also been mapped on chromosomes 6A and 2D, respectively. However, in the Apache/Balance population these markers were linked with *Xgpw4440, Xgwm325, Xgpw4350*, *Xgpw43* and *Xgm469*, which are positioned on chromosome 6DS in the aforementioned linkage map databases. In the mapping process the marker alignment of chromosome 6DS was sorted by a LOD score of 4, indicating a 10,000 fold higher likelihood of linkage. It is therefore concluded that *Stb18* and its closest flanking markers *Xgpw3087* and *Xgpw5176*, are mapped on chromosomes 6DS.

Table 5. Quantitative trait loci (QTL) associated with resistance to STB evoked by inoculations with *Mycosphaerella graminicola* IPO323, earliness and tallness in the adult plant stage under field conditions.

		Flag leaf lesion									Earliness						Plant Tallness					
	Location	Seasem			Serasem			Florimond Desprez			Serasem			Florimond Desprez			Serasem			Florimond Desprez		
		2007			2008			2008			2008			2008			2008			2008		
Closest Marker	Chromosome	PD[1]	LOD	Exp %	PD	LOD	Exp %	PD	LOD	Exp %	PD	LOD	Exp %	PD	LOD	Exp %	PD	LOD	Exp %	PD	LOD	Exp %
Xgpw332b	2DS	2	8.9	30.9	6	3.5	7.8	11.9	6.7	30.9	5	24	77.5	3	27.3	79.3	6	5.6	24.8	7	6.56	23.3
wPt-0797	3AS	0	6.2	19.1																		
wPt-0836	3AS				1	14.2	35.6	1	8.6	28.6												
Xgpw5176-Xgpw3087[2]	6DS				8.3- 0	5.9	12.5															

[1]PD = QTL peak distance in cM.

[2]Flanking markers.

Another major QTL was detected and mapped on chromosome 1BS using isolate IPO87016. Previously, (Chartrain et al. 2005c) mapped *Stb11* on chromosome 1BS in the wheat line TE9111 and determined the linked SSR marker *Xbarc008* using the Mexican isolate IPO90012. In the Apache/Balance map, the identified 1BS QTL is associated with DArT marker *wPt-2019 (v2.3)* that is mapped next to DArT marker *wPt-5562 (v2.3)* (3.4 cM), which is tightly linked to *Xbarc008* (1.2 cM) in the Conan/Reeder reference map at the Grain Genes data base (Anonymous 2010e,f). Phenotypic interaction between IPO87016 and the *Stb* differential set of cultivars, also confirmed that IPO87016 is avirulent on TE9111 (Tabib Ghaffary et al. unpublished data) that is reported to carry *Stb11, Stb6* and *Stb7*, which are mapped on chromosomes 1BS, 3AS and 4AL, respectively (Chartrain et al. 2005c). The isolate IPO87016 is virulent on *Stb6* but avirulent on *Stb7* (Tabib Ghaffary et al. unpublished data). Therefore, the observed resistance in cv. Apache can be due to *Stb7* or *Stb11*. As the only detected QTL was positioned on chromosome 1BS and not on chromosome 4AL, we conclude that the QTL in cv. Apache represents *Stb11*, which was also confirmed by map comparison and additional phenotypic data. The QTL associated to *Stb11* in the Apache/Balance population is linked to DArT marker *wPt-2019* that can be used in addition to *Xbarc008* as an alternative for marker assisted selection.

The QTL on chromosome 3AS is associated with DArT marker *wPt-0836 (v2.3)*. This marker is clustered with *wPt-2478* that is also mapped in the Avalon/Cadenza reference map (Anonymous 2010d) close (2.1cM) to marker *Xgwm369* that was determined as a closely linked marker of *Stb6* (Brading et al. 2002). This gene confers resistance to isolate IPO323 and is prevalent among a worldwide set of cultivars and breeding lines (Arraiano and Brown 2006; Chartrain et al. 2005b; Eriksen et al. 2003). As no other gene has been mapped on chromosome 3AS in the Apache/Balance population, the 3AS QTL must represent *Stb6* in cv. Balance. Additional evidence is provided by the screening with the other isolates that did not detect the 3AS QTL and are all virulent on cv. Shafir that carries *Stb6* (Tabib Ghaffary et al. unpublished data). Unfortunately, the DArT marker *wPt-0836* cannot be used for detecting *Stb6*, as it is associated with susceptibility to isolate IPO323 in cv. Apache.

Adhikari et al. (2004a) and Arraiano et al. (2001b) have reported *Stb4* and *Stb5* on chromosome 7DS, respectively, and linkage with SSR marker *Xgwm111*,

which is recognized as a specific marker for *Stb4* (0.7 cM). This marker is also present on the Apache/Balance 7D linkage group and is associated with QTLs that was detected with isolates IPO98046 and IPO98022. IPO98046 is avirulent on cv. Tadinia (Tabib Ghaffary et al. unpublished data) that carries *Stb4* and *Stb6* (Arraiano and Brown 2006; Chartrain et al. 2005b), but virulent on cv. Shafir that carries *Stb6* (Brading et al 2002, Tabib Ghaffary et al. unpublished data). The present data confirm this observation, as IPO98046 did not, but IPO323 did detect *Stb6* on chromosome 3AS. Hence, the 7DS QTL that was detected with IPO98046 in cv. Apache seems identical with *Stb4*. *Stb5*, present in CS/synthetic 6x, is also reported on chromosome 7DS (Arraiano et al. 2001b). Despite isolate IPO98022 is avirulent on CS/synthetic 6x and detected a QTL on chromosome 7DS that is linked to SSR marker *Xgwm111*, it is dissimilar with *Stb5* as this isolate is virulent on cv. Tadinia that carries both *Stb4* and *Stb6* (Tabib Ghaffary et al. unpublished data). The QTL detected with IPO89011 is associated with DArT marker *wPt-1859* that is positioned amidst SSR markers on chromosome 7DL (Fig. 2D). No *Stb* genes have been mapped to this chromosome arm and hence, cv. Apache carries one or more unknown *Stb* genes on chromosome 7DL that require further characterization.

Finally, the data show that accumulation of QTL associated markers incrementally contributes to higher and broader levels of STB resistance. Chartrain et al. (2005a; 2004; 2005c) thoroughly analyzed STB resistance in cvs. KK4500 and TE9111. They describe several *Stb* genes in these cultivars and suggested that gene pyramiding might be an effective method of resistance breeding, but neither interactions between these genes nor phenotype/genotype associations were addressed. Still, KK4500 and TE9111 have relatively broad efficacy (Kema et al., 1996a, 1996b). This accords with our findings that *Stb* resistance gene accumulation is a valid strategy to breed for wide efficacy resistance in wheat to STB as was also shown in many other breeding programs dealing with other crops and various single or multiple biotic stresses (Barloy et al. 2007; Song et al. 2009). Therefore, a detailed characterization of known and new *Stb* genes is indispensable and contributes greatly to their deployment in marker assisted stacking strategies in commercial breeding programs.

QTL analysis in adult plants

The field experiments confirmed the presence of the 3AS and 6DS QTLs that were identified as *Stb6* and *Stb18*. The latter is inconsistently expressed in the presence of *Stb6*, which also provides mature plant resistance to specific *M. graminicola* isolates, as reported earlier (Arraiano and Brown 2006; Brading et al. 2002; Chartrain et al. 2005b). The new QTL on chromosome 2D was consistently and exclusively expressed in adult plants in both years at both locations. However, this QTL is strongly associated with earliness and tallness and regression analyses did not show a significant residual effect on STB resistance. We are therefore reluctant to assign STB resistance to the 2D QTL and rather suggest that it indirectly influences STB resistance by regulating earliness and tallness that are known to affect STB severity (Arama et al. 1999; Arraiano et al. 2009; Simon et al. 2005). The associated SSR marker *Xgpw332* is also associated with *Rht8* and *Pp1* that are involved in the regulation of wheat tallness and earliness (Korzun et al. 1998; Worland et al. 1988; Anonymous 2010g). These physiological parameters also influence FHB resistance (Somers et al. 2003; Steiner et al. 2004). Interestingly, a QTL for FHB resistance was mapped on the same position in the Apache/Balance population. Previously, Handa et al. (2008) identified a possible multidrug resistance associated protein (MRP) at this 2D chromosomal location that is involved in the wheat-Fusarium interaction. We tentatively conclude that the 2D QTL confers earliness/tallness in wheat and therefore indirectly contributes to multiple pathogen resistance.

This project showed that new *Stb* loci can still be identified in contemporary commercial wheat cultivars by using panels of carefully characterized *M. graminicola* isolates. Such screens also demonstrate the efficacy of *Stb* genes in various production environments and therefore contribute to STB resistance management.

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Chapter 5

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

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Molecular Breeding(in press)

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 on 4AL conditional on 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.

Introduction

Septoria tritici blotch (STB), caused by the ascomycete fungus *Mycosphaerella graminicola* (Fuckel) J. Schröt. (anamorph: *Septoria tritici*), is one of the most serious foliar diseases in wheat worldwide and may result in severe yield losses through reduction of the photosynthetic area (Eyal et al. 1987). High humidity and moderate temperature conditions are conducive to the spread of asexual pycnidiospores in the field and disease development (Palmer and Skinner 2002). Field populations of *M. graminicola* are genetically diverse due to a high level of sexual recombination (Zhan et al. 2003). Fungal resistance to strobilurins (Fraaije et al. 2005) and azoles (Zhan et al. 2006) has hampered the chemical control of the disease

by fungicides. Therefore deployment of effective resistance genes in wheat varieties plays a key role in the control of STB. The inheritance of resistance to STB has been described as quantitative, incomplete and non-specific to fungal isolates, as well as qualitative, monogenic or oligogenic, and complete (Rillo and Caldwell 1966; Chartrain et al. 2004). In diallel analyses, general combining ability (GCA) effects were found to be more important for resistance expression than specific combining ability (SCA) (van Ginkel and Scharen 1987; Jlibene et al. 1994; Simón and Cordo 1998). Kema et al. (2000) found an avirulence gene in the *M. graminicola* isolate IPO323, and identification of the corresponding resistance gene *Stb6 (*Brading et al. 2002) in the wheat variety Flame provided the first evidence for a gene-for-gene relationship.

To date, fifteen isolate-specific resistance genes with major effects against STB have been mapped in hexaploid wheat. A thorough review on identification and mapping of these *Stb* genes was given by Goodwin (2007). With QTL analysis, Eriksen et al. (2003) identified in addition to *Stb6* a QTL for seedling resistance with minor effects on 3BL and QTL for adult plant resistance on 2B and 7B. QTL with minor and major effects in the adult plant and seedling stage were mapped to 3AS, different from *Stb6* (Eriksen et al. 2003) and to 6B (Chartrain et al. 2004). Further minor QTL for seedling resistance were found to be located on chromosomes 1D, 2D and 7DS, and for adult plant resistance on 3D and 7B (Simón et al. 2004; Arraiano et al. 2007). In a genetic and physical mapping study, Raman et al. (2009) suggested allelism to *Stb11* for a major QTL on 1BS accounting for 60% to 98% of the phenotypic variance. Interaction between genes or QTL was not investigated so far.

In disease assessment both, the loss of photosynthetic activity by necrosis and the production of pycnida, the asexual fructifications which play an important epidemiological role, are relevant to characterize STB resistance. Kema et al. (1996a; 1996b) suggested a different genetic control for both traits. There are only few reports on the underlying mechanisms of STB resistance. Histological observations by Kema et al. (1996a) showed a different degree of colonization in terms of both, necrosis and pycnidia formation, between a resistant and a susceptible variety.

Arraiano and Brown (2006) investigated the distribution and frequency of STB resistance genes in 238 European cultivars and breeding lines using seven

isolates in a detached-leaf assay, and identified resistance to IPO88004 (*Stb15*) and IPO323 (*Stb6*) as the most frequent. Resistance that follows a gene-for-gene relationship is prone to breakdown by isolates with novel virulence specificities. Collapse of field resistance was observed in cultivars Gene and Tadinia carrying *Stb4* (Cowger et al. 2000; Jackson et al. 2000). Krenz et al. (2008) demonstrated the adaptation of *M. graminicola* on a moderate resistant cultivar. However, it is still unclear why resistance conferred by some isolate-specific genes is more durable than that of others. For managing STB resistance it has been proposed to pyramid effective genes in single varieties or to assemble genes by the use of cultivar mixtures in the field. Indeed, a decrease of disease severity in cultivar mixtures could be observed (Mille et al. 2006) but appeared to be inconsistent (Cowger and Mundt 2002). Stacking of isolate-specific STB genes requires the availability of molecular markers. Validation of such markers in different genetic backgrounds and their applicability to high-throughput analysis is crucial for marker-assisted selection (MAS) strategies.

In this study we carried out a QTL analysis of STB resistance to seven isolates of *M. graminicola* at the seedling stage using a DH population derived from a cross between the German bread wheat cultivar Solitär and the susceptible Hungarian cultivar Mazurka. Since its release in 2004 Solitär expresses the highest level of STB resistance in the field among the registered varieties in Germany (Anonymous 2004). The aims of the study were (1) to identify isolate-specific resistance in the parental cultivars with a diverse set of *M. graminicola* isolates, (2) to locate QTL with major and minor effects conferring STB resistance at the level of necrosis and pycnidial coverage using a subset of isolates, and (3) to study epistatic interactions among resistance QTL.

Materials and Methods

Plant and Fungal Materials

The German winter wheat (*Triticum aestivum* L.) cultivar Solitär, resistant to STB in the field, was crossed with the susceptible Hungarian winter wheat cultivar Mazurka. A DH population consisting of 134 lines was generated from F_1 seed by the KWS-Lochow breeding company (Bergen, Germany). All lines of this population,

referred to as SxM DH population, were used for linkage map construction. Due to limited seed availability 128 DH lines were screened for seedling resistance to *M. graminicola* at Plant Research International (PRI, Wageningen, The Netherlands), and 128 to 132 DH lines, varying between replications, at the Department of Plant Breeding, Martin-Luther-University (MLU, Halle, Germany).

The two parents were screened for STB resistance using a set of 30 *M. graminicola* isolates of *T. aestivum* collected from 15 countries worldwide (Table 1). All isolates were received as mycelia or spore culture except German isolates Ma3, Ma4, Ta1 and Hungarian isolates Hu1, Hu2 and Hu3. These were collected as single pycnidia from leaf samples either from the field nursery in Halle (varieties Mazurka and Taras) or from the breeding nursery at the Agricultural Research Institute (Martonvásár, Hungary).

Pathogenicity assays

Seedling assays with *M. graminicola* isolates were conducted in a greenhouse cabinet (PRI) or a growth chamber (MLU). Parental screening with IPO isolates were performed in three replications at PRI and in two replications with German and Hungarian isolates at MLU. Ten plants per DH line (including the parents) and isolate were sown in pots containing a peat/sand mixture, and grown for seven to ten days under 16 h light per day at a temperature of 18/16°C (day/night) and 70% relative humidity. Plants were inoculated before emergence of the second leaf. Inoculum preparation and inoculation with IPO isolates were according to procedures described by Kema et al. (1996a). To produce inoculum of the German and Hungarian isolates, monopycnidial spore ranks of infested leaf samples were spread on malt yeast agar (MYA) plates (1% malt, 0.4% yeast , 0.4% glucose, 2% agar w/v) and incubated at 20°C for several days. *S. tritici* spores were scraped off the agar plate and stored at -80°C. For inoculation, thawed isolates were spread on MYA plates and floated with distilled water after 7 days of growth. The inoculum was adjusted to a final concentration of 1 x 10^7 spores per ml. Two to three drops of Tween 20 surfactant were supplemented and plants sprayed with approximately 2 ml of inoculum per plant and isolate until run-off occurred. Inoculated plants were kept at ≥ 98% relative humidity and in dark conditions for 48 h by covering with black plastic foil bags in

Table 1. List of *M. graminicola* isolates originating from 15 countries that were used for the seedling test of the parents of the SxM DH population, Solitär and Mazurka. Isolates in bold letters were selected to analyze resistance to STB in the SxM DH population.

Isolate	**Origin**	**Source**[a]
IPO00003	USA	PRI
IPO00005	USA	PRI
IPO02159	Iran	PRI
IPO02166	Iran	PRI
IPO86013	Turkey	PRI
IPO86086	Argentina	PRI
IPO87016	Uruguay	PRI
IPO88004	Ethiopia	PRI
IPO88018	Ethiopia	PRI
IPO89011	Netherlands	PRI
IPO90006	Mexico	PRI
IPO90015	Peru	PRI
IPO92004	Portugal	PRI
IPO92034	Algeria	PRI
IPO94218	Canada	PRI
PO94269	Netherlands	PRI
IPO95036	Syria	PRI
IPO99015	Argentina	PRI
IPO323	Netherlands	PRI
IPO95054	Algeria	PRI
Ma3	Germany (Mazurka)	MLU
Ma4	Germany (Mazurka)	MLU
Ta1	Germany (Taras)	MLU
Hu1	Hungary	ARI
Hu2	Hungary	ARI
Hu3	Hungary	ARI
BBA22	Germany	JKI
BBA27	Germany	JKI
BBA39	Germany	JKI
BASF27	Germany	BASF

[a] PRI = Plant Research International, Wageningen, The Netherlands; MLU = Martin-Luther-University, Halle, Germany; JKI = Julius-Kühn-Institute, Braunschweig, Germany; ARI = Agricultural Research Institute, Martonvasar, Hungary; BASF = BASF SE, Ludwigshafen, Germany

the greenhouse or by switching off the lights in the growth chamber. After inoculation the temperature and humidity was increased to 21°C and ≥ 85%, respectively. Disease development on the primary leaves was promoted by clipping the second and third leaf 10 days post inoculation (dpi) and by the application of a compound fertilizer. Seven isolates were selected to analyse the SxM DH population (Table 1). All experiments were conducted in a randomized complete block design. As isolates IPO90015, IPO99015 and IPO92034 were tested together in a series of experiments, we applied a split-plot design with isolates as whole plot treatment and DH lines as split-plot treatment.

Disease assessment

Symptoms of STB were visually rated on the primary leaf as (1) percentage of necrotic leaf area (NEC) and (2) percentage of pycnidial coverage (PYC) on each experimental units (10 plants). Symptoms were assessed at intervals of two to six days during a period of 12 to 21 dpi depending on disease development and isolate.

Molecular marker analysis

DNA was extracted from leaves of 10-day old seedlings by the CTAB method (Doyle and Doyle 1990). For molecular mapping, simple sequence repeat (SSR) markers developed by IPK Gatersleben (*Xgwm*, *Xgdm*; Röder et al. 1998; Ganal and Röder 2007; Pestsova et al. 2000), Wheat Microsatellite Consortium (*Xwmc,* Gupta et al. 2002), USDS-ARS Beltsville, Agricultural Research Center (*Xbarc,* Song et al. 2005), Genoplant (*Xgpw,* Sourdille et al. 2004a; *Xcfa*/*Xcfd*, Guyomarc'h et al.2002) were used in the SxM DH population.

PCR reactions were carried out in a PT200 thermocycler (MJ Research; BIO-RAD, Munich, Germany) in a final volume of 25 µl containing 1x PCR buffer (including 1.5 mM $MgCl_2$), 0.2 mM of each dNTP, 0.4 µM of each primer, 1 U Taq polymerase (QIAGEN, Hilden, Germany), and 50 to 100 ng template DNA. Cycling conditions were: 3 min initial denaturation at 94°C, and 45 cycles of 1 min at 94°C, 1 min annealing at 60°C, 55°C or 50°C depending on the primer pair, 2 min extension

at 72°C. A final extension step was performed for 10 min at 72°C. One primer of each microsatellite primer pair was 5'-labelled with Cy5.5 and amplicons electrophoretically separated on an ALF Express sequencer (GE Healthcare, Freiburg, Germany).

Amplified Fragment Length Polymorphism (AFLP) analysis followed the protocol of Vos et al. (1995) except that *Eco*RIor *Pst*I selective primers were 5'-labelled with FAM or HEX. PCR amplicon pools generated from each of a FAM- and HEX-labelled primer combination were purified using a centrifugation clean-up step with MultiScreen 96 HV well filter plates (Millipore GmbH, Schwalbach, Germany) loaded with Sephadex® G-50 (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) according to a procedure described in http://www.genome.ou.edu/protocol_book/protocol_partIV.html (validated on 22th November 2010). Amplification products were separated on a MegaBACE 1000 capillary DNA sequencer (GE Healthcare, Freiburg, Germany) and analyzed with MegaBACE Fragment Profiler v1.2 software (GE Healthcare, Freiburg, Germany). Mapped AFLP loci were named based on the nomenclature of Keygene N.V. (Wageningen, Netherlands).

Data analysis and QTL mapping

The mean disease severity in terms of NEC (in %) and PYC (in %) was calculated by averaging the AUDPC (area under the disease progress curve) values (Shaner and Finney 1977) by the period of disease assessment. Analysis of variance (ANOVA) was conducted for IPO90015, IPO99015 and IPO92034 which were tested together in the same series of experiments. For this dataset, isolate, genotype and genotype x isolate interaction effects were estimated. Experiments (blocks) were considered as random effects, genotype and isolates as fixed effects. With isolates IPO323, Hu2, Hu1 and BBA22 only the genotype effect could be determined. Correlations between PYC and NEC were calculated with Kendalls tau rank correlation coefficient. All statistics were calculated using the statistical programming environment R v2.8 (R Development Core Team 2009).

A genetic map of the SxM DH population was generated with MAPMAKER/EXP Version 3.0 (Lincoln et al. 1993). For the assignment of linkage groups to chromosomes *Xgwm* microsatellite loci were used as anchor markers according to their chromosomal location in the ITMI population (Ganal and Röder 2007). Linkage was established at a minimum LOD threshold of 3.0. Marker orders were obtained by three-point and subsequent multi-point analysis supposing an *a priori* genotyping error of 1%. Only markers which could be placed in the most likely map order at a minimum LOD of 2.0 were included for the subsequent QTL analysis. Multipoint maximum-likelihood recombination fractions were converted into map distances by the Kosambi mapping function. Charts of linkage groups were drawn with Mapchart v2.1 (Voorrips 2002).

All QTL analyses were carried out with the R/qtl package 1.11-12 (Broman et al. 2003) in the R environment using whole-genome interval mapping (Lander and Botstein 1989). Initially, all QTL analyses were performed for each experiment and isolate separately. First, in a single-QTL model a search for individual QTL was performed using maximum-likelihood estimation. If the phenotypic distribution exhibited a marked spike, a two-part model, composed of a binary and a normal model, was applied as described by Broman (2003), and DH lines with mean disease severities $\leq$ 2.5% of PYC and NEC, respectively, were considered resistant. Evidence for pairwise epistatic QTL interactions was tested by a two-dimensional genome scan with a two-QTL model using Haley-Knott regression (Haley and Knott 1992). LOD significance thresholds of P=0.05 for the single- and two-QTL models were determined by running 10,000 permutations on the phenotypic data. Finally, all significant single QTL and QTL involved in interactions were considered and their map positions refined in the context of a multiple-QTL model (MQM). From these refined QTL positions the QTL confidence ranges, defined by a 1.5 LOD drop from the maximum LOD position, were estimated. The overall fit of the full model against the null model was tested by ANOVA. In a second step each QTL term was dropped from the model one at a time and a comparison was made between the full model relative to the model with the term omitted (reduced model). If the omitted QTL also occurred in the interaction with another QTL, the interaction was dropped as well. From the drop-one ANOVA table the heritability of a QTL term, defined as the proportion of the phenotypic variance explained by the term, was calculated, and the

effect of a QTL was estimated as the difference in the mean between the two homozygous QTL genotypes. Interaction effects were estimated as the deviation of the combined effect of alleles at two QTL from the sum of its individual effects (Fisher 1918).

A joint MQM analysis using DH line means of phenotypic data from all experiments included only those QTL and QTL interaction terms which were significant in at least two single experiments (Table 3).

Results

Parental screening for STB resistance with M. graminicola isolates

A total of 30 *M. graminicola* isolates originating from 15 countries throughout the world (Table 1) were used for the seedling assay with the two parents of the SxM DH population, Solitär, a German variety with outstanding field resistance to several fungal diseases, and Mazurka, a Hungarian variety with tolerance to drought and frost. Both wheat genotypes clearly differentiated in their response to STB for the majority of isolates (Fig. 1). On average, Solitär showed a lower percentage of PYC in comparison to Mazurka. With isolates IPO323, IPO86068, IPO99015 and Hu2, complete resistance was observed in Solitär. Amongst other isolates Solitär exhibited the highest disease symptoms after infection with the German isolates BASF27, BBA22, BBA27, BBA39, Ma3 and Ma4. In contrast, Mazurka appeared to be moderately resistant to these isolates, and highly resistant to isolate IPO90015. Due to the distinct response observed in the parental genotypes, IPO323, IPO90015, IPO92034, IPO99015, BBA22, Hu1 and Hu2 were chosen for analysing STB resistance in the SxM DH population.

Phenotypic distribution of STB resistance in the SxM DH population

Between 128 and 132 DH lines were tested with the subset of isolates for STB resistance. Scatter plots and associated histograms of mean disease severity shown in Fig. 2 for IPO92034 and IPO90015 indicate a broad phenotypic variation in the SxM DH population for both NEC and PYC. Generally, two different patterns of

distributions could be observed which were more pronounced for NEC (Fig. 2). One pattern, as illustrated by isolate IPO92034, describes a symmetric continuous distribution when STB was measured by NEC and became right-skewed on the basis of PYC. The relationship between the two disease parameters appeared to be linear and only moderately correlated (Kendall rank correlation coefficient $\tau = 0.50$). Segregation patterns of response to BBA22, Hu1 and Hu2 also suggested a right-skewed (PYC) or normal distribution (NEC) of the DH population (data not shown). A different distribution pattern is exemplified by isolate IPO90015 (Fig. 2). DH lines bearing no pycnidia and no or low necrotic area on the first true leaf stood out as a distinct spike. This distribution points to the action of a major gene superimposed on quantitative inheritance of STB resistance. If DH lines corresponding to the spike were excluded from correlation analysis the obvious relationship between PYC and NEC became visible ($\tau = 0.57$). Such mixture distributions were also revealed with

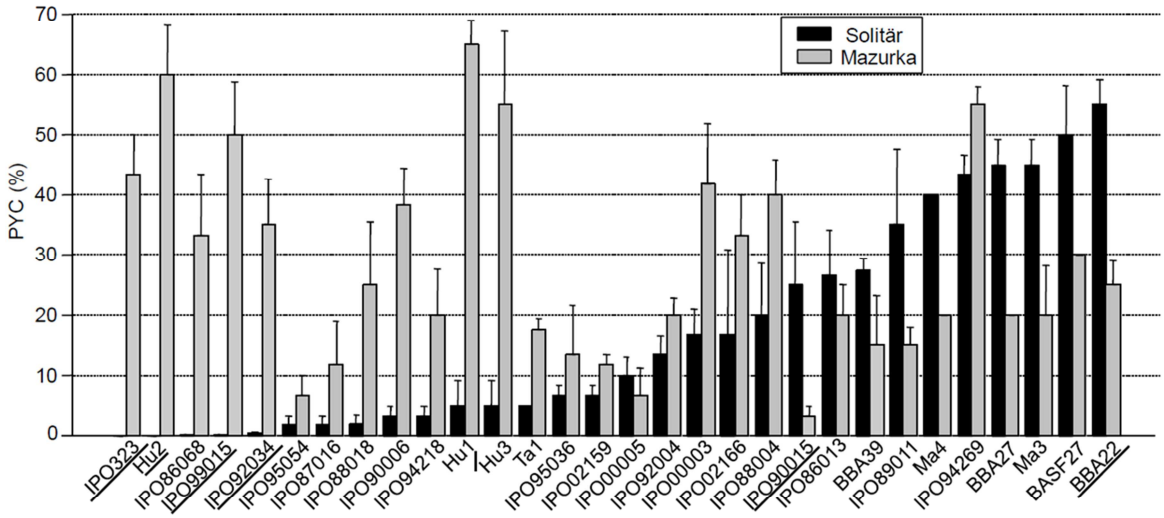

Figure 1. Means and standard errors of pycnidial coverage (PYC, in %) in the parental screening of Solitär and Mazurka with 30 worldwide *M. graminicola* isolates. Underlined isolates were chosen for analysing the doubled-haploid lines of the SxM population. IPO isolates: mean of three replicates determined at 21dpi; BASF27, BBA isolates, Hu1, Hu2, Hu3, Ma3, Ma4, Ta1: means of two replicates determined at 29dpi

isolates IPO99015 and IPO323 (data not shown). DH lines with low PYC (≤ 2.5 %) but high NEC were also found, particularly with isolate IPO92034.

A significant genotype-by-isolate interaction in response to IPO90015, IPO99015 and IPO92034 pointed to isolate-specific reactions to STB in the SxM DH population (Table 2). These results imply QTL mapping needs to be carried out on single isolates. Although IPO323, Hu1, Hu2 and BBA22 were tested in separate experiments, indirect evidence from correlation analyses also suggested genotype-by-isolate interactions (Appendices; ESM 1).

QTL mapping of seedling resistance to STB

A genetic framework map constructed with 145 SSRs has been augmented with 120 AFLP loci. The entire map comprised 31 linkage groups which could be assigned to all 21 wheat chromosomes. Finally, 120 SSR loci, 58 AFLP and one phenotypic marker (*B1*) arranged in statistically reliable orders were chosen for QTL interval mapping. The linkage map covers 2272.8 cM with an average marker density of 12.7 cM.

The single-QTL analysis of resistance to isolates IPO90015, IPO99015, IPO323 employed a two-part QTL model (Broman 2003) whereas for isolates IPO92034, Hu1, Hu2, BBA22 a normal model was applied. Significant pairwise QTL interactions, i.e. deviations from purely additive effects, could be established for five isolates in a two-QTL analysis. In Table 3, the given QTL parameters from the MQM analyses are based on the means of the three experiments. Two QTL interaction pairs, one detected with isolate IPO92034, the other with isolate Hu2 closely missed the significance level of P=0.05 (P=0.06 and P=0.07, respectively) in one experiment each. The confidence ranges of QTL for different isolates and disease parameters are shown in Fig. 3. If a map region affected resistance to more than one isolate a single QTL name was assigned to overlapping ranges.

Seedling resistance to IPO323, conferred by Solitär, was predominantly controlled by a QTL located distally on chromosome arm 3AS. This locus explained

Table 2. Analysis of Variance (ANOVA) of isolate (IPO90015, IPO99015 and IPO92034) and line effects conducted in a split-plot experiment. Computations were done separately for pycnidial coverage (PYC, in %) and necrotic leaf area (NEC, in %)

Source of variation	Degrees of freedom	PYC		NEC	
		Mean Square	F-value[a]	Mean Square	F-value[a]
Isolate	2	32.0	0.2	7576.7	26.3**
Error (block*isolate)	4	213.9		288.5	
DH line	127	276.6	18.4***	851.4	24.5***
Error (block*DH line)	254	15.1			
DH line*isolate	254	121.1	10.1***	280.3	13.1***
Error (block*isolate*DH line)	508	12.0		21.4	

[a] ** P = 0.01; *** P = 0.001

68.8% (PYC) or 84.1% (NEC) of the phenotypic variance, respectively. On average, *QStb.3AS* caused a difference in PYC of 19.1%. In the two-part model, a QTL with small effects on PYC (6.2%) and NEC (6.8%) was identified on 4AL, proximately linked to *Xwmc313*. Conditional on observations above 2.5% disease severity *QStb.4AL* accounted for 5.0% (NEC) to 14.7% (PYC) of the phenotypic variance. QTL *QStb.4AL* was also detected with IPO90015 but with a QTL heritability ranging from 47.9% (PYC) to 75.8% (NEC) and a decrease in disease severity by 24.8% (NEC) and 12.8% (PYC). This QTL is responsible for the spike of resistant DH lines in the distribution of PYC and NEC (Fig. 2).*QStb.4AL* was also identified in response to the German isolate BBA22, although the maximum LOD positions differed slightly among the two disease traits (Table 3). There it accounted for 11% (NEC) or 18% (PYC), respectively, of the phenotypic variance.

Solitär imparted the main component of resistance to IPO99015. This locus, *QStb.1B.*a, linked to *Xgwm11*, could be assigned to 1BS based on a deletion bin (Sourdille et al. 2004b) and explained between 30% (NEC) and 42.8% (PYC) of the phenotypic variance. Two further QTL with minor effects on PYC and NEC could be detected on chromosomes 3D and 7D (Table 3). QTL overlapping with the *QStb.1B.a* interval were also found upon infection with Hu1, Hu2 and IPO92034, and resistance was mediated by Solitär likewise. QTL heritabilities and effects of *QStb.1B.a* were found to be higher for Hu2 than Hu1 suggesting less favourable infection conditions for the latter isolate.

Each of the above QTL was evident in either disease trait. In contrast, resistance specific to PYC and NEC was observed for IPO92034 as the formation of pycnidia was remarkably affected by a QTL located on chromosome 3B and the size of necrotic lesions by a QTL on 1A (Table 3). *QStb.3B* amounts to 38.4% of the phenotypic variance. Whereas Solitär conferred resistance at *QStb.3B*, Mazurka carried the resistant allele at *QStb.1A* which contributed only 11.5% of the variance associated with NEC. A further QTL, denoted as *QStb.1B.b*, controlling PYC-specific resistance to BBA22 was detected on 1B, but in a different position than *QStb.1B.*a (Fig. 3). Only a small proportion of the phenotypic variance (7.0%) could be attributed to *QStb.1B.b*. Resistance of this PYC-specific locus is mediated by Solitär.

Epistatic QTL effects on STB resistance

Epistatic interactions were detected for both resistance traits with IPO323 and Hu2. However, three interactions showed specificity to PYC (IPO90015, IPO92034) and one interaction specificity to NEC (IPO99015) (Table 3, Fig. 3). Two types of epistasis that could be distinguished by presence or absence of a single locus effect were observed in the SxM DH population, and these are illustrated for IPO323 and IPO90015 (Fig. 4). The two QTL involved in the epistatic interaction in response to IPO323, *QStb.3AS* (marker *Xgwm369*) and *QStb.4AL* (marker *Xwmc313*) were also identified in the single-QTL model via a two-part analysis. Yet, inclusion of the interaction effect yielded a significantly better model fit. The presence of the Solitär allele at *Xgwm369* (= epistatic) ensures seedling resistance independent of the allelic state at *QStb.4AL* (= hypostatic). Lines carrying the Mazurka alleles at loci *Xgwm369*

and *Xwmc313* were less susceptible. On average, this interaction explained 5.4% (PYC) and 1.9% (NEC) of the phenotypic variance (Table 3). The PYC-specific QTL *QStb.3B* detected with IPO92034 interacted with each of two single QTL (*QStb.6B* and *QStb.1B.a*) with smaller effects on pycnidia formation (Appendices; ESM 2 Fig 1). The Solitär allele at *QStb.3B* (= epistatic) conferred a high level of resistance to PYC, but when absent the Solitär allele at *QStb.6B* or *QStb.1B.a* (= hypostatic) still reduced pycnidia formation. Thus, apart from an additive mode of action, epistatic effects of *QStb.3B* and *QStb.1B.a* (explained phenotypic variance = 4.2%) and between *QStb.3B* and *QStb.6B* (explained phenotypic variance = 4.7%) were involved in resistance to pycnidia formation.

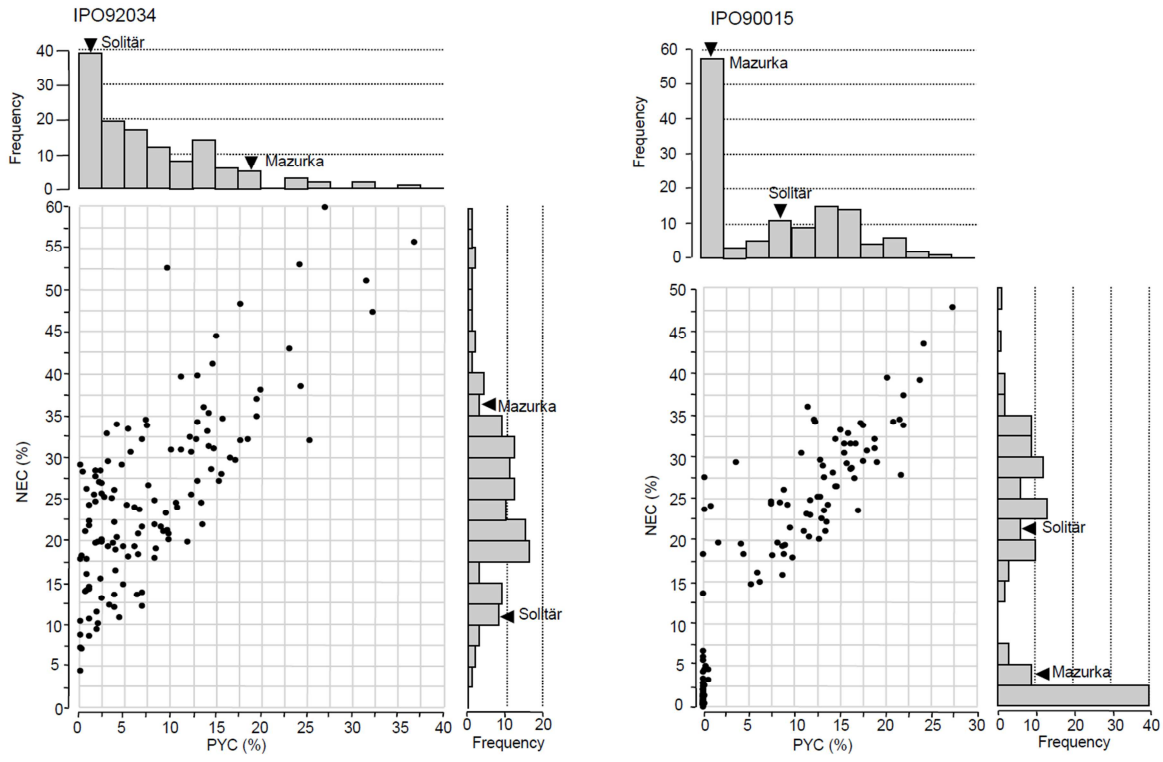

Figure 2. Scatterplots with marginal histograms for necrotic leaf area (NEC, in %) and pycnidial coverage (PYC, in %) in the seedling stage (mean of three replicates) of Solitär, Mazurka and their doubled-haploid offspring (n = 128). Data are given for *M. graminicola* isolates IPO92034 (left) and IPO90015 (right).

QTL-by-QTL interactions without marginal single-QTL effects were uncovered with isolates IPO90015, IPO99015 and Hu2. This crossover interaction, sometimes termed as duplicate epistasis, only gave rise to resistance (or susceptibility) in genotypes with opposite allelic configurations in a pair of QTL. As an example, the interaction between *QStb.1B.c* (tightly linked to *Xgwm806*) and *QStb.2AL* (closely linked to *Xgpw2046*) conditional on the allelic state of *QStb.4AL* (locus *Xwmc313*) in response to IPO90015 is presented in Fig. 4. Genotypes carrying the Mazurka allele at *Xwmc313* respond with disease severities of PYC ≤ 2.5% regardless of the alleles at *QStb.1B.c* and *QStb.2AL*, with only few exceptions. The interaction between the latter

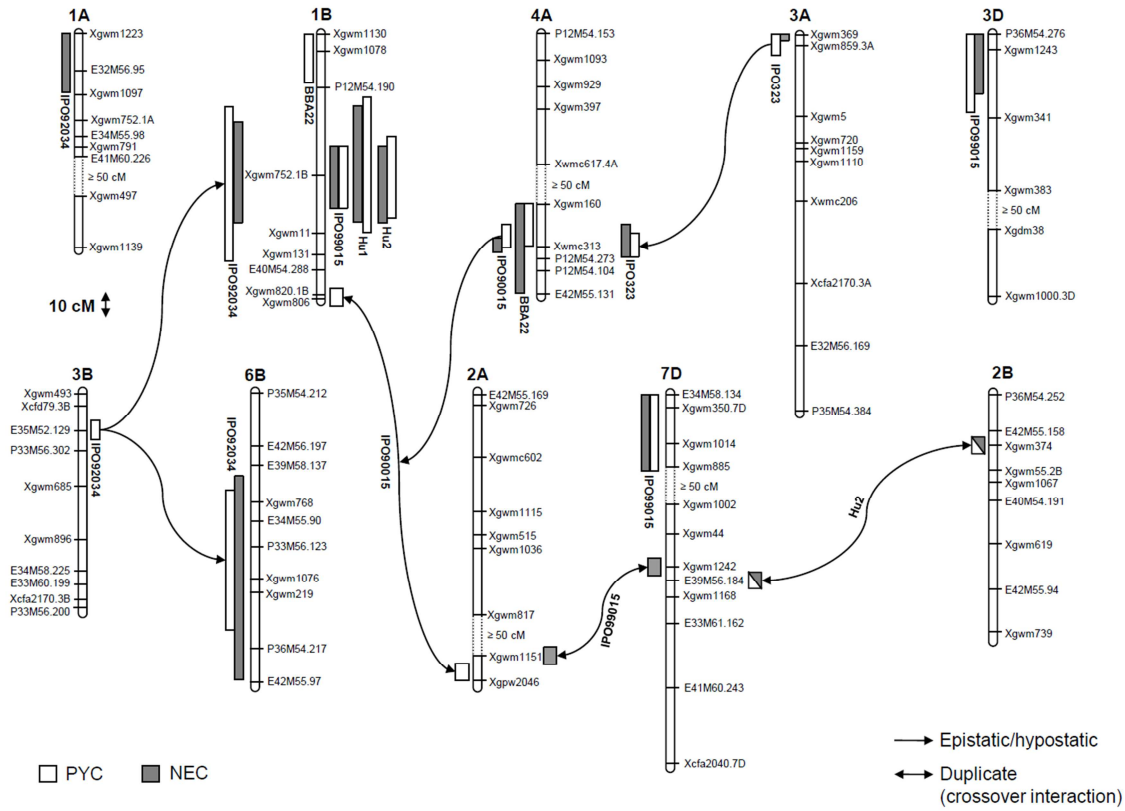

Figure 3. Location of main resistance QTL effects for STB on the genetic map of the SxM DH population detected with 7 isolates in the seedling stage for pycnidial coverage (PYC) and necrotic leaf area (NEC. The bar size indicates the max LOD - 1.5 LOD. An epistatic/hypostatic QTL-by-QTL interaction is indicated by a single arrow, duplicate (crossover) interaction by a double arrow. For the latter, confidence ranges could not be determined and boxes next to the closest linked marker were used instead.

Table 3. QTL and QTL-by-QTL interactions for STB resistance (means of three experiments) to seven *M. graminicola* isolates in the seedling stage identified in the SxM doubled haploid population by multiple QTL mapping

Isolate	Disease trait	No. of experiments[b]	QTL / QTL pair[c]	Resistance donor[d]	Position(s) (cM)[e]	Nearest marker / marker pair	QTL heritability[f] (%)	Genotypic effect[g] (%)	F-value[h]	Putative gene
IPO90015	PYC	3	*QStb.4AL*	M	12	*Xwmc313*	47.9	12.8	165.8 ***	*Stb7/Stb12*
		3	*QStb.1B.c:QStb.2AL*	S:M; M:S	108:8	*Xgwm806:Xgpw2046*	3.2	6.6	11.2 **	
	NEC	3	*QStb.4AL*	M	18	*Xwmc313*	75.8	24.8	393.9 ***	*Stb7/Stb12*
IPO323	PYC	3	*QStb.3AS*	S	0	*Xgwm369*	68.8	19.1[i]	152.0 ***	*Stb6*
		3	*QStb.4AL*	M	18	*Xwmc313*	14.7	6.2[i]	32.5 ***	*Stb7/Stb12*
		3	*QStb.3AS:QStb.4AL*	S:M	0:18	*Xgwm369:Xwmc313*	5.4	13.3[i]	24.0 ***	
	NEC	3	*QStb.3AS*	S	0	*Xgwm369*	84.1	42.7[i]	329.2 ***	*Stb6*
		3	*QStb.4AL*	M	18	*Xwmc313*	5.0	6.8[i]	19.7 ***	*Stb7/Stb12*
		3	*QStb.3AS:QStb.4AL*	S:M	0:18	*Xgwm369:Xwmc313*	1.9	14.7[i]	14.6 ***	
IPO99015	PYC	3	*QStb.1B.a*	S	66	*Xgwm752.1B*	42.8	11.7	120.6 ***	*Stb11*
		3	*QStb.3DS*	S	12	*Xgwm1243*	4.6	3.7	12.9 ***	
		3	*QStb.7DS*	S	2	*E34M58_134*	1.4	1.9	4.0 *	*Stb4/Stb5*
	NEC	3	*QStb.1B.a*	S	64	*Xgwm752.1B*	30.0	17.8	103.4 ***	*Stb11*
		3	*QStb.3DS*	S	10	*Xgwm1243*	8.0	8.5	27.7 ***	
		3	*QStb.7DS*	S	33	*Xgwm885*	1.8	3.9	6.2 *	*Stb4/Stb5*
		3	*QStb.2AL:QStb.7DL*	S:M; M:S	0:28	*Xgwm1151:Xgwm1242*	3.3	10.4	11.2 **	

Table 3 continued

Isolate	Disease trait	No. of experiments[b]	QTL / QTL pair[c]	Resistance donor[d]	Position(s) (cM)[e]	Nearest marker / marker pair	QTL heritability[f] (%)	Genotypic effect[g] (%)	F-value[h]	Putative gene
IPO92034	PYC	3	*QStb.3B*	S	15	*E35M52_129*	38.4	9.0[i]	32.0 ***	*Stb2/Stb14*
		3	*QStb.1B.a*	S	76	*Xgwm752.1B*	11.5	4.7[i]	14.4 ***	*Stb11*
		3	*QStb.6B*	S	72	*Xgwm1076*	10.2	4.0[i]	12.8 ***	
		3	*QStb.3B:QStb.1B.a*	S:S	15:76	*E35M52_129:Xgwm752.1B*	4.2	7.3[i]	10.4 **	
		2	*QStb.3B:QStb.6B*	S:S	15:72	*E35M52_129:Xgwm1076*	4.7	7.2[i]	11.8 ***	
	NEC	3	*QStb.1B.a*	S	66	*Xgwm752.1B*	19.7	11.1	37.4 ***	*Stb11*
		3	*QStb.6B*	S	88	*Xgwm219*	7.4	6.5	14.0 ***	
		3	*QStb.1A*	M	14	*E32M56_95*	10.1	7.1	19.2 ***	
Hu1	PYC	3	*QStb.1B.a*	S	46	*Xgwm752.1B*	12.2	3.7	18.1 ***	*Stb11*
	NEC	3	*QStb.1B.a*	S	48	*Xgwm752.1B*	16.1	4.8	24.9 ***	*Stb11*
Hu2	PYC	3	*QStb.1B.a*	S	68	*Xgwm752.1B*	32.6	7.0	75.1 ***	*Stb11*
		2	*QStb.2B:QStb.7DL*	S:M; M:S	20:34	*Xgwm374:E39M56_184*	6.7	5.9	15.3 ***	

Table 3 continued

Isolate	Disease trait	No. of experiments[b]	QTL / QTL pair[c]	Resistance donor[d]	Position(s) (cM)[e]	Nearest marker / marker pair	QTL heritability[f] (%)	Genotypic effect[g] (%)	F-value[h]	Putative gene
	NEC	3	*QStb.1B.a*	S	68	*Xgwm752.1B*	26.0	7.4	54.5 ***	*Stb11*
		3	*QStb.2B:QStb.7DL*	S:M; M:S	20:34	*Xgwm374:E39M56_184*	9.6	8.4	20.1 ***	
BBA22	PYC	3	*QStb.4AL*	M	6	*Xgwm160*	18.3	4.1	32.1 ***	*Stb7/Stb12*
		3	*QStb.1B.b*	S	4	*Xgwm1078*	7.0	2.4	12.3 ***	*Stb11*
	NEC	3	*QStb.4AL*	M	18	*Xwmc313*	11.2	2.9	16.2 ***	*Stb7/Stb12*

[a]PYC = pycnidial coverage, NEC = necrotic leaf area

[b] Number of experiments in which a single QTL and QTL-by-QTL effect identified, respectively[c] QTL name described by chromosome or chromosome arm; a lower-case character indicates different QTL on the same chromosome

[d] single QTL allele, QTL-by-QTL interaction allele combination(s) conferring resistance; S = cv. Solitär; M = cv. Mazurka

[e] QTL position(s) determined by refined MQM analysis

[f] QTL heritability defined as phenotypic variance explained by the QTL or QTL-by-QTL interaction

[g] QTL effect was estimated as the difference in the mean between the two homozygous QTL genotypes

[h] * P = 0.05; ** P = 0.01; *** P = 0.001

[i] Estimated single QTL effect and QTL-by-QTL interaction effect not unambiguously distinguishable

resulted in a higher resistance to PYC whenever one QTL carried the Solitär allele and the other QTL the Mazurka allele, and explained 3.2% of the phenotypic variance independent of the major effect of 4AL. A crossover interaction could also be observed between *QStb.2B* (linked to *Xgwm374*) and *QStb.7DL* (linked to *E39M56_184*) in response to Hu2, and amounts to 6.7% (PYC) or 9.6% (NEC) of the phenotypic variance, respectively. A NEC-specific crossover-interaction has been detected with IPO99015 between *QStb.2AL* (linked to *Xgwm1151*) and *QStb.7DL* (linked to *Xgwm1242*), and explained 3.3% of the phenotypic variance (Appendices; ESM 2 Fig 1). Chromosome arm 2AL, covered by only two markers, was already involved in the interaction with *QStb.1B.c*. *Xgpw2046* and *Xgwm1151* are separated by only 8 cM. Therefore it cannot be ruled out that this part on 2AL is involved in multiple interactions. This might also be true for the QTL region on 7DL since *Xgwm1242* is only 6 cM apart from *E39M56_184*, linked to *QStb.7DL*, which has already been shown to interact with *QStb.2B* in response to Hu2.

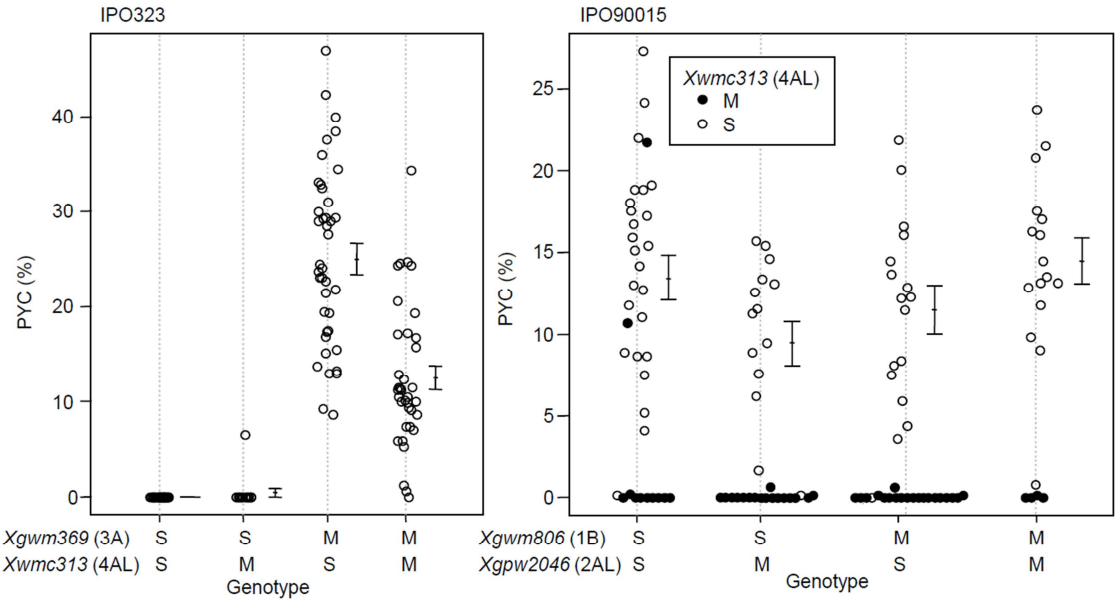

Figure 4. Epistatic effects revealed in the SxM DH population. Means and standard errors of pycnidial coverage (PYC, in %) for the Solitär (S) and Mazurka (M) allele pairs at loci *Xgwm369* (3A) and *Xwmc313* (4L) determined by testing with IPO323 (left) and allele pairs at *Xgwm806* (1B) and *Xgpw2046* (2AL) after infection with IPO90015 (right). In the right panel filled circles represent the Mazurka allele, open circles the Solitär allele at locus *Xwmc313*. For isolate 90015, standard errors were calculated conditional on the Solitär allele at this locus.

Discussion

Differential parental responses to M. graminicola isolates

In several studies, specificity in the *T. aestivum* - *M. graminicola* pathosystem has been identified as significant isolate-by-genotype interaction in experiments using differential sets (Kema et al. 1996a; 1996b). Solitär and Mazurka, the parents of the SxM DH population, were included in a larger differential set of thirteen *T. aestivum* genotypes representing all fifteen mapped *Stb* resistance genes (Tabib Ghaffary et al. 2008). Among the twenty IPO isolates tested at PRI four isolates were postulated to be avirulent to cultivars carrying *Stb6* (IPO323), *Stb5* (IPO94269), *Stb9* (IPO89011) or *Stb15* (IPO88004), respectively (Brading et al. 2002; Arraiano et al. 2001; Chartrain et al. 2009; Arraiano et al. 2007). From the screening results we hypothesize that Mazurka probably possesses none of the four *Stb* genes whereas Solitär carries *Stb6* and other resistance genes not covered by the set of isolates. Quite often differentiation for STB between the two cultivars is not as clear-cut to distinguish between qualitative and quantitative resistance to single isolates. In addition to the IPO isolates we also tested locally adapted German and Hungarian fungal isolates. It is remarkable that the German fungal isolates BBA22, BASF27, BBA39, Ma3 and Ma4,the latter two collected from Mazurka, caused lower PYC on Mazurka but were aggressive on Solitär. Conversely, while Solitär was resistant to the three Hungarian isolates Hu1, Hu2 and Hu3, Mazurka was highly susceptible. These findings apparently indicate adaptation of *M. graminicola* isolates to German and Hungarian, respectively. Adaptations of *M. graminicola* to resistant and moderate resistant wheat cultivars are known and well documented (Jackson et al. 2000; Krenz et al. 2008) as high sexual recombination in *M. graminicola* populations increases the chance of generating novel virulence combinations. The low acreage of Solitär in Germany in combination with isolate non-specific resistance might explain the high field resistance of this variety.

Isolate-specific major and minor QTL identified in the SxM DH population

In many studies major resistance genes, designated as *Stb* genes, to specific *M. graminicola* isolates have been identified because in a single-isolate assay almost

complete resistance was conditioned by a corresponding gene pair (Goodwin 2007). Yet, owing to the concerted action of several genes and environmental effects, resistance to single isolates appeared also as a quantitative character (Eriksen et al. 2003, Simón et al. 2004). QTL mapping exploits the total observed variation to dissect the genetics of STB resistance including minor genetic effects and, as with classical genetics, to disclose epistatic relationships. In the SxM DH population we detected QTL explaining the bulk of the phenotypic variance, depending on the isolate, on chromosomes 3A, 4A, 1B for both resistance traits and on 3B with specificity to PYC. Besides these major genes, QTL which contributed moderately or little to the phenotypic variance were localized on chromosomes 1A, 1B, 3D, 6B and 7D. . *Stb6* was characterized by conferring resistance to IPO323 but susceptibility to IPO94269 and its co-segregation with SSR locus *Xgwm369*. Our pathogenicity assays and QTL analyses demonstrate that Solitär possesses *Stb6* and *QStb.3AS*corresponds to *Stb6*. Varieties carrying *Stb6* still show genetic variation in disease severity (Arraiano et al. 2006), and Chartrain et al. (2005c) assumed allelic variation in the *Stb6* gene itself or gene modifiers. Kema et al. (2000) provided evidence that besides the *Stb6* matching avirulence gene IPO323 carries more *Avr* genes. Chartrain et al. (2005a) showed that the spring wheat line Kavkaz-K4500 L.6.A.4 (KK),besides *Stb6,* has an additional gene for resistance to IPO323. In our study QStb.4AL also contributed to resistance against IPO323 but was not as effective as *Stb6,* and the underlying gene acts downstream of the epistatic *Stb6* gene. The fact that the Mazurka allele at *QStb.4AL* not only enhanced resistance to IPO323 but also to IPO90015 and BBA22 points to a single gene or a complex of linked genes. It is likely that among the published *Stb* genes, *Stb7* and *Stb12*, both located distally on chromosome arm 4AL, are candidates for *QStb.4AL*. *Stb7* has been mapped in proximity to *Xwmc313* in crosses with the spring wheat variety Estanzuel Federal (McCartney et al. 2003) and independently in a population derived from a cross between KK and cv. Shafir (Chartrain et al. 2005a). *Stb12*, first mentioned in the latter study, has been distinguished from *Stb7* by the differential response of the parents to two Israeli isolates and was found to be closer linked to *Xwmc219* than to *Xwmc313*. According to pedigree data (L. Láng, personal communication) it is unlikely that Mazurka could have received *Stb12*. Based on this evidence and the strong linkage to *Xwmc313* we assume that Q*Stb.4AL* identified in the SxM DH population is likely to correspond to *Stb7*.

A large LOD confidence interval on chromosome 1B defined as *QStb.1B.a* conferred resistance to four isolates in the SxM DH population, with the positive allele being contributed by Solitär. The phenotypic effects that vary with the isolate could reflect action of a major gene modulated by interacting genes, or, as suspected for Hu1, less favourable conditions for disease development. Until now, the only gene mapped to 1B is *Stb11* identified in the Portuguese breeding line TE9111 (Chartrain et al. 2005b). By physical mapping Raman et al. (2009) could refine the location of *Stb11* to the flanking markers *Xwmc230* and *Xbarc119b*. In our study, *QStb.1B*.a is closely linked to *Xgwm752.1B* and by comparison with the consensus map (Sourdille et al. 2004b) its confidence range includes *Stb11*.

Two minor QTL were localized with IPO99015 on the short arms of chromosomes 3D and 7D. *QStb.3DS* should be different from a QTL for adult plant resistance that has been mapped to the long arm of chromosome 3D by Simón et al. (2004). Hence, *QStb.3DS* constitutes a newly identified QTL. Two published genes, *Stb4* and *Stb5*, are clustered on the short arm of 7D. *Stb5* can be excluded as a candidate because of the susceptibility of Solitär to IPO94269 being indicative for the absence of *Stb5* (Arraiano et al. 2001). *Stb4*, first described by Somasco et al. (1996), exhibited good resistance in field and greenhouse experiments and mapped near the centromere closely linked to *Xgwm111*(Adhikari et al. 2004b). As yet, no *Stb* gene has been mapped to the distal end of 7DS (Goodwin 2007). However, a QTL on 7DS with minor effects was identified by Arraiano et al. (2007) in the Swiss wheat cv. Arina and its location is distal to *Stb4. QStb.7DS*could be unambiguously mapped to a 31 cM interval between the AFLP marker *E34M58_134* and *Xgwm885* demonstrating that *QStb.7DS* is not identical with *Stb4* but possibly located in the same region on 7DS as the QTL identified by Arraiano et al. (2007).

A QTL with minor effect on the long arm of chromosome 6B was identified in all replicates in response to IPO92034, and the most likely position is between *Xgwm219* and *Xgwm1078*. While none of the known *Stb* genes mapped to this chromosome, some studies reported several QTL on 6B. Eriksen et al. (2003) located two different minor QTL on 6BS in the seedling stage after inoculation with IPO323 and a Danish isolate, respectively. With IPO323 a QTL on 6B could not be detected in the SxM DH population indicating that *QStb.6B* is another QTL. In the ITMI mapping

population Simón et al. (2004) found a minor QTL on 6BS in the seedling stage for two independent isolates. In adult plant tests with three isolates Chartrain et al. (2004a) revealed a QTL with minor effects linked to *Xgwm133* and *Xgwm219*. Possibly this QTL coincides with *QStb.6B* because they cover roughly the same region. Unfortunately, a conclusive comparative QTL analysis is often complicated by the lack of common polymorphic markers between different mapping populations.

QTL with specificity to necrosis and pycnidia formation

Separate analyses were carried out for the parameters NEC and PYC in order to disclose resistance QTL involved in different stages of disease development. The positive relationship between NEC and PYC detected in the SxM DH population was expected since pycnidia formation usually relies on the presence of necrotic lesions (Simón et al. 2005). In the *T. aestivum – M. graminicola* pathosystem, pycnidia formation is conditioned by collapsed but not necessarily necrotic plant tissue (Kema and van Silfhout 1997). Ten to 14 dpi the fungus switches from a symptomless to a necrotrophic stage by the induction of cell collapse, release of nutrients and formation of pycnidia. Assessment of the disease using necrotic leaf area is not always reliable as other biotic and abiotic stress-related factors may mimic chlorotic or necrotic symptoms thereby overestimating the actual infestation.

In this study we worked with whole seedlings under optimal growing conditions in the greenhouse and senescence was only visible on mock plants 21 dpi after scoring was already finished on inoculated DH lines. The loose relationship between PYC and NEC found in isolates IPO92034 (Fig. 2) and BBA22 already indicated the occurrence of development-specific resistance mechanisms. Likewise, Chartrain et al. (2005b) determined a moderate correlation between necrosis and pycnidia formation in a mapping population screened with IPO323 and suspected that partial resistance of one parent, TE 9111, to be the cause. In contrast strong necrosis was always accompanied with high pycnidial coverage in the SxM DH population, i.e. PYC-specific resistance factors are absent in Mazurka. Two PYC-specific QTL, both contributed by Solitär, were mapped to chromosome arm 3BS with isolate IPO92034 and to chromosome arm 1BS with isolate BBA22 (Table 3). It is evident that *QStb.1B.b* is different from *QStb.1B.a* asit resides at a more distal region(Fig. 3).

QStb.3B had a major effect on pycnidia formation. As a possible candidate gene for *QStb.3B* we considered *Stb2*, first identified by Wilson (1985) under natural conditions and mapped by Adhikari et al. (2004a) to the short arm of chromosome 3B, tightly linked to *Xgwm389* and proximal to *Xgwm493*. Since *QStb.3B* is located distal to *Xgwm493* in the SxM DH population it is evident that a different gene is involved. Eriksen et al. (2003) mentioned a QTL with minor effects on 3BL in the seedling stage. Unfortunately the authors did not consider measures of pycnidia formation. Another *Stb* gene, *Stb14*, also mapped to 3BS. However, no further information on this gene is available in the catalogue of gene symbols (McIntosh et al. 2007). It appears that *QStb.3B*affects initial pycnidia formation whereas *QStb.1B.a* and *QStb.6B* are more generally involved in the suppression of the infection process. Besides PYC-specific QTL, one NEC-specific QTL with minor effects, also obtained with isolate IPO92034, could be identified. Its position on 1A does not coincide with any known *Stb* gene or QTL and hence this is the first report of a QTL on this chromosome.

Epistatic relationships in STB seedling resistance

Complete epistasis could be shown for *QStb.3AS* and *QStb.4AL* with isolate IPO323. Epistatic effects up to 13.3% for PYC were observed which do not differ greatly from the single main effect at 3AS of 19.1%. This means that epistasis can make an important contribution to the genetic variance of STB resistance. Setting up an appropriate statistical model in such a situation is challenging because effects are confounded. Firstly, the epistatic locus should rather be considered as binomial variate, and the residual genetic variation accounted for by the hypostatic locus be approximated as normally distributed. This can be handled roughly by composite interval mapping (Zeng 1994) or exactly as a two-part model as suggested by Broman et al. (2003). We have found 2.5% disease severity to be a reasonable cut-off point to separate the phenotypic spike from the residual distribution (Fig. 2) and slightly different values did not affect the outcome. Secondly, QTL main effects are not easily interpretable in the presence of interaction and are prone to bias. The effect of the epistatic locus (*QStb.3AS*) is less affected than the effects of the hypostatic locus (*QStb.4AL*) and the interaction. Meaningful estimates for *QStb.4AL* are obtained conditional on the *QStb.3AS* genotype. The situation is even more intricate for

crossover interactions when resistance alleles at a locus pair originate from different parents and therefore single locus effects cancel out each other. We detected only few of such effects and due to their marginal contribution they can be neglected in breeding programs.

Efficacy of a resistance gene, i.e. whether it is considered a major or minor gene, strongly depends upon the presence of specific alleles at other resistance loci. In the same way efficacy is affected by the frequency of corresponding allele combinations at avirulence determining loci in the pathogen population. For instance, *QStb.4AL* had a major effect on resistance to IPO90015 and probably matches *Stb7* or *Stb12 w*hereas its effect is masked in individuals carrying the resistant allele at *QStb.3AS* (*Stb6)* when exposed to IPO323. When challenged with IPO90015, *QStb.4AL* is a major QTL which is epistatic to the PYC-specific crossover interaction between *QStb.1B.c* and *QStb.2AL*. These interrelationships constitute a three-way interaction. Combining the results of the IPO323 and IPO90015 assays, we hypothesize a resistance control pathway in which *Stb6* is hierarchical over *Stb7* (or *Stb12*) which again acts on top of the *QStb.1B.c – QStb.2AL* interaction.

Evidence of epistatic and disease development specific gene action possibly reflects differences at the histological, biochemical and molecular levels found between susceptible and resistant genotypes in early and late events of the infection process (Shetty et al. 2003; Adhikari et al. 2007; Keon et al. 2007). Isolate IPO323 has been shown to hijack plant resistance signalling of a susceptible host by accelerating programmed cell death (PCD) (Keon et al. 2007). Possibly *Stb6* is active during the penetration stage and shortly after, thereby preventing PCD and as a consequence necrosis and pycnidia formation is suppressed. *Stb7*, when challenged to IPO90015, may act like *Stb6*, or attenuated after infection with IPO323 and BBA22, by a reduction of fungal growth, accompanied with less necrotization and pycnidia formation. The PYC-specific *QStb.1B.c – QStb.2AL* interaction might interfere in a later stage on pycnidia formation and influence pycnidia maturation by inhibiting fungal synthesis of reactive oxygen species. Likewise, such responses can be assumed for interactions detected with Hu2 and IPO99015.

Concluding remarks and outlook

By adoption of multiple QTL models to a set of isolates we unravelled seedling resistance to STB as an intricate pathway involving genes at different stages of the infection process. How *Stb* genes, which usually have large effects, relate to QTL with small effects is still a matter of discussion. It is evident that with major effects found in this study coincide with previously described *Stb* genes. One hypothesis introduced the notion of QTL with minor effects being weak alleles of 'major' resistance genes as a result of gene erosion due to pathogen co-evolution (Poland et al. 2008).

QTL analysis revealed that Solitär carries at least two Stb genes and few minor QTL. Whether any of these resistance factors, single or in combination, is involved in the remarkable field resistance remains to be demonstrated. Field testing the SxM DH population is currently under way. Breeding of resistance to STB relies on efficacy and durability of employed resistance genes in the field, and a strong effect by pyramiding *Stb* genes has not been reported to this day. Knowledge of additive and epistatic action of Stb genes (or QTL) might allow MAS to be more efficient and targeted. Taking into account the dynamic virulence structure of *M. graminicola*, breeding for field resistance to STB yet remains a challenging task.

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Chapter 6

General discussion

General Discussion

Gene for gene (GFG) concepts in host-pathogen interactions are basal for co-evolutionary resistance gene and pathogenicity effector evolvement. *Mycosphaerella graminicola* is considered to be a high-risk pathogen due to its biology. It frequently undergoes sexual and asexual reproduction (Hunter et al. 1999; Kema et al. 1996c; Ponomarenko et al. 2011), has spore dissemination strategies that favor gene flow and is therfore considered to easily circumvent resistance genes(Linde et al. 2002). Each scientific investigation provides fundamental results as a basis for next steps and future research. In this section we discuss the results of the current project, draw conclusions and put these into a broader context in order to optimize phenotyping and genotyping scenarios for septoria tritici blotch (STB) resistance improvement in practical breeding programs.

Thus far, in contrast to the hundreds of resistance genes to other cereal diseases and pests, only 15 resistance genes (*Stb*) have been identified to STB (Komugi, 2011). All of these have been mapped in bread wheat and none in durum wheat, despite the dramatic severity of STB in this crop, particularly in the Mediterranean area (Goodwin et al. 2003). In this thesis we have followed a comprehensive strategy to identify new sources of resistance to STB. Previously, *Stb* identification largely concentrated on already known sources of resistance. These however, have been sparsely used in commercial breeding programs, due to their narrow efficacy and hence, provided the importance of STB in virtually all wheat growing areas and certainly in Europe where concurrently pesticide reduction programs are widely implemented by national governments. Thus there is an urgent need to identify more *Stb* genes.

For screening purposes it is essential that *M. graminicola* isolates be well characterized. The best procedure is to phenotype a *M. graminicola* strain on a suite of isogenic lines. These are, however, not available and thus the next best option is to screen isolates on wheat cultivars with mapped *Stb* genes. After initial analyses (Wilson 1979, 1985) 15 *Stb* genes were identified and mapped with well-characterized *M. graminicola* isolates (Adhikari et al. 2003; Adhikari et al. 2004a; Adhikari et al. 2004b; Adhikari et al. 2004c; Arraiano et al. 2007; Arraiano et al. 2001; Brading et al. 2002; Chartrain et al. 2005a; Chartrain et al. 2004; Chartrain et

al. 2005c; Chartrain et al. 2009; McCartney et al. 2003). Here, we have expanded these analyses by careful characterization of the pathogenicity patterns of 50 isolates on 98 wheat accessions (**Chapter 2)**. This provided us with a unique suite of isolates that were used to test six recombinant inbred line (RIL) and double haploid (DH) mapping populations that resulted in the identification of three new *Stb* genes in two populations. This, however is an effort that should be continued in order to monitor new pathogenic variants that occur in growers field due to the biology of the pathogen that continuously undergoes sexual recombination leading to novel gene combinations (Kema et al. 1996c; Wittenberg et al. 2009; Zhan et al. 2003). Due to the fact that all studies have addressed bread wheat cultivars, there is an urgent need to launch a similar program for durum wheat. It can be broadly stated that the majority of the well-characterized *M. graminicola* strains with specific virulence for mapped *Stb* genes are useless in durum wheat screens as the far majority is avirulent on these tetraploids (Kema et al. 1996b). Hence, durum wheat breeding for STB resistance has to start from scratch, unless we are able to translate the advanced know-how from the bread wheat pathosystem to durum wheat by designing new phenotyping protocols. For any analyses, though, it is essential to study biparental mapping populations with such a suite of isolates rather than single isolates in order to verify the efficacy of individual resistance factors to STB. This then also contributes to effective isolation of individual *Stb* genes in segregating DH or RIL populations that can be used as additional so-called differential lines and eventually can replace the current *Stb* 'differentials'. This would strongly contribute to improved phenotyping of *M. graminicola* strains, certainly with an eye on the massive investment in such tools in cereal rusts research (Bockus et al. 2007; Goodwin 2007; Kolmer et al. 2009; Ordoñez and Kolmer 2009; Visser et al. 2009; Cereal disease laboratory 2011; Zeven et al. 1983).

Throughout the history of wheat research aiming at cereal disease improvement, wild relatives have been considered as very valuable resources for new resistance genes. A gene for stripe rust resistance (*Yr8*) was introduced from *Triticum comosum* into cv. Chinese Spring and has been used for decades in differential sets for this disease (Riley et al. 1968). Research from Sears and co-workers delivered aneuploid wheat stocks that have been globally used for genetic studies, but were primarily aimed at the introgression of genes from wild resources (Feldman and Sears

1981). Reduced genetic diversity in wheat germplasm has been asserted as a consequence of breeding elite modern wheat cultivars (Fu et al. 2006; Hao et al. 2006; Roussel et al. 2004). Several analyses indicated the close genetic relationship of European germplasm (Bohn et al. 1999; Plaschke et al. 1995) and the genetic diversity of modern wheat germplasm was significantly lower than in landraces (Hao et al. 2006; Roussel et al. 2004). As such, wheat domestication resulted in an erosion of genetic diversity from wild wheat D genome donors to wheat landraces and subsequently from landraces to contemporary wheat cultivars (Raman et al. 2010; Reif et al. 2005). However, this process is not merely driven by breeding programs, but is also due to the limited number of wheat progenitor accessions that were involved in wheat evolution (Dvorak et al. 2006; Dvorak et al. 1998; Reif et al. 2005; Talbert et al. 1998). White et al. (2008) showed a significantly lower diversity for DArT markers in the D genome than in the A and B genomes of wheat germplasm originating from the UK and the US, suggesting that the number of D genome accessions that was involved in the evolution of allopolyploid wheat is perhaps lower than the number of A and B genome donors.

Our data confirm these findings as the mapping process of the Apache/Balance population (**Chapter 4**) showed that 44, 36.3 and 19.7 % of the identified SSR and DArT markers resided on the A, B and D genomes, respectively. Mapping genes is only possible when sufficient linkage groups are determined that cover the genome of an organism as much as possible due to optimal recombination events, which will contribute to genetic diversity (Huang et al. 2002). Genetic studies using closely related wheat lines, therefore, result in poor recombinant populations that may also suffer from uneven recombination frequencies along chromosomes, such that even hotspots for recombination have been reported closer to telomeres rather than centromeres (Sourdille et al. 2004). Gene-rich regions are mainly located in distal rather than proximal regions and are highly decondensed facilitating recombination and thus the occurrence of polymorphisms (Faris et al. 2000; Schnable et al. 1998). Ever since the elucidation of wheat evolution and domestication, breeders started to introgress material from wild relatives (Valkoun 2001; Zhang et al. 2009; Zohary et al. 1969). Programs started that directly crossed wild relatives and related grasses to bread wheat cultivars for gene transfer (Anderson et al. 2010; Hajjar and Hodgkin 2007; Mujeeb-Kazi and Hettel 1995), which eventually resulted in

commercial cultivars including the Dutch bread wheat cv. Bristol. Alternatively, synthetic hexaploids were developed that avoid structural chromosomal rearrangements and fertility problems in such gene enrichment programs (Gill and Raupp 1987; Inagaki and Mujeeb-Kazi 1998; Mujeeb-Kazi et al. 2006; Mujeeb-Kazi et al. 2000; Mujeeb-Kazi et al. 2007; van Ginkel and Ogbonnaya 2007; Xu et al. 2004; Yang et al. 2009). This latter strategy has been increasingly and widely adopted since it enables the rapid transfer of genes from a broad gene pool by direct crosses with common wheat and, hence, such lines directly and significantly contribute to commercial breeding programs (Mujeeb-Kazi et al. 1996; Ogbonnaya et al. 2008; Warburton et al. 2006). In **Chapter 2** we describe the remarkably broad resistance of germplasm that is derived from synthetic hexaploid wheat accessions that were developed at CIMMYT. In **Chapter 3** we studied the genetic basis of this resistance following the above mentioned approach and pre-screened the synthetic hexaploid line 'M3' and cv. Kulm with 20 *M. graminicola* isolates. Subsequently, the 'M3'/'Kulm' mapping population was initially tested with four distinctive isolates and final analyses involved two strains. This, eventually, resulted in the discovery of *Stb16* and *Stb17*, which is a convincing token of efficiently combining pathogen characteristics along with evolutionary aspects of wheat development to open a new pool of *Stb* genes. These multiple pathotype analyses also helped us to discern whether all these different isolates detected one and the same QTL or that a combination of QTLs was providing this broadly effective resistance in line 'M3'. QTL analyses of previously reported *Stb* genes only used a single isolate per population leading to single gene identifications (Adhikari et al. 2003; Adhikari et al. 2004a; Adhikari et al. 2004b; Adhikari et al. 2004c; Arraiano et al. 2007; Chartrain et al. 2009; McCartney et al. 2003), and sometimes to the identification of multiple *Stb* genes (Chartrain et al. 2005a; Chartrain et al. 2005c). However, none of the previous reports addressed interactions between QTLs, let alone QTL stacking as a strategy to develop broad resistance to STB as we discuss in **Chapter 4**. Surprisingly, these studies also did not contribute to the development of differential lines by singling out lines with individual *Stb* genes. Indeed, marker assisted selection cannot be considered for all *Stb* genes as some of them map on the same position, like *Stb12* and *Stb7* (Chartrain et al. 2005a; McCartney et al. 2003), or too close to each other, such as *Stb4* and *Stb5*(Adhikari et al. 2004a; Arraiano et al. 2001), but future studies should also address this issue that will serve the community. Based on the data presented in

Chapter 3, we continued our studies and tried to single out *Stb16* and *Stb17* which were uniquely expressed in the adult plant stage in order to deliver lines that can be used for future *M. graminicola* phenotyping. We also tested whether individual RILs from the 'M3'/'Kulm' population expressed the same broad efficacy as the 'M3' parent by selecting - based on genetic markers and phenotypic reactions - lines for analyses with the full panel of *M. graminicola* isolates that was used in the pre-screening (Table 1). This confirmed the broad resistance for the majority of these RILs as the absence of the associated marker resulted in broad susceptibility of the selected lines. However, contrary to the expectation, some of the lines that carried the marker were not universally resistant, but expressed a different efficacy pattern to the set of *M. graminicola* isolates. This raises the question whether *Stb16* on its own explains the broad resistance of 'M3'. Alternatively, it could come from a cluster of several genes at the 3DL QTL position that carries *Stb16*. At this stage, we cannot conclusively analyze these data due to the recombination suppression in this QTL region, but we have started work using other synthetic hexaploid derivatives to resolve this question. This example, however, clearly underscores that future genetic studies (*i*) should work with multiple isolates, (*ii*) should also test the resistance spectrum of individual RILs or DH lines to a broad(er) set of isolates and (*iii*) should validate marker positions with publicly available wheat maps. This in order to avoid erroneous *Stb* positions (Adhikari et al. 2004b; Table 2) for polyploid wheat species originated from interspecific hybridization of wild diploid wheat progenitors (Dubcovsky and Dvorak 2007) that resulted in a greatly similar gene order and content of the A, B and D homeologous chromosomes (Chao et al. 1989; Dvorak et al. 2006; Gu et al. 2006). This may practically even result in multiple marker positions on the wheat genomes (Deynze et al. 1995; Nelson et al. 1995a; Nelson et al. 1995b; Song et al. 2005). To ascertain map positions in our study, we used the reported positions of SSR and DArT markers - either by Diversity Arrays Technology Pty Ltd or publicly accessible wheat map databases such as INRA/Genoplant (2011), Triticarte (2011) and GrainGenes (2011) - and revised the marker names using the concatenate option of EXCEL before analyses with mapping software. This approach facilitates the choice of appropriate LOD values and increases the accuracy of constructed linkage groups by monitoring the map alignment and chromosomal location of the markers. Hence we confidently can claim that the reported QTLs in our study have been mapped on the right position. Embracing these guidelines enables

Table 1. Phenotyping of individual RILs and parents of the 'Kulm'/'M3' mapping population with 20 *Mycosphaerela graminicola* isolates.

Specifically resistance < 5% Pycnidia < intermediate -susceptible

	Flanking Markers[1]		Bread wheat isolates																		Durum isolates	
RIL/cv.	3D																					
	Xwmc494	***Xbarc125***	**IPO94218**	**IPO00003**	**IPO00005**	**IPO90006**	**IPO90015**	**IPO87016**	**IPO86068**	**IPO99015**	**IPO89011**	**IPO92004**	**IPO95054**	**IPO92034**	**IPO88018**	**IPO88004**	**IPO95036**	**IPO86013**	**IPO02166**	**IPO02159**	**IPO95052**	**IPO86022**
'M3'	**M**	**M**	0	0	0	0	0	0	0	0	0	0	5	0	5	0	0	0	0	0	0	0
KM 20	**M**	**M**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KM 7	**M**	**M**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KM 8	**M**	**M**	5	0	0	0	0	0	0	0	0	20	0	0	10	0	5	0	0	0	0	0
KM 32	**M**	**M**	0	0	0	0	0	5	0	5	0	0	50	0	10	0	5	0	0	0	0	0
KM 88	**M**	**M**	20	10	0	20	0	35	5	35	0	25	55	45	40	10	20	0	15	0	0	5
KM 14	**M**	**M**	25	0	5	0	0	5	0	0	0	15	0	5	5	0	10	10	0	0	0	0
KM 15	**K**	**K**	40	70	75	75	60	80	70	80	30	30	70	60	45	50	100	30	75	60	0	0
KM 41	**K**	**K**	35	55	50	50	80	90	45	30	15	60	25	40	50	80	50	55	50	40	0	0
KM 21	**K**	**K**	25	35	60	50	80	60	45	50	40	25	45	60	25	20	70	40	35	30	0	0
KM 63	**K**	**K**	55	95	30	50	50	80	5	70	55	50	30	80	50	65	75	55	70	50	0	0
KM 73	**K**	**K**	25	50	80	25	35	50	20	30	40	60	40	30	10	30	100	60	70	50	5	0
'Kulm'	**K**	**K**	25	50	75	30	75	30	80	30	60	40	75	50	20	0	100	50	10	60	0	0
'Taichung 29'	Sus. Ch [2]		50	100	50	45	80	40	85	90	80	40	70	40	85	40	100	80	100	50	0	5

[1] K and M representing alleles of 'Kulm' and 'M3', respectively

[2] Susceptible check

Table 2. Additional differential lines derived from the Apache/Balance (A/B) double haploid population and the Kulm/M3 (K/M3) recombinant inbred population. Specifically resistance < 5% Pycnidia < intermediate -susceptible

							Mycosphaerella graminicola isolates							
	Stb genes						Tested on A/B seedlings					Tested on K/M3 seedlings		Tested on K/M3 adult plants
RILs/DH lines	*Stb4*	*Stb6*	*Stb11*	*Stb18*	*Stb16*	*Stb17*	IPO98046	IPO323	IPO87016	IPO89011[1]	IPO98022	IPO88018	IPO94218	IPO 88018
A/B-01015.3HD-166		+	+	+			20	2	2	5	3			
A/B-01015.3HD-131	+		+	+			5	7	0	0	0			
A/B-01015.3HD-124	+			+			5	8	43	2	0			
A/B-01015.3HD-120		+	+				67	2	3	73	53			
A/B-01015.3HD-137				+			72	12	63	15	5			
A/B-01015.3HD-138				+			33	10	47	27	5			
A/B-01015.3HD-149		+					67	0	65	73	67			
A/B-01015.3HD-126			+				40	30	3	50	50			
A/B-01015.3HD-108	-	-	-	-			63	50	60	67	60			
K/M3-KM20					+	+						0	0	2
K/M3-KM7					+							0	0	15
K/M3-KM41						+						45	45	3
K/M3-KM73					-	-						52	32	45

[1] Isolates IPO89011 and IPO98022 are both considered as avirulent on *Stb18* (see responses of RILs 01015.3HD-166, 01015.3HD-131 and 01015.3HD-124), but not for RILs 01015.3HD-137 and 01015.3HD-138. Therefore, perhaps two QTLs are positioned on the *Stb18* locus and are detected by IPO89011 and IPO98022. '+' is for present and '-' is for absent of *Stb* gene. Empty cells indicating lack of the this *Stb* genes in the tested RIL or DH population.

the selection of lines with individual *Stb* genes and will greatly contribute to a sound characterization of *M. graminicola* isolates and in turn to improved QTL analyses in wheat which will greatly support practical breeding for STB resistance.

Another important aspect of phenotyping segregating populations or germplasm is the threshold between resistance and susceptibility. Too many times it is just an arbitrary threshold, which is not objective. Compared to the rust diseases, where agreed scales are being used, based on scientific evidence (McIntosh et al. 1995; McNeal et al. 1971; Peterson et al. 1948), the threshold between compatibility and incompatibility in the wheat - *M. graminicola* pathosystem is hardly addressed (Kema et al. 1996d; Shetty et al. 2009; Shetty et al. 2003; Shetty et al. 2007). In general, the separation of resistance and susceptible plants in segregating populations was not transparent and only a few reports proposed arbitrary thresholds in different scales (Adhikari et al. 2003; Chartrain et al. 2005b; McCartney et al. 2003). It is urgently required to install an agreed methodology to phenotype populations, but it is even more difficult to propose decisive methodologies for screening germplasm, which are not stable over geographical and temporal scales (Kema et al. 1996a; Kema et al. 1996b; Kema and vanSilfhout 1997; Kema et al. 1996d; Shetty et al. 2009). In segregating populations, validation of QTLs can be easily addressed by defining (in)compatibility by the extreme STB severity levels of plants with and without the co-segregating markers. This clearly depends on environmental situations and may differ over laboratories, but is founded in genetic facts (**Chapter 3).** From that starting point we can also address the individual action of QTLs. In **Chapter 4** we have shown that the LOD values of QTLs not only depend on the applied *M. graminicola* isolates, but also on the action of other QTLs. The Apache/Balance mapping population resulted in the discovery of the new *Stb18* gene, with a rather narrow efficacy, but has importantly shown interactions between QTLs. Hence, it is ultimately incorrect to designate QTLs as minor or major QTLs as this clearly depends on the genetic background, the used *M. graminicola* strains and (variable) environmental effects. Most importantly, this study showed that the accumulation of QTLs, does contribute to broad(er) efficacy of resistance to STB, which aligns with GFG concepts. Thus, the identification of new *Stb* genes and their accumulation in germplasm will significantly contribute to STB management. This is also illustrated by the fact that the majority of differential cultivars with a broad resistance spectrum

(Brown et al. 2001; Chartrain et al. 2005a; Chartrain et al. 2005c; Kema et al. 1996a; Kema et al. 1996b) turned out to carry up to four *Stb* genes. Nevertheless, despite the current number of identified *Stb* genes, alternative phenotyping methods are urgently required to support practical breeding for STB resistance. These might also be derived from capitalizing on related Dothideomycete-wheat pathosystems such as *Stagonospora nodorum* and *Pyrenophora tritici-repentis*. In these systems two major findings are very relevant for the *M. graminicola* - wheat pathosystem. First, the effect of light on symptom expression should be understood. The historical instability of phenotyping assays over laboratories is most likely due to these effects. Unpublished data from our laboratory have confirmed the positive effects of light on symptom development and showed that some cultivars, such as Veranopolis (*Stb2+6*), are very sensitive to such fluctuations, whereas others, such as Courtot (*Stb9*), do not seem to be affected. Secondly, despite the fact that the *M. graminicola*-wheat pathosystem is characterized by hemibiotrophy, and not necrotrophy such as the afore mentioned systems, the results from (functional) genomic programs point clearly in the direction of small-secreted proteins that play a crucial role in pathogenesis. In line with these preliminary data, it is important to consider that chloroplast disruption is among the very first responses of mesophyll cells to the presence of *M. graminicola* in the apoplast (Cohen and Eyal 1993; Grieger 2001; Kema et al. 1996d; Shetty et al. 2009; Shetty et al. 2003). Brading et al. (2002) have shown that the wheat-*M. graminicola* pathosystem complies with the GFG theory, the question now is whether it also follows inverse GFG characteristics (Friesen et al. 2007). Resolving these imminent and basic pathological issues will greatly contribute to sound phenotyping protocols that eventually will significantly contribute to breeding for resistance to STB and also open windows towards association genetic approaches in order to speed-up *Stb* gene discovery.

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Summary in English

Cultivated wheat is the most important food and feed commodity, with an annual production of over 600 million tons and globally contributing 19% of human dietary energy. The human population is projected to increase to nine billion people in 2050, however, the annual growth rate of global cereal production -including wheat- is below one percent, which eventually cannot meet the demands of the four decades ahead. Therefore, increasing global wheat yield calls for generation of cultivars with adequate and durable resistance to biotic and abiotic stresses.

Septoria tritici blotch (STB), caused by the ascomycete fungal agent *Mycosphaerella graminicola* (Fuckel) J. Schröt., is a foliar wheat disease that reduces the green leaf area index significantly affecting photosynthesis and thus reducing yields up to 50% under conducive environmental conditions. STB management has strongly focused on chemical control and *M. graminicola* is currently the main target of the agrochemical industry. However, the increasing incidence of fungicide resistance underscores the need for and importance of breeding approaches aiming at STB resistance.

The first genetic study of resistance to STB in wheat was performed in 1957 and subsequently the first resistance gene (now designated as *Stb* genes) was reported in 1966. Since that time 18 *Stb* genes (including three described in this thesis) have been characterized. This number is very low compared to the 88, 96, 64, 104 and 33 resistance genes that have been identified to yellow rust, leaf rust, stem rust, powdery mildew and hessian fly, respectively. Therefore, exploring more wheat germplasm is crucial in order to identify new *Stb* genes. The aim of the research presented in this thesis was to identify and characterize known and new *Stb* genes and to identify molecular markers facilitating their deployment in breeding. **Chapter 1** is a general introduction to the thesis and covers the biology of *M. graminicola*, its interaction with wheat as well as its management under field conditions to prevent yield losses. In **Chapter 2** the genetic diversity of *Mycosphaerella graminicola* is described. Isolates originating from geographically very diverse regions were characterized in phenotyping and genotyping assays. The interaction between the isolates and a differential set of cultivars, carrying reported *Stb* genes, enabled the identification of specific interactions that can be used in *Stb* gene postulations in wheat germplasm. These analyses also demonstrated *Stb* gene efficacy, which supports

decisions on their deployment in breeding programs. Finally, these experiments enabled the selection of isolates for detailed genetic analyses and mapping studies. **Chapter 3** describes the unusual broad resistance to *M. graminicola* and the underlying new *Stb* genes in synthetic hexaploid wheats (SHs). Analyses of recombinant inbred lines (RILs) derived from the cross between the SH M3 and the highly susceptible bread wheat cv. Kulm revealed two novel resistance loci on chromosomes 3DL and 5AL. The 3DL resistance was designated as *Stb16* and is expressed in the seedling and adult plant stages, whereas the specific adult *Stb* resistance gene on chromosome 5AL, was designated as *Stb17q*. **Chapter 4** describes the genetic analysis of STB resistance in the French commercial wheat cvs. Apache and Balance. Five *M. graminicola* isolates were used to detect four QTLs on chromosomes 3AS, 1BS, 6DS and 7D (7DS/7DL switch) in seedlings and two QTLs on chromosomes 3AS and 2DS in adult plants. The QTL on chromosome 6DS is a novel QTL that was designated *Stb18.* Since multiple *M. graminicola* isolates were used, individual gene action could be estimated and was shown to depend on the used strains. In addition, the LOD-scores of effective QTLs, thus tested with different avirulent *M.* graminicola strains, indicated strong epistatic and additive effects between QTLs and the potential of pyramiding strategies in practical breeding. The 2DS QTL indirectly contributes to STB resistance as it largely controls earliness and tallness of wheta plants. **Chapter 5** describes the genetic analysis of STB resistance in the German cvs. Solitär and cv. Mazurka. Seven *M. graminicola* isolates were used and enabled the identification of major effect QTLs on chromosomes 3AS, 1BS and 4AL and minor effect QTLs on chromosomes 1B, 3D, 6B and 7D that were contributed by both parental cultivars. The major QTLs were tightly linked to previously reported *Stb* gene positions. Interaction between QTLs were reliably detected, but contributed less to the total variance. Seedling analyses showed a complex inheritance of STB resistance. Identified QTLs had various isolate-specificities and seemed to control different resistance mechanisms, thus complicating marker development and gene deployment. **Chapter 6** puts the results of Chapters 2-5 in a broader context and provides a critical review of past methodologies and the current alternatives providing a better characterization and higher resolution of STB resistance. Finally, the chapter anticipates on improved phenotyping protocols to stabilize data generation, which will contribute to enhanced genotyping and mapping analyses and hence to the successful commercial deployment of *Stb* genes.

Samenvatting

Tarwe is het belangrijkste voedsel- en voedergewas en met een jaarlijkse productie van meer dan 600 miljoen ton draagt het voor 19% bij aan de menselijke energiebehoefte. De wereldpopulatie zal naar verwachting tot negen miljard mensen toenemen in 2050, maar de jaarlijkse toename van de globale graanproductie – inclusief tarwe – is minder dan één procent en zal niet toereikend zijn om de vraag gedurende de komende vier decennia te beantwoorden. Het is daarom van belang te zorgen dat de globale tarweproductie toe zal nemen door het maken en op de markt brengen van tarwerassen met voldoende en duurzame resistentie tegen biotische en abiotische stress factoren. Septoria tritici bladvlekkenziekte (STB), die wordt veroorzaakt door de ascomyceet *Mycosphaerella graminicola* (Fuckel) J. Schröt., is een schimmelziekte van tarwe die de hoeveelheid beschikbaar blad voor de fotosynthese verminderd en daardoor de opbrengst onder slechte omstandigheden tot wel 50% kan reduceren. De beheersing van STB is sterk afhankelijk van gewasbeschermingsmiddelen waardoor *M. graminicola* momenteel het belangrijkste doel is van de agrochemische industrie. Het optreden van fungicidenresistentie heeft echter het belang van resistentieveredeling onderstreept. De eerste genetische studie naar de overerving van resistentie tegen STB werd in 1957 uitgevoerd en het eerste resistentiegen (nu aangeduid met *Stb* genen) werd gerapporteerd in 1966. Sinds die tijd zijn er 18 *Stb* genen (inclusief de drie dit in dit proefschrift worden beschreven) geïdentificeerd. Dit aantal is erg laag ten opzichte van het aantal genen tegen gele roest (88), bruine roest (96), zwarte roest (64), meeldauw (104) en tarwestengelgalmug (33). Het is daarom cruciaal om nieuwe *Stb* genen te vinden in tarwemateriaal. Het doel van het in dit proefschrift beschreven onderzoek was het identificeren en karakteriseren van bestaande en nieuwe *Stb* genen en het ontwikkelen van moleculaire merkers die behulpzaam zijn bij het introduceren van deze genen in veredelingsprogramma's. **Hoofdstuk 1** is een algemene inleiding op het proefschrift waarin de biologie van *M. graminicola*, de interactie met tarwe en het management van STB in het veld om opbrengstverliezen te voorkomen worden beschreven. In **Hoofdstuk 2** wordt de genetische diversiteit van *M. graminicola* beschreven. Isolaten van geheel verschillende geografische herkomst werden gekarakteriseerd met behulp van fenotypische en genotypische methoden. De interactie tussen isolaten en een differentiële set tarwerassen die alle tot nog toe gerapporteerde *Stb* genen bezit

maakte het mogelijk een set isolaten te identificeren die erg behulpzaam is bij genpostulaties in onbekende tarwerassen. Deze analysen gaven ook een indruk van het resistentiespectrum van deze genen dat het gebruik in veredelingsprogramma's ondersteunt. Tenslotte maakten deze experimenten het mogelijk om de juiste isolaten te identificeren voor toekomstige genetische studies en karteringsexperimenten. **Hoofdstuk 3** beschrijft de ongebruikelijk breed werkzame resistentie, en de onderliggende *Stb* genen, tegen *M. graminicola* in synthetische hexaploïden (SHs). Uit analysen van recombinante inteeltlijnen (RILs) die werden verkregen uit een kruising tussen de SH 'M3' en het vatbare tarweras 'Kulm' kwamen twee nieuwe resistentieloci op de chromosomen 3DL en 5AL naar voren. De eerstgenoemde resistentie werd *Stb16* genoemd en komt zowel in kiemplanten als volwassen planten tot expressie terwijl het gen dat alleen in dit laatste stadium tot expressie kwam *Stb17q* wordt genoemd. **Hoofdstuk 4** beschrijft de genetische analyse van STB resistentie in de Franse commerciële tarwerassen Apache en Balance. Vijf *M. graminicola* isolaten werden gebruikt om vier gebieden die coderen voor kwantitatieve resistentie (QTLs) op chromosomen 3AS, 1BS, 6DA en 7D (7DS/7DL omwisseling) in kiemplanten en twee QTLs op de chromosomen 3AS en 2DS in volwassen planten te karteren. Het QTL op chromosoom 6DS betreft een nieuw QTL dat *Stb18* werd genoemd. Omdat er gebruik werd gemaakt van meerdere isolaten kon ook de individuele bijdrage per QTL worden geschat en die bleek samen te hangen met het gebruikte *M. graminicola* isolaat. Daarnaast kwamen uit de waarschijnlijkheidsanalysen voor koppeling (LOD waarden) van individuele QTLs, die werden gemeten in onafhankelijke tests met verschillende *M. graminicola* isolaten, epistatische en additionele effecten tussen QTLs naar voren die mogelijk een effect hebben op stapeling van QTLs in praktische veredelingsprogramma's. Het 2DS QTL draagt indirect bij aan STB resistentie omdat het voor een groot gedeelte betrokken is bij het bloeitijdstip en de lengte van tarwerassen. **Hoofdstuk 5** beschrijft de genetische analyse van STB resistentie in de Duitse tarwerassen Solitär en Mazurka. Zeven *M. graminicola* isolaten werden gebruikt en maakten het mogelijk om QTLs met grote en kleine effecten te identificeren op respectievelijk chromosomen 3AS, 1BS, 4AL en chromosomen 1B, 3D, 6B en 7D, die van beide tarwerassen afkomstig waren. De QTLs met grote effecten waren nauw gekoppeld met reeds bekende *Stb* genen. Interacties tussen QTLs werden betrouwbaar gedetecteerd maar droegen niet veel bij aan de algemene genetische variatie.

Kiemplantanalysen lieten een complex overervingspatroon van STB resistentie zien. De geïdentificeerde QTLs vertoonden verschillen in isolaatspecificiteit en leken verschillende resistentiemechanismen aan te sturen. Dit is een complicerende factor bij het ontwikkelen van moleculaire merkers en het gebruik van deze genen. **Hoofdstuk 6** plaats de resultaten van de hoofdstukken 2-5 in een bredere context en voorziet in een kritische analyse van tot nu toe gebruikte methoden en recente alternatieven die leiden tot een betere karakterisering en een hogere resolutie van resistentie tegen STB. Tenslotte anticipeert dit hoofdstuk op verbeterde fenotyperings-protocollen die resulteren in stabielere data sets en zo bijdragen aan preciezere genotyperingsmethoden en karteringsstudies waardoor *Stb* genen beter kunnen worden ingezet in commerciële veredelingsprogramma's.

Résumé

Le blé cultivé est la principale matière première pour l'alimentation, avec une production annuelle de plus de 600 millions de tonnes. Cette céréale contribue, en moyenne, pour 19% de l'apport énergétique de l'homme dans le monde. La population humaine devrait atteindre 9 milliards de personnes en 2050, toutefois le taux de croissance annuel de la production mondiale des principales céréales – y compris le blé - est inférieur à 1%, ce qui ne suffira pas à répondre à la demande pour les quatre prochaines décennies. Par conséquent, accroitre le rendement global du blé nécessite de nouvelles variétés avec un excellent niveau de résistance aux stress biotique et abiotique.

La septoriose (STB) causée par le champignon ascomycète *Mycosphaerella graminicola* (Fuckel) J. Schröt, est une maladie biotique foliaire, qui réduit la surface verte des feuilles et supprime la photosynthèse, ce qui implique une réduction sévère du rendement du blé, qui peut aller jusqu'à 50%. La gestion de la septoriose a été axée sur la lutte chimique aussi bien que sur la résistance des hôtes pour diminuer les dommages au champ. Bien que la septoriose soit une cible principale des industries agrochimiques, l'apparition fréquente de nouvelles souches résistantes aux fongicides dans la population de *M. graminicola* augmente la nécessité et l'importance des approches de sélection pour améliorer la résistance des variétés de blé.

La première étude génétique de la résistance à la Septoriose dans le blé a été effectuée en 1957 et le premier gène résistance *Stb* a été identifié en 1966. Depuis cette date, 18 gènes de résistance, au total, (y compris les trois présentés dans cette thèse) ont été caractérisés. Ce nombre est très faible par rapport aux 88, 96, 64, 104 et 33 gènes de résistance identifiés, respectivement, pour les rouilles jaune, brune et noire, l'oïdium et la mouche de Hesse. C'est pourquoi, l'étude de germplasme supplémentaire est cruciale afin d'identifier de nouveaux gènes de résistance à la Septoriose. L'objectif de la recherche présentée dans cette thèse était d'identifier, de caractériser de nouveaux gènes de résistance à la Septoriose et d'identifier des marqueurs moléculaires liés à ces gènes pour faciliter leur utilisation sélection. Le **chapitre 1** présente *M.graminicola* et son interaction avec le blé ainsi que les différents aspects des recherches qui ont été effectuées pour contrôler la Septoriose et pour réduire les pertes de rendement du blé. Dans le **chapitre 2**, la diversité génétique

des isolats de *Mycosphaerella graminicola,* provenant de régions géographiquement très diverses, a été décrite sur la base de tests de phénotypage et de génotypage SSR. L'interaction entre les isolats et une série de variétés différentielles portant des gènes de Septoriose connus a permis de mettre en évidence des interactions spécifiques de résistance particulièrement utiles pour l'identification de gène Septoriose dans le germplasme de blé. Ces analyses ont également montré la pertinence de l'utilisation des gènes *Stb* par les sélectionneurs. Enfin, l'interaction des isolats sur les lignées parentales des RIL et des populations HD a permis d'identifier des isolats révélant des interactions parentales contrastées indispensables pour effectuer des analyses de cartographie de QTL. Le **chapitre 3** est ciblé sur les blés synthétiques hexaploïdes (SHs), qui sont une source importante de nouveaux gènes de résistance à la Septoriose. Ces gènes révèlent généralement une efficacité peu commune envers un large panel d'isolats de *M. graminicola*. L'analyse d'une population de RIL issue d'un croisement entre le blé synthétique M3 et la variété de blé tendre sensible Kulm a permis d'identifier deux nouveaux loci de résistance sur les chromosomes 3DL et 5AL. La résistance 3DL, qui a été désigné comme *Stb16*, est exprimée aux stades juvénile et adulte. Le gène *Stb* de résistance adulte présent sur 5AL chromosome, a été nommé *Stb17*. Le **chapitre 4** décrit l'analyse génétique de la résistance à la septoriose dans les variétés françaises de blé Apache et Balance. Cinq isolats de *M. graminicola* ont été utilisés pour détecter quatre QTL sur les chromosomes 3AS, 1BS, 6DS et 7D (7DS/7DL inversé) au stade juvénile et un QTL sur 2DS au stade adulte. Le QTL sur le chromosome 6DS est un nouveau QTL qui a été nommé *Stb18*. L'utilisation de plusieurs isolats de *M. graminicola* a permis de montrer que l'action individuelle de ces gènes dépend des souches utilisées. En outre, de forts effets épistatiques et additifs entre QTL efficaces (testé avec des souches avirulentes de *M. graminicola*) ont entraîné des valeurs de LOD très variables pour les analyses d'un même gène *Stb* avec des isolats de *M. graminicola* différents. Le QTL 2DS, qui a été identifié dans des tests de résistance adulte au champ, est probablement une composante génétique majeure dans la régulation de la précocité et la hauteur des plantes. Il contribue ainsi indirectement à la résistance à la Septoriose. Le **chapitre 5** décrit l'analyse génétique de la résistance à la Septoriose dans la variété allemande Solitär et la variété Mazurka. Sept isolats de *M. graminicola* ont été utilisés et ont permis d'identifier des QTL à effet majeur sur les chromosomes 3AS, 1BS et 4AL et des QTL à effet mineur sur les chromosomes 1B, 3D, 6B et 7D provenant des deux

variétés parentales. Les QTL majeurs sont étroitement liée aux positions des gènes de Septoriose précédemment décrits et des effets épistatiques encore ont été détectés de manière fiable, mais ils contribuent moins à la variance totale. Les tests de résistance juvénile ont montré une héritabilité complexe de la résistance à la Septoriose en matière de mécanismes et de spécificité d'isolats, ce qui complique l'utilisation à grande échelle de ces gènes par sélection assistée par marqueurs. Le **chapitre 6** intègre les résultats des Chapitres 2-5 dans un contexte plus large et présente un examen critique des méthodes passées et des solutions alternatives actuelles qui offrent une meilleure résolution et une meilleure caractérisation de la résistance à la Septoriose. En outre, le chapitre démontre que l'amélioration des protocoles de phénotypage permettra l'obtention de données stables qui contribueront à améliorer le génotypage et les analyses de cartographie et qui faciliteront, ainsi, une utilisation commerciale réussie des gènes *Stb*.

چکیده :

گندم یکی از محصولات مهم کشاورزی می باشد که تولید جهانی آن بیش از 600 میلیون تن در سال بر آورد شده است و بر اساس آمار سازمان خوار و بار جهانی (FAO) بطور متوسط 19% انرژی غذائی روزانه مورد نیاز هر فرد را تامین می نماید. پیش بینی می شود که جمعیت روز افزون بشر در سال 2050 میلادی به بیش از نه میلیارد نفر افزایش یابد، در حالیکه رشد سالانه تولید غلات اساسی از جمله گندم در حال حاضر کمتر از 1% می باشد. بدیهی است که این روند افزایش تولید، جوابگوی نیاز غذای بشر در چهار دهه آتی نخواهد بود. در نتیجه به منظور افزایش تولید، به نژادی و معرفی ارقام گندم با بیشترین میزان مقاومت ممکن نسبت به تنش های زنده و غیر زنده امری اجتناب ناپذیر است.

سپتوریوزبرگی از جمله بیماری های مهم گندم و یکی از تنش های زنده ای است که عامل آن قارچ *Mycosphaerella graminicola* می باشد که در شرایط مطلوب محیطی تا 50 % باعث کاهش عملکرد گندم می شود. کنترل شیمیائی و مقاومت ارقام گندم دو راهبرد اصلی مبارزه با این بیماری است، ولی توسعه مقاومت به قارچ کش ها در جمعیت *M. graminicola* از یک طرف و فشار های سیاسی و اجتماعی برای کاهش استفاده از سموم شیمیائی در محصولات کشاورزی از طرف دیگر، اهمیت به نژادی گندم برای مقاومت به سپتوریوز را بیش از پیش نموده است.

اولین ارزیابی سرشت مایه (Genetic study) برای مقاومت گندم به سپتوریا در سال 1957 میلادی انجام شد و متعاقب آن اولین تک سرشت (Gene) مقاومت به این بیماری در سال 1966 گزارش گردید. با احتساب نتایج ارائه شده در این رساله جمعا 18 تک سرشت مقاومت به بیماری سپتوریوز برگی (*Stb* gene) در گندم گزارش شده است که در مقایسه با تک سرشت های مقاومت گندم به سایر بیماری ها بسیار ناچیز است. تا به امروز 88، 96، 64، 104 و 33 تک سرشت به ترتیب برای مقاومت به زنگ زرد، زنگ قهوه ای، زنگ سیاه، سفیدک پودری و حشره گندم خوار(Hessian Fly) معرفی شده است. بنابراین کاوش برای شناسائی و معرفی منابع جدید مقاومت به سپتوریوز بسیار ضروری می باشد.

پژوهش ارائه شده در این رساله با هدف معرفی و نمایش منش تک سرشت های جدید مقاومت و همچنین شناسائی نشانگرهای مولکولی وابسته به این تک سرشت ها انجام شده است. در **فصل اول** ضمن نشان دادن اهمیت گندم در تغذیه بشر، یک نمای کلی از اهمیت این بیماری و تاریخچه پژوهش های انجام شده در همکنش (Interaction) گندم و قارچ *M.graminicola* ارائه گردیده است. **فصل دوم** به بررسی میزان مقاومت ارقام افتراقی گندم (Differential set of cultivars) نسبت به سویه های(Isolates) مختلف قارچ *M.graminicola* که از نقاط مختلف جغرافیائی گرد آوری شده اند اختصاص یافته است. تنوع سویه های قارچ با کمک رخ نمود (Phenotype) ارقام افتراقی و همچنین با کمک نشانگرهای مولکولی نشان داده شده است. رخ نمود ارقام افتراقی (Differential set phenotype) در همکنش با سویه های قارچ یک الگوی اولیه شبیه سازی پیش بینی تک سرشت های مقاومت به سپتوریا (*Stb* genes) را در سایر ارقام گندم ارائه می نماید. این مطالعه همچنین سودمندی و پایداری مقاومت هر یک از *Stb* های مورد بررسی را مشخص نموده و امکان تصمیم گیری برای بهره گیری از آنها را در برنامه های به نژادی آسان تر می سازد. اما

مهمترین نتیجه این مطالعه را می توان دستیابی به والدین مقاوم و حساس برای دورگ گیری و شناسائی سویه های متمایز کننده این والدین برای بررسی و تجزیه سرشت سازه (Genetic map) قلمداد کرد. در **فصل سوم** دو تک سرشت جدید مقاومت به سپتوریوز برگی با دامنه گسترده ای از مقاومت به سویه های مختلف قارچ معرفی و نامگذاری گردید. سر چشمه هر دوی این تک سرشت ها از گندم های شش دسته ای بشر زاد (Synthetic hexaploid wheats) می باشد. ارزیابی ارقام یک دست نوترکیب (Recombinant Inbred lines) بدست آمده از دو رگ گیری بین والد مقاوم M3 و والد بسیار حساس Kulm ، منجر به شناسائی و نامگذاری *Stb16* و *Stb17q* به ترتیب روی سرشت میله های (Choromosome) 3DL و 5AL گردید. در **فصل چهارم** مطالعه جمعیت بدست آمده از ارقام گندم های فرانسوی Apache و Balance با استفاده از پنج سویه قارچ *M.graminicola* گزارش شده است. در این بررسی چهار جایگاه صفت کمی((Quantitative Trait loci(QTL) روی سرشت میله های 3AS ، 1BS، 6DS و (7DL/7DS Switch) 7D در مرحله گیاهچه ودو QTL روی سرشت میله های 3AS و 2DS در مرحله آزمون مزرعه ای و گیاه کامل یافت شد که در بین آنها 6DS به عنوان یک تک سرشت نو یافته به نام *Stb18* نامگذاری گردید. اثرات افزایشی و پوشانندگی (Additive and Epistatic effects) این QTL ها در مرحله گیاهچه مورد بررسی قرار گرفت و مشخص شد که کوهان سازی از تک سرشت ها (Gene Pyramiding) برای افزایش دامنه مقاومت ارقام گندم به بیماری سپتوریوز برگی می تواند به عنوان یک راهبرد کاربردی مورد استفاده قرار گیرد. **فصل پنجم** مقاومت به سپتوریوز برگی را در جمعیت حاصل از ارقام آلمانی Solitär و Mazurka مورد بحث قرار داده است. در این بررسی سه QTL بزرگ اثر (Major) روی سرشت میله های 3AS، 1BS و 4AL و چهارQTL کوچک اثر(Minor) روی سرشت میله های 1B، 6B، 3Dو 7D یافت شد. هر سهQTL بزرگ اثر دارای پیوستگی با تک سرشت هائی (*Stb* Genes) بود که پیش از این گزارش شده بودند. تجزیه داده ها در مرحله گیاهچه بیانگر پیچیدگی وراثت پذیری مقاومت به سپتوریوز برگی بود. همکنش بین QTL ها در این جمعیت به عنوان یک عامل موثر در مقاومت شناخته شد هر چند سهم آن در دامنه کل تغییرات در این جمعیت ناچیز بود. **فصل ششم** نتایج فصل های پژوهشی دوم، سوم، چهارم و پنجم را دست مایه یک نگاه کلی ونقادانه قرار داده تا با بهبود روش های ارزیابی مقاومت ارقام گندم به بیماری سپتوریوز برگی، ضمن افزایش دانسته ها و داشته ها، بتوانیم در آینده بهترین بهره وری را در به نژادی گندم برای مقاومت به این بیماری داشته باشیم.

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About the author

Seyed Mahmod Tabib Ghaffary was born in Dezful, Iran on 27 January 1968, where he received elementary, secondary and high school education. He was accepted in the Agricultural Faculty of Chamran University, Ahvaz, Iran, in 1987 and obtained his BSc degree in Agronomy and Plant Breeding in 1991. In 1992, he was appointed as plant breeder at the Safiabad Agricultural Research Center in Dezful with a tight programmatic connection to the national wheat breeding program. In 1999, he continued his studies at the Karaj Islamic Azad University and acquired his MSc degree in plant breeding in 2001. His first international experience was through a pathology-breeding course on small grain cereals crops in 2003 at CIMMYT (International Center of Development of Wheat and Maize), Mexico, where he was recommended as PhD student. The Agricultural Research and Education Organization (AREO) of Iran seconded him in 2006 to the wheat-*Mycosphaerella graminicola* PhD project that was funded through Bioplante and the Fonds de Soutien à l'Obtention Végétale (FSOV) in France and performed at Wageningen University and Research Center - Plant Research International (PRI) in The Netherlands. He will continue his career in wheat breeding and pathology in national and international breeding programs in Iran.

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S. Mahmod Tabib Ghaffary, Justin D. Faris, Timothy L. Friesen, Richard G.F. Visser, Theo A.J. van der Lee, Olivier Robert, Gert H.J. Kema (2011). New broad-spectrum resistance to septoria tritici blotch derived from synthetic hexaploid wheat. *Submitted*

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Wageningen University & Research Centre

1) Start-up phase	*date*
► **First presentation of your project**	
Summary of Wheat National Breeding Program In IRAN	May 09, 2006
► **Writing or rewriting a project proposal**	
Unraveling and exploitation of diversity of resistance to *Mycosphaerella graminicola* and Fusarium graminearum in Wheat and its progenitors	Jun 27, 2006
► **Writing a review or book chapter**	
► **MSc courses**	
► **Laboratory use of isotopes**	
Subtotal Start-up Phase	*7,5 credits**

2) Scientific Exposure	*date*
► **EPS PhD student days**	
PhD student day, Wageningen University	Sep 13, 2007
PhD student day, Leiden University	Feb 26, 2009
► **EPS theme symposia**	
EPS Theme 2 symposium 'Interactions between plants and biotic agents', Utrecht university	Jan 22, 2009
EPS Theme 2 symposium 'Interactions between plants and biotic agents', Utrecht university	Jan 15, 2010
► **NWO Lunteren days and other National Platforms**	
KNPV-Working group Cereal diseases, Wageningen	Feb 12, 2009
KNPV-Fast Forward Spring meeting, Wageningen	May 25, 2009
KNPV-Recent development, Wageningen	Jun 16, 2010
► **Seminars (series), workshops and symposia**	
Micro array workshop, Leiden University	Sep 21, 2007
Genomic Momentum 2007, Amsterdam	Nov 28, 2007
Marker assisted breeding for disease resistance in wheat, Karaj (IRAN)	May 10-12, 2009
Technology workshop on Marker assisted selection : from discovery to application, Wageningen (The Netherlands)	Feb 09-10, 2010
First INRA-WUR workshop on septoria disease, Versailles (France)	Sep 16-17, 2010
► **Seminar plus**	
► **International symposia and congresses**	
9th European Fusarium Seminar, Wageningen (The Netherlands)	Sep 19-22, 2006
7th European Symposium on septoria and stagonospora diseases of cereals, Ascona (Switzerland)	Aug 18-22, 2008
8th international wheat conference(8iwc), St. Petersburg (Russia)	Jun 01-04, 2010
► **Presentations**	
PhD student day, poster *'Evaluation of French Mycosphaerella graminicola Isolates on wheat cultivars with mapped resistance genes'*	Sep 13, 2007
Summer school, poster *'Screening for resistance to septoria tritici blotch, the major wheat disease in Western Europe I'*	Jun 19, 2008
7th European Symposium on septoria and stagonospora diseases of cereals, poster *'Screening for resistance to septoria tritici blotch, the major wheat disease in Western Europe II'*	Aug 18-22, 2008
KNPV spring meeting 2009, oral *'Finding more resistance sources to septoria tritici blotch of wheat'*	May 25, 2009
Bayer PhD course 2009, poster *'Identification of new resistance genes to septoria tritici blotch in wheat'*	Jul 06, 2009
KNPV spring meeting 2010, oral *'Identification of a new resistance gene to septoria tritici blotch in wheat'*	Jun 16, 2010
8th Intern. Wheat Conference, poster *'Identification of a new resistance gene to septoria tritici blotch in wheat'*	Jun 01-04, 2010
INRA-WUR workshop, poster *'Efficient breeding approach for development resistant wheat cultivars to Mycosphaerella graminicola'*	Sep 17, 2010
► **IAB interview**	Dec 05, 2008
► **Excursions**	
Subtotal Scientific Exposure	*17,4 credits**

3) In-Depth Studies	*date*
► **EPS courses or other PhD courses**	
Genetic linkage mapping	Mar 21-23, 2007
QTL Analysis	Mar 26-28, 2007
Summer school 'On the Evolution of Plant Pathogen Interactions: from Principal to Practice', Wageningen, NL	Jun 18-20, 2008
PhD course 'Natural Variation in Plants', Wageningen, The Netherlands	Aug 26-29, 2008
Basic and Advanced Statistics	Dec 17-21, 2007; Feb 25-29, 2008
► **Journal club**	
Participant in literature discussion group (weekly)	2006-2010
► **Individual research training**	
Subtotal In-Depth Studies	*9,9 credits**

4) Personal development	*date*
► **Skill training courses**	
English Basic user II	Jan-Apr, 2007
Academic Writing I	Sep-Nov, 2007
Bayer PhD course 2009	Jul 05-09,2009
► **Organization of PhD students day, course or conference**	
► **Membership of Board, Committee or PhD council**	
Subtotal Personal Development	*4,5 credits**

TOTAL NUMBER OF CREDIT POINTS*	**39.3**

Herewith the Graduate School declares that the PhD candidate has complied with the educational requirements set by the Educational Committee of EPS which comprises of a minimum total of 30 ECTS credits

** A credit represents a normative study load of 28 hours of study.*

Appendices

Chapter 2 extra tables and figures

Chapter 4 Apache/Balance Genetic map

Chapter 5 extra tables and figures

Chapter 2

Extra tables and figures

Table S1. Results of inoculation experiments with 29 *Mycosphaerella graminicola* isolates - arranged according to hierarchical sampling at five geographically different locations in France (see Fig. S1) - on 11 wheat differential cultivars that carry 12 *Stb* genes (EXP1). Figures represent pycnidia data. Colors indicate resistant (not significantly different from 0P, greenboxes), intermediate significantly different from 0P as well as maxP, yellow boxes) and susceptible (not significantly different from maxP, red boxes).

	Netherlands		Aires d'Havrincourt						Beauce					Cappelle-en-Pévèlle				Saint Pol de Léon						Villaines la Gonais					
Cultivar	IPO323	IPO94269	IPO98031	IPO98047	IPO98094	IPO98097	IPO98099	IPO98113	IPO99018	IPO99031	IPO99032	IPO99038	IPO99048	IPO98032	IPO98033	IPO98035	IPO98034	IPO98028	IPO98075	IPO98038	IPO98046	IPO98050	IPO98078	IPO98001	IPO98021	IPO98051	IPO98057	IPO98022	IPO98072
Bulgaria	1	7	4	43	6	35	1	6	17	2	4	11	1	10	5	6	11	10	17	8	2	1	8	6	3	29	14	37	22
Veranopolis	1	17	5	16	12	17	2	25	1	2	3	7	1	4	2	2	8	3	4	10	2	1	1	0	0	9	1	26	16
ISR493	1	11	4	41	14	15	5	13	3	1	3	10	1	4	14	1	7	8	9	5	6	3	5	0	0	6	6	3	9
Tadinia	1	20	1	36	14	39	3	9	21	15	15	12	1	5	7	5	4	1	25	5	5	1	17	0	0	8	12	16	13
Cs/synthetic 7D	1	8	3	9	6	4	2	4	1	2	4	2	1	1	3	1	8	2	4	3	17	1	3	0	1	3	3	6	2
Shafir	1	44	15	61	43	34	12	38	28	14	24	26	1	6	15	20	23	16	18	26	36	16	17	1	2	3	3	55	22
E. Federal	64	56	21	50	60	56	8	40	8	33	43	44	24	38	26	9	13	26	60	30	47	14	21	13	31	56	36	52	30
W7984	21	54	10	58	71	48	5	36	32	34	40	37	3	3	5	6	17	17	24	24	42	1	15	8	41	11	15	24	14
Courtot	83	80	17	60	57	91	23	45	74	54	49	81	62	49	44	12	48	35	73	58	47	39	64	61	66	64	72	71	33
KK4500	1	6	2	36	1	10	1	23	11	1	3	17	1	15	4	19	14	1	6	3	27	1	1	0	2	2	1	2	1
TE9111	1	10	6	60	62	34	27	21	10	41	13	38	1	28	21	20	42	4	28	13	41	1	2	1	0	14	10	5	24

Table S2. Results of inoculation experiments with 30 *Mycosphaerella graminicola* isolates – arranged according to hierarchical sampling at five geographically different locations in France (see Fig. S1) - on 40 French wheat breeding lines, nine cultivars and a wild tetraploids relative accession (EXP2). Figures represent pycnidia data. Colors indicate resistant (not significantly different from 0P, green boxes), intermediate significantly different from 0P as well as maxP, yellow boxes) and susceptible (not significantly different from maxP, red boxes).

	Netherlands		Aires d'Havrincourt						Beauce						Cappelle-en-Pévèlle				Saint Pol de Léon						Villaines la Gonais					
Cultivar	IPO323	IPO94269	IPO98031	IPO98047	IPO98094	IPO98097	IPO98099	IPO98113	IPO99018	IPO99031	IPO99032	IPO99038	IPO99042	IPO99048	IPO98032	IPO98033	IPO98035	IPO98034	IPO98028	IPO98075	IPO98038	IPO98046	IPO98050	IPO98078	IPO98001	IPO98021	IPO98051	IPO98057	IPO98022	IPO98072
FD1	0	25	40	19	38	2	20	7	21	21	21	10	25	0	10	14	2	14	5	40	7	25	3	14	1	0	5	10	10	3
FD10	0	25	14	40	10	5	20	21	18	40	10	10	35	0	40	29	3	17	16	33	14	35	40	33	1	0	21	6	25	10
FD11	0	45	30	25	56	14	40	25	8	25	33	30	40	0	30	18	30	21	14	55	10	40	50	44	1	0	25	26	44	21
FD12	30	14	20	25	43	2	35	10	5	25	21	10	50	16	18	21	5	7	16	40	1	25	35	38	3	21	10	5	10	2
FD13	45	40	18	14	21	3	25	16	18	19	35	20	50	33	21	35	7	13	10	33	2	40	55	50	2	25	7	7	18	14
FD14	5	33	29	10	40	7	21	40	13	25	18	5	45	5	20	14	10	10	7	20	3	25	14	35	1	14	2	7	3	1
FD15	0	33	29	7	44	10	25	5	13	5	2	25	45	1	7	14	1	2	1	40	3	35	25	40	1	0	5	13	29	7
FD16	25	19	3	7	29	7	29	7	3	14	6	1	40	5	3	14	1	5	3	10	1	14	14	22	1	7	3	7	7	0
FD17	55	45	29	45	43	6	40	25	40	35	29	14	45	21	35	50	20	18	33	56	29	35	44	60	25	35	8	14	22	10
FD18	2	67	40	10	10	2	5	25	30	13	10	10	50	1	29	14	6	2	7	25	17	29	40	44	1	0	20	10	18	7
FD19	2	50	29	10	40	2	25	10	26	25	3	2	33	0	18	10	14	14	9	40	10	25	2	20	1	0	2	5	14	12
FD2	50	50	25	5	35	10	55	5	6	29	25	25	33	25	5	25	10	10	19	25	10	50	60	45	2	18	10	7	20	7
FD20	0	25	14	10	9	2	25	2	6	3	2	1	30	0	3	10	1	2	3	18	3	20	1	21	1	0	2	2	10	2
FD3	50	45	50	18	50	14	40	25	8	34	33	20	45	7	29	9	7	20	9	65	7	18	67	45	4	21	6	10	40	14
FD4	50	43	35	25	56	12	40	30	29	20	30	29	40	25	14	6	10	18	5	55	14	18	50	45	13	33	18	5	40	25
FD5	56	40	16	25	40	3	35	14	6	26	5	5	25	3	2	34	10	14	5	29	16	18	45	33	2	10	3	2	14	2
FD6	0	14	3	1	29	2	40	7	2	14	18	10	32	0	14	5	14	7	18	25	1	2	10	30	1	0	3	5	25	3
FD7	75	40	20	50	29	6	45	35	6	25	10	18	56	29	20	20	7	5	3	45	10	40	56	45	7	38	5	13	29	35
FD8	0	30	35	45	25	13	44	29	18	25	29	30	40	0	25	43	14	21	40	44	25	25	33	56	1	0	14	25	35	25
FD9	67	25	9	38	62	10	45	38	11	29	29	25	45	33	40	33	21	25	22	43	14	40	71	55	1	29	10	25	35	30

Table S2. Results of inoculation experiments with 30 *Mycosphaerella graminicola* isolates – arranged according to hierarchical sampling at five geographically different locations in France (see Fig. S1) - on 40 French wheat breeding lines, nine cultivars and a wild tetraploids relative accession (EXP2). Figures represent pycnidia data. Colors indicate resistant (not significantly different from 0P, green boxes), intermediate significantly different from 0P as well as maxP, yellow boxes) and susceptible (not significantly different from maxP, red boxes).

	Netherlands		Aires d'Havrincourt						Beauce						Cappelle-en-Pévèlle				Saint Pol de Léon						Villaines la Gonais					
Cultivar	IPO323	IPO94269	IPO98031	IPO98047	IPO98094	IPO98097	IPO98099	IPO98113	IPO99018	IPO99031	IPO99032	IPO99038	IPO99042	IPO99048	IPO98032	IPO98033	IPO98035	IPO98034	IPO98028	IPO98075	IPO98038	IPO98046	IPO98050	IPO98078	IPO98001	IPO98021	IPO98051	IPO98057	IPO98022	IPO98072
Frontana	50	10	0	5	10	2	14	0	1	3	0	1	18	2	1	3	1	0	2	2	2	5	5	10	1	25	0	2	7	0
Iassul20	30	7	20	10	35	5	10	0	5	4	10	0	33	18	5	14	1	7	2	3	2	7	18	7	1	33	20	25	25	1
Kavkaz	0	33	60	29	35	29	5	25	2	29	19	55	50	1	9	43	25	21	7	33	21	55	25	16	1	0	13	33	44	14
Olaf	10	25	30	45	45	14	45	25	20	45	35	1	60	0	5	20	10	13	45	45	16	45	40	29	1	0	25	25	50	20
SE1	35	25	5	25	35	3	18	10	8	30	35	14	35	5	5	30	5	14	18	50	7	20	18	14	1	3	10	2	25	10
SE10	0	1	3	5	21	1	20	10	5	8	18	10	25	0	2	21	2	14	4	14	6	20	16	7	1	0	2	2	10	1
SE11	0	0	0	1	20	2	1	0	7	8	25	5	1	0	2	0	1	7	0	2	0	1	0	0	1	0	1	2	10	2
SE12	20	0	1	40	44	3	25	16	5	25	18	14	25	13	10	2	10	10	10	33	14	18	21	29	1	5	10	1	21	1
SE13	0	2	20	25	25	3	13	10	2	25	13	14	20	0	3	6	3	1	3	9	10	14	14	25	1	0	5	2	10	1
SE14	0	2	10	25	14	1	14	2	1	16	21	9	14	0	2	9	1	10	3	5	10	14	20	18	1	0	3	2	7	2
SE15	1	3	14	26	21	3	10	7	5	10	6	7	44	1	5	33	3	7	3	22	10	29	40	29	1	0	5	1	8	1
SE16	0	0	3	3	33	2	14	10	13	25	2	5	25	0	3	14	2	7	3	18	1	18	3	14	1	0	3	2	10	5
SE17	2	1	40	10	35	7	25	30	25	29	7	25	50	18	5	18	5	5	2	40	29	34	21	9	1	10	8	10	57	2
SE18	0	2	25	29	21	13	29	21	13	25	10	5	25	0	5	21	2	14	35	55	20	30	29	33	1	0	21	1	10	1
SE19	10	0	10	14	21	1	25	7	3	3	3	3	40	2	7	7	3	3	4	9	2	7	20	50	1	4	2	1	0	1
SE2	0	25	2	25	40	10	33	1	7	7	13	20	55	0	14	3	3	5	14	55	5	2	21	20	1	0	3	10	2	10
SE20	0	21	14	16	5	2	20	14	21	22	3	9	35	0	10	2	1	3	3	20	1	10	7	3	1	0	3	2	2	7
SE3	0	0	7	0	0	1	5	2	1	2	2	3	7	0	2	0	2	2	2	2	0	3	7	7	1	0	2	1	10	0
SE4	3	14	6	14	33	5	14	14	18	18	5	7	40	5	10	18	3	7	3	14	2	10	14	9	2	4	2	5	10	2
SE5	0	3	14	40	38	10	35	5	4	40	9	18	45	0	5	25	1	1	6	25	10	18	21	40	1	0	3	5	3	10

Table S2. Results of inoculation experiments with 30 *Mycosphaerella graminicola* isolates – arranged according to hierarchical sampling at five geographically different locations in France (see Fig. S1) - on 40 French wheat breeding lines, nine cultivars and a wild tetraploids relative accession (EXP2). Figures represent pycnidia data. Colors indicate resistant (not significantly different from 0P, green boxes), intermediate significantly different from 0P as well as maxP, yellow boxes) and susceptible (not significantly different from maxP, red boxes).

	Netherlands		Aires d'Havrincourt						Beauce						Cappelle-en-Pévèlle				Saint Pol de Léon						Villaines la Gonais					
Cultivar	IPO323	IPO94269	IPO98031	IPO98047	IPO98094	IPO98097	IPO98099	IPO98113	IPO99018	IPO99031	IPO99032	IPO99038	IPO99042	IPO99048	IPO98032	IPO98033	IPO98035	IPO98034	IPO98028	IPO98075	IPO98038	IPO98046	IPO98050	IPO98078	IPO98001	IPO98021	IPO98051	IPO98057	IPO98022	IPO98072
SE6	10	1	10	10	8	2	20	3	8	21	2	12	40	2	14	4	1	2	3	6	0	2	29	10	2	10	14	2	2	2
SE7	50	25	25	14	33	13	14	30	21	44	10	29	62	19	10	29	7	1	14	45	20	30	50	50	2	29	18	9	25	29
SE8	29	6	1	29	44	2	45	29	25	40	5	10	40	2	7	2	3	20	25	50	14	25	20	25	1	3	2	5	14	10
SE9	0	45	50	10	18	25	40	10	13	60	21	18	34	0	10	50	3	20	25	60	13	55	50	55	1	0	33	25	40	18
Bulgaria	2	5	4	3	10	3	10	0	40	4	2	1	25	0	3	3	7	3	38	33	2	1	1	2	1	0	4	21	13	3
Veranopolis	0	38	2	0	35	0	2	0	0	1	0	2	10	0	2	0	1	2	0	2	10	0	0	0	1	0	0	0	2	0
Tadinia	0	5	0	2	19	2	18	2	14	21	9	2	29	0	14	2	1	1	4	45	1	0	5	13	1	0	6	5	5	2
Shafir	0	50	40	25	35	18	18	2	10	20	40	10	18	0	7	5	1	10	2	44	10	45	43	45	1	0	1	2	40	1
T29	80	7	56	29	45	50	25	14	35	25	25	60	67	45	26	25	40	13	18	71	21	13	80	57	2	55	45	71	71	60
T._polonicum	0	2	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	2	0	0

Table S3. Phenotypic comparison of *Mycosphaerella graminicola* isolates IPO98034 and IPO98035 on 40 French wheat breeding lines, nine cultivars and a wild tetraploid accession seedling experiment (EXP1). Both isolates originated from the same wheat field and had identical genotypes according to SSR genotyping.

	P% logit transformed data				P% Back transformed data	
Germplasm	98034-CEP	98035-CEP	Difference	LSD 5%= 2.281 LSD 1%= 3	98034-CEP	98035-CEP
Bulgaria	-3.396	-2.571	0.825	ns	3	7
FD1	-1.792	-3.77	1.978	ns	14	2
FD10	-1.604	-3.396	1.792	ns	17	3
FD11	-1.301	-0.847	0.454	ns	21	30
FD12	-2.571	-2.944	0.374	ns	7	5
FD13	-1.896	-2.571	0.675	ns	13	7
FD14	-2.197	-2.197	0.000	ns	10	10
FD15	-3.77	-4.595	0.825	ns	2	1
FD16	-2.944	-4.595	1.651	ns	5	1
FD17	-1.522	-1.386	0.136	ns	18	20
FD18	-4.119	-2.721	1.398	ns	2	6
FD19	-1.792	-1.792	0.000	ns	14	14
FD2	-2.197	-2.197	0.000	ns	10	10
FD20	-3.77	-4.944	1.174	ns	2	1
FD3	-1.386	-2.571	1.185	ns	20	7
FD4	-1.522	-2.197	0.675	ns	18	10
FD5	-1.792	-2.197	0.405	ns	14	10
FD6	-2.571	-1.792	0.779	ns	7	14
FD7	-2.991	-2.571	0.420	ns	5	7
FD8	-1.301	-1.792	0.490	ns	21	14
FD9	-1.099	-1.301	0.203	ns	25	21
Frontana	-5.293	-4.944	0.349	ns	0	1
Iassul20	-2.571	-4.944	2.373	*	7	1
Kavkaz	-1.301	-1.117	0.185	ns	21	25
Olaf	-1.896	-2.197	0.301	ns	13	10
SE1	-1.792	-2.991	1.199	ns	14	5
SE10	-1.792	-3.77	1.978	ns	14	2
SE11	-2.571	-4.595	2.024	ns	7	1

Table S3. Phenotypic comparison of *Mycosphaerella graminicola* isolates IPO98034 and IPO98035 on 40 French wheat breeding lines, nine cultivars and a wild tetraploid accession seedling experiment (EXP1). Both isolates originated from the same wheat field and had identical genotypes according to SSR genotyping.

	P% logit transformed data				P% Back transformed data	
Germplasm	98034-CEP	98035-CEP	Difference	LSD 5%= 2.281 LSD 1%= 3	98034-CEP	98035-CEP
SE12	-2.165	-2.165	0.000	ns	10	10
SE13	-4.595	-3.396	1.199	ns	1	3
SE14	-2.165	-4.595	2.430	*	10	1
SE15	-2.571	-3.396	0.825	ns	7	3
SE16	-2.571	-3.77	1.199	ns	7	2
SE17	-2.991	-2.944	0.046	ns	5	5
SE18	-1.792	-3.77	1.978	ns	14	2
SE19	-3.396	-3.396	0.000	ns	3	3
SE2	-2.944	-3.396	0.452	ns	5	3
SE20	-3.396	-4.595	1.199	ns	3	1
SE3	-3.77	-3.77	0.000	ns	2	2
SE4	-2.571	-3.396	0.825	ns	7	3
SE5	-4.595	-4.944	0.349	ns	1	1
SE6	-3.77	-4.944	1.174	ns	2	1
SE7	-4.595	-2.571	2.024	ns	1	7
SE8	-1.386	-3.396	2.010	ns	20	3
SE9	-1.386	-3.396	2.010	ns	20	3
Shafir	-2.197	-4.595	2.398	*	10	1
T29	-1.896	-0.405	1.490	ns	13	40
T._polonicum	-5.293	-5.293	0.000	ns	0	0
Tadinia	-4.944	-4.944	0.000	ns	1	1
Veranopolis	-3.77	-4.944	1.174	ns	2	1

Table S4. Phenotypic comparison of *Mycosphaerella graminicola* isolates IPO98034 and IPO98035 on 11 wheat cultivars carrying mapped *Stb* genes in seedling experiment (EXP2). Both isolates originated from the same wheat field and had identical genotypes according to SSR genotyping.

	P% logit transformed data				P% Back transformed data	
Germplasm	98034-CEP	98035-CEP	Difference	LSD 5%= 2.045 LSD 1%= 2.691	98034-CEP	98035-CEP
Bulgaria	-2.128	-2.734	0.606	ns	11	6
Veranopolis	-2.494	-3.716	1.222	ns	8	2
ISR493	-2.539	-4.25	1.711	ns	7	1
Tadinia	-3.205	-2.941	0.264	ns	4	5
Cs/synthetic 7D	-2.423	-5.049	2.626	*	8	1
Shafir	-1.224	-1.413	0.189	ns	23	20
E. Federal	-1.897	-2.35	0.453	ns	13	9
W7984	-1.576	-2.734	1.158	ns	17	6
Courtot	-0.064	-2.002	1.938	ns	48	12
KK4500	-1.807	-1.453	0.354	ns	14	19
TE9111	-0.335	-1.387	1.052	ns	42	20

Table S5. Genotypic diversity of 50 *Mycosphaerella graminicola* isolates used in EXP1-4 tested with 7 polymorphic microsatellite markers identified from EST sequences.

		Microsatellite markers[1] (allele sizes are indicated in base pairs)						
Isolate	Origin	ag-0003	ac-0001	caa-0005	caa-0003	ag-0009	ac-0002	tcc-0009
IPO86022	Turkey	210	185	272	157	198	188	164
IPO94269	Netherlands	226	171	272	154	198	192	176
IPO98072	France	226	185	263	154	194	190	164
IPO98097	France	228	185	263	154	194	190	164
IPO99018	France	228	185	263	154	198	190	164
IPO92004	Portugal	230	185	263	154	194	188	164
IPO95036	Syria	230	185	272	151	198	190	164
IPO88018	Ethiopia	230	185	272	154	198	190	164
IPO95052	Algeria	230	185	272	157	198	188	164
IPO02159	Iran	230	187	263	154	198	190	164
IPO98033	France	230	187	275	154	194	188	164
IPO88004	Ethiopia	230	187	275	154	194	190	164
IPO98001	France	230	199	278	151	194	188	164
IPO98038	France	238	185	272	154	194	188	164
IPO98047	France	238	227	275	154	198	188	164
IPO98032	France	242	199	278	157	194	190	164
IPO99038	France	242	201	263	139	194	188	164
IPO98113	France	244	185	272	157	198	188	164
IPO98022	France	244	185	272	157	198	190	164
IPO98046	France	244	187	263	151	194	188	164
IPO86013	Turkey	244	203	272	151	194	188	164
IPO87016	Uruguay	246	185	263	139	198	190	164
IPO98034[2]	*France*	*246*	*185*	*263*	*154*	*194*	*188*	*164*
IPO98035[2]	*France*	*246*	*185*	*263*	*154*	*194*	*188*	*164*
IPO00003	USA	246	185	263	154	194	188	167
IPO99048	France	246	185	263	154	194	190	164
IPO90006	Mexico	246	185	263	154	194	190	170
IPO90015	Peru	246	185	263	154	198	190	167
IPO99032	France	246	185	272	151	200	188	164
IPO98094	France	246	185	272	154	194	190	164
IPO98057	France	246	185	290	151	198	190	164
IPO98051	France	246	185	290	151	200	190	164
IPO02166	Iran	246	187	263	154	194	190	164
IPO89011	Netherlands	246	201	275	157	194	188	164
IPO86068	Argentina	248	185	263	154	198	190	164
IPO98050	France	248	185	263	157	194	188	164
IPO99031	France	248	185	272	157	196	188	164
IPO92034	Algeria	248	185	275	139	198	188	164

Table S5. Genotypic diversity of 50 *Mycosphaerella graminicola* isolates used in EXP1-4 tested with 7 polymorphic microsatellite markers identified from EST sequences.

		Microsatellite markers[1] (allele sizes are indicated in base pairs)						
Isolate	Origin	ag-0003	ac-0001	caa-0005	caa-0003	ag-0009	ac-0002	tcc-0009
IPO99015	Argentina	248	185	275	154	200	190	164
IPO94218	Canada	248	185	281	154	194	188	164
IPO00005	USA	248	195	272	154	194	190	167
IPO98021	France	248	213	263	151	194	188	164
IPO98078	France	248	215	263	154	194	188	164
IPO98028	France	250	185	263	154	194	188	164
IPO98075	France	252	185	263	154	194	188	164
IPO323	Netherlands	252	185	272	154	194	188	164
IPO98031	France	252	185	278	157	194	188	176
IPO95054	Algeria	254	185	275	151	198	188	164
IPO98099	France	—[3]	—	—	—	—	—	—
IPO99042	France	—	—	—	—	—	—	—

[1]Detailed information on the microsatellite markers is available in Goodwin et al. (2007)

[2]The two isolates in *italics* have the same alleles for all microsatellite markers tested

[3]Not tested because no DNA was available

Figure S1. Five locations (underlined) where wheat leaves were collected from individual wheat field for hierarchical sampling of the French *Mycosphaerella graminicola* isolates used in this study.

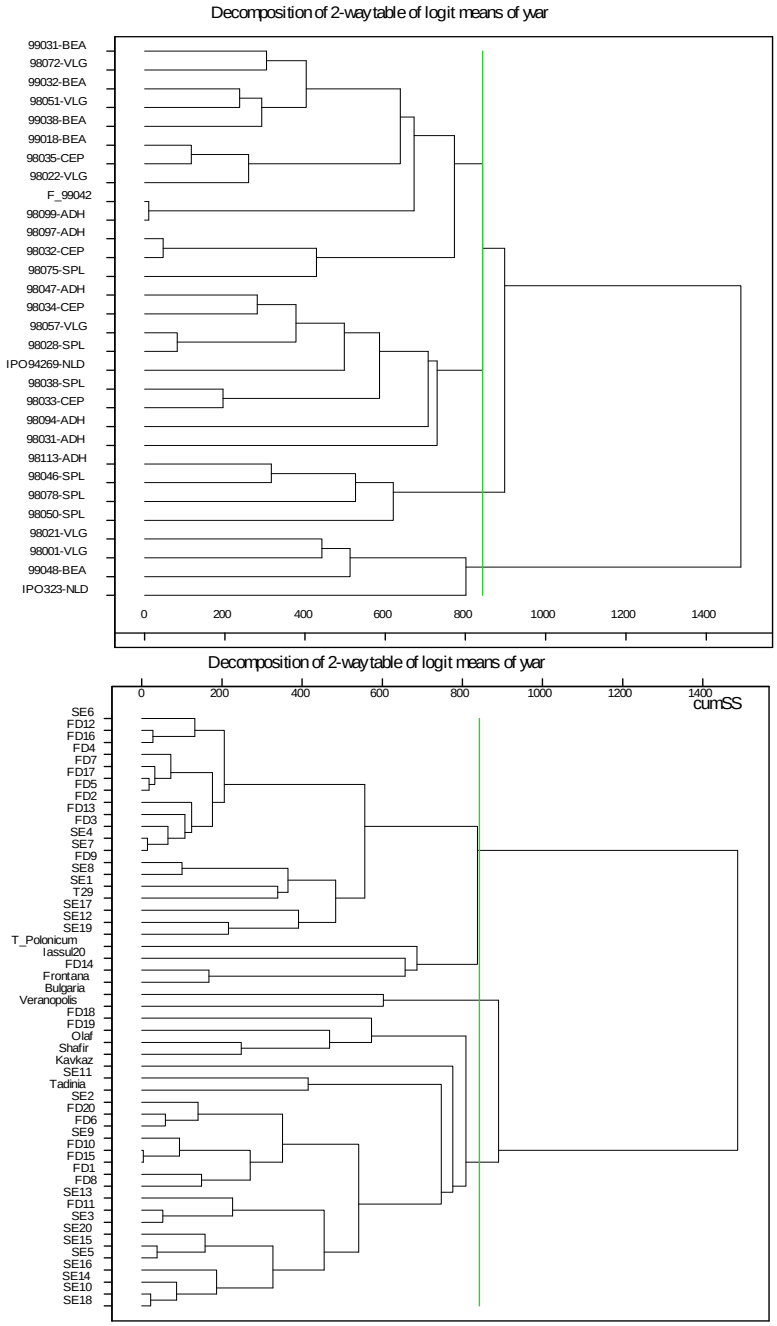


Figure S2. CINTERACTION output of EXP1 where 50 wheat cultivars and breeding lines were inoculated with 28 French and 2 Dutch *Mycosphaerella graminicola* isolates. Data analysis was based on N, the green line shows the threshold at P=0.05 for cluster assembly based on the cumulative sum of squares.

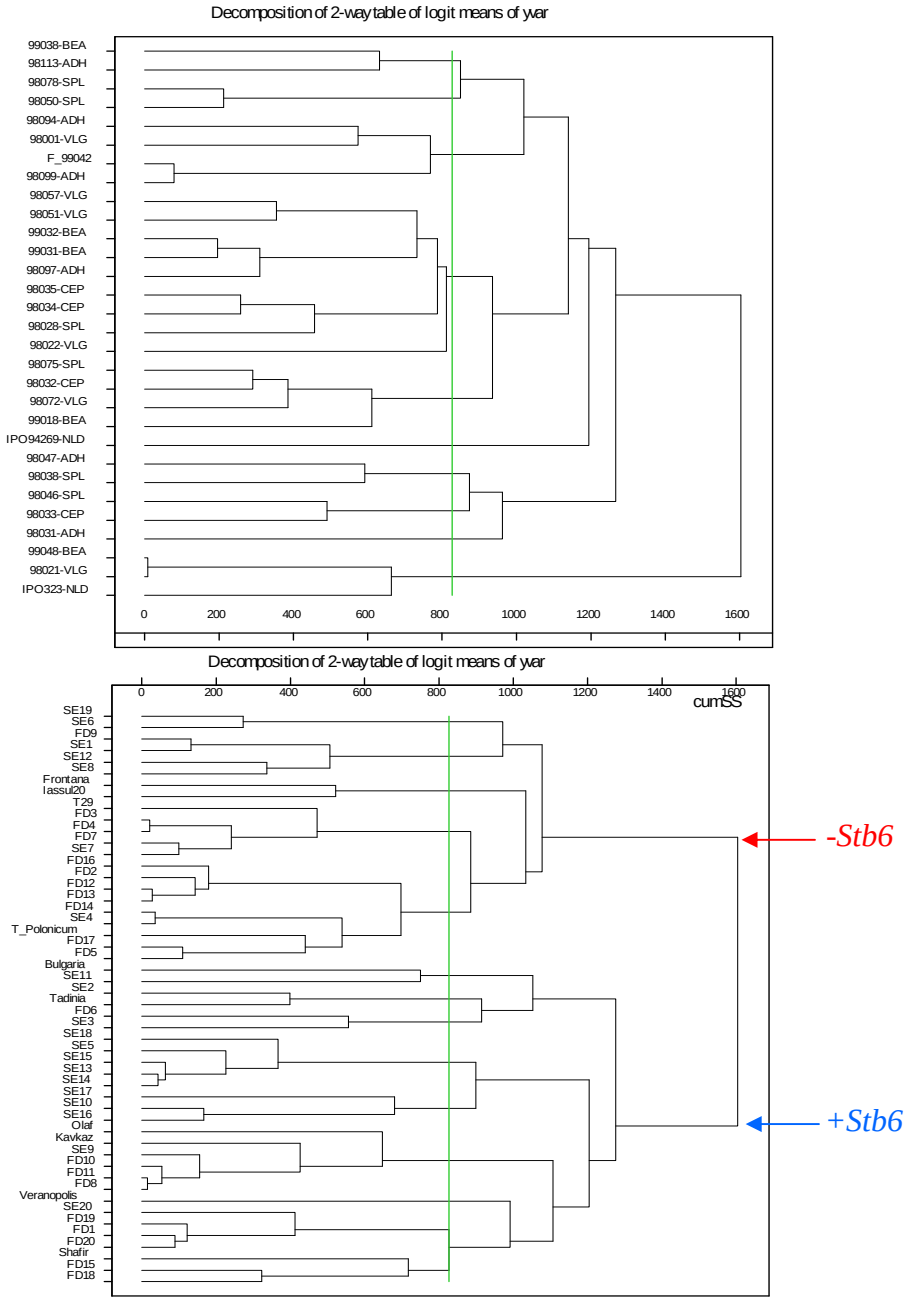


Figure S3. CINTERACTION output of EXP1 where 50 wheat cultivars and breeding lines were inoculated with 28 French and two Dutch *Mycosphaerella graminicola* isolates. Data analysis was based on P, the green line shows the threshold at P=0.05 for cluster assembly based on the cumulative sum of squares.

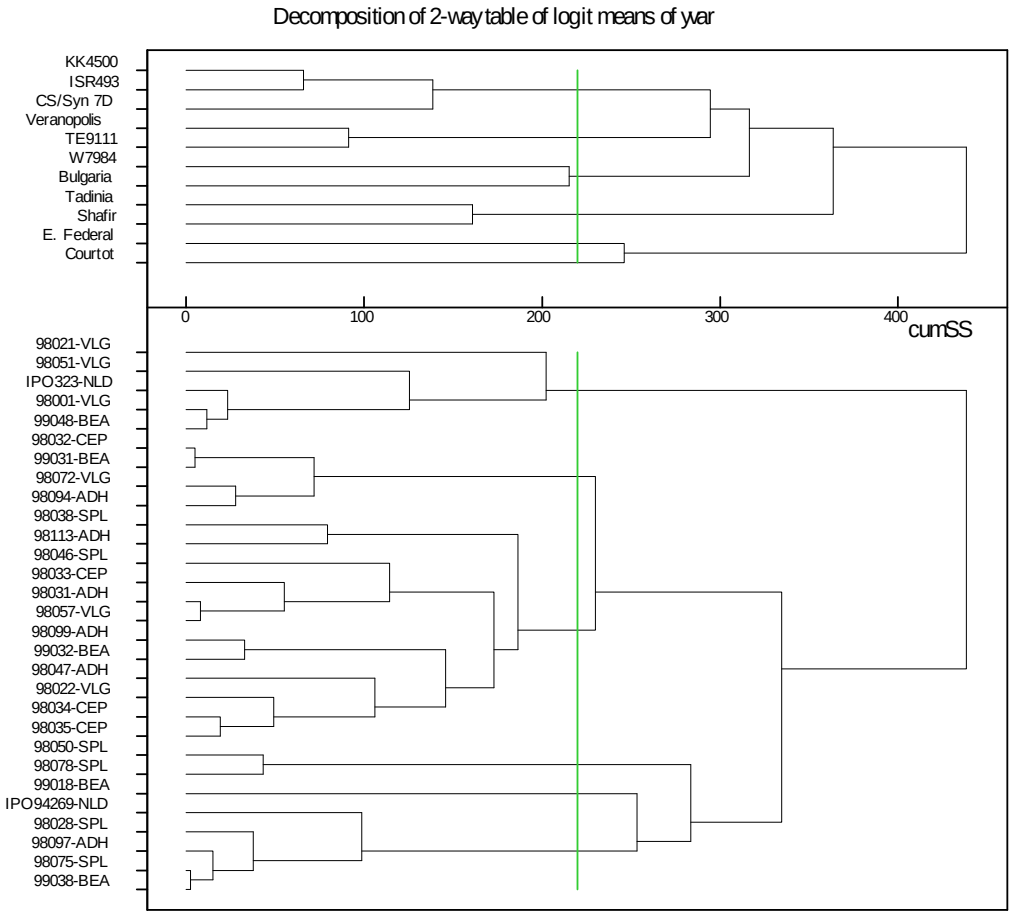


Figure S4. CINTERACTION output of EXP2 where 11 wheat differential cultivars carrying 12 *Stb* genes were inoculated with 27 French and two Dutch *Mycosphaerella graminicola* isolates. Data analysis was based on N, the green line shows the threshold at P=0.05 for cluster assembly based on the cumulative sum of squares.

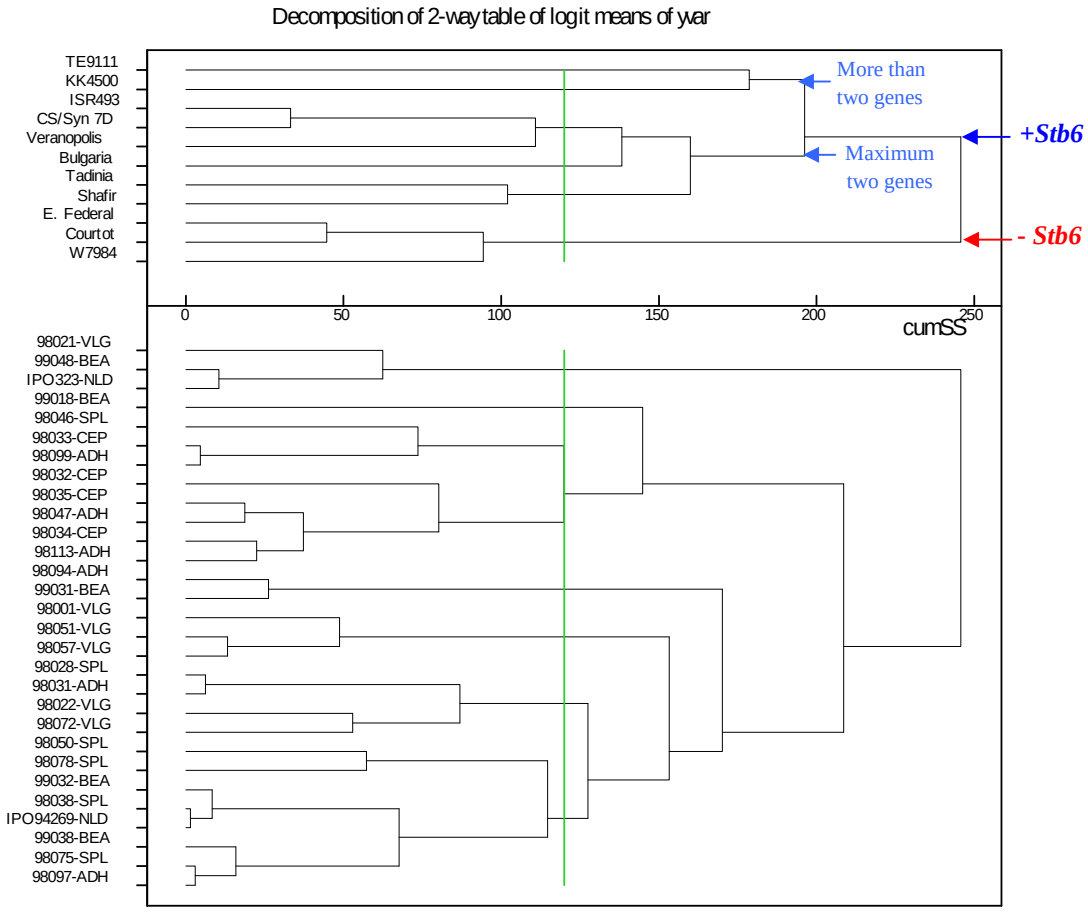


Figure S5. CINTERACTION output of EXP2 where 11 wheat differential cultivars carrying 12 *Stb* genes were inoculated with 27 French and two Dutch *Mycosphaerella graminicola* isolates. Data analysis was based on P, the green line shows the threshold at P=0.05 for cluster assembly based on the cumulative sum of squares. The differential set of cultivars was mainly distributed by the postulated presence/absence of *Stb6* in the genetic background as well as by the number of *Stb* genes.

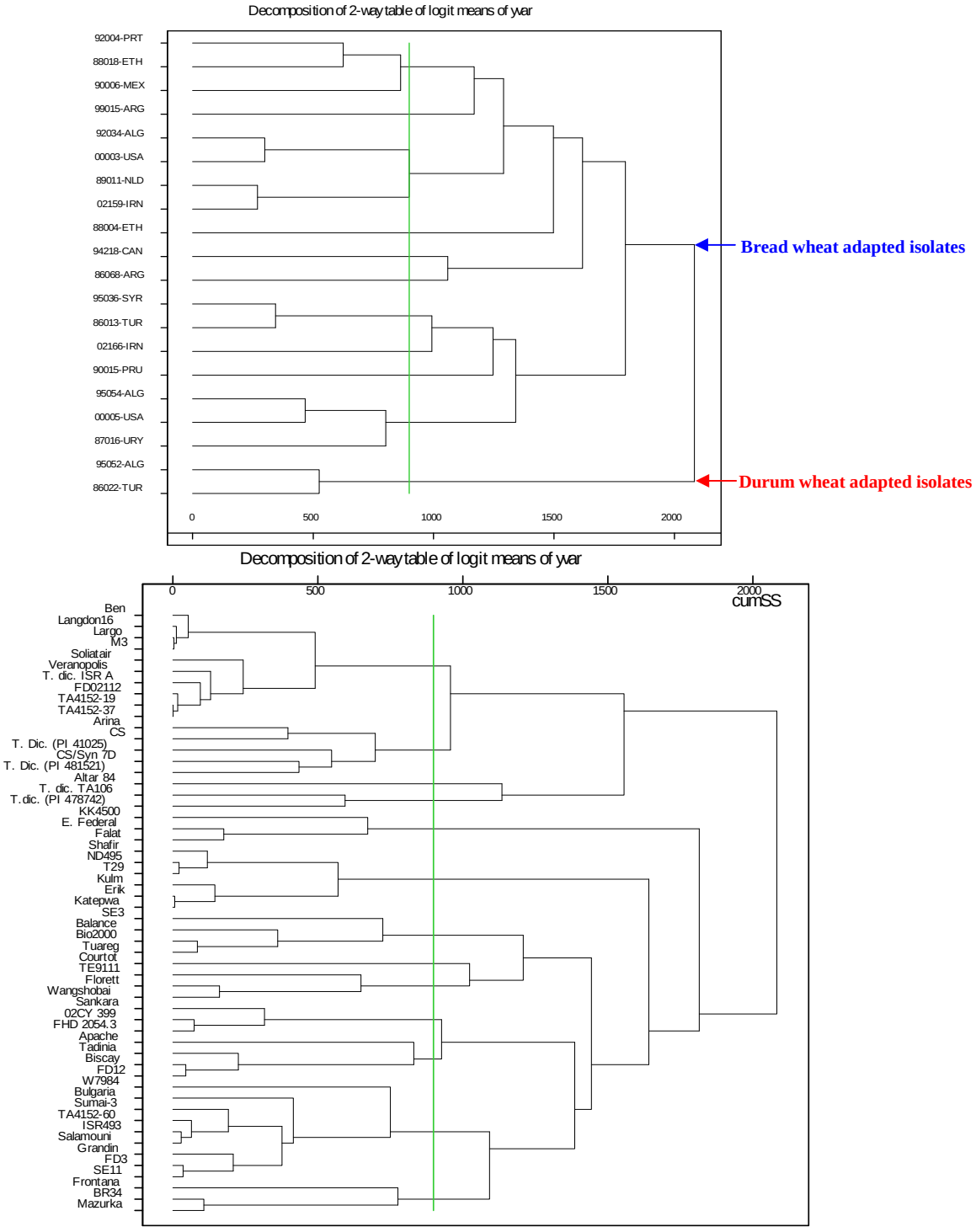


Figure S6. CINTERACTION output of EXP3 where 54 wheat cultivars and breeding lines carrying 15 *Stb* genes were inoculated with 20 global *Mycosphaerella graminicola* isolates. Data analysis was based on N, the green line shows the threshold at P=0.05 for cluster assembly based on the cumulative sum of squares. Two durum adapted *M. graminicola* isolates clustered separately from all other isolates.

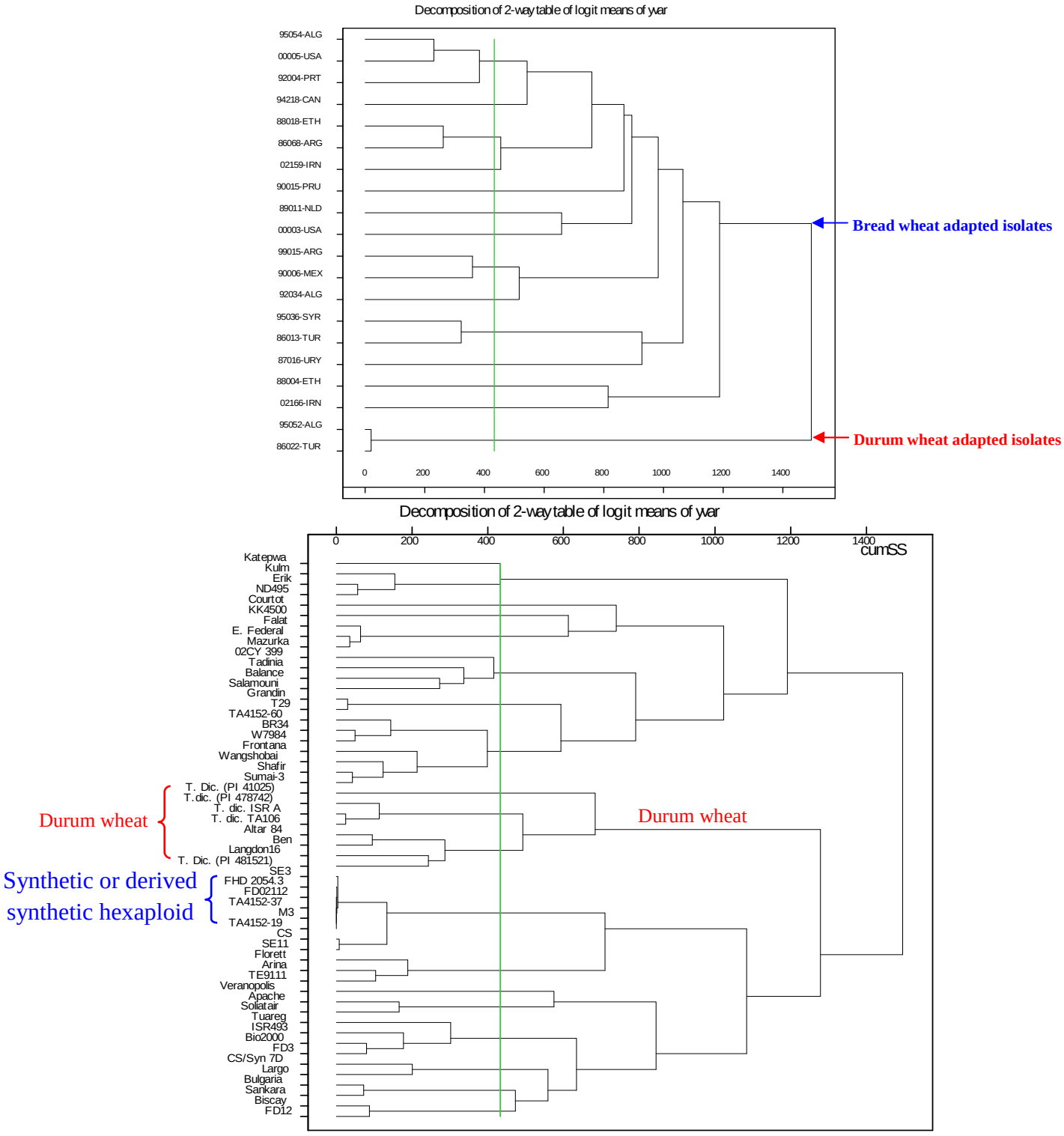


Figure S7. CINTERACTION output of EXP3 where 54 wheat cultivars and breeding lines carrying 15 *Stb* genes were inoculated with 20 global *Mycosphaerella graminicola* isolates. Data analysis was based on P, the green line shows the threshold at P=0.05 for cluster assembly based on the cumulative sum of squares. Two durum adapted *M. graminicola* isolates clustered separately from all other isolates.

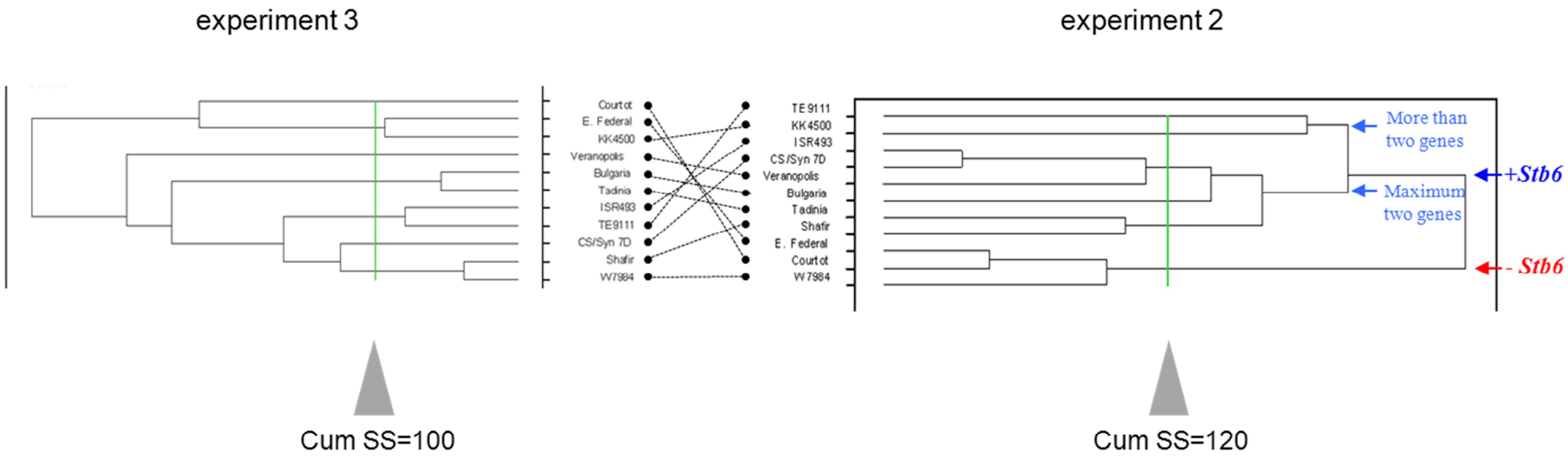


Figure S8. Significant ranking/clustering differences of wheat cultivars and lines carrying 12 *Stb* genes by using a French or global panel of *Mycosphaerella graminicola* isolates in EXP2 and EXP3

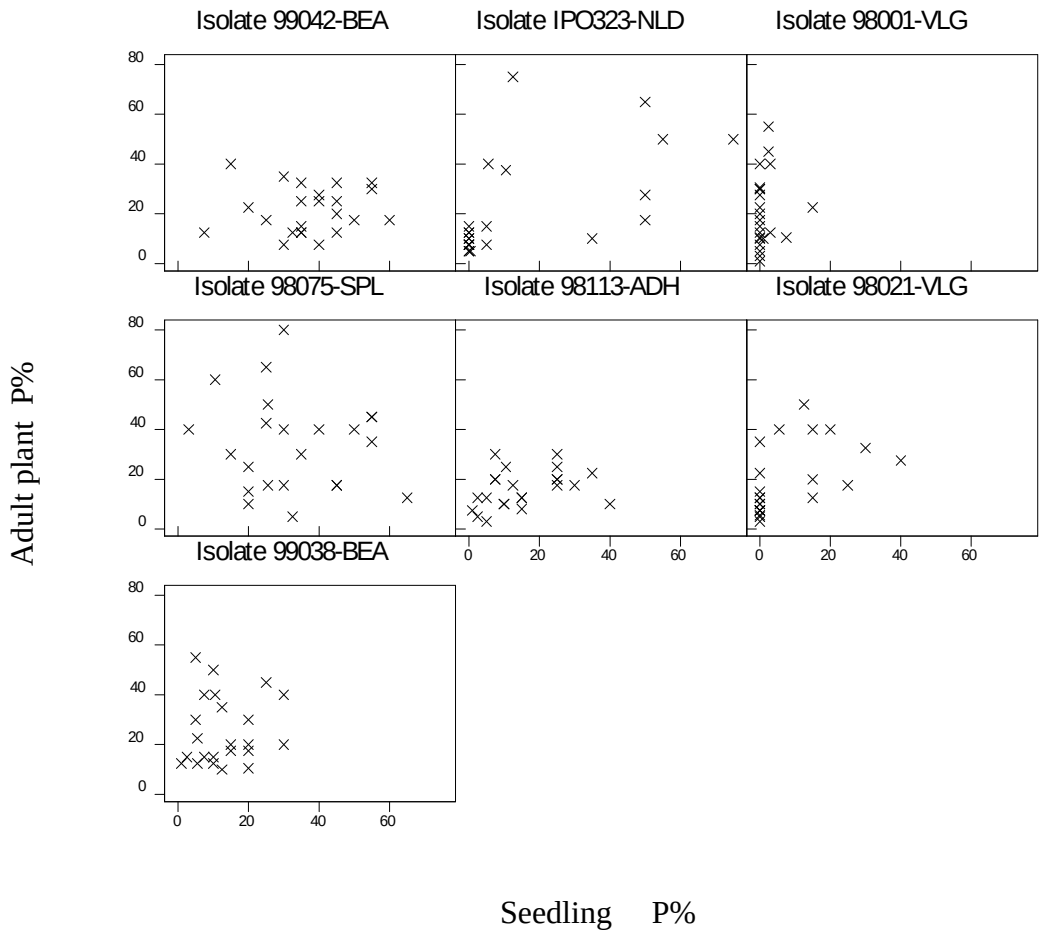
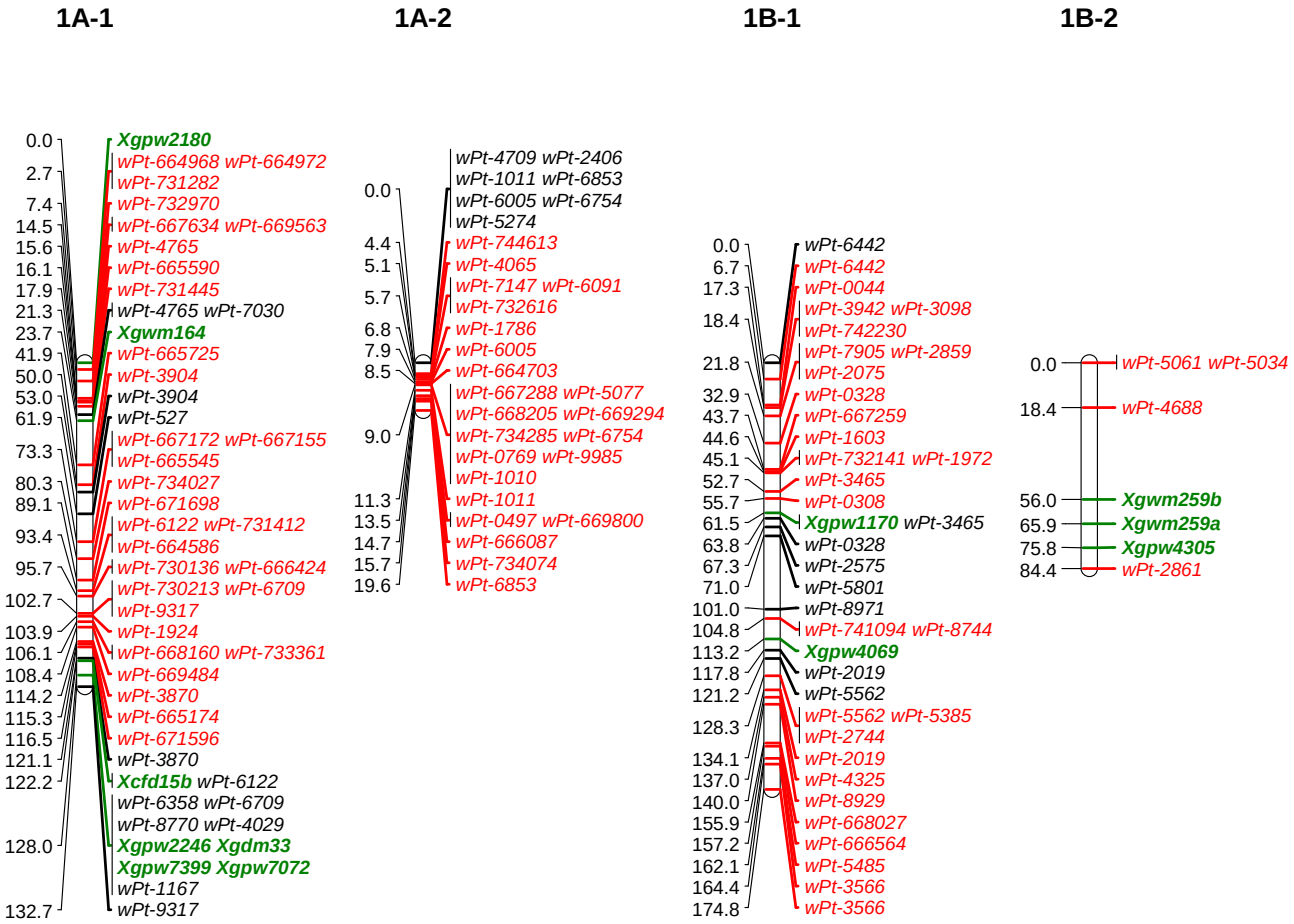


Figure S9. Output of the comparative seedling-adult plant experiment (EXP4). Seedling P values are plotted along the x-axis and adult plant P levels along the Y-axis. Experiments involved 23 French breeding lines that were inoculated with seven *Mycosphaerella graminicola* isolates.

Chapter 4

Apache/Balance Genetic map

Black, red and green fonts represent DArT *(V2.3)*, DArT *(V3)* and SSR markers, respectively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17.9 wPt-665590
21.3 wPt-731445
23.7 wPt-4765 wPt-7030
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73.3 wPt-527
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93.4 wPt-734027
95.7 wPt-671698
102.7 wPt-6122 wPt-731412
103.9 wPt-664586
106.1 wPt-730136 wPt-666424
108.4 wPt-730213 wPt-6709
114.2 wPt-9317
115.3 wPt-1924
116.5 wPt-668160 wPt-733361
121.1 wPt-669484
122.2 wPt-3870
128.0 wPt-665174
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wPt-3870
Xcfd15b wPt-6122
wPt-6358 wPt-6709
wPt-8770 wPt-4029
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Xgpw7399 Xgpw7072
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14.7 wPt-664703
15.7 wPt-667288 wPt-5077
19.6 wPt-668205 wPt-669294
wPt-734285 wPt-6754
wPt-0769 wPt-9985
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44.6 wPt-667259
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101.0 wPt-5801
104.8 wPt-8971
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117.8 Xgpw4069
121.2 wPt-2019
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wPt-3566
wPt-3566
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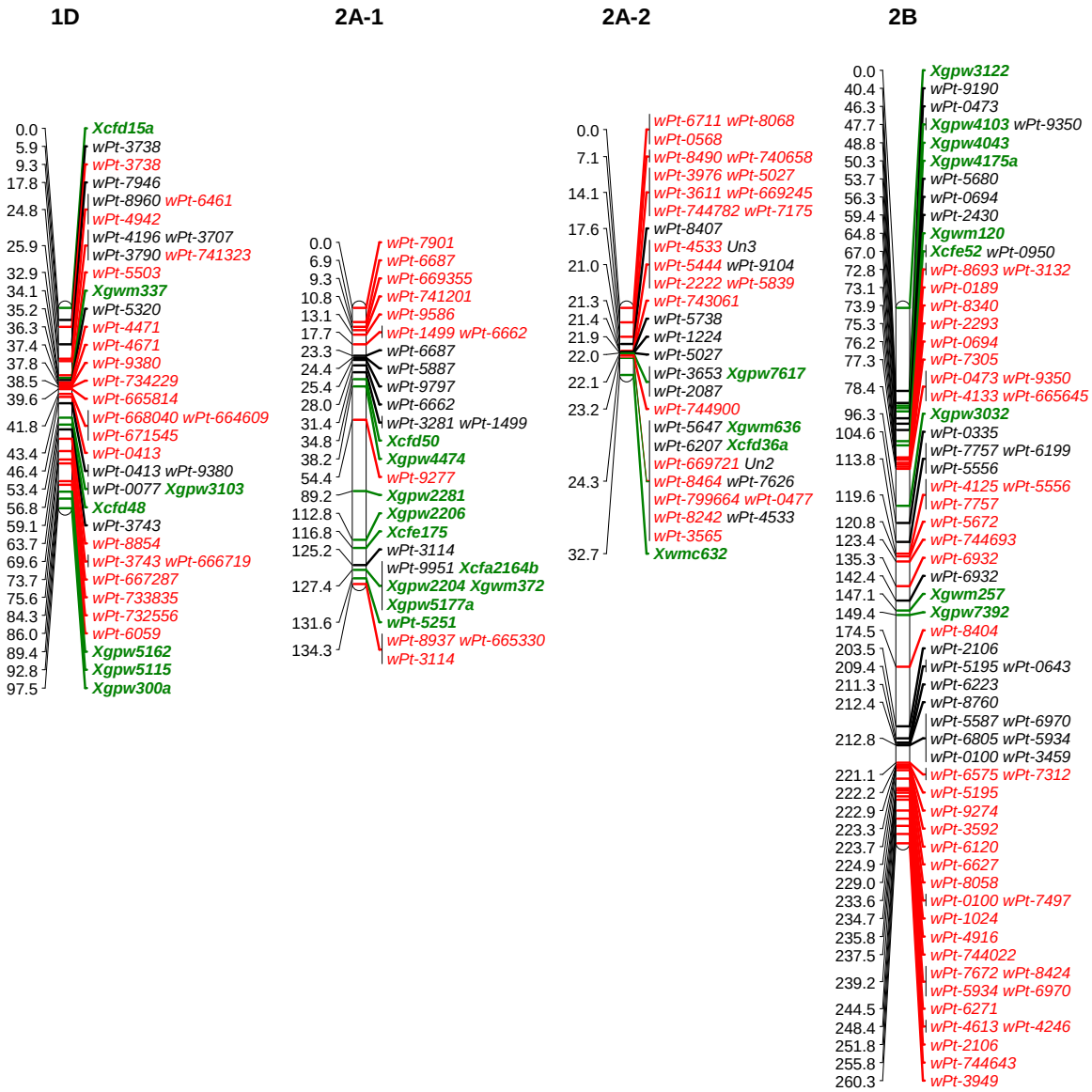
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39.6 wPt-665814
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24.4 wPt-5887
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Xgpw5177a
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67.0 Xcfe52 wPt-0950
72.8 wPt-8693 wPt-3132
73.1 wPt-0189
73.9 wPt-8340
75.3 wPt-2293
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wPt-4133 wPt-665645
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wPt-5556
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wPt-8760
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wPt-0100 wPt-3459
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222.9 wPt-9274
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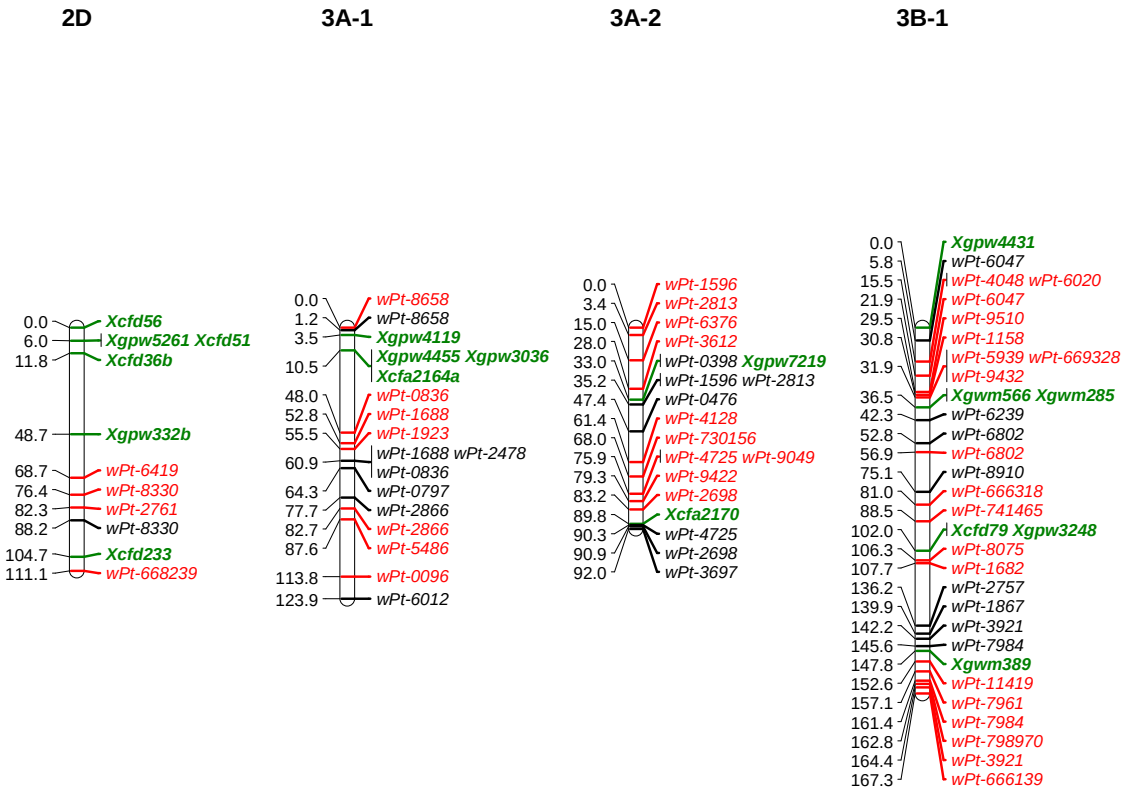
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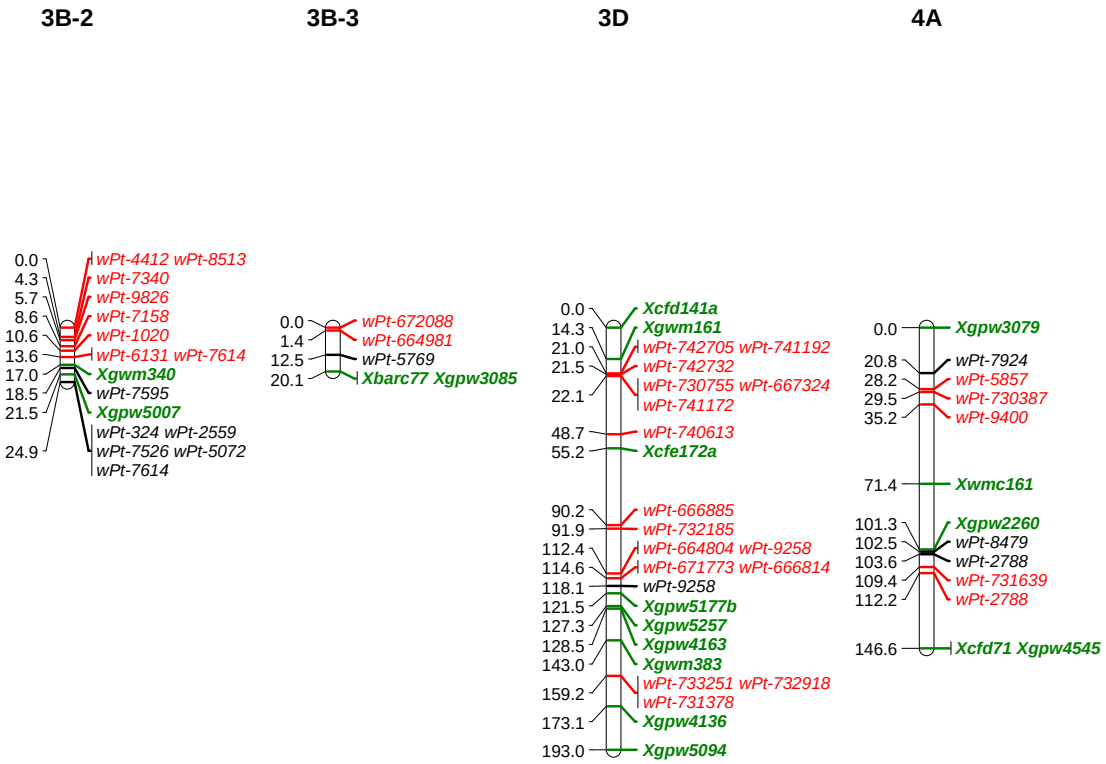
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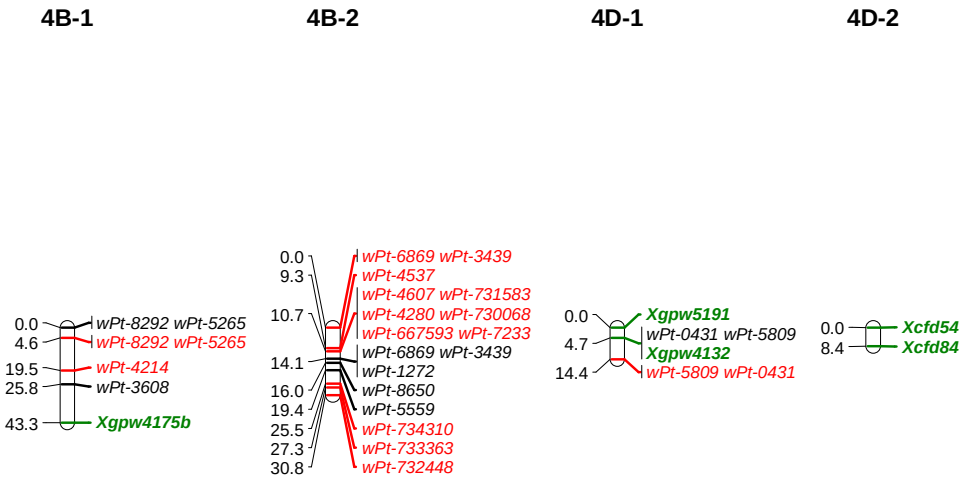
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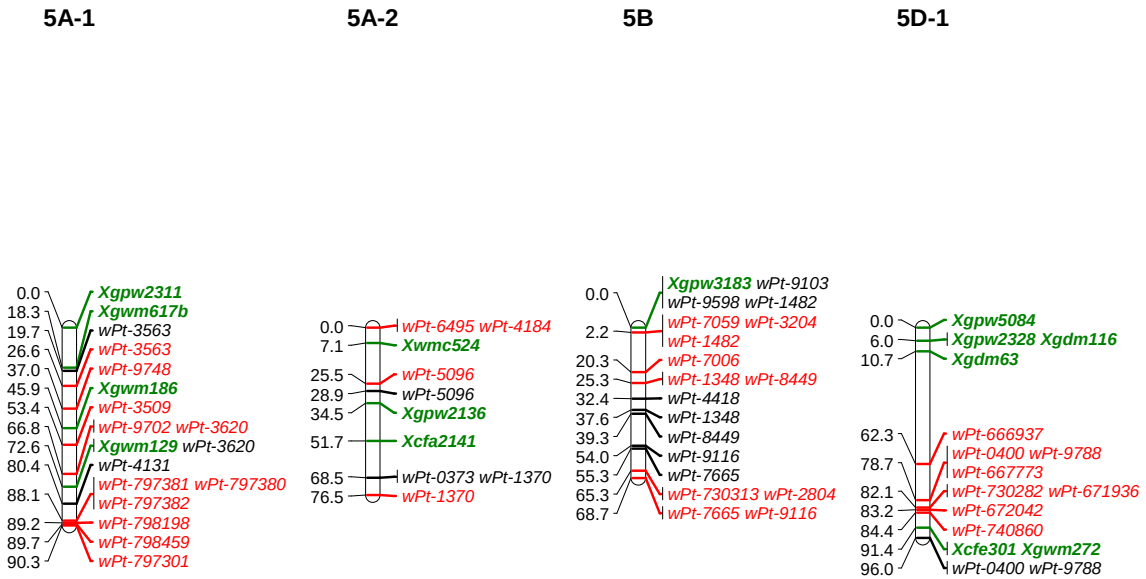
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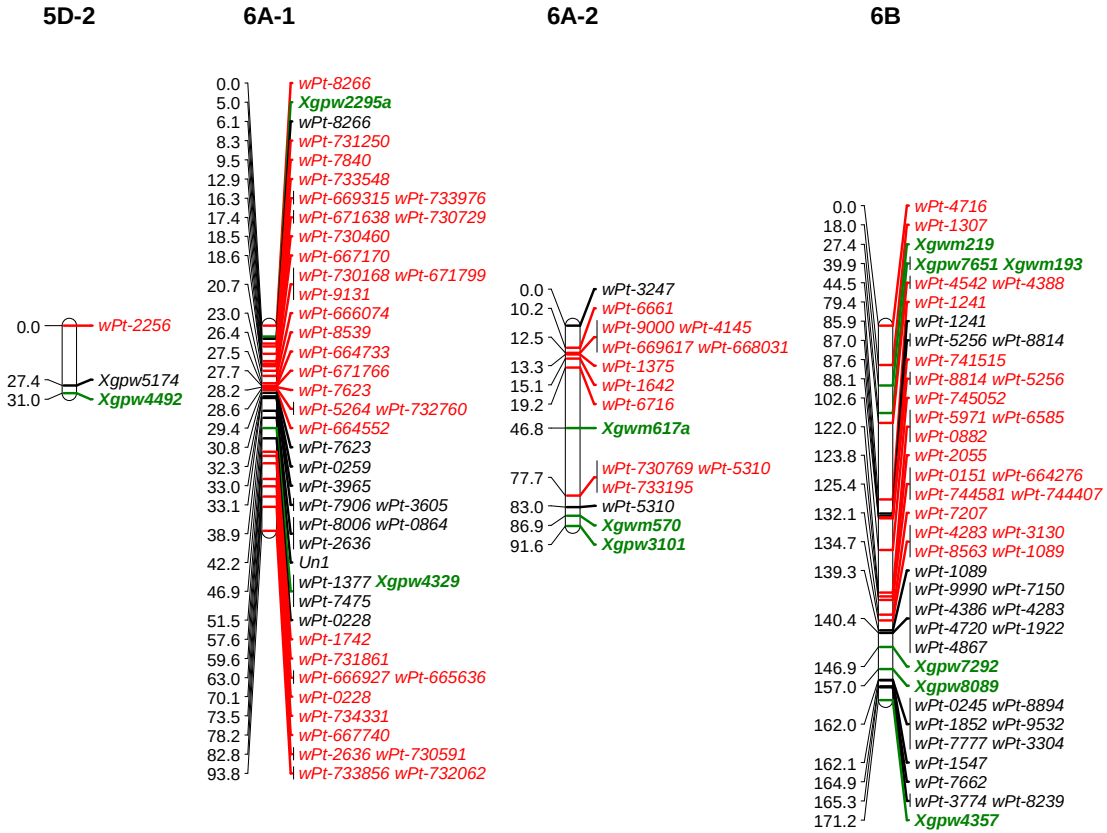
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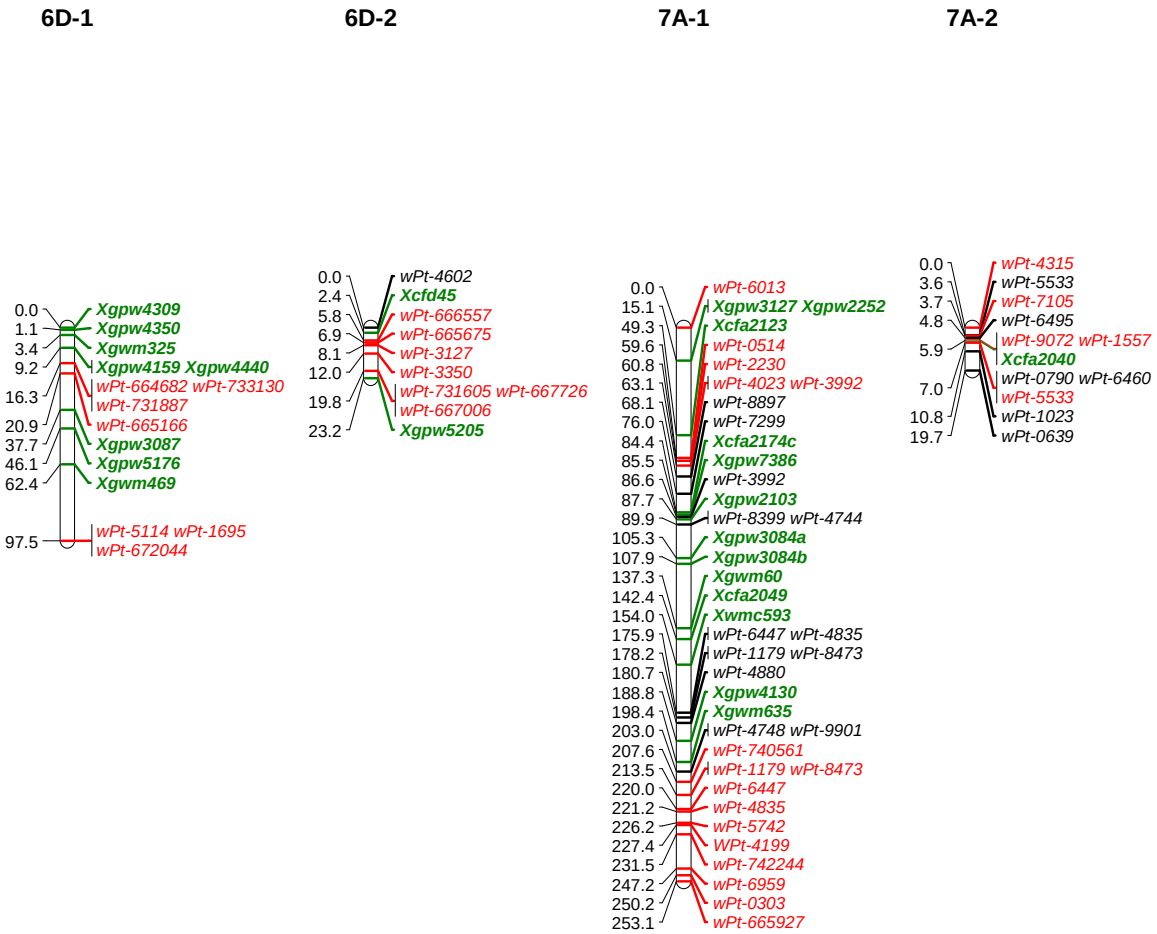
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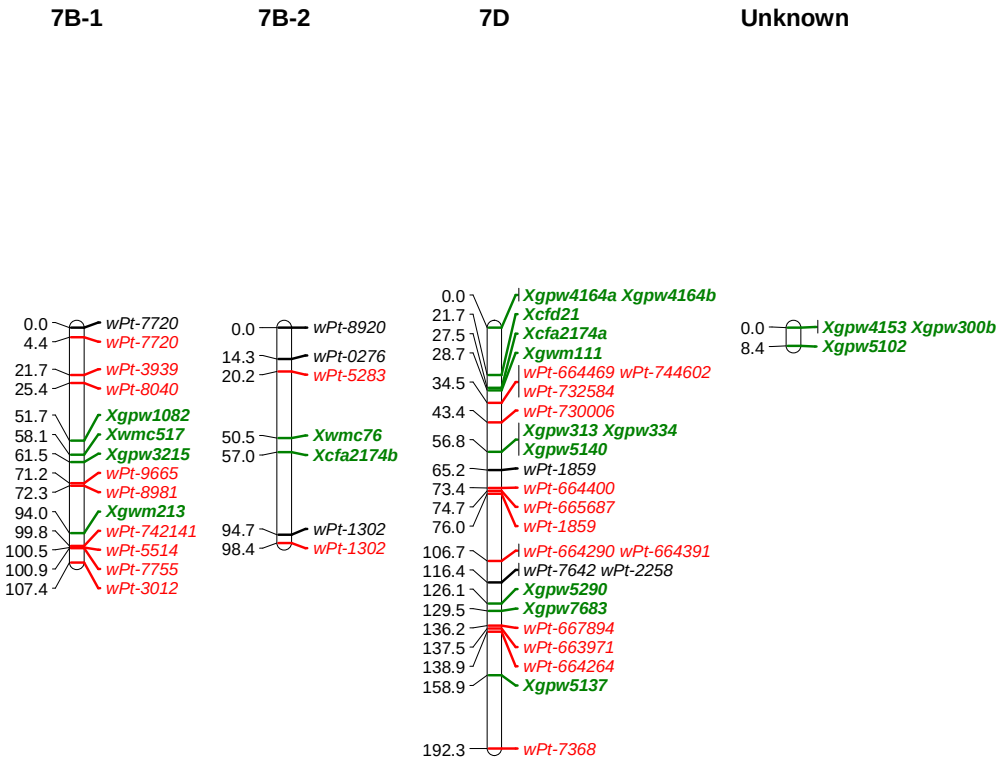
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Chapter 5

Extra tables and figures

ESM 1 Table 1. Correlation coefficients of pycnidial coverage (PYC) (upper triangle) and necrotic leaf area (NEC) (lower triangle) between isolates PO90015, IPO99015, IPO92034 and IPO323 in seedling assays (three experiments)

		IPO90015				IPO99015				IPO92034				IPO323			
	experiment no.	1	2	3	mean	1	2	3	mean	1	2	3	mean	1	2	3	mean
IPO90015	1 (n=128)		0.84***	0.85***	0.94***	0.11	0.19*	0.12	0.15	0.23**	0.19*	0.24**	0.24**	0.29**	0.28**	0.23**	0.27**
	2 (n=128)	0.89***		0.88***	0.96***	0.14	0.25**	0.15	0.20*	0.29***	0.26**	0.25**	0.29**	0.18*	0.20*	0.15	0.18*
	3 (n=128)	0.89***	0.91***		0.95***	0.10	0.26**	0.13	0.18*	0.30***	0.34***	0.35***	0.36***	0.22*	0.23**	0.16	0.21*
	mean	0.90***	0.97***	0.96***		0.13	0.25**	0.14	0.19*	0.29**	0.27**	0.29***	0.31***	0.24**	0.25**	0.19*	0.23**
IPO99015	1 (n=128)	0.19*	0.26**	0.24**	0.24**		0.77***	0.80***	0.92***	0.36***	0.33***	0.36***	0.38***	0.21*	0.21*	0.22*	0.22*
	2 (n=128)	0.20*	0.30***	0.28**	0.27**	0.85***		0.80***	0.93***	0.28**	0.35***	0.33***	0.35***	0.17*	0.18*	0.16	0.17*
	3 (n=128)	0.11	0.19*	0.18*	0.17	0.85***	0.87***		0.93***	0.36***	0.41***	0.39***	0.42***	0.16	0.15	0.17	0.16
	mean	0.18*	0.20**	0.25**	0.24**	0.94***	0.96***	0.95***		0.36***	0.39***	0.39***	0.41***	0.20*	0.20*	0.20*	0.20*
IPO92034	1 (n=128)	0.27**	0.20***	0.30***	0.30***	0.63***	0.62***	0.55***	0.63***		0.73***	0.81***	0.90***	-0.09	-0.05	-0.05	-0.06
	2 (n=128)	0.27**	0.34***	0.36***	0.33***	0.57***	0.65***	0.61***	0.64***	0.73***		0.82***	0.92***	-0.01	0.00	-0.02	-0.01
	3 (n=128)	0.21*	0.27**	0.32***	0.28**	0.65***	0.71***	0.68***	0.72***	0.78***	0.84***		0.95***	0.11	0.13	0.10	0.12
	mean	0.27**	0.32***	0.35***	0.33***	0.67***	0.71***	0.67***	0.72***	0.90***	0.93***	0.95***		0.01	0.03	0.02	0.02
IPO323	1 (n=128)	0.12	0.01	0.10	0.08	0.27**	0.19*	0.15	0.21*	0.16	0.15	0.21*	0.19*		0.93***	0.93***	0.97***
	2 (n=128)	0.12	0.02	0.11	0.08	0.27**	0.21*	0.16	0.22*	0.18*	0.18*	0.24**	0.22*	0.97***		0.93***	0.98***
	3 (n=128)	0.10	0.02	0.09	0.07	0.28**	0.21*	0.16	0.23*	0.18*	0.18*	0.23*	0.21*	0.95***	0.97***		0.97***
	mean	0.11	0.02	0.10	0.08	0.28**	0.20*	0.16	0.22*	0.17*	0.17	0.23**	0.21*	0.98***	0.99***	0.99***	

* P=0.05, ** P=0.01, ***P=0.001

ESM 1 Table 2. Correlation coefficients of pycnidial coverage (PYC) (upper triangle) and necrotic leaf area (NEC) (lower triangle) between isolates Hu1, Hu2 and BBA22 in seedling assays (three experiments)

		Hu1				Hu2				BBA22			
	experiment no.	1	2	3	mean	1	2	3	mean	1	2	3	mean
Hu1	1 (n=131)		0.35***	0.30***	0.51***	0.29***	0.42***	0.43***	0.44***	0.11	0.04	0.07	0.08
	2 (n=130)	0.41***		0.33***	0.55***	0.32***	0.37***	0.45***	0.44***	0.11	0.14	0.08	0.12
	3 (n=131)	0.31***	0.38***		0.84***	0.60***	0.42***	0.37***	0.57***	0.22*	0.13	0.05	0.15
	mean	0.55***	0.61***	0.83***		0.56***	0.47***	0.41***	0.58***	0.18*	0.13	0.08	0.15
Hu2	1 (n=132)	0.28**	0.37***	0.65***	0.58***		0.42***	0.43***	0.78***	0.17*	0.12	0.18*	0.18*
	2 (n=131)	0.46***	0.40***	0.47***	0.57***	0.46***		0.75***	0.84***	0.07	0.01	0.08	0.06
	3 (n=131)	0.38***	0.39***	0.54***	0.49***	0.48***	0.62***		0.86***	0.12	0.06	0.02	0.07
	mean	0.45***	0.47***	0.68***	0.66***	0.81***	0.82***	0.84***		0.15	0.08	0.12	0.14
BBA22	1 (n=131)	0.14	0.1	0.37***	0.26**	0.42***	0.23**	0.33***	0.40***		0.68***	0.57***	0.86***
	2 (n=131)	0.12	0.08	0.37***	0.27**	0.40***	0.17	0.34***	0.38***	0.62***		0.62***	0.89***
	3 (n=131)	0.11	-0.07	0.22*	0.16	0.36***	0.26**	0.33***	0.39***	0.46***	0.46***		0.85***
	mean	0.15	0.04	0.38***	0.28**	0.48***	0.27**	0.41***	0.48***	0.82***	0.84***	0.80***	

* P=0.05, ** P=0.01, ***P=0.001

ESM 1 Table 3. Correlation coefficients of pycnidial coverage (PYC) between isolates Hu1, Hu2, BBA22 and IPO90015, IPO99015, IPO92034, IPO323 in seedling assays (three experiments)

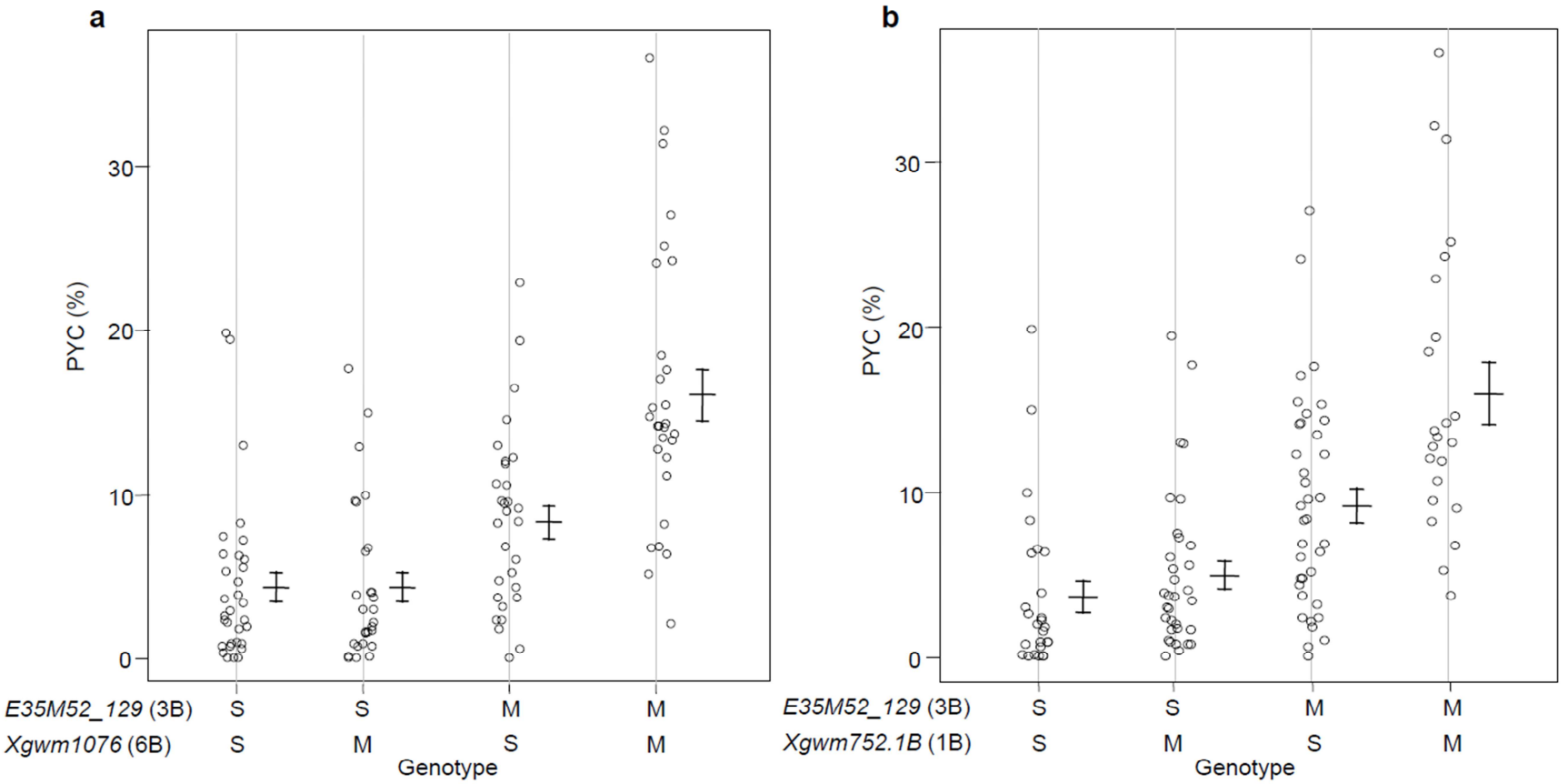
		Hu1				Hu2				BBA22			
	experiment no.	1 (n=131)	2 (n=130)	3 (n=131)	mean	1 (n=132)	2 (n=131)	3 (n=131)	mean	1 (n=131)	2 (n=131)	3 (n=131)	mean
IPO90015	1 (n=128)	0.08	0.06	0.15	0.13	0.21*	0.03	0.08	0.15	0.42***	0.46***	0.40***	0.50***
	2 (n=128)	0.12	0.12	0.10	0.09	0.18*	0.04	0.15	0.16	0.33***	0.43***	0.34***	0.43***
	3 (n=128)	0.13	0.11	0.21*	0.19*	0.19*	0.11	0.15	0.20*	0.37***	0.43***	0.35***	0.45***
	mean	0.12	0.10	0.16	0.14	0.20*	0.06	0.13	0.18*	0.39***	0.46***	0.38***	0.48***
IPO99015	1 (n=128)	0.45***	0.42***	0.32***	0.36***	0.45***	0.63***	0.67***	0.68***	0.02	-0.03	0.02	0.00
	2 (n=128)	0.42***	0.50***	0.38***	0.40***	0.51***	0.61***	0.67***	0.71***	0.11	0.05	0.09	0.10
	3 (n=128)	0.32***	0.45***	0.37***	0.35***	0.51***	0.66***	0.65***	0.71***	0.05	-0.02	0.09	0.05
	mean	0.44***	0.49***	0.38***	0.40***	0.53***	0.68***	0.72***	0.76***	0.07	0.00	0.07	0.05
IPO92034	1 (n=128)	0.30***	0.26**	0.27**	0.27**	0.31***	0.28**	0.31***	0.37***	0.28**	0.19*	0.09	0.21*
	2 (n=128)	0.35***	0.23**	0.38***	0.38***	0.39***	0.26**	0.26**	0.37***	0.28**	0.21*	0.19*	0.26**
	3 (n=128)	0.31***	0.26**	0.26**	0.26**	0.25**	0.33***	0.27**	0.36***	0.22*	0.19*	0.17	0.22*
	mean	0.35***	0.27**	0.33***	0.33***	0.34***	0.31***	0.30***	0.39***	0.28**	0.21*	0.16	0.25**
IPO323	1 (n=128)	-0.02	0.04	0.07	0.01	0.15	0.24**	0.23**	0.26**	0.07	0.14	0.15	0.14
	2 (n=128)	-0.09	0.04	0.05	-0.02	0.17	0.19*	0.20*	0.24**	0.06	0.11	0.12	0.11
	3 (n=128)	-0.04	0.01	0.08	0.00	0.17	0.24**	0.21*	0.26**	0.04	0.10	0.11	0.10
	mean	-0.05	0.03	0.07	0.00	0.17	0.23*	0.22*	0.26**	0.06	0.12	0.13	0.12

* P=0.05, ** P=0.01, ***P=0.001

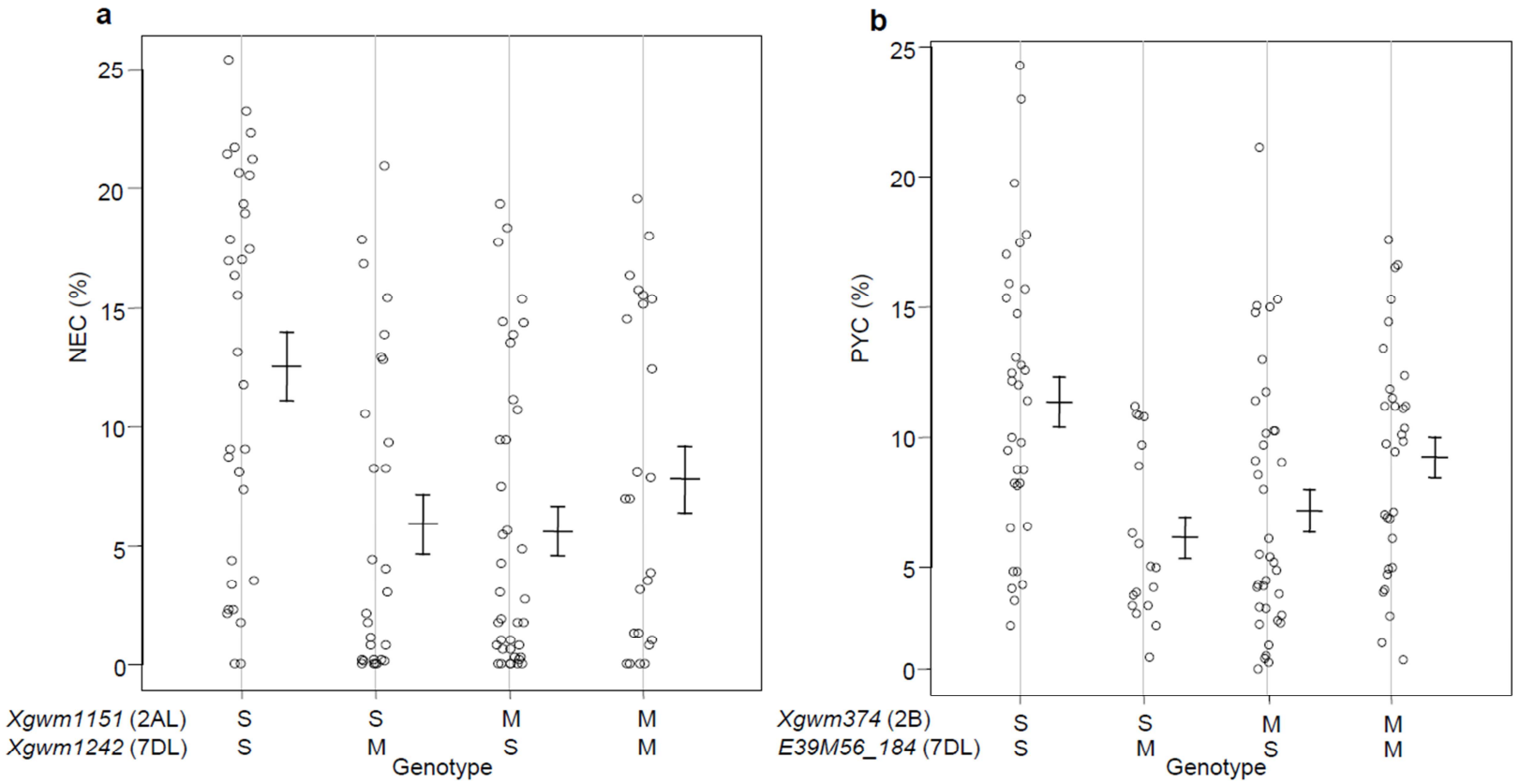
ESM 1 Table 4. Correlation coefficients of necrotic leaf area (NEC) between isolates Hu1, Hu2, BBA22 and IPO90015, IPO99015, IPO92034, IPO323 in seedling assays (three experiments)

		Hu1				Hu2				BBA22			
	experiment no.	1 (n=131)	2 (n=130)	3 (n=131)	mean	1 (n=132)	2 (n=131)	3 (n=131)	mean	1 (n=131)	2 (n=131)	3 (n=131)	mean
IPO90015	1 (n=128)	0.06	0.09	0.22*	0.19*	0.25**	0.07	0.11	0.18*	0.35***	0.43***	0.20*	0.40***
	2 (n=128)	0.14	0.09	0.17	0.15	0.25**	0.09	0.09	0.18*	0.34***	0.40***	0.26**	0.41***
	3 (n=128)	0.09	0.09	0.20*	0.15	0.24**	0.10	0.14	0.20*	0.35***	0.37***	0.24**	0.39***
	mean	0.10	0.09	0.20*	0.17	0.26**	0.09	0.11	0.20*	0.36***	0.42***	0.24**	0.41***
IPO99015	1 (n=128)	0.49***	0.48***	0.28**	0.35***	0.43***	0.59***	0.47***	0.59***	0.10	0.08	0.09	0.11
	2 (n=128)	0.47***	0.54***	0.37***	0.42***	0.51***	0.57***	0.51***	0.64***	0.15	0.12	0.12	0.16
	3 (n=128)	0.44***	0.56***	0.38***	0.42***	0.52***	0.61***	0.51***	0.66***	0.11	0.05	0.12	0.12
	mean	0.49***	0.56***	0.36***	0.42***	0.51***	0.62***	0.52***	0.67***	0.13	0.09	0.12	0.14
IPO92034	1 (n=128)	0.32***	0.31***	0.22*	0.25**	0.43***	0.34***	0.21*	0.40***	0.16	0.19*	0.09	0.17
	2 (n=128)	0.41***	0.37***	0.34***	0.36***	0.51***	0.35***	0.34***	0.49***	0.25**	0.26**	0.21*	0.29***
	3 (n=128)	0.39***	0.39***	0.30***	0.32***	0.46***	0.43***	0.35***	0.50***	0.19*	0.15	0.16	0.21*
	mean	0.40***	0.39***	0.31***	0.33***	0.50***	0.40***	0.33***	0.51***	0.21*	0.21*	0.17	0.24**
IPO323	1 (n=128)	0.08	0.07	0.02	0.01	0.06	0.12	0.16	0.13	0.02	0.01	0.05	0.03
	2 (n=128)	0.06	0.09	0.05	0.02	0.08	0.10	0.16	0.13	0.03	0.01	0.05	0.04
	3 (n=128)	0.08	0.09	0.07	0.04	0.11	0.14	0.19*	0.17	0.06	0.01	0.08	0.06
	mean	0.07	0.08	0.05	0.02	0.09	0.12	0.17	0.15	0.03	0.01	0.06	0.05

* P=0.05, ** P=0.01, ***P=0.001



ESM Figure 1 Mean epistatic effects and standard errors revealed in the SxM DH population with isolate IPO92034. **(a)** pycnidial coverage (PYC, in %) of the Solitär (S) and Mazurka (M) allele combinations at *E35M53_129* (3B) and *Xgwm1076* (6B). (**b)** parental allele combinations at *E35M53_129* (3B) and *Xgwm752.1B (1B)*



ESM Figure 2. Mean epistatic QTL effects and standard errors revealed in the SxM DH population. (a) necrotic leaf area (NEC, in %) of the Solitär (S) and Mazurka (M) allele combinations at *Xgwm1151* (2AL) and *Xgwm1242* (7DL) discovered with IPO99015. (b) pycnidial coverage (PYC, in %) of the parental allele combinations at *Xgwm374* (2B) and *E39M56_184* (7DL) discovered with Hu2