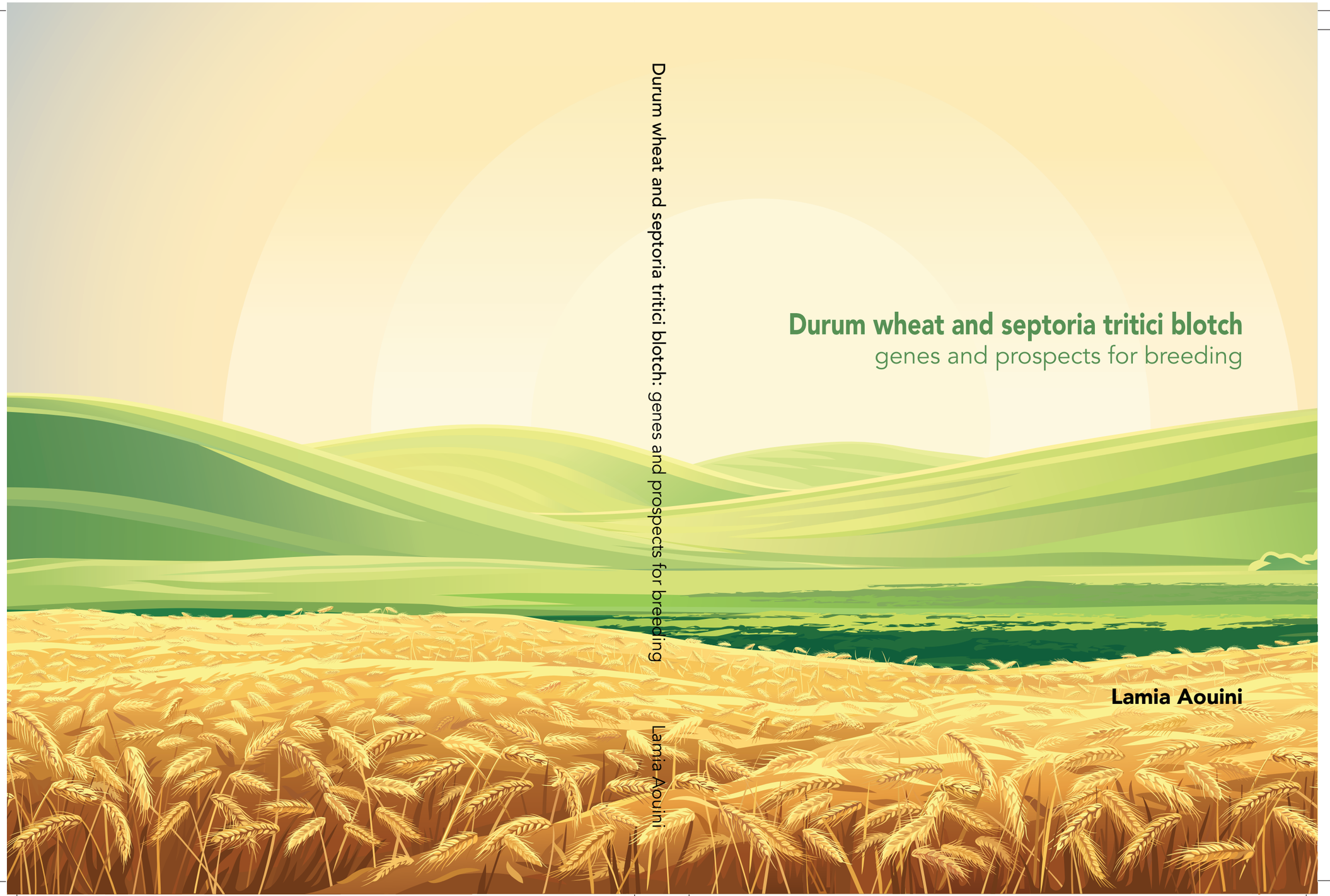

Durum wheat and septoria tritici blotch: genes and prospects for breeding

Lamia Aouini

Durum wheat and septoria tritici blotch

genes and prospects for breeding

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INVITATION

To attend the public defense of my PhD-thesis entitled:

Durum wheat and septoria tritici blotch
genes and porspects for breeding

Lamia Aouini

Tuesday 17th of April 2018
at 13:30 hours

The Aula,
Wageningen university,
Generaal Foulkesweg 1,
6703 BG, Wageningnen.

There will be a reception held afterwards,
you are all cordially invited.

paranymphs

Nani Maryani Martawi
nani.maryanimartawi@wur.nl

Florencia Lucca
Flolucca@gmail.com

Propositions

1. The stress-induced sexual preference of *Zymoseptoria tritici* is incompatible with boom-and-bust cycles and rapid deterioration of host resistance.

(this thesis)

2. The overall susceptibility of durum wheat to *Zymoseptoria tritici* is likely due to a major bottle-neck that narrowed the genetic basis of contemporary varieties.

(this thesis)

3. Striving for optimal cultivars damages nature.

4. Since the abundance of data limits integration, multidisciplinarity is needed to make progress.

5. Science needs perseverance rather than outstanding skills.

6. Future generations will only be better nourished by empowering women in agriculture.

Propositions belonging to the PhD thesis, entitled

"Durum wheat and septoria tritici blotch: genes and prospects for breeding"

Lamia Aouini

Wageningen, 17 April 2018

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Lamia Aouini

Thesis

Submitted in fulfilment of the requirements
for the degree of doctor at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr. A.P.J. Mol
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Tuesday 17 April 2018
at 1.30 pm in the Aula

Thesis committee

Promotors

Prof. Dr. ir G.H.J. Kema
Professor of Tropical Phytopathology
Wageningen University

Prof. Dr. R.G.F. Visser
Professor of Plant Breeding
Wageningen University

Other members

Prof. Dr. ir. P.C. Struik, Wageningen University

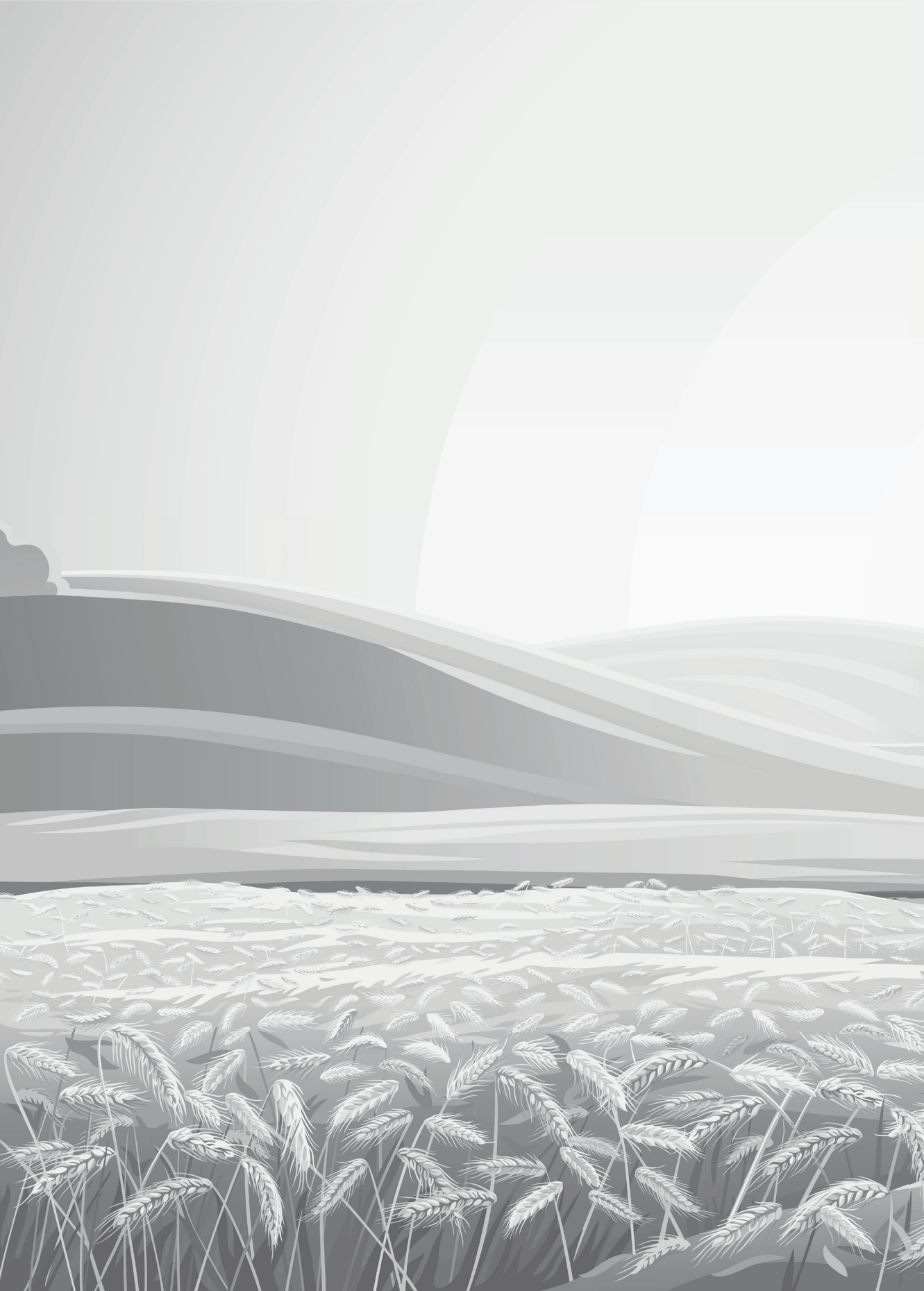
Prof. Dr. B.J. Zwaan, Wageningen University

Dr. M. Rep, University of Amsterdam

Dr. T.L. Friesen, USDA, North Dakota

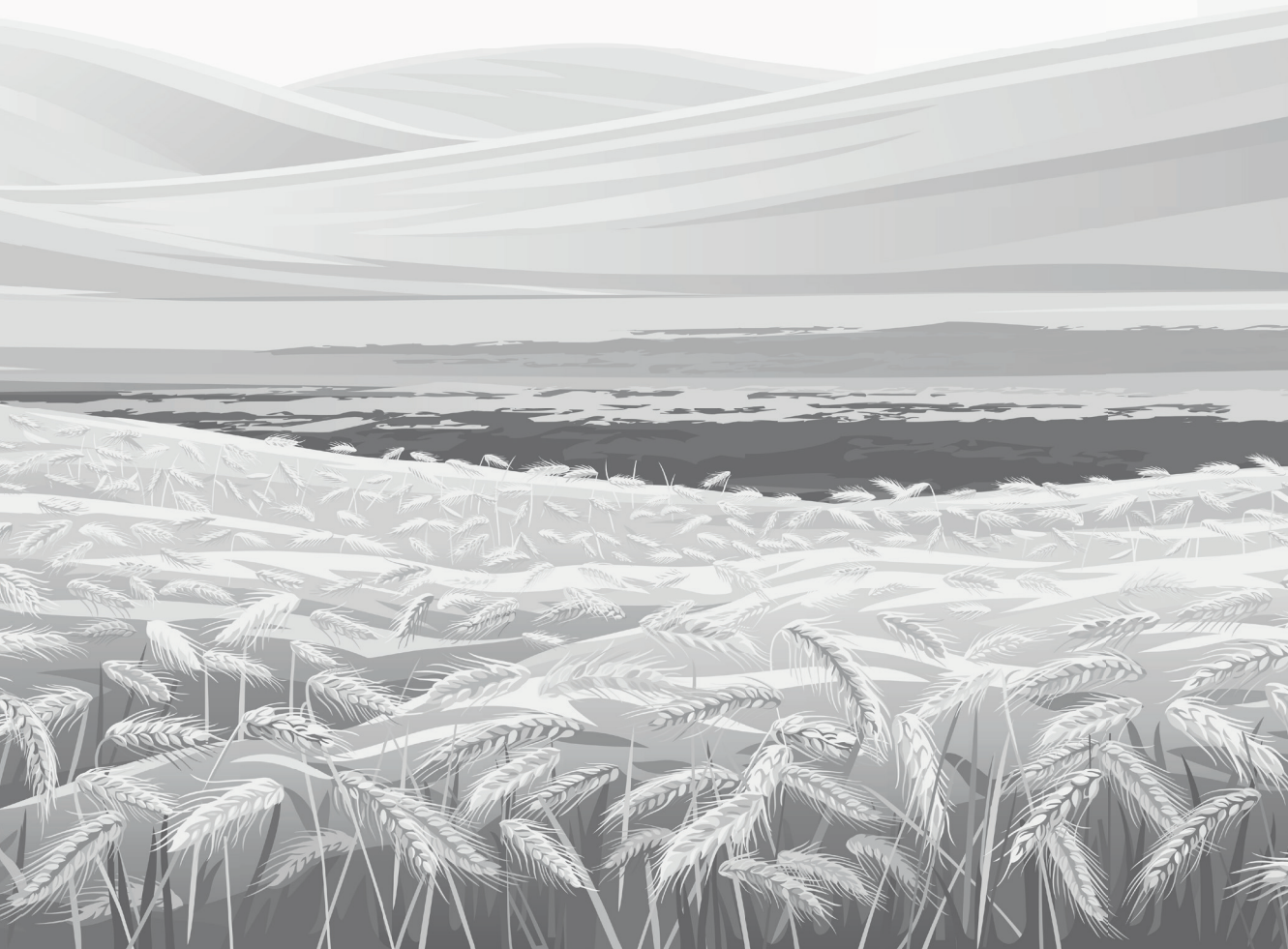
This thesis was conducted under the auspices of the Graduate school of "Experimental Plant Sciences"

Contents



Chapter 1

General Introduction



The domestication of tetraploid wheat and the onset of modern agriculture

Describing the history of mankind requires a range of books, but in short one can state that it coincided with major changes in lifestyle and also resulted in massive demographic events, often displacing one culture by the other involving huge social and economic battles, eventually resulting in our contemporary civilization (Harman 2008). A major move that enabled the development of agriculture was the transition from the hunter-gatherer to a sedentary life style. This shift occurred between 10,000 and 5,000 years B.C and is known as the Neolithic Revolution that gave rise to the first agrarian communities (Faris 2014; Harman 2008). Inhabitants of the Levant pioneered farming as a main food supply and spread agricultural practices throughout the Fertile Crescent (Faris 2014), which is therefore considered as the cradle of the agriculture and a rich niche of biodiversity, particularly for cereal crops (Gepts 2010; Zeder 2008). Early human settlers in the region likely harvested the wild forms of wheat, and other cereals, from natural stands before domestication of tetraploid wheats and agriculture developed into deliberate cultivation (Salamini et al. 2002) that started around 7.000 B.C (Harlan and Zohary 1966; Newton et al. 2010). The factors driving domestication and the change from hunter-gatherers to growers remain enigmatic, but one of the possibilities is a ~1,000-year episode of a cold and dry climate, known as the Younger Dryas, 10,000 – 9,000 B.C, which restricted and impaired vast natural stands of wheat, thereby constraining primary human civilizations and hence initial domestication of plants and animals. However, regardless on the precise events and timing, the start of agriculture was enabled by a set of domesticated primitive landraces of cereals, many of which are still in use in various parts of the world in contemporary rural areas (Gepts 2010; Salamini et al. 2002). The domestication of wild tetraploid wheat species, as well as other cereals, and their adoption as potential staple crops was not only a crucial step for the onset of agriculture, but also paved the way for a durum wheat based diet in the Mediterranean basin that lasts until now in well-known dishes such as pasta, couscous and bulgur (Elias 1995).

The evolution of tetraploid wheat and its genetic diversity

Tetraploid wheats (*Triticum turgidum* ssp.) are believed to be among the first cereal grains that were domesticated during the Neolithic era (Tadesse et al. 2016). Archaeological studies revealed that tetraploid emmer (*T. turgidum* ssp. *dicoccum*) was among the first forms of wheat that was domesticated from wild emmer, *T. dicoccoïdes* (2n = 2x = 28, A^uA^uBB) (Heun et al. 1997; Newton et al. 2010). This species emerged from natural hybridizations

between diploid wheat species (Tadesse et al. 2016), that subsequently gave rise to a wide range of sub-species, which widened the genetic diversity of wheat and hence, its wide adaptation and cultivation across the globe (Feuillet et al. 2008; Newton et al. 2010; Serrão et al. 2016), particularly after amphidiploidization with *Aegilops squarrosa*, the D genome donor that has a vast natural distribution area compared to the diploid ancestors of tetraploid wheats (Feldman and Levy 2005; Feldman and Sears 1981; Zohary et al. 2012). Such an amphidiploidization took place between the parental diploid, einkorn *T. urartu* (2n = 2x = 14, A^uA^u) and the B genome progenitor, *Ae. speltoides* (2n=2x= 14, SS) giving rise to wild emmer wheat (Dvorak and Akhunov 2005; Feldman and Levy 2005; Johnson and Dhaliwal 1976). Several other tetraploid wheats have also been domesticated and cultivated, even though to a limited extent including *T. araraticum* (2n=4x= 28; A^uA^uGG) and *T. timopheevii* (2n = 4x = 28, A^tA^tGG). The former containing a pair of A genomes from *T. urartu* and a pair of G genomes, which is believed to be a divergent form of the S genome of the *Aegilops* progenitor (Rodriguez et al. 2000). *Triticum timopheevii* is assumed to be domesticated from the wild emmer *T dicoccoides* ssp. *armeniacum* (William et al. 2011), and most probably has resulted from a secondary domestication due to its limited significance as a crop in the Georgia region (Nesbitt et al. 1996). The *T. turgidum* subspecies emerged due to a species-specific translocation in *T. timopheevii* involving the 6At, 1G, and 4G chromosomes that distinguished it from *T. turgidum* (Jiang and Gill 1994; Naranjo 1990). The former contains a translocation involving chromosomes 4A, 5B and 7B (Devos et al. 1995). *Triticum turgidum* subspecies have also been progenitors of the hexaploid bread wheats, which developed into one of the top global staple foods (Harlan and Zohary 1966; Tadesse et al. 2016; Zohary et al. 2012). Hexaploid wheat is solely known in its domesticated form as no direct hexaploid wild progenitor is recognised (Charmet 2011; Kilian et al. 2010; Newton et al. 2010; Qin et al. 2017).

During domestication tetraploid wheats have undergone a suite of anatomical and morphological changes that marked its divergence from its wild ancestor(s) (Charmet 2011; Newton et al. 2010). The genetic modifications have been described as the domestication syndrome (Harlan 1971; Harlan 1992; Harlan et al. 1973; Meyer et al. 2012) comprising characteristics such as seed retention (non-shattering), increased seed size, changes in branching and stature, change in reproductive strategy, and changes in secondary metabolism. The first and foundational morphological divergence occurred in wild emmer wheat; non-brittle rachis prevents the natural seed dispersal mechanisms, enabling the harvest of entire heads, which must have contributed to enhanced grain yields (Faris 2014; Peleg et al. 2011) (Figure1).

Other domestication-related traits include larger seeds, loss of seed dormancy, the free-threshing character, and enhanced grain quality (Faris 2014; Harlan et al. 1973; Nevo et al. 2013). These changes were mainly driven by mutations in the major hallmark genes of wheat evolution; *Br*, *Tg* and *q,* conferring brittle rachis, tenacious glume, and a hulled seed, respectively (Avni et al. 2017; Dubcovsky and Dvorak 2007; Faris 2014; Peng et al. 2011) (Figure 1). These changes eventually increased the geographical diffusion of wheat, which intimately contributed to the rise of human civilization (Nevo et al. 2013). Archaeological records revealed that free-threshing tetraploids first appeared around 8,000-9,000 BC in Tell Aswad and other Syrian sites as well as Can Hassan III in southern Turkey (Colledge and Conolly 2010; Faris 2014). Free-threshing derivatives of domesticated emmer, such as the extinct tetraploid *T. turgidum* ssp. *parvicoccum*, appear in the archaeological record shortly after emmer was domesticated. Free-threshing durum wheat replaced the domesticated emmer around 1,000 BC, when it disseminated to the Middle-East, the Mediterranean basin, and Europe (Feldman and Levy 2005). It appears that free-threshing durum, emmer, and probably other tetraploids were cultivated in mixtures for many years until free-threshing durum wheat was established as a prominent crop in the Mediterranean basin and the Near East almost 3,000 years ago (Faris 2014; Thuillet et al. 2005; Vigouroux et al. 2011). Ever since, durum wheat has spread to even more remote geographical areas, and its expansion has intimately followed human migration. From the ancient Egypt, durum wheat disseminated to Europe, and from there across the entire continent by the end of the 15th century. It then reached the American continent in 1492 with the first European settlers (Baloch et al. 2017; Capparelli et al. 2005; Ren et al. 2013). Nowadays, durum wheat is mainly cultivated in the Mediterranean basin that encompasses Southern Europe and North Africa, the Norther Great Plains of the U.S. and more recently in Southern Asia (Baloch et al. 2017; Faris 2014) and represents approximately 10% of the global cultivated wheat area (Magallanes-López et al. 2017).

Evidently, wheat domestication has not only shaped the genetic structure of the crop but also contributed to micro-environmental changes that affected microbes and pests, which enforces selection pressure and together with the influence of mankind completed the disease triangle of the crop. One of the pathogens that co-evolved with wheat crops is the damaging foliar blight *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous (formerly *Mycosphaerella graminicola* [Fuckel] J. Schröt. in Cohn) (Stukenbrock et al. 2006).

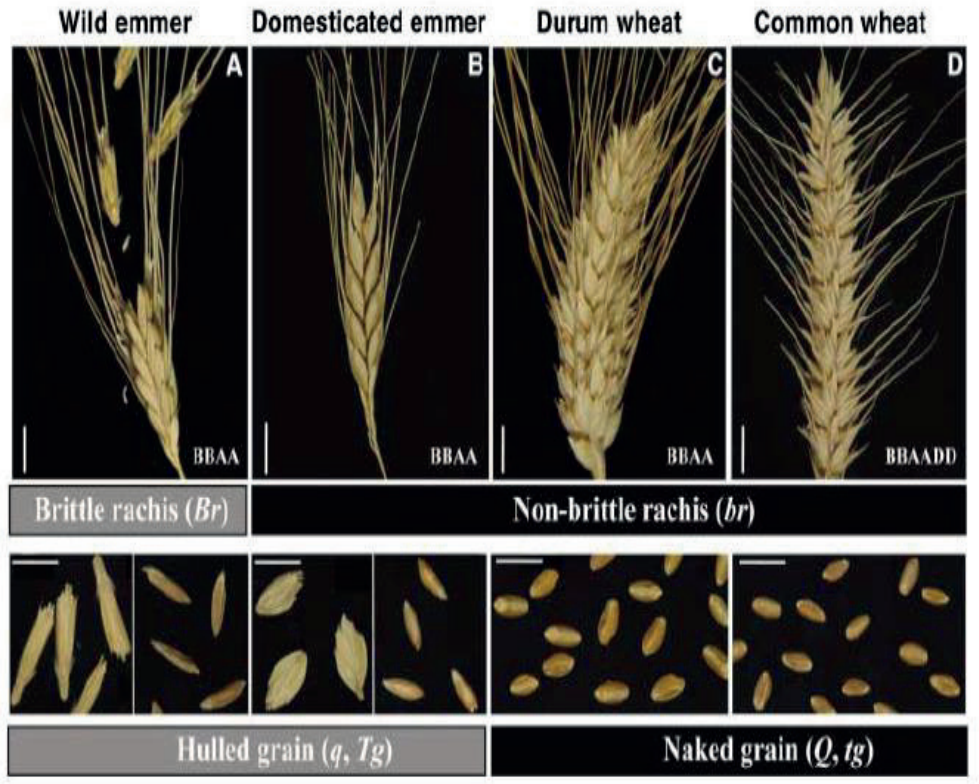


Figure 1. Major morphological divergence of the wheat spike occurred during wheat domestication. Figure (A) corresponds to the wild emmer wheat (*T. dicoccoides*) characterized by a brittle rachis and a hulled grain. Figure (B) shows the domesticated emmer (*T. dicoccum*) divergent from the *T. dicoccoides* by its non-brittle rachis. Alike *T. dicoccoides*, *T. dicoccum* (B) is characterized by a hulled grain. Figures (C) and (D) represent cultivated forms of wheat, the durum (*T. durum*) (C) and the common or bread wheats (*T.aestivum*) (D) characterized by a non-brittle rachis and a naked grain. White scale bars represent 1 cm. The genome formula of each type of wheat is indicated at the lower right corner. Genes conferring brittle rachis (*Br*), tenacious glume (*Tg*) and hulled grain (*q*) are major genes that marked wheat evolution (adopted from (Dubcovsky and Dvorak 2007)).

Zymoseptoria tritici: The intimate perilous tracker of wheat

Several studies have recognised that the origin of *Z. tritici* coincided with wheat domestication. This foliar fungal pathogen has been an intimate tracker and emerged in parallel with wheat in the Fertile Crescent from its ancestors *Z. ardabiliae* and *Z. pseudotritici* that were

isolated from the wild grasses *Elymus repens*, *Dactylis glomerata* and *Lolium perenne* in Iran (Stukenbrock et al. 2006; Stukenbrock et al. 2011; Stukenbrock and Croll 2014; Stukenbrock and McDonald 2008; Stukenbrock et al. 2012; Torriani et al. 2011). The newly evolved *Z. tritici* pathogen, causal agent of septoria tritici blotch (STB), has diverged towards a specialized pathogen of wild and cultivated wheats.

Zymoseptoria tritici is currently globally distributed, threatening durum and bread wheat production that might decline to 50% under conducive conditions (O'Driscoll et al. 2014; Ponomarenko et al. 2011). In Europe, STB is the most economically important disease of wheat, with an estimated ~€1 billion per year in fungicide expenditure directed toward its control (Kettles and Kanyuka 2016). Septoria tritici blotch drew international attention after an epidemic in North-Africa in 1968–1969, following the introduction of semi-dwarf wheat cultivars and the inherent use of artificial fertiliser (Brown et al. 2015; Saari and Wilcoxson 1974). The high genetic plasticity and diversity, and the active sexual reproduction of *Z. tritici* have hampered the implementation of an efficient strategy to control STB (Goodwin et al. 2011; Torriani et al. 2015; Wittenberg et al. 2009).

Zymoseptoria tritici: the art of know-how of a fungus

Zymoseptoria tritici is an ascomycete belonging to the family of the *Mycosphaerellaceae* in the class of the *Dothideomycetes*. It was formerly known as *septoria tritici* that was discovered by Desmazières in 1842 and its teleomorph *Mycosphaerella graminicola* was described 150 years later by (Sanderson 1976). Recently, the fungus has been renamed as *Zymoseptoria tritici* following the one-name-one-fungus taxonomy (Quaedvlieg et al. 2011). *Zymoseptoria tritici* has a heterothallic bipolar mating system with two mating type alleles, *mat1-1* and *mat1-2* (Waalwijk et al. 2002), and actively sporulates through asexual and sexual fructifications that release splash-borne pycnidisopores and air-borne ascospores, respectively (Ponomarenko et al. 2011), both contributing to epidemics. Ascospores can be formed year-round, and constitute the primary inoculum released from wheat debris. During the growing season, disease progress is ensured by the pycnidisopores (Figure2) (Eyal 1999; Eyal et al. 1987; Hunter et al. 1999; Kema et al. 1996b; McDonald and Linde 2002; Ponomarenko et al. 2011).

Albeit *Zymoseptoria tritici*'s lifestyle has been considered as hemibiotrophic, a late necrotroph has been suggested to be more accurate (Sanchez-Vallet et al. 2015) and recently Fones et al. (2017) described a role for epiphytic growth of the fungus prior to penetration. In

any case, the infection cycle includes a biotrophic symptomless phase during which the fungus colonizes the extracellular space surrounding the mesophyll cells without any apparent damage to the host. Upon landing on a wheat leaf surface and under conditions of high humidity, germinating ascospores and conidia produce hyphae that penetrate through the stomata to access the apoplast without developing haustoria or any other visible feeding structures, where it slowly grows in a close contact with the plant cell wall (Kema et al. 1996a; Marshall et al. 2011; Mehrabi 2006). The life strategy of *Z. tritici* during the initial symptomless colonization remains enigmatic. Despite the macoscopical lack of symptoms, microscopic observations by Kema et al. (1996a) showed that chloroplasts are in constant move towards the cell wall during this phase, which suggests that *Z. tritici* acts on the plant cell physiology by altering its functionality. The release of small secreted proteins may explain these observations (Ben M'Barek et al. 2015; Gohari 2015; Kettles et al. 2017; Kettles and Kanyuka 2016; Mirzadi Gohari et al. 2015; Palma-Guerrero et al. 2016; Rudd et al. 2015), and contrary to other ascomycete cereal pathogens, *Z. tritici* also encodes a few carbohydrate-active enzyme family proteins, but abundant peptidase and alpha-amylase enzymes, which suggests an alternative nutrient acquisition of *Z. tritici* from its host environment (Fones et al. 2017; Goodwin et al. 2011). Moreover, genes encoding for lipases, cutinases and fatty acid metabolism enzymes are up-regulated during this phase (Keon et al. 2007; Palma-Guerrero et al. 2017; Rudd et al. 2015), which suggests that *Z. tritici* relies upon stored energy in germinating spores that are conceivably supplemented by cuticular waxes rather than tapping directly from host resources (Kettles et al. 2017; Kettles and Kanyuka 2016; Rudd et al. 2015). Host defence mechanisms are most probably supressed during this latent phase (Hammond-Kosack and Rudd 2008; Palma-Guerrero et al. 2016; Rudd 2015). The secretion of LysM effectors by *Z. tritici* prevents chitin recognition, which also supports its invasion and one LysM effector plays a role in virulence, suggesting various roles of these effectors (Lee et al. 2014; Marshall et al. 2011).

In a compatible interaction, the necrotrophic phase occurs 10-14 days after inoculation, depending on environmental conditions, and is characterized by the appearance of macroscopically visible chlorotic lesions that turn into larger necrotic blotches bearing the pycnidia, the asexual fructifications of the fungus (Duncan and Howard 2000; Kema et al. 1996a; Shetty et al. 2003). Genetic factors that trigger the switch from a biotrophic lifestyle to the ramifying necrotrophic state are still unidentified (Mirzadi Gohari et al. 2015; Palma-Guerrero et al. 2016). It is associated with the induction of host defences, but not with the classical hypersensitive response (HR) despite characteristics of HR-associated programmed

cell death (PCD) and the regulation of wheat Mitogen-Activated Protein Kinase (MAPK). Other mechanisms also occur during this switch that are mainly associated with Effector-Triggered Immunity (ETI), which essentially play a role in reducing cell permeability leading to a nutrient leakage from dying plant cells that enables fungal proliferation in the apoplast (Keon et al. 2007; Rudd et al. 2008). The subsequent extended necrotrophic phase characterizes the final infection phase, during which the pathogen feeds on nutrients released from dying host tissue and produces the asexual pycnidia that contribute significantly to the development of epidemics (Shaw 1987; Shaw and Royle 1989).

Zymoseptoria tritici has proven its high adaptability to diverse agricultural ecosystems (Croll et al. 2017; Croll and McDonald 2017; McDonald et al. 1995; McDonald and Mundt 2016). It is omnipresent in temperate climates where bread wheat cultivation is significant (O'Driscoll et al. 2014), as well as in the hot dry climate of North Africa and the Mediterranean basin, suitable for durum wheat cultivation (Brown et al. 2015; Meamiche Neddaf et al. 2017), as well as the cold areas of North America (Banke and McDonald 2005; Eyal et al. 1985; Linde et al. 2002; Zhan et al. 2003). The high genome plasticity of *Z. tritici*, illustrated by the high number of accessory chromosomes that can be lost without any apparent impact on the pathogenic fitness (Stukenbrock et al. 2010; Wittenberg et al. 2009), has likely contributed to its survival, adaptation and speciation to various agro-ecosystems (Croll et al. 2017; Croll and McDonald 2012; Croll and McDonald 2016), including its resistance to fungicides that limits the efficacy of the chemical control (Brunner et al. 2013; Torriani et al. 2009; Torriani et al. 2015).

Interestingly, breeding for resistance to *Z. tritici* in bread wheat has taken off once *Stb* genes were identified thanks to the use of well-characterized *Z. tritici* isolates (Adhikari et al. 2003; Adhikari et al. 2004a; Adhikari et al. 2004b; Arraiano et al. 2001a; Brading et al. 2002; Brown et al. 2001; Brown et al. 2015; Chartrain et al. 2005b; Chartrain et al. 2004). Hence, breeding for resistance in durum wheat is promising despite its current standing. In this thesis the foundation for such an endeavour is presented and discussed.

Figure 2. A schematic description of the septoria tritici leaf blotch (STB) disease dissemination on wheat (outer ring) overlain with the commercial wheat production cycle (inner ring) (adopted from (O'Driscoll et al. 2014)).

Zymoseptoria tritici specificity: the mysterious black box

Species specificity in the *Z. tritici*- wheat pathosystem generated a nearly 50 years' long debate in the Septoria community. Although denied in some studies (Johnson 1992; Parlevliet 1993; Van Ginkel and Scharen 1988), cultivar specificity has been widely reported in bread wheat (Ahmed et al. 1995; Kema et al. 1996a; Kema et al. 1996b; Kema et al. 1996c; Kema and van Silfhout 1997; Kema et al. 1996d), as well as in durum wheat (Ghaneie et al. 2012; Kema et al. 1996c; Medini and Hamza 2008), suggesting a gene-for-gene (GFG) interaction between wheat and *Z. tritici* isolates. This model was supported by the single gene inheritance

of avirulence in *Z. tritici* (Kema et al. 2000) and further detailed by Brading et al. in (2002) in a genetic study carried out on the cross between the *Z. tritici* isolates IPO323 avirulent on cv. Shafir (carrying the *Stb6* resistance gene) and IPO94269 (virulent on cv. Shafir). The (a)virulence gene was subsequently mapped on the distal part of chromosome 5, and co-segregates with the genomic region responsible for the host specificity, widely reported in a range of studies (Eyal et al. 1973; Kema et al. 1996a; Mirzadi Gohari et al. 2015; Van Ginkel and Scharen 1988; Ware 2006). Albeit early reported, host specificity and cultivar specificity in the *Z. tritici* pathosystem remain inscrutable as so far only genes controlling cultivar specificity have been identified (this thesis;(Zhong et al. 2017)). Nevertheless, whilst the reported GFG model has greatly enhanced gene postulation in bread wheat resulting into the identification of 21 *Stb* major resistance genes (Brown et al. 2015), the dichotomy in *Z. tritici* is a burden for gene postulation in durum wheat, hampering its improvement for STB resistance.

Wheat-*Zymoseptoria tritici* interaction: The incomplete story

The increased awareness about the destructive effects of *Z. tritici* in wheat that peril global food security has urged the need to adopt sustainable strategies for STB control. The repeatedly reduced efficacy of fungicides due to the emergence of resistance among natural populations of *Z. tritici* (Torriani et al. 2009*;* Torriani et al. 2015*;* Torriani et al. 2011), has directed wheat producers to associate chemical control with the use of germplasm with enhanced resistance to STB and to adopt better cultural practices (Omrane et al. 2015). Tremendous efforts have been made to improve resistance to *Z. tritici* in bread wheat. This has started early in 1957 with the identification of the first *Stb* gene, *Stb1* in the winter wheat cv. Bulgaria 88, which was the first commercially deployed gene in cvs. Oasis and Sullivan (Goodwin 2007; Narvaez and Caldwell 1957; Tabib Ghaffary 2011). Ever since, numerous other *Stb* resistance genes have been identified and mapped in various bread wheat cultivars (Cuthbert 2011; Tabib Ghaffary 2011). The formal elucidation of an operational GFG interaction by Brading et al. in (2002) has greatly advanced the identification of additional resistance genes, most importantly the *Stb6* gene in cv. Shafir, proven to interact in a GFG model with the *Avrstb6* effector gene mapped in *Z. tritici* isolate IPO323 (Arraiano and Brown 2006). The widespread distribution of *Stb6* in the European bread wheat cultivars highlights its importance as a valuable source for *Z. tritici* resistance (Arraiano et al. 2007b). Since 2003, nine new resistance genes (*Stb7- Stb15*) were characterized and mapped in bread wheat (Tabib Ghaffary 2011). A unique wide spectrum resistance gene, *Stb16q* was identified in the synthetic hexaploid accession M3 (W-7976) in 2011, together with *Stb17* that confers adult plant stage

resistance to a limited number of isolates (Tabib Ghaffary et al. 2012). The resistance gene *Stb18*, which also has a limited efficacy and a largely partial effect, was mapped in the French contemporary bread wheat cv. Apache (Brown et al. 2015; Tabib Ghaffary et al. 2011). All abovementioned *Stb* resistance genes, together with *StbWW*, identified in three mapping populations in Australia (Raman et al. 2009), constitute the 20 *Stb* genes that were in bread wheat. In addition, *TmStb1* was derived from the *T. monococcum* accession MDR043 (Jing et al. 2008) (Table1). In addition to these qualitative resistance sources, 167 quantitative trait loci (QTLs) contributing to STB resistance were also identified (Brown et al. 2015). This arsenal of qualitative and quantitative resistances have enhanced bread wheat resistance breeding to manage *Z. tritici* and to sustain the yield potential of contemporary bread wheat cultivars (Arraiano and Brown 2016). Although the number of *Stb* genes and identified QTLs is limited compared to the high number of resistance genes to for instance the rusts diseases of wheat (McIntosh et al. 1995), in durum wheat not a single *Stb* gene has been identified, which contributes to the overall vulnerability of the far majority of durum germplasm to *Z. tritici*.

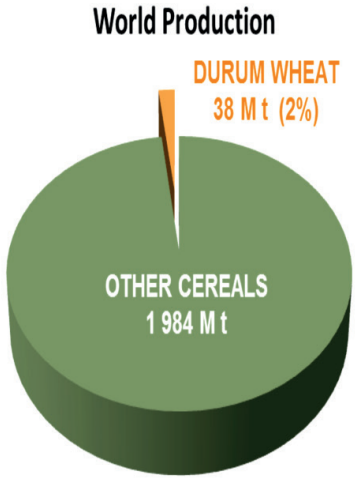
Despite its historical and dietary importance in the Mediterranean basin, durum wheat has received very limited attention compared to bread wheat (Royo et al. 2007). During the last 25 years, durum wheat resistance to *Z. tritici* has been hardly investigated leading to a poor understanding of the *Z. tritici*-durum wheat interaction. This staple crop, is largely grown under the constraining climatic conditions of the Mediterranean, where 75% of the global durum wheat production is located (Baloch et al. 2017; Faris 2014; Zapata et al. 2004). Worlwide durum wheat production is estimated to be around 36 to 38 million tonnes (Magallanes-López et al. 2017) (Figure 3). Nonetheless, this estimate does not accurately reflect the importance of durum wheat in various developing countries, which are often excluded from the statitics compiled by the wheat industry mainly due to unaccurate or unavailable data (Cakmak et al. 2010). Moreover, durum wheat is commonly conceived in statistics along with bread wheat and other cultivated wheat species. Hence, credible data to calculate statistics such as per capita intake are rather hard to define (Cakmak et al. 2010).

A closer look at the durum wheat production estimated at different regional scales indicates an unbalanced yield potential between developing countries where the crop is grown under low input in semi-arid regions and other marginal areas characterized by sharp annual fluctuations in cropping conditions, and non-developing countries where moisture and other resources are less obstructive (Abdalla et al. 1992) (Figure 3). Durum wheat in developing countries often constitutes the livestock of small holder farmers, grown under harsh, drought-

prone, and even marginal conditions with very low chemical inputs (Cakmak et al. 2010). These vulnerable environments are often subject to high production variability due to annual variation in rainfall (Gharbi et al. 2008). Juxtaposed to the frequent (a)biotic limiting factors, fungal diseases, particularly STB, constitute an additional threat to yield stability in durum wheat production.

Thus, studying the genetic basis of the *Z. tritici* - durum wheat interaction is required to enhance the resistance level of the durum germplasm to STB, that would unquestionably help small holder farmers to maintain yield potential of their durum wheat crops. Taking into account the history, strategies and most recent advances - particularly for the determinants of the GFG interaction - for a better understanding of the bread *Z. tritici* - wheat interaction, it is necessary to investigate and dissect the genetic diversity in the durum wheat – *Z. tritici* interaction. The identification of resistance genes by using well-characterized *Z. tritici* isolates is a foundational starting point to resolve specificity in this system and to enable marker assisted breeding to advance the improvement of STB resistance in durum wheat.

(A)



(B)

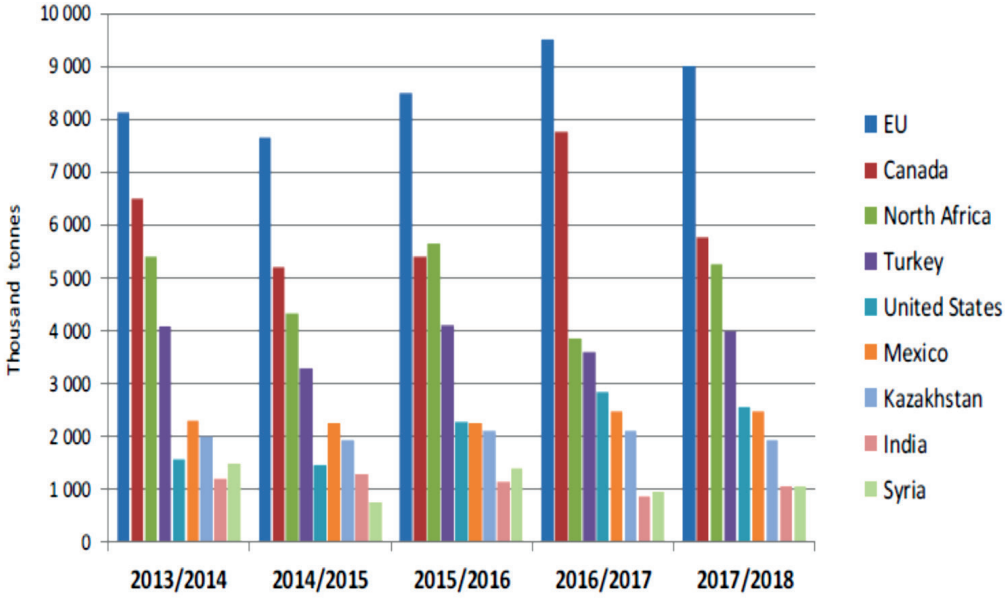


Figure 3. Estimate of the durum wheat world production in million tonnes compared to other cereals based on 5 years trimmed average date (A) and shares of the main durum producer countries (B) (adopted from Willems, 2017)

Table 1. Major genes for resistance to Septoria tritici blotch (*Stb*) identified in bread wheat (*Triticum aestivum*) with their corresponding chromosomal position, nearest marker and the avirulent *Zymoseptoria tritici* isolate with which the gene have been identified.

***Stb* Gene**	**Resistance source**	**Chromosomal position**	**Avirulent *Z. tritici* isolate**	**Associated markers (distance to gene)**	**References**
Stb1	Bulgaria 88[1]	5BL	IN95-Lafayette-1196-WW 1-4 & Purdue local (USA)	Xbarc74 (2.8cM), Xgwm335 (7.4cM)	(Adhikari et al. 2004a)
Stb2	Veranopolis[1]	1BS	Paskeville local (Australia) (and IPO92034)	Xwmc406 (6cM), Xwmc230 (5cM)	(Liu et al. 2013)
Stb3	Israel 4931	7AS	Paskeville local isolate (Australia)	Xwmc83	(Goodwin and Thompson 2011)
Stb4	Tadinia[1]	7DS	IN95-Lafayette-1196-WW-1-4, I-89, IPBr1	Xgwm111 (0.7cM)	(Adhikari et al. 2004a)
Stb5	Synthetic 6x[1]	7DS	IPO94269	Xgwm44 (7.2cM)	(Arraiano et al. 2001b)
Stb6	Flame, Hereward, Shafir	3AS	IPO323	Xgwm369 (2cM)	(Brading et al. 2002)
Stb7	ST6, Estanzuela Federal	4AL	MG2 (Canada) (and IPO87019)	Xwmc313 (0.3 to 0.5cM), Xwmc219 (1cM)	(McCartney et al. 2003)
Stb8	Synthetic W7984	7BL	IN95-Lafayette-1196-WW 1-4	Xgwm146 (3.5cM), Xgwm577 (5.3cM)	(Adhikari et al. 2003)
Stb9	Courtot, Tonic	2BL	IPO89011	Xfbb226 (3.6cM), Xwmc317, Xbarc0129	(Chartrain et al. 2009)
Stb10	Kavkaz-K4500[2]	1Dc	IPO94269 and ISR8036	Xgwm848	(Chartrain et al. 2005a)
Stb11	TE9111[2]	1BS	IPO90012	Xbarc008 (1cM)	(Chartrain et al. 2005c)
Stb12	Kavkaz-K4500[2]	4AL	ISR398 and ISR8036	Xwmc219	(Chartrain et al. 2005a)
Stb13	Salamouni	7BL	MG96-36, MG2 (Canada)	Xwmc396 (7-9cM)	(Cowling 2006)
Stb14	Salamouni	3BS	MG2 (Canada)	Xwmc500 (2cM), wmc632 (5cM)	(Cowling 2006)
Stb15	Arina[1], Riband	6AS	IPO88004	Xpsr904 (14cM)	(Arraiano et al. 2007a)
StbSm3	Salamouni	3AS	MG96-36, MG2 (Canada)	barc321 (1.9cM)	(Cuthbert 2011)
Stb16q	Synthetic Hexaploid M3	3DL	IPO88018 and IPO94218	Xgwm494 (4.3cM), Xbarc128 (9.9cM)	(Tabib Ghaffary et al. 2012)
Stb17	Synthetic Hexaploid M3	5AL	IPO88018	Xhbg247 (3.1cM), Xgwm617 (38.3cM)	(Tabib Ghaffary et al. 2012)
Stb18	Balance[1]	6DS	IPO323, IPO98022, IPO89011, IPO98046	Xgpw5176, Xgpw3087	(Tabib Ghaffary et al. 2011)
StbWW	WW1842, WW2449, WW2451	1BS	79, 2, 1A	Xbarc119b (0.9–4.1cM)	(Raman et al. 2009)
TmStb1	MDR043 (*T. monococcum*)	7AmS	IPO323	Xbarc174 (23.5cM)	(Jing et al. 2008)

[1] Lines carrying *Stb6*
[2] Lines carrying *Stb6* and *Stb7*

Scope of the thesis

This thesis aims at elucidating the genetic basis of resistance to *Z. tritici* in tetraploid wheats and at identifying the fungal genes involved in the GFG interaction between wheat and *Z. tritici* and understanding there epidemiological consequences that are intimately linked with the reproductive biology of the pathogen in natural and agricultural environments. Together, these objectives will be a solid foundation to further research in the *Z. tritici* – durum wheat interaction to support effective resistance breeding.

Chapter 1 provides an historical overview of the evolution of tetraploid wheats and the importance of the domesticated form as a food source for the Mediterranean basin. Futhermore, it describes the advances in *Z. tirtici* research, but also highlights the lack of information and limited translation of the generated know-how to durum wheat.

Chapter 2 describes the cloning and functional analysis of the first *Z. tritici* effector *Avrstb6* and the translation of the observed ubiquitous sexual reproduction, despite one of the *Z. tritici* parents is unable to infect the host in the *in-planta* crossing protocol. The reproductive consequence of this observation is developed into a new epidemiological model that we present as exclusive paternal parenthood (**EPP**), which likely has a wide application in natural and agricultural settings, thereby providing a new view on durability of host resistance. This is important for newly identified resistance genes, which are dercribed in the following chapters.

In **Chapter 3,** the resistance to *Z. tritici* in the *Triticum dicoccum* accession PI41025 is identified and characterized. Subsequently, the inheritance of the resistance was determined in a mapping population generated from the cross between the durum wheat cultivar cv. Ben and PI41025. This resulted in the identification of the first major quatitative trait locus (QTL) confering a wide-spectrum resistance to *Z. tritici* in durum wheat. The QTL was mapped on chromosome 3AL and was derived from PI41025 and was designated as *Stb22q*. Furthermore, another novel locus mapped on chromosome 5A and provides an isolate-specific resistance with a narrow efficacy and was derived from cv. Ben.

In **Chapter 4** the resistance to *Z. tritici* in a suite of Tunisian durum landraces was investigated. One of the most outstanding landraces was “Agili 39”, which was crossed with the contemporary cv. Khiar that is high-yielding, but very susceptible to *Z. tritici.* Analyses of the greenhouse and field data indicated that the broad spectrum resistance of “Agili 39” results form the natural pyramiding of several minor effect QTLs. Albeit that no new mapping

locations were identified, the strongest effect were generated by QTLs on chromosome 2BL and 2BS. The latter was exclusivly associated with adult plant resistance, whereas the former co-locolizes with *Stb9* that has a very low efficacy in bread wheat, but is crucial in "Agili 39".

Chapter 5, embarks on the characterization of STB resistance in high yielding contemporary durum wheat cultivars that are currently preferred by farmers. From a suite of durum wheat cultivars that were screened with a panel of *Z. tritici* isolates, the cvs. Simeto, Levante, Kofa and Svevo were chosen for further analayses. Recombinant inbred populations were generated between Simeto/Levante and Kofa/Svevo and recombinant inbred lines (RILs) were tested with four *Z. tritici* isolates under greenhouse conditions and with one strain in the field. After analyses of the generated data it was shown that the STB resistance in these cultivars results from the synergic effect of several minor effect QTLs on several new genomic locations, providing an acceptable level of STB resistance.

Chapter 6 is a general discussion placing all generated data in an overarching context. It highlights the importance of the EPP model and the lack of boom-and-bust cycles in the *Z. tritici* – wheat pathosystem, thereby tailoring the STB epidemiological model and differentiating it from other cereal pathogens. The EPP model applies to both bread wheat and durum wheat models, and the remainder of the discussion therefore revolves around the new data generated for durum wheat resistance to *Z. tritici* and their importance for advanced and improved resistance breeding programs in this crop.

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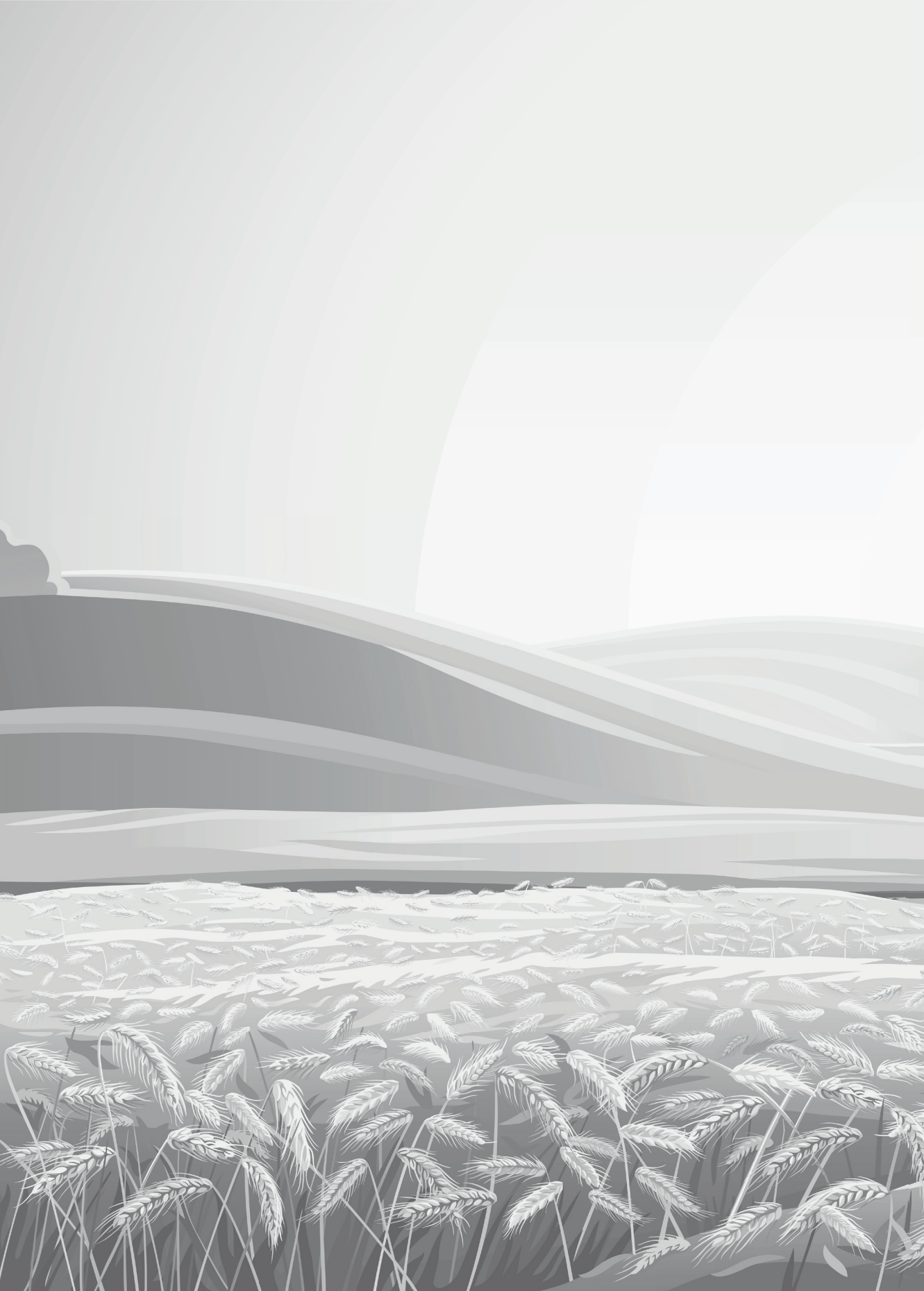

Chapter 2

Stress and sexual reproduction affect the dynamics of the wheat pathogen effector AvrStb6 and strobilurin resistance

Ware, Frank Van den Bosch, Robbie Manning-Smith , Vasthi Alonso-Chavez , Joe Helps , Sarrah Ben M'Barek , Rahim Mehrabi , Caucasella Diaz-Trujillo , Elham Zamani , Henk Schouten , Theo van der Lee , Cees Waalwijk , Maarten de Waard , Pierre de Wit , Els Verstappen , Bart P.H.J. Thomma , Harold J.G. Meijer , and Michael F. Seidl

[§]These authors contributed equally

Abstract

Host resistance and fungicide treatments are cornerstones for plant disease control. Here, we show these do not prevent sex and modulate parenthood in the fungal wheat pathogen *Zymoseptoria tritici*. We prove that the *Z. tritici*-wheat interaction complies with the gene-for-gene model by identifying the effector AvrStb6 that is recognized by the wheat resistance protein Stb6. Recognition triggers host resistance, implying removal of avirulent strains from pathogen populations. However, *Z. tritici* crosses on wheat reveal that sex occurs even with an avirulent parent, thereby retaining avirulence alleles in forthcoming populations. Crossing fungicide sensitive and resistant isolates under fungicide pressure shows a rapid increase of the resistance allele frequency. Isolates under selection always act as male donor, and thus disease control modulates parenthood. Modelling these observations for agricultural and natural environments reveals extended durability of host resistance and rapid emergence of fungicide resistance. Therefore, fungal sex has significant implications for disease control.

Sexual reproduction is common in nearly all branches of the eukaryotic tree of life, including microbial organisms like fungi [1,2], and has been considered an important driver for rapid adaption to novel or changing environments[3]. Dothideomycete fungi represent the largest and most ecologically diverse group of ascomycetes with approximately 20,000 species[4], and most of them reproduce sexually and asexually. One of them is the plant pathogen *Zymoseptoria tritici* that causes septoria tritici blotch in wheat. At the onset of the wheat growing season, *Z. tritic* produces air-borne sexual ascospores, thereby releasing genetically diverse founding populations in commercial wheat fields[5-7], and splash-dispersed asexual conidia that drive epidemics during the growing season[8]. Fungicides and host resistance are paramount for disease control. Until now, 21 resistance genes to septoria tritici blotch (*Stb* genes) have been identified (Table S1) and mapped, and *Stb6,* which is ubiquitous in European wheat cultivars[9], is the first resistance gene that was recently cloned[10]. However, the molecular processes underlying the *Z. tritici*-wheat interaction are still relatively poorly understood[4,11-13].

Gene-for-gene (GFG) interaction models have been suggested for a plethora of plant-pathogen interactions[14], but genetic proof was only provided for a limited number of pathosystems[15,16]. After more than a decade of genetic studies[4,6,12,17-20], we report the map-based

cloning of the first *Z. tritici* avirulence effector *AvrStb6,* which triggers *Stb6*-mediated immunity[10] that underlies GFG in the *Z. tritici*-wheat interaction. We previously developed a mapping population between *Z. tritici* isolates IPO323 and IPO94269[4,12,17,19] that we saturated here with Diversity Array Technology (DarTseq) markers (Table S2-4, Fig. S1) and mapped a putative avirulence effector gene on the tip of chromosome 5 (Table 1, Fig. 1, Supplementary Note). Public RNAseq data[21] were used to predict a single gene candidate (four exons; Supplementary Note, Figs. S2-4), which was highly expressed *in planta,* encoding a small secreted protein (82 amino acids [aa], 12 cysteines, mature size 63 aa; Fig. 1). Deletion in the avirulent strain IPO323 resulted in compatibility on cv. Shafir that carries *Stb6*, identifying the candidate as *AvrStb6*. Introducing *AvrStb6* into the compatible strain IPO94269 resulted in incompatibility on cv. Shafir, thereby demonstrating that AvrStb6 is recognized by Stb6 (Figs. 1, S5). Recently, *AvrStb6* was also identified in a genome-wide association study and subsequent ectopic integration in a virulent *Z. tritici* strain[22]. Analyses of the IPO323/IPO94269 mapping population and a panel of *Z. tritici* isolates suggests that pathogenicity on cultivars carrying *Stb6* is consistent with two aa changes in the *AvrStb6* protein (Table S5, Fig. S6).

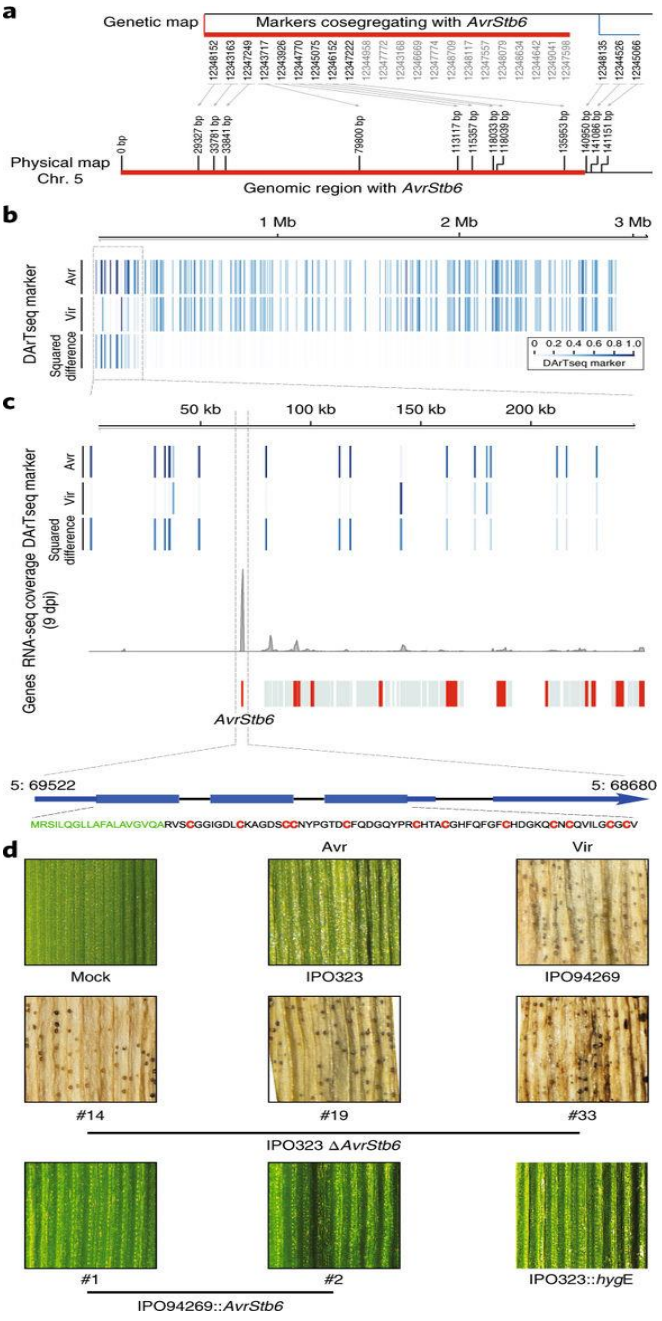

Figure 1. DArTSeq markers mapping combined with RNAseq revealed the *Avr* effector gene of *Zymoseptoria tritici*. (**a**) Illustration of the genetic and physical map on the tip of chromosome 5

of *Z. tritici* isolate IPO323. A cluster of 22 DArTSeq markers fully co-segregating with *AvrStb6* is highlighted in red, and DArTSeq markers flanking *AvrStb6* are highlighted in blue. The physical locations of DArTSeq markers (excluding markers with non-unique mapping; grey) on chromosome 5 are indicated by arrows, and the genomic location harboring the avirulence effector *AvrStb6* is highlighted in red. (**b**) Genomic location of DArTSeq markers on chromosome 5 of *Z. tritici* reference isolate IPO323 are indicated by coloured lines. For each location, the fraction of DArTSeq markers in the avirulent or virulent *Z. tritici* progeny is colour-coded (scale 0 [white] to 1 [blue]), and the squared difference between these fractions is shown. The dashed rectangle on the tip of this chromosome highlights the only polymorphic region in the genome of *Z. tritici* characterized by a high squared difference. (**c**) Magnification of the polymorphic region (the first 250 kb on chromosome 5) with the genomic location of the DArTSeq markers (color code as in (**b**)). The position of predicted genes in this region is indicated by grey bars, and red bars highlight genes encoding secreted proteins. RNAseq reads mapped to *Z. tritici* reference isolate IPO323 derived from wheat cv. Riband infection[21] indicate a single, highly expressed gene, designated *AvrStb6*, which encodes a secreted, cysteine-rich effector protein. The *AvrStb6* gene model is schematically displayed, where the blue line indicates exon-intron structure and the coding region is highlighted by extended line width. Green and red colored amino acids highlight the predicted signal peptide and the cysteines, respectively. (**d**) Functional analysis of *AvrStb6.* Top row: mock and wt *Z. tritici* isolates IPO323 and IPO94269, which are avirulent and virulent on cv. Shafir, respectively, carrying resistance gene *Stb6.* Middle row: Three independent knock-out mutants of *AvrStb6* in IPO323, which are consequently virulent on cv. Shafir. Bottom row: Two independent introductions of *AvrStb6* into the virulent strain IPO94269, resulted in two avirulent strains (#1 and #2). The ectopic integration of the deletion construct in IPO323 resulted in IPO323::*hyg* E, which had the same phenotype as the wt strain on cv. Shafir.

Z. tritici isolates are crossed *in planta* (see also Supplementary Note), which is similar to sex in nature, to generate segregating populations[4-7,12,15,17-19]. Apart from demonstrating a GFG interaction between wheat and *Z. tritici*, we observed unexpected sexual reproduction between IPO323 and IPO94269 on cv. Shafir, despite the presence of *AvrStb6* in the avirulent parent IPO323 (Table 1, see also Supplementary Note). Sexual reproduction was further confirmed by crossing IPO323 and IPO95052 on the cvs. Obelisk or Inbar, which are susceptible to IPO323 and

resistant to IPO95052 or *vice versa,* respectively (Table 1). We analyzed the four progenies with three nuclear markers (the avirulence gene *AvrStb6;* the mating type alleles *mat1-1* or *mat1*-2; a random nuclear SSR marker) and a mitochondrial SSR marker (mt-SSR) and conclude that IPO323, despite its avirulence, undergoes sexual reproduction with isolates IPO94269 or IPO95052 (Table 1, Fig. S7). Thus, although IPO323 cannot infect cvs. Shafir and Inbar, it completes a sexual cycle, thereby maintaining *AvrStb6* in subsequent populations (Figs. 2-3, S8). Moreover, crosses between sexually compatible *Z. tritici* strains never fail unless both parents are avirulent (Table S3). Notably, IPO323 is the exclusive paternal donor in the cross with the virulent isolate IPO94269 on cv. Shafir, but swaps to the exclusive maternal - and virulent - donor in the cross with the avirulent isolate IPO95052 on cv. Obelisk, as shown by the mt-SSR marker that is only maternally inherited[21] (Table 1). Thus, isolate IPO323 circumvents unfavorable host conditions (i.e. resistance) via sexual reproduction as male partner; a mechanism that we here call exclusive paternal parenthood (EPP, Table 1, Fig. 2). Hence, we conclude that host resistance is a biotic stress factor that modulates parenthood in fungal sex. Therefore, our data challenge the common belief[14,16] that avirulent individuals disappear from natural populations since they can neither infect nor reproduce on resistant host.

Table 1. Summary of segregation ratios for the nuclear markers for avirulence (AvrStb6), mating type (mat), and the microsatellite ag-0006, as well as the mitochondrial microsatellite marker mtSSR in four populations derived from crosses between *Zymoseptoria tritici* isolate IPO323 (adapted to bread wheat) with isolates IPO94269 (adapted to bread wheat) or IPO95052 (adapted to durum wheat) on three wheat cultivars with different modes of resistance (cv. Obelisk, generally susceptible to *Z. tritici* isolates derived from bread wheat; cv. Shafir carries Stb69; cv. Inbar is a tetraploid durum wheat cultivar that is resistant to virtually all *Z. tritici* from bread wheat, including IPO323 and IPO94269, but susceptible to the majority of Z. tritici isolates from durum wheat, including IPO950524). Segregation ratios that differ significantly (P<0.0001) from a 1:1 are indicated by an asteris

Marker	IPO323	IPO94269	Progeny on bread wheat cv. Obelisk n = 74	χ^2, P<0.05	Progeny on bread wheat cv. Shafir n = 87	χ^2, P<0.05	IPO323	IPO95052	Progeny on bread wheat cv. Obelisk n = 99	χ^2, P<0.05	Progeny on durum wheat cv. Inbar n = 58	χ^2, P<0.05
AvrStb6	+	-	42:32	1.351	48:39	0.931	+	-	53:46	0.495	28:30	0.690
mat	*mat1-1*	*mat1-2*	41:33	1.108	47:40	0.563	*mat1-1*	*mat1-2*	55:44	1.222	31:27	0.276
ag-0006	B[a]	A[a]	36:38	0.054	52:35	3.322	B	A	43:56	1.707	28:30	0.690
mtSSR	B[b]	A[b]	19:55	17.514*[c]	0:87	87.000*	B	C[b]	99:0	99.000*	43:15	13.517*[d]

[a]A and B represent the different alleles of ag-0006 in the parental isolates; [b]A, B and C represent the different alleles of mtSSR in the parental isolates. [c]Both parents have expected equal chance on paternal and maternal parenthood, but the skewed segregation of mtSSR is likely caused by competition of both isolates during pathogenesis on the susceptible cv. Obelisk, similar with Fig. 3a - see also Fig. 2 - where both isolates were inoculated on cv. Taichung 29. [d]The expected segregation ratio is 0:58, but durum wheat cv. Inbar allows - atypically – pycnidia production by some bread wheat isolates, such as IPO323[40].

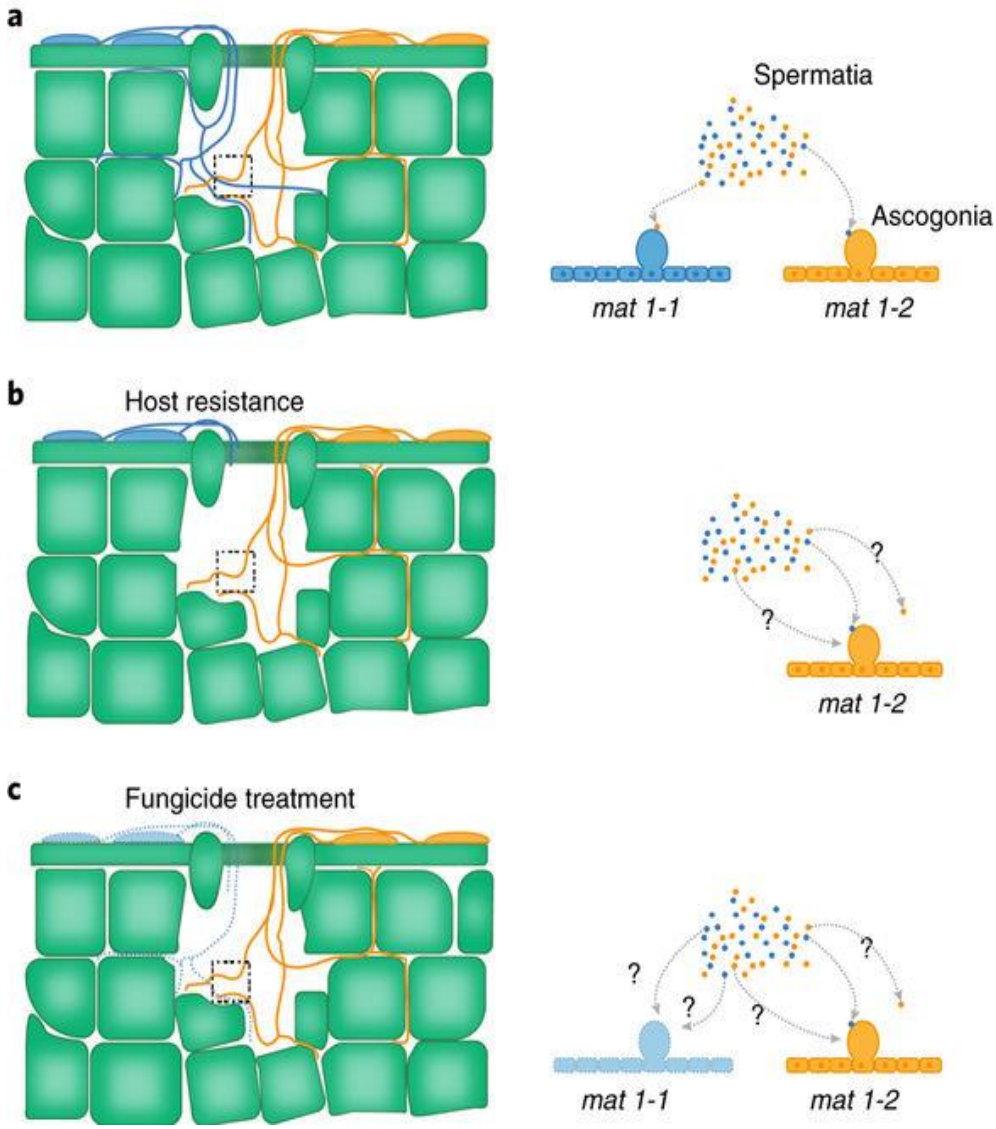

Figure 2. Sex in *Zymoseptoria tritici.* The fungus has a heterothallic bipolar mating system. Each strain has a unique mating type, either *mat1-1* (blue) or *mat1-2* (orange). When both strains infect the same host, they produce female (ascogonia) and male (microconidia or spermatia[37,38]) reproductive organs. Both strains have equal chances for maternal or paternal parenthood. Heterothallism defines that *mat1-1* ascogonia are exclusively fertilized by *mat1-2* spermatia and *vice versa*. (**a**) Optimal conditions for two pathogenic strains. (**b**) An avirulent strain (*mat1-1,* blue) encounters biotic stress

on resistant wheat, despite penetration[39]. The virulent strain (*mat1-2,* orange) colonizes the mesophyll. Biotic stress reduces biomass of the avirulent strain, but allows the production of spermatia. Exclusive paternal parenthood (EPP) determines that ascogonia of the virulent strain are exclusively fertilized by the avirulent strain. Consequently, avirulence genes are transmitted to the progeny and distributed by airborne ascospores. (**c**) The sensitive isolate (*mat1-1,* blue dotted line) is under abiotic stress, while the resistant strain (*mat1-2,* orange solid line) colonizes the host after strobilurin application. Sensitive strains are shown during colonization or just after penetration for strobilurin applications under field conditions are preventive/curative. Abiotic stress reduces biomass of the sensitive strain but allows the production of spermatia. EPP determines that mating is exclusively accomplished by fertilizing the ascogonia of the resistant strain. Consequently, the entire progeny caries the *cytb* gene with the G143A mutation (fungicide resistance), which is maternally transferred and further disseminated by airborne ascospores.

To generalize these observations, we considered fungicides as abiotic stress factors for *Z. tritici* and hypothesized that they result in EPP of sensitive strains. We used the strobilurin fungicide Amistar® and resistance as the maternally inherited marker (Figs. 3, S9). Six *Z. tritici* field isolates originating from Germany and The Netherlands with equal pathogenicity, opposite mating types and contrasting fungicide resistance were crossed in three sets (Tables 2, S6, Figs. 3, S10, Supplementary Note). We produced 42 progenies under various concentrations of Amistar® (Table 2) and the percentage of resistant ascospores was determined through either visual observation (9,025 ascospores) or by PCR assays on 2,100 progeny isolates (50 per cross) (Table 2, Supplementary Note). Despite the use of fungicides, we confirmed sexual reproduction for all crosses (Table 2, Fig. S11). Sensitive strains were outcompeted in each crossing assay (Figs. 3, S10). Under normal and double azoxystrobin concentrations all progenies were entirely fixed for resistance in one generation (Table 2). Thus, Amistar® applications direct resistant and sensitive isolates into maternal and paternal parenthood, respectively, leading to a rapidly increasing frequency of resistance alleles in the generated progenies. In conclusion, we observed that biotic and abiotic stresses may hamper or restrict host colonization but cannot preclude sexual reproduction as male gametes (spermatia) presumably survive (a)biotic stresses (Fig. 2)

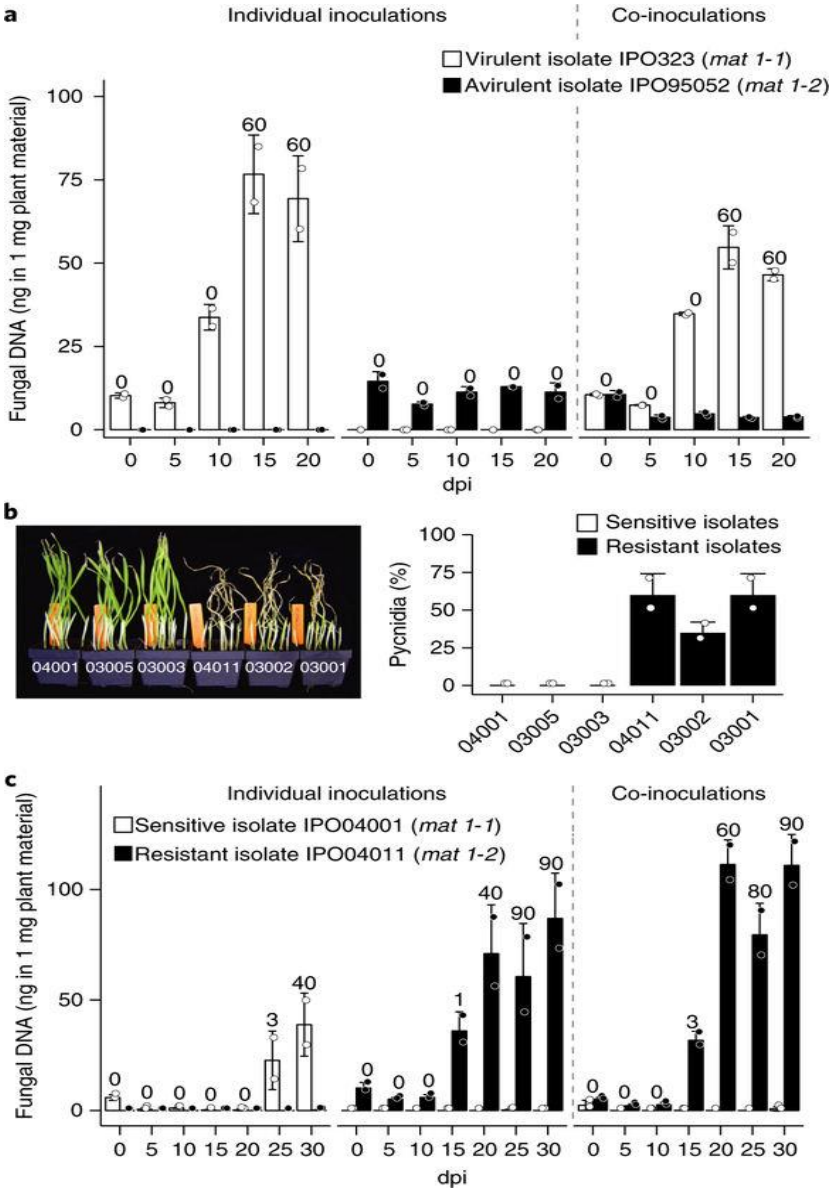

Figure 3. Inoculation and mating/competition assays using *Zymoseptoria tritici* strains with opposite virulence characteristics or fungicide sensitivity. (**a**) Quantitative fungal biomass detection of *Z. tritici* isolates IPO323 (left, virulent) and IPO95052 (middle, avirulent) and their mixture (right) on the bread wheat cv. Taichung 29 at 0, 5, 10, 15, and 20 dpi (bars; average of two biological replicates; dots indicate the individual measurements and whiskers the standard deviations). Percent leaf area covered by

pycnidia at each time point is shown as numbers over each bar. (**b**) Fungicide sensitivity screen at 20 dpi with *Z. tritici.* Plants of wheat cv. Taichung 29 were treated (48h prior to inoculation) with the full recommended rate of the strobilurin fungicide Amistar® (active ingredient [ai] azoxystrobin) and then inoculated with the sensitive *Z. tritici* isolates 04001, 03005 or 03003 and with the resistant isolates 04011, 03002 or 03001 (right panel; Percent pycnidia (leaf area covered) based on visual observations (average of two biological replicates, whiskers indicate standard deviations)). This resulted in significantly different disease severities between the sensitive and resistant *Z. tritici* strains (both panels). (**c**) Quantitative fungal biomasses detection of *Z. tritici* isolates on cv. Taichung 29 after preventative treatment (48 h prior to inoculation) with the full recommended field rate of Amistar® at 0, 5, 10, 15, 20, 25, and 30 dpi (three independent crossing experiments for the phenomenon; bars are averages of two technical replicates; dots indicate the individual measurements and whiskers the standard deviations). Plants were inoculated with the sensitive isolate IPO04001 (left), the resistant isolate IPO04011 (middle), and their mixture (right). Percent leaf area covered by pycnidia at each time point is shown as numbers over each bar.

Table 2. Non-Mendelian inheritance of resistance to azoxystrobin in ascospore progeny populations of *Zymoseptoria tritici*. A, The percentage of strobilurin-resistant progeny was determined by monitoring the germination of 9,025 ascospores, originating from 21 *in planta* crosses between strobilurin resistant and sensitive *Z. tritici* isolates on seedlings of wheat cv. Taichung 29 that were preventively treated with six doses of Amistar® and then discharged onto water agar amended with 1 ppm (MIC value) Amistar® normalized to germination frequencies of 15,975 ascospores from the same crosses that were discharged to unamended water agar. B, The percentage of strobilurin resistant progeny determined by a strobilurin sensitivity PCR screen in 42 *Z. tritici* progenies.

A	IPO03001 [R] x IPO03003 [S]		IPO03002 [R] x IPO03005 [S]		IPO04011 [R] x IPO04001 [S]	
Rate	Total #	% germination	Total #	% germination	Total #	% germination
0	630	93	856	15	2,108	49
1/32	85	89	390	48	709	64
1/16	188	92	183	78	556	100
1/8	191	76	135	88	607	100
1/4	237	100	166	88	336	99
1/2	496	99	105	93	349	100
full dose	186	96	NA	NA	512	100
Total	2,013		1,835		5,177	

B	% resistant in PCR					
Rate	1st round	2nd round	1st round	2nd round	1st round	2nd round
0	100	100	0	33	100	38
1/32	100	100	98	49	96	71
1/16	100	100	100	100	65	98
1/8	100	100	100	98	100	100
1/4	100	100	96	98	100	100
1/2	100	100	92	92	100	100
full dose	*	100	*	100	*	100
2x full dose	*	100	*	100	*	100

NA = Not assessed due to limited leaf material, * = Concentration not used in the first series of crosses.

We developed a population genetic model by incorporating EPP into Leonard's seminal model of GFG coevolution of a plant-pathogen system[23] (see Supplementary Note). In this model, a plant has one locus with alleles for resistance and susceptibility, and the pathogen has a corresponding locus with alleles for avirulence and virulence. The proportion of each allele in a well-mixed population is modelled over time. In real-life cases alleles often co-exist in stable or cyclic polymorphisms, however in Leonard's model the frequency of resistance and virulence alleles in the respective population only results in fixation of one of the genes – coexistence is not possible (Fig. 4). Because of this Leonard's model forms the theoretical framework to identify traits whose inclusion can result in stable or cyclic polymorphisms (such as having multiple pathogen cycles per plant cycle, including a seed bank, or incorporating spatial structure)[4]. We explore the consequences of incorporating the EPP reproduction mechanism into the Leonard model under two scenarios: firstly, when the frequency of the plant alleles is constant, as can be assumed in an agricultural system, and secondly, when the frequency of the plant is free to vary, as in a natural ecosystem. In the agricultural scenario, the frequency of virulence in the pathogen population increases slower when avirulent strains partake in sexual reproduction on resistant hosts (Fig. 4). Additionally, polymorphisms (where the two alleles can coexist indefinitely) are possible, although unlikely (see Supplementary Note), which is not the case without the EPP mechanism[24]. This implies that resistance in crop cultivars will erode slower, which can have significant consequences for the sustainability of disease control in crop production systems. In the natural scenario, our model (Fig. 4) shows stable or cyclic polymorphisms occurring across a wide range of parameter values (see Supplementary Note). We therefore showed that the presence of sex under biotic stress allows the occurrence of stable polymorphisms simply as a result of the pathogens' genetic system. Moreover, the model confirms that when fungicide sensitive strains partake in sex, the mitochondrially inherited *cytb* resistance allelle invades faster than any nuclear inherited fungicide resistance allelle (not shown).

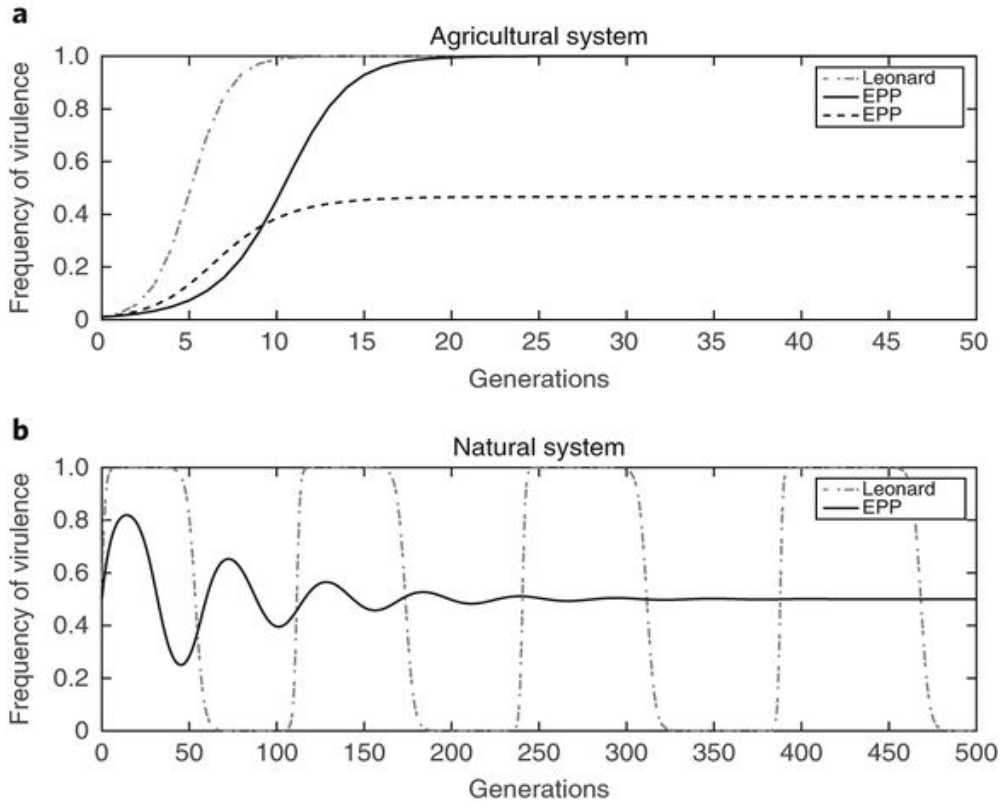

Figure 4. Comparison of the EPP model and Leonard's model. In the figure showing the agricultural scenario (**a**) the frequency of the resistance allele in the plant population is kept constant – in agricultural systems this frequency is under the control of the growers and not affected by the selection pressures imposed by the pathogen. In the natural scenario (**b**) the frequency of the resistance allele is dynamic and under control of the selection pressures in the system. The parameters of the model represent various fitness costs and allele frequencies. Briefly, c represents the cost to the host plants' fitness from being infected, d represents the cost of having a resistance allele, k represents the cost of virulence, σ represents the probability that an avirulent pathogen will mate with a virulent individual on the resistant cultivar, and the parameter q represents the frequency of the susceptible allele, which is fixed in the agricultural system (see also Supplementary Note). In the agricultural scenario (a) a comparison of the solid line of the EPP model and the dotted line of Leonard's model demonstrates the reduced rate of build-up of virulence. The parameters used for these simulations are k=0.5, q=0.45 and in the EPP model σ=0.1. Additionally, the dashed line of the EPP model represents a different set of parameters, k=0.7 q=0.3 and σ=0.6, and demonstrate a polymorphis in the pathogen population.

In the natural scenario (b) the stable internal equilibrium point of the EPP model is demonstrated. In this simulation, the parameters used are c=0.2, d=0.1, k=0.35 and in the EPP model, σ=0.5. A comparison between the dotted line of Leonard's model and the solid line of the EPP model demonstrates the possible stability of the internal equilibrium point in the EPP model, and the instability of the corresponding internal equilibrium point in Leonard's model. In the Supplementary Note we show that the model output shows stable and cyclic polymorphisms for a wide range of parameter values.

The experimental data and our theoretical model provide explanations for practical observations. Slow decline of host resistance is commonly observed in the wheat-*Z. tritici* pathosystem matching the unanticipated, but ubiquitous presence of *Stb6* in many old and contemporary wheat cultivars around the world (Table S1)[9,25]. Compared to the typical boom-and-bust cycle in the yellow rust pathogen *Puccinia striiformis*, resistance to septoria tritici blotch declined significantly slower over a period of 10 years in the United Kingdom[26]. Strobilurin fungicides were commercially introduced in 1996 and showed initially excellent control of a wide range of plant pathogens including *Z. tritici*. However, in 1998 resistance appeared for powdery mildew in wheat, caused by *Blumeria graminis*[27], in 2002 for *Z. tritici,* which then occurred throughout Europe one year later and presently strobilurin resistance is fixed in the vast majority of *Z. tritici* populations[28,29]. A similar trend for strobilurin resistance dynamics was observed in *Pseudocercospora fijiensis*, the banana Black Sigatoka fungus[30,31].

Plant disease management mostly relies on host resistance or fungicide applications[32,33]. Therefore, our observations on fungal sex have a broad relevance for developing resistant host varieties and shaping disease control strategies. This not only applies to plant pathogens, but also to human fungal pathogens such as *Aspergillus fumigatus,* where sex probably also contributes to the development of new life-threatening resistance mechanisms[34,35]. We conclude that fungal sex is an underestimated aspect in disease control that requires much more attention.

Materials and Methods

Primer development and PCR conditions

We developed a mismatch amplification mutation assay (MAMA)[41] on part of the cytochrome b (*cytb*) gene to determine azoxystrobin sensitivity or resistance among generated *Zymoseptoria tritici* ascospore progenies. Primers were designed with a mismatch on the penultimate nucleotide and the ultimate nucleotide was at position 143 of *cytb*. The primer set

to specifically amplify a DNA fragment in sensitive isolates used a sense primer StrobSNP2fwd [5'-3' (404-428)] with a mismatch of T instead of G at nucleotide 427 of *cytb* and an antisense primer StrobSNP1rvs [5'-3' (1024-1043)]. The primer set to specifically amplify a DNA fragment in resistant isolates used an antisense primer StrobSNPrcF7[5'-3'(428-453)] with a mismatch of T instead of G at nucleotide 429 and a sense primer StrobSNPrcR1 [5'-3' (152-173)]. One and 0.5 µl of DNA were used for the MAMA and mating-type PCR assays, respectively.

Mating type PCR primers and thermal cycling conditions were as previously described[20]. Amplicons were analyzed on 1.2% agarose gels using 25 µl aliquots of the PCR products. PCRs to amplify simple sequence repeats (SSRs) were in a 20 µl volume containing 20 ng DNA, 2 µl 10X PCR buffer with $MgCl^{2+}$, 2 µl each forward and reverse primers (2 µM), 0.8 µl dNTPs (5 mM), 0.2 µl Taq DNA polymerase (5U/ µl), and x µl sdd water. Thermal cycling was as follows: cycle 1; 94°C for 2 mins., cycle 2 (repeated 12x); 94°C for 30 sec then 66°C for 30 secs. minus 1°C per cycle, then 72°C for 30 secs., cycle 3 (repeated 27x); 94°C for 30 secs., then 53°C for 30 secs., then 72°C for 30 secs. and cycle 4; 72°C for 7 mins., followed by a cooling-off step to 10°C. Fragments were separated on a Mega-Gel Dual High-Throughput Vertical Electrophoresis Unit (CBS Scientific, Del Mar, CA, USA) with 6% non-denaturing acrylamide gels stained with ethidium bromide during the run.

To monitor biomass of isolates in crossing and infection assays, we designed specific TaqMan® probe/primer combinations for quantitative PCRs (qPCR) based on the *mat1-1* and *mat1-2* idiomorph sequences of the two reference *Z. tritici* isolates IPO323 and IPO94269, respectively[20]. Primers that specifically amplify DNA fragments in *mat1-1* isolates were Mmat1F3/Mmat1R3, with a FAM-fluorescent probe IP3, and primers to specifically amplify DNA fragments in *mat1-2* isolates were Mmat2F7/Mmat2R7, with a YY-fluorescent probe 2P4. Both quantitative real-time amplifications were performed in a single PCR on an Applied Biosystems 7500 Real-time PCR System (Foster City, CA, USA). Total reaction volumes were 25 µl, including 3 µl DNA, 12.5 µl Premix Ex Taq™ (2X) (TaKaRa, Shiga, Japan), 1 µl each forward and reverse primers (6 µM), 0.67 µl for each probe (5 mM), 0.5 µl ROX Reference Dye II (50x), and 8.33 µl ultraPURE™ nuclease-free water (Gibco, Paisley, Scotland). Thermal cycling was as follows: cycle 1; 50°C for 2 mins., cycle 2; 95°C for 10 mins., cycle 3 (repeated 39x); 95°C for 15 secs., then 60°C for 20 secs. Results were analyzed using Sequence Detection Software version 1.2.3 (Applied Biosystems, Foster City, CA, USA). Standard curves from serial dilutions of known concentrations of pure fungal DNA of the six parental isolates plus

the DNA from the reference isolates (Table S6) gave highly similar results in CT values. Therefore, serial dilutions of DNA from isolates IPO323 and IPO94269 were included in each TaqMan® PCR run to calculate the unknown concentrations of fungal DNA in inoculated wheat seedlings. The standard curves had very high R2 values (0.990-0.996) for all data points from 3 pg to 30 ng and, therefore, CT values within this range were reliable (data not shown). See Table S7 for all used probes and primers.

Generation and analyses of segregating *Zymoseptoria tritici* populations

Crossing assays. We used an *in planta* crossing protocol for all mating assays[6]. For mapping, we extended the existing *Z. tritici* mapping population IPO323/IPO94269 to 400 progeny isolates and the IPO323/IPO95052 population to 165 progeny isolates by manually collecting individual ascospores. For the EPP-biotic stress validation, we independently performed six crosses between avirulent and virulent isolates (IPO323, IPO94269, IPO95052) on five wheat cultivars (Obelisk, Shafir, Taichung 29, Inbar or Volcani 447) in multiple (>=2) biological replications. In addition, we used eight isolates in 19 crosses on nine wheat varieties (seven bread wheat and two durum wheat) and one barley accession (Tables S2, S3) to test the occurrence of sex despite one of the parents is avirulent. For the EPP-abiotic stress validation we conducted 42 crosses between three sets of fungicide resistant and sensitive isolates on cv. Taichung 29 (Figs. 3 and S8, S10; Table S6). Single sequence repeat (SSR) genotyping was routinely used to either confirm that segregating populations resulted from the applied parental isolates (Table 1, Figs. S7, S11) or to determine the genotype of asexual fructifications that appeared in crossing assays (Figs. S12, S13). Populations were maintained at -80°C[42] for further detailed analyses, including DArTSeq as well as MAMA, diagnostic PCRs for mating type determinations[20] and the maternal/paternal contributions to sexual development, sequencing/phenotyping to determine (a)virulence in progeny and wild type strains (Tables 1, 2, S4, S5, Fig. S8) and qPCR (Figs. 3, S8, S10).

Phenotyping. We prepared inoculum following published procedures[19] and performed seedling assays at growth stage (GS) 11-12[43] either by painting a spore suspension using a soft brush (mapping populations) or by atomizing a spore suspension onto the potted seedlings that were placed at the perimeter of a circular rotary table in an inoculation cabinet, adjusted at 15 rpm, which is equipped with interchangeable atomizers and a water cleaning device to avoid cross-contamination between isolates (all other assays). Infected plants were incubated in transparent plastic bags for 48h at 100% RH in the aforementioned greenhouse. Disease

severity was assessed at 21 days post-inoculation using necrosis and pycnidial development estimated as percentage of the total primary leaf area of individual seedlings. Following these procedures, we screened 190 IPO323/IPO94269 offspring isolates, partly in three independent replicates (81 isolates) or singular tests (Fig. S14) with the parental strains as controls, on cv. Shafir, carrying *Stb6*, and the susceptible control cv. Taichung 29.

Genetic mapping. Fungal genomic DNA was isolated using a standard CTAB-chloroform protocol. The parents and off-spring (N=282) of the *Z. tritici* mapping population (IPO323/IPO94269)[4,12], were assayed of which 171 isolates showed distinct avirulence/virulence phenotypes on cv. Shafir. We used DArTSeq™, a genotyping-by-sequencing (GBS) method that combines diversity-arrays-technology (DArT) and next generation sequencing platforms[44]. In total 5,392 polymorphic DArTSeq markers *Z. tritici* isolates were obtained. Marker sequences (max 69 nt) were placed on the *Z. tritici* reference genome (Fig. 1), using NCBI BLASTn (megablast)[45] and visualized using the GViz package[46] (Fig. 1). Multi-mapping markers were only placed on the genome at the best position if there was a considerable difference in bit-scores (difference ≥5).

For fine mapping, the 5,392 generated DArTSeq markers were sorted according to their discrimination power for avirulent/virulent isolates by calculating the squared differences of genotype frequencies, and 60 DArTSeq markers linked with avirulence were selected. These markers were sorted into a genetic linkage map, using JoinMap® 4 software with settings LOD (Log of Odds) ≥3 for grouping, and the maximum likelihood mapping option for linkage group generation[47]. Since the segregation of avirulence fitted the model of single gene inheritance (Fig. S14)[18], phenotypic data were converted to an appropriate marker (*AvrStb6*) using scoring codes that are required for JoinMap, and this was integrated in the mapping procedure.

Offspring isolates with more than 10% missing genotypic values were removed from the analysis. Moreover, isolates without recombination near the (a)virulence locus, and eight showing discrepancies between the genotyping and phenotyping were not considered for analysis. To delimit the physical region harboring *AvrStb6*, we deployed a graphical mapping approach using the recombinant offspring isolates and clustered the markers that co-segregated with *AvrStb6* into bins with the marker order as estimated by JoinMap as a reference. The generated genetic linkage map was compared to the IPO323 reference genome sequence by aligning the DArTSeq (http://genome.jgi.doe.gov/Mycgr3/Mycgr3.home.html) to determine the physical position of *AvrStb6*.

Gene annotation

Gene annotation was performed on the *Z. tritici* reference genome isolate IPO323[4] using the Maker2 pipeline[48], combining *ab initio* protein-coding gene evidence from SNAP[49], Augustus[50], and GeneMark-HMM[51]. Additionally, Maker2 was provided with protein alignments to 35 predicted fungal proteomes, *Z. tritici* reference gene models annotated by the Joint Genome Institute (JGI)[4], and transcriptome data (assembled transcripts and splice-junctions) derived from two previously published RNA-seq datasets[21,36]. For gene annotation, RNA-seq data (single-end) were mapped to the *Z. tritici* reference genome with TopHat (version 2.0.13) (--min-intron-length 20 --max-intron-length 2000 --max-multihits 5)[52]. *Z. tritici* transcripts were assembled using Cufflinks[53]. Gene models predicted with Maker2 were manually evaluated and refined[54], for example by excluding protein-encoding genes <60 aa or lacking a starting methionine.

Identification of effector candidates

Gene expression, expressed as fragments per kilobase of exon per million fragments mapped (FPKM), during wheat colonization for newly predicted protein-coding genes was inferred using Cuffdiff (version 2.2.1)[53]. Similar to previous observations[21,55], the third replicate of the RNA-seq experiment of Rudd et al.[21] behaved differently and was therefore excluded from all further analyses. Pair-wise log2-fold expression changes as well as multiple-testing corrected p-values ($P < 0.05$) were inferred for *in planta* RNA-seq samples compared to CDB[21]. N-terminal secretion signals were predicted in all proteins using SignalP (version 4.1)[56]. Protein domains were predicted with InterProScan[57].

Functional analyses of *AvrStb6*

Strains, media and growth conditions. *Z. tritici* strains IPO323 and IPO94269, which are avirulent and virulent on cv. Shafir, were used as wild type strains (WTs) and recipient strains for gene deletion and ectopic expression (Fig. S15). The WTs and all deletion strains were kept at -80°C and were re-cultured on potato dextrose agar (PDA) (Sigma-Aldrich Chemie, Steinheim, Germany) at 15°C once desired for experimentation. Yeast-like spores were produced in yeast glucose broth (YGB) medium (yeast extract 10 $g.L^{-1}$, glucose 30 $g.L^{-1}$) after placement in an orbital shaker (Innova 4430, New Brunswick Scientific, Nijmegen, The Netherlands) at 15°C. For *in vitro* expression analyses in *Z. tritici* blastospores we used YGB and MM[58] under similar conditions, whereas we adjusted the conditions in YGB to 25°C for

expression in mycelium. *Escherichia coli* DH5α was used for general plasmid transformation and *Agrobacterium tumefaciens* strain AGL-1 was used for all fungal transformations.

Fungal transformation. All transformations were performed using *A. tumefaciens* mediated transformation (ATMT) as described previously[59,60]. Genomic DNA of stable transformants was extracted according to standard protocols[61]. For ectopic complementation, the same procedure was utilized with minor modifications, including the use of 250 µg $m.L^{-1}$ geneticin for the selection of mutants.

RNA isolation and qRT-PCR. *In vitro* and *in planta* expression profiling of *AvrStb6* was performed using quantitative real-time PCR (qRT-PCR). For *in planta* analyses, wheat cv. Shafir was inoculated, in triplicate, with the WT isolates as described[60], and leaf samples were collected at seven hours post-inoculation, and subsequently at 1, 2, 4, 8, 12, 16 and 20 dpi, followed by flash freezing and grinding in liquid nitrogen using a mortar and pestle. Total RNA was extracted either from ground leaves or fungal biomass produced in YGB using the RNeasy plant mini kit (Qiagen, MA, USA). DNA contamination was removed with the DNAfree kit (Ambion, Cambridgeshire, U.K.). First-strand cDNA was synthesized from approximately 2 µg of total RNA primed with oligo(dT) using the SuperScript III following manufacturers' instructions. One µl of the resulting cDNA was used in a 25 µl PCR reaction using a QuantiTect SYBR Green PCR Kit and run and analyzed using an ABI 7500 Real-Time PCR System. The relative expression of each gene was initially normalized with the constitutively expressed *Z. tritici* beta-tubulin gene[62] and then calculated based on the comparative C (t) method described previously (Fig. S2)[63].

Pathogenicity assays and quantitative fungal biomass analyses. All assays were conducted as described above using wheat cvs. Shafir and Taichung 29 (Figs. S5, S16). Disease development was monitored and recorded every three days and leaves of cv. Taichung 29 were harvested at 2, 4, 8, 12, 16 and 20 dpi for qRT-PCR expression analyses and for qPCR fugal biomass determination of all WTs and transformed *Z. tritici* strains[20,64] (Fig. S16). Genomic DNA was extracted from approximately 100 mg of infected leaves using a standard phenol/chloroform DNA extraction[61].

Generation of gene deletion and ectopic integration constructs. To generate the *AvrStb6* deletion construct, pKOZtAvrStb6, the multisite Gateway® three-fragment vector construction kit was used, enabling the cloning of three fragments into the destination vector, which was compatible with the ATMT procedure. A 2 kb upstream and downstream sequence of *AvrStb6*

was cloned in pDONR™P4-P1R and pDONR™P2R-P3. The generated constructs along with pRM250[13] containing the hygromycin phosphotransferase (*Hph*) gene as a selection marker were cloned into the destination vector, pPm43GW, via the LR reaction. In order to make the *AvrStb6* ectopic integration construct (pZtAvrStb6.com), the full ORF of *AvrStb6*, including a 1,020 bp upstream stretch as its promoter and 552 bp stretch downstream as terminator, were cloned into pDONR™P221 (Invitrogen, CA, USA) resulting in the generation of p221-ZtAvrStb6.com. The p221-ZtAvrStb6.com as well as two entry vectors pRM245 and pRM234[13], were used to clone these three fragments into the destination vector, pPm43GW, through the LR reaction.

Determining exclusive paternal parenthood

EPP-biotic stress. To determine parenthood in the conducted crosses, we analyzed four crosses (Table 1; Figs. S6, S7) using four markers (*AvrStb6*, *mat*, ag-0006 and mt-SSR) and monitored fungal biomass development by qPCR (Figs. 3, S8).

EPP-abiotic stress. Strobilurin sensitivity was assayed in six strains (Table S6) on potato dextrose agar (PDA) plates that were amended with kresoxim-methyl (BASF, Ludwigshafen, Germany) and trifloxystrobin (Bayer CropScience, Monheim am Rhein, Germany) and determined minimal inhibitory concentrations (MICs) of two different technical samples of the fungicides by spotting isolates on strobilurin amended PDA plates. The concentrations for kresoxim-methyl were 0.0025, 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, and 1.0 ppm, and the concentrations for trifloxystrobin were 0.00025, 0.0005, 0.001, 0.0025, 0.005, 0.01, 0.025, 0.05, 0.1 and 0.25 ppm. All isolates were spotted in triplicate in a volume of 5 µl per spot at a concentration of 4 x 10^5 spores ml^{-1}. As a positive control for growth isolates were also plated on PDA amended with the strobilurin solvent (1% methanol). Plates were placed at 18°C in the dark for 10 days, after which MIC values were assessed. A test progeny was generated by crossing *Z. tritici* isolates IPO03001 and IPO03003 and analyzed it on amended PDA plates and with MAMA assays to conclude that both methods are congruous.

MIC values for the six parental isolates (Table S6) for the commercially available fungicide Amistar™ (Syngenta, Roosendaal, Netherlands) were determined, containing the active ingredient azoxystrobin, at 0.1, 1.0, and 10 ppm and then determined which concentrations of azoxystrobin to use for infection and crossing assays using an *in planta* dose response curves for the sensitive *Z. tritici* isolates using different preventive applications of azoxystrobin (250 $g.L^{-1}$ a.i. of azoxystrobin; 50% E.C.) on 10 day-old seedlings of cv. Taichung 29 that were preventatively treated (48h) using a track sprayer that was calibrated to deliver the

recommended application of 1 L.ha^{-1} sprayed at a rate of 250 L.ha^{-1}, with the following percentages of the full recommended dose: 0, 3.125, 6.25, 12.5, 25,50, 100 and 200% (which correspond with fungicide solutions of 0, 0.03125, 0.0625, 0.125, 0.25, 0.5, 1, and 2 g azoxystrobin.L^{-1}, respectively). We then inoculated with *Z. tritici* and percentages of leaf area covered by pycnidia were recorded at 20, 23, 26, and 29 dpi for dose response curve experiments, at 20 dpi for infection assays, and at 0, 5, 10, 15, 20, 25 and 30 dpi for qPCR biomass monitoring over time (Figs. 3, S9, S10). Finally, three sets of *Z. tritici* field isolates IPO03001/IPO03003, IPO03002/IPO03005 and IPO04001/IPO04011, with equal pathogenicity, opposite mating types and contrasting sensitivity to azoxystrobin (Table 2, Figs. 3, S9) were used for the generation of 42 *in planta* ascospore progenies and fungal biomass development of each isolate in each crossing assay (Figs. 3, S10) individually and in pairwise mixtures on untreated and preventatively treated (48h, 100% azoxystrobin) seedlings of the wheat cv. Taichung 29 was monitored. Leaf samples were collected at 0, 5, 10, 15, 20, 25 and 30 dpi and were immediately frozen in liquid nitrogen before storage at -80°C until lyophilization, subsequent DNA extraction, and qPCR analyses. Two extractions were made from each sample (technical repeats), and the mean results were expressed in ng of fungal DNA.mg^{-1} dry weight leaf material. A first set of 18 crosses was performed in seedlings of cv. Taichung 29 that were preventively treated (48h) with Amistar™ at 0 (control), 3.125, 6.25, 12.5, 25 and 50% of the full rate. In a second set of 24 crosses we repeated these conditions but added two concentrations; full rate (100%), and the double rate (200%) (Tables 2, S6). From six through 12 weeks after inoculation, material was harvested for ascospore discharge and collection[6]. Ascospores were isolated as much as possible from diverse locations within a plate or within several plates from each cross to obtain random ascospore progenies. Baseline germination frequencies on unamended WA plates for all 42 progeny sets (N=15,975) and randomly selected ascospores were determined. Germination frequencies of the 24 ascospore progenies for the second series of crosses were also determined on WA amended with 1 ppm active ingredient azoxystrobin (N=9,025), and these frequencies were expressed as percentages relative to the mean of the control germination frequencies on unamended water agar. We evaluated the percentage of resistant offspring by 2,100 independent MAMA PCRs (Table 2).

Developing the new population genetics model

The model equations were numerically solved in C^{++}. Output was plotted using the graphics package Sigmaplot. The calculation showing that, independent of the parameter

values, the frequency of virulence increases slower when the avirulent strain takes part in the sexual reproduction was done by hand and checked using the package Maple. We modelled the population genetic consequences of this new observation using an allele frequency model as introduced by Leonard[23].

Data Availability and Accession Code Availability Statements

All data are available and deposited in NCBI Genbank under accession number ACPE00000000[4], in Gene Expression Omnibus under the accession number GSE54874[36], and as a BioProject with the accession number PRJEB8798[21].

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Supplementary Information

Specificity and mating in the *Zymoseptoria tritici* – wheat pathosystem

Zymoseptoria tritici has a bipolar heterothallic mating system, each partner has a unique mating type (*mat1-1* or *mat1-2*) (Waalwijk et al. 2002), but can produce female (ascogonia) as well as male (spermatia) gametes. However, thus far crosses can only be made *in planta*, by co-inoculating the mating partners on a susceptible wheat cultivar (Kema et al. 1996a). To our surprise, we noticed that crosses are also successful in case one of the mating partners is avirulent on the used wheat cultivar, e.g. the cross between the avirulent isolate *Z. tritici* IPO323 (carrying *AvrStb6*) with the virulent isolate IPO94269 on cv. Shafir (carrying *Stb6*) (Table 1, Fig. S7). We then further demonstrated and generalized this observation by a range of additional and routine crosses (at least two pots with >10 seedlings per cross; Tables S2, S3), including crosses between *Z. tritici* isolates that are specific for either bread wheat (AABBDD, 2x=42) or durum wheat (AABB, 2x=28). This pathogenic dichotomy for either bread wheat or durum wheat is a remarkable phenomenon in *Z. tritici* that thus far has not been elucidated(Kema et al. 1996). Since there are no wheat cultivars that are highly susceptible to both types of isolates, we used wheat cultivars with high susceptibility for either of them in the *in planta* crossing protocol. All crosses resulted in substantial progeny (not counted, but accumulative estimations

are at least >1,000 ascospores per cross), except in attempts to cross two avirulent isolates (Table S3). In all these cases, no ascospores were observed in multiple ascospore discharge experiments.

Albeit that specificity in the *Z. tritici*-wheat pathosystem was already expected (Eyal et al. 1973; Kema and van Silfhout 1997; Kema et al. 1996; Kema et al. 1996a) the elucidation of the mating system was the necessary step to eventually confirm that avirulence to cv. Shafir – and other wheat cultivars – was controlled by a single locus (Kema et al. 2000). This was the onset of a range of new experiments that eventually resulted in claiming a gene-for-gene relationship between *Z. tritici* and wheat (Brading et al. 2002) and the identification of 21 *Stb* genes (Brown et al. 2015) by using well characterized pathogen isolates, both under greenhouse and field conditions (Table S1).

In the current suite of experiments, we confirmed single gene inheritance of avirulence in *Z. tritici* isolate IPO323. All the phenotyped IPO323/IPO94269 offspring showed high pathogenicity towards the susceptible cv. Taichung 29 with very high necrosis and pycnidia levels across all replicates (Fig. S14). Both parental strain showed a differential response on cv. Shafir, carrying *Stb6,* and the progeny consequently segregated 1:1 for avirulence: virulence (Figs. 1, S14; avirulence: virulence = 101:93; $\chi^2 = 0.164$, P= 0.05), thereby confirming the monogenic inheritance of *AvrStb6*.

Genetic (fine) mapping of *AvrStb6*

In total 5,392 polymorphic DArTSeq markers (max length 69nt) for a cross between avirulent IPO323 and virulent IPO94269 *Z. tritici* isolates were obtained. As virulence and avirulence segregated clearly into two distinct groups, we used avirulence as a phenotypic marker, allowing selection of 60 DArTSeq markers that co-segregated with avirulence. We sorted these markers, thus developing a local genetic linkage map. From these 60 markers we selected a cluster of 22 DArTSeq markers that perfectly co-segregated with the *AvrStb6* locus without any recombination with avirulence. This cluster could be positioned at the tip of chromosome 5, spanning the physical interval from 0 to 140 kbp of this chromosome in the reference genome (Fig.1).

A graphical positioning of the *AvrStb6* was displayed by sorting offspring isolates with recombination events closely linked to the *AvrStb6* gene. A plausible genomic position of the

AvrStb6 was thus defined and a graphical representation of the recombination events was generated (Fig. S17).

Alignment of the closest markers to *AvrStb6* to the reference genome IPO323 assembly version 2 for *Mycosphaerella graminicola*, available on the JGI database (http://genome.jgi.doe.gov/pages/blast-query.jsf?db=Mycgr3) showed that the co-segregating markers with *AvrStb6* had either a hit with the distal part of chromosome 5 or in another genomic region with lower score and E-values. We, therefore, considered only markers having a perfect hit with the reference genome to physically delimit the genomic region carrying *AvrStb6*. Consequently, and by combining the QTL, graphical and physical mapping approaches, we located *AvrStb6* at the distal part of chromosome 5 between 0 bp and 140,950 bp on the reference genome IPO323 (Table S4, Fig. 1).

Functional analysis of *AvrStb6*

Deletion and ectopic integration of AvrStb6. To verify the function of *AvrStb6* disruption and recombinant strains were generated in *Z. tritici* IPO323 and IPO4269, respectively. We generated three independent disruption strains of *AvrStb6* in IPO323, IPO323Δ*AvrStb6*#14, #19 and #33 (Fig. S15), which were all pathogenic on cv. Shafir (carrying *Stb6*) and showed similar phenotypes as IPO94269 (Figs. 1, S5). Alternatively, a fragment of 1,937 bp corresponding to the entire open reading frame of *AvrStb6*, a promoter region of 1,020 bp and a terminator region of 552 bp was ectopically integrated in the virulent IPO94269 by *Agrobacterium tumefaciens*-mediated transformation (ATMT). This resulted in two independent transformants expressing the *AvrStb6*, designated as IPO94269::*AvrStb6*#1 and IPO94269::*AvrStb6*#2, which are avirulent on cv. Shafir, showing similar phenotypes as IPO323. We, thereby, have formally shown that *AvrStb6* is a single avirulence factor explaining gene-for-gene relationship in the wheat-*Z. tritici* pathosystem.

Expression profiling of *AvrStb6* during compatible and incompatible interactions with wheat

The *in vitro* expression of *AvrStb6* in IPO323 conidial blastospores declined from 44x (fold change compared to *beta*-tubulin) to 16x in mycelium and finally to 12x in MM, whereas the expression in IPO94269 was naught. Expression *in planta* was much lower during compatible (IPO94269) and incompatible (IPO323) interactions with cv. Shafir and varied

between 2x-8x, with slight (8x at 1 dpi with IPO323), but overall differential peaks between both isolates at virtually all sampling moments (except at 12 dpi) (Fig. S2). At 8 dpi expression of *AvrStb6* was significantly higher in IPO94269 than in IPO323, which may suggest a role during the transition from biotrophy to necrotrophy (Mirzadi Gohari et al. 2015). However, the expression of *AvrStb6* in the susceptible cv. Riband, as derived from RNAseq experiments(Rudd et al. 2015) was much higher and showed a completely different pattern, with major peaks at 10 dpi and 14 dpi (Fig. S3). We currently cannot explain these differences, but the data were clearly independently collected during different experimental conditions, which would confirm that *Z. tritici* is very sensitive to environmental fluctuations (Ben M'Barek et al. 2015). However, notwithstanding these differences, *AvrStb6* - as a classical avirulence effector - determines the outcome of the interaction as shown by the current data and by previous data (Kema et al. 2000; Zhong et al. 2017).

We quantified fungal biomass during compatible interactions of the WT strains IPO323 and IPO94269 along with the transformants IPO323*ΔAvrStb6-33* and IPO94269Δ*AvrStb6*-1 on cv. Taichung to determine any possible virulence function of *AvrStb6* (Fig. S16). As we did not observe any significant difference, except at 16 dpi in the comparison between IPO323 and IPO323Δ*AvrStb6*-33, we conclude that *AvrStb6* has no virulence function, at least not in these interactions. Tests were performed in three biological replicates and repeated thrice.

Crosses elucidating exclusive paternal parenthood: host resistance as a biotic stress factor

We used the established crossing protocol developed by Kema et al.[2] that is the basis for all genetic studies published thus far. In short, crosses are initiated on plants by co-inoculation of strains with opposite mating types. After initial symptom development for approximately 14 days, plants are placed in the outside, natural environment – crosses can be made throughout the year – and after six to seven weeks deteriorated primary leaves are collected, submerged in water, for 30 min. and then placed on filter paper in one quarter of the lid of a Petri dish and the excess of water is blotted away. The Petri plate is then closed and placed upside down - thus agar up - to prevent contamination and to capture discharged ascospores from the agar after 24h germination. Discharges start within 15 min. after closing the Petri dish and were continued for one hour, by turning the plate every 15 min. To our surprise all crosses were successful irrespective of the avirulence of one parent that could not establish a pathogenic relationship with the used wheat cultivar. The only crosses that failed were those between isolates that are both avirulent on the used wheat cultivar or on a non-host

crop such as barley (Table S3). In total eight isolates, either virulent or avirulent on wheat cultivars that were used to perform *in planta* crosses, from different origin (Table S2) were used in 19 crosses. All crosses were successful, except for the six crosses between two avirulent partners (Table S3). This is fundamentally different from the current paradigm in plant disease epidemiology where avirulent isolates are thought to be eliminated from the population as they do not colonize the host, do not reproduce and consequently do not contribute to the epidemic (Van der Plank 1982; Zadocs and Schein 1979). However, we show that in *Z. tritici* avirulent strains can successfully enter a sexual cycle, and consequently transmit their genes to subsequent populations. Thus, genotypes are lost, but their genes - here avirulence genes - are saved. This would temporally affect adaptation to host resistance and hence the longevity of host resistance (Brown 2015).

To determine female and male contributions to the mating process we deployed several nuclear and mitochondrial markers in 46 crosses. In four crosses - *Z. tritici* IPO323/IPO94269 and IPO323/IPO95052 each on two wheat cultivars (Tables 1, S4, Fig. S7) – the nuclear markers (*AvrStb6*, *mat*, SSR ag-0006) segregated according to the expected 1:1 ratios. However, the segregation of the mitochondrial SSR (mt-SSR) marker was significantly skewed, confirming that the partaking of non-pathogenic or avirulent isolates affects the expected 1:1 ratio for maternal or paternal parenthood when both parents are pathogenic (Table 1, Fig. 2). The 19:55 mt-SSR segregation in the *Z. tritici* IPO323/IPO94269 cross on the susceptible bread wheat cv. Obelisk shows that both isolates were either paternal or maternal donors, the ration probably depending on sexual fitness or slight differences in aggressiveness. However, conducting the same cross on cv. Shafir that carries *Stb6* (Kettles et al. 2017; Saintenac et al. This issue) and is resistant to *Z. tritici* IPO323 (Brading et al. 2002; Kema et al. 2002), the segregation was 0:87. Hence, *Z. tritici* IPO323 was the exclusive paternal donor. We then further substantiated this observation by the *Z. tritici* IPO323/IPO95052 cross on cv. Obelisk. This cultivar is completely resistant to *Z. tritici* IPO95052, which is a durum wheat strain, and observed a 99:0 ratio indicating the exclusive paternal contribution of IPO95052 to the successful sexual reproduction. This clearly demonstrates that *Z. tritici* IPO323 swaps exclusive paternal or maternal parenthood in sexual reproduction, depending on its avirulence or virulence towards the resistant or susceptible crossing host, respectively. Repetition of the *Z. tritici* IPO323/IPO95052 cross on the durum wheat cv. Inbar indicated mutual parenthood as *Z. tritici* IPO323 has low pathogenicity on this cultivar (see Table 1, subscript c).

We determined the fungal biomass of both mating partners of four crosses over time through qPCR (Table 1, Figs. 3, S8). First, we quantified biomass of each individual isolate, and then each isolate during the *in planta* mating process. While virulent isolates in compatible interactions increase in biomass and (eventually) develop pycnidia, avirulent isolates do not or hardly exceed the biomass of the applied starting inoculum in incompatible interactions and never reproduce asexually (no pycnidia). In mixed inoculations avirulent isolates always show a similar pattern. They do not increase in biomass and are outcompeted by the virulent isolates that exclusively produce the asexual fructifications (Figs. 3, S8, S12). Hence, we conclude that the avirulent parent is the exclusive paternal donor.

Crosses elucidating exclusive paternal parenthood: azoxystrobin as an abiotic stress factor

We subsequently considered that fungicides may cause a similar effect as they disable either germination or fungal development. Strobilurin fungicides were chosen as abiotic stress factor as the target *cytb* gene is on the mitochondrion. This also enabled the analysis of parenthood of either sensitive or resistant *Z. tritici* isolates in crosses on plants that were treated with Amistar® (ai azoxystrobin) prior to co-inoculations, as mitochondria are usually exclusively maternally inherited in filamentous fungi (Basse 2010). Furthermore, strobilurin resistance is qualitative, due to the G143A QoI single nucleotide polymorphism (SNP), which can be easily detected with the developed MAMA assay that enabled precise genetic analyses of the generated progenies. In total, we performed 42 additional crosses on Amistar® treated cv. Taichung 29 plants tested whether sensitive strains still participate in sexual reproduction. All crosses were between sensitive and resistant strains on plants that were preventatively treated with various doses of the fungicide. Three sets of *Z. tritici* field isolates IPO03001/IPO03003, IPO03002/IPO03005 and IPO04001/IPO04011, with equal pathogenicity, opposite mating types and contrasting azoxystrobin resistance (Figs. 3, S9, S10) were crossed in two rounds of crossing experiments comprising 18 (round 1) and 24 (round 2) crosses using the same azoxystrobin concentrations, but the latter had two additional concentration (100% and 200%). Despite the used azoxystrobin concentrations, all crosses generated offspring (Table 2). Thus, effective azoxystrobin concentrations for disease management do not prevent sexual reproduction of *Z. tritici*. We subsequently evaluated the percentage of resistant offspring by determining the germination patterns in all offspring populations on water agar with (N=9,025) and without (N=15,975) azoxystrobin as well as by 2,100 independent diagnostic MAMA assays (Table 2). The percentage of resistant off-spring

rapidly increases to 100% under suboptimal azoxystrobin concentrations and at the recommended full dose all progenies were entirely fixed for resistance in one generation (Table 2). This shows that azoxystrobin applications direct resistant and sensitive *Z. tritici* isolates into maternal and paternal parenthood, respectively.

In order to further substantiate these results, we also determined the biomass of the parental strains by qPCR in plants that were preventatively treated with azoxystrobin and subsequently used for individual or co- inoculation assays as part of the *in planta* crosses (Figs. 3, S10). The individually inoculated resistant parents developed biomass and symptoms whereas the sensitive parents hardly developed, but eventually produced pycnidia, indicating that the used azoxystrobin concentrations are not lethal but fungistatic for sensitive *Z. tritici* strains (Fig. S9). In the co-inoculations, however, the sensitive parents were undetectable and apparently completely out-competed by the resistant strains. Still all co-inoculations resulted in successful sexual reproduction (Table 2, Figs. 3, S10).

Genotyping of the developed asexual pycnidia from co-inoculated isolates with opposite mating types for crosses aiming at understanding the effect of biotic stress, through host resistance, confirmed that all these fructifications were merely produced by the virulent strains (Fig. S13).

The population genetic consequences

Our model consists of an extension of the model developed by Leonard (Leonard 1969), to include the mechanism described here, i.e. an avirulent pathogen is able to undergo sexual reproduction with a virulent pathogen strain on a resistant crop within the framework of a well-mixed *Z. tritici*-wheat system. The model describes the dynamics of a haploid pathogen infecting a population of a diploid plant. Both the plant and pathogen have a single bi-allelic locus conferring resistance and virulence, respectively, and the frequency of the pathogen virulence allele and the plants' resistance allele is simulated over time. The frequency of the virulent (V) allele in the pathogen population is represented by the number $0 < n < 1$. The frequency of the avirulent allele (A) is $1 - n = m$. Similarly, for the plant population the frequency of the resistant (R) allele in the host species is represented by the number $0 < p < 1$, while the frequency of the susceptible (S) allele is $1 - p = q$. For simplicity of analyses we assume that the resistance gene in the plant population is fully dominant, and therefore consider only two plant genotypes: SS and $R-$. As with the typical GFG model, we assume that the avirulent strain of the pathogen can only develop on the susceptible plant, while the

virulent strain can develop on both the susceptible and resistant plants. In addition, we include the mechanism described here, i.e. if an avirulent pathogen lands on a resistant plant, although unable to develop into a lesion, it is able to sexually reproduce with a virulent strain of the pathogen.

In our model, we simulate the dynamics of the frequency of each pathogen and plant strain. As in Leonard's model, we assume that selection pressures, such as the cost to a pathogen of having a virulence allele (k), the reduced fitness of a plant infected by a pathogen (c), and the cost of having a resistance allele to a resistant plant's fitness (d), direct the change in allele frequency in the pathogen population. In addition, we consider the effect of the newly observed mechanism of pathogen reproduction (EPP) on the co-evolution of alleles. We introduce a new parameter, σ, representing the probability of an avirulent individual on a resistant cultivar mating with a virulent individual. We consider two scenarios regarding the plant population. Firstly, we assume the frequency of the plant population is under the control of growers, and therefore not governed by the evolutionary forces, instead, we assume a constant frequency of resistance in the plant. We term this the 'agricultural scenario'. Secondly, in the 'natural scenario', we allow the frequency of susceptible and resistance alleles in the plant population to vary as a result of the selection pressure of being infected and/or resistant.

We first describe the derivation of the equations governing the frequency of virulence in both scenarios, before deriving the equations governing the frequency of resistance in the plant population.

The dynamics of the frequency of virulence

The frequency of virulent pathogens following one cycle of selection (n_{i+1}) can be determined by:

$$n_{i+1} = \frac{\sum_H (\text{genotype V fitness}) \cdot (\text{V allele freq.on host})}{\sum_H [(\text{genotype V fitness} \cdot \text{V allele freq.on host}) + (\text{genotype A fitness} \cdot \text{A allele freq.on host})]} \qquad \text{(Y1)}$$

where the summation is over the host genotypes (H). The numerator in (Y1) represents the sum of the virulent pathogen strain infecting all host genotypes, while the denominator is the sum of all pathogen strains on all host genotypes. In each case the change in the frequency of a pathogen genotype on a host genotype is given by the frequency of that pathogen genotype on the host (hereafter denoted $Freq(X\ on\ YY)$, for pathogen allele X on host genotype YY) multiplied by the fitness of that pathogen on that host (hereafter denoted $Fit(X\ on\ YY)$).

The frequency of each pathogen strain on each plant genotype in genotype i is given by: $Freq(A\ on\ SS) = q_i^2 m_i$, $Freq(A\ on\ R-) = (1 - q_i^2) m_i$, $Freq(V\ on\ SS) = q_i^2 n_i$, and

$Freq(V\ on\ R-) = (1-q_i^2)n_i$. The fitness of each pathogen strain on each plant genotype can be specified similarly: $Fit(A\ on\ SS) = 1$, $Fit(A\ on\ R-) = 0$, and $Fit(V\ on\ SS) = Fit(V\ on\ R-) = (1-k)$. The denominator therefore becomes:

$$(1-k)(1-{q_i}^2)n_i + (1-k){q_i}^2 n_i + {q_i}^2 m_i = {q_i}^2 + n_i((1-{q_i}^2)-k)$$

To calculate the numerator, the number of new pathogen infections of each genotype is determined from the sexual recombination of strains on the same host genotype. The proportion of pathogen lesions that are on susceptible hosts is $\xi = q_i^2 \cdot \big((1-k)n_i + m_i\big)$, and the proportion on resistant hosts is $\varphi = (1-q_i^2) \cdot ((1-k) \cdot n_i)$. In each case we determine the proportion of reproduction events on each host that produce virulent offspring: f_V and g_V on the susceptible and resistant hosts respectively.

$$g_{v=} = \frac{Fit(VV\ on\ SS)\times Freq(VV\ on\ SS) + \frac{1}{2}Fit(VA\ on\ SS)\times Freq(VA\ on\ SS)}{Fit(VV\ on\ SS)\times Freq(VV\ on\ SS) + Fit(VA\ on\ SS)\times Freq(VA\ on\ SS) + Fit(AA\ on\ SS)\times Freq(AA\ on\ SS)}$$

$$f_v = \frac{Fit(VV\ on\ R-)\times Freq(VV\ on\ R-) + \frac{1}{2}Fit(VA\ on\ R-)\times Freq(VA\ on\ R-)}{Fit(VV\ on\ R-)\times Freq(VV\ on\ R-) + Fit(VA\ on\ R-)\times Freq(VA\ on\ R-) + Fit(AA\ on\ R-)\times Freq(AA\ on\ R-)}$$

For the proportion of mating events on susceptible plants that result in virulent lesions, g_v, $Fit(VV\ on\ SS) = (1-k)^2$, $Fit(VA\ on\ SS) = (1-k)$, $Fit(AA\ on\ SS) = 1$, $Freq(VV\ on\ SS) = {n_i}^2$, $Freq(VA\ on\ SS) = 2n_i m_i$ and $Freq(AA\ on\ SS) = {m_i}^2$. With substitution and simplification, we establish g_v as:

$$g_v = \frac{(1-k)n_i}{(1-k)n_i + m_i}$$

However, in order to calculate the proportion of reproduction events on resistant plants that result in virulent offspring, f_v, we now account for the possibility that avirulent pathogen strains are involved in reproduction. Therefore, $Fit(VV\ on\ R-) = (1-k)^2$, $Fit(VA\ on\ R-) = (1-k)$, $Fit(AA\ on\ R-) = 0$, $Freq(VV\ on\ R-) = {n_i}^2$, $Freq(VA\ on\ R-) = 2\sigma n_i m_i$ and $Freq(AA\ on\ R-) = {m_i}^2$. The parameter σ describes the proportional decrease in participation in sexual reproduction by the avirulent males on the resistant plant. With substitution and simplification, f_v is found to be:

$$f_v = \frac{\sigma + (1-k-\sigma)\,n_i}{2\sigma + (1-k-2\sigma)\,n_i}$$

The numerator of (Y1) is therefore:

$$\varphi f_v + \xi g_v = (1-q_i^2)\big((1-k)n_i\big)\frac{\sigma + (1-k-\sigma)\,n_i}{2\sigma + (1-k-2\sigma)\,n_i} + q_i^2\big((1-k)n_i + m_i\big)\frac{(1-k)n_i}{(1-k)n_i + m_i}$$

$$= (1 - q_i^2)\big((1-k)n_i\big)\left[\frac{\sigma + (1-k-\sigma)n_i}{2\sigma + (1-k-2\sigma)n_i}\right] + q_i^2(1-k)n_i$$

Therefore, the following equation determines the frequency of the virulent pathogen allele at generation $i + 1$:

$$n_{i+1} = \frac{(1 - q_i^2)\big((1-k)n_i\big)\left[\frac{\sigma + (1-k-\sigma)n_i}{2\sigma + (1-k-2\sigma)n_i}\right] + q_i^2(1-k)n_i}{{q_i}^2 + n_i(({1 - q_i}^2) - k)} \quad \text{(Y2)}$$

When there is no chance for avirulent pathogen spores to mate with virulent lesions on a resistant host, then $\sigma = 0$ and our model is reduced to a form of Leonard's model, where there is no advantage of virulent race on hosts with corresponding gene for resistance, and resistance is completely effective:

$$n_{i+1} = \frac{(1 - q_i^2)\big((1-k)n_i\big) + q_i^2(1-k)n_i}{{q_i}^2 + n_i\,(({1 - q_i}^2) - k)} \equiv \frac{n_i(1-k)}{{q_i}^2 + n_i\,(({1 - q_i}^2) - k)} \quad \text{(Y3)}$$

The dynamics of the frequency of resistance:

As stated previously, we model two scenarios for the frequency of resistance. In the agricultural scenario, the frequency of resistance in the plant population is not dependent on evolutionary dynamics, but rather on the planting frequency, and therefore:

$$p_{i+1} = p_i = p_0$$

In the natural scenario, we allow the frequency of resistance in the plant population to vary as a result of the fitness of each strain; the susceptible plants have a cost of being infected (c) by both strains of the pathogen, while the resistant plants have a cost of carrying a resistant allele (d). Compared to the model described by Leonard, we assume there is no advantage of a virulent strain developing on hosts with the corresponding gene for resistance ($a = 0$) and that resistance is absolute ($t = 1$). There is random mating between hosts, so the genotype frequencies are given by RR is p_i^2, RS is $2p_iq_i$, SS is q_i^2. In this case we assume that the resistance allele is not fully dominant. We establish the equation for the frequency of resistance using the following:

$$\frac{\Sigma(\text{resistant host fitness})\times(\text{allele frequencies})}{\Sigma(\text{resistant host fitness})\times(\text{allele frequencies})+(\text{susceptible host fitness})\times(\text{allele frequencies})} \quad \text{(Y4)}$$

Host fitness is affected by the pathogen population, depending on which allele variants are present in the host. The allele frequencies of the pathogen population also affect the allele frequency of resistance. We thus describe the frequency of resistance as:

$$p_{i+1} = \frac{a}{b}$$

Where:

$$a = Fit(RR\ w.A) \times Freq(RR\ w.A) + Fit(RR\ w.V) \times Freq(RR\ w.V) + Fit(RS\ w.A) \times Freq(RS\ w.A) + Fit(RS\ w.V) \times Freq(RSw.V)$$

$$b = a + Fit(SS\ w.A) \times Freq(SS\ w.A) + Fit(SS\ w.V) \times Freq(SS\ w.V)$$

We define $Fit(XX\ w.Y)$ as the fitness of host with an XX allele pairing infected by a pathogen of genotype Y, similarly $Freq(XX\ w.Y)$ is the frequency of host with an XX allele pairing infected by a pathogen of genotype Y. Here $Fit(RR\ w.A) = Fit(RS\ w.A) = 1 - d$, $Fit(RR\ w.V) = Fit(RS\ w.V) = 1 - d - c(1-k)$, $Fit(SS\ w.A) = 1 - c$, $Fit(SS\ w.V) = 1 - c(1-k)$; $Freq(RR\ w.A) = p_i^2 \cdot m_i$, $Freq(RR\ w.V) = p_i^2 \cdot n_i$, $Freq(RS\ w.A) = 2p_iq_i \cdot m_i$, $Freq(RS\ w.V) = 2p_iq_i \cdot n_i$, $Freq(SS\ w.A) = q_i^2 \cdot m_i$ and $Freq(SS\ w.V) = q_i^2 \cdot n_i$. With substitution and simplification, the change in the resistant allele is modelled by:

$$p_{i+1} = \frac{p_i[1 - d - n_i c(1-k)]}{1 - c + n_i kc + (1 - q_i^2)[c - d - n_i c]} \quad \text{(Y5)}$$

Systems summary

We thus have two model variants for the system simulated (agricultural and natural) and two model variants simulating the classical sexual reproduction where the avirulent strains does not partake in the sexual reproduction on the resistant plant ($\sigma = 0$) and the newly discovered system where the avirulent strain does partake in the reproduction ($\sigma > 0$). The equations that describe the entirety of each model are summarised in Table S8. The model incorporating the classical genetics is identical to the model described by Leonard and the model with the newly discovered genetics will be termed the **exclusive paternal parenthood (EPP) model**. Our model results in Fig. 4a and shows that in agricultural systems, the possibility of virulent pathogen strains mating with avirulent ones reduces the rate of virulence selection when compared with the traditional Leonard's model (Leonard 1969). Moreover, we observe that for large values of σ the possibility of a polymorphism in the pathogen population can arise. This contrasts Leonard's model results where this could not occur.

Our analyses for the natural system show that the dynamics of the system can lead to stable or cyclic polymorphisms. Fig. 4b shows that in our model a stable polymorphism is obtained (solid line) whereas Leonard's model shows that although there can be internal steady states in a natural setting, these are always unstable (Tellier and Brown 2007).

The model results explain the reduction in the rate of virulence build-up and the existence of polymorphisms in agricultural systems, and the existence of stable and cyclic polymorphisms in natural systems which have been observed in other studies (Tellier and Brown 2007), but cannot be explained through Leonard's GFG model. The stable and cyclic polymorphisms occur for a wide range of parameter values. Fig. S18 shows sub-sets of parameter space that result in stable polymorphisms. The practical implication of these outcomes is that resistance of wheat to septoria tritici blotch in the field will show a 'durable' effect, being an absence of boom-and-bust phenomena and sustained longevity of resistance. This is exactly what was observed and calculated in a recent overview where the durability of resistance of various wheat pathogens was compared (Brown 2015). The current data and analyses show that this phenomenon is due to the unique ability to sexually reproduce irrespective of host resistance. Evidently, this maintains effector genes in natural populations that are by definition genetically diverse due to the heterothallic bipolar mating system and a virtual continuum of sexual reproduction (Kema et al. 1996a). Based on our model and data, we also conclude that the model confirms that when QoI sensitive strains still partake in sexual reproduction (Table 2), the mitochondrially inherited *cytb* resistance allele invades faster than any nuclear inherited fungicide resistance allele (Torriani et al. 2009), a mechanism that might well account for similar observations in the related banana pathogen *Pseudocercospora fijiensis* (Amil et al. 2007; Arango Isaza et al. 2016).

We observe that our data address a major flaw in epidemiological models and considerations (McDonald and Mundt 2016) which have GFG models as a basis, and anticipate that similar mechanisms are operational for many other Dothideomycetes. Hence, our observations have a very broad application and open a new window in disease epidemiology.

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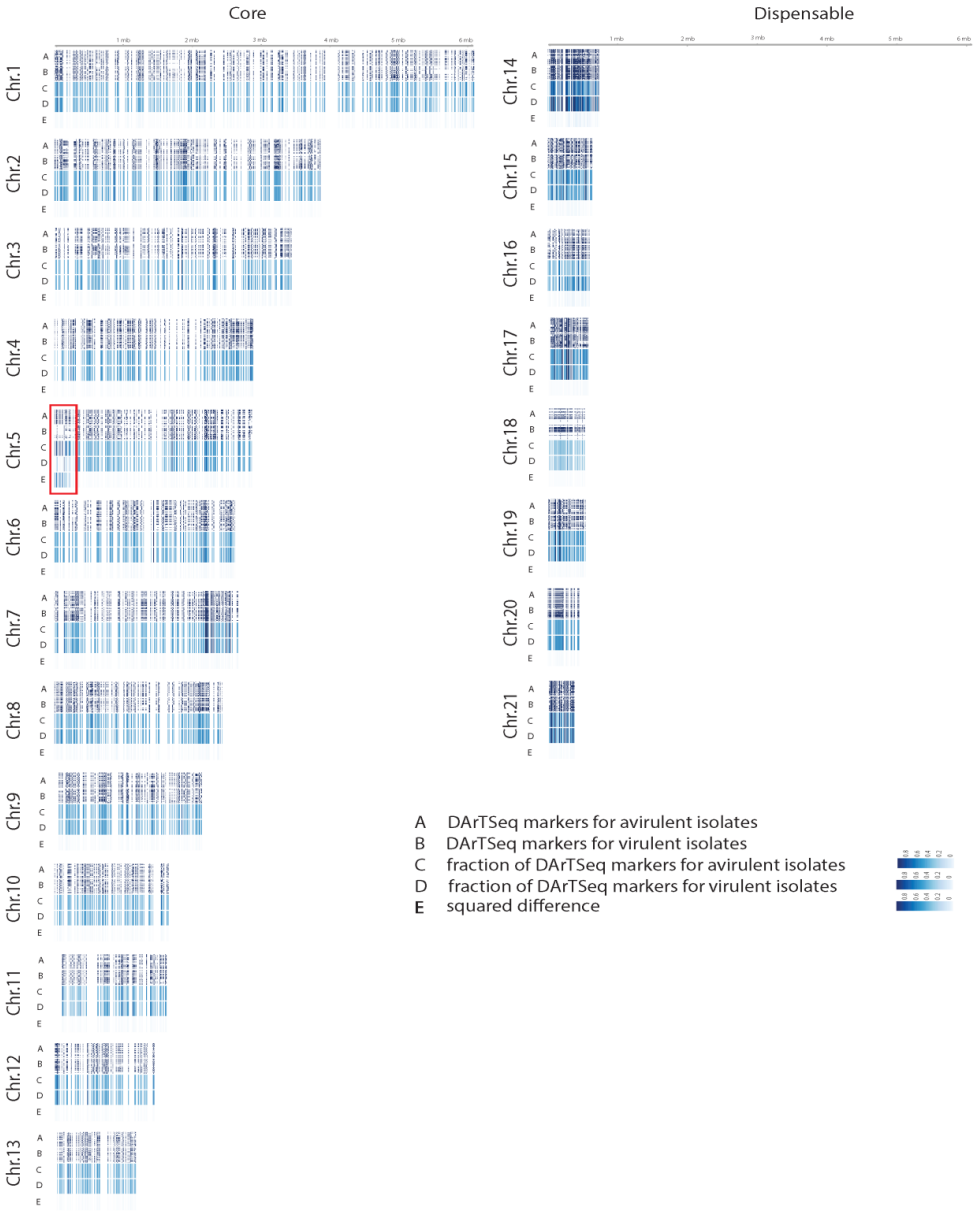
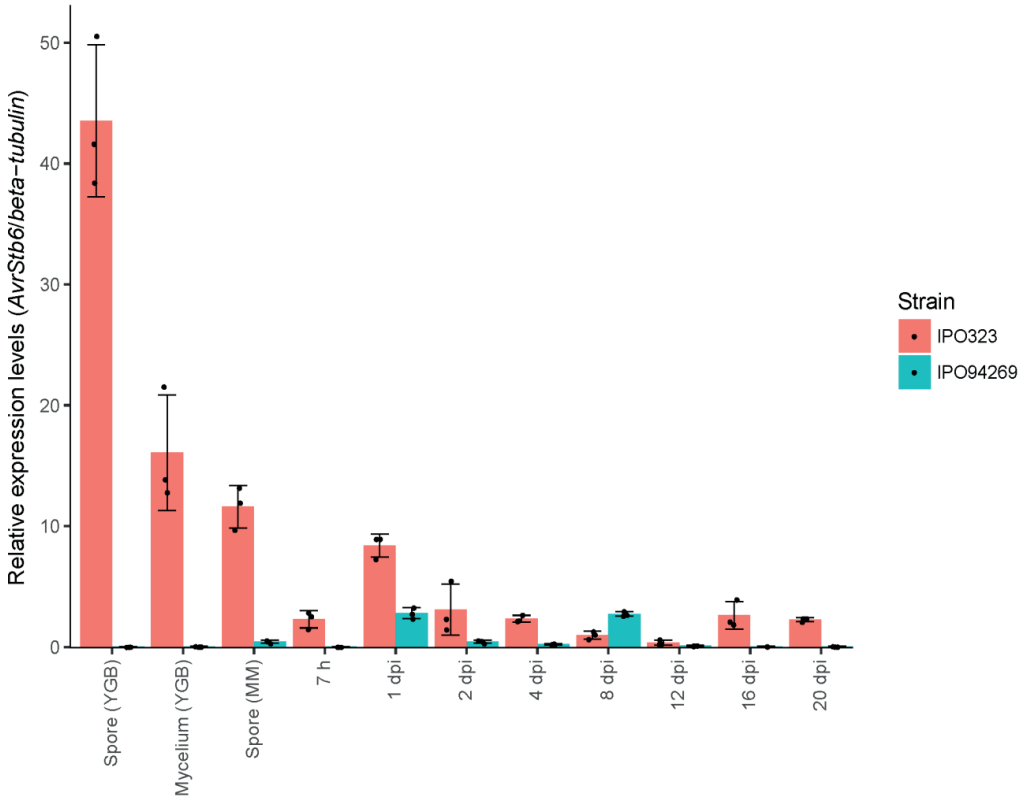
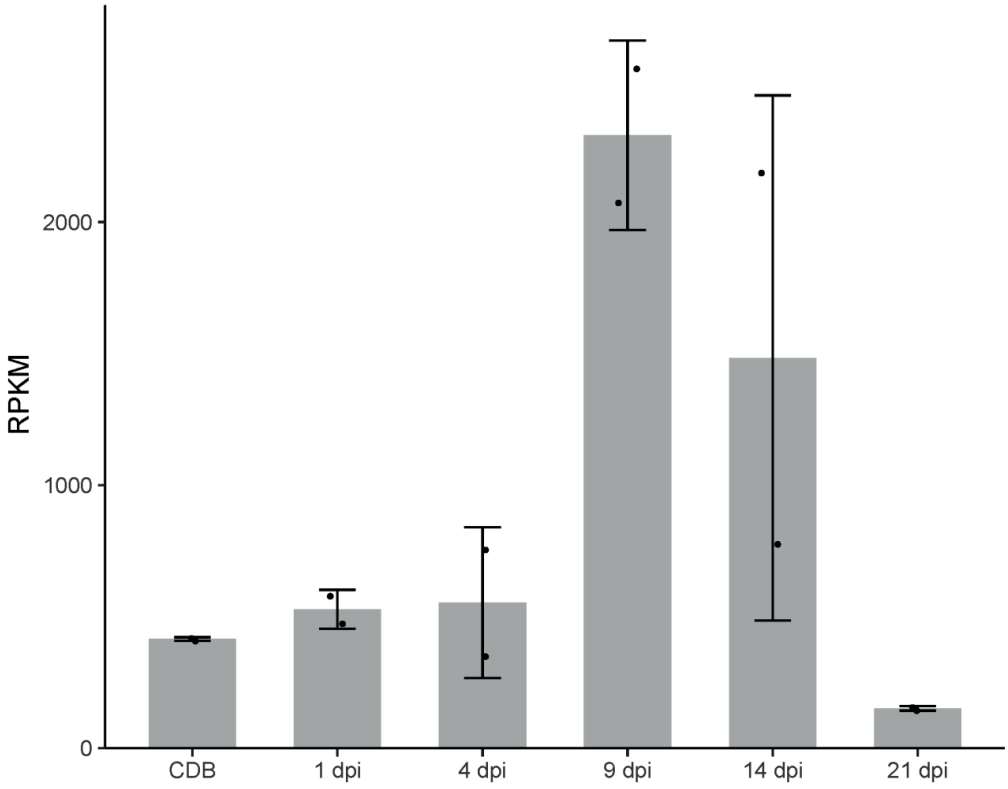


Supplementary Figure 1. Genome positions of the mapped DArT markers. Genome positions for individual DArT markers that are present (blue bars) or absent in the avirulent (A) or virulent (B) progeny (282 *Z. tritici* isolates have been genotyped of which 158 phenotyped isolates are shown) is displayed using the core chromosomes (Chr. 1-13; left) and the dispensable chromosomes (Chr.14-21; right) of *Z. tritici* IPO323 as a reference. The values of presence (1)

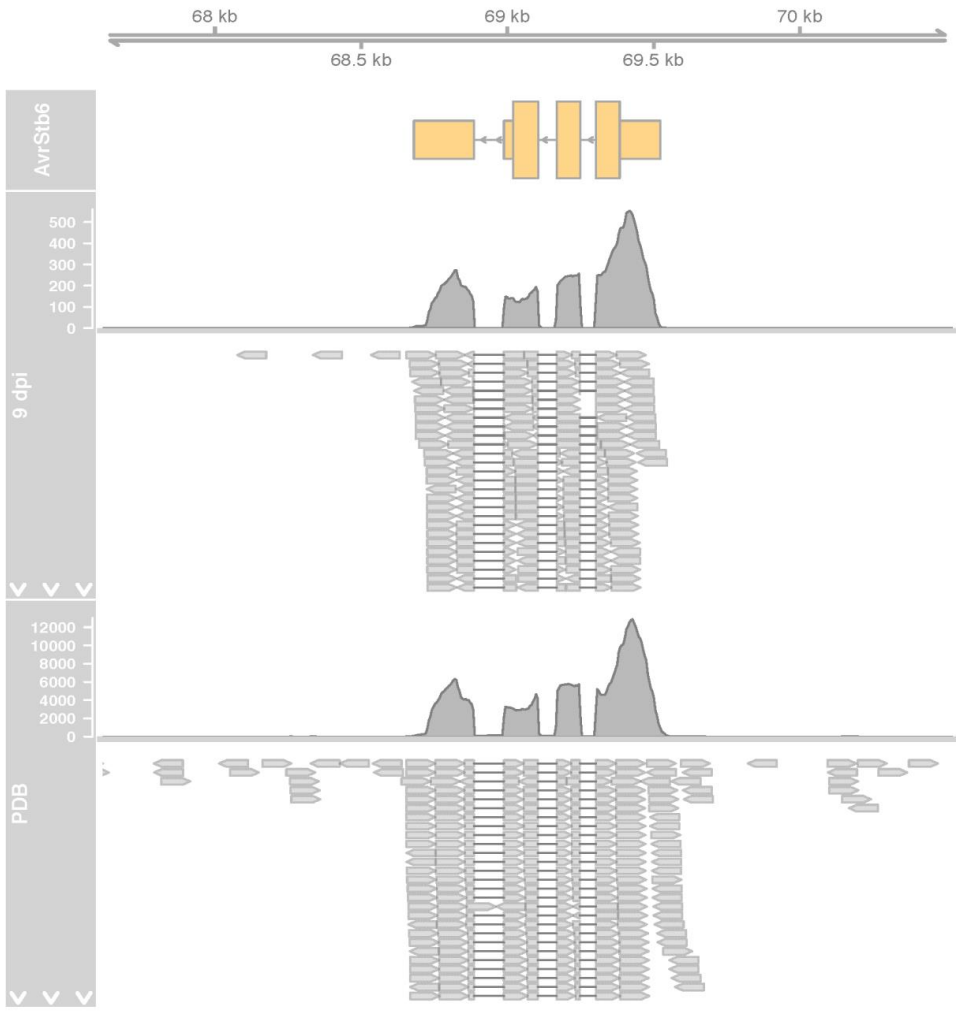
or absence (0) of individual DArT markers was summarized for all avirulent (C) or virulent (D) progeny. This was calculated by taking the fraction of DArT marker present in the avirulent or virulent progeny (range between 0 and 1). The squared difference (E) between the fractions of DArT markers present in the avirulent or virulent progeny is calculated, identifying a single polymorphic region in the genome of *Z. tritici* located on the tip of chromosome 5.



Supplementary Figure 2. *In vitro* and *in planta* expression of *AvrStb6*. For *in vitro* conditions, *AvrStb6* expression was profiled in spores and mycelium produced in either YGB or MM medium, respectively. For *in planta* expression, leaves of cv. Shafir were inoculated with the wt strains (IPO323 and IPO94269) and harvested 7 hours, 1, 2, 4, 8, 12, 16 and 20 days post-inoculation (dpi). Data were normalized with the constitutively expressed *Z. tritici* beta-tubulin gene. Bar plots display mean expression of three independent experiments; whiskers show standard deviations

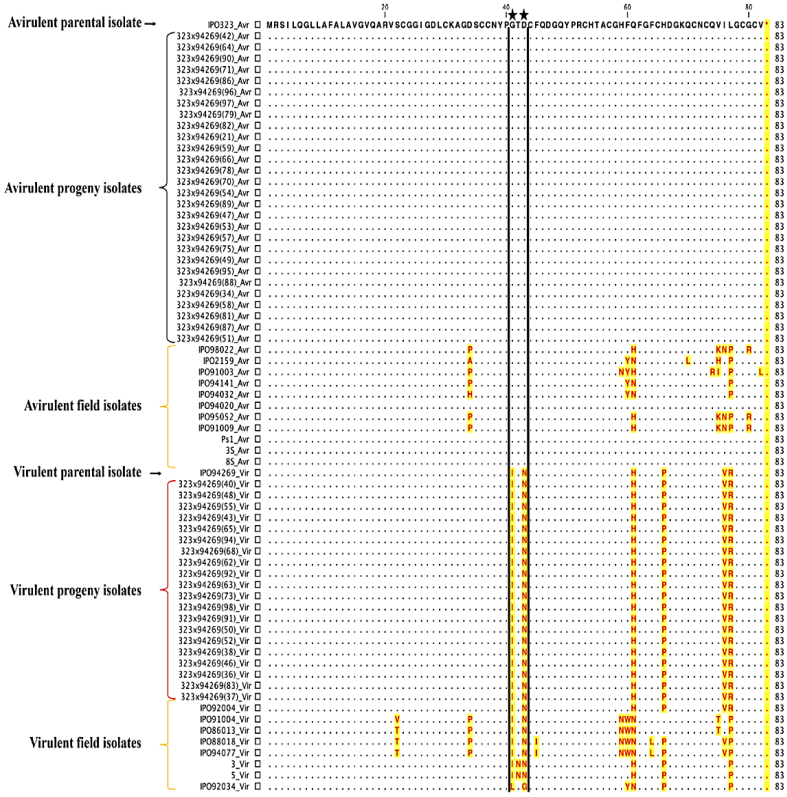


Supplementary Figure 3. Expression of *AvrStb6* as measured in an RNAseq experiment using various *in vitro* and *in planta* conditions (Rudd et al. 2015). Bar plots display the mean expression based on two independent experiments (see Online Methods); whiskers show standard deviations

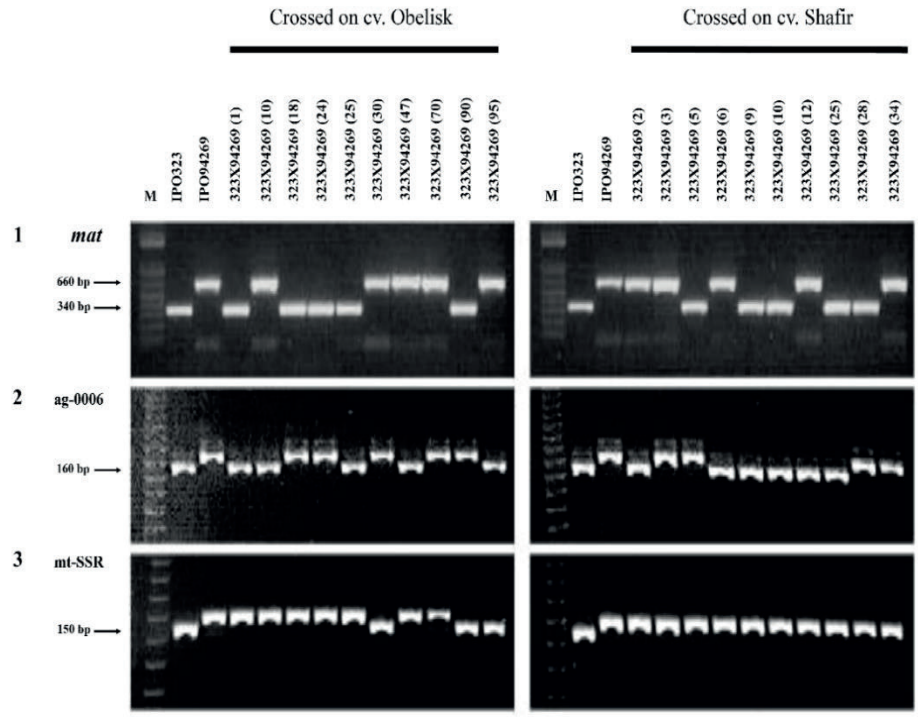


Supplementary Figure 4. Genomic location of *AvrStb6* on chromosome 5. The gene model (exon-intron structure) of *AvrStb6* is displayed. Wide bars represent the coding DNA regions, the narrow bars are the 5' and 3' untranslated regions. Mapping of RNA sequencing reads to the reference genome of *Z. tritici* IPO323 is shown as a coverage and as a read alignment track. The transcriptomic data was derived from *in planta* (9 dpi) and *in vitro* (PDB) condition, respectively (Rudd et al.,2015).

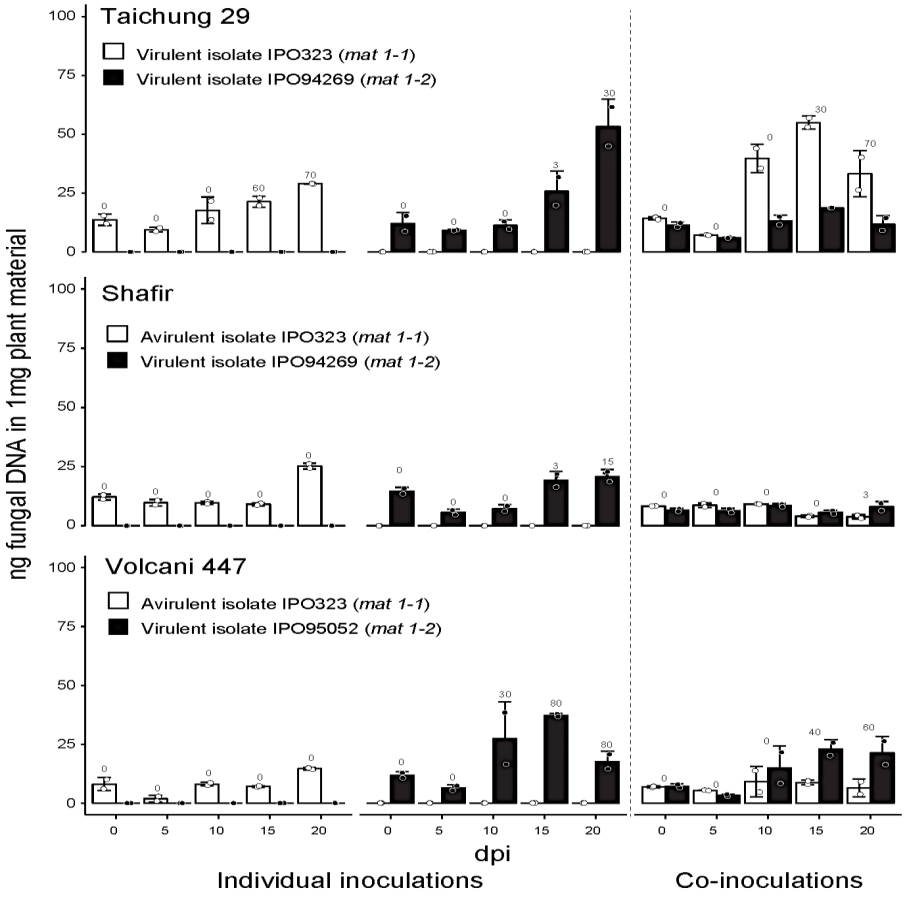
Supplementary Figure 5. The effect of *Zymoseptoria tritici AvrStb6* deletion on disease development in the wheat cv. Shafir (*Stb6*). Primary leaves were inoculated with (from left to right) *Z. tritici* IPO323 and IPO94269 (WTs), and the deletion strains IPO323Δ*AvrStb6*#14-19-33, the complemented strains IPO94269::*AvrStb6*#1-2 and the ectopic strain IPO323::*hyg* E. Experiments were triplicated and photographs were taken at 20 days post-inoculation.



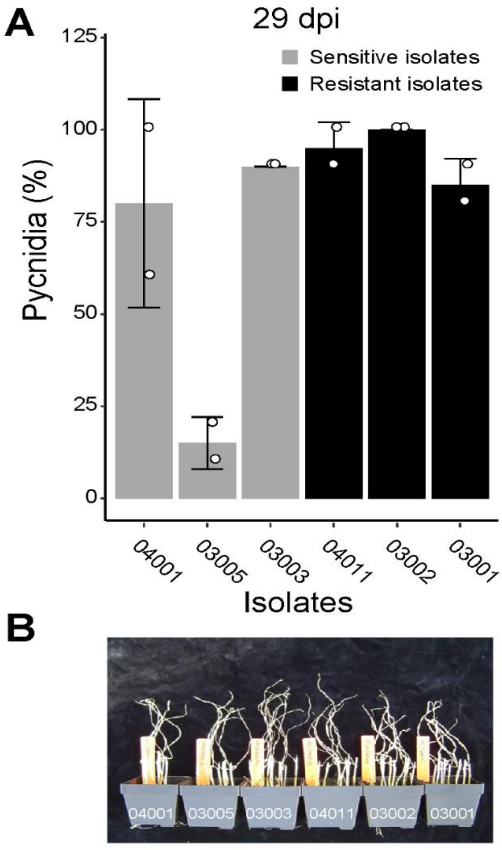
Supplementary Figure 6. Protein alignment highlighting amino acid differences between virulent and avirulent *Zymoseptoria tritici* wild type isolates and IPO323/IPO94269 progeny isolates. In the alignment amino acid substitutions are indicated, identical amino acids are denoted with "**.**".



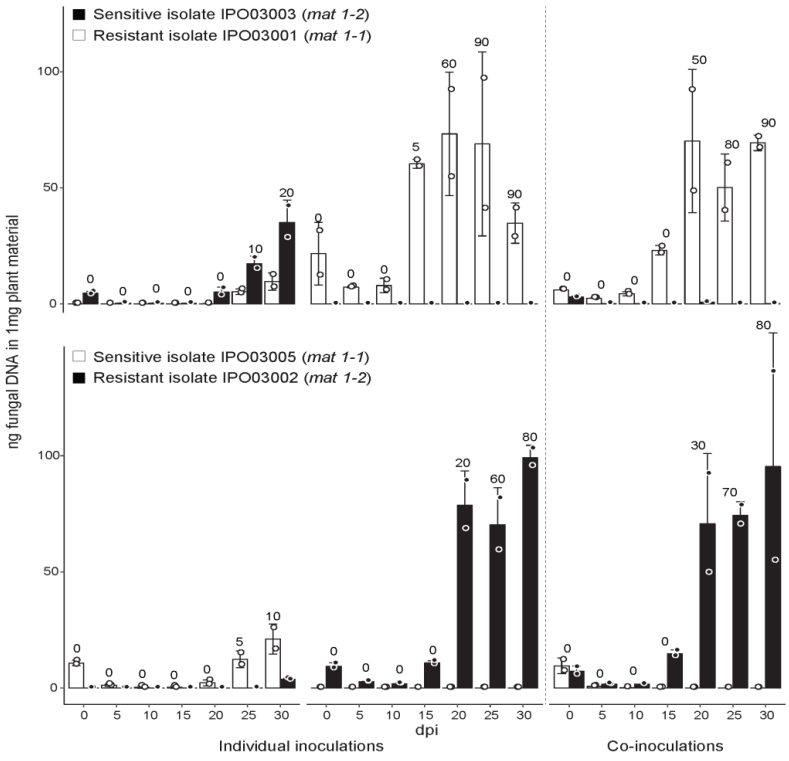
Supplementary Figure 7. Examples of segregating markers in populations that were derived from *in planta Zymoseptoria tritici* crosses. *Z. tritici* isolates IPO323 and IPO94269 crossed on the bread wheat cvs. Obelisk (left) and Shafir (right). 1, *mat*. Upper band = *mat 1-1*, lower band = *mat 1-2*. 2, ag-0006. 3, mt-SSR (see also Table1).



Supplementary Figure 8. Quantitative fungal biomass detection of *Zymoseptoria tritici* isolates IPO323 and IPO94269 on bread wheat cvs. Taichung 29 and Shafir, and isolates IPO323 and IPO95052 on durum wheat cv. Volcani 447 at 0, 5, 10, 15, and 20 dpi (bars; average of two independent experiments; whiskers indicate standard deviations) and percent leaf area covered by pycnidia at each time point (numbers above each bar).

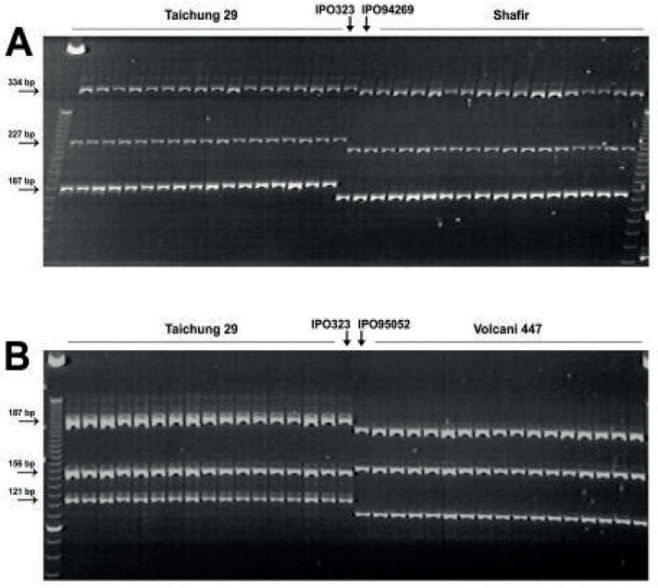


Supplementary Figure 9. Responses of seedlings of wheat cv. Taichung 29 at 29 dpi after inoculation with sensitive (04001, 03005 and 03003) or resistant (04011, 03002 and 03001) isolates of *Zymoseptoria tritici* after a pre-treatment (48h prior to inoculation) with the full recommended rate of Amistar®. (A) Percent pycnidia (leaf area covered) based on visual observations (average of two independent experiments, whiskers indicate standard deviations). (B) Overall view of seedlings.

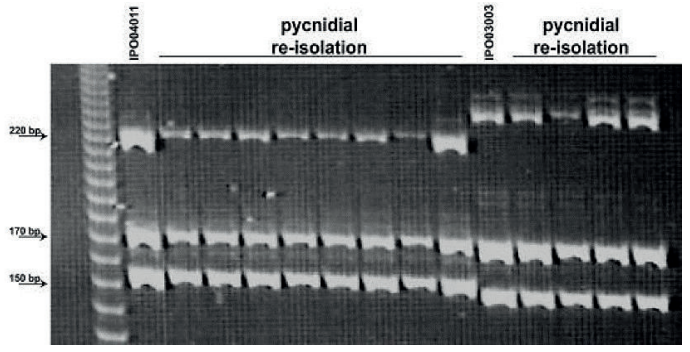


Supplementary Figure 10. Fungal biomasses and percent pycnidia of parental isolates of *Zymoseptoria tritici* inoculated individually and in mixtures on cv. Taichung 29 after preventative treatment (48h prior to inoculation) with the full recommended field rate of Amistar® at 0, 5, 10, 15, 20, 25, and 30 dpi (bars; average of two technical replicates; whiskers indicate standard deviations). Left to right, upper panel: IPO03003 (sensitive), IPO03001 (resistant), and mixture of IPO03001 and IPO03003. Lower panel: IPO03005 (sensitive), IPO03002 (resistant), and mixture of IPO03002 and IPO03005. Pycnidial percentages based on visual observations are shown above each bar (numbers above each bar).

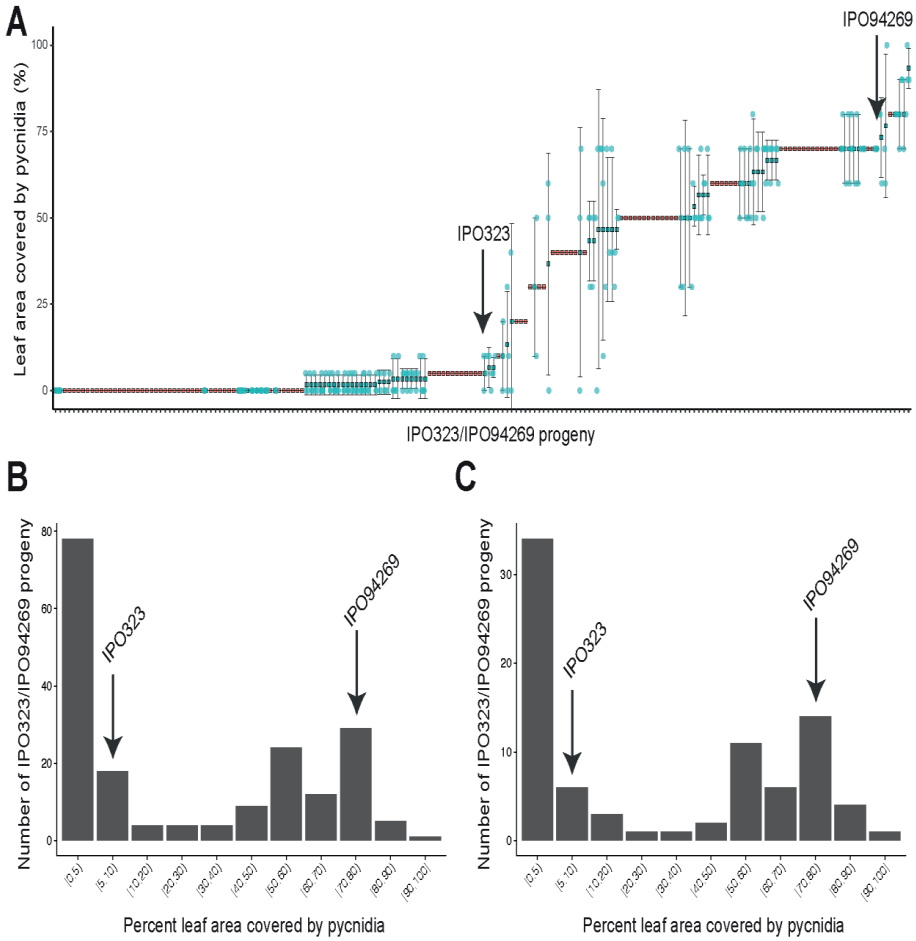
Supplementary Figure 11. Example of SSR genotyping of progeny from crosses between *Zymoseptoria tritici* strains with opposite Amistar® phenotypes (resistant and sensitive). Isolates IPO04001 (sensitive) and IPO04011 (resistant) generated off spring on wheat seedlings preventatively treated with Amistar® in doses ranging from 0-200%. Multi-plexed PCRs using the differentiating SSR marker primer sets ag-0003, tcc-0006 and tcc-0008 revealed recombinant SSR profiles in progeny.



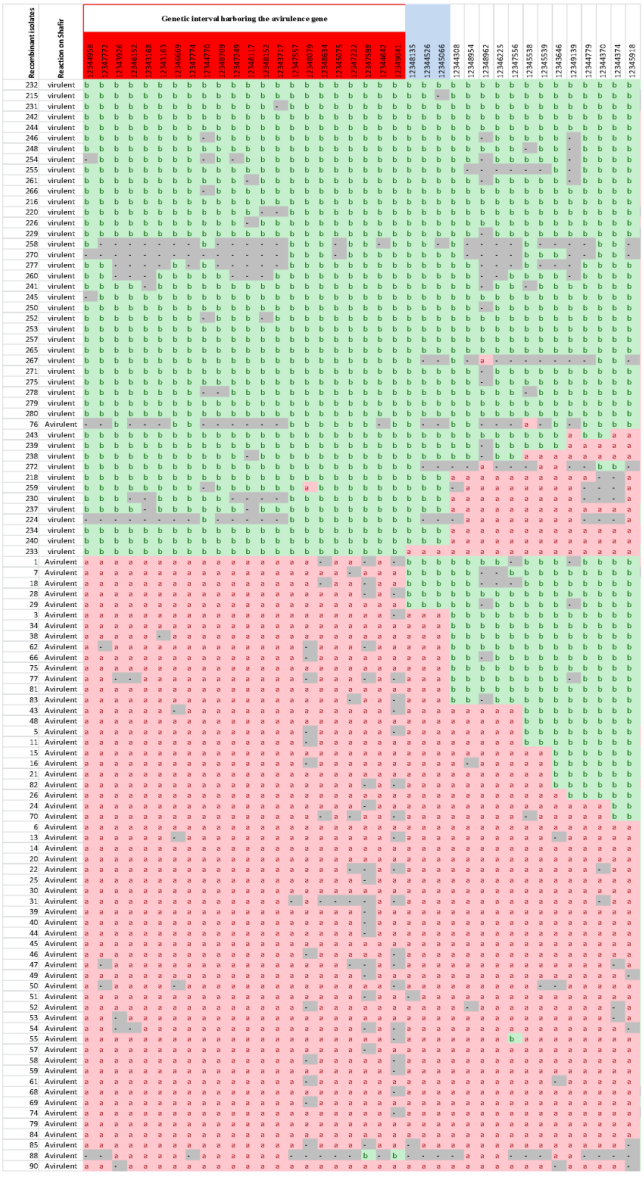
Supplementary Figure 12. SSR genoyping of re-isolations of asexual pycnidial isolates from wheat leaves that were co-inoculated with *Zymoseptoria tritici* isolates. (a) From mixtures of IPO323 and IPO94269 on cvs. Taichung 29 and Shafir using SSR markers (from top to bottom) ac-0001, ggc-0001, and caa-0002. (b) From mixtures of IPO323 and IPO95052 on cvs. Taichung 29 and Volcani 447 using SSR markers (from top to bottom) ag-0003, ag-0006, and ac-0007.



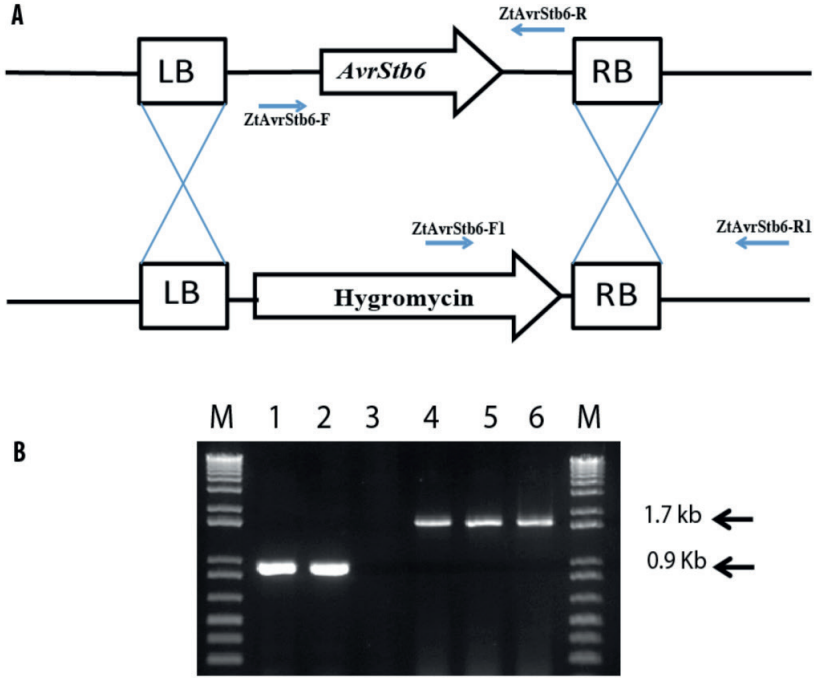
Supplementary Figure 13. SSR genotyping of pycnidial isolates of *Zymoseptoria tritici* recovered from wheat seedlings preventatively treated with Amistar® at half and full doses. All SSR patterns are clonal like the sensitive pycnidial isolates IPO04001 or IPO03003. Multi-plexed PCRs using the differentiating SSR marker primer sets ag-0003, tcc-0006 and tcc-0008 revealed no recombinant SSR patterns in recovered pycnidial isolates.



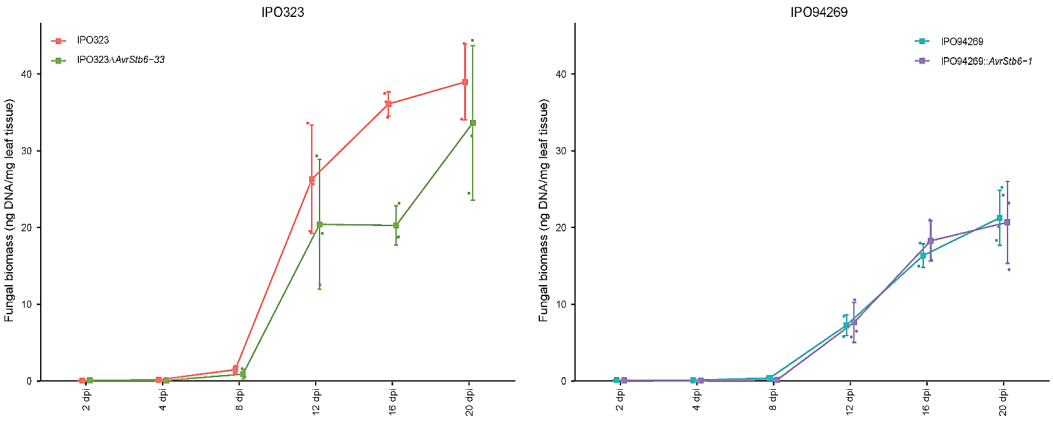
Supplementary Figure 14. Segregation of the F1 IPO323/IPO94269 progeny isolates for pycnidia development on cv. Shafir carrying the *Stb6* resistance gene at 21 days post-inoculation. (A) Average leaf area covered by pycnidia for each IPO323/IPO94269 progeny isolates and their parental isolates, is shown. Individual isolates were ordered along the x-axis based on their average leaf area covered by pycnidia. Isolates for which more than one up to three independent experiments have been performed are highlighted in green (whiskers indicate standard deviations), while isolates used in a single experiment are shown in red. Distributions of pycnidia development (median leaf area covered) for (B) all isolates and (C) isolates with >1 independent experiment are shown. Arrows indicate average parental pycnidia development on cv. Shafir.



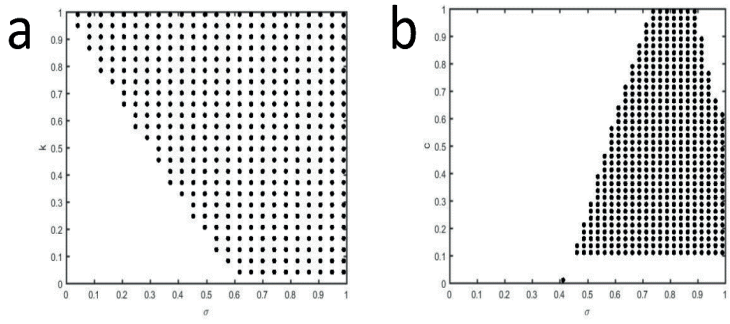
Supplementary Figure 15. Graphical genotyping of DArTseq markers and (a)virulence to 'Shafir'. By sorting the progeny isolates from the F1-population from IPO323 x IPO94269 according to the positions of recombination events on chromosome 5 of *Zymoseptoria tritici*, the avirulence gene *AvrStb6* could be positioned between the blue flanking markers as shown on the picture. The isolates are haploid, and therefore have either the maternal locus from the avirulent parent or the paternal locus from the virulent parent. Red color indicates loci inherited from the avirulent parent IPO323, whereas loci inherited from the virulent parent IPO94269 are displayed in green. Grey dashes indicate missing values. The yellow markers fully co-segregated with the (a)virulence. We only display the isolates that showed recombination near the (a)virulence locus.



Supplementary Figure 16. Replacement strategy for *AvrStb6* in *Zymoseptoria tritici*. (a) Diagram showing the replacement by the hygromycin phosphotransferase (*hph*) resistance cassette through homologous recombination. The dotted line depicts the flanking regions used for homologous recombination. (b) Identification of replacement mutants by PCR; Lane M, 1-kb-plus ladder. The gel shows the correct amplification (900 bp), using primers ZtAvrStb6-F and ZtAvrStb6-R, of *AvrStb6* in IPO323 (Lane 1) and the ectopic strain IPO323E*AvrStb6*#2 (Lane 2), but no amplification in the mutant strain IPO323Δ*AvrStb6*#33 (Lane 3). Amplification with primers ZtAvrStb6-F1 and ZtAvrStb6-R1, which are located in the middle of the *hph* gene and downstream of *AvrStb6,* respectively, result in a 1.7 kb amplicon in the three independent replacement mutants IPO323Δ*AvrStb6*#14, IPO323Δ*AvrStb6*#19 and IPO323Δ*AvrStb6*#33.



Supplementary Figure 17. Fungal biomass quantifications of *Zymoseptoria tritici* in the susceptible wheat cv. Taichung 29 at 2, 4, 8, 12, 16 and 20 days post inoculation. A, Fungal biomass comparison of *Z. tritici* IPO323 (wt) and the knock-out strain IPO323Δ*AvrStb6*-33, B, Fungal biomass comparison of *Z. tritici* IPO94269 (wt) and the *AvrStb6* random integration strain IPO94269::*AvrStb6*-1. Squares display average fungal biomass based on three independent experiments, whiskers indicate standard deviations.



Supplementary Figure 18. (a) The parameter space σ vs k. The dotted area of the graph shows the pair of (σ,k) parameters where the internal equilibrium point is stable. In this plot, the fitness cost to the plant by being infected was 0.2, the fitness cost to the plant of resistance was 0.1. (b)The parameter space σ vs c. The dotted area of the graph shows the pair of (σ,c) parameters where the internal equilibrium point is stable. In this plot, the fitness cost to the pathogen of being virulent was 0.2, the fitness cost to the plant of resistance was 0.1

Supplementary 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.

	Stb	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Stb*	*Tm*	References
Bulgaria 88[a]	■					■																(Adhikari et al. 2004c)
Veranopolis[a]		■				■																(Liu et al. 2013)
Israel 493[a]			■			■																(Goodwin and Thompson 2011)
Tadinia[a]				■		■																(Adhikari et al. 2004a; Somasco et al. 1996)
CS/synthetic(6x) 7D					■																	(Arraiano et al. 2001b)
Shafir						■																(Brading et al. 2002)
Estanzuela Federal							■															(McCartney et al. 2003)
M6 Synth(w7984)								■														(Adhikari et al. 2003)
Courtot									■													(Chartrain et al. 2009)
Kavkaz - K45002						■	■			■		■										(Chartrain et al. 2005a)
TE9111[b]						■	■				■											(Chartrain et al. 2005c)
Salamouni													■	■		■						(Cowling 2006; Cuthbert 2011)
Arina[a]						■									■							(Arraiano et al. 2007)
M3 (Synthetic)																	■	■				(Tabib Ghaffary et al. 2012)
Balance						■													■			(Tabib Ghaffary et al. 2011)
WW1842, WW2449, WW2451																				■		(Raman et al. 2009)
MDR043 (*T. monococcum*)																					■	(Jing et al. 2008)

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

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Supplementary Table 2. Summary information about the *Zymoseptoria tritici* isolates that were used in the *in planta* crossing protocol on wheat cultivars with various levels of resistance.

Isolate	Year	Origin	Host	Virulent on	Mating type
IPO001	unknown	Netherlands	Bread Wheat[1]	Bread Wheat	*mat 1-1*
IPO323	1981	Netherlands	Bread Wheat	Bread Wheat	*mat 1-1*
IPO87019	1987	Uruguay	Bread Wheat	Bread Wheat	*mat 1-2*
IPO88004	1988	Ethiopia	Durum Wheat	Durum Wheat	*mat 1-2*
IPO94269	1994	Netherlands	Bread Wheat	Bread Wheat	*mat 1-2*
IPO95054	1995	Algeria	Bread Wheat	Bread Wheat	*mat 1-2*
IPO95050	1995	Algeria	Durum Wheat	Durum Wheat	*mat 1-1*
IPO95052	1995	Algeria	Durum Wheat	Durum Wheat	*mat 1-2*

[1]derived from a hexaploid derivative of a cross between bread wheat and wild emmer wheat (*T. dicoccoïdes*, AABB, 2n=28).

Supplementary Table 3. Crosses between isolates of *Zymoseptoria tritici* using an *in planta* protocol of co-inoculation of two strains with opposite mating types on both bread wheat and durum wheat cultivars and on barley. V = virulent; A = avirulent.

Isolates Crossed[1]	Cultivar	Species	Progeny
bread wheat strains			
IPO323 (V) x IPO94269 (V)	Obelisk	Bread Wheat	yes
IPO323 (V) x IPO94269 (V)	Taichung 29	Bread Wheat	yes
IPO323 (A) x IPO94269 (V)	Shafir	Bread Wheat	yes
IPO323 (A) x IPO94269 (A)	Volcani 447	Durum Wheat	no
IPO323 (A) x IPO94269 (A)	Topper 33	Barley	no
IPO001 (V) x IPO94269 (V)	Obelisk	Bread Wheat	yes
IPO001 (V) x IPO94269 (A)	Lakhish	Bread Wheat	yes
IPO001 (A) x IPO94269 (V)	Clement	Bread Wheat	yes
IPO323 (A) x IPO87019 (A)	Kavkaz-K4500	Bread Wheat	no
IPO323 (A) x IPO88004 (A)	Veranopolis	Bread Wheat	no
durum wheat strains			
IPO95050 (V) x IPO95052 (V)	Volcani 447	Durum Wheat	yes
IPO95050 (A) x IPO95052 (A)	Obelisk	Bread Wheat	no
bread wheat x durum wheat strains			
IPO323 (A) x IPO95052 (V)	Inbar	Durum Wheat	yes
IPO323 (V) x IPO95052 (A)	Obelisk	Bread Wheat	yes
IPO323 (A) x IPO95052 (A)	Shafir	Bread Wheat	no
IPO94269 (A) x IPO95050 (V)	Inbar	Durum Wheat	yes
IPO94269 (V) x IPO95050 (A)	Obelisk	Bread Wheat	yes
IPO95054 (A) x IPO95050 (V)	Inbar	Durum Wheat	yes
IPO95054 (V) x IPO95050 (A)	Obelisk	Bread Wheat	yes

[1]Mutiple crosses, on at least two pots with wheat seedlings. The number of ascospores retrieved per cross was not counted or estimated but is usually >1,000. We only observed significant reductions in progeny size in crosses between *Z. tritici* isolates with reduced numbers of dispensable chromosomes, but this is not addressed in the current paper.

Supplementary Table 4. DArT Seq genotyping of the *Zymoseptoria tritici* IPO323 x IPO94269 mapping population. (Please refer to the online version: (https://www.nature.com/articles/s41588-018-0052-9#Sec4) or at doi:10.1038/s41588-018-0052-9)).

Supplementary Table 5. Wild type *Zymoseptoria tritici* isolates (shaded) and progeny isolates from crosses between IPO323 and IPO94269 on different wheat cultivars and their host specificity, phenotypes on cv. Shafir that carries *Stb6,* and the mutations in *AvrStb6*

solate number	Origin	Host specificity	Avirulent/virulent on cv. Shafir (*Stb6*)	1st/2nd mutation (amino-acid)
IPO323	The Netherlands	Bread wheat	avirulent	GGG (Glycine)/GAC (Aspartic acid)
IPO95052	Algeria	Durum wheat	avirulent	GGG (..)/GAC (..)
IPO94141	The Netherlands	Bread wheat	avirulent	GGG (..)/GAC (..)
IPO94020	The Netherlands	Bread wheat	avirulent	GGG (..)/GAC (..)
IPO2159	Iran	Bread wheat	avirulent	GGG (..)/GAC (..)
IPO98022	France	Bread wheat	avirulent	GGG (..)/GAC (..)
IPO94032	The Netherlands	Bread wheat	avirulent	GGG (..)/GAC (..)
IPO91009	Tunisia	Durum wheat	Not tested	GGG (..)/GAC (..)
IPO91004	Syria	Durum wheat	Not tested	ATA (Isoleucine)/AAC (Asparagine)
IPO91003	Syria	Durum wheat	Not tested	GGG (Glycine)/GAC (Aspartic acid)
8S	Iran	Isolate from *Phalaris minor*	Not tested	GGG (..)/GAC (..)
3S	Iran	Isolate from *P. minor*	Not tested	GGG (..)/GAC (..)
Ps1	Iran	Isolate from *P. paradoxa*	Not tested	GGG (..)/GAC (..)
IPO94269	The Netherlands	Bread wheat	virulent	ATA (Isoleucine)/AAC (Asparagine)
5	USA	Bread wheat	virulent	ATC (..)/AAC (..)
3	USA	Bread wheat	virulent	ATC (..)/AAC (..)

IPO86013	Turkey	Bread wheat	virulent	ATA (..)/AAC (..)
IPO92034	Algeria	Bread wheat	virulent	TTG (Leucine)/GGC (Glycine)
IPO94077	The Netherlands	Bread wheat	virulent	ATA (Isoleucine)/AAC (Asparagine)
IPO88018	Ethiopia	Bread wheat	virulent	ATA (..)/AAC (..)
IPO92004	Portugal	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(86T)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (Glycine)/GAC (Aspartic acid)
323x94269(96T)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(97)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(82T)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(78)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(70)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(89)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(47)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(57)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(49)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(95)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(88)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(90 T)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(34)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)

323x94269(58)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(81)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(87)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(51)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(21)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(66)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(71T)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(75)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(54)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(53)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(64T)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(42)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(79)	Progeny from IPO323/IPO94269[1]	Bread wheat	avirulent	GGG (..)/GAC (..)
323x94269(59)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (Isoleucine)/AAC (Asparagine)
323x94269(48)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(55)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x9426(46)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(36)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(43)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)

323x94269(65)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(37)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(40T)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(94T)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(68)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(62)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(92)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(63)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(73)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(83)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(98)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(91)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(50)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(52)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(38)	Progeny from IPO323/IPO94269[1]	Bread wheat	virulent	ATA (..)/AAC (..)
323x94269(21)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (Glycine)/GAC (Aspartic acid)
323x94269(34)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(42)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(47)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)

323x94269(49)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(54)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(57)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(58)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(64S)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(70)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(71S)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(75)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(78)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(79)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(81)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(82S)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(84)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(86S)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(87)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(88)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(89)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(90S)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(95)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)

323x94269(96S)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (..)/GAC (..)
323x94269(36)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (Isoleucine)/AAC (Asparagine)
323x94269(37)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(38)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(40S)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(43)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(46)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(48)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(50)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(52)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(55)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(59)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(62)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(63)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(65)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(66)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(68)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(73)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(83)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)

323x94269(91)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(92)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(98)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (..)/AAC (..)
323x94269(51)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ACT (Threonine)/CTT (Leucine)
323x94269(53)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (Glycine)/GAT (Aspartic acid)
323x94269(56)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	CCT (Leucine)/ACC (Threonine)
323x94269(93)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	GGG (Glycine)/GGA (Glycine)
323x94269(94S)	Progeny from IPO323/IPO94269[2]	Bread wheat	Not tested	ATA (Isoleucine)/AAA (Lysine)

[1]Progeny derived from a cross between *Z. tritici* isolates IPO323 and IPO94269 on the susceptible cvs. Taichung or Obelisk; [2]Progeny derived from a cross between *Z. tritici* isolates IPO323 and IPO94269 on the differential cv. Shafir.

Supplementary Table 6. Summary information about the *Zymoseptoria tritici* isolates used in the *in planta* crossing protocol under various levels of preventative strobilurin applications.

Pycnidial isolate	Location	Year	Strobilurin application	Strobilurin phenotype	Mating type
IPO03001 (BCS3R)[a]	Germany	2003	unknown	Resistant	*mat1-1*
IPO03002 (BCS8S)[b]	Germany	2003	unknown	Resistant	*mat1-2*
IPO03003 (BCS16S)	Germany	2003	unknown	Sensitive	*mat1-2*
IPO03005 (BCS17S)	Germany	2003	unknown	Sensitive	*mat1-1*
IPO04001	Netherlands	2004	No	Sensitive	*mat1-1*
IPO04011	Netherlands	2004	No	Resistant	*mat1-2*
IPO323 (reference)	Netherlands	1981	No	Sensitive	*mat1-1*
IPO94269 (reference)	Netherlands	1994	No	Sensitive	*mat1-2*

[a] Isolate code from Bayer CropScience; [b]Phenotype of isolate was mislabeled. Sequence information and additional Phenotyping confirmed its resistance to strobiluri

Supplementary Table 7. Probes and primers for *Zymoseptoria tritici* used in this study

Name	Sequence (5' to 3')
MAT1-1 F	CCGCTTTCTGGCTTCTTCGCACTG
MAT1-1 R	TGGACACCATGGTGAGAGAACCT
MAT1-2 F	GGCGCCTCCGAAGCAACT
MAT1-2 R	GATGCGGTTCTGGACTGGAG
StrobSNP2fwd	CTTATGGTCAAATGTCTTTATGATG
StrobSNP1rvs	GGTGACTCAACGTGATAGC
StrobSNPrcF7	CAATAAGTTAGTTATAACTGTTGCGG
StrobSNPrcR1	CTATGCATTATAACCCTAGCGT
Mmat1P3	FAM- CGCAGTCTGCTTTGAATGAGAAGTTATC –Darquencher
Mmat1F3	GGCATTTCGCAGTATGTG
Mmat1R3	CTGCGCATTTCTCGTC
Mmat2P4	YY- CCTCGCAAGCCATCGGAGA -Darquencher
Mmat2F7	GCATCCGGGATACCAGTA
Mmat2R7	CTTGGTCATGCGACGTT
ag-0003 F	ACTTGGGGAGGTGTTGTGAG
ag-0003 R	ACGAATTGTTCATTCCAGCG
gca-0004 F	TAACGGTAACGGCAACAACC
gca-0004 R	GTGTACCCTTGAATCGCAGC
tcc-0008 F	AAAAGACATGACGCCCGAC
tcc-0008 R	ACGAGGAATAATCGCGGAAC
ag-0006 F	TAACCAACACCAGGGGAATG
ag-0006 R	CATCAGTTGTCAGCGAATGG
ag-0009 F	GACTCCATTTACCTGTGGCG
ag-0009 R	TGTGAAGGACACGCAAAGAG
tcc-0006 F	ATCTGGACACCATCCACCAG
tcc-0006 R	GTAGGTGGGAGGGTTCATGC
ac-0001 F	CACCACACCGTCGTTCAAG
ac-0001 R	CGTAAGTTGGTGGAGATGGG
ggc-0001F	GATACCAAGGTGGCCAAGG
ggc-0001R	CACGTTGGGAGTGTCGAAG
caa-0002 F	TCTGCAGAGATCCCGTTACC
caa-0002 R	ATCCATCACATGACGCACAC
ac-0007 F	TGCTCGCAAGACATAAAACG
ac-0007 R	CTCTTAGCATTGGTCGGTGG
ZtAvrSt6-F	TTCCACACTTCTTTCCACAACTCC
ZtAvrSt6-R	CATGCAATGGAGGTATGTATGGG
ZtAvrStb6-F1	GTACACTTGTTTAGAGGTAATCCTTC
ZtAvrStb6-R1	GTCGTCGTCGTCGCAATTGATAA
Q- ZtAvrSt6-F	TTCCAGGACGGGCAATATC
Q- ZtAvrSt6-R	AGCCACAACCAAGAATGACC
Mt-SSR-F	CTCAGTTCAAGTCTGAGTGC
Mt-SSR-R	GACGCACGCATTTCCACTCTA

Supplementary Table 8. Natural and agricultural systems for Leonard's model and EEP model

	Leonard's model	EEP model
Natural system	$n_{i+1} = \frac{n_i(1-k)}{{q_i}^2 + n_i((1-{q_i}^2)-k)}$ $p_{i+1} = \frac{p_i[1-d-n_i c(1-k)]}{1-c+n_i kc+(1-{q_i}^2)[c-d-n_i c]}$	$n_{i+1} = \frac{(1-q_i^2)(1-k)n_i\left[\frac{\sigma+(1-k-\sigma)n_i}{2\sigma+(1-k-2\sigma)n_i}\right]+q_i^2(1-k)n_i}{{q_i}^2+n_i((1-{q_i}^2)-k)}$ $p_{i+1} = \frac{p_i[1-d-n_i c(1-k)]}{1-c+n_i kc+(1-{q_i}^2)[c-d-n_i c]}$
Agricultural system	$n_{i+1} = \frac{n_i(1-k)}{q^2 + n_i((1-q^2)-k)}$ $q_{i+1} = q_i = q$	$n_{i+1} = \frac{(1-q^2)(1-k)n_i\left[\frac{\sigma+(1-k-\sigma)n_i}{2\sigma+(1-k-2\sigma)n_i}\right]+q^2(1-k)n_i}{q^2+n_i((1-q^2)-k)}$ $q_{i+1} = q_i = q$

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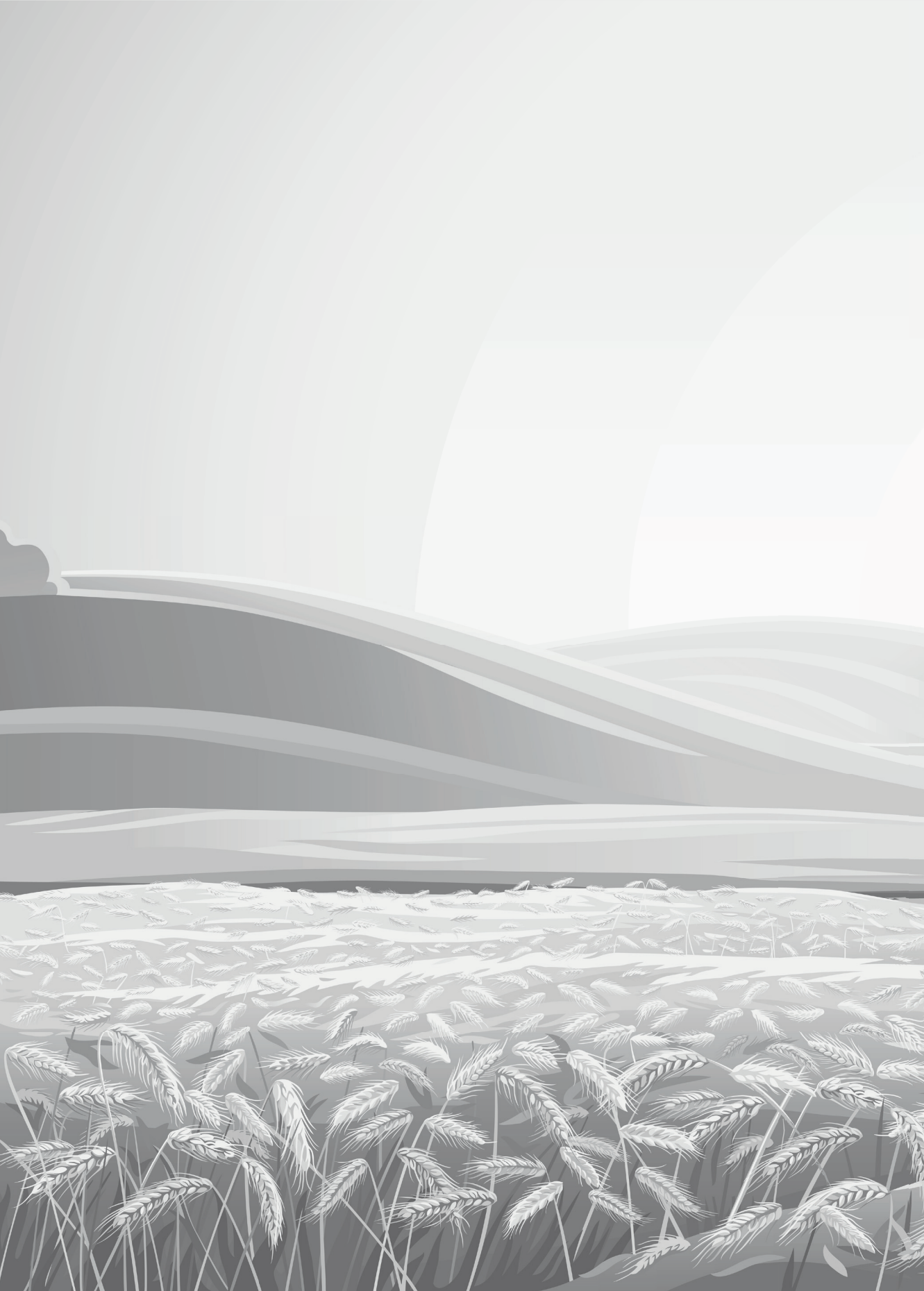

Chapter 3

Broad spectrum resistance to Zymoseptoria tritici in the tetraploid emmer wheat accession PI41025

Lamia Aouini, Justin Faris, Ruud F. Grootens, Richard G.F. Visser, Steven S. Xu, Gerrit H.J. Kema

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Abstract

Durum wheat varieties are notoriously susceptible to *Zymoseptoria tritici* (*Z. tritici*), the septoria tritici blotch (STB) fungus. This might be one of the reasons why research into the *Z. tritici* - wheat pathosystem has almost entirely focused on bread wheat. The identified *Stb* resistance genes in bread wheat, however, do not protect durum wheats from STB, due to the pathogenic dichotomy of *Z. tritici* to tetraploid and hexaploid wheat germplasm. Thus far, no *Stb* genes have been identified or mapped in durum wheat. Here, we describe the broad efficacy of resistance to STB in the tetraploid emmer wheat accession PI41025 to a panel of 31 diverse *Z. tritici* isolates originating from durum wheat. A recombinant inbred population was developed by crossing PI41025 with the susceptible durum wheat cv. Ben. The phenotyping of this population resulted in the identification and mapping of the first resistance gene in tetraploid wheat. We designated this gene as *Stb22q,* which is located on chromosome 3AL. A minor QTL on chromosome 5A provides partial resistance to one of the used *Z. tritici* isolates. Despite its efficacy, *Stb22q* does not protect PI41025 from necrosis development, a phenomenon that is commonly observed in durum wheats, but remains to be elucidated. Nonetheless, *Stb22q* is a valuable resource for STB resistance breeding in durum wheat.

Key words: Emmer wheat, durum wheat, *Zymoseptoria tritici, Stb22q*, resistance gene

Introduction

Wheat is a staple food throughout the ages and has been crucial for the development of mankind (Faris 2014; Tadesse et al. 2016) and was first cultivated about 12,000 years ago in the Fertile Crescent, where emmer wheat (*T. turgidum* ssp. *dicoccum*) was domesticated from its wild ancestor *Triticum turgidum* ssp. *dicoccoïdes* (or wild emmer) (Ren et al. 2013). Although, we lack accurate archaeological records (Nesbitt and Samuel 1998; Zeder et al. 2006; Zohary et al. 2012), it is likely that the transition from the hunter-gatherer lifestyle to primitive forms of agriculture were accompanied by the selection and cultivation of altered populations of wild grasses into domesticated varieties of wheat, which were characterized by anatomical and morphological changes oriented towards new environments (Charmet 2011; Stukenbrock et al. 2006). The major morphological features that drove wheat domestication were the mutations in three major genes during wheat evolution that ultimately generated fully domesticated wheats with non-brittle rachis preventing shattering and allowing farmers to harvest entire heads. These genes are the *Br*, *Tg* and *q* genes which confer brittle rachis, tenacious glume, and a hulled seed, respectively (Avni et al. 2017; Dubcovsky and Dvorak 2007). In addition, ongoing selection also resulted in larger and more plump seeds, loss of seed dormancy and improved grain quality, which resulted in of the transition of cultivated emmer wheat into fully-domesticated landraces of durum wheat (Faris 2014).

The first free-threshing tetraploid wheats occurred between 8,000 - 9,000 BC in the Damascus basin in Southern Syria. Durum wheat first appeared in Can Hassan III, Turkey 6.200-7500 years ago, and disseminated later in the eastern Mediterranean, replaced emmer wheat and developed into the major cultivated form of allotetraploid wheat by the second millennium BC (Dvorak et al. 2011; Ren et al. 2013). The geographical expansion of durum wheat intimately followed human migration (Baloch et al. 2017). It was initially introduced to Europe and North Africa during the Neolithic period, but remained most preferred in the western Mediterranean (Zapata et al. 2004). By the end of the 15th century, durum wheat spread out in Europe after the replacement of the staple emmer in Ancient Egypt by durum wheat during the Hellenistic Period (Moragues et al. 2007; Ren et al. 2013); and from there to the 'new world' when Europeans touched the shores of the Americas across the Atlantic in 1492 (Capparelli et al. 2005) .

Hereafter, many durum wheat-derived dishes developed and became a trade mark of the Mediterranean diet. Nevertheless, dishes such as “pasta” and “couscous” were most likely introduced to the Mediterranean. A common hypothesis is that Marco Polo brought noodles or noodle recipes to Italy from China in the 13th century, where it developed into pasta. However, pasta was already gaining popularity in other areas of Italy during the 13th century, making it very unlikely that Marco Polo introduced these dishes to Italy (Sher 2015). Cous-cous is a staple in the North African “Maghreb region “where it was the ideal food for nomadic tribes. Charles de Clairambault, a naval commissioner from Brittany, reported a Moroccan delicacy in a letter dated January 12, 1699, but the traveler Jean-Jacques Bouchard described already in a letter from Toulon, Provence, France, in 1630 a “certain kind of pasta which is made of little grains like rice, and which puffs up considerably when cooked” (Wright 1999).

Nowadays, durum wheat accounts for 5% of the cultivated wheat acreage, mainly concentrated in the marginal areas of Mediterranean region, the Norther Great Plains of the U.S., Southern Europe, and North Africa, and more recently in Southern Asia (Baloch et al. 2017; Faris 2014) . It plays a key role in traditional farming practices and is a staple food and the basis for many typical dishes in the Mediterranean region, which represents approximately 75% of the global durum wheat production area (Zapata et al. 2004). Despite its limited geographical distribution when compared to the hexaploid bread wheat, which represents 95% of the global wheat acreage (Shewry 2009), durum wheat has provided a range of sub-species that were widely cultivated across the globe for thousands of years, and is therefore considered to be a major contributor to the current wheat diversity (Feuillet et al. 2008; Newton et al. 2010). Nonetheless, as in many other crops, domestication and intensive selection have reduced the genetic diversity of durum wheat. Emmer wheat and durum landraces became almost extinct due to the introduction and wide adoption of semi-dwarf so-called ‘elite’ germplasm during the Green Revolution (Serrão et al. 2016). Albeit the Green Revolution, which occurred in the 20th century at the mid-1960s (Borlaug 2002; Hedden 2003), greatly contributed to an increase in wheat productivity and mitigation of hunger for millions of people, the trade-offs of reduced diversity and genetic uniformity of the most productive contemporary well-adapted durum cultivars cannot be neglected (Serrão et al. 2016). The bottle-neck of ‘genetic erosion’ was addressed in several studies (Autrique et al. 1996; Maccaferri et al. 2005; Soleimani et al. 2002), which demonstrated that successful modern wheat varieties usually originate from a limited

number of ancestors (Serrão et al. 2016). This has contributed significantly to the vulnerability of durum wheat to abiotic and biotic threats (Newton et al. 2010).

Fungal diseases are among the most frequent biotic constraints to durum wheat production. Coalescence analyses suggest that *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous (formerly *Mycosphaerella graminicola* [Fuckel] J. Schröt. in Cohn) has a relatively recent origin that overlaps with the known domestication of wheat in the Fertile Crescent (Stukenbrock et al. 2006), and emerged as a host-specialized wheat pathogen (Stukenbrock and McDonald 2008) during the domestication of wheat (McDonald and Mundt 2016; Torriani et al. 2011). *Zymoseptoria tritici* is currently globally distributed and causes septoria tritici blotch (STB) on wheat (O'Driscoll et al. 2014). The first STB epidemic occurred in North Africa in 1968-1969 and coincided with the introduction of semi-dwarf wheat cultivars and the intensive use of fertilizers (Brown et al. 2015). These epidemics raised international awareness of the threat of STB as *Z. tritici* is omnipresent throughout the temperate regions (Fones and Gurr 2015). Under conducive conditions, STB may reduce yields by 35 to 50% (Ponomarenko et al. 2011) and disease management mostly has relied on fungicide applications combined with sustainable agricultural practices (Omrane et al. 2015). However, fungicide efficacy regularly fails or continuously drops (Cools and Fraaije 2008; Torriani et al. 2009; Torriani et al. 2015), as a result of the high adaptability of *Z. tritici* populations (Goodwin et al. 2011; Stukenbrock et al. 2011). Therefore, breeding for resistance to *Z. tritici* is the most sustainable alternative to manage STB.

Thus far, 21 *Stb* resistance genes have been identified (Brown et al. 2015), but none have been identified in durum wheat. The emphasis of research into the *Z. tritici* – wheat relationship has been dominated by bread wheat, despite the high and overall susceptibility of durum wheats. Therefore, breeding for resistance to *Z. tritici* in durum wheat is hampered by a major lack of information, which is largely due to the biological phenomenon that *Z. tritici* populations show a remarkable pathogenic dichotomy to either bread wheat or durum wheat (Ware 2006). This nullifies the applicability of results derived from bread wheat research to durum wheat research and breeding. Hence, efforts to characterize the *Z. tritici* - durum wheat pathosystem are important and urgently required (Brown et al. 2015; Gharbi et al. 2008). Recently, a few Tunisian landraces with resistance to STB were identified (Ferjaoui et al. 2015; Ferjaoui et al. 2011) and the screening of contemporary Tunisian and Italian durum wheat cultivars resulted in the characterization of partial resistance to STB (Berraies et al. 2014;

Gharbi et al. 2000). In bread wheat breeding, valuable genes for resistance have been identified and deployed that originate from wild ancestors (Mujeeb-Kazi et al. 1996; Tabib Ghaffary et al. 2012; Yang et al. 2009). For instance, the *Stb5* gene was identified in a synthetic hexaploid derived from *T. turgidum* ssp. *dicoccoïdes* and *Ae. tauschii* (Arraiano et al. 2001), and *Stb16q* was identified in the synthetic hexaploid M3 (W-7976) and has a wide efficacy to over 100 different *Z. tritici* pathotypes (Makhdoomi et al. 2015; Mehrabi et al. 2015). Here, we identified broad resistance to *Z. tritici* isolates from durum wheat in the emmer accession PI41025 and mapped the responsible genes by screening recombinant inbred populations from crosses with the commercial durum wheat cv. Ben with a range of isolates. This is the first report of a major QTL for resistance to durum wheat-derived *Z. tritici* isolates and an important starting point to improve the understanding of STB disease management in an under-investigated, important, but largely neglected crop.

Materials and Methods

Plant and fungal materials, and experimental conditions

The cultivated emmer wheat accession PI41025 was collected near Samara, Russia in 1909, and it is characterized by a moderate resistance to Fusarium head blight (FHB), caused by *Gibberella zeae* (Schweinty) Petch anamorph *Fusarium graminearum* Schwabe) (Oliver et al. 2008) and by an adult susceptibility to stripe rust as described in the U.S. National Plant Germplasm System. The modern durum wheat cv. Ben (PI596557) (https://npgsweb.arsgrin.gov/gringlobal/accessiondetail.aspx?id=1105105) is an amber variety developed in North Dakota and was released in 1996. In addition to its high yield potential, cv. Ben is characterized by its resistance to most races of wheat stem rust (caused by *Puccinia graminis* Pers.), a good level of resistance to tan spot (caused by *Pyrenophora tritici-repentis*) and a moderate level of resistance to FHB (Elias and Miller 1998). Seeds of PI41025 and 'Ben' were provided by the NDSU durum breeding program in Fargo, North Dakota, and the USDA-ARS National Small Grains Collection (NSGC) in Aberdeen, Idaho, respectively. We developed a recombinant inbred population (RIL) by crossing cv. Ben with PI41025 as described in (Faris et al. 2014; Zhang et al. 2014). The study of the *Z. tritici* resistance inheritance in the BP025 population was performed using the F6 RILs.

With slight modifications due to the origin of the materials, we followed similar protocols and used the same materials for plant management, inoculum production and inoculations as described in chapter 4 (Aouini et al. 2017).

Experimental design, data collection and QTL analyses

As no predisposed information was available on the resistance of the PI41025 and cv. Ben to *Z. tritici*, we used a diverse set of 31 *Z. tritici* isolates for the initial screening of the parents of the BP025 population (experiment 1, three replicates, Table 1). We then reduced the screening panel to the six most differentiating *Z. tritici* isolates in a single screen of the 193 RILs (experiment 2, one replication, Table 1), and subsequently to the two isolates (IPO91009 and 2B123), which showed the highest logarithm of the odds (LOD) values in the preliminary QTL analysis for an additional screening (experiment 3, two replicates, Table 1).

Seedlings of the 193 BP025 RILs were tested following a split plot design with trays as whole plots. Individual pots were the experimental units and they were randomly arranged for each isolate/replicate combination on separate parallel greenhouse tables. The parents PI41025 and cv. Ben were included in all replicates as checks.

Seedling disease severities were evaluated by assessing the quantitative (%) presence of necrosis (N) and pycnidia (P) on the primary inoculated leaves at 15, 18 and 21 days post-inoculation (dpi), which enabled the calculations of the Area Under the Disease Progress Curve (AUDPC) for quantitative analyses of temporal differences in disease progress (Madden et al. 2007).

The Fisher's unprotected Least Significant Difference (LSD) test was used to determine significant differences between means of the parents at a probability of P=0.05. LSD-values were determined and applied to the table of means. The mixed models were analyzed using the restricted maximum likelihood REML procedure in GenStat (Searle et al. 1992). Fixed effects were tested by computing approximate F-tests following (Kenward and Roger 1997) and were used to test for main effects of isolate and line and their interaction. In case a significant line by isolate interaction was determined, the agglomerative hierarchical clustering procedure (Corsten and Denis 1990) implemented in the XLstat was used for identifying simultaneously groups of isolates and groups of lines in the two way table of isolate by line predicted means, such that interaction is due to interaction between those groups. A normality test for distribution of the pycnidia residuals was also performed using GenStat. The homogeneity of error variances among replications was checked by inspecting the error plot and the equal variance plot. As

error variances were homogeneous among replications, the scores of each replicate were used to calculate the overall mean per *Z. tritici* isolate for each RIL. Mean values were subsequently used for QTL analyses.

QTL analyses were performed using two datasets: (i) The first replicate of the BP025 RILs screen and (ii) the mean values of two *Z. tritici* isolates of the first, second, and the third replicates of the RILs screen. QTL analysis was performed using a high-density genetic map comprised of 2,461 SNP, 128 SSR, and four EST–STS markers spanning a genetic distance of 2,444.4 cM with an average marker density in the whole genome of 0.94 cM between each marker (Faris et al. 2014). Multiple interval mapping (MIM) was used to identify significant associations using the software program QGenev.4.3 (Joehanes and Nelson 2008). A permutation test with 1,000 permutations was performed in order to determine the critical LOD threshold at the $P = 0.05$ level of probability to be 3.3.

Table 1. Thirty-One *Zymoseptoria tritici* isolates that were derived from durum wheat in the Mediterranean basin and were used to phenotype the cultivated emmer wheat accession PI41025 and the durum wheat cv. Ben as well as a recombinant inbred population derived from a cross between these tetraploid wheats.

					Experiment		
Region	Isolate ID	Country	Location	Year	1	2	3
Middle-East	IPO91004	Syria	Lattakia	1991	+		
	IPO95004	Syria	Lattakia	1995	+		
	IPO95005	Syria	Lattakia	1995	+		
	IPO86022	Turkey	Altinova	1986	+		
North Africa	IPO92042	Algeria	Oum Bouachi	1992	+	+	
	IPO95042	Algeria	Menzel el abtal	1995	+		
	IPO95052	Algeria	Menzel el abtal	1995	+	+	
	IPO95053	Algeria	Berrahal	1995	+		
	IPO07001	Tunisia	Bejá	2007	+		
	IIB123	Tunisia	Bejá	2005	+	+	+
	Tun1	Tunisia	Qued bagrat	-	+	+	
	Tun6	Tunisia	Sidi Nsir	-	+		
	IPO91009	Tunisia	Bejá	1991	+	+	+
	IPO91012	Tunisia	Sidi Nsir	1991	+		
	IPO91016	Tunisia	Bejá	1991	+		
	IPO91018	Morocco	Jenica Shaim	1991	+	+	
	IPO91020	Morocco	Doukkala	1991	+		
Europe	IPO92003	Portugal	-	1992	+		
	IPO13001	Italy	Emilia Romagna	2013	+		
	IPO13010	Italy	Bologna	2013	+		
	IPO13013	Italy	Bologna	2013	+		
	IPO13014	Italy	Bologna	2013	+		
	IPO13015	Italy	Bologna	2013	+		
	IPO13018	Italy	Sicily	2013	+		
	IPO13022	Italy	Sicily	2013	+		
	IPO13024	Italy	Sicily	2013	+		
	IPO13025	Italy	Sicily	2013	+		
	IPO13057	Italy	Toscani	2013	+		
	IPO13058	Italy	Toscani	2013	+		
	IPO13059	Italy	Toscani	2013	+		
	IPO13061	Italy	Toscani	2013	+		

Results

Resistance to Zymoseptoria tritici in the parents of the BP025 population

To understand the efficacy of STB resistance in cv. Ben and PI41025, we screened these accessions with 31 *Z. tritici* isolates originating from durum wheat in the Mediterranean basin. The selected isolates grew successfully under laboratory conditions, resulting in optimal inoculum quality, and after application and incubation the disease developed as expected. Necrosis development at 21 dpi was 100% for all *Z. tritici* isolates (not shown), but pycnidia development differed significantly (Table 2), and a subsequent analysis of the mean variances indicates significant host/isolate interactions (Table 3, Figure S1). Overall, PI41025 was not significantly different from 0P for the far majority of isolates in the *Z. tritici* panel, except for isolates IIB123 and the IPO91004, where it developed an intermediate response (significantly different from 0P<actual score<maxP). The scores of cv. Ben were not significantly different from maxP for nearly all isolates, except for *Z. tritici* isolate IPO13058, which is avirulent on both accessions (Table 2).

BP025 RILs screen and pycnidia distribution on the BP025 population.

The first RIL screen was conducted on a single replicate using six *Z. tritici* isolates (Table 1, Figure 1). Disease developed well but was not normally distributed and the scores indicated negative and positive transgressive segregation, as some RILs were either more susceptible than cv. Ben, or more resistant than PI41025, respectively (Figure 1). The BP025 population means for disease parameter P ranged between 25.49% and 46.52%, with the lowest and the highest values prompted by *Z. tritici* isolates IPO95052 and IPO91009, respectively (Figure 1). Eventually, we determined that over 50 % (N=105) of the screened BP025 RILs exceeded the susceptibility of cv. Ben with *Z. tritici* isolate IPO91009, whereas 102 RILs were more resistant than PI41025 with isolate IIB123. Therefore, we used these strains to conduct second and third replicate screening of the BP025 RIL population. Error variances were homogeneous across replications and, hence, average scores across the replicates could be used for the QTL analyses (not shown). Analysis of mean variances of the BP025 RILs with the IPO91009 and IIB123 *Z. tritici* isolates showed a significant difference between the isolates, indicating that the observed variation in the data is accounted for by the diverging isolates' pathogenicity (Table 4).

Table 2. Percentage of pycnidia (%P) on the primary leaves of the durum cv. Ben and the cultivated emmer wheat accession PI41025 after phenotyping with 31 *Zymoseptoria tritici* durum derived isolates originated from the Mediterranean Basin. Significant differences are based on Least Significant Differences of %P (5.5≤P≥24.2; for P=0.05).; colors indicate resistant (not significantly different from 0P; green), intermediate (significantly different from 0P and maxP; yellow) and susceptible (not significantly different from maxP; red) responses

	Isolates																													
	Middle-East				North Africa														Europe											
Accessions	IPO91004-Syria	IPO95004-Syria	IPO95005-Syria	IPO86022-Turkey	IPO92042-Algeria	IPO95042-Algeria	IPO95052-Algeria	IPO95053-Algeria	IPO07001-Tunisia	IIB123-Tunisia	Tun1-Tunisia	Tun6-Tunisia	IPO91009-Tunisia	IPO91012-Tunisia	IPO91016-Tunisia	IPO91018-Morocco	IPO91020-Morocco	IPO92003-Portugal	IPO13001-Italy	IPO13010-Itlay	IPO13013-Italy	IPO13014-Italy	IPO13015- Italy	IPO13018-Italy	IPO13022-Italy	IPO13024-Italy	IPO13025-Italy	IPO13057-Itlay	IPO13058-Italy	IPO13059-Italy	IPO13061-Italy
Ben	85	75	60	30	70	65	65	70	75	70	70	75	55	70	55	60	50	40	55	70	55	75	70	55	70	60	75	100	0	15	35
PI41025	25	0	0	2,5	5	2,5	2,5	5	0	10	5	5	5	0	5	5	5	5	2,5	0	2,5	0	0	5	0	0	5	2,5	0	2,5	0

Table 3. Results of REML analysis of pycnidia (P) seedling data of the cv.Ben and PI41025 accession inoculated with 31 *Zymoseptoria tritici* isolates

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
isolate	1610.63	30	53.69	124.0	<0.001
accession	11036.73	1	11036.73	124.0	<0.001
Isolate x accession	1306.96	30	43.57	124.0	<0.001

Table 4. Results of REML analysis of pycnidia (P) seedling data of the BP025 population RILs inoculated with the IPO91009 and IIB123 *Zymoseptoria tritici* isolates

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Isolate	34.46	1	34.46	772.0	<0.001
RIL	276.30	192	1.44	772.0	<0.001
Isolate X RIL	102.60	192	0.53	772.0	1.000

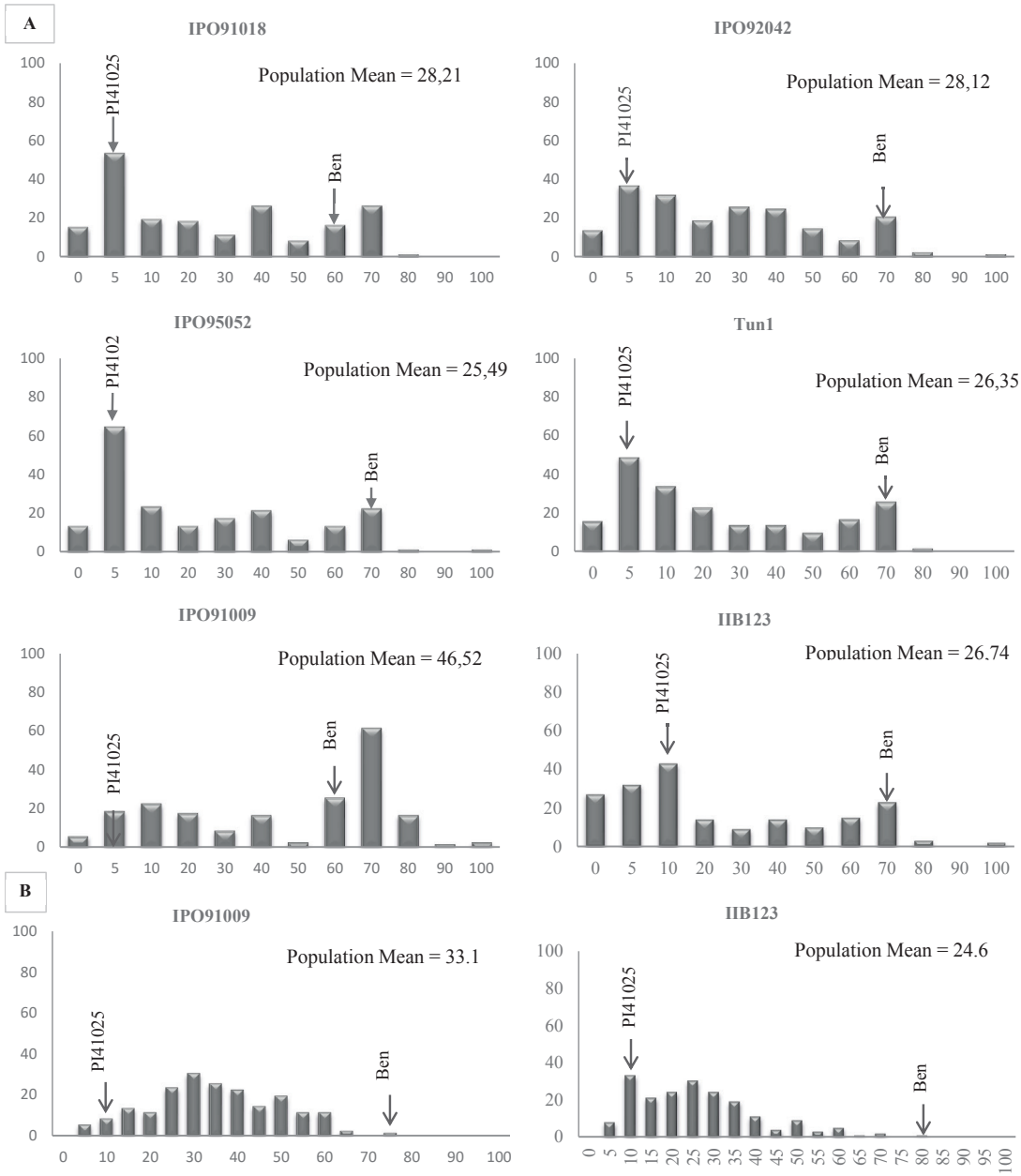

Figure 1. Distribution of the percent of the leaf area covered by pycnidia (x-axis) for the PB025 recombinants inbred lines (y-axis) for six *Zymoseptoria tritici* isolates in the prescreening (A panels) and the average of three replicates with two isolates (B panels).

QTL mapping of resistance to Zymoseptoria tritici *in the BP025 population*

QTL analysis revealed three genomic regions associated with resistance to *Z. tritici* in the BP025 population, and these three regions were located on chromosomes 3A, 5A, and 6A. The QTL identified on chromosome 6A was significantly associated with necrosis development at 14 dpi only, but as all RILs scored 100N at 21dpi we did not consider it any further (not shown).

The QTL on chromosome 3A (LOD range 4.4-18; explained variance between 14-29%) was located in the distal region of the long arm, and resistance effects at this locus were derived from PI41025. This QTL was significantly associated with STB caused by all isolates, and it peaked at position 177.4 cM between the two SNP markers *IWA7812* and *IWA3949* for isolates IPO91009, IPO91018, IPO92042, IPO95052 and IIB123, and at 175.8 cM for isolate Tun1 (Flanking markers: *IWA4905-IWA3031*). This locus represents a new genomic location associated with *Z. tritici* seedling resistance, and it is the first major QTL identified in tetraploid wheat. Therefore, we propose to designate this QTL as *Stb22q* (Table 5; Figure 2).

In addition, one minor QTL was identified on the long arm of chromosome 5A, associated with resistance to isolates IPO91009 and IIB123 (LOD range 5.5 – 5.9, explained variance range - 8%). Resistance effects for this QTL were derived from Ben, and it peaked at position 169.9 cM between the two SNP markers *IWA1942* and *IWA5154* (Table 5; Figure 2). This genomic region has not previously been associated with a *Z. tritici* resistance, therefore it is a novel location conferring partial resistance to *Z. tritici* in durum wheat.

Table 5. Summary of the QTL analysis for percentage of pycnidia (%P) on the primary leaves of the BP025 recombinant inbred population derived from a cross between the durum cv. Ben and the cultivated emmer wheat accession PI41025 after phenotyping with *Zymoseptoria tritici* isolates IIB123 and IPO91009.

Isolate	Chromosome	Peak marker position (cM)	LOD	$R^2 \times 100$	Marker interval	Additive effects[a]
95052	3A	177.4	11.0	22.0	*IWA7812-IWA3949*	68.37
91018	3A	177.4	4.5	8.3	*IWA7812-IWA3949*	75.37
92042	3A	177.4	14.0	23.0	*IWA7812-IWA3949*	75.23
91009	3A	177.4	16.0	28.0	*IWA7812-IWA3949*	104.31
Tun1	3A	175.8	4.4	9.9	*IWA4905-IWA3031*	61.13
IIB-123	3A	177.4	14.0	29.0	*IWA7812-IWA3949*	93.67
91009 average reps. 1-3	3A	177.4	9.4	17.1	*IWA7812-IWA3949*	38.61
	5A	169.9	5.9	7.9	*IWA1942-IWA5154*	-25.89
IIB-123 average reps. 1-3	3A	177.4	18.0	29.0	*IWA7812-IWA3949*	49.99
	5A	169.9	5.5	6.4	*IWA1942-IWA5154*	-22.07

[a]A positive additive effect indicates that resistance is derived from PI 41025, and a negative effect indicates resistance is derived from Ben.

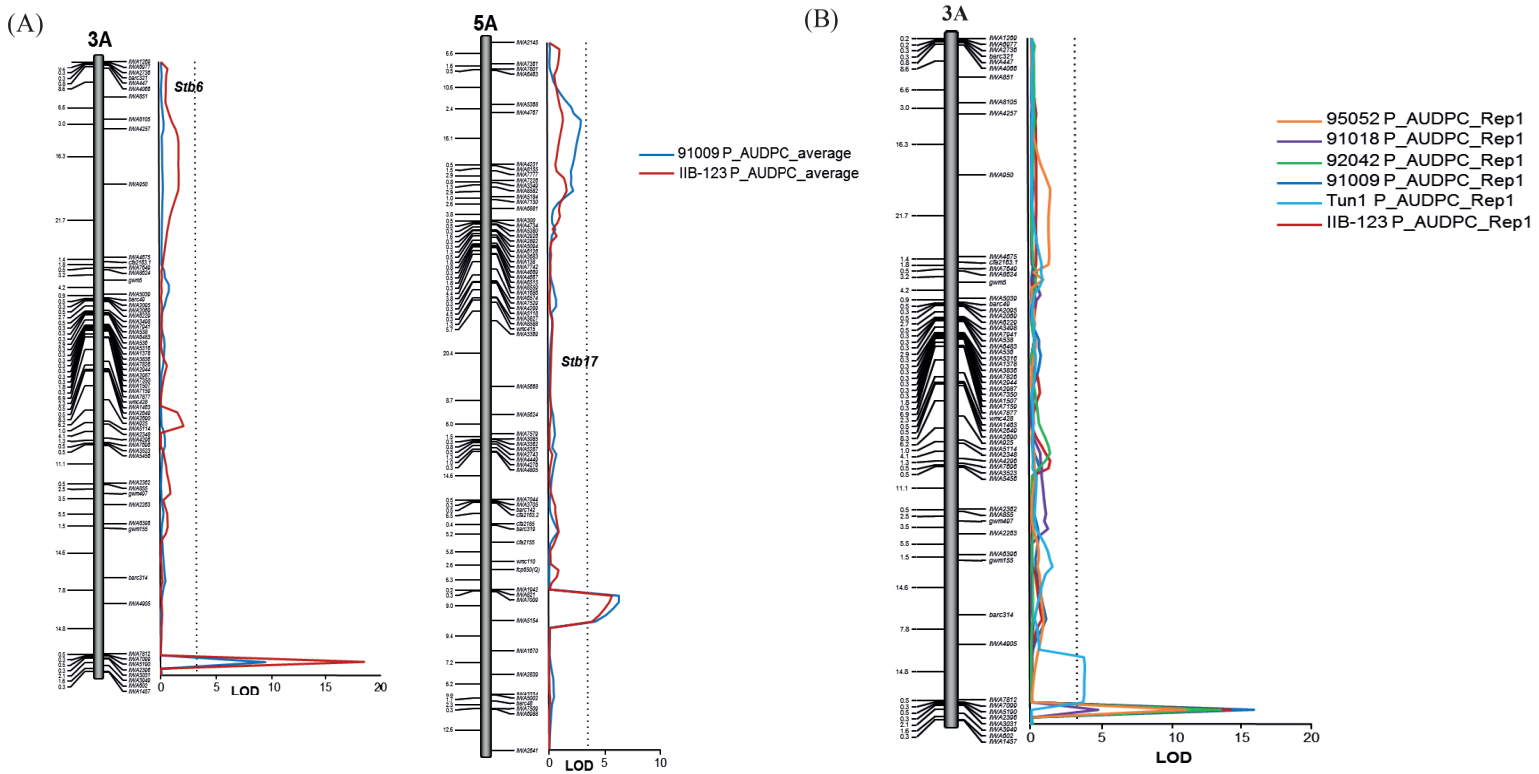

Figure 2. Regions of the linkage maps for chromosomes 3A and 5A associated with *Zymoseptoria tritici* seedling resistance detected in the BP025 population (***Panel A***). The QTL detected on chromosome 5A is associated with the IPO91009 and IIB123 pycnidia resistance (***Panel A***). The QTL detected on chromosome arm 3AL referred to as the *Stb22* is a novel resistance gene that governs a wide spectrum of pycnidia resistance (***Panel B***). The centiMorgan (cM) distances between marker loci and the positions of marker loci are on the left and right sides of the linkage maps, respectively. The logarithm of the odds (LOD) significance threshold of 3.3 is represented by a vertical dotted line.

Discussion

It is remarkable that durum wheat, despite its importance as a staple crop in North Africa and importance for pasta delicacies around the world has gained such little attention with regard to disease resistance (Prat et al. 2014). Septoria tritici blotch is no exception. Virtually all progress in this research area has entirely focused on the interaction between the causal agent *Z. tritici* and bread wheat (Brown et al. 2015). Throughout the history of plant breeding, expanding the genetic diversity by introgression of genes from wild ancestors of agricultural crops has been crucial to generate superior genotypes (Blanca et al. 2015; McCouch 2004). Wheat domestication has particularly benefited from polyploidization (Dubcovsky and Dvorak 2007), and deploying the rich diversity from wild ancestors has substantially contributed to managing biotic threats, particularly the cereal rusts (Edae et al. 2016; Gyani et al. 2017; Zhang et al. 2016). Such resources have been used in direct hybridizations or by making these gene pools available through developed synthetic hexaploid wheats (Dreisigacker et al. 2008; Yang et al. 2009). Also for *Z. tritici,* the most efficacious *Stb* genes were derived from wild wheat relatives through synthetic hexaploids, such as *Stb16* (Tabib Ghaffary et al. 2012), which is already used in commercial bread wheat cultivars (Dalvand et al. 2016). Other broad-spectrum resources in *T. monococcum* and various *Aegilops* species have been identified, but not commercially deployed (Jing et al. 2008). Hence, the strategy to enhance disease resistance in durum wheat by exploring wild ancestors is valid and entails the identification of new sources of quantitative resistance and major genes in durum wheat landraces and progenitors. Oliver et al.(2008) reported moderate FHB resistance in the emmer accession PI41025, and Zhang et al. (2014) mapped the underlying QTLs on chromosomes 3A and 5A.

Here, we used the same accession to investigate resistance to *Z. tritici.* In contrast to the durum cv. Ben, which was susceptible to all tested *Z. tritici* isolates, PI 41025 showed resistance to all but one of these *Z. tritici* isolates (pycnidia level not significantly different from 0P). Only isolate IPO91004 from Syria induced pycnidia production, but still at a moderate level (25%). Clearly, this could be explained by an adaptation of the *Z. tritici* population from the Middle-East to domesticated emmer wheats, which emerged in the same geographical area as the pathogen (Stukenbrock et al. 2006). Subsequent analyses of the RIL population identified the underlying QTL that we designate *Stb22* on chromosome arm 3AL, which provides broad-spectrum resistance to a plethora of *Z. tritici* isolates and is closely linked to the threshability QTL '*QFt.fcu-3A*'(Faris et al. 2014). Chromosome 3AS has been associated with STB resistance and carries the recently cloned *Stb6* (Saintenac 2017) that is omnipresent in European

wheat varieties (Chartrain et al. 2005b), as well as *StbSm3,* which is derived from the hexaploid landrace Salamouni and confers resistance to two Canadian *Z. tritici* isolates (Cuthbert 2011). The minor QTL on chromosome 5A is distinct from *Stb17* and provides resistance to isolates IPO91009 and IIB123, hence has a narrow efficacy. Recent studies have shown that pyramiding such QTLs also leads to acceptable levels of STB resistance (Aouini et al. 2017; Berraies et al. 2014).

Contrary to the *Z. tritici* – bread wheat pathosystem, interactions with durum wheat and isolates retrieved from this species almost exclusively occur for pycnidia development (P), but not for necrosis (N) (Aouini et al. 2017; Kema et al. 1996a) . Similarly, the broad efficacy of *Stb22q* is demonstrated for P, but not for N. We do not know why durum wheats exhibit extensive necrosis as a result of *Z. tritici* infections, but the current data also demonstrate that N and P are under different genetic control (Kema et al. 1996b). The recent cloning of *AvrStb6* (Kema et al. 2017; Zhong et al. 2017); will likely help to resolve more mechanistic details of *Z. tritici* pathogenesis in wheat (Kettles et al. 2017), as it also unveiled the exclusive paternal parenthood phenomenon that contributes to overall durability of resistance to STB.

Despite the demonstrated value of *Stb22q,* subsequent field trials and adult plant tests will be conducted to verify its broad efficacy in the adult plant stage. Most *Stb* genes were identified in seedling assays (Brown et al. 2015), but a few comparative seedling/adult mapping studies showed specificity for growth stage, such as *Stb17* (Chartrain et al. 2004; Ghaffary et al. 2012; Ghaffary et al. 2011); and recently also in durum wheat (Aouini et al. 2017). Therefore, field trials may reveal more insight into the specificity of *Stb22q* to *Z. tritici* isolates. It is also possible that PI41025 carries additional resistance factors for specific adult plant resistance, which could contribute to its resilience under STB selection pressure (Gieco et al. 2004). The observation that *Z. tritici* isolate IPO91004 caused moderate disease severity suggests that the commercial deployment of *Stb22q* should be done in conjunction with other STB resistance QTLs to protect its efficacy. This can be done most efficiently with the aid of molecular markers, which are available for most STB resistance genes and QTLs. Gene pyramiding has proven to be an efficient approach in plant breeding to manage the damaging effects of diverse and rapidly evolving pathogens, including *Z. tritici* and stem rust caused by *Puccinia graminis* (Mundt 2014). Examples of broadened resistance to STB are available in well characterized germplasm such as in the bread wheat differentials 'KK4500' and 'TE11' (Chartrain et al. 2005a; Chartrain et al. 2004). We recently showed that co-occurring QTLs with limited efficacy also occur in durum wheat landraces, likely explaining their renowned

resilience to STB (Aouini et al. 2017). The identification of *Stb22,* therefore*,* is an important discovery, which may facilitate the release of new STB resistant durum wheat varieties.

Acknowledgments

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Supplementary information

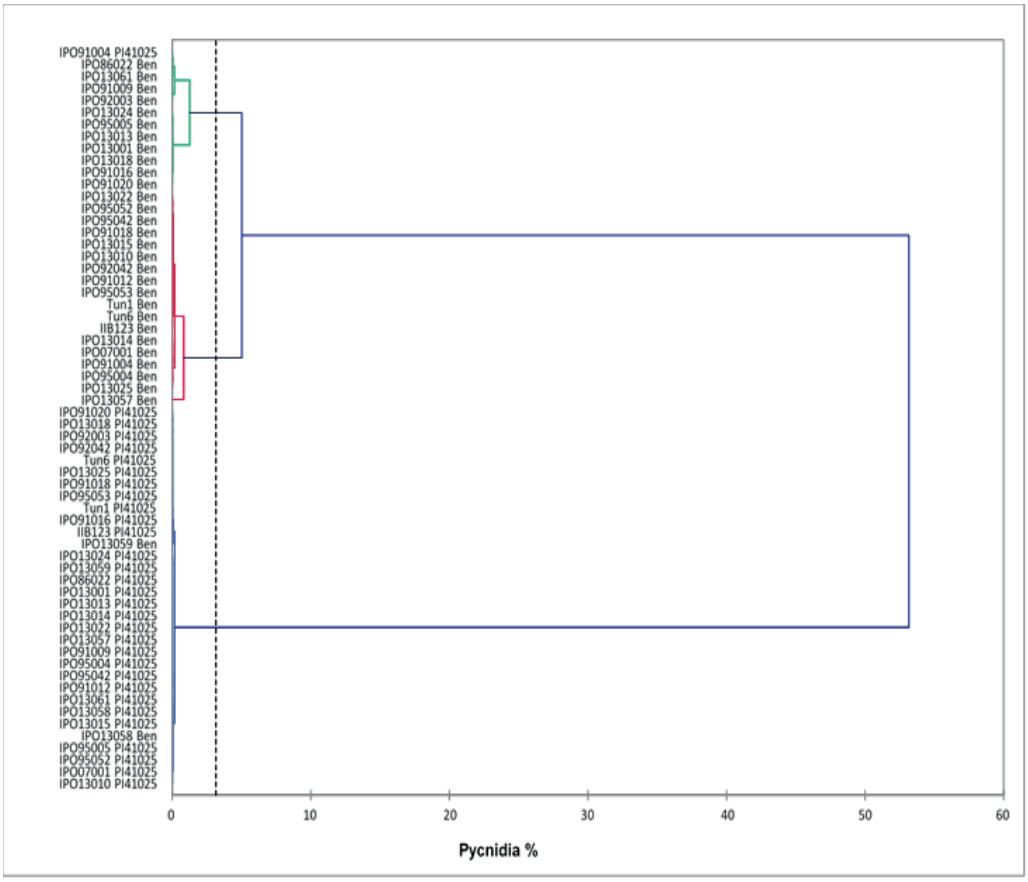
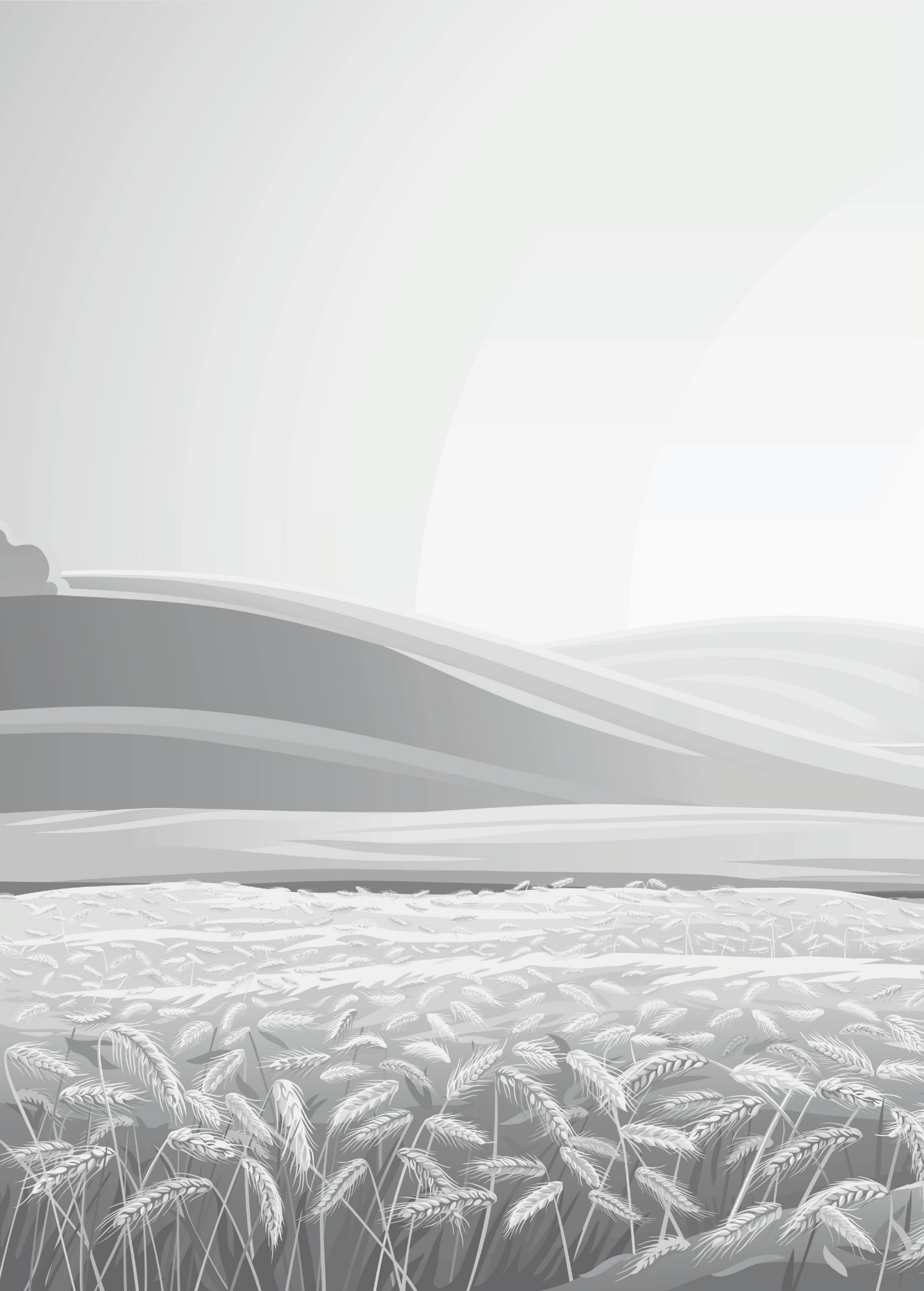


Figure S1. Agglomerative hierarchical clustering of the interaction between the PI41025 accession and the cv. Ben with 31 *Zymoseptoria tritici* isolates. Ward 's method was used to assess the dissimilarities between the pycnidia means. Comparisons between the (isolate x accession) means were performed in GenStat by using the Fisher's unprotected least significant difference test. The dotted line shows the threshold at P= 0.05 for cluster assembly based on the Pycnidia means.



Chapter 4

Deciphering durable resistance to Zymoseptoria tritici in the Tunisian durum wheat landrace 'Agili 39'

Lamia Aouini[§], Sahbi Ferjaoui[§], Rym Ben Slimane, Karim Ammar, Suzanne Dreisigacker, Sarrah Ben M'Barek, Henk J. Schouten., Richard G.F. Visser, Gert H.J. Kema, Sonia Hamza.

[§]These authors contributed equally

Submitted for publication

Abstract

Septoria tritici blotch (STB), caused by the Dothideomycete *Zymoseptoria tritici,* is the most important disease of bread wheat in Europe, and a very important biotic threat to durum wheat in the entire Mediterranean Basin. The majority of durum wheat cultivars are very susceptible to *Z. tritici,* but most efforts in understanding the pathosystem have focused on the *Z. tritici* – bread wheat interaction. Hence, research in durum wheat has been limited and no resistance genes to *Z. tritici* were identified until now. Here, we have identified resistance to a wide array of *Z. tritici* isolates in the Tunisian durum wheat landrace 'Agili 39'. In subsequent analyses, recombinant inbred populations were developed and tested under greenhouse conditions at seedling stage with eight *Z. tritici* isolates and for five years under field conditions with three *Z. tritici* isolates. Mapping of quantitative trait loci (QTL) resulted in the identification of one major QTL at chromosomes 2BL (highest LOD 17.7, explained variance 38%) for seedling resistance to pycnidia development and effective against five *Z. tritici* isolates out of the eight used isolates and one for adult plant resistance on chromosome 2BS (highest LOD 25.7, explained variance 42.%) that has been effective against the three used Z. tritici isolates at the field trials. In addition, we identified two minor QTLs on chromosomes 1A and 7AS (highest LODs 7.5 and 5, and explained variances of 18 % and 14%, respectively) that were specific to three and two *Z. tritici* isolates, respectively, as well as a QTL that contributed to STB susceptibility on chromosome 2A (LOD 6.0, explained variance 13%) derived from the susceptible parent cv. Khiar and detected with two Z. tritici isolates. We provide evidence that the broad efficacy of the resistance to STB in 'Agili 39' is due to a natural pyramiding of these QTLs. Some of the identified QTLs, such as the one on chromosome 2BL, map on the same positions as *Stb9,* which was identified with *Z. tritici* isolates from bread wheat. However, its efficacy to *Z. tritici* isolates from durum wheat is much better than to isolates from bread wheat, which illustrates the dichotomy of pathogenicity in *Z. tritici* for thesetetraploid durum and hexaploid bread wheat hosts.

Key Words: durum wheat-, landraces, *Zymoseptoria tritici*, major quantitative trait loci, gene efficacy, susceptibility locus, gene pyramiding.

Introduction

Wheat has been, for centuries, the prime food and feed crop for humanity (Arzani and Ashraf 2017) and currently – after rice and maize (FAOSTAT 2017)- supplies 20% of the human calorie intake, and is thereby a major component for global food security (Shiferaw et al. 2013). The genus *Triticum* L. comprises several wheat species with various ploidy levels, but global wheat production is almost entirely based on bread wheat, *T. aestivum* L. em. Thell. (2n=6x = 42, AABBDD), and durum wheat, *T. turgidum L. var durum* (2n=4x = 28, AABB), also known as macaroni wheat (Charmet 2011). Durum wheat accounts for about 8% to the global wheat production, and its cultivation is concentrated in latitudes ranging from 55°N to 40°S (Palamarchuk 2005; Royo et al. 2014), corresponding mostly to the Mediterranean Basin, the North American Great Plains, India and the former USSR (Royo et al. 2014). Northern Africa has been the cradle of wheat production for centuries and was the bread basket for the Roman Empire (Fabricant 1998; Oliveira et al. 2012) with locations such as Dougga in Tunisia, as exquisite trading zones for wheat and other commodities until the late 500's AD (Davis 2007). Nowadays, the Maghreb zone of North Africa is still the major durum wheat production region covering 17 million ha, which is a basis for many traditional dishes such as couscous, but also for the popular pastas. In Tunisia, durum wheat approximates 49% of the total annual cereal area, with an average yield of 1.6 tons/ha between 2000 and 2012 (Nefzaoui et al. 2012; Rastoin and Benabderrazik 2014). However, ongoing climate events, such as the regular, but infrequent El Niño episodes significantly affect global wheat production and particularly durum wheat production due to abiotic stress conditions - mostly drought - and the emergence of more aggressive pathogens (McDonald and Mundt 2016). Throughout the Maghreb region, the foliar blight septoria tritici blotch (STB), caused by the hemibiotroph *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous (formerly *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn), is among the major threats, particularly since durum wheat isoverall very susceptible to this disease. Estimated yield losses amount up to 385 $kg.ha^{-1}$ in 2008-2009, which is more than 30% in most regions (Berraies et al. 2014). Recent research increased the general understanding of the *Z. tritici* epidemiology in the Maghreb. Hamada (2014) reported the occurrence of the teleomorph of the fungus in Tunisia, despite the arid conditions in the region, and Meamiche Neddaf et al. (2017) determined an equal distribution of both mating types in Algeria, indicating regular sexual reproduction, which likely contributes to the vast genetic diversity in this region. The use of fungicides has been slowly adopted by durum wheat growers as compared to bread wheat producers in Europe, and the first occurrences of strobilurin resistance have been reported in Tunisia and Algeria (Boukef et al. 2012; Meamiche Neddaf et al. 2017).

One of the best management strategies for virtually all plant diseases is the generation of new excelling disease resistant germplasm through plant breeding. The huge genetic diversity in wheat and its ancestors has provided new varieties for almost a century (Charmet 2011). Even, re-emerging and upcoming threats such as stem rust caused by the strain Ug99 (Bajgain et al. 2016; Chen et al. 2015; Saintenac et al. 2013), have been managed during the last decade by releasing new germplasm that turned the potential havoc into a manageable problem (Singh et al. 2014; Singh et al. 2011). Before the upcoming of plant breeding, improved crops frequently resulted from farmers' selections of outperforming genotypes in terms of yield stability. Often, such so-called landraces contained a variety of closely related lines that quenched biotic threats. During the onset of breeding, these landraces were often the starting material for targeted efforts to improve for instance disease resistance (Kingsbury 2009; Lopes et al. 2015; Mondal et al. 2016). In this study, we have surveyed resistance to *Z. tritici* in a suite of Tunisian landraces, which could be the basis for durum wheat improvement.

Studies into the genetic basis of resistance to *Z. tritici* have exclusively been addressed in bread wheat. Until now, up to 21 septoria tritici blotch (*Stb*) resistance genes have been identified and mapped (Brown et al. 2015). However, due to the apparent dichotomy in natural *Z. tritici* populations for either bread wheat or durum wheat (Kema et al. 1996a; Kema et al. 1996b; Kema and van Silfhout 1997), the presence of these mapped *Stb* genes in durum wheat cannot be determined using well characterized *Z. tritici* strains originating from bread wheat. Thusfar, the substantial progress in understanding the wheat-*Z. tritici* pathosystem (Arraiano and Brown 2017; Kema et al. 2000; Kema et al. 2017; Tabib Ghaffary 2011; Zhong et al. 2017) is entirely based on the *Z. tritici* – bread wheat pathosystem. Therefore, resistance breeding to *Z. tritici* in durum wheat has hardly progressed over the last 25 years. This affects many small growers who produce this wheat as a staple crop in an area that is severely struck by septoria tritici blotch. We have embarked on increasing the understanding of the *Z. tritici* - durum wheat pathosystem. Here, we have first screened for diversity for pathogenicity in the pathogen and resistance in Tunisian landraces. Subsequently, we developed a mapping population between the resistant landrace 'Agili39' and the susceptible modern cv. Khiar to identify the genetic basis of resistance to *Z.tritici* and identify and map the involved genes under greenhouse and field conditions.

Materials and Methods

Experimental design, Plants management and growth conditions

Eleven durum wheat accessions (Table 1) and a bi-parental recombinant inbred population derived from a single seed descent cross between the Tunisian landrace 'Agili 39' and the commercial cv. Khiar, were screened for resistance to septoria tritici leaf blotch. We performed a total of three experiments (Table 2). The first experiment was repeated three times and comprised the screening of the 11 Tunisian landraces with the panel of 20 *Z. tritici* isolates to understand overall resistance patterns to STB and to select potential parents for further detailed genetic analyses. In addition, we used these data to select eight differential *Z. tritici* isolates for the genetic analysis of the Agili 39/Khiar population, which was performed thrice in the second experiment. Finally, in the third experiment we tested the Agili 39/Khiar population under field conditions in Oued-Bejá, located in North-Western Tunisia, over a period of five years, 2011 – 2014 and 2016, with three different *Z. tritici* isolates. This region belongs to the sub-humid bioclimatic zone of Tunisia with an average rainfall ranging from 500 to 850 mm and a daily mean temperature between 10-28°C, and is particularly known as a hot spot for STB (Ferjaoui et al. 2015).

For the seedling assay of the RIL population, we followed a split plot design with trays as whole plots. Individual pots were the experimental units and were randomly arranged in the trays for each isolate/replicate combination on separate parallel greenhouse tables. Several checks were included with both parents 'Agili 39' and cv. Khiar (Table1). Seedlings of the durum wheat accessions were linearly grown, five seeds per pot, in VQB 7x7x8 cm plastic pots (TEKU®, Lohne, Germany), whereas 157 F6 recombinant inbred lines (RILs) of the "Agili 39"/Khiar mapping population were planted in round peat pots (Jiffy, Moerdijk, Netherlands), also five seeds per pot, using a special mixture for growing seeds (Substraat Zaai) provided by the greenhouse facility Unifarm of Wageningen University and Research (WUR), The Netherlands. Pre-inoculation plant development was allowed for 10 days in a greenhouse adjusted at a temperature of 18/16°C (day/night rhythm) and relative humidity (RH) of 70%. Post-inoculation conditions were set at a temperature of 22/±2°C and RH of ≥95%. Light intensity (son-T Agro 400 W lamps) and day length (16/8 h light/dark) were similar during pre- and post- inoculation conditions. Ten days after inoculation, seedlings were trimmed for the second and subsequent leaves to enable sufficient light on the inoculated primary leaves for

appropriate disease development. Fertilizer (Sporumix PG®, Rotterdam, Netherlands; 0.5 $g.l^{1}$) was applied to maintain plant condition.

For the field trials, we used an augmented randomized complete block design. Five blocks with 1.5 m width and spaced 1.5 m were linearly drilled with 30 to 35 RILs per block. Each RIL was sown as one row per spike of 1.5 m length and spaced 25 cm. We randomized all RILs, the parents and four additional checks modern durum wheat cvs. Karim, Nasr, Maali and Salim, important in Tunisian breeding programs and showing different levels of susceptibility in each block. For the 2016 field trial we used a complete random block design with three replicates with both parents 'Agili 39' and 'Khiar' as checks.

Zymoseptoria tritici isolates and inoculation procedures

Initial screening of durum wheat germplasm was performed with 20 *Z. tritici* isolates (Table 2). Finally, eight isolates were selected to study the inheritance of resistance to STB in the RIL population in the seedling stage and three *Z. tritici* isolates were individually used under field conditions. Pre-cultures of each isolate 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 with frozen isolate samples that were directly taken from the *Z. tritici* isolate collection at WUR that is maintained at -80°C and subsequently placed in an incubated rotary shaker (Innova 4430, New Brunswick Scientific, USA) set at 125 rpm and 15°C for 5–7 days. These pre-cultures were then used to inoculate two 1L Erlenmeyer flasks containing 500 ml YG media per isolate that were incubated under the aforementioned conditions to provide sufficient inoculum for the seedling inoculation assays at growth stage (GS) 11 (Zadoks et al. 1974). Spores were collected after overnight settling in static cultures, concentrated by decanting the supernatant medium, adjusted to 1.10^{7} $spores.ml^{-1}$ in a total volume of 40 ml for a set of 18 plastic pots or 24 Jiffy® pots and supplemented with two drops of Tween 20 surfactant (MERCK®, Nottingham, UK). Inoculations were conducted by spraying the inoculum over the seedlings that were placed in an inoculation cabinet on a rotary table, adjusted at 15 rpm, which is equipped with interchangeable atomizers and a water cleaning device to avoid cross- contamination. Infected plants were incubated in transparent plastic bags for 48 h under 100% RH in the aforementioned greenhouse.

Field inoculations were conducted with three isolates (Tun1, Tun6 and IIB123) across the F6-F10 RILs generations. We used *Z. tritici* isolate Tun6 during three years to screen the

F6 (N=164), the F8 (N=158) and the F9 (N=157) RILs in 2011, 2013 and 2014, respectively and isolate Tun1 to screen the F7 (N=158) in 2012 and the F9 (N= 157) in 2013. In 2016 we screened the F10 (N=155) with *Z. tritici* isolate IIB123. In all field trials and across all generations, plants were inoculated twice at the three leaf stage and at stem elongation GS 37, (Zadoks et al. 1974), adjusted to 10^7 spores/ml of the corresponding Z. tritici isolates using a CO2-pressurized knapsack sprayer with a 1 m hand-held boom till run-off.

Table 1. Nine Tunisian durum wheat landraces and two cultivars that were investigated for resistance to *Zymoseptoria tritici*.

Name	Habitus	Source	Empiric evaluation of septoria tritici blotch under field conditions
Agili 37	landrace	INAT[2]	Resistant
Agili 38	landrace	INAT	Resistant
Agili 39 (P)[1]	landrace	INAT	Resistant
Agili 41	landrace	INAT	Resistant
Azizi 27	landrace	INAT	Resistant
Derbessi 12	landrace	INAT	Resistant
Jneh Khotifa 85	landrace	INAT	Resistant
Mahmoudi 101	landrace	INAT	Resistant
Sbei 99	landrace	INAT	Resistant
Khiar (P)[1]	cultivar	INRAT[3]	Susceptible
Karim	cultivar	INRAT	Susceptible

[1]Parents of the recombinant inbred population.

[2]National Institute of Agronomy-Tunis, Tunisia.

[3]National Institute of Agronomical Research-Tunis, Tunisia

Table 2. Origin of 20 *Zymoseptoria tritici* isolates that were isolated from durum wheat in the Mediterranean Basin and that were used for phenotyping in the seedling and adult plant stage.

					Experiment		
Region	Isolate ID	Country	Location	Year	1	2	3
Middle-East	IPO91004	Syria	Lattakia	1991	+	+	
	IPO95002	Syria	Lattakia	1995	+		
	IPO95003	Syria	Lattakia	1995	+		
North Africa	IPO91009	Tunisia	Bejá	1991	+	+	
	IIIB-123	Tunisia	Bejá	2005	+	+	+
	Tun1	Tunisia	Qued bagrat	-	+	+	+
	Tun6	Tunisia	Sidi Nsir	-	+	+	+
	IPO91019	Morocco	Jenica Shaim	1991	+	+	
	IPO95052	Algeria	Berrahal	1995	+	+	
Europe	IPO92003	Portugal	-	1992	+	+	
	IPO13001	Italy	Emilia Romagna	2013	+		
	IPO13003	Italy	Emilia Romagna	2013	+		
	IPO13006	Italy	Emilia Romagna	2013	+		
	IPO13007	Italy	Emilia Romagna	2013	+		
	IPO13008	Italy	Emilia Romagna	2013	+		
	IPO13018	Italy	Sicily	2013	+		
	IPO13019	Italy	Sicily	2013	+		
	IPO13023	Italy	Sicily	2013	+		
	IPO13024	Italy	Sicily	2013	+		
	IPO13056	Italy	Tuscany	2013	+		

Data collection and analyses

In the seedling assays, disease severities were evaluated at 15, 18 and 21 days post-inoculation (dpi). These multiple observations enabled Area Under the Disease Progress Curve (AUDPC) calculations for quantitative analyses of temporal differences in disease progress.

We estimated the quantitative presence of necrosis (N) and pycnidia (P) on the inoculated seedling leaves in percentages. AUDPC calculations for seedling scores followed the trapezoidal method, which approximates the time variable and calculates the average disease intensity between each pair of adjacent time points (Madden et al. 2007). For the field evaluations, we scored pycnidial classes at 28 days post the second inoculation (Zadoks scale 51) that were later transformed to P values (0 = no pycnidia, 1=12%, 2=25%, 3=50%, 4=75% and 5=87%) (Adhikari et al. 2004a; Eyal and Brown 1976).

The RIL N and P scores were transformed to the logit scale for statistical analysis and subsequently a residual maximum likelihood (REML) variance component analysis, using Genstat (13th edition,) (VSN International 2013) was performed (Alvey et al. 1982; Searle et al. 1992). Significant differences were determined using the least significant difference (LSD) of back-transformed N and P values. The data transformations resulted in minor changes between observed and processed data to cope with zero N or P scores. Homogeneity of the seedling replicates was checked and homogeneous data across replications were subsequently averaged and used for the seedling QTL analysis (Chu et al. 2010). However, individual field year scores where considered for the field QTL analysis.

For QTL analyses we used MapQTL® 6.0 (Van Ooijen 2004) for interval mapping (IM) for QTL positioning, followed by multiple QTL analysis (MQM) after cofactor selection by manually investigating the marker alignment on the linkage groups where the peaks of IM QTLs were detected. A permutation test with 1,000 permutations was conducted to determine the critical LOD threshold for this mapping population and the QTL profiles were plotted against their LOD and drawn using MapChart 2.3 (Voorrips 2002).

Genotyping by sequencing

Genomic DNA was extracted from fresh leaves using a modified CTAB (cetyltrimethylammonium bromide) method and quantified using NanoDrop 8000 spectrophotometer V 2.1.0. Whole-genome profiling was performed using DArT-Seq™ technology by Diversity Arrays Technology Pty Ltd, Australia, as described by Kilian et al. (2012) and Raman et al. (2014). In brief, the DArT-Seq™ technology was optimized by selecting the most appropriate complexity reduction method for wheat (*Pst*I-*Mse*I restriction enzymes). DNA fragments digested with restriction enzymes were ligated with *Pst*I adaptors and unique barcodes, and then amplified following PCR. Amplicons were pooled and

sequenced in a 96-multiplex on a HiSeq2000 (Illumina, USA) resulting in a total of 5,891 GBS SNP markers, which subsequently filtered according to their polymorphism between the parents 'Agili 39' and 'Khiar' and then used to generated a genetic linkage map using JoinMap ® 4 software (Van Ooijen 2006).

Results

Phenotyping of RILs, landraces and modern cultivars.

The selected 20 *Z. tritici* isolates grew successfully under the laboratory conditions enabling appropriate inoculum production and phenotyping assays. None of the tested durum landraces and cultivars was resistant to the entire suite of *Z. tritici* isolates (Table 3), but the landraces showed a broader efficacy compared to the cvs. Khiar and Karim, resulting in a significant isolate by cultivar/landrace interaction, indicating specific gene action (Table S1; Figure 1). Interestingly, for necrosis values were high and ranged between 72 and 97 % and hence these interactions were not observed (Figure 1). The parents of the developed recombinant inbred population, 'Agili 39' and cv. Khiar showed highly significantly different P values (ranging between 6-96%, respectively) and henceforward we selected a set of eight *Z. tritici* isolates that discriminated between 'Agili39' and cv. Khiar for subsequent phenotyping of the developed F6 RILs population (Table 2).

The seedling screening of the RILs resulted in non-symmetric frequency distributions. Overall, a skewed distribution was recorded, indicating substantial transgressive segregation towards susceptibility (Figure 2). Subsequent REML analyses revealed no significant difference between replicates and the RIL-by-isolate interaction term was large and highly significant, indicating that the observed variation in the data is accounted for by host and pathogen genes conditioning specificity (Table 4).

Table 3. Phenotypic response, in percentage pycnidia on the inoculated primary leaves, of durum wheat landraces and cultivars with 20 *Zymoseptoria tritici* isolates. Coloured cells indicate significant differences (LSDs; P=0.05) with resistant in green (not significantly different from 0P), intermediate in yellow (significantly different from 0P and maxP) and susceptible in red (not significantly different from maxP).

	IPO13001	IPO13018	IPO13003	IPO95002	IPO13008	IPO95003	Tun1	IPO92003	IPO13024	IPO13023	IPO13007	IPO13056	IPO13019	IPO1300	IPO91009	IPO91004	IPO95052	IPO91018	IIB-123	Tun6	Genotype Average
Agili 37	0	0	0	0	0	0	0	13	0	3	0	0	0	0	0	2	5	0	7	3	2
Agili 39	0	0	0	0	3	17	0	20	2	3	13	10	0	40	0	0	0	3	0	0	6
Agili 38	0	0	0	0	0	17	2	10	2	3	2	3	2	23	17	5	13	3	17	0	6
Sbei 99	0	2	0	0	0	0	0	10	0	0	27	0	0	17	3	2	3	10	3	57	7
Derbessi 12	0	0	5	0	5	2	20	13	0	17	2	13	8	0	0	0	0	43	2	0	7
Mahmoudi 101	2	3	0	2	2	0	0	0	0	7	13	5	2	0	0	13	43	43	30	2	8
JK 85	2	0	10	2	17	2	2	3	2	17	13	13	13	27	0	0	10	27	33	0	10
Azizi 27	0	2	0	0	0	0	3	0	2	5	0	3	2	0	20	27	60	30	63	5	11
Agili 41	2	10	10	2	3	2	0	10	0	5	40	13	27	15	47	77	40	67	43	97	25
Khiar	2	2	13	13	17	17	37	17	50	30	20	47	57	40	77	73	47	30	53	87	36
Karim	13	2	13	33	17	23	40	23	67	37	30	63	70	47	73	87	63	47	70	77	45
Isolate Average	2	2	5	5	6	7	9	11	11	12	15	16	16	19	22	26	26	28	29	30	

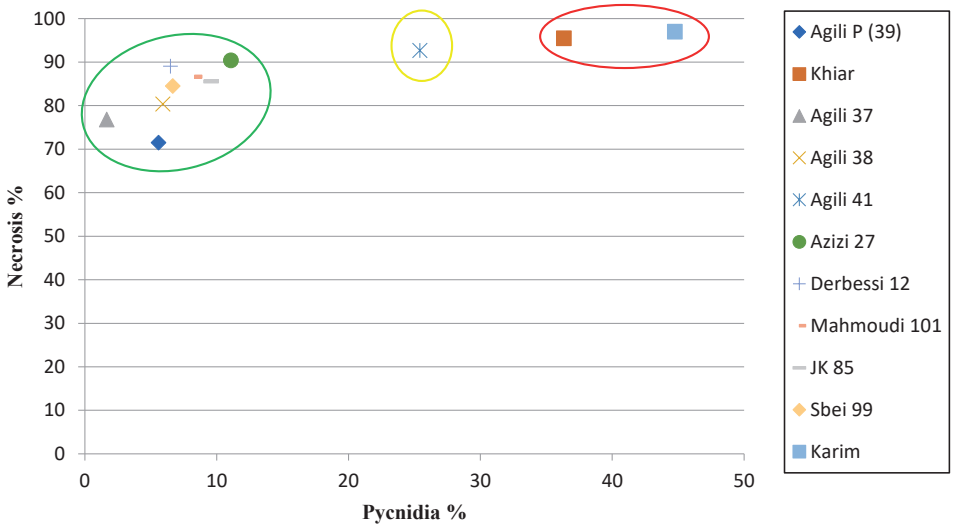


Figure 1. Scatter plot of necrosis and pycnidia of the durum wheat landraces and cultivars after inoculation with 20 *Zymoseptoria tritici* isolates. The three clusters show significantly different groups; green = not significantly different from 0P, yellow = significantly different from 0P and maxP and red = not significantly different from maxP.

Table 4. Results of REML analysis of necrosis (N) and pycnidia (P) seedling data from the Agili39/Khiar recombinant inbred lines (RILs).

Fixed terms	Wald statistic		df[1]	Wald/d.f.		Chi-square probability	
	N	P		N	P	N	P
Replicate	5.14	8.63	2	2.57	4.31	0.077	0.013
Isolates	5.14	12.02	7	0.86	2.00	0.522	0.061
RILs	1772.97	3314.45	157	11.29	21.11	<0.001	<0.001
Isolates x RILs	2087.60	3169.60	942	2.22	3.36	<0.001	<0.001

[1]df- degrees of freedom

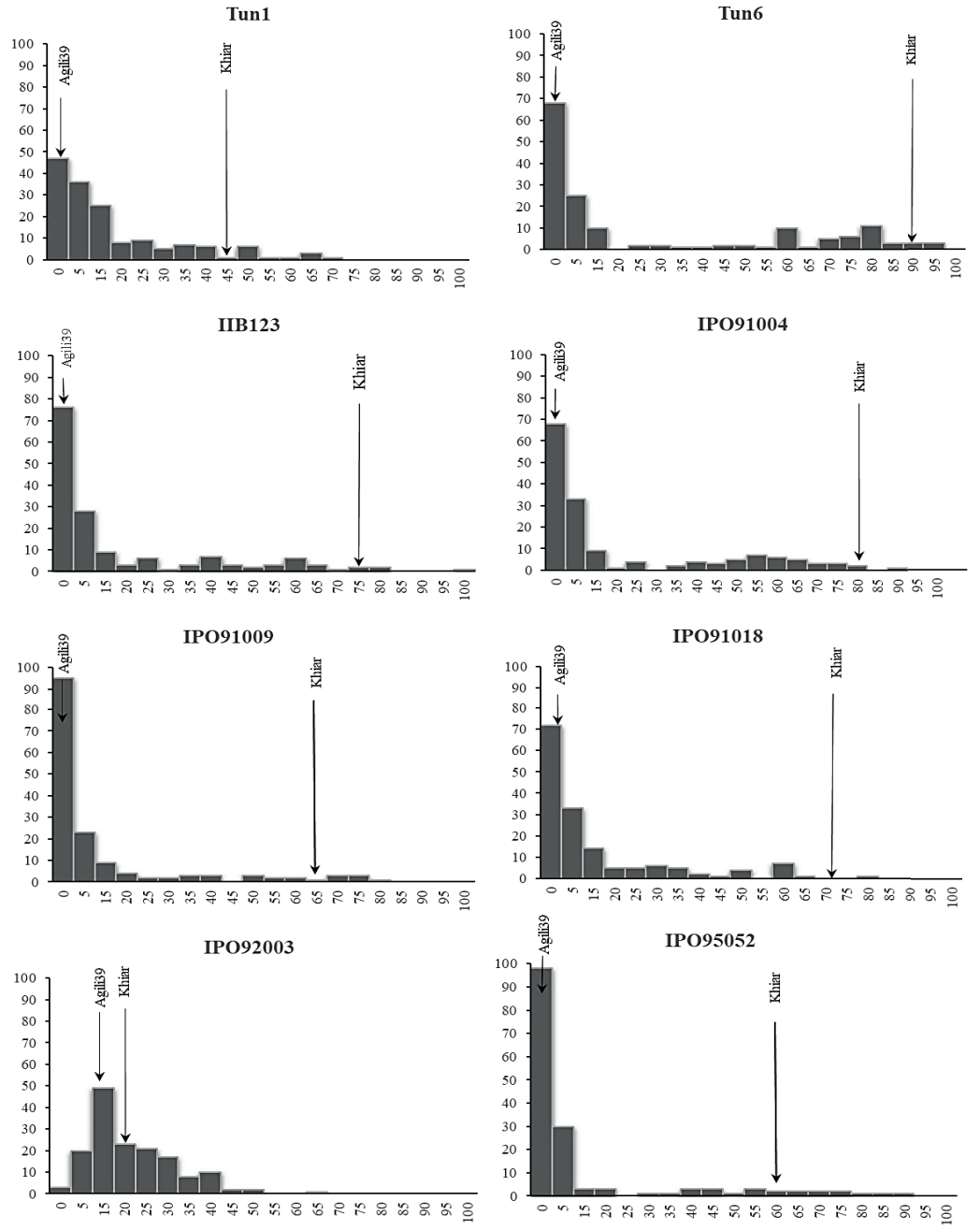


Figure 2. Distribution of the percent of the leaf area covered by pycnidia (x-axis) for the "Agili39"/Khiar recombinants inbred lines (y-axis) for eight *Zymoseptoria tritici* isolates at the seedling stage.

During all field trials, STB developed well after the inoculations, but only pycnidia coverage was assessed. Cultivar Khiar showed high disease severities throughout the trials (rate 4.1-5 for Tun6 and IIB123, and 3.1-4 for Tun1), whereas 'Agili 39' remained free of disease (0P) (Figure 3). The analysis of variance revealed no significant variation between blocks, indicating no variation in the micro-environment and the homogeneity of the field inoculation and - similar to the seedling assays – the interaction component was significant, indicating also specificity in the adult plant stage (Table 5). The F6-Tun6 data correlated well with subsequent F8 tests, which was also observed by comparing the F9 and F10 RILs after inoculation with isolates Tun6 and IIB123, respectively, indicating stable performance of the entries in the field assays across the years (Table S2). The comparative single isolate 2016 trial showed significant main effects for the tested RILs, indicating important differences in genetic make-up resulting in varying levels of STB severity (data not shown).

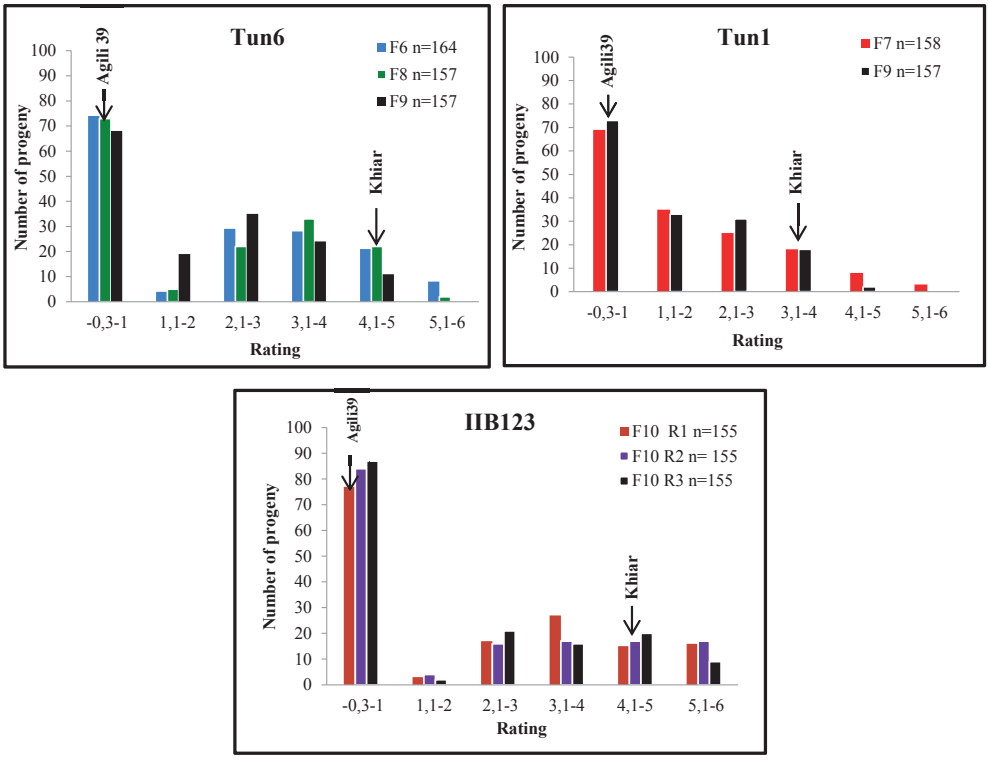


Figure 3. Frequency distributions of disease severity, assessed as percentage pycnidia and processed into pycnidia formation classes, in adult plants of the F6-F10 recombinant inbred lines of Agili39/Khiar with three *Zymoseptoria tritici* under field conditions.

Table 5. Analysis of variance for adult plant disease severity scores in the F6-F9 Agili39/Khiar recombinant inbred lines (RILs) that were inoculated with *Zymoseptoria tritici* isolates Tun1 or Tun6 *Z. tritici* isolates.

Source of variation	df[1]	MS[2]	P-value
Block	4	0.37333	0,408
Check	5	10.74141	<0.0001
Isolate	1	34.06406	<0.0001
RILs	153	5.33980	<0.0001
Isolate. RILs	153	3.04266	<0.0001
Error	593	0.37443	

R^2 = 98%.
[1]df = Degree of freedom
[2]MS = Mean of squares

Identification of quantitative trait loci for resistance to Zymoseptoria tritici in the Agili39/Khiar population at the seedling and adult stages

A genetic linkage map carrying 2,425 GBS markers with an average length of 88.10 cM and 30 linkage groups that represent the 14 chromosomes of durum wheat was used to map resistance/susceptibility to *Z. tritici*. The permutation test was used to define a significant threshold LOD value at 3.5, hence only QTLs with a LOD ≥ 3.5 were considered, which excluded all detected QTLs with isolate IPO92003. In total, we identified five significant QTLs on four chromosomes. None of these QTLs was mapped with every tested *Z. tritici* isolate, which underscores specificity of the interaction between *Z. tritici* and durum wheat. Two QTLs were identified on the long and short arm of chromosome 2B (Table 6; Figure 4). QTL-2BL was effective in both the seedling stage - particularly against isolates Tun6, IIB123, IPO91009, IPO95052 and IPO91004, but not for isolates Tun1, IPO91018 and IPO92003 - and adult plant stage, where it provided resistance to *Z. tritici* isolates Tun6 and IIB-123, but not to Tun1. Overall, the 2B-QTLs had the widest efficacy, the highest LOD values and the largest explained variance, particularly to Tun6 (LOD 32.57; explained variance 57.6%) (Table 6, Figure 4). Finally, three additional QTLs with lower LODs and explained variances were mapped on chromosomes 1A and 7AS and showed specificity for *Z. tritici* isolates Tun1, Tun6, IPO91018 and IPO95052 (Table 6). All abovementioned QTLs were derived from 'Agili 39', but a minor QTL on chromosome 2A, was contributed by cv. Khiar, and significantly increased disease

severity (Figures 5 and 6). We also noticed that only QTL-1A and QTL-2BL were effective to both N and P, whereas all others were detected for either N or P. The 1A (with IPO91018) and 7AS QTLs were specifically restricted to N in the seedling stage (Table 6).

Interactions between QTLs

Several *Z. tritici* isolates detected multiple QTLs. With *Z. tritici* isolate Tun6 we identified QTLs on chromosomes 1A, 2A, 2BL and 7AS, with isolate IPO91009 we detected QTLs on chromosomes 2A and 2BL and with IPO95052 we found QTLs on chromosomes 2BL and 7AS. We selected the three QTLs that were detected for P with isolate Tun6 on chromosomes 1A, 2A and 2BL in the seedling stage (Table 6) for two-way and three-way interaction tests. RILs lacking the 1A and 2BL markers had an average P score of 61.90%. RILs with the 1A QTL marker showed a significantly reduced P score of 21.13% and those with the 2BL QTL marker had an average P score of 5.76 %. Both QTLs showed a clear additive effect as the presence of markers for QTL-1A and QTL-2BL reduced STB severity to a P score of 2.3%. However, the presence of the marker for QTL-2A always significantly increased the P scores, except in the presence of QTL-2BL, showing a dominant effect of reducing disease severity (Figure 5). Hence, QTL-2A seems to be associated with a susceptibility locus derived from the susceptible modern cv. Khiar.

Finally, we investigated the three-way interactions between QTLs on chromosomes 1A, 2A and 2BL by calculating the average P scores of RILs carrying the identified markers for these QTLs in the various combinations (Figure 6). Absence of all marker resulted in a P score of 55.67%, whereas adding the QTL-2A maker significantly increased disease severity to a P score of 69.3%. Individual markers for the QTLs on chromosomes 1A and 2BL as well as their combination resulted in a significant disease reduction of P=3.17%. Addition of the QTL-2A marker again did not increase disease severity in this case, supporting the abovementioned observation that QTL-2BL has a dominant effect over QTL-2A.

At the adult plant stage, we mapped one QTL on each arm of chromosome 2B with *Z. tritici* isolates Tun6 and IIB-123 and one QTL on chromosome 2BS with isolate Tun1, which provides specific partial resistance to this isolate that is overcome by isolate Tun6. Absence of the 2BL and 2BS QTLs results in in high P scores for isolates Tun6 and IIB123, respectively 54.81% and 43% (Figure 7). The average P score of RILs with QTL-2BL is zero for both isolates. We could not determine the effect of QTL-2BS separately as no RILs were identified

that uniquely carries the associated marker, suggesting that both QTLs are closely linked, likely in the proximity of the centromere. Also, here QTL-2BL showed a dominant effect as adding the markers for QTL-2BS did not lower disease severity for isolate Tun6, but slightly increased the P score for isolate IIB-123 (Figure 7).

Table 6. Detected quantitative trait loci for necrosis (N) and pycnidia (P) in the seedling and adult plant stage in the Agili39/Khiar recombinant inbred population along with the percentage of explained variation, their physical chromosomal positions, associated molecular marker positions and their origin.

Chromosome	Isolate	LOD value			% explained variance			Position (cM)			Clone ID number		Source
		Seedling		Adult	Seedling		Adult	Seedling		Adult	Seedling	Adult	
		N	P	P	N	P	P	N	P	P			
1A	Tun1	7,49	5,79	-[1]	17.8	13,6	-	63,69	63,69	-	1127081	-	Agili39
	Tun6	-	4.58	-	-	9.4	-	-	63,69	-	1127081	-	
	IPO91018	4.53	-		13.1	-		63,69	-		1127081		
2A	Tun6	-	6	-	-	13.2	-	-	162,1	-	1247908	-	Khiar
	IPO91009	-	5.77		-	14.6		-	162,1		1247908		
7As	Tun6	4.96	-	-	13.7	-	-	52,82	-	-	2277193	-	Agili39
	IPO95052	3.71	-		10.3			52,82	-		2277193		
2BL	Tun6	9,17	17.67	32.57	23,8	38.4	57.6	73,93	73,93	73,93	1056626	1056626	Agili39
	IIB123	14,12	15.93	9.09	32,6	37	22.6	73,93	73,93	73,93	1056626	1056626	
	IPO91009	7,08	9.53		17,7	21.9		73,93	73,93		1056626		
	IPO95052	6,7	7.95		18,1	19.5		73,93	73,93		1056626		
	IPO91004	13,24	17.36		31,1	39.2		73,93	73,93		1056626		
2BS	Tun1	-	-	7.83	-	-	18.8	-	-	111,91	-	100031118	Agili39
	Tun6	-	-	25.74	-	-	42.4	-	-	111,91	-	100031118	
	IIB123	-	-	7.83	-	-	20.7	-	-	111,91	-	100031118	

[1]- = QTL not detected.

Figure 4. Region of the linkage map for chromosome 2B associated with *Zymoseptoria tritici* seedling and field resistances detected in the 'Agili39'/Khiar population. QTLs detected on chromosome 2B govern a wide spectrum of resistance. Markers linked to these QTLs are written in red. The centiMorgan (cM) distances between marker loci and the positions of marker loci are on the left and right sides of the linkage maps, respectively.

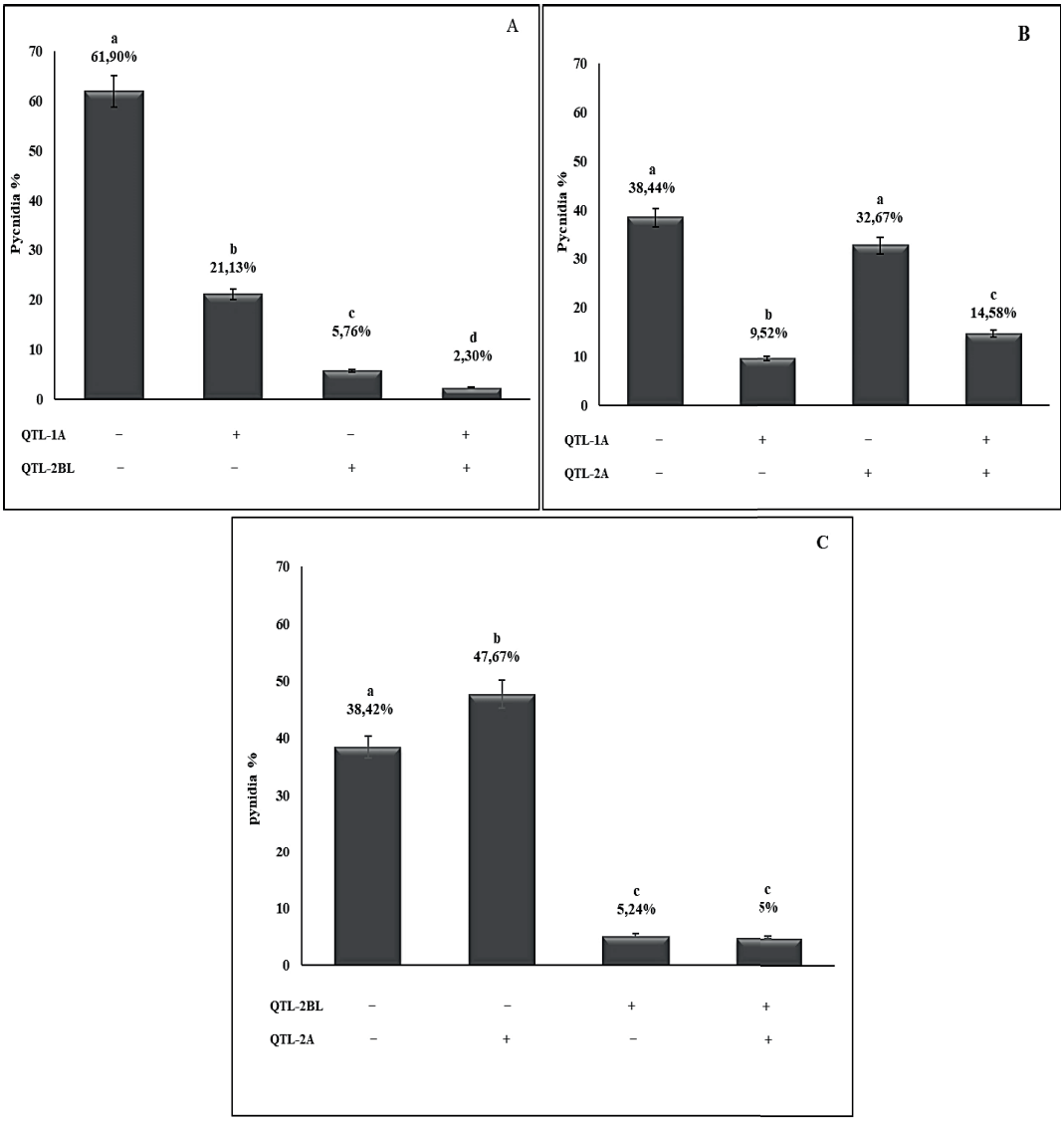


Figure 5. Two-way interactions between quantitative trait loci (QTL) controlling pycnidia formation (P) of *Zymoseptoria tritici* isolate Tun6 in the seedling stage in the Agili 39/Khiar F6 recombinant inbred population determined by the presence/absence of markers for QTLs on chromosomes 1A, 2A and 2BL. A: QTL-1A and QTL-2BL have additive gene action. Each QTL contributes to reduced disease severity, but their combination shows least disease; B: QTL-1A and QTL-2A show epistatic gene action. Presence of QTL-2A does not significantly reduce P, but the presence of QTL-1A does lower P. Addition of QTL-2A increases the susceptibility of lines carrying QTL-1A; C: A similar trend as in (B), presence of QTL-2A increases susceptibility, but not in the presence of QTL-2BL, which shows a dominant effect.

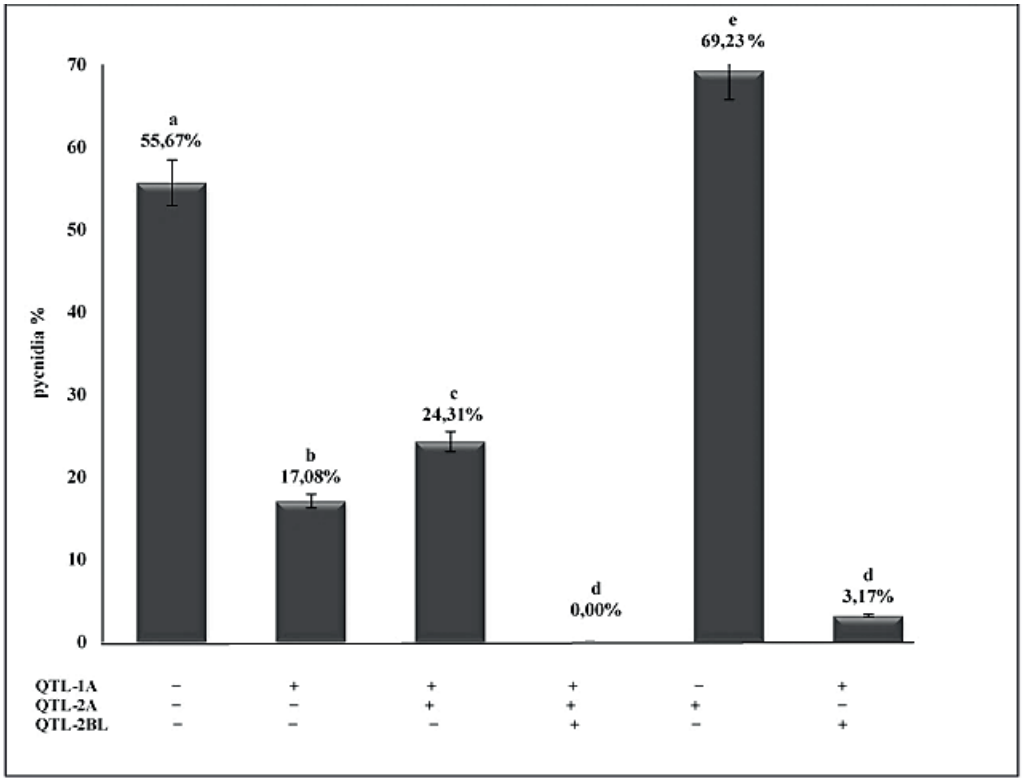


Figure 6. Three-way interactions between quantitative trait loci (QTL) controlling pycnidia formation (P) of *Zymoseptoria tritici* isolate Tun6 in the seedling stage in the Agili 39/Khiar F6 recombinant inbred population determined by the presence/absence of markers for QTLs on chromosomes 1A, 2A and 2BL. QTLs on chromosomes 1A and 2BL have strong and significantly different reducing effects on disease severity, whereas their combination results in the lowest disease severity, independent of the disease promoting QTL on chromosome 2A. The latter is particularly significant in two-way combinations with QTLs on chromosomes 1A or 2BL, confirming that the QTL on chromosome 2BL has a dominant gene action.

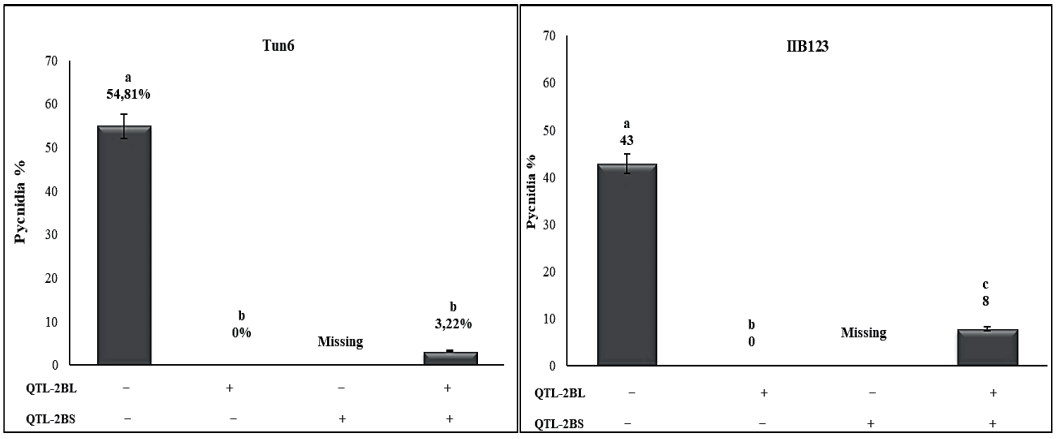


Figure 7. Two-way interactions, determined by the presence/absence of markers for the quantitative trait loci that were detected on chromosomes 2BL and 2BS with *Zymoseptoria tritici* isolates Tun6 and IIB-123 in Agili 39/Khiar F6, F8, F9 (Tun6 inoculated) and F10 (IIB-123 inoculated) recombinant inbred lines in the adult plant stage in the field. Both QTLs have a significant reducing effect on disease severity, but their combination shows least disease severity with isolate Tun6, whereas the presence of the QTL on chromosome 2BS slightly increases disease severity.

Discussion

Cereal diseases threaten food security (Roelfs and Bushnell 2014). Increasing demand and changing climatic conditions cause extreme events that strongly affect yield stability (Newton et al. 2011; Strange and Scott 2005). *Zymoseptoria tritici* is a major threat to European and Mediterranean bread and durum wheat production (Fones and Gurr 2015). However, the emphasis in research has been skewed towards bread wheat (Brown et al. 2015).The complexity of mapping resistance genes in durum wheat is twofold; (i) the majority of durum wheat varieties is highly susceptible to *Z. tritici* and (ii) mapping resistance genes requires using specific isolates with pathogenicity to durum wheat as the majority of bread wheat derived *Z. tritici* isolates is non-pathogenic on durum wheat. Therefore, we made an effort to investigate the genetics of resistance by collecting and using *Z. tritici* strains from durum growing countries after determining that *Z. tritici* has a typical dichotomy in pathogenicity for either bread or durum wheat (Kema et al. 1996a; Ware 2006). Here, we report effective QTLs mapped for resistance to STB in durum wheat after exploring diversity for resistance in a suite of Tunisian landraces. We then furthered these studies by genetic analyses that revealed five QTL on four chromosomes. All data indicated and confirmed significant host x pathogen interactions - both

in the seedling and adult plant stages - as determined in earlier studies (Arraiano et al. 2001; Kema et al. 1996a; Kema et al. 1996b); and recently proven in the bread wheat – *Z. tritici* pathosystem where both *Stb6* and *AvrStb6* were cloned (Kema et al. 2017; Zhong et al. 2017).

Four of the QTL affected disease severity towards specific isolates under seedling and adult plant conditions in multiple field trials, but one QTL on chromosome 2A significantly contributed to susceptibility and was derived from the modern cv. Khiar. Resistance breeding has been associated with removing such alleles (Arraiano and Brown 2016) and hence negative selection for the identified marker could help to increase disease resistance as shown in all interactions between the resistance QTL on chromosomes 1A, 2BS and 2BL. In addition we also determined QTLs that are specific for either the seeding or adult plant stage as well as a QTL that was expressed at both stages, similar to other cereal diseases , namely to rust (Hou et al. 2015; Lin et al. 2014). Overall, our data contrast the observations and conclusions of Van Ginkel and Scharen (1988) who concluded that resistance to *Z. tritici* in durum wheat is explained by additive gene action and general combining ability, thus denying specificity. Since the cloning of the first *Stb* gene and the corresponding avirulence effector, the controversy - see also (Johnson 1992) and (Parlevliet 1993) as well as many other studies (Ben M'Barek et al. 2015; Kema et al. 2000; Mirzadi Gohari et al. 2015; Zhong et al. 2017) - on specificity in the *Z. tritici* – wheat pathosystem has finally ended.

The initial screening of the Tunisian landraces showed a remarkable genetic diversity for STB resistance. Eight landraces (Agili 37; Agili 38; Agili 39, Sbei99; Derbessi 12, Mahmoudi 101, JK85 and Azizi 27) were highly resistant and one landrace showed an intermediate response ('Agili 41'). The different 'Agili' landrace accessions reacted differently to the deployed *Z. tritici* isolates, suggesting a different genetic background, which is in accord with Ferjaoui et al. (2015) who hypothesized that the tested 'Agili' accessions most likely carry different *Stb* genes. Our data, however, contrast the findings of these authors, which is most likely due to the fact that we used different and many more *Z. tritici* isolates, underscoring the specificity in the durum wheat pathosystem (Ghaneie et al. 2012; Kema et al. 1996a; Kema et al. 1996b; Medini and Hamza 2008).

We eventually selected 'Agili 39' for a detailed analysis of the genetic basis of its resistance to *Z. tritici* by crossing it to cv. Khiar, a contemporary high yielding durum wheat developed by INRAT (Tunisian National Research Institute of Agronomy) and CIMMYT in 1992, formerly reported to be susceptible to *Z. tritici* (Ferjaoui et al. 2015; Ferjaoui et al. 2011; Gharbi et al. 2008; Medini et al. 2014). Previously, Ferjaoui et al. (2011) reported a single gene

determining the adult plant resistance in 'Agili 39' to Tun6 under field conditions based on F_2 and F_3 analyses. Our data do not support that conclusion as we identified two QTLs on the short and long arm of chromosome 2B that are required for adult plant resistance. One of these, QTL-2BL was also expressed at the seedling stage - for both N and P - and is a major determinant of resistance to five *Z. tritici* isolates, including Tun6 with a LOD value of 17.67 and explained variance of 38.4, IPO91004 with a LOD of 17.36 and an explained variance of 39.2 and IIB-123 with a LOD value of 15.93 and explained variance of 37%. The remaining two QTLs for seedling resistance on chromosomes 1A and 7AS had lower LOD values, explained the observed variance to a lesser extent and were effective for either N or P, except for QTL-1A for Tun1. However, it clearly demonstrates that the broad efficacy of the observed STB resistance in 'Agili 39' is due to several stacked QTLs, both for the seedling as well as the adult plant stage, which was also commonly observed in inheritance studies in bread wheat (Brown et al. 2015; Ghaffary et al. 2012). Despite the susceptibility of cv. Khiar, transgressive segregation towards resistance was also observed for several isolates, which is likely due to the absence of the susceptibility QTL on chromosome 2A in some RILs. Recently, (Arraiano and Brown 2016) reported three alleles on chromosomes 3A, 6A and 2D that contribute STB susceptibility in a suite of cultivars bred and released by the former Plant Breeding Institute (PBI, Cambridge, UK) between the mid-1960s and the mid-1980s. Introduction of these alleles may be a trade-off from using germplasm to increase yield, rust resistance and eyespot resistance in UK wheat breeding programs between the 1950s and 1980s. The overall STB susceptibility in durum wheat may also result from such events. Cultivar Khiar was generated from a cross between Chen "S" and Altar 84 – CD 57 005-1Y-2B-5Y-1M-0Y and was successfully introduced to Tunisia because of its high yield potential (Gilchrist et al. 1999; Maccaferri et al. 2005).

For the four QTLs contributing to resistance, we compared the linked GBS markers with the publically available database 'Ensemble genome' by aligning them to the reference genome of *Triticum aestivum*
(http://plants.ensembl.org/Triticum_aestivum/Info/Index?db=core.)
A QTL for resistance to *Z. tritici* was mapped on chromosome 1A by Goudemand et al. (2013) in the bread wheat Apache/Balance population, which could co-localize with the QTL we mapped in the 'Agili 39'/Khiar population. However, we cannot discern whether the identified 1A-QTL in the 'Agili 39'/Khiar population is the same as in the Apache/Balance population, as the sequences of the linked markers in the Apache/Balance population are not publicly

available. The 7AS-QTL particularly conferred reduced necrosis values to *Z. tritici* isolates IPO95052 and Tun6 and co-localizes with *Stb3* that was mapped in the bread wheat cultivar Israel 493 (Adhikari et al. 2004b; Goodwin et al. 2015). Also, markers for the chromosome 2BL QTL co-align with the known major gene *Stb9* that was mapped in the French bread wheat cv. Courtot (Chartrain et al. 2009). The 2BL-QTL is effective in the seedling and adult plant stages, whereas *Stb9* in cv. Courtot is mostly ineffective in the seedling stage (Chartrain et al. 2009; Goudemand et al. 2013; Tabib Ghaffary 2011). Nevertheless, we conclude that it is likely that the 2BL-QTL is identical with *Stb9*, but acts differently in a tetraploid background. Finally, chromosome arm 2BS was also associated with *Z. tritici* resistance in the mapping populations Apache/Balance and FD3/Robigus (Goudemand et al. 2013) for both necrosis and pycnidia development in the adult plant stage. However, due to the unavailability of the GBS tag sequences, we cannot conclude that the 'Agili39'/Khiar 2BS-QTL is the same locus that was mapped in the aforementioned bread wheat mapping populations.

Thus, the identified QTLs in 'Agili 39' co-align with previously mapped QTLs for STB resistance in bread wheat, hence we cannot claim a new *Stb* gene in 'Agili 39'. However, we clearly have identified the first QTLs conferring resistance to a wide range of *Z. tritici* isolates under artificial inoculation conditions in seedlings and adult plants, known as field resistance (Arraiano et al. 2009; Ghaffary 2011). Thus far, only partial resistance to *Z. tritici* was reported (Berraies et al. 2014; Tuberosa 2014). Here, we derived a QTL from "Agili 39" that provides resistance to at least five *Z. tritici* isolates, but likely more.

Pyramiding genes for disease resistance has been an effective strategy in preventing boom-and-bust cycles, and is now amenable through marker assisted breeding as a strategy to maintain disease resistance durability, such as for wheat stem rust where various resistance gene combinations have well controlled the disease since the mid-1950s and more recently to the devastating Ug99 race (Mundt 2014; Singh et al. 2011). A concrete illustration for *Z. tritici* is effective resistance to a wide range of isolates in the bread wheat germplasm 'KK4500' and 'TE11' which is conferred by stacking several known *Stb* genes (Chartrain et al. 2005a; Chartrain et al. 2005b; Chartrain et al. 2004) and also in other germplasm several QTLs have contributed to broad efficacy of resistance (Ghaffary et al. 2011). Our data also confirm that stacking QTLs in durum wheat results in broad efficacy of STB resistance. The QTL interaction analyses clearly showed the additive effect of most QTLs, which is promising news for durum wheat breeding because deciphering ancient broad-based resistance to *Z. tritici* in a durum wheat landrace now indicates that negative selection for the 2A-QTL along with positive

selection for the other markers may result in new high yielding durum wheat cultivars with wide resistance to *Z. tritici* reminiscent of the durable resistance to STB in landraces. Provided the overall high susceptibility to STB in modern durum wheat cultivars, our data shed new light on disease resistance breeding in durum wheat.

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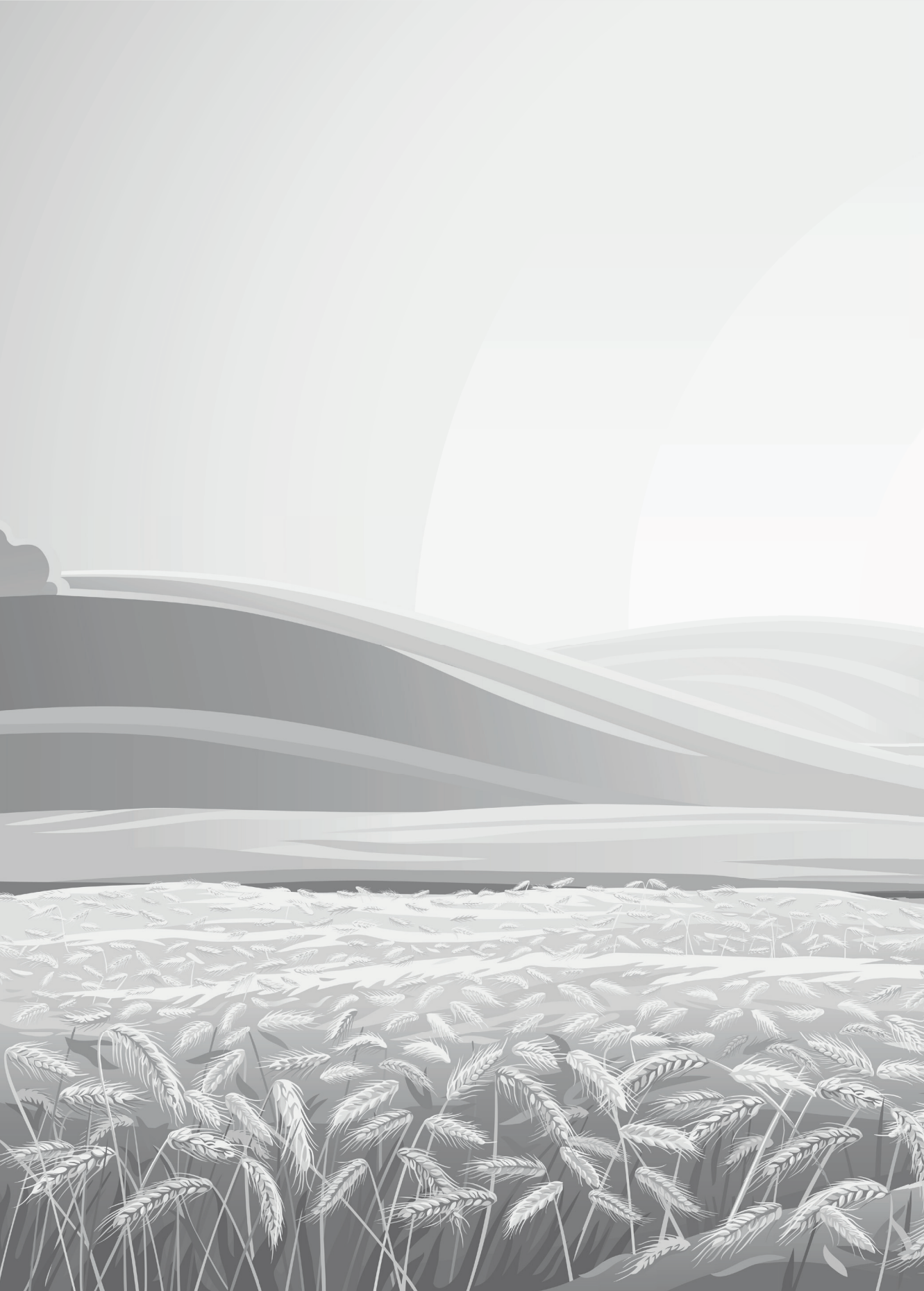
Supplementary Information

Table S1. Analysis of variance of pycnidia percent of nine Tunisian durum landraces and two modern varieties inoculated with a diverse range of twenty durum derived *Zymoseptoria tritici* isolates

Fixed terms	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Isolate	19	54958	2893	97.16	<2e-16	***
accessions	10	69442	6944	233.26	<2e-16	***
Isolate x accessions	119	69149	581	19.52	<2e-16	***
Residuals	221	6579	30			

Table S2. Adult plant correlations of the Agili39/Khiar RILs tested with the Tun6 and IIB123 Z. tritici isolates under field conditions for five years (2011-2016)

RIL generation /Z.tritici isolate / Year	F6/Tun6/2011	F8 /Tun6 /2013	F9 /Tun6/-2014	F10/IIB123/ 2016
F6/Tun6/2011	1			
F8/Tun6/2013	0,8	1		
F9/Tun6/2014	0,8	0,8	1	
F10/IIB123/2016	0,4	0,5	0,5	1



Chapter 5

Resistance to Zymoseptoria tritici in elite durum wheat is defined by major and minor, breeding-exploitable quantitative trait loci

Lamia [§] Aouini , Marco [§] Maccaferri, Elisabetta Frascaroli, Antonio Prodi, Roberto Tuberosa, Sandra Stefanelli, Simona Corneti, Paola Mantovani, Andrea Massi, Mahmoud Sayed Tabib Ghaffary, Els CP Verstappen, Richard GF Visser, Gert HJ Kema

§These authors contributed equally

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Abstract

Zymoseptoria tritici causes septoria tritici blotch (STB), a primary biotic threat for durum wheat production in the Mediterranean Basin, the cradle of durum wheat. Here, we report on the dissection of STB resistance in two bi-parental recombinant inbred line (RIL) mapping populations derived from crosses between the Italian cultivars Simeto and Levante as well as between the premium quality Italian durum cv. Svevo and the DesertDurum® cv. Kofa. We assessed the RILs for STB response to four *Z. tritici* isolates in the greenhouse and under field conditions. QTL analysis revealed that STB resistance resulted from the additive effect of several loci providing various levels of quantitative resistance. This involved some novel genetic regions, including those on chromosomes 6B and 4B. Two QTLs identified in the Simeto/Levante population on chromosome 4B conferred resistance to four *Z. tritici* isolates, both in the greenhouse and in field trials. In addition, in the Kofa/Svevo population we identified loci mapped closely to known *Stb* resistance genes such as *Stb2* that conferred resistance at adult plant and seedling stages. Our study demonstrates that contemporary durum wheat cultivars are sources of novel, quantitative and partial resistance alleles that can be cumulated by targeted breeding in segregating progenies to enhance STB resistance.

Key words: durum wheat, recombinant inbred lines, septoria, *Zymoseptoria tritici*, durable resistance, QTL, SNP, Marker-Assisted Selection.

Introduction

Food security has always been a major concern in many civilizations. Hence, increasing the resilience of staple crops to biotic and abiotic stresses in diverse environments is key to enhance food security. Wheat is the third staple crop after rice and maize (FAOSTAT 2017). Over 600 million tons of wheat are harvested each year (Shewry 2009), and its global production mainly relies on hexaploid bread wheat or *Triticum aestivum* L. (2n = 6x = 42, AABBDD genomes) and tetraploid durum or pasta wheat or *T. turgidum* L. ssp. *durum* (2n = 4x = 28; AABB genomes). In terms of cultivated land, bread wheat occupies approximately 90% of the global production area, whereas durum wheat (Dixon et al. 2009) covers not more than 8%, with an estimated production of 36 million tons (Magallanes-López et al. 2017) of which about 35% are produced in North Africa and West Asia, 25% in North America, 30% in the EU and 10% in India. Nonetheless, the global durum wheat area does not reflect its importance as a staple crop in the Mediterranean Basin, the West Asian and the North African

countries, the so-called WANA region, where durum wheat plays a key role in classical farming practices and is the basis for many typical dishes such as pasta, couscous and burgul. Even though 75 and 50% of the worldwide acreage and production, respectively, are concentrated in the Mediterranean Basin, the production of durum wheat has recently expanded to southern and Central Asia underlining its importance as a regional staple crop (Baloch et al. 2017). Furthermore, durum wheat has a historical legacy for wheat diversity, including a range of sub-species that were widely cultivated across the globe for thousands of years (Newton et al. 2010).

Regardless of its historical and dietary significance, durum wheat has received far less attention compared to the bread wheat from the scientific community (Royo et al. 2007). The limited scientific efforts and the so-called 'genetic erosion' underlined by several studies (Maccaferri et al. 2005; Soleimani et al. 2002) gave rise to a higher vulnerability of durum wheat germplasm to the majority of biotic constrains, in particular to fungal diseases such as Fusarium head blight and leaf rust (Prat et al. 2017).

Septoria tritici blotch (STB) caused by the foliar fungal pathogen *Zymoseptoria tritici* (formerly *Mycosphaerella graminicola*) is no exception and the same case scenario has been noticed and reported for the durum wheat-*Z. tritici* interaction (Brown et al. 2015). This devastating fungus has caught the attention of the scientific community after damaging epidemics occurred in North Africa between 1968 and 1969, in coincidence with the introduction of novel semi-dwarf cultivars and the intensive use of fertilizers (Saari and Wilcoxson 1974). Even though precise grain yield losses due to STB are still not precisely defined, loss reports have always claimed to range between 35 to 50% under conducive conditions (Ponomarenko et al. 2011). Thus far, disease management has largely relied on chemical control and more recently on selection of resistant wheat varieties once *Stb* genes were discovered and mapped (Brown et al. 2001; Orton et al. 2011), particularly after elucidating the *Z. tritici* – wheat interaction and the underlying genetics (Kema et al. 1996a; Kema and van Silfhout 1997; Orton et al. 2017). However, effectiveness of the chemical control has rapidly declined with the frequent and the rapid emergence of fungicide resistance (Cools and Fraaije 2008; Torriani et al. 2009; Torriani et al. 2015), mainly due to the high adaptability of the *Z. tritici* populations and to its high genome plasticity (Goodwin et al. 2011). Furthermore, the accessibility of chemical control is restricted by its high cost for smallholder farmers in less developed regions (Altieri 2004; Sahri et al. 2014) and for European growers by new EU

regulations restricting fungicide use (Kettles and Kanyuka 2016). Since the increased incidence over the years, STB eventually became one of the major wheat diseases in Europe, hence breeding for resistance was prioritized and research intensified (Fones and Gurr 2015) leading to the identification of 21 major genes (*Stb*) and to 167 quantitative trait loci (QTLs) associated with *Z. tritici* resistance (Brown et al. 2015). However, most studies that led to the identification of major resistance genes and more recently QTLs for partial resistance only targeted bread wheat (Brown et al. 2015; Dreisigacker et al. 2015; Mirdita et al. 2015), leaving fewer resources for understanding the basis of resistance in durum wheat. The reported dichotomy and speciation in the *Z. tritici* populations (Kema et al. 1996a; Kema and van Silfhout 1997; Ware 2006) prevented the use of well-defined *Z. tritici* isolates for gene postulation in durum wheat, further limiting resistance breeding in durum wheat. Nonetheless, few efforts to dissect STB resistance in contemporary tetraploid wheats and durum landraces showed an abundance of partial resistance in modern cultivars (Gharbi et al. 2000; Gharbi et al. 2008; Kidane et al. 2017). More recently, the presence of loci for quantitative STB partial resistance has been studied using mapping by association in modern durum wheat germplasm (Maccaferri et al. 2010) and recombinant inbred line (RIL) populations from the cross between the susceptible high-yielding cv. Karim and the moderately resistant cv. Salim (Berraies et al. 2014). Partial resistance to STB was also reported in Italian durum wheat germplasm (Tuberosa 2014). The first major QTL for STB resistance in durum wheat was identified in the Tunisian landrace 'Agili' (Ferjaoui et al. 2015; Ferjaoui et al. 2011), which was recently mapped on chromosome 2B by Ferjaoui et al. (Personnal communication).

The current study focused on analyzing STB resistance in well-adapted and high-yielding contemporary durum wheat cvs. Simeto, Levante, Kofa and Svevo using a range of *Z. tritici* isolates in greenhouse assays as well as in field trials. We show that the inheritance of resistance to STB in contemporary durum wheat germplasm is complex and results from the additive effects of multiple *Z. tritici* strain-specific major and minor effect QTLs.

Materials and Methods

Plant materials and Zymoseptoria tritici isolates

Two bi-parental mapping populations were obtained from crosses between the durum wheat cvs. Simeto and Levante and between cvs. Kofa and Svevo (Table 1), all considered as modern, semi-dwarf and highly productive cultivars. Cultivar Simeto (pedigree Capeiti 8/Valnova) was released by "Stazione Sperimentale di Granicoltura per la Sicilia", Caltagirone,

Italy in 1974. This cultivar has been derived from the local Mediterranean germplasm (North African and Syrian origin), with Valnova parent considered as representative of the innovation based on the first successful introduction of the *Rht-B1b* semi-dwarf allele from the early Mexican germplasm (Vallega and Zitelli 1974). Simeto has been the Italian reference for the first generation of semi-dwarf, early-heading genotypes and is characterized by high end-use quality (semolina) and a well-defined adaptability to Central-Southern Italy and Mediterranean countries, such as Spain and Greece. Levante is a modern high-yielding Italian durum wheat bred from the North American germplasm introduction, renowned for its excellent agronomic features, pasta quality and SBCMV resistance (Maccaferri et al. 2012; Rubies-Autonell et al. 2009). The third Italian cv. Svevo was derived from a cross between a CIMMYT line (pedigree rok/fg//stil/3/dur1/4/sapi/ teal//hui) related to the widely utilized Yavaros79 genetic background (Jori/Anhinga//Flamingo) and cv. Zenit, originating from a cross between Italian and American accessions (Valriccardo/Vic). Desert Durum® cv. Kofa originates from the United States and was derived from a population based on multiple parents (dicoccum alpha pop-85 S-1) mainly related to US and CIMMYT germplasm, with the inclusion of emmer accessions, and was released by Western Plant Breeders (Arizona, US). Both cvs. Kofa and Svevo are well adapted to the Mediterranean climate and can be classified as early-flowering genotypes in such conditions (Maccaferri et al. 2008). The Simeto/Levante population was developed by single seed descent (SSD) up to the F5 generation in the greenhouse and subsequently, a single spike from each F6 family was harvested and sown in the field as single spike/row progeny to produce F7 RIL foundation seed and DNA for the molecular analysis (Maccaferri et al. 2012). The Kofa/Svevo population of 249 RILs was developed by "Società Produttori Sementi" (Bologna, Italy), through SSD from (Maccaferri et al. 2008).

All parents were pre-screened at the seedling stage with 22 *Z. tritici* isolates obtained from durum wheat and randomly selected from the available collection at Wageningen University and Research (WUR). Eventually, four isolates were used to study the response of the RIL populations (Table 2).

Seedling assays

- *Plant and* Zymoseptoria tritici *management, growth conditions and inoculation procedures*

Seedlings of the four parents were sown in VQB TEKU® 7x7x8 cm plastic pots, using five seeds per pot, whereas smaller, round peat pots (Jiffy, Moerdijk, Netherlands) were used

to accommodate all RILs, also with five seeds per pot, of the Simeto/Levante and Kofa/Svevo populations. Parents and RILs were sown in a special mixture for growing seeds (Substraat Zaai) provided by Unifarm, WUR, The Netherlands and maintained in a greenhouse with a similar pre- and post-inoculation environment with respect to light (son-T Agro 400 W lamps) and day length (16/8 h light/ dark). Temperature and relative humidity (RH) were adjusted for 10 days to 18/16 °C (day/night) and 70%, respectively, prior to inoculation. Post-inoculation conditions, including 48 h incubation, were set at a temperature of 22/±2 °C and a RH > 95%. Ten days after inoculation, all seedlings were trimmed for the second and subsequent leaves to enable sufficient light on the inoculated primary leaves for appropriate disease development. Fertilizer (Sporumix PG®, a commercial fertiliser containing microelements, Rotterdam, Netherlands; 0.5 g L^{-1}) was applied to maintain healthy plant conditions.

Z. tritici isolates were pre-cultured in autoclaved 100 ml Erlenmeyer flasks containing 50 ml yeast glucose (YG) liquid medium (30 g glucose, 10 g yeast per liter of demineralized water). The flasks were inoculated with frozen isolate samples directly taken from the -80 °C maintained collection and subsequently placed in an incubated rotary shaker (Innova 4430, New Brunswick Scientific, USA) set at 125 rpm and 15 °C for 5-7 days. These pre-cultures were then used to inoculate 1L Erlenmeyer flasks containing 500 ml YG media per isolate that were incubated under the aforementioned conditions to provide sufficient inoculum for the seedling inoculation assays at growth stage (GS) 11 (Zadoks et al. 1974). Spores were collected, and the inoculum concentration was adjusted to 1×10^7 spores ml^{-1} in a total volume of 40 ml for a set of 18 plastic pots or 24 Jiffy® pots and supplemented with two drops of Tween 20 surfactant (MERCK®, Nottingham, UK). Inoculations were conducted by spraying the spore suspension over the seedlings placed in an inoculation cabinet on a rotary table, adjusted at 15 rpm, which is equipped with interchangeable atomizers and a water-cleaning device to avoid cross-contamination. Infected plants were incubated in transparent plastic bags for 48 h under 100% RH in the greenhouse.

The same inoculation protocol reported above was applied to assay in the growth chamber the Simeto/Levante and Kofa/Svevo recombinant inbred line populations with four selected isolates and three replicates per RIL population.

- *Experimental design, data collection and statistical analysis*

The Simeto/Levante and Kofa/Svevo RILs were tested in three independent replicates in time following a split-plot design with isolates as main plots and genotypes as subplots. Subplots were randomly arranged for each isolate and replicate on greenhouse tables in trays

each containing 54 subplots. The parents were included throughout all replicates and trays as checks. Disease severities were evaluated by assessing the quantitative presence of necrosis (*NEC*) and pycnidia (*PYC*) on the inoculated leaves in percentages at three stages post-inoculation, at 15, 18 and 21 days post-inoculation (dpi), which enabled the calculation of the Area Under the Disease Progress Curve (AUDPC) values (Shaner and Finney 1977). Performing three consecutive disease severity assessment allowed us to obtain more precise estimates as compared to recording the disease severity at the end of disease cycle (21 dpi) only.

For the initial parental screening experiment, analysis of variance (ANOVA) was conducted on *NEC* and *PYC* scores of the tested parents based on the mean values of the experimental units and Fisher's Least Significant Difference (LSD) was used to detect significant disease severity differences among parents at a 0.05 probability. For the RIL experiments, ANOVA was performed based on mixed linear models built in PROC MIXED in SAS v. 9.3 (SAS Institute Inc., Cary, NC, USA). Initially, the whole experiment, including the four isolates, was jointly analyzed for each RIL population. The mixed model included replicates, isolates, isolates × replicates, genotypes, isolates × genotypes as random effects, trays as random nested within isolates × replicates. Subsequently, data from each isolate (specifically selected to maximize the differential response between parents, therefore to be considered as fixed effects) were analyzed separately. The model included replicates and genotypes as random effects, trays as random nested within replications. Estimates of variance component were obtained using the Restricted Maximum Likelihood (REML) method. Repeatability (h^2) within each isolate was computed based on the variance components using the following formula:

$$h^2 = \sigma^2_G / [\sigma^2_G + \sigma^2_e / n]$$

where σ^2_G is the variance component due to genotypes, σ^2_e is the variance due to residual effcts and n is the number of replicates per isolate.

For subsequent QTL analysis, Best Linear Unbiased Estimators (BLUEs) for RILs were calculated for each population and each isolate considering genotypes as fixed effects.

Field trials

- *Field experimental design; inoculation procedures and data collection*

Field experiments to assess the RILs response to STB at adult plant stages were conducted at the experimental station of the University of Bologna located in Cadriano (44°35' N 11°27' E), Po Valley, Italy, in the 2010, 2013 and 2014 seasons. The Kofa/Svevo RIL

population was evaluated together with the two parents in 2010 under high natural inoculation, and in 2013 under artificial inoculation (isolate IPO92003) while the Simeto/Levante RIL population, together with its two parents and susceptible checks, was evaluated in 2014 under artificial inoculation (isolate IPO92003).

In all experiments, the genotypes were evaluated as 2-m-long double row (twin) plots and experimental fields were equipped with a mist supplementary irrigation that aided the disease spread and development in flag leaves after inoculation. The mist irrigation was applied starting one week before inoculation until disease assessment. A randomized complete block design was adopted in all trials with two replicates for the Kofa/Svevo population and three replicates for the Simeto/Levante population.

Artificial inoculation was carried out according to Ghaffary et al. (2011) on adult plants with developed flag leaves at the booting and early heading stages. Heading date and plant height were recorded and used as covariates in the ANOVA model and to obtain adjusted BLUEs for the RILs. Disease severity assessment and AUDPC computation were carried out as reported for seedling experiments. The statistical analysis was performed using PROC MIXED in SAS v. 9.3 (SAS Institute Inc., Cary, NC, USA). The mixed linear model included heading date and plant height as covariates, genotypes and replicates as random effects, field columns and rows as random nested within replicates.

Additionally, for the Kofa/Svevo population only, a combined analysis of the two years was carried out in a model including heading date and plant height as covariates, genotypes as random, years, replicates nested within years, field columns and rows nested within replicates and genotype x year as random. Adjusted BLUEs for the RILs were obtained by considering genotypes as fixed.

QTL analysis

The two dense linkage maps included up to 5,000 markers as follows: i) simple sequence repeats, SSRs (Maccaferri et al. 2003; Röder et al. 1998), Diversity Array Technology markers (DArT®, Yarralumla, Australia; (Mantovani et al. 2008) and Illumina transcript-derived single nucleotide polymorphism (SNP) markers (Maccaferri et al. 2015; Wang et al. 2014). Up to 13,823 SNPs were informative in a durum wheat panel and were genetically ordered based on a SNP consensus map (Maccaferri et al. 2012; Maccaferri et al. 2015; Maccaferri et al. 2008) (Table1). QTL analyses were computed based on the BLUEs of the AUDPC of *NEC* (=N_{audpc}) and of the relative *PYC* AUDPC square root (=P_{audpc}), hereafter referred as N and P, respectively, and analyses were performed using the MapQTL® 6.0 software (Van Ooijen

2009). QTLs were initially positioned based on interval mapping (IM) analyses, followed by a multiple QTL model (MQM) analysis after cofactor selection by manually investigating the marker alignment on the linkage groups where the peaks of IM QTLs were detected. A permutation test with 1,000 permutations was conducted to determine the critical logarithm of the odds (LOD) threshold for each *Z. tritici* isolate in each mapping population. All identified QTLs were projected on a consensus map comprising 30,144 markers (including 26,626 SNPs and 791 SSRs) and spanning 2,631 cM (Maccaferri et al. 2015) by using the BioMercator V4.2 ® software (Arcade et al. 2004; Sosnowski et al. 2012), (https://urgi.versailles.inra.fr/Tools/BioMercator-V4). Two input files, a map file and a QTL file, were prepared for the BioMercator V 4.2 ® software according to its requirements. The QTL profiles were plotted against their LOD values and drawn using MapChart 2.3 (Voorrips 2002).

Table 1. Details of the two *Triticum durum* bi-parental mapping populations screened for resistance to *Zymoseptoria tritici*.

Mapping population			**Molecular markers**					**Linkage group**		
Parents	Acronym	Size no.	SSR[(a)] no.	DArT[(b)] no.	SNP[(c)] no.	Others[(d)] no.	Total no.	Linkage group no.	Total length cM	Intermarker distance cM/marker
Simeto x Levante	Sm x Lv	180	142	335	5,315	6	5,798	30	2,185	0.40
Kofa x Svevo	Kf x Sv	249	205	-	-	38	243	18	1,256	5.17

[(a)] Single sequence repeat

[(b)] Diversity Array Technology

[(c)] Single Nucleotide Polymorphism

[(d)] Include sequence tagged sites, morphological and biochemical markers

Table 2. *Zymoseptoria tritici* isolates derived from durum wheat originated from diverse geographical areas that were used in seedling assays and field trials in Italy.

					Experiment			
Region	Isolate ID	Country	Location	Year	Parental screen	Sm x Lv Seedling assays	Kf x Sv Seedling assays	Field trials
Middle-East	IPO91004	Syria	Lattakia	1991	+	–	–	–
	IPO86022	Turkey	Altinova	1986	+	+	+	–
North Africa	IPO92042	Algeria	Oum Bouachi	1992	+	–	+	–
	IPO95052	Algeria	Berrahal	1995	+	+	+	–
	IPO95062	Algeria	Berrahal	1995	+	–	–	–
	IPO91018	Morocco	JenicaShaim	1991	+	–	–	–
	IPO91020	Morocco	Doukkala	1991	+	+	–	–
	IPO91009	Tunisia	Bejá	1991	+	–	–	–
	IPO91014	Tunisia	Mateur	1991	+	–	–	–
	IIIB-123	Tunisia	Bejá	2005	+	–	–	–
Europe	IPO92003	Portugal	-	1992	+	+	+	+
	IPO13001	Italy	Emilia R.	2013	+	–	–	–
	IPO13003	Italy	Emilia R.	2013	+	–	–	–
	IPO13006	Italy	Emilia R.	2013	+	–	–	–
	IPO13007	Italy	Emilia R.	2013	+	–	–	–
	IPO13008	Italy	Emilia R.	2013	+	–	–	–
	IPO13018	Italy	Sicily	2013	+	–	–	–
	IPO13019	Italy	Sicily	2013	+	–	–	–
	IPO13023	Italy	Sicily	2013	+	–	–	–
	IPO13024	Italy	Sicily	2013	+	–	–	–
	IPO13056	Italy	Tuscany	2013	+	–	–	–
North America	07-MG-020	Canada	Saskatchewan	2008	+	–	–	–

Results

Phenotyping

Parental screens. Inoculation of the selected *Z. tritici* isolates allowed for good development of the disease symptoms and accurate phenotyping of the parental cultivars and the derived RIL populations. The initial screening of the parental cvs. Kofa, Svevo, Simeto and Levante was carried out with 22 *Z. tritici* isolates to obtain an overall estimate of their differential resistance/susceptibility to STB and to select individual *Z. tritici* isolates informative for the genetic analyses of the RILs (Table 3). The necrosis (*NEC)* level was equal

in all *Z. tritici*/parent combinations and reached the maximum of 100% (data not shown). However, pycnidia (*PYC*) scores varied and showed significant differences and cultivar-by-isolate interactions (Table 3, Supplemental Table 1). None of the tested cultivars was resistant to all *Z. tritici* isolates; one isolate (IPO13007, Italy) was pathogenic on all cultivars, whereas the Canadian strain 07-MG-020-Canada was found to be avirulent on all of them. This likely indicates that this isolate, though collected from durum wheat, is a bread wheat strain. Four *Z. tritici* isolates (IPO86022, IPO92042, IPO95052, IPO91020) that differentiated the parental cultivars were selected for the progeny trials in the greenhouse and isolate IPO92003 was used for the field experiments at the Cadriano station for the Simeto/Levante population (2014) and the Kofa/Svevo population (2013) which was previously (2010) evaluated at the same site under natural infection conditions.

RIL screens. The ANOVA of the *NEC* and *PYC* values for the seedling screening showed significant interactions between the RILs of both mapping populations and the *Z. tritici* isolates (Supplemental Tables 2 and 3). The observed phenotypic variations were in large portion explained by the specific interactions between the applied isolates and the RILs (combined ANOVA analysis, Supplemental Tables 2 and 3). Thus, the single isolates chosen for the QTL analysis were considered separately in the subsequent statistical analysis.

At the seedling stage, parents and population means and ranges for *NEC* and *PYC* were variable between the tested isolates, which were five in total, considering the two populations (Table 4). Overall, the repeatability was higher for *PYC* (from 40.48 to 71.02%) than for the *NEC* (from 22.78 to 48.75%) in both mapping populations. Repeatability values mainly ranged between ca. 40 and 70%, except for the Kofa/Svevo population for the N_{audpc}/IPO86022 and for the N_{audpc}/IPO95052 combination (h^2 = 28.72 and 22.78%, respectively). However, for the latter isolates, the same Kofa/Svevo population showed much higher repeatability values when considering P_{audpc} (h^2 = 71.02 and 43.55%, respectively).

For the RILs tested with both isolates, the genetic component of the observed variation for P_{audpc} were high in the case of IPO86022 and IPO92003, and lower for IPO95052. On the contrary, the variation for the N_{audpc} in Simeto/Levante inoculated with IPO92042 and in Kofa/Svevo after inoculation with IPO95052 was poorly associated with genotypic variation, indicating strong environmental effects contributing to the observed phenotypic variation (Supplemental Tables 3 and 4).

In the field trials, differences were non-significant in the Kofa/Svevo population for *PYC* under natural infection in 2010 and for the inoculated trial in 2013. However, *NEC* scores were higher in the inoculated trial and reached 54.04, whereas the maximum score was 48.09 under natural infection. Repeatability of both traits was significantly higher in the inoculated trials than under natural infection (Table 5). A similar trend was observed in the Simeto/Levante population, where *NEC* showed much more variation than *PYC*, was highly associated with the genotypic variation, as the observed h^2 was equal to 0.76. Environmental effects strongly impacted *PYC* (Table 5). Transgressive segregation was observed towards both resistance and susceptibility in all populations/traits and isolate combinations (Figure 1).

Table 3. Pycnidia averages of the primary inoculated leaves of the durum modern wheat cultivars; parents of the generated bi-parental mapping populations; and inoculated with twenty-two durum derived *Zymoseptoria tritici* (*Z. tritici*) isolates. Significant differences are based on Least Significant Differences of mean values of Pycnidia (*PYC*) percentages. Colors indicate resistant (not significantly different from 0P; green box); intermediate (significantly different from 0P and maxP; yellow box) and susceptible (not significantly different from maxP, red box). (11.12≤P≥30.81; for P=0.05).

		Middle-East			North-Africa								Europe									North-America
	Isolate ID-Origine	IPO91004-Syria	IPO86022-Turkey	IPO92042-Algeria	IPO95052-Algeria	IPO95062-Algeria	IPO91018-Morocco	IPO91020-Morocco	IPO91009-Tunisia	IPO91014-Tunisia	IIIB-123-Tunisia	IPO92003-Portugal	IPO13001-Italy	IPO13003-Itlay	IPO13006-Itlay	IPO13007-Italy	IPO13008-Italy	IPO13018-Italy	IPO13019-Italy	IPO13023-Italy	IPO13024-Italy	IPO13056-Italy	07-MG-020-Canada
	Exp.		c	b	c			a				d											
Trait	Cultivar																						
PYC	Simeto	27.5	2.5	0	7.5	2.5	10	0	10	7.5	2.5	37.5	2.5	25.8	24.2	40	3.8	4.2	15.8	20	8.3	25.8	0
	Levante	22.5	15	25	27.5	10	10	22.5	0	15	2.5	42.5	10	55	25.5	41	20	3.5	48	36.5	38	34.5	0
	Svevo	32.5	0	10	12.5	0	20	25	22.5	25	47.5	2.5	12.5	37.5	35	45	20	7.5	40	15	7.5	25	0
	Kofa	57.5	0	47.5	7.5	2.5	17.5	20	32.5	42.5	45	52.5	7.5	40	15	60	35	20	65	65	45	35	0

a : Isolates used on the Sm x Lv seedlings

b: Isolates used on the Kf x Sv seedlings

c : Isolates used on seedlings of both mapping populations

d : isolates used on seedlings of both mapping populations and in the field

Table 4. Mean, range (minimum and maximum) and repeatability (h^2) of Necrosis Audpc (N_{audpc}) and Pycnidia square-root audpc (P_{audpc}) values of the durum parent cultivars and their derived recombinant inbred lines at the seedling stage.

Z.tritici isolates	Parent	Mean		Mapping population	Mean		Min		Max		h^2	
		Trait										
		N_{audpc}	P_{audpc}		N_{audpc}	P_{audpc}	N_{audpc}	P_{audpc}	N_{audpc}	P_{audpc}	N_{audpc}	P_{audpc}
IPO86022	Sm [(a)]	115.0	2.82	Sm x Lv [(e)]	73.3	2.61	13.2	0.93	137.0	6.3	48.8	57.8
	Lv [(b)]	101.7	4.88									
	Kf [(c)]	184.2	8.73	Kf x Sv [(f)]	167.8	4.86	19.3	2.09	361.1	11.9	28.7	71.0
	Sv [(d)]	115.4	2.86									
IPO95052	Sm [(a)]	245.8	11.19	Sm x Lv [(e)]	195.5	10.79	107	5.20	323.0	15.9	46.1	43.8
	Lv [(b)]	249.2	11.98									
	Kf [(c)]	399.1	15.01	Kf x Sv [(f)]	419.6	15.32	203.6	7.39	558.7	20.3	22.8	43.6
	Sv [(d)]	437.1	15.62									
IPO92003	Sm [(a)]	196.7	4.09	Sm x Lv [(e)]	143.6	4.13	67.0	1.39	234.3	8.5	40.6	40.5
	Lv [(b)]	162.9	4.38									
	Kf [(c)]	348.4	7.46	Kf x Sv [(f)]	307.1	6.18	84.8	2.11	519.3	15.4	48.3	57.3
	Sv [(d)]	210.8	3.57									
IPO91020	Sm [(a)]	252.9	9.95	Sm x Lv [(e)]	218.2	10.7	111.3	5.74	318.9	16.7	44.0	43.9
	Lv [(b)]	230.3	12.52									
IPO92042	Kf [(c)]	421.4	16.39	Kf x Sv [(f)]	408.5	15.24	167.0	6.79	566.9	22.8	39.9	49.4
	Sv [(d)]	314.3	9.72									

[(a)] Sm = Simeto

[(b)] Lv = Levante

[(c)] Kf= Kofa

[(d)] Sv= Svevo

[(e)] Sm x Lv = the bi-parental mapping population generated by the cross between Simeto and Levante

[(d)] Kf x Sv = The bi-parental mapping population generated by the cross between Kofa and Svevo

Table 5. Mean, range (minimum and maximum) and repeatability (h^2) of the relative Necrosis Audpc (N_{audpc}) and Pycnidia square-root audpc (P_{audpc}) values of the durum parent cultivars and their derived recombinant inbred lines at the adult plant stage.

Z.tritici isolates	Parent	Mean		Mapping population	Mean		Min		Max		h^2	
				Trait								
		N_{audpc}	P_{audpc}		N_{audpc}	P_{audpc}	N_{audpc}	P_{audpc}	N_{audpc}	P_{audpc}	N_{audpc}	P_{audpc}
Cadriano 2010												
Natural infection	Kf	77.5	9.89	Kf × Sv	48.1	8.17	19.7	5.43	87.7	10.7	68.4	51.2
	Sv	33.4	5.77									
Cadriano 2013												
Artificial inoculation	Kf	62.7	9.62	Kf × Sv	54.0	8.41	16.4	5.55	114.1	11.4	76.3	73.3
	Sv	25.4	6.53									
Cadriano 2010/2013												
Combined data	Kf	76.3	8.51	Kf × Sv	57.1	7.01	31.1	4.30	92.4	9.01	81.2	75.6
	Sv	36.0	4.97									
Cadriano 2014												
Artifical inoculation	Sm	55.1	5.62	Sm × Lv	57.1	6.67	38.4	4.02	87.5	9.52	76.9	31.7
	Lv	49.4	4.21									

[a] Sm = Simeto

[b] Lv = Levante

[c] Kf= Kofa

[d] Sv= Svevo

[e] Sm x Lv = the bi-parental mapping population generated by the cross between Simeto and Levante

[d] Kf x Sv = The bi-parental mapping population generated by the cross between Kofa and Svevo

Assessment of the frequency distributions at both the seedling and adult plant stages indicated a polygenic inheritance of the *Z. tritici* response (Figure 1; Supplemental Figures S1 and S2). A double-oriented transgressive segregation towards susceptibility and resistance was observed for both populations indicating that both parents contributed to the observed genetic variation with beneficial/susceptibility alleles in different combinations. In particular, the Simeto/Levante population segregated positively towards resistance in the tests with isolates IPO86022 and IPO95052 at the seedling stage, with a noticeable number of RILs falling outside the parental range. A similar trend was observed for the Simeto/Levante RILs in the test with IPO92003, but more specifically for *NEC*. A tendency towards segregation for susceptibility was more rarely observed in both populations. Similarly, normal distributions indicating polygenic inheritance for STB resistance was observed in the field trials for both populations (Figure 1), but contrary to the seedling assays, transgressive segregation was more evident towards susceptibility, particularly in the Simeto/Levante population, which showed a remarkably high number of RILs that were more susceptible than the susceptible parent Simeto (Figure 1).

Overall, significant, albeit weak to moderate, correlations were obtained for the RIL response phenotypes among isolates, between seedling and field trials and among field trials (Table 6), suggesting that the responses to different *Z. tritici* isolates and the performance at different physiological stages are – in these trials – driven by different genetic factors. In particular, the response to different isolates cannot be generalized, indicating the lack of strong resistance genes effective across the majority of isolates.

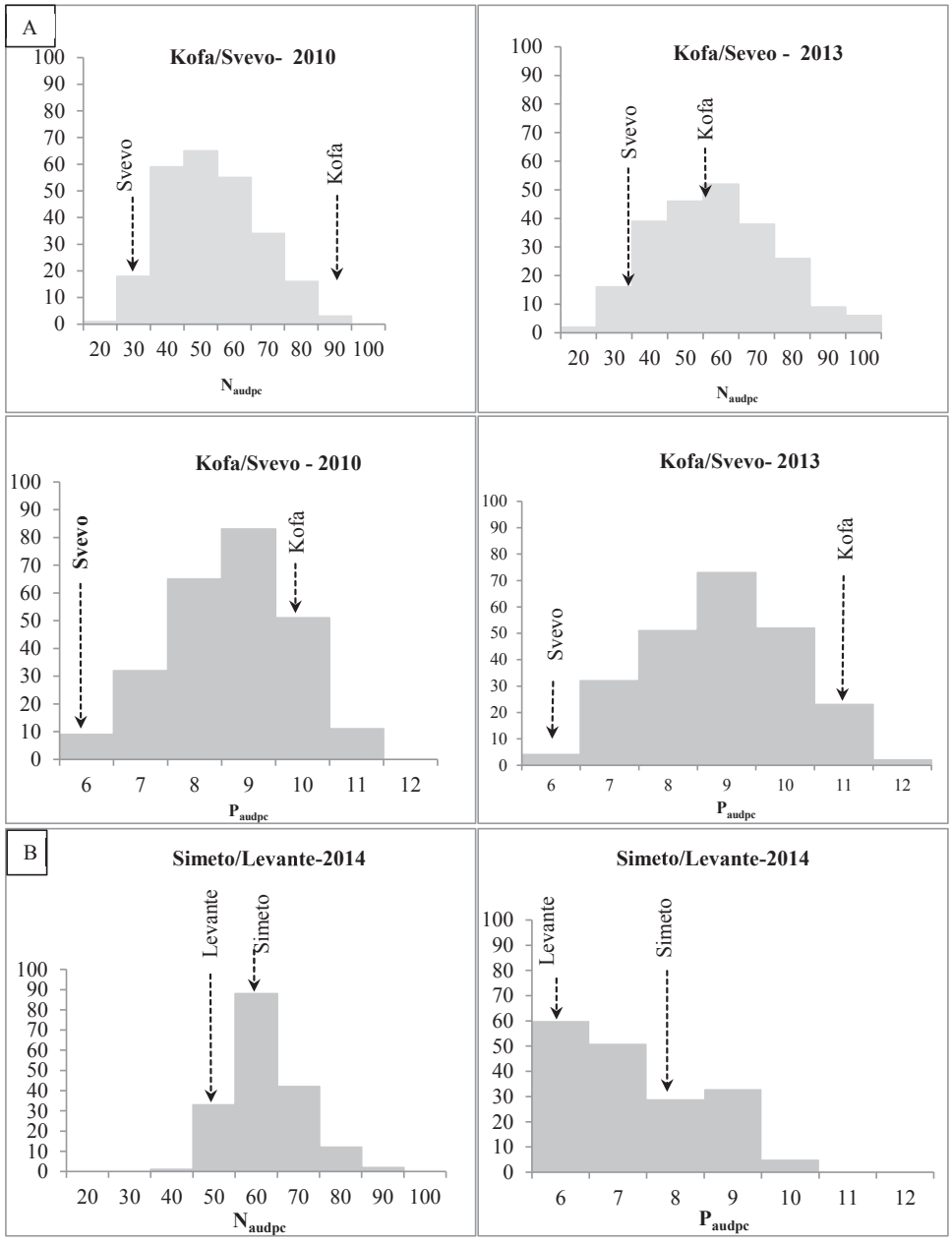

Figure 1. Phenotypic variation for resistance to *Zymoseptoria tritici* under field conditions, scored as N_{audpc} (N_{audpc}) and PaudpcSQRT (P_{audpc}), of recombinant inbred lines (RILs) of the Kofa/Svevo population (A) in 2010 and 2013 (y-axis) and the Simeto/Levante population (B) in 2014 (y-axis).

Table 6. Correlation coefficients (*r*) between the seedlings trials and the field trials of the Simeto (Sm) x Levante (Lv) and the Kofa (Kf) x Svevo (Sv) mapping populations.

	Z. tritici isolates	IPO86022		IPO91020		IPO92003		IPO95052		IPO92042	
Isolate / Trial year	Trait	N_{audpc}	P_{audpc}	N_{audpc}	P_{audpc}	N_{audpc}	P_{audpc}	N_{audpc}	P_{audpc}	N_{audpc}	P_{audpc}
IPO86022	Sm × Lv [(a)]	1.00	1.00								
	Kf × Sv [(b)]	1.00	1.00								
IPO91020	Sm × Lv [(a)]	0.27**	0.24**	1.00	1.00						
	Kf × Sv [(b)]	-	-	-	-						
IPO92003	Sm × Lv [(a)]	0.29**	0.42***	0.30**	0.30**	1.00	1.00				
	Kf × Sv [(b)]	0.21*	0.10[ns]	-	-	1.00	1.00				
IPO95052	Sm × Lv [(a)]	0.30**	0.26**	0.44***	0.28**	0.33***	0.20*	1.00	1.00		
	Kf × Sv [(b)]	0.09[ns]	0.09[ns]	-	-	0.26**	0.29**	1.00	1.00	0.36***	0.42***
IPO92042	Sm × Lv [(a)]	-	-	-	-	-	-	-	-	-	-
	Kf × Sv [(b)]	-0.01[ns]	0.03[ns]	-	-	0.32**	0.29**	0.36***	0.42***	1.00	1.00
Cadriano-2013	Kf × Sv [(b)]	-	-	-	-	0.08[ns]	0.05[ns]	-	-	-	-
Cadriano-2014	Sm × Lv [(a)]	-	-	-	-	0.24**	-	-	-	-	-
		N_{audpc} Cadr-2010	Cadr-2013	P_{audpc} Cadr-2010	Cadr-2013						
Cadriano-2010	Kf × Sv [(b)]	1.00		1.00							
Cadriano-2013	Kf × Sv [(b)]	0.26**	1.00	0.21*	1.00						

Chromosomes associated with quantitative responses to Zymoseptoria tritici *in contemporary durum wheat cultivars*

Quantitative trait loci (QTLs) for resistance to *Z. tritici* were identified using the dense genetic linkage maps available for both populations. Comparative analyses with known *Stb* resistance genes, mainly mapped in hexaploid wheat, were possible due to the presence of common markers in both populations and the consensus maps available for both tetraploid and hexaploid wheat. The comparative analysis was based on the recent review by Brown et al. (2015) on currently mapped sources of resistance to *Z. tritici*. Consequently, QTLs were projected along major reported resistance genes (*Stb*) in the consensus map (Figure 2).

Permutation tests indicated that the threshold for significant LOD differentiation should be set at LOD ≥ 3 for both mapping populations. Thus, QTLs with a LOD ≥ 3 were considered as *effective* and QTLs with LOD ranging between 2 and 3 were considered as *putative*. Subsequently, a total of 52 effective and putative QTLs were identified on the durum wheat populations which were associated to nearly all chromosomes, except for chromosomes 2A and 6A where no resistance loci were detected (Tables 7, 8, S5 and S6; Figure 2). None of the detected QTLs showed a significant effect to all tested *Z. tritici* isolates; nonetheless, some QTLs showed a better efficacy than others and were detected for multiple isolates at the seeding stage or at both seedling and field trials for *NEC* and *PYC*.

In the Simeto/Levante population, chromosome 4B was associated to *Z. tritici* resistance to all tested isolates at the seedling stage and to adult plants when inoculated with IPO92003 (Tables 7 and S5, Figure 2). The comparative analysis of the identified QTLs revealed that chromosome 4B contains two closely linked genomic regions that confer a wide spectrum of resistance to *Z. tritici* (Figure 2). The first QTL spanned a genetic region ranging between 83.8 and 115.5 cM corresponding to the interval between SNP *IWB73770* and SSR *WMC47*. This region carried a QTL identified with *Z. tritici* isolates IPO86022, IPO95052 and IPO91020, controlling seedling *NEC* for the two first isolates and *PYC* development for the former isolate. The highest LOD and explained variance of this QTL were those determined with IPO91020 (8.65 and 20%, respectively), while the lowest LOD (2.23) and explained variance (5%) were observed with IPO86022. The second QTL was located 2.3 cM distal from the first QTL and spanned a genetic distance ranged between 86.1 and 101.3 cM in the genetic linkage map of chromosome 4B flanked by the SNP marker *IWB72179* and the DArT marker wPt-8291. This QTL was detected with isolate IPO92003 for *PYC* and *NEC* development and with IPO91020

for necrosis and was confirmed for *PYC* and *NEC*in the adult stage test with IPO92003. The highest LOD (5.38) and explained variance (12.8%) were determined with isolate IPO92003 for pycnidia development at the seedling stage. Under the field trial, this QTL had a LOD score of 4.55 and explained 9.9% of variance for *NEC*, slightly higher than for *PYC* (Tables 7 and S5). The region covered a minor effect QTL for *NEC* with isolate IPO91020 (LOD 2.09 and explained variance 5.3%). Chromosome 4B also carries two unique and closely linked QTLs for field resistance under natural conditions to *NEC* and *PYC*development detected in the Kofa/Svevo (Tables 8 and S6, Figure 2). These QTLs are 40 cM distal from the Simeto/Levante QTL and explained up to 5.3% of the observed *NEC* phenotypic variance (Tables 8 and S6). All mapped QTLs on chromosome 4B represent new locations not yet reported in bread wheat (Figure 2, Tables 7, 8, S5 and S6).

For the Kofa/Svevo population, chromosome 1B is associated with seedling resistance to two isolates and with field resistance, thereby presenting a location with broader effects compared to other QTLs that were either exclusively detected in the seedling or the adult plant stage (Table 8; Figure 2). One QTL spanned a genetic distance ranged between 27.4 and 48.5 cM, is flanked by the SSR markers *BARC119* and *KSUM28*, and controls *NEC* (highest LOD 6.51 and explained variance 11.5%), and *PYC* development under natural conditions as well as *PYC* development after inoculation with isolate IPO92003. In addition, it controls *NEC* seedling resistance with isolate IPO86022 (Tables 8 and S6; Figure 2). An additional QTL, positioned 13.1 cM distal from the abovementioned QTL controls resistance to *NEC* (LOD 8.47, explained variance 14.2%) and *PYC* (LOD 8.08, explained variance 14.6%) with IPO92003 (Tables 8 and S6). Contrary to the novel chromosome 4 region detected in the Simeto/Levante population, the Kofa/Svevo 1B region also carries *StbWW* and *Stb2/Stb11* that were reported in bread wheat (Liu et al. 2013; Raman et al. 2009).

Our analyses also revealed QTLs identified for specific individual isolates, such as the 6B-QTL that was mapped with isolate IPO95052 for *NEC* and *PYC*development and for field resistance to *PYC* in the Simeto/Levante (Tables 7 and S5). A closely linked QTL (8.8 cM distal) was detected in the Kofa/Svevo population with isolates IPO95052 and IPO86022 for *NEC* and *PYC*resistance, respectively (Tables 8 and S6). These QTLs also represent new locations not reported in bread wheat and provide sources for quantitative resistance to STB in durum wheat (Figure 2, Tables 7, 8, S5 and S6). Other isolate-specific QTLs herein detected co-aligned with known *Stb* genes, such as *Stb7/Stb12* mapped in the Simeto/Levante population

with IPO86022 and IPO92003 at seedling and adult plant stage, respectively. The latter QTL was also detected in the Kofa/Svevo population under field conditions after inoculation with isolate IPO92003. Overall, neither of the detected QTLs have a major effect and explained variances ranged between 5 and 30.8%, with LODs ranging between 2 and 15.44. The QTLs with the highest LOD and explained variance were new, have an isolate-specific mode of action and mapped on chromosomes 6B (LOD = 15.44, 30.8%) and 7B (LOD = 15.75; 24.7%) in the Simeto/Levante and Kofa/Svevo populations (Tables 7 and 8), respectively.

In total, our study revealed several new locations contributing to isolate specific resistance to STB in durum wheat (Tables 7, 8, S5 and S6, S7; Figure 2), of which the majority was derived from cvs. Simeto, Levante and Svevo. Cv. Kofa confirmed to be a reliable susceptible test genotypes to map resistance as well as partial resistance QTLs in durum wheat.

Table 7. QTLs with a LOD > 3 mapped in the Simeto x Levante mapping population at the seedling and adult stages.

	N_{audpc}	P_{audpc}	Resistance source [a]	LOD (unit)	Effect	PEV (%)	Confidence Interval-QTL peak (cM) [b]	QTL projection on tetraploid consensus map (cM) [b]	Putative known *Stb* locus
Seedling response (greenhouse, single isolate inoculation)									
IPO86022									
Qstb.ubo-3A		×	Sm	3.26		6.3	0-5.2-12.4	1.3-7.2-11.4	15.5 cM to *Stb6*
Qstb.ubo-4A	×		Sm	3.59		8.9	77.9-86.1-91.2	170-172.3-173.6	*Stb7/Stb12*
Qstb.ubo-7A	×		Sm	5.39		11.0	126.8-130.6-135.0	107.6-108.9-112.7	26.7 cM to *Stb3*, 17.6 cM to *TmStb1*
Qstb.ubo-7A		×	Sm	7.83		16.2	126.8-130.6-135.0	107.6-108.9-112.7	Novel
Qstb.ubo-7B	×		Lv	3.83		7.8	7.9-13.7-19.6	188-204-211.5	Novel
IPO91020									
Qstb.ubo-4B	×		Lv	8.65		20.02	17.54-24.7-28.9	87.0-91.7-92.9	Novel
Qstb.ubo-5B	×		Lv	4.1		10.1	20.0-30.2-36.9	132.6-154.5-145.9	Novel
IPO92003									
Qstb.ubo-3A		×	Sm	6.46		13.1	0-3.4-12.35	1.3--11.4	15.5 cM to *Stb6*
Qstb.ubo-1B	×		Sm	3.75		9.1	27.74-32.7-43.1	43.6-23.2-48.7	16.5 cM to *Stb11*, 14.8 cM to *StbWW*
Qstb.ubo-4B	×		Lv	5.38		12.8	17.54-24.3-28.9	86.5-91.7-97.5	Novel
Qstb.ubo-4B		×	Lv	4.36		9.6	17.54-24.3-28.9	83.0-85.7-87.0	Novel

(Table 7. Continued)

(Table 7. Continued)

	N	P	Resistance source [a]	LOD (unit)	Effect	PEV (%)	Confidence Interval-QTL peak (cM) [b]	QTL projection on tetraploid consensus map (cM) [b]	Putative known *Stb* locus
Seedling response									
IPO95052									
Qstb.ubo-7A		×	Sm	3.35		5.8	58.28-66-73.89	79.9-84.6-84.6	*Stb3*
Qstb.ubo-1B	×		Sm	4.06		6.09	27.74-36.4-43.05	42.2-47.1-53.9	16.5 cM to *Stb11*, 14.8 cM to *StbWW*
Qstb.ubo-4B	×		Lv	4.31		7.3	1.85-8-14.25	83.8-110.2-113.8	Novel
Qstb.ubo-5B	×		Lv	5.22		8.7	20.0-31.7-36.92	145.9-152.1-157.5	Novel
Qstb.ubo-6B	×		Sm	15.44		30.8	0-5.4-11.42	13.1-15.2-22.14	Novel
Qstb.ubo-6B		×	Sm	6.00		13.3	0-5.4-11.42	11.9-15.2-27.1	Novel
Adult plant response (mist-irrigated field conditions)									
Cadriano-2014 (artificial inoculation, IPO92003)									
Qstb.ubo-4A	×		Sm	5.53		13.0	77.96-81.4-91.24	165.2-170-171.3	*Stb7/Stb12*
Qstb.ubo-4B	×		Lv	4.55		9.9	17.54-24.3-28.95	86.1-91.7-101.3	Novel
Qstb.ubo-4B		×	Lv	4.09		10.0	17.54-24.3-28.95	86.8-91.7-103.3	Novel
Qstb.ubo-5B	×		Lv	6.54		11.1	20-24-36.92	155.5-160.1-166.1	Novel
Qstb.ubo-6B		×	Sm	3.06		4.6	0-5.4-11.42	11.9-15.2-27.1	Novel
Qstb.ubo-7B	×		Lv	6.50		13.8	7.94-14.4-19.55	200.1-200.1-204.8	Novel

[a] Sm = Simeto, Lv = Levante

[b] Confidence Interval left- QTL peak-Confidence Interval right

Table 8. QTLs with a LOD > 3 mapped in the Kofa x Svevo mapping population at the seedling and adult stages.

	N	P	Resistance source [a]	LOD (unit)	Effect	PEV (%)	Confidence Interval-QTL peak (cM) [b]	QTL projection on tetraploid consensus map (cM) [b]	Putative known *Stb* locus
Seedling response (greenhouse, single isolate inoculation)									
IPO86022									
Qstb.ubo-2B	×		Sv	4.40		7.9	3.22-7.7-12.2	132.8-138.2-140.5	Novel
Qstb.ubo-6B		×	Sv	6.91		12.6	0-0-6.54	0-0-3.1	Novel
IPO92042									
Qstb.ubo-3B	×		Sv	5.29		8.3	5.67-9.9-14.23	63.3-74-75.4	Novel
Qstb.ubo-3B		×	Sv	5.95		8.1	5.67-9.9-14.23	63.3-74-75.4	Novel
Qstb.ubo-7B	×		Sv	8.59		14.2	66.17-68.6-71.05	74.1-86.2-86.4	Novel
Qstb.ubo-7B		×	Sv	15.79		24.7	66.17-68.6-71.05	74.1-86.2-86.4	Novel
IPO92003									
Qstb.ubo-5A	×		Sv	3.27		5.8	12.81-18.7-24.77	86.9-96.3-87.9	Novel
Qstb.ubo-1B	×		Sv	8.47		14.2	2.96-5.4-11.03	0-0-8.3	8.9 cM to *Stb2/Stb11*
Qstb.ubo-1B		×	Sv	8.08		14.6	2.96-5.4-11.03	0-0-8.3	8.9 cM to Stb2/Stb11
Qstb.ubo-2B	×		Sv	4.97		7.7	3.22-7.72-12.23	132.8-138.2-148.1	Novel

(Table 8. Continued)

(Table 8. Continued)

	N	P	Resistance source [a]	LOD (unit)	Effect	PEV (%)	Confidence Interval-QTL peak (cM) [b]	QTL projection on tetraploid consensus map (cM) [b]	Putative known *Stb* locus
Seedling response									
IPO95052									
Qstb.ubo-3B		×	Sv	3.72		6.9	27.62-32.6-37.67	75.4-89.5-100.7	Novel
Qstb.ubo-6B	×		Sv	3.3		5.3	0-0-6.54	0-0-3.1	Novel
Adult plant response (mist-irrigated field conditions)									
Cadriano-2010 (natural infection)									
Qstb.ubo-4A	×		Sv	4.05		6.10	0-0-5.37	91.6-91.6-98.2	Novel
Qstb.ubo-1B	×		Sv	6.51		11.5	42.73-45.5-48.58	29.9-36.7-43.0	Novel
Qstb.ubo-1B		×	Sv	5.95		10.09	42.73-45.5-48.58	29.9-36.7-43.0	Novel
Qstb.ubo-4B	×		Sv	3.30		5.30	12.83-19-25.18	33.2-39.4-46.1	Novel
Cadriano-2013 (articial inoculation, IP92003)									
Qstb.ubo-4A	×		Sv	4.70		8.2	89.72-93.9-98.1	141.1-159.8-165.2	*Stb7/Stb12*
Qstb.ubo-1B	×		Sv	3.37		5.9	24.65-30.52-36.41	14.3-23.2-27.4	*Stb11/StbWW*
Qstb.ubo-1B		×	Sv	3.56		6.6	24.65-30.52-36.41	14.3-23.2-27.4	*Stb11/StbWW*

[a] Kf = Kofa, Sv = Svevo

[b] Confidence Interval left- QTL peak-Confidence Interval right.

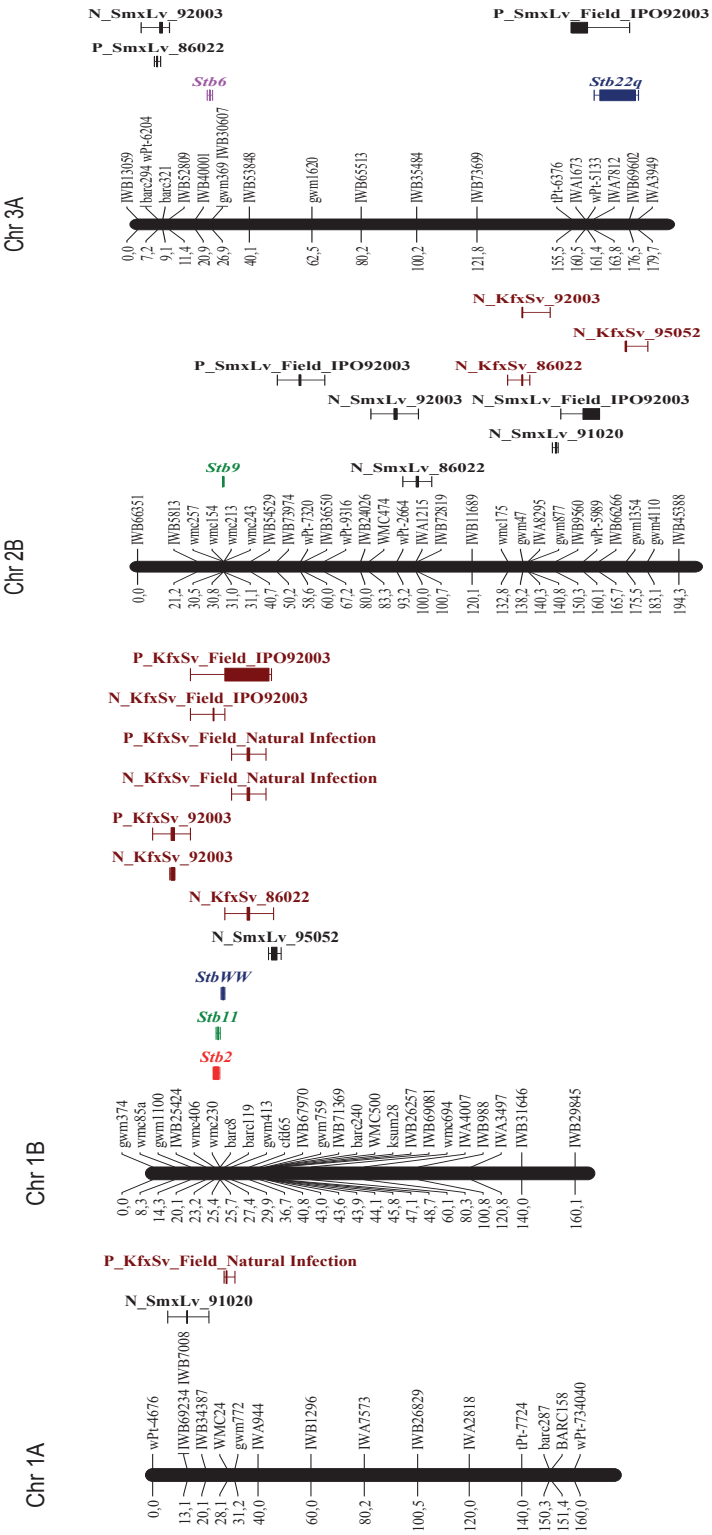

Figure 2. Regions of the linkage maps for chromosomes of the tetraploid consensus map associated to *Zymoseptoria tritici* seedling and field resistance detected in the Simeto/Levante and Kofa/Svevo mapping populations. Quantitative trait loci (QTLs) detected in the Simeto/Levante population are reported in bold black font, whereas QTLs detected in the Kofa/Svevo population are written in bold brown font. Reported major *Stb* resistance genes were projected on the consensus map and displayed in different colours. The centiMorgan (cM) distances between marker loci and the positions of marker loci are on the left and right sides of the linkage maps, respectively.

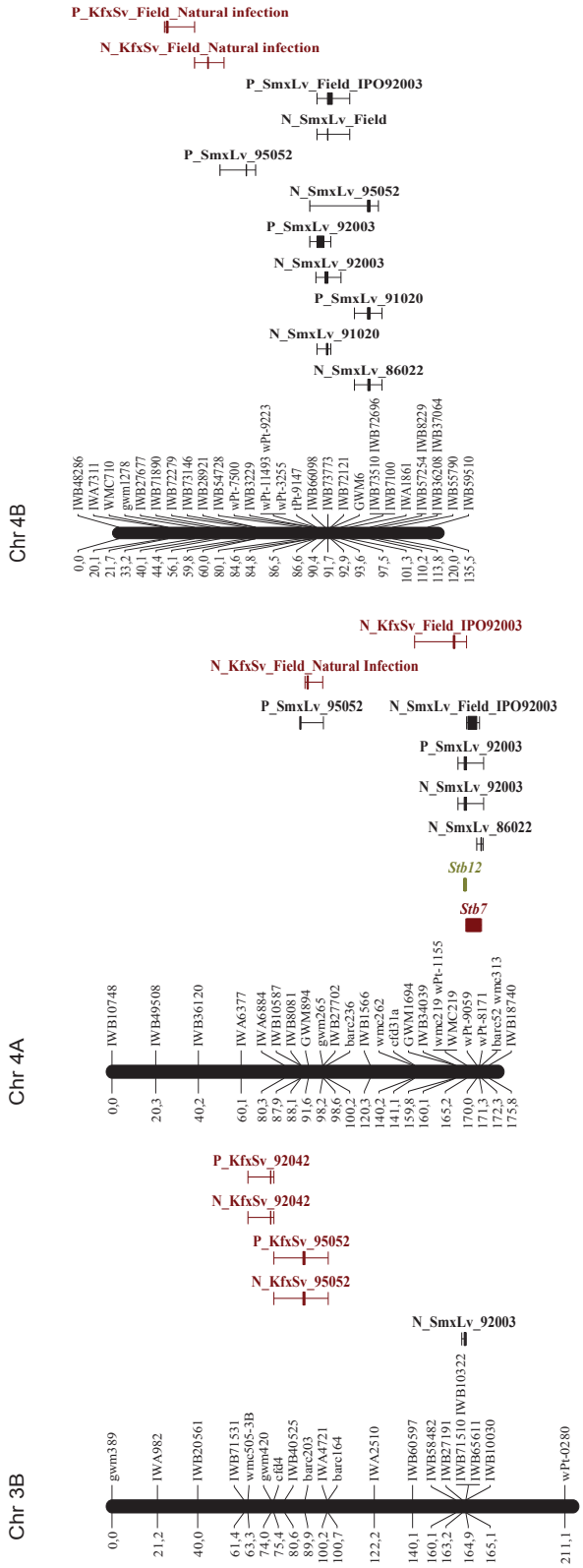

Figure 2. Continued.

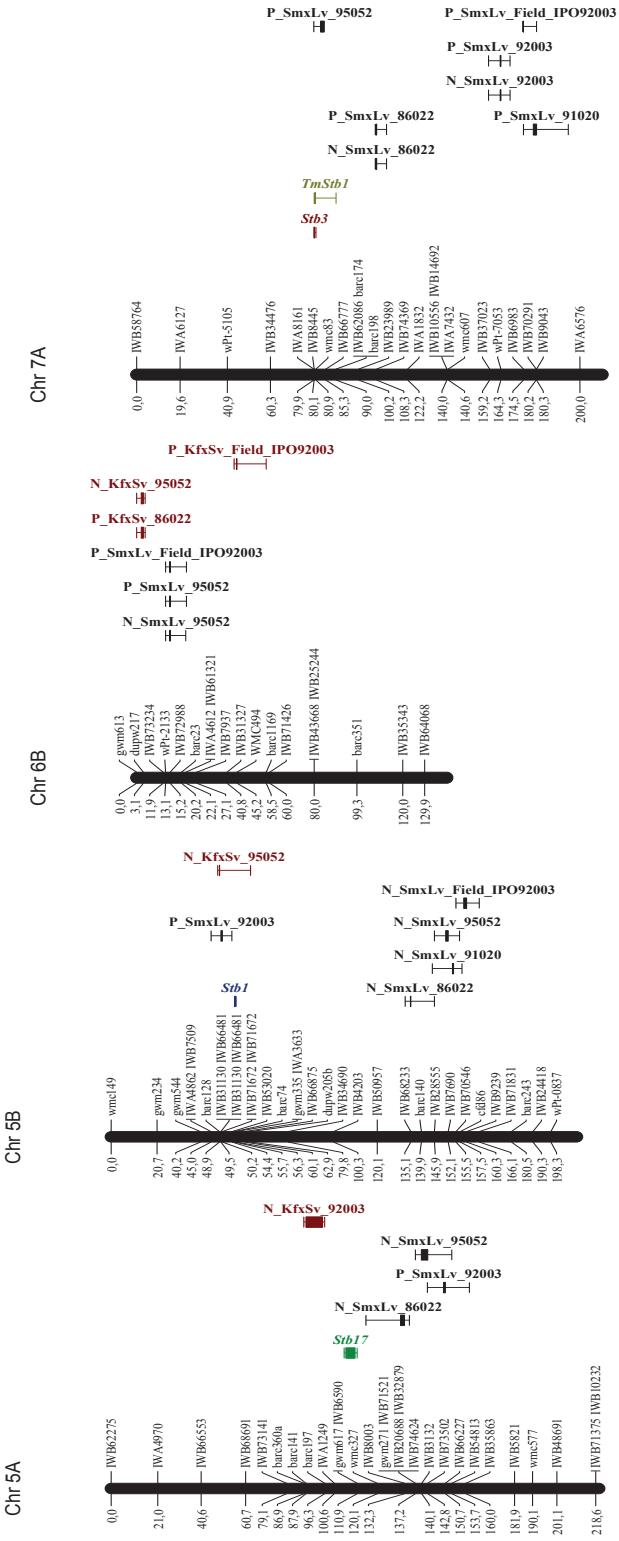

Figure 2. Continued.

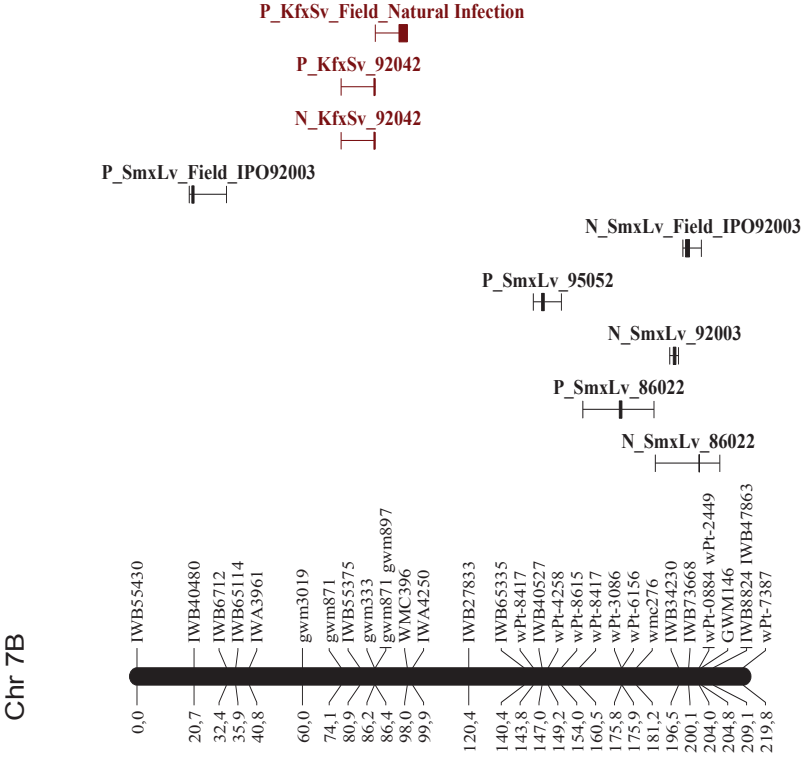

Figure 2. Continued.

Discussion

Durum wheat is widely grown in marginal agricultural environments in the Mediterranean Basin where septoria and other fungi often compromise productivity and continuously challenge the host immune response (Bevan et al. 2017). *Zymoseptoria tritici* is one of the most relevant biotic threats with a notably rapid evolution to adapt to the constant environmental fluctuations (Goodwin et al. 2011; Stukenbrock et al. 2011; Stukenbrock and Croll 2014; Stukenbrock and Francis 2014), a threat for the release of new wheat varieties (Eckhoff et al. 2017; Gharbi et al. 2000; Gharbi et al. 2008). Regrettably, STB in durum wheat has been largely neglected, unlike in bread wheat (Brown et al. 2015).

Our study sheds light on the genetic architecture of resistance to *Z. tritici* in contemporary durum wheat varieties widely adopted in Mediterranean environments. We show that the Italian cvs. Simeto, Levante and Svevo harbor several and diverse, novel QTLs for partial resistance to STB. Altogether, we identified several new QTLs for STB resistance in durum wheat that can be deployed via marker-assisted selection to enhance STB resistance. The QTLs in the Italian cultivars partially control STB resistance in the seedling and adult plant stages and are mostly isolate-specific. However, chromosomes 1B and 4B were associated with QTLs effective to at least three *Z tritici* isolates out of four at the seedling stage and also to natural population and individual isolates in the adult plant stage.

In the Kofa/Svevo population, two QTLs were identified on chromosome 1B. The first QTL contributes to the field resistance for *NEC* and *PYC* development under natural conditions as well as to *PYC* development after inoculation with isolate IPO92003 and to necrosis resistance at the seedling stage when tested with isolate IPO86022. The second closely linked QTL is mapped 13.1 cM distal from the first and contributes to seedling resistance to isolate IPO92003. In the Simeto/Levante population, two QTLs were identified in chromosome 4B. The first QTL contributes to seedling STB resistance to *Z. tritici* isolates IPO86022, IPO95052 and IPO91020, and the second 4B QTL was detected with the latter isolate and with IPO92003 in the adult plant stage.

All four QTLs covered genetic regions that were previously not associated with resistance to *Z. tritici,* unlike the two 1B QTLs that co-aligned with *StbWW* (Raman et al. 2009) and *Stb2/Stb11*(Liu et al. 2013). In bread wheat, the efficacy to *StbWW* is limited to a single *Z. tritici* isolate (79.2.1A) at the seedling stage, whereas *Stb2* has a much wider efficacy (Cuthbert 2011; Ghaffary 2011; Goudemand et al. 2013; Liu et al. 2013). Other minor QTLs with a narrow efficacy co-aligned with *Stb7* (4A)*, Stb12* (4A)*, Stb3* (7A) and *Stb6* (3A), all showing a much

wider efficacy in bread wheat (Brown et al. 2015; Cuthbert 2011). However, we cannot claim that these known *Stb* genes providing resistance to *Z. tritici* isolates are derived from durum wheat. The reported dichotomy in *Z. tritici* pathogenicity for bread wheat and durum wheat (Kema et al. 1996a; Kema et al. 1996b; Kema et al. 1996c; Kema and van Silfhout 1997; Kema et al. 1996d; Ware 2006) has largely contributed to the limited progress in the identification of resistance genes in the latter species, as even the most susceptible durum wheats are resistant to *Z. tritici* isolates derived from bread wheat. Therefore, the use of well-characterized *Z. tritici* isolates for the *Z. tritici* – bread wheat pathosystem (Ghaffary 2011; Ghaffary et al. 2012; Ghaffary et al. 2011) appears to be unsuitable for durum wheat research and breeding.

Even though many of the identified QTLs have a narrow spectrum, their combination or pyramiding showed their value in attaining broader spectrum resistance, as demonstrated by the wide transgressive segregation for resistance under field conditions noticed in both RIL mapping populations. This has also been recently demonstrated in the Tunisian landrace 'Agili 39' (Ferjaoui et al., unpublished) as well as in several bread wheat cultivars, including cv. 'Apache' (Ghaffary et al. 2011), and the breeding lines KK4500 and TE11 whose resistance is conferred by a combination of several known *Stb* genes (Chartrain et al. 2005a; Chartrain et al. 2005b; Chartrain et al. 2004). Therefore, as demonstrated in this study, contemporary durum wheat cultivars can reveal new loci for STB resistance that, despite their limited efficacy when considered individually, can provide broader resistance spectrum once introgressed in new elite germplasm.

Partial resistance to STB has been reported in contemporary durum wheat (Berraies et al. 2014; Gharbi et al. 2000; Gharbi et al. 2008; Kidane et al. 2017; Tuberosa 2014), in Ethopian durum Landraces (Kidane et al. 2017) and in bread wheat germplasm (Arraiano and Brown 2017; Tabib Ghaffary et al. 2011). Possibly, partial resistance could also be associated with increased durability, as in other wheat pathogens such as the cereal rusts (Bansal et al. 2014; Lowe et al. 2011; Mundt 2014). Although causal links between the level of resistance and durability remain unravelled (Krattinger and Keller 2016), several studies showed partial resistance to be more durable than major-gene driven resistance (Brown 2015; Ellis et al. 2014; Krattinger et al. 2009), particularly when partial resistance QTLs are pyramided. Also, highly diverse and rapidly evolving fungal pathogen populations, such as *Z. tritici* (Goodwin et al. 2011; Stukenbrock et al. 2011; Stukenbrock and Croll 2014; Stukenbrock and McDonald 2008), are frequently considered to rapidly overcome major resistance genes (Croll and McDonald 2016; McDonald and Linde 2002; McDonald and Stukenbrock 2016). However, it is necessary

to place each of these projections into the correct biological and epidemiological context. Resistance genes that are circumvented by mutations in *Z. tritici* effectors not necessarily disseminate rapidly in natural populations resulting in boom and bust scenarios.

Therefore, rather than advocating any specific form of resistance, particularly given the urgency for STB breeding in durum wheat, each and every QTL should be embraced to improve the overall low level of STB resistance. Our study contributes a suite of well-characterized *Z. tritici* isolates and confirms specific host-pathogen interactions (Chartrain et al. 2004; Kema et al. 1996a; Kema et al. 1996b) whose genetic basis was recently elucidated (Zhong et al. 2017). Based on the relatively high number of QTLs identified in this study, breeding for STB partial resistance appears feasible and its efficiency could be possibly enhanced by the use of genomic selection (Bassi et al. 2016; Juliana et al. 2017; Varshney et al. 2005), which allows to simultaneously account for the quantitative loci segregating in the breeding germplasm more effectively than classic marker-assisted selection.

Conclusions and perspectives

Our study provides a more detailed and complete view of the wheat QTLome for STB resistance while reporting a suite of novel QTLs for STB partial resistance, present in the durum wheat germplasm currently providing the basis of breeding populations. This finding supports the possibility to enhance STB partial resistance through both transgressive conventional and marker-aided breeding, as demonstrated by the high level of STB resistance reached by recombinant inbred lines in field experiments. Some of the identified QTLs showed appreciable effects and PEV values, such as in the case of QTLs identified in chromosomes 1B, 4B and 6B. These QTLs are good candidates for a positional cloning approach that will eventually facilitate the identification of rare beneficial haplotypes while paving the way to their editing to assemble even more beneficial alleles (Salvi and Tuberosa 2015). The recent release of the emmer genome assembly of wild wheat (Avni et al. 2017) and durum wheat (http://www.unibo.it/en/notice-board/durum-wheat-assembly-can-lead-to-better-and-more-resilient-durum-for-more-nutritious-food: Cattivelli et al. unpublished) coupled with the use of high-density consensus maps of tetraploid wheat including also markers from bread wheat will further accelerate genomics-based approaches to enhance STB resistance in cultivated wheat.

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Supplementary Information

Supplemental Table 1. ANOVA analysis of the durum wheat cultivar parents assessed with the twenty-two *Zymoseptoria tritici* isolates.

Source of Variation	**Necrosis**				***df***	**Pycnidia**			
	Var	*F*	*P-value*	*F crit*		*Var*	*F*	*P-value*	*F crit*
Isolate	1862.	50.81	3.2E-40	1.68	21	1351.01	48.90	1.5E-39	1.68
Genotype	374.4	10.22	7.7E-06	2.71	3	2934.08	106.20	3.8E-29	2.71
Isolate-by-Genotipe	146.4	3.99	1.6E-09	1.46	63	279.75	10.13	7.5E-22	1.46
Residual	36.65				88	27.63			
Total					175				

Var=variance component
df= Degree of freedom

Supplemental Table 2. Variance components (Var) and Wald test significance the durum wheat Simeto × Levante Recombinant Inbred Line (RIL) populations assessed with the four isolates at the seedling stage.

Source of Variation	Necrosis		*df*	Pycnidia	
	Var	*P-value*		*Var*	*P-value*
Simeto/Levante RIL - combined analysis of four isolates					
Isolate	3913.6	0.1475		3198.59	0.1277
genotype	363.04	<.0001		115.31	0.0001
Isolate × Genotype	151.79	0.0029		230.11	<.0001
Residual	1994.43			1273.19	
IPO 86022					
Genotype	267.19	<.0001		0.8033	<.0001
residual	892.10			1.8896	
IPO 91020					
Genotype	1993.95	0.1602		1.4276	<.0001
residual	2553.84			5.789	
IPO 92003					
Genotype	441.71	0.0001		0.766	0.0003
residual	1977.05			3.4299	
IPO 95052					
Genotype	692.04	<.0001		1.4849	<.0001
residual	2488.41			5.8501	

Supplemental Table 3. ANOVA analysis of the durum wheat Kofa/Svevo Recombinant Inbred Line (RIL) populations assessed with the four isolates at the seedling stage.

Source of Variation	Necrosis		*df*	Pycnidia	
	Var	*P-value*		*Var*	*P-value*
Kofa/Svevo RIL-STB isolate combined analysis					
Isolate	1625.39	0.0234		4523.45	0.256
genotype	251.20	<.0001		125.6	0.0001
Isolate × Genotype	202.3	0.0030		230.11	<.0001
Residual	1256.9			1273.19	
IPO 86022					
Genotype	926.91	0.0013		2.7776	<.0001
residual	8897.2			4.9702	
IPO 92003					
Genotype	3048.11	<.0001		3.1337	<.0001
residual	13388			8.9098	
IPO 92042					
Genotype	1604.48	0.0001		3.4089	<.0001
residual	9158.69			15.4705	
IPO 95052					
Genotype	672.96	0.0271		1.9397	<.0001
residual	8324.17			11.9596	

Supplemental Table 4. ANOVA analysis of the two durum wheat Recombinant Inbred Line (RIL) populations assessed under field trials at the adult plant stage.

Source of Variation	Necrosis		*df*	Pycnidia	
	MS	*P-value*		*MS*	*P-value*
Simeto/Levante					
Cadriano-2014					
Genotype	50.35	<.0001		0.4858	<.0001
residual	47.78			3.1355	
Kofa/Svevo					
Cadriano-2010/2013 combined analysis					
Genotype	47.89	0.0011		0.3616	<.0001
Years	624.23	0.2621		1.2943	0.335
Years × Geno	106.03	<.0001		0.3499	0.0002
residual	168.38			1.3104	
Kofa/Svevo					
Cadriano-2010					
Genotype	116.02	<.0001		0.6039	<.0001
Residual	13.5187			1.5842	
Cadriano-2013					
Genotype	196.44	<.0001		0.8569	<.0001
Residual	187.48			1.1665	

Supplemental Table 5. Putative QTLs with a LOD between 2 and 3 mapped in the Simeto x Levante population at the seedling and adult stages.

Isolate IPO86022-Seedling stage			**Chromosomes**											
QTL parameter	**Trait**		**1A**	**3A**	**4A**	**5A**	**7A**	**1B**	**2B**	**3B**	**4B**	**5B**	**6B**	**7B**
LOD	N				3,59	2,09	5,39		2,4		2,23	2,57		3,83
	P			3.26			7.83							2.81
% Expl	N				8,9	4,6	11		5,2		5	5,7		7,8
	P			6.3			16,2							5.1
Linked Marker	N				IWB43779-wmc313	IWB8003	IWB74369		IWB72819		IWB8229	IWB68233		wPt-2449
	P			wPt-6204	-		IWB74369							wPt-6156
CI in cM	N	CI left			81,07	69,58	126,79		115,29		0	37,38		7,94
		CI right			91,24	89,26	135,02		132,7		17,11	53,26		19,55
	P	CI left		0			128,11							23,23
		CI right		12,35			133,7							40,98
CI_{CM}[1] in cM	N	CI left			170	115,5	107,6		95,2		103,3	132,6		188
		CI right			173,6	134,5	112,7		105,6		115,5	145,9		211,5
	P	CI left		1,3			107,6							161,7
		CI right		11,4			112,7							187,5
Putative Stb gene				15,5 cM away from Stb6	Stb7/stb12	4,6 cM away from Stb17	26,7 cM away from Stb3 and 17,6 cM away from TmStb1		New position		New position	New position		New position
Resistance Source				Simeto	Simeto	Levante	Simeto		Levante		Levante	Levante		Levante

Supplemental Table 5. Continued

Isolate IPO91020-Seedling stage			Chromosomes											
QTL parameter	**Trait**		**1A**	**3A**	**4A**	**5A**	**7A**	**1B**	**2B**	**3B**	**4B**	**5B**	**6B**	**7B**
LOD	N		2.01						2.43		8.65	4.1		
	P						2.22				2.09			
% Expl	N		5.1						6.1		20.2	10.1		
	P						5.6				5.3			
Linked Marker	N		IWB69234						IWB9560		IWB73773	wPt-8125		
	P						IWB9043				IWB8229			
CI in cM	N	CI left	0						2,11		22,55	25,79		
		CI right	17,07						16,96		27,04	34,76		
	P	CI left					24,12				0			
		CI right					40,29				16,59			
CI_{CM}[1] in cM	N	CI left	5,7				174,5		150,3		87	144,8		
		CI right	21,3				194,5		151		92,9	158,3		
	P	CI left									103,3			
		CI right									115,5			
Putative Stb gene			New position				New position		New position		New position	New position		
Resistance Source			Levante				Simeto		Levante		Levante	Levante		

Supplemental Table 5. Continued

Isolate IPO92003-Seedling stage			Chromosomes											
QTL parameter	**Trait**		**1A**	**3A**	**4A**	**5A**	**7A**	**1B**	**2B**	**3B**	**4B**	**5B**	**6B**	**7B**
LOD	N				2.05		2.41	3.75	2.13	2.05	5.38			2.6
	P			6.46	2.25	2.62	2.9				4.36	2.55		
% Expl	N				5.1		6	9.1	5.3	4.4	12.8			5.7
	P			13.1	5.1	6.5	6.5				9.6	6.1		
Linked Marker	N				wmc219		wPt-7053a	wmc500b	wPt-2664	IWB71510	IWB72379			IWB34230
	P			IWB7037	wmc219	IWB66227	wPt-7053a				IWB72121	IWB71672		
CI in cM	N	CI left			68,65		50,2	27,74	106,4	42,73	20,84			8,54
		CI right			86,41		65,29	37,69	123,48	63,31	27,92			24,43
	P	CI left		0	68,65	94,75	50,78				18,02	21,62		
		CI right		12,35	86,41	108,68	64,71				27,45	36,47		
CI_{CM}[1] in cM	N	CI left			161,2		158,8	42,2	83,8	164,9	86,5	45		181,2
		CI right			173,2		168,4	53,9	100,7	173,6	97,5	54,4		209,1
	P	CI left		1,3	161,2	142,6	158,8				83			
		CI right		11,4	173,2	161,6	168,4				87			
Putative Stb gene				15,5 cM away from Stb6	Stb7/stb12	32 cM away from Stb17	New position	16,5 cM away from Stb11 and 14,8 cM away from StbWW	New position	New position	New position	10 cM away from Stb1		New position
Resistance Source				Simeto	Simeto	Simeto	Simeto	Simeto	Levante	Levante	Levante	Levante		Levante

Supplemental Table 5. Continued

Isolate IPO95052-Seedling stage			Chromosomes											
QTL parameter	**Trait**		**1A**	**3A**	**4A**	**5A**	**7A**	**1B**	**2B**	**3B**	**4B**	**5B**	**6B**	**7B**
LOD	N					2.47		4.06			4.31	5.22	15.44	
	P				2.83		3.35				2.96		6	2.25
% Expl	N					5.5		6.9			7.3	8.7	30.8	
	P				6.5		5.8				5.5		13.3	4.7
Linked Marker	N					IWB73503		IWB26257			IWB8229	IWB7690	IWB72988	
	P				IWB8081		IWB66777a				IWB72279		IWB72988	wPt-8615
CI in cM	N	CI left				87,76		29,930,003			1,85	26,51	4	
		CI right				104,23		43,053,997			14,25	36,92	6,94	
	P	CI left			0		58,28				64,45		2,06	45,26
		CI right			6,965,812		73,89				80,92		8,87	64,53
CI_{CM}[1] in cM	N	CI left				137,2		43,6			83,8	145,9	13,1	
		CI right				153,7		48,7			113,8	157,5	22,14	
	P	CI left			88,1		79,9				44,4		11,9	143,8
		CI right			98,6		84,6				60		27,1	146,8
Putative Stb gene					New position	New position	Stb3	16,5 cM away from Stb11 and 14,8 cM away from StbWW			New position	New position	New position	New position
Resistance Source					Simeto	Levante	Simeto	Simeto			Levante	Levante	Simeto	Simeto

Supplemental Table 5. Continued

Isolate IPO92003- Adult plant stage			Chromosomes											
QTL parameter	**Trait**		**1A**	**3A**	**4A**	**5A**	**7A**	**1B**	**2B**	**3B**	**4B**	**5B**	**6B**	**7B**
LOD	N				5.53				2.81		4.55	6.54		6.5
	P			2.26			2.75		2.87		4.09		3.06	2.48
% Expl	N				13				4.2		9.9	11.1		13.8
	P			3.9			6.5		6.8		10		7.6	6.2
Linked Marker	N				wPt-9059				IWB66266		IWB72379	IWB9239		IWB73668
	P			wPt-5133			IWB6983		wPt-7320		gwm6		IWB72988	IWB40480
$CI_{(SmxLv)}$ in cM	N	CI left			77,96				0		19,81	20		11,12
		CI right			84,93				10,78		28,95	28,16		17,68
	P	CI left		19,11			35,8		59,36		17,54		0	161,54
		CI right		42,33			49,73		72,68		26,6		11,42	176,15
$CI_{(Cs)}$ in cM	N	CI left		155,5	165,2				151		86,1	155,5		200,1
		CI right		176,5	171,3				165,7		101,3	166,1		204,8
	P	CI left					174,5		50,2		86,8		11,9	20,7
		CI right					180,3		67,2		103,3		27,1	32,4
Putative Stb gene				Stb22	Stb7/Stb12		New position		New position		New position	New position	New position	New position
Resistance Source				Levante	Simeto		Simeto		Levante		Levante	Simeto	Simeto	Levante

[1]CM=consensus map.

QTL not detected

Supplemental Table 6. Putative QTLs with a LOD between 2 and 3 mapped in the Kofa x Svevo mapping population at the seedling and adult stages

Isolate IPO86022-Seedling stage			**Chromosomes**									
QTL parameter	**Trait**		**1A**	**4A**	**5A**	**1B**	**2B**	**3B**	**4B**	**5B**	**6B**	**7B**
LOD	N					2.55	4.4					
	P										6.91	
% Expl	N					4.5	7.9					
	P										12.6	
Linked Marker	N					cfd65	gwm47					
	P										gwm613	
CI in cM	N	CI left				37,87	3,34					
		CI right				53,28	12,12					
	P	CI left									0	
		CI right									2,75	
CI_{CM}[1] in cM	N	CI left				27,4	132,8					
		CI right				48,5	140,5					
	P	CI left									0	
		CI right									3,1	
Putative Stb gene						StbWW	New position				New position	
Resistance Source						Svevo	Svevo				Svevo	

Supplemental Table 6. Continued

Isolate IPO92042-Seedling stage			Chromosomes									
QTL parameter	**Trait**		**1A**	**4A**	**5A**	**1B**	**2B**	**3B**	**4B**	**5B**	**6B**	**7B**
LOD	N							5.29				8.59
	P							5.95				15.79
% Expl	N							8.3				14.2
	P							8.1				24.7
Linked Marker	N							gwm420				gwm333
	P							gwm420				gwm333
CI in cM	N	CI left						5,77				66,17
		CI right						14,13				71,05
	P	CI left						5,67				67,21
		CI right						14,23				70,01
$CI_{CM}{}^{1}$ in cM	N	CI left						63,3				74,1
		CI right						75,4				86,4
	P	CI left						63,3				74,1
		CI right						75,4				86,4
Putative Stb gene								New position				New position
Resistance Source								Svevo				Svevo

Supplemental Table 6. Continued

Isolate IPO92003-Seedling stage			Chromosomes									
QTL parameter	**Trait**		**1A**	**4A**	**5A**	**1B**	**2B**	**3B**	**4B**	**5B**	**6B**	**7B**
LOD	N				3.27	8.47	4.97					
	P					8.08						
% Expl	N				5.8	14.2	7.7					
	P					14.6						
Linked Marker	N				barc197	gwm33	gwm47					
	P					gwm374						
CI in cM	N	CI left			12,81	2,96	3,22					
		CI right			24,77	7,84	12,23					
	P	CI left				6,28						
		CI right				11,03						
CI_{CM}[1] in cM	N	CI left			86,9	0	132,8					
		CI right			87,9	8,3	148,1					
	P	CI left				0						
		CI right				14,3						
Putative Stb gene					New position	8,9 cM away from Stb2	New position					
Resistance Source					Svevo	Svevo	Svevo					

Supplemental Table 6. Continued

Isolate IPO95052-Seedling stage			Chromosomes									
QTL parameter	**Trait**		**1A**	**4A**	**5A**	**1B**	**2B**	**3B**	**4B**	**5B**	**6B**	**7B**
LOD	N						2.25	2.44		2.26	3.3	
	P							3.72				
% Expl	N						3.8	4.7		3.9	5.3	
	P							6.9				
Linked Marker	N						gwm1354	barc203		barc128a	gwm613	
	P							barc203				
CI in cM	N	CI left					0	25,26		0	0	
		CI right					9,13	40,02		8,89	6,54	
	P	CI left						27,62				
		CI right						37,67				
CI_{CM}[1] in cM	N	CI left					175,5	75,4		48,9	0	
		CI right					183,1	100,7		62,9	3,1	
	P	CI left						75,4				
		CI right						100,7				
Putative Stb gene							New position	New position		stb1	New position	
Resistance Source							Kofa	Svevo		Kofa	Kofa	

Supplemental Table 6. Continued

Natural infaction (2010)-Adult plant stage			Chromosomes									
QTL parameter	**Trait**		**1A**	**4A**	**5A**	**1B**	**2B**	**3B**	**4B**	**5B**	**6B**	**7B**
LOD	N			4.05		6.51			3.3			
	P		2.48			5.95			2.93			2.97
% Expl	N			6.1		11.5			5.3			
	P		4.2			10.9			4.9			5
Linked Marker	N			gwm894		cfd65			gwm368			
	P		wmc24			cfd65			wmc710			wmc396
CI $_{(SmxLv)}$ in cM	N	CI left		0		42,73			12,83			
		CI right		5,37		48,42			25,18			
	P	CI left	0			42,57			0			72,32
		CI right	13,52			48,58			6,68			85,41
CI $_{(Cs)}$ in cM	N	CI left		91,6		29,9			33,2			
		CI right		98,2		43			46,1			
	P	CI left	28,1			29,9			21,7			86,4
		CI right	31,2			43			33,2			98
Putative Stb gene			New position	New positions		2,5 cM away from StbWW			New position			New position
Resistance Source			Kofa	Svevo		Svevo			Svevo			Svevo

Supplemental Table 6. Continued

IPO92003 (2013)-Adult plant stage			Chromosomes									
QTL parameter	**Trait**		**1A**	**4A**	**5A**	**1B**	**2B**	**3B**	**4B**	**5B**	**6B**	**7B**
LOD	N audpc			4.7		3.37						
	PaudpcSQRT					3.56					2.45	
% Expl	N audpc			8.2		5.9						
	PaudpcSQRT					6.6					4.6	
Linked Marker	N audpc			gwm1694		wmc500a						
	PaudpcSQRT					wmc500a					wmc494	
CI $_{(SmxLv)}$ in cM	N audpc	CI left		89,72		24,65						
		CI right		98,18		36,41						
	PaudpcSQRT	CI left				25,27					101,27	
		CI right				35,78					116,35	
CI $_{(Cs)}$ in cM	N audpc	CI left		141,1		14,3						
		CI right		165,2		27,4						
	PaudpcSQRT	CI left				14,3					45,2	
		CI right				27,4					58,5	
Putative Stb gene				Stb7/Stb12		StbWW					New position	
Resistance Source				Svevo		Svevo					Svevo	

[1]CM=consensus map.

QTL not detected

Supplemental Table 7. New genomic regions associated with the *Zymoseptoria tritici* resistance in the Simeto/Levante and kofa/Svevo mapping populations

IPO86022-Seedling Stage			Chromosomes																			
			1A		5A		7A		2B		3B		4B		5B		6B		7B			
QTL parameter	**Trait**		Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo
LOD	N				2,09		5,39		2,4	4.4			2,23		2,57				3,83			
	P						7.83											6.91	2.81			
% Expl	N				4,6		11		5,2	7.9			5		5,7				7,8			
	P						16,2											12.6	5.1			
CI_{CM}[1] in cM	N	CI left			115,5		107,6		95,2	132,8			103,3		132,6				188			
		CI right			134,5		112,7		105,6	140,5			115,5		145,9				211,5			
	P	CI left					107,6											0	161,7			
		CI right					112,7											3,1	187,5			
Resistance source					Levante		Simeto		Levante	Svevo			Levante		Levante			Svevo	Levante			

Supplemental Table 7. Continued

IPO91020-Seedling stage			Chromosomes																			
			1A		5A		7A		2B		3B		4B		5B			6B		7B		
QTL parameter	**Trait**		Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Sime/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo		
LOD	N		2.01						2.43				8.65		4.1							
	P						2.22						2.09									
% Expl	N		5.1						6.1				20.2		10.1							
	P						5.6						5.3									
CI_{CM}[1] in cM	N	CI left	5,7						150,3				87		144,8							
		CI right	21,3						151				92,9		158,3							
	P	CI left					174,5						103,3									
		CI right					194,5						115,5									
Resistance source			Levante				Simeto		Levante				Levante		Levante							

Supplemental Table 7. Continued

IPO92003-Seedling stage			Chromosomes																			
			1A		5A		7A		2B		3B		4B		5B				6B		7B	
QTL parameter	**Trait**		Simeto/Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo		
LOD	N					3.27	2.41		2.13	4.97	2.05		5.38						2.6			
	P				2.62		2.9						4.36		2.55							
% Expl	N					5.8	6		5.3	7.7	4.4		12.8						5.7			
	P				6.5		6.5						9.6		6.1							
CI_{CM}[1] in cM	N	CI left				86,9	158,8		83,8	132,8	164,9		86,5		45				181,2			
		CI right				87,9	168,4		100,7	148,1	173,6		97,5		54,4				209,1			
	P	CI left			142,6		158,8						83									
		CI right			161,6		168,4						87									
Resistance source					Simeto	Svevo	Simeto		Levante	Svevo	Levante		Levante		Levante				Levante			

Supplemental Table 7. Continued

IPO92042-Seedling stage			Chromosomes																	
			1A		5A		7A		2B		3B		4B		5B		6B		7B	
QTL parameter	**Trait**		Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simet/ Levante	Kofa/ Svevo
LOD	N											5.29								8.59
	P											5.95								15.79
% Expl	N											8.3								14.2
	P											8.1								24.7
CI_{CM}[1] in cM	N	CI left										63,3								74,1
		CI right										75,4								86,4
	P	CI left										63,3								74,1
		CI right										75,4								86,4
Resistance source												Svevo								Svevo

Supplemental Table 7. Continued

IPO95052-Seedling stage			Chromosomes																	
			1A		5A		7A		2B		3B		4B		5B		6B		7B	
QTL parameter	**Trait**		Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simet/ Levante	Kofa/ Svevo
LOD	N				2.47					2.25		2.44	4.31		5.22		15.44	3.3		
	P						3.35					3.72	2.96				6		2.25	
% Expl	N				5.5					3.8		4.7	7.3		8.7		30.8	5.3		
	P						5.8					6.9	5.5				13.3		4.7	
CI_{CM}[1] in cM	N	CI left			137,2					175,5		75,4	83,8		145,9		13,1	0		
		CI right			153,7					183,1		100,7	113,8		157,5		22,14	3,1		
	P	CI left					79,9					75,4	44,4				11,9		143,8	
		CI right					84,6					100,7	60				27,1		146,8	
Resistance source					Levante		Simeto			Kofa		Svevo	Levante		Levante		Simeto	Kofa	Simeto	

Supplemental Table 7. Continued

IPO92003-Adult plant stage		Chromosomes																	
		1A		5A		7A		2B		3B		4B		5B		6B		7B	
QTL parameter	**Trait**	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo
LOD	N							2.81				4.55		6.54				6.5	
	P					2.75		2.87				4.09				3.06	2.45	2.48	
% Expl	N							4.2				9.9		11.1				13.8	
	P					6.5		6.8				10				7.6	4.6	6.2	
CI_{CM}[1] in cM	N CI left							151				86,1		155,5				200,1	
	N CI right							165,7				101,3		166,1				204,8	
	P CI left					174,5		50,2				86,8				11,9	45,2	20,7	
	P CI right					180,3		67,2				103,3				27,1	58,5	32,4	
Resistance source						Simeto		Levante				Levante		Simeto		Simeto	Svevo	Levante	

Supplemental Table 7. Continued

Natural infection -Adult plant stage			Chromosomes																	
			1A		5A		7A		2B		3B		4B		5B		6B		7B	
QTL parameter	**Trait**		Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo	Simeto/ Levante	Kofa/ Svevo
LOD	N							3.3												
	P			2.48				2.93												2.97
% Expl	N							5.3												
	P			4.2				4.9												5
CI_{CM}[1] in cM	N	CI left						33,2												
		CI right						46,1												
	P	CI left		28,1				21,7												86,4
		CI right		31,2				33,2												98
Resistance source				Kofa				Svevo												Svevo

[1]CM=consensus map.

Isolate not tested

QTL not detected

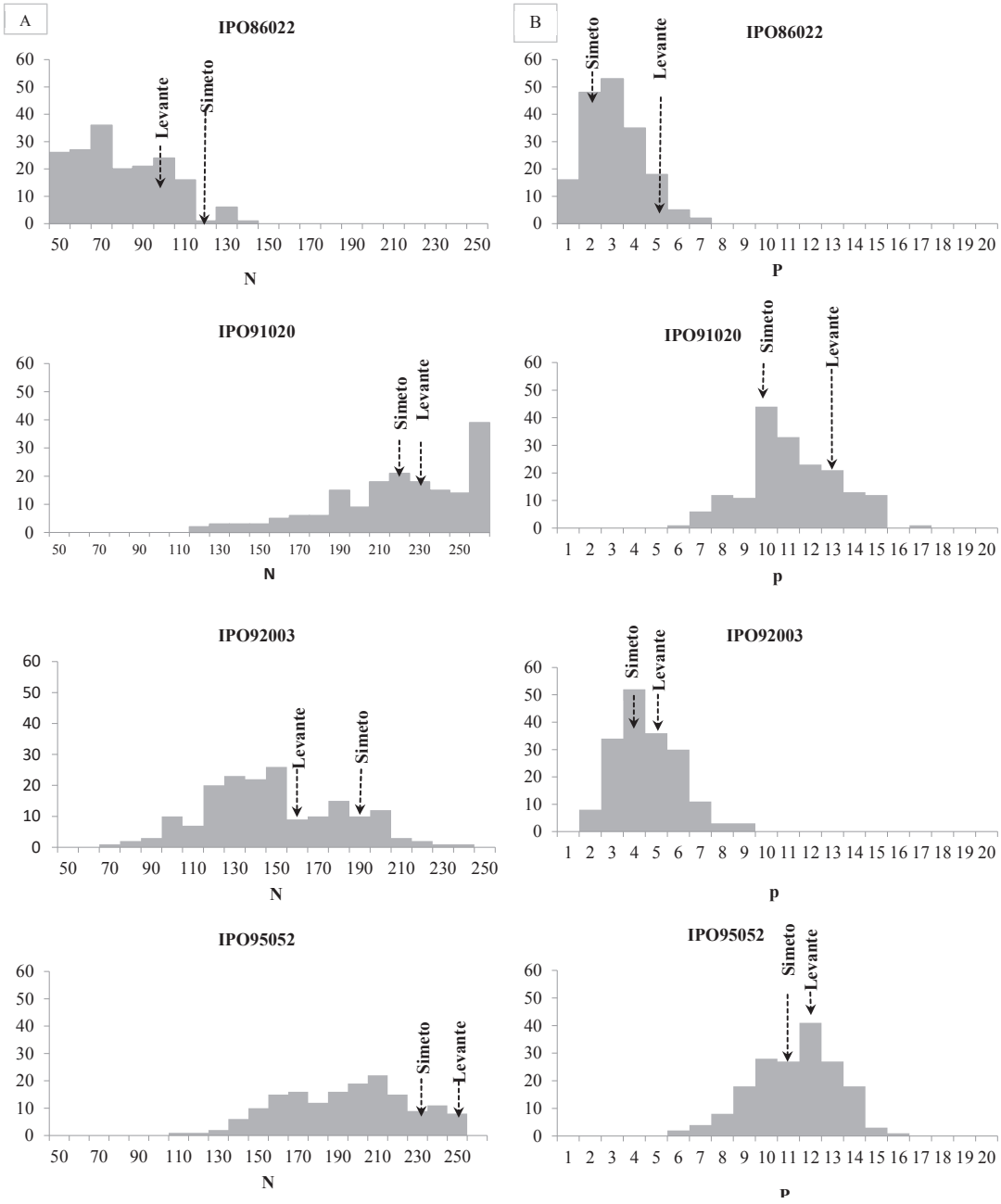


Figure S1. Phenotypic variation, scores as Naudpc (N) **(a panel)** or PaudpcSQRT (P) **(b panel)** of recombinant inbred lines (RILs) of the Simeto/Levante population (y-axis) to four *Zymoseptoria tritici* isolates in the seeding stage. The scores for the parents are indicated by arrows.

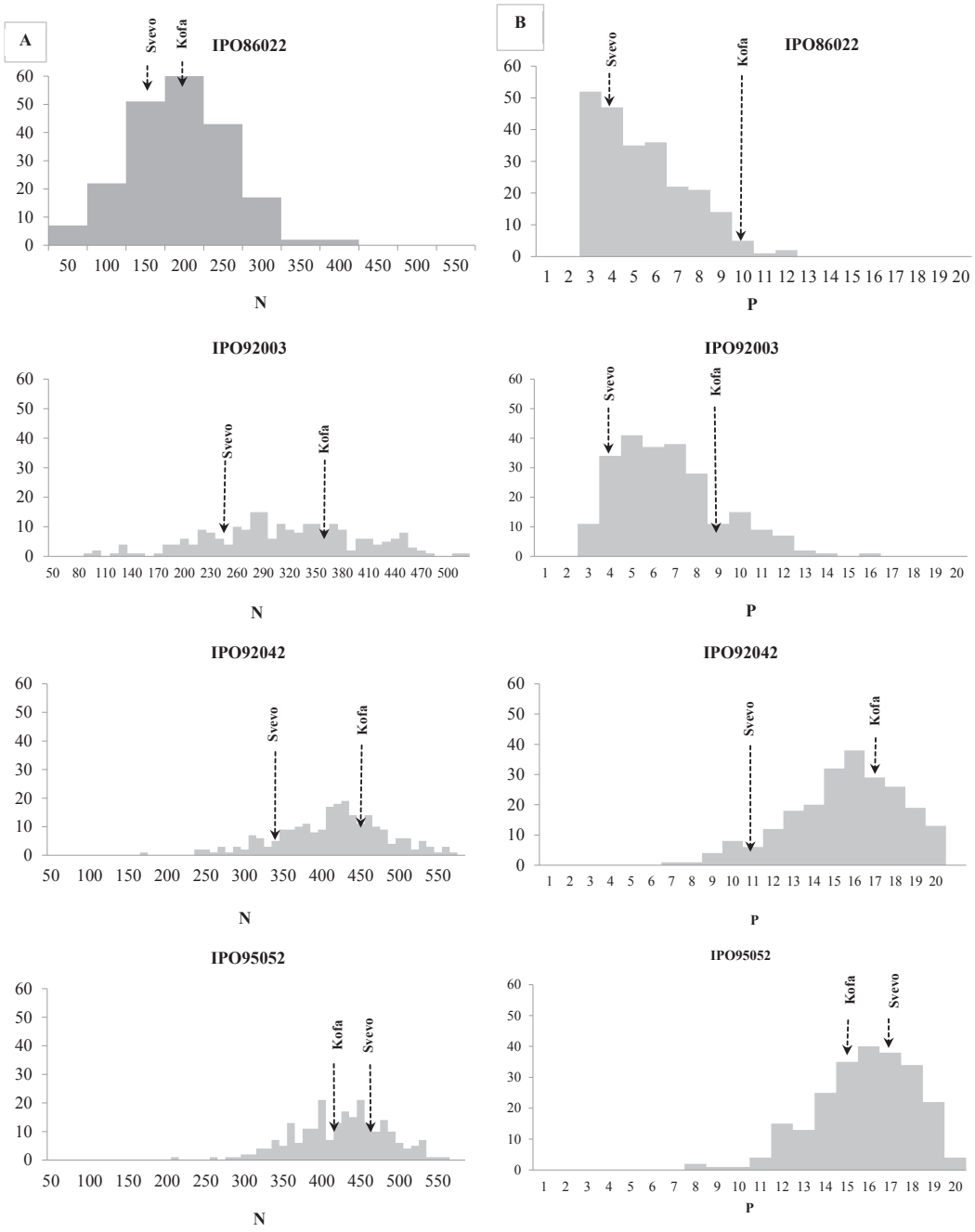
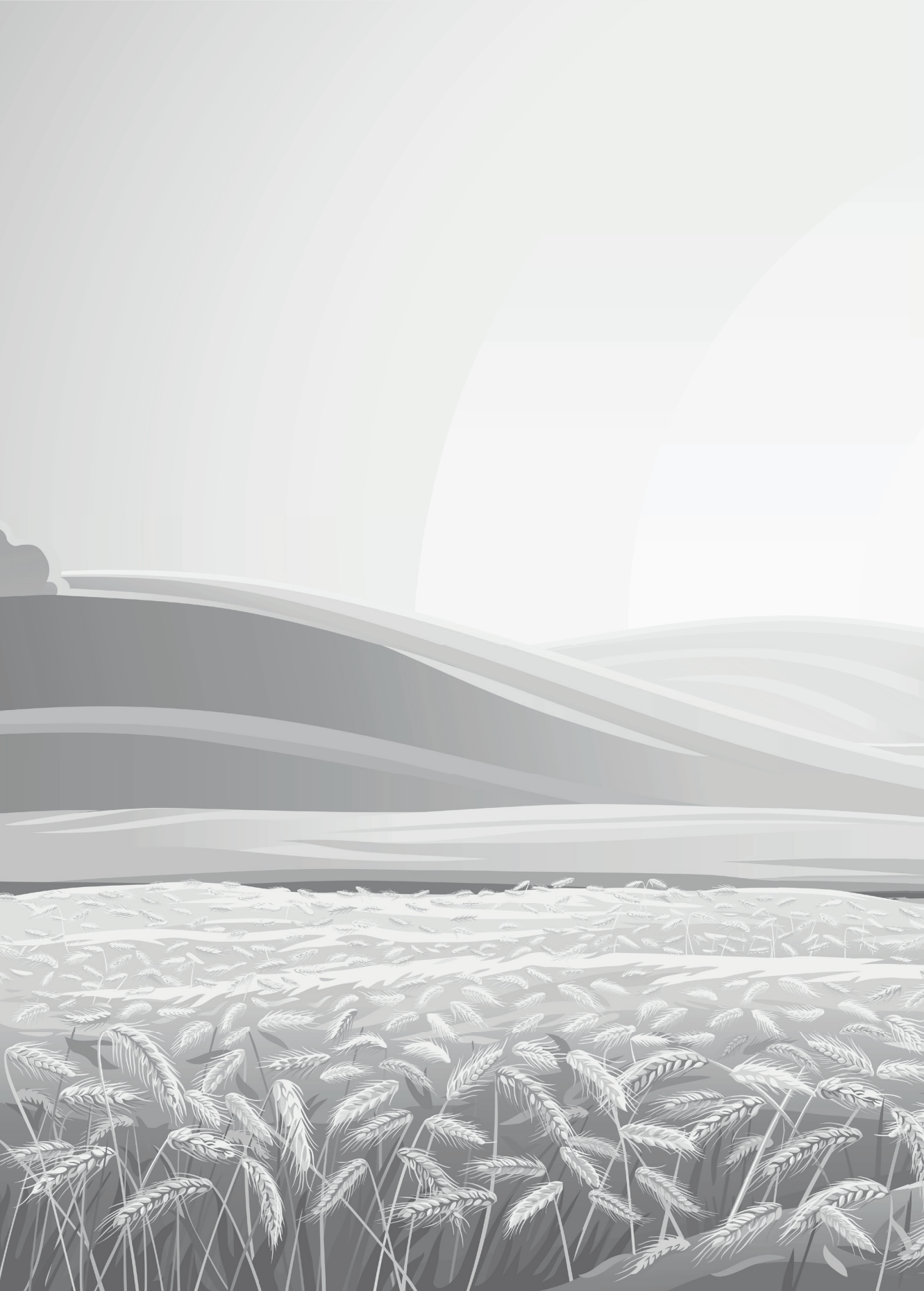
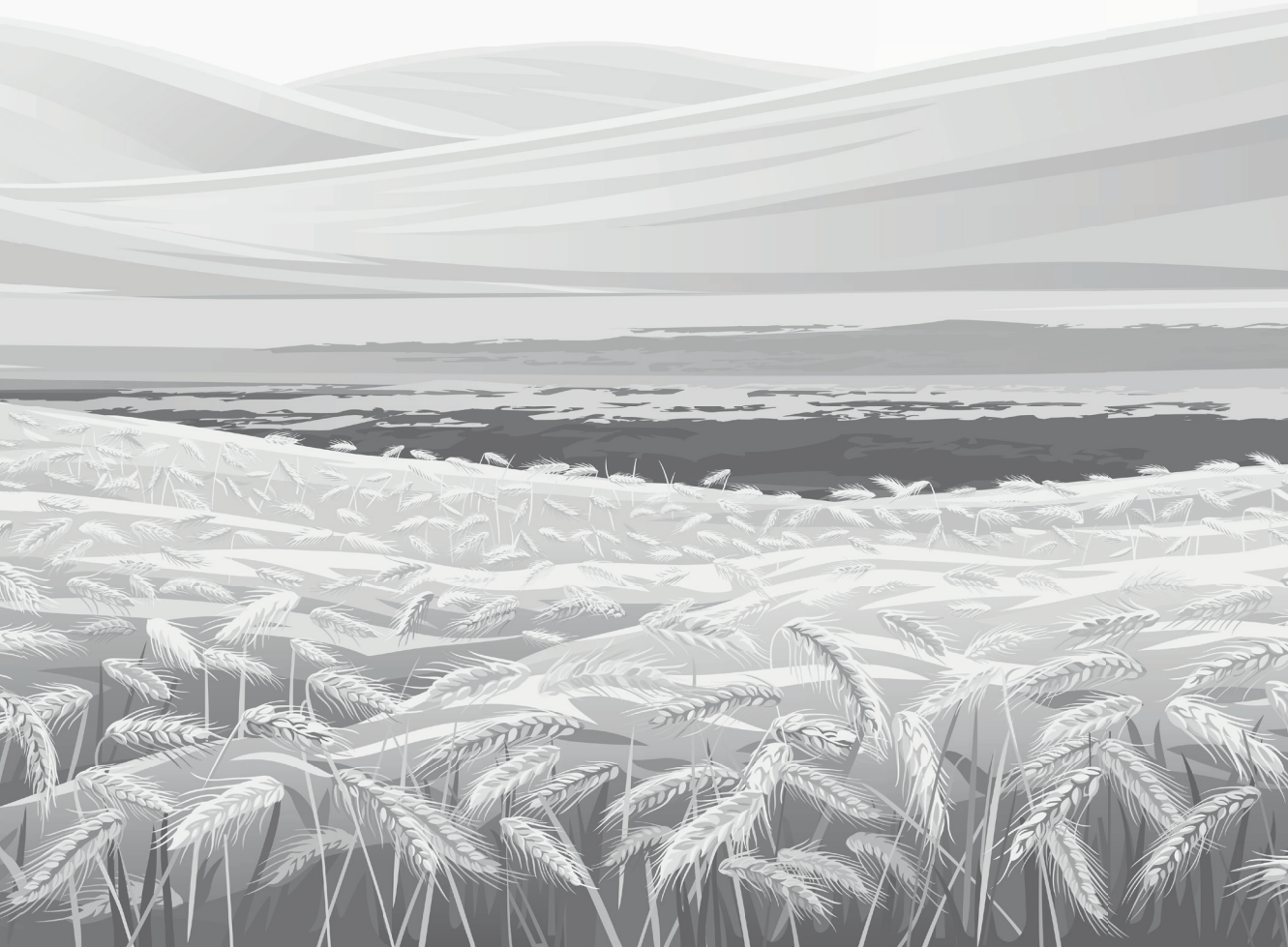


Figure S2. Phenotypic variation, scores as Naudpc (N) **(a panel)** or PaudpcSQRT (P) **(b panel)** of recombinant inbred lines (RILs) of the Kofa/Svevo population (y-axis) to four *Zymoseptoria tritici* isolates in the seeding stage. The scores for the parents are indicated by arrows.



Chapter 6

General discussion



Albeit enormous progress has been achieved worldwide in improving human welfare, much remains to be done to fulfil the vision of the Food and Agriculture Organization of the United Nations (FAO) to create a world free of hunger and malnutrition, and one in which agriculture contributes to improve the living standards of all, especially the poorest, in an economically, socially and environmentally sustainable manner (FAO 2017). Improving and sustaining staple crop production such as wheat in a briskly expending population are important challenges. Even though world population growth has slowed down over the last five decades, populations will continue to expand well beyond 2050 in some regions, surely in Africa, leading to an increasing global demand for food of 70 %, making reduced hunger and malnutrition unreachable and burdened prospects (Bhat 2017; FAO 2017). Above and beyond the expected population explosion, there are stark facts evidencing the perceived challenges such as the growing threat of plant pathogens to global food security. Such crop-destroyers account for persistent yield losses of up to 20% of the world's harvest (Bebber and Gurr 2015; Strange and Scott 2005). Moreover, climate change is prompting a rapid plant pathogens' evolution to shifting ecosystems (Croll and McDonald 2017; McDonald and Stukenbrock 2016; Nejat et al. 2017).

Durum wheat, a fundamental crop for many people in the Mediterranean basin, is no exception. This crop contributes up to 20 % of the daily calorie intake (Shewry 2009) and is subject to diverse constraints that hamper its production. The continuous co-evolutionary battle between durum wheat and *Zymoseptoria tritici* (*Z. tritici*) (Desm.) Quaedvl. & Crous (formerly known as *Mycosphearella graminicola*), the cause of septoria leaf blotch (SLB), constitutes a juxtaposed fact that needs to be considered in sustaining durum wheat production and that cannot be ignored by breeders that are in a constant search for excelling genotypes (Chen et al. 2017; Stukenbrock et al. 2011; Stukenbrock and Croll 2014; Stukenbrock and Francis 2014; Stukenbrock and McDonald 2008). The co-evolutionary history between wheat and *Z. tritici* has resulted into various levels of resistances that have increased the fitness of germplasm when encountering *Z. tritici* attacks, which are roughly divided in qualitative and quantitative resistance (Arraiano et al. 2009; Brown et al. 2015; Cowger et al. 2000; Eyal and Brown 1976; Kema et al. 1996a; Kema et al. 1996c; Kema and van Silfhout 1997; Kema et al. 2000; Simón 2010; Simón et al. 2016). Despite a range of studies that confirmed qualitative host resistance in the *Z. tritici*-wheat pathosystem (Brading et al. 2002; Kema et al. 1996a; Kema et al. 1996c; Kema and van Silfhout 1997; Kema et al. 2000; Kema et al. 2017),expressed as compatible or incompatible interactions between hosts and pathogen genotypes complying with the gene-for-

gene (GFG) model, it remained controversial, particularly due to different epidemiological perceptions (Van Ginkel and Scharen 1988). Recently, however, the first resistance gene and its cognate effector have been cloned thereby functionally proving that GFG underlies the interaction between *Z. tritici* and wheat (Saintenac et al. 2017; Zhong et al. 2017).

Despite enormous progress over the last decades, the co-evolutionary study between wheat and *Z. tritici* will remain partial if it is not completed with a better underpinning of durum wheat resistance to *Z. tritici*, which is also characterized by presumed GFG interactions (Ghaneie et al. 2012; Kema et al. 1996a; Kema et al. 1996b; Kema et al. 1996c; Medini and Hamza 2008; Medini et al. 2005). However, notwithstanding the devastating impact of *Z. tritici* on durum wheat production (Brown et al. 2015; Gharbi et al. 2000; Gharbi et al. 2008; Sebei and Harrabi 2008), the number of studies on its interaction with *Z. tritici* is limited. Durum wheat has been treated as an orphan crop by the scientific community, which entirely focussed on the relationship between bread wheat and *Z. tritici*. This skewed interest has resulted in 21 major *Stb* resistance genes and 167 quantitative trait loci (QTLs) all identified in bread wheat and some wild relatives leading to virtually extinct resistance sources in durum wheat (Brown et al. 2015).

Therefore, this thesis has its emphasis on durum wheat resistance and also presents a new perception of the fungal biology and (a)biotic stresses in shaping the coevolution between *Z. tritici* and wheat.

The wheat - *Zymoseptoria tritici* interaction: a door opened

A long coevolutionary history existst between plants and their associated pathogens in which the plant-pathogen interaction is considered to be a biological battlefield where both organisms attempt to outwit each other (Stukenbrock and McDonald 2009; Tan et al. 2010). Whilst the host builds-up an effective defence response upon the recognition of the pathogen, the pathogen coordinates its pathogenicity arsenal to facilitate a successful colonisation of the host tissue (Tan and Oliver 2017). The long co-evolutionary history has been a major driving force of diversification and speciation in fungal plant pathogens (Ravensdale et al. 2011; Stukenbrock and McDonald 2008), where natural selection has resulted in a diverse array of recognition and resistance mechanisms in plants (Hammond-Kosack and Parker 2003), and in the evolution of pathogen genes to counteract plant defences as well as to promote virulence (Stukenbrock and McDonald 2008; Stukenbrock and McDonald 2009). The interactions

shaping the age-long molecular arms race between pathogens and their hosts could be proven and genetically elucidated by deciphering the GFG model (Rouxel and Balesdent 2017; Thrall et al. 2016). Although other mechanisms, such as the matching allele model (MA) (Thrall et al. 2016), might elucidate host-pathogen interactions, the GFG model has been largely supported by genetic data and most convincingly by the isolation and mechanistic understanding of genes governing plant immune responses to biotrophic and hemibiotrophic pathogens (Dodds and Rathjen 2010; Thrall et al. 2016).

Since GFG was hypothesized by Flor (1947), based on his foundational work on flax and flax rust, tremendous efforts have been directed to quest for molecular and biochemical proof of GFG (de Wit et al. 2009; Mirzadi Gohari et al. 2015). The GFG model is defined by the congenial interaction of a dominant (a)virulence (*Avr*) gene in the pathogen, with its corresponding (*R*) resistance gene in the host. The recognition of the *Avr* protein by its cognate *R* receptor leads to the activation of a cascade of host defence responses that hamper colonisation of host tissue by the pathogen (de Wit et al. 2009; Ravensdale et al. 2011). Upon contact with their invasive pathogens, several layers of plant defences are activated, starting with the recognition of conserved pathogen structures referred to as pathogen-associated molecular patterns (PAMPs) by plant receptors that trigger basal defences or PAMP-triggered immunity (PTI) (Rouxel and Balesdent 2010, 2017). Successful pathogens could overcome this first defence barrier by blocking PTI via the secretion of specific effector molecules (Jones and Dangl 2006; Rouxel and Balesdent 2017). At a specific phase, the plant defence surveillance machinery is activated to recognize the effectors, by the so-called effector-triggered immunity (ETI), where classically a plant resistance (*R*) protein directly or indirectly interacts with a cognate pathogen (a)virulence (*Avr*) effector protein (Rouxel and Balesdent 2017).

Effectors are commonly defined as small secreted molecules from a microbe that can alter host cell structure and function, facilitating infection, such as host-selective toxins in necrotrophic fungi (Friesen et al. 2008), and/or trigger defence responses, known as (a)virulence factors, such as in biotrophic fungi (Selin et al. 2016; Tan and Oliver 2017). Several bacterial, fungal and oomycetes effector genes have been studied and functionally characterized. The first *Avr* effector gene was cloned in 1984 and was derived from the bacteria *Pseudomonas syringae* pv. *glycinea,* (de Wit et al. 2009; Staskawicz et al. 1984). The first fungal avirulence gene *Avr9* of *Cladosporium fulvum*, the causal agent of tomato leaf mold, was cloned in 1991 (de Wit et al. 2009; van Kan et al. 1991). After a decade, the *Avr1b* locus of the oomycete, *Phytophthora sojae*, was identified and cloned (de Wit et al. 2009; Shan et al.

2004). Ever since and with the prodigious advances in "omics" technologies, the identification of (a)virulence genes has been accelerating. At present, at least 35 *Avr* effector genes have been cloned from filamentous fungi infecting a wide variety of agronomically important crops (Bourras et al. 2016b; Zhong et al. 2017).

Several levels of complexity are detected from the standard GFG model elucidated by the cloned avirulence genes in *C. fulvum* and *Leptosphaeria maculans*, where single *Avr* genes are recognized by their cognate resistance genes (Bourras et al. 2016a; Hayward et al. 2012; Wulff et al. 2009) to the recognition of a single *Avr* by multiple R genes such as the case for the *Avr-Pik/km/kp* in *Magnaporthe oryzae* (Yoshida et al. 2009) and in *Melampsora lini* secreting the *AvrL567* and *AvrP123* effector genes (Ravensdale et al. 2012). Other effector genes have, however, a divergent action such as the suppression of the recognition of other resistance genes as illustrated by the *Avr1* effector gene encoded by *Fusarium oxysporum* f. sp. *lycopersici,* which is recognized by the tomato gene *I-1,* but also acts as a suppressor of the recognition of *Avr2* and *Avr3* by *I-2* and *I-3*, respectively (Bourras et al. 2016a; Houterman et al. 2008). Therefore, abundant studies have led to an extended body of knowledge about the role of effectors as an expression of the "extended phenotype" and their impact in the host plants (Oliver and Solomon 2010; Vleeshouwers and Oliver 2014). The translation of this research has successfully embraced effectors for breeding purposes as illustrated in potato breeding for *P. infestans* resistance, and many other examples (Nejat et al. 2017; Zhang and Coaker 2017), in which "effectoromics" plays a potent role in *R* gene postulation (Vleeshouwers and Oliver 2014; Vleeshouwers et al. 2011),

Nonetheless, effector-driven wheat breeding for *Z. tritici* has yet to start. *Zymoseptoria tritici* produces diverse arrays of small secreted proteins throughout the interaction with its host, which have been partly functionally analysed and characterized (Kettles and Kanyuka 2016; Mirzadi Gohari et al. 2015; Morais do Amaral et al. 2012; Rudd et al. 2015). Quite a few proteins have been described that contribute to *Z. tritici* pathogenesis on wheat (Kettles et al. 2017). Two effectors, MgNLP and Mg3LysM, have been identified in *Z. tritici* with a confirmed implication during the two lifestyle phases that are characteristic of the hemibiotrophic nature of *Z. tritici.* MgNLP represents a unique gene and is a member of the necrosis- and ethylene-inducing peptide 1 (Nep1)-like protein family (NLP). It is highly expressed during the immediate pre-symptomatic phase of colonization in a susceptible host, with a subsequent drastic decrease during disease lesion formation. However, deletion of this gene in *Z. tritici* does not affect pathogenicity or virulence, suggesting that this gene does not play a major direct

role during the fungal infection of its host (Motteram et al. 2009). The *Z. tritici* genome contains also three homologs of the extracellular protein 6 (Ecp6), the Lysin (LysM) domain-containing effector from the biotrophic tomato leaf mould fungus *C. fulvum*, which interferes with chitin-triggered immunity in plants. The molecular and functional characterization of the three LysM homologs in *Z. tritici* have revealed that *Mg3LysM* and *Mg1LysM* were specifically up-regulated during the symptomless phase. Uniquely, *Mg3LysM* blocks the elicitation of chitin-induced plant defences, and has shown to be a virulence factor (Marshall et al. 2011). Recently, a study that combined bioinformatics approaches with expression profiling (Mirzadi Gohari et al., (2015) has revealed a number of putative effectors that are up-regulated during pathogenesis of *Z. tritici* and two top candidates, SSP15 and SSP18, were functionally analysed but appeared to be dispensable for pathogenicity. The first proteinaceous toxins ZtNIP1 and ZtNIP2 of *Z. tritici* were described by Ben M'Barek et al. (2015a) and induce cell death and chlorosis, respectively, on some wheat cultivars, although the mechanism and contribution to virulence has yet to be elucidated (Kettles et al. 2017).

In spite of these substantial efforts, the molecular details of the *Z. tritici* – wheat interaction remain to be unravelled and many questions require a deeper understanding of this atypical pathosystem (Rudd 2015). Elucidating the hidden genetic factors underlying GFG in the *Z. tritici* – wheat pathosystem is among these quests that have been studied for long and was finally opened-up during this thesis work. In **chapter 2**, we describe an in-depth study of the *Z. tritici* effector *Avrstb6,* that interacts with the widely disseminated resistance gene *Stb6* (Brading et al. 2002) by using classical genetics, bioinformatics and functional characterization approaches. Resistance gene *Stb6* is located at the distal end of chromosome 3AS, and confers resistance to the Dutch *Z. tritici* isolate IPO323, which is present in cvs. Flame and Hereward (McCartney et al. 2003), and was subsequently also identified in about 15% of the European germplasm as well as in Chinese Spring, a selection from a landrace that is the model variety of wheat cytogenetics (Arraiano and Brown 2006; Chartrain et al. 2005). Our investigations in the architecture of the *Z. tritici* resistance in contemporary Italian durum wheat cultivars has also proven the presence of genetic regions associated with *Stb6* (**chapter 5**). Hence, the abundance of this gene in bread wheat cultivars and durum wheats made the identification of *AvrStb6* even more interesting, particularly since *Stb6* was recently cloned (Saintenac et al. 2017) .

Previous studies to identify *Z. tritici* effectors were biased by inappropriate techniques, unfitting standard qualifiers for the effectors, and most prominently a poor quality of the

genome annotation (Mirzadi Gohari et al. 2015; Rudd et al. 2015). During our study, we combined a map-based approach, DArT sequencing and functional analysis using a corrected genome annotation which almost immediately yielded the successful identification of the *Z. tritici AvrStb6* effector gene. Previous studies deploying genetics approaches have been also effective in mapping QTLs containing *Z. tritici* effectors (Lendenmann et al. 2015; Lendenmann et al. 2016). Thus, an effective unveiling of *Z. tritici* effectors requires an improved annotation of the *Z. tritici* genome and a mixed suite of genetic techniques and "omics" techniques. The improved annotation of the Z. *tritici* genome also facilitates genome wide association (GWAS) approaches that were recently shown to be very successful in the identification of *AvrStb6* (Zhong et al. 2017), which surely will lead to the discovery of a plethora of new effectors in the *Z. tritici* genome.

The unveiled *AvrStb6* effector gene encodes a small, cysteine-rich, secreted protein, located in a highly polymorphic distal part of chromosome 5, which is packed with transposable elements and shares effector structures described in other fungi (De Wit 2016; de Wit et al. 2009; Rouxel and Balesdent 2010; Stergiopoulos and de Wit 2009). The highly polymorphic region comprising *AvrStb6* fits the "two-speed genome" hypothesis (Faino et al. 2016) involved in rapid evolution to overcome the *Stb6* gene (Zhong et al. 2017). Expression profiling of the identified effector suggests that it is operating during the latent phase of the infection, which suggests an important role during the switch from the biotrophic phase to the necrotrophic phase of pathogenesis. However, a firm conclusion cannot be drawn and further investigations are required to answer whether *AvrStb6* plays a key role in inducing necrosis and thus to the switch between both stages. A protein infiltration assay would be an alternative to give us more insights and to answer this question (Solomon 2017).

Undoubtedly, the characterization of the *AvrStb6* effector gene provides the first key step towards dissecting this complex quantitative disease (Solomon 2017). The recent cloning of *Stb6* revealed that it is a wall-associated receptor kinase (Saintenac et al. 2017), which emphasizes the hypothesis that *AvrStb6* is recognized at the plant cell surface leading to an Effector-Triggered-Defence (ETD). Therefore, further investigations into the molecular interaction between *AvrStb6* and *Stb6,* which was not addressed in our study, are required to increase our understanding of STB.

The wheat - *Zymoseptoria tritici* interaction: Survival of the fittest

Host and pathogen diversity are driven by their constant and long evolutionary interaction (Ravensdale et al. 2011; Rouxel and Balesdent 2017). Despite substantial advances in our understanding of these interactions at both the molecular and population levels, major questions are unresolved regarding the mechanisms of host resistance and pathogen virulence, their variation in space and time, and their long-term effect on host–pathogen co-evolution (Ravensdale et al. 2011). Our findings in **chapter 2** have shed new light on the epidemiology of *Z. tritici* and most likely on many other pathogens. The Exclusive Paternal Parenthood (EPP) mechanism, includes the sexual reproduction of (a)virulent *Z. tritici* strains that comes down to the loss of the genotype, but the maintenance of the *Avr* loci in the population. This contradicts the assumed aspect in botanical epidemiology that (a)virulent strains are removed from the population as they cannot reproduce asexually. Hence, the classical view on botanical epidemiology, where allele frequencies of plants and pathogens are subject to variation relying on the selection forces that shape their abundance, resulting in boom-and-bust cycles such as for the wheat rusts (Brown and Tellier 2011), does not apply for *Z. tritici* and likely many other Dothideomycetes and has important implications for durability of resistance. The EPP model better explains several real-world farming observations as overcoming host resistance takes more time and host resistance would only be defeated when the frequency of virulence isolates passes a critical threshold and becomes established on a hitherto resistant cultivar (Brown 2015). Thus, we could better relate the longevity of *Stb* genes and QTLs, where hardly an abrupt collapse has been reported or observed, resulting in the observation that an *Stb* resistance gene would be effective for an average of a decade, before its breakdown (Brown 2015; Cuthbert 2011; Czembor et al. 2011). Nonetheless, agricultural landscapes based on monoculture and the intensive use of identical resistance sources would facilitate adaptation of *Z. tritici,* which has a very active sexual cycle, through the development of recombinant genotypes that eventually could overcome resistance (Croll and McDonald 2016; McDonald and Linde 2002a, b; McDonald and Mundt 2016; McDonald and Stukenbrock 2016). However, this should also be addressed in epidemiological studies and the careful monitoring of the occurrence of virulent strains upon hitherto effective *Stb* genes. Since *Stb16q* has now been introduced in commercial wheat cultivars in Europe, such as in cv. Cellule, these studies now become feasible. The EPP model better enlightens the observations of Tellier and Brown (2007) where the virulence build-up rate is observed together with the occurrence of polymorphisms in an agricultural system as

a result of human selection forces, but a stable and cyclic polymorphism occurs in natural systems, where external selection forces are almost extinct. Similar observations were reported in several other studies (Brown 2015; McDonald and Linde 2002a) and are supported by the EPP model. The model also better explains the rapid dissemination of the QoI resistance in European *Z. tritici* populations (Torriani et al. 2009) as our data prove that when QoI sensitive strains still contribute to the sexual reproduction, the mitochondrially inherited *cytb* resistance allele invades faster than any nuclear inherited fungicide resistance.

Recently, Grandaubert et al. (2017) showed that sexual reproduction plays a central role in *Z. tritici* protein evolution and thus in the rate of genomic adaptation. Positive selection for genes encoding secreted proteins and putative virulence determinants is crucial to successfully overcome host defences or avoid host recognition. This observation matches the EPP model where diversifying selection for *Avr* effector genes would occur when an (a)virulent isolate can partake in sexual recombination with a virulent isolate. A similar observation has also been made in other fungal pathogens (Dodds and Thrall 2009; Endo et al. 1996) as pathogens can undergo sexual reproduction to accede favorable genes that will increase their fitness to invade and circumvent host resistances. A recent study illustrates the battlefield between wheat and *Z. tritici* by investigating the effect of host resistance on the genetic structure of an Irish *Z. tritici* populations. It showed that the effect of host resistance selection is minor compared with sexual reproduction, thus denying the assumption that R genes constitute selection forces to remove avirulent isolates from the population (Welch et al. 2017).

In conclusion, it is time to initiate studies on the impact of EPP on the structure of natural *Z. tritici* populations and other cereal pathogens, which truly will help to improve disease and resistance management in various crops.

Zymoseptoria tritici and wheat: How knowledge of pathogen genetics supports breeding for STB resistance

Understanding the genetic architecture of individual pathogens and subsequently the detailed genetic dynamics of whole populations has been the focus of efforts to develop more effective means of controlling the long-term disease resistance to crop pathogens (Jacobs and Parlevliet 2012). Over nearly 30 years, several studies have been conducted to elucidate the population biology of *Z. tritici*, their epidemiological cycles and the fungal genetic factors

deployed to invade and cause diseases in wheat (McDonald and Mundt 2016). Several studies have dissected the molecular characterization of the major events occurring during the *Z. tritici* infection of wheat which has yielded a more thorough understanding of disease progress (Ben M'Barek et al. 2015a; Ben M'Barek et al. 2015b; Gohari 2015; Kettles et al. 2017; Kettles and Kanyuka 2016; Mirzadi Gohari et al. 2015). Albeit that the mode of action of *Z. tritici* effectors to infect plant cells remains intriguing, characterized isolates have furnished the postulation for resistance genes in bread wheat (Tabib Ghaffary 2011). Substantial progress has been made over the last 20 years in the genetics of resistance to *Z. tritici*. Nowadays, bread wheat germplasm accounts for several sources of resistance and additionally a number of exotic as well as synthetic wheat genotypes have been identified as good sources of STB resistance (Kettles and Kanyuka 2016). Alike many other fungal diseases, both qualitative and quantitative resistances to STB occur in wheat (Arraiano et al. 2009; Arraiano and Brown 2006). At least 20 distinct *Stb* genes have been identified, which frequently have an isolate-specific efficacy. Up-to-date, solely the *Stb16q* gene has revealed a wide spectrum of resistance to a plethora of *Z. tritici* isolates. *Stb6* is one of the better-characterized resistance genes that was recently cloned (Saintenac et al. 2017). Throughout the *Z. tritici* breeding history, some major *Stb* genes have been noted to last longer than others. For instance, US wheat breeders have been actively deploying two major resistance genes, *Stb1* and *Stb4*, since the early 1970s (Goodwin 2007). Whilst *Stb1* provided a long-lasting resistance to wheat in the central USA, *Stb4* only remained effective in California for about 15 years before a noted reduced effectiveness of this gene (Jackson et al. 1990). Efficacy of the other known *Stb* genes against natural populations of *Z. tritici* are undetermined (Kettles and Kanyuka 2016). It is also still enigmatic what makes some *Stb* genes more effective than others, but our developed EPP model could very well explain these differences. Moreover, the recent isolation of *Stb6* and *AvrStb6* enables research into the cost of fitness of (a)virulent isolates to resistant host genotypes. Evidently, assessing the frequencies of avirulence towards known *Stb* genes in field populations of the fungus, that currently are unknown, would provide us with better insights on the durability of *Stb* resistance genes and their combinations (Kettles and Kanyuka 2016; Leach et al. 2001). Notably, some bread wheat genotypes carrying more than three isolate-specific *Stb* genes have been considered as major sources of resistance to STB, such as cvs. Kavkaz-K4500, TE9111 and Veranopolis (Chartrain et al. 2004; Kollers et al. 2013), which emphasizes the importance of pyramiding as an effective strategy to ensure durability of disease resistance (Brown 2015; Mundt 2014). Moreover, the 167 QTLs that have been identified and mapped in a total of 19 bi-parental

mapping populations (Brown et al. 2015; Goudemand et al. 2013; Kettles and Kanyuka 2016), contribute to valuable genetic variation for resistance breeding, despite the limited efficacy to just a few *Z. tritici* isolates or the partial resistance.

Despite the significance of the detected STB resistance loci, it remains a challenge to enhance the resistance levels of wheat cultivars because of the mostly quantitative nature of wheat-*Z. tritici* interactions and of the very high level of genetic diversity within pathogen populations (Ghaffary 2011; Marcel et al. 2017). This holds for bread wheat breeding and even more for durum wheat breeding, where the initial data on diversity are presented in this thesis. Translating the advanced known-how from the bread wheat pathosystem to durum wheat firstly required a better characterisation of the pathogenicity and specificity of durum wheat derived isolates. The availability of the fully sequenced genome of the durum wheat derived reference isolate IPO95052 is a good starting point for in-depth studies focusing on the genetics of the durum wheat *Z. tritici* interaction and populations genetics. A major point of attention should be the species specificity that was observed since the 1970s (Eyal et al. 1973; Kema et al. 1996a; Kema et al. 1996c; Kema and van Silfhout 1997) and can be addressed by the further analyses of crosses between *Z. tritici* isolates that are specific for durum wheat or bread wheat such as the cross between the reference strains IPO323 and IPO95052 (Ware 2006).

Genetic makeup of durum wheat resistance to STB: The drastic effect of an intensive selection pressure

This thesis is a start of unveiling durum wheat resistance to *Z. tritici.* An efficient use of biodiversity in breeding programs is imperative for improving STB resistance in these wheats. Therefore, we had planned to decipher STB resistance in tetraploid wheats, representing part of the evolutionary track of durum wheat from cultivated emmer (**Chapter 3**) to landraces (**Chapter 4)** to contemporary cultivars (**Chapter 5)**. During these trials, we also surveyed a large number of durum derived *Z. tritici* isolates, which comprised a first characterisation of durum pathotypes and also enabled an accurate selection of the deployed strains for the genetic studies.

Wild wheat relatives have been frequently deployed in breeding programs to improve the fitness of contemporary germplasm to encounter (a)biotic stresses (Monneveux et al. 2000; Prat et al. 2014; Reynolds et al. 2007; Sheikh et al. 2017; Wang et al. 2017; Wulff and Moscou

2014). Remarkably, a large number of resistance genes to the most hazardous fungal diseases has been derived from the cultivated emmer *Triticum tugidum* ssp. *dicoccum* (Buerstmayr et al. 2012; Faris et al. 2010; Piarulli et al. 2012; Zhang et al. 2014). It has been recognized that 12 leaf rust resistance genes and 20 stem rust resistance genes originate from progenitors of cultivated wheat species (Monneveux et al. 2000). Emmer wheat is currently grown as a minor crop in Ethiopia, India, Italy, Turkey, France and Iran and used primarily for cultural reasons. However, the interest in these species has recently increased because of the demand for speciality breads and beers as well as due to lower requirements of nitrogen fertilizers and crop protection chemicals (Gooding 2009; Shevkani et al. 2017). In our attempt to better understand STB resistance in durum wheat, we firstly investigated the genetics of *Z. tritici* resistance in a mapping population (BP025 population) derived from a cross between the emmer wheat (PI41025) and the contemporary cultivar Ben (**chapter 3**). The pre-screening of PI41025 and cv. Ben with a plethora of durum derived *Z. tritici* isolates has revealed a broad resistance of the emmer accession, in contrast to the contemporary cv. Ben that exposed an overall high susceptibility. A subsequent study in the derived Recombinant Inbred Lines (RILs) resulted in the identification of a major and novel quantitative trait locus, designed as *Stb22q* that is derived from PI41025, and mapped at chromosome 3AL. However, *Stb22q* only confers a broad resistance spectrum for pycnidia coverage in the seedling stage as necrosis levels were invariably high. An additional minor QTL on chromosome 5A, distinct from *Stb17*, has been also identified in the BP025 population and provides resistance to isolates IPO91009 and IIB123, hence has a narrow efficacy, and is derived from the susceptible contemporary cv. Ben. As shown in **chapters 4 and 5**, pyramiding such QTLs also leads to acceptable levels of STB resistance, similar to other studies (Berraies et al. 2014; Ghaffary 2011; Ghaffary et al. 2011; Kelm et al. 2012; Kidane et al. 2017; Tuberosa 2014).

Our findings emphasize that wild relatives of wheat represent a diverse gene pool containing novel and potentially effective resistances to wheat diseases. Breeding for *Z. tritici* resistance in bread wheat using synthetic wheat hexaploids, derived from tetraploid ancestors and the recently sequenced *Aegilops tauschii* (Zimin et al. 2017), has also been proven as a valuable resource for common wheat improvement (Yang et al. 2009). For *Z. tritici, Stb16q* that originates from the synthetic hexaploid M3 (W-7976) (Ghaffary et al. 2012) has the widest efficacy. The result of **chapter 3**, is a proof that major and wide spectrum resistance do simultaneously occur in tetraploid wheat. *Stb22q* is the first major QTL identified in durum wheat with a wide efficacy and is therefore an important discovery that will facilitate the release

of new STB resistant durum wheat varieties. Albeit the demonstrated value of *Stb22q,* subsequent field trials and adult plant tests are required to verify its broad efficacy in the adult plant stage.

Deciphering the durum wheat resistance to STB has been subsequently investigated in durum wheat landraces (**Chapter 4**) that are commonly perceived as the outcome of natural and human selection, thus an intermediate stage in domestication between wild wheat and elite cultivars. Smallholder farmers in traditional agrosystems used to select and maintain excelling genotypes to form traditional varieties adapted to local conditions with an adequate level of resistance to biotic stress and high tolerance to abiotic stresses (Giraldo et al. 2016; Sahri et al. 2014). These landraces are treasured sources that were often used in wheat improvement (Lopes et al. 2015; Newton et al. 2010; Soriano et al. 2016). Alike wild durum progenitor (**chapter 3**), landraces convey valuable resistance genes that were used to enhance durum wheat resistance to various fungal diseases (Prat et al. 2017; Rahman et al. 2017), which thus stirred our interest. In **chapter 4,** we studied resistance to *Z. tritici* genetic in a range of Tunisian landraces, previously proven to contain valuable sources of resistance (Ferjaoui et al. 2015; Ferjaoui et al. 2011; Medini et al. 2014). Analogous with the observation in **chapter 3** the tested landrace accessions showed a broad spectrum of resistance to *Z. tritici*, which contrasts with the contemporary high yielding durum cvs. Khiar and Karim that are largely susceptible to all deployed isolates. Studying the RILs from the cross between the resistant landrace "Agili39" and the susceptible cv. Khiar at the seedling and adult stages have shown that *Z. tritici* is governed by distinct factors, which is in accord with previous studies (Brown et al. 2015; Tabib Ghaffary et al. 2012; Tabib Ghaffary 2011; Tabib Ghaffary et al. 2011). The broad resistance in "Agili39" is mainly caused by the natural pyramiding of several QTLs with partial and major effects in the seedling and adult stage, which provide an improved fitness to withstand *Z. tritici* encounters. The 2BL QTL is effective at both stages, with a large spectrum of resistance to the used *Z. tritici* isolates, and most likely more, and explains up to 57% of the phenotypic variance in the adult stage during field testing with isolate Tun6. Unexpectedly, this QTL co-aligns with *Stb9* that is identified in bread wheat cv. Courtot (Chartrain et al. 2009) and commonly deceived by most *Z. tritici* bread wheat isolates (Tabib Ghaffary 2011; Tabib Ghaffary et al. 2011). The confirmed effectiveness of *Stb9* in durum wheat could result from the cost of virulence for *Stb9* in durum wheat derived *Z. tritici* isolates. Nevertheless, longevity of *Stb9* is also endangered in durum wheat, as we showed that virulent isolates for *Stb9* already occur in the natural durum wheat isolates (eg in isolate IPO92003 from Portugal, **chapter 3**). Thus, the

effectiveness of the putative *Stb9* in durum wheat is loomed and its breakdown would just be a matter of time if this gene is not wisely commercialized and used. Under the EPP model, however, its effectiveness may sustain for substantial time.

Unfortunately, inaccessibility of DArT marker sequences disabled ascertaining whether the identified QTLs in the "Agili39"/Khiar population (1A and 2BS) overlap with reported *Stb* positions, and we could thus not confirm the identification of novel locations in this population. However, we clearly demonstrated that stacking QTLs contributed to wide spectrum resistance observed in "Agili39", showing that gene pyramiding is an effective strategy for widening efficacy as evidenced in many other studies (McDonald and Mundt 2016; Mundt 2014; Singh et al. 2017; Singh et al. 2014; Singh et al. 2011; Singh et al. 2015; Singh et al. 2005; Singh et al. 2016). Albeit that two-way and three-way interactions (**chapter 4)** not always effectively reduce disease severity - as further demonstrated in our analyses of resistance in contemporary durum cultivars (**Chapter 5**) - we showed that accumulating the markers for the putative *Stb9* allele and 1A-QTL considerably reduced disease in the seedling stage with a dominant effect of the *Stb9* over the 1A-QTL. However, in the adult stage this positive effect was reduced by the presence of the 2BS-QTL marker, acting as a negative epistatic factor. Clearly, QTL interactions should be considered in order to plan combining favourable QTLs in a gene pyramiding approach (Vanderplank 2012).

Unexpectedly, the susceptible high yielding Tunisian cv. Khiar, used in the cross with the landrace 'Agili39', shares a QTL on chromosome 2A that increased its susceptibility to *Z. tritici* in the derived RILs. This cultivar was introduced by CIMMYT to increase the yield potential in Tunisian durum wheats. Thus, one should be increasingly aware that introducing alleles for better yields could also drag other yield compromising susceptibility alleles into a breeding program, as was also observed among European bread wheats where susceptibility genes for *Z. tritici* were unconsciously introduced from CIMMYT germplasm (Arraiano and Brown 2016). Moreover, Arraiano and Brown (2016) also showed that such alleles are closely linked to partial resistance QTLs for *Z. tritici,* which further complicates the removal of such unfavourable loci in contemporary breeding programs. Thus, trade-offs should be considered in plant breeding when several traits are important. A counterbalance between the studied traits and the use of non-adapted germplasm could underlie gene drag leading to undesired genes that are only later to be discovered, for instance due to changes biotic stresses. The situation discussed by Arraiano and Brown (2016) also refers to the time that *Z. tritici* was not considered

to be of much importance for European wheat breeding (Arraiano and Brown 2016; Reif et al. 2005).

Studying the genetics of resistance to *Z. tritici* in durum wheat cannot exclude contemporary durum cultivars or the so called semi-dwarf cultivars that are notably the most desired materials for farmers due to their improved agronomical traits, their high productivity and their genetic uniformity (Soriano et al. 2016; Tadesse et al. 2016) (**Chapter 5**). Modern cultivars constitute the outstanding outcome of the green revolution. They are - even after twenty years - the touchstone in international agricultural development that prevented famine at a time when it seemed imminent (Wolf 1986). During the late 1960s and early 1970s, new varieties with semi-dwarf stature conferred by the reduced height genes *Rht1* and *Rht2* were introduced into Asia and Latin America along with fertilizers, pesticides and mechanized farm equipment, and dramatically increased harvests without any perceived change in the wheat cultivation area (Smale 1997; Tadesse et al. 2017; Tadesse et al. 2016). Meanwhile, landraces - once abundant – became extinct and disappeared largely from the modern farming system due to their undesirable agronomic characteristics with respect to plant height, general late flowering and low harvest index (Soriano et al. 2016). The adoption of modern wheat cultivars rapidly expanded in developing countries, and studies of CIMMYT/ICARDA indicated that in the 1990s, semi-dwarf wheats covered nearly 50 million ha., which was 70% of the total wheat area in the developing world, excluding China (Byerlee 1993). Albeit, the Green Revolution has been controversial in terms of the distribution of its benefits, the general consensus is that adoption of modern cultivars has, in most cases, been favourable in terms of income distribution (Reynolds and Borlaug 2006). Therefore, these benefits should be embraced and high-yielding contemporary durum wheats should be continuously developed and improved by adequate breeding programs using the latest practices and know-how to reduce the risk of imminent food shortages.

Therefore, **Chapter 5**, focuses on deciphering the genetic basis of resistance in contemporary durum wheats from Italy, which is one of the leading durum wheat producing countries (Álvaro et al. 2008; Royo et al. 2007). In contrast **chapters 3** and **4** where major QTLs were identified, the QTLs in cvs. Simeto, Levante, Kofa and Svevo have only minor effects on resistance, which suggests a deterioration of STB resistance compared to landraces and wheat progenitors as discussed in the previous chapters and similar to other studies in durum wheat (Berraies et al. 2014; Tuberosa 2014) and in bread wheat cultivars (Arraiano and Brown 2016;

Kelm et al. 2012; Tabib Ghaffary et al. 2011). Nevertheless, the Simeto/Levante and Kofa/Svevo mapping populations revealed new locations for partial STB resistance on chromosomes 4B, 5B, 6B and 7B. Apart from QTLs mapped on chromosome 4B which showed a wide efficacy in the seedling and adult stage, most of these were also specific to one or a few *Z. tritici* isolates. This is reminiscent of **chapters 3** and **4** where - with the omission of the *Stb22q* locus - the putative *Stb9* and the 2BS-QTL loci together contributed to the wide resistance in the landrace "Agili39", despite their independent limited efficacy. Hence, besides researching and discussing GFG in clearly qualitative interactions (Eyal et al. 1973; Kema et al. 1996a; Kema et al. 1996b; Kema et al. 1996c; Kema and van Silfhout 1997; Kema et al. 1996d; Medini and Hamza 2008), specificity has now been confirmed and explained by cloning the first (a)virulence effector *Avrstb6* (**Chapter 2)**. In addition, however, we show the value of genes with smaller effects and that their qualitative expression is also based on specific interactions with *Z. tritici* isolates. However, it is clear that the apparent dichotomy in *Z. tritici* adds another layer of complexity. The identified QTL on chromosome 1B in the Kofa/ Svevo population co-align with *StbWW* (Raman et al. 2009) and *Stb2* (Adhikari et al. 2004). Other minor effect QTLs with a narrow efficacy co-aligned with *Stb7* (4A), *Stb12* (4A), *Stb3* (7A) and *Stb6* (3A) that all have been mapped and showed a much wider efficacy in bread wheat (Cuthbert 2011; Kelm et al. 2012; Tabib Ghaffary 2011).

Identifying the genetic factors determining this dichotomy is evidently important as it is plausible that (a)virulence effectors diverge pathogenicity for durum and bread wheat. Since this dichotomy seems to have the widest possible efficacy, which also translates in e.g. the very high levels and wide resistance to *Z. tritici* in *T. monococcum* (Jing et al. 2008), discovery of the underlying mechanisms has substantial fundamental and practical implications for resistance breeding in wheat and bread wheat. For now, it us useful to consider the frequency of (a)virulent loci in natural populations in planning wheat breeding efforts, particularly since we expect that soon many more effectors will be discovered and validated.

Unravelling resistance to *Z. tritici* in durum wheat has shown the drastic decrease of gene efficacy, from a broad spectrum of resistance derived from a cultivated emmer wheat (**chapter 3**), to durum landraces (**chapter 4**) until a partial and narrow efficacy in contemporary durum cultivars (**chapter 5**). Hence, natural and human selection and active breeding for high-yielding genetically uniform varieties led to the use of ultimately a small fraction of the genetic reservoir (Lopes et al. 2015; Maccaferri et al. 2005; Mangini et al. 2017; Royo et al. 2005a;

Royo et al. 2005b; Soriano et al. 2016). Our data demonstrate how domestication and modern plant breeding have presumably narrowed the genetic base of durum wheat. Through the centuries, genetic variation was reduced early from the natural genetic drift to the early selection of traditional farmers, which eventually resulted in landrace cultivars adapted to specific conditions of their habitats (Reif et al. 2005). Many of the traditional landraces were replaced by modern wheat cultivars that were bred with a limited number of landraces in their pedigree, and it is postulated that contemporary cultivars contain less genetic diversity than traditional landraces or wild relatives, as evidenced in our data, which could jeopardize future wheat improvement (Reif et al. 2005).

Thus, gene introgression from wild progenitors and landraces can greatly enhance the fitness of contemporary cultivars to *Z. tritici* and maintain their high yield potential (Blanco et al. 2008; Merchuk-Ovnat et al. 2017; Rong et al. 2000; Sheikh et al. 2017; Valkoun 2001; Xie and Nevo 2008). In this thesis, several new and valuable QTLs were identified that show a broad efficacy that is mainly derived from the cultivated emmer accession (PI41025) (**Chapter 3**) and the Tunisian landrace 'Agili39' (**Chapter 4**). These are likely only the "tip-of-the-iceberg" of potential sources for *Z. tritici* improvement in durum wheat. Nonetheless, recent studies unveiling detrimental genes that were unconsciously introduced in modern wheat breeding programs should be taken into consideration to avoid future disappointments. However, it also raises the awareness that screening should not be limited to the contemporary diseases and threats, but that conscious breeding considers a broad phenotyping with a wide array of pests and pathogens. The costs aspect, however, likely will not support such a strategy and hence, we are dealing with a perpetual cycle of incompleteness. However, expanding data sets and ever increasing genomic information enabling genomic selection will likely counteract such developments. Hence, direct effect of an R gene on for instance yield, suggests a fundamental mechanistic relationship, as the genes that are linked to this R gene may also affect yield and therefore hamper the selection of commercially successful resistant cultivars (Brown 2002). There are numerous examples of yield penalties upon the introduction of R genes, such as the unbroken linkage between yield depression and the *Lr9* R gene from *Ae. umbellulata*, which confers resistance to wheat brown rust (also known as leaf rust, caused by *Puccinia triticina*, syn. *Puccinia recondita* f. sp. *tritici*). Other R genes on introgressed segments that are associated with reduced yield are *Wsm1* for resistance to wheat streak mosaic virus from *Thinopyrum intermedium*, associated with a yield reduction of 21%, and three genes for stem rust (*Puccinia graminis* f. sp. *tritici*) resistance, notably *Sr26* from *Agropyron*

elongatum, which resulted in a 9% yield penalty. Of course reduced yield is not the only cost of resistance, but is considered as most important as it hampers commercialization of a new resistant wheat cultivars (Cox 1997; Feuillet et al. 2008). Overall, any new disease resistance needs to be placed in a genetic background that meets current requirements for yield, quality, agronomy and resistance to other diseases and stresses (Summers and Brown 2013). Any deficiency in one of the abovementioned requirements will definitely affect commercialization.

Our data show also that despite the value of the identified loci for increasing the resistance to *Z. tritici* in the cultivated emmer and the durum landraces, they could be defeated by some virulent *Z. tritici* isolates, right away revealing their limited longevity, which requires precautious management of the detected resistance. Uniformity of the resistance sources in agroecosystems often results in huge selection forces that eventually nullify valuable resistance whose discovery and introgression has taken many years and intrinsically high costs (van de Wouw et al. 2009). Despite the value of the EPP taking place in *Z. tritici* and likely in many more Dothideomycetes, we better take the above serious into consideration until EPP has been further validated in real life situations. In any case, gene pyramiding and the use of diverse sources of resistance would ensure a better longevity and durability of resistance genes (McDonald and Linde 2002a, b; McDonald and Mundt 2016). Using the available markers, this strategy is no longer a pipedream.

Albeit that *Z. tritici* research is relatively young, compared to for instance the attention for rusts diseases, the data have shown that a poorly understood disease has risen to an interesting academic research area that also has resulted in the postulation and identification of important R genes. Our latest results (**chapter 2**) will undoubtedly increase the general understanding of the *Z. tritici*-wheat pathosystem. Therefore, we should not take these lessons for granted and actively lay the foundation under effective durum wheat breeding for resistance to *Z. tritici*.

Concluding remarks and future perspectives

The characterisation of the first (a)virulent effector gene and the elucidation of EPP resulting in a new epidemiological model will contribute to developing new and efficient breeding strategies for *Z. tritici* resistance that can predict the durability of a given resistance gene based on the frequency of the virulence alleles in natural populations. Despite these new

insights, support from field trials and observations are required to further implement this knowledge in breeding programs. It should start with a detailed monitoring program for *Stb16q* virulence as the first virulent strains have appeared in France, and hence this provides the ultimate situation to demonstrate and validate EPP, thereby predicting the commercial lifetime of a given *Stb* gene.

Albeit, this research enabled the virtual elucidation of the GFG mechanism in the *Z. tritici*- pathosystem by the cloning and the isolation of the *Avrstb6* effector gene, it is still unclear how *Avrstb6* interacts with its cognate *Stb6* gene in the *Z. tritici* – wheat pathosystem. The cloning of *Stb6* will undoubtedly ease the elucidation of this mechanistic interaction, and will open new perspective in the understanding and the characterisation of bread and durum wheat resistance genes.

The first resolved resistance to *Z. tritici* in durum wheat has proven that so far, all tested germplasm, despite resistance to pycnidia development - which is essential for epidemic development - displays an invariable high level of necrosis. This is puzzling and not understood, which requires further studies into the potential role of a selective sweep for sensitivity to - yet to be discovered - host selective toxins that may be produced by *Z. tritici.*

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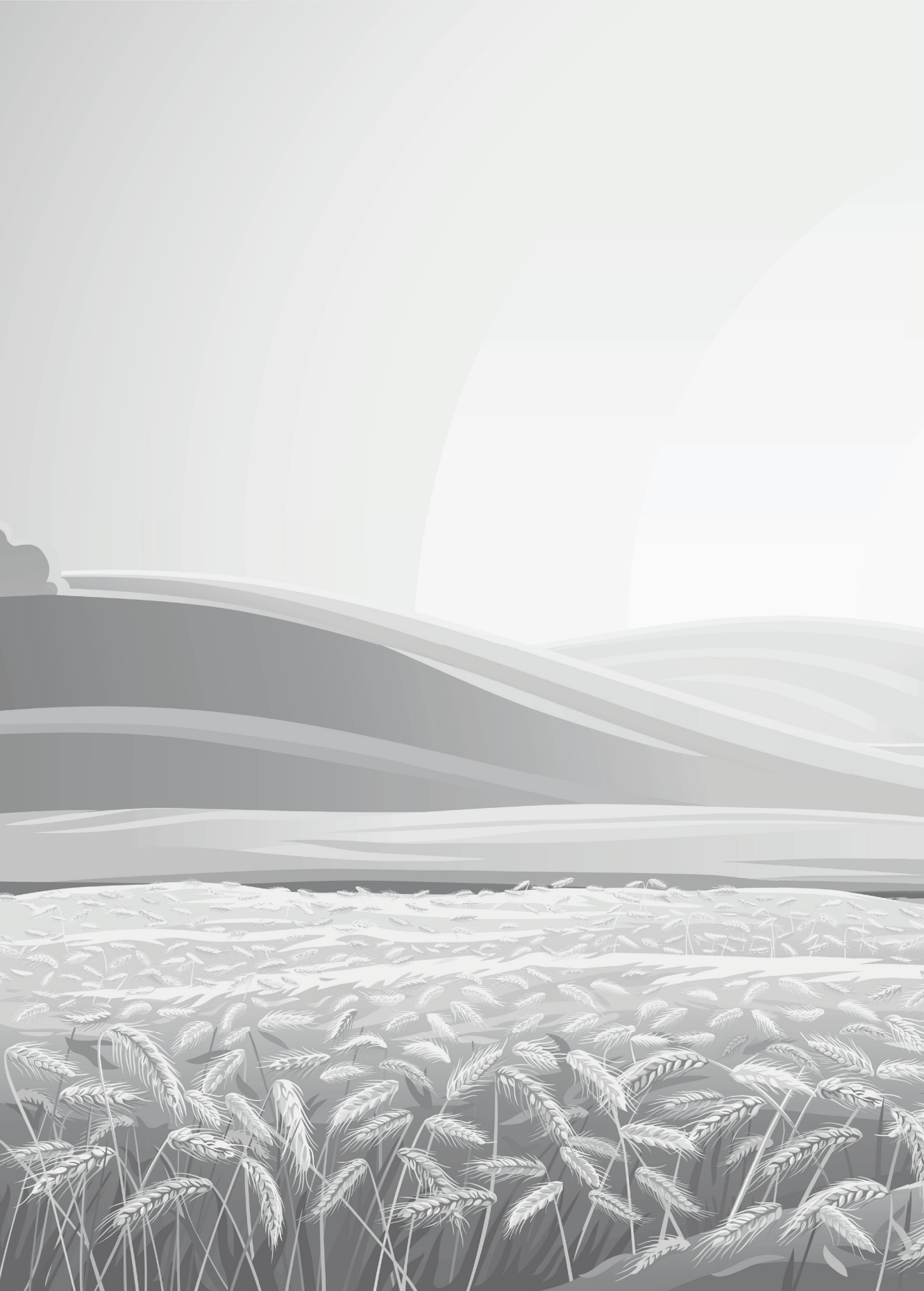
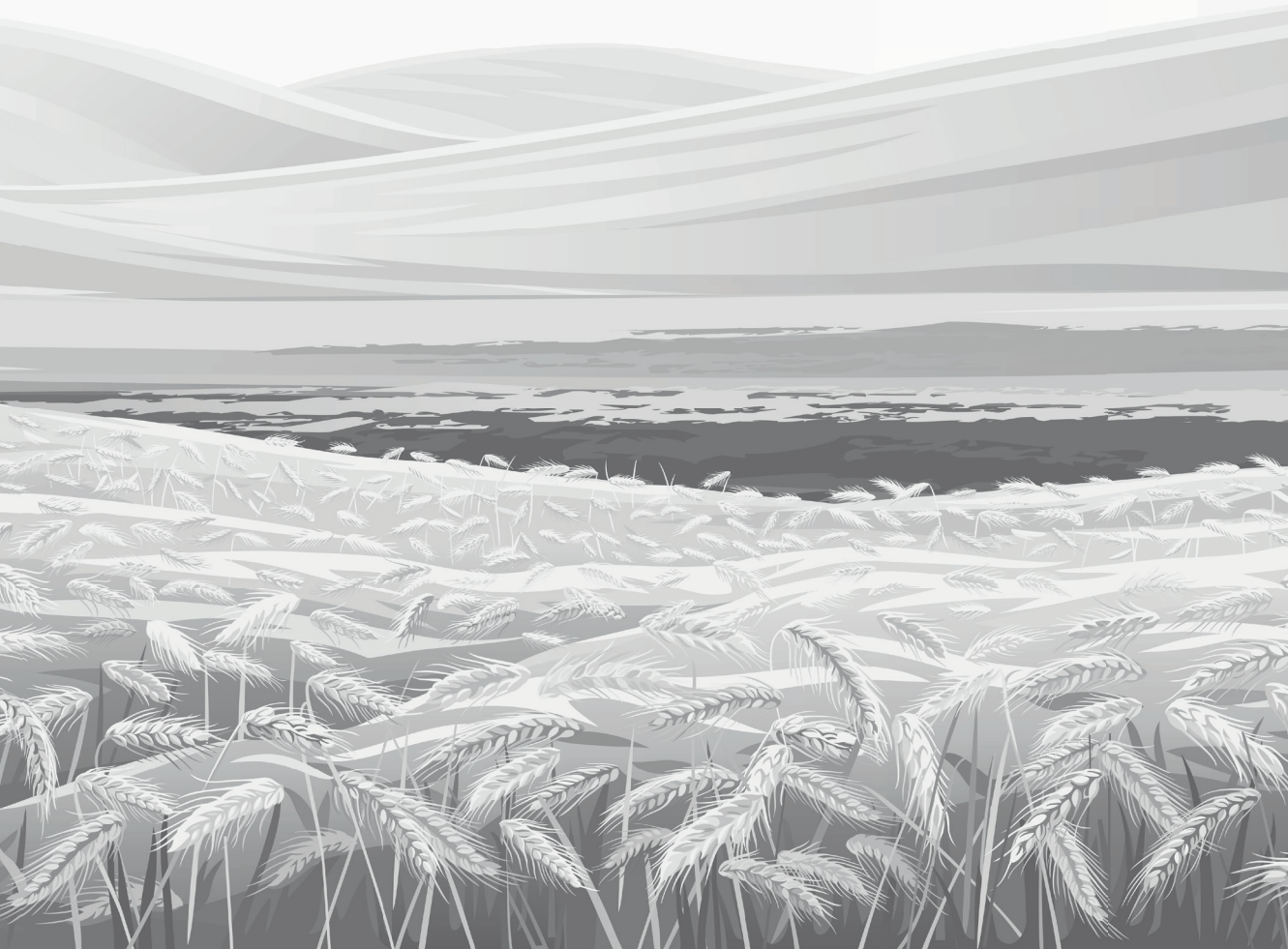


Summary



Summary

Durum wheat (*Triticum durum*) is among the most important food crops of the Mediterranean Basin, encompassing regions in Southern Europe and North Africa, as well as the Northern Great Plains of the U.S. and parts of Southern Asia. Particularly in North Africa it is the prime economical and dietary crop for small holder farmers in marginal areas and has greatly contributed to the existing genetic diversity of bread wheat. Despite its standing as a staple crop, primarily in North Africa and Southern Europe, the overall vulnerability of durum wheat germplasm to fungal diseases is well known and frequently reported. Among those is septoria tritici blotch (STB), the major foliar disease of wheat in Europe that is caused by *Zymoseptoria tritici.* However, the scientific community has limited attention for this crop and mostly focused on bread wheat. Therefore, very little is known about the genetic basis of the resistance to STB in tetraploid wheat. In bread wheat, 21 *Stb* major genes and manifold quantitative trait loci (QTLs) have been identified and intensively deployed in breeding programs, whereas *Stb* genes are neither recognized nor mapped in the largely under-investigated tetraploid wheats. One of the reasons is the reported dichotomy of the pathogenicity of *Z. tritici* isolates towards bread and durum wheat. This has been an additional hurdle hampering breeding for resistance in durum wheat because well characterized *Z. tritici* isolates that are pathogenic on bread wheat cannot be used in durum wheat phenotyping assays (and *vice versa)*. On top of that, specificity in either of these wheat systems has been questioned for a long time and therefore hindered effective breeding strategies. Thus, deciphering the genetics of the wheat-*Z. tritici* interaction, specifically for tetraploid wheats, greatly contributes to enhancing our understanding of this important pathosystem and thereby to more effective breeding strategies in this important cereal staple crop.

Chapter 1 is the introduction of the thesis and provides an historical overview of the emergence of the current forms of durum wheat and their vulnerability to *Z. tritici*. This fungal pathogen has evolved in close association with wheat, thereby deploying an arsenal of effector genes, and has a very strategic life style which generated abundant diversity. Therefore, *Z. tritici* has evolved as a major pathogen of wheat. The chapter concludes with an overview of the thesis.

Chapter 2 describes the map-based cloning and functional analysis of the first *Z. tritici* effector gene *AvrStb6* that interacts in a gene-for-gene manner with the first cloned and widespread major resistance gene *Stb6*. An even more important discovery represents the new exclusive paternal parenthood (**EPP**) epidemiological model. This shows that host resistance indeed precludes the developement of biomass of avirulent strains, but cannot stop sexual reproduction. Hence, the avirulence genes of avirulent parents are maintained in natutal populations, which extends the longevitiy of resistant wheat cultivars. The EPP model confirms many observations in agricultural and natural environments and is therefore most likely applicable to several other pathosystems.

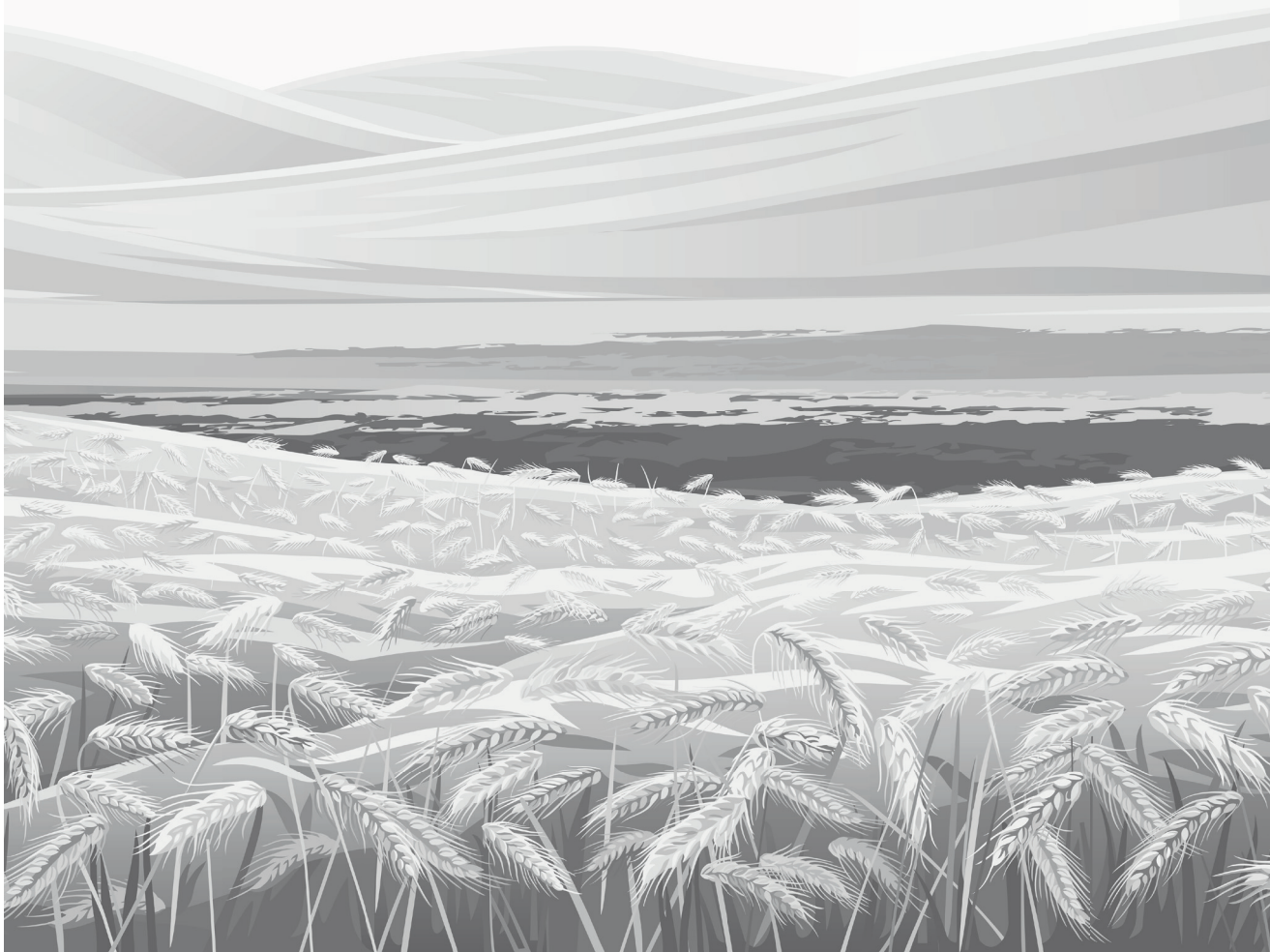
Chapter 3 unveils the genetic basis of resistance to *Z. tritici* in the cultivated emmer wheat (*Triticum dicoccum*) accesion PI41025. Mapping populations generated from crosses between PI41025 and the contemporary cv. Ben were used to unravel the reistance in the former accession. This resulted in a first QTL confering wide-spectrum resistance to *Z. tritici* in durum wheat. The QTL was mapped on chromosome 3AL, was derived from PI41025 and designated as *Stb22q*. In addition, another novel locus was mapped on chromosome 5A of cv. Ben, which provides an isolate-specific resistance, hence with a limited efficacy.

Chapter 4 takes the reader to more recent times by focusing on the resistance to *Z. tritici* in a suite of Tunisian durum wheat landraces. The oustanding landrace accession 'Agili39' was crossed to the contemporary high-yielding cv. Khiar that is very susceptible to *Z. tritici*. The analyses of the resistance in the generated recombinant inbred population revealed that the broad spectrum resistance of 'Agili39' results from the natural pyramiding of several minor effect QTLs. Nonetheless, QTLs on chromosome 2BL and 2BS exerted a strong effect on 'Agili39' resistance. The latter was exclusivly associated with adult plant resistance, whereas the former co-locolizes with *Stb9* that has a very low efficacy in bread wheat, but is crucial in 'Agili 39'.

Chapter 5 brings the reader to the present time by investigating STB resistance in contemporary durum wheat cultivars, which are preferred by farmers due to their high-yielding potential. Recombinant inbred populations were subsequently generated from crosses between cvs. Simeto and Levante and cvs. Kofa and Svevo and were tested with four *Z. tritici* isolates under greenhouse conditions and with one strain in the field. The analyses of the generated data showed that the STB resistance in these cultivars results from the synergic effect of several minor effect QTLs on several new genomic locations that collectively provide an acceptable level of STB resistance.

Chapter 6 is the final piece of this thesis and is a general discussion that puts the results in a boarder prespective and places all generated data in an overarching context. The newly elucidated epidemiological model applies for bread and durum wheat. Along with the newly discovered *Stb* genes and QTLs, this will lead to more effective (durum) wheat breeding programs that aim for resistance to *Z. tritici*.

Samenvatting



Samenvatting

Durum tarwe (*Triticum durum*) is een van de belangrijkste voedselgewassen van het Middellandse Zeegebied, dat gebieden in Noord-Afrika en Zuid-Europa omvat, alsmede van de prairies in Noord-Amerika en van Zuidoost-Azië. Met name in Noord-Afrika is het gewas van groot economisch belang en ook een belangrijk ingrediënt in het dagelijks menu van vele kleine boeren. Daarnaast heeft het een grote bijdrage geleverd aan de grote genetische diversiteit van broodtarwe. Ondanks het feit dat durumtarwe een basisvoedsel is voor vele mensen, met name in Noord-Afrika en Zuid-Europa, heeft het de reputatie gevoelig te zijn voor schimmelziekten waarover ook regelmatig wordt gerapporteerd. Eén van deze ziekten is septoria tritici bladvlekkenziekte (STB), de belangrijkste tarweziekte in Europa, die wordt veroorzaakt door *Zymoseptoria tritici.* De wetenschappelijke gemeenschap heeft hier nauwelijks aandacht voor gehad en heeft zich vooral beziggehouden met broodtarwe. Daarom is er erg weinig bekend over de genetische basis van resistentie tegen STB is tetraploïde tarwe. In broodtarwe zijn 21 *Stb* resistentiegenen geïdentificeerd, naast vele loci die een bijdrage leveren aan kwantitatieve resistentie (QTLs). Deze worden veelvuldig gebruikt in veredelingsprogramma's, maar geen enkel *Stb* gen is ooit herkend laat staan gekarteerd in de nauwelijks onderzochte tetraploïde tarwe. Eén van de redenen is het feit dat *Z. tritici* òf broodtarwe òf durumtarwe aantast. Dit vormt een belangrijke drempel in de resistentieveredeling van durumtarwe omdat goed gekarakteriseerde *Z. tritici* isolaten die pathogeen zijn op broodtarwe niet gebruikt kunnen worden in infectieproeven met durumtarwe (en *vice versa*). Daarnaast is specificiteit van de interactie tussen *Z. tritici* en beide tarwesoorten gedurende lange tijd in twijfel getrokken waardoor de vooruitgang in effectieve veredelingstrategieën ook vertraging heeft opgelopen. Het ontcijferen van de genetica van de tarwe-*Z. tritici* interactie, vooral voor tetraploïde tarwe, zal daarom enorm bijdragen aan het begrip van dit belangrijke pathosysteem en daarmee de effectiviteit van de veredeling in dit cruciale voedselgewas verbeteren.

Hoofdstuk 1 is een introductie van het proefschrift en geeft een historisch overzicht van de ontstaansgeschiedenis van de huidige durumtarwerassen en hun kwetsbaarheid voor *Z. tritici.* Deze pathogene schimmel heeft zich in nauwe samenhang met het gewas ontwikkeld, daarbij gebruik makend van een arsenaal van effector genen, waarbij de strategische levenscyclus heeft geresulteerd in een enorme genetische diversiteit. Daarom heeft *Z. tritici*

zich ontwikkeld tot een van de belangrijkste pathogenen van tarwe. Het hoofdstuk besluit met een overzicht van de inhoud van het proefschrift.

Hoofdstuk 2 beschrijft het gebruik van de genetische kaart van *Z. tritici* om het eerste avirulentiegen, *AvrStb6,* te kloneren en functioneel te analyseren. Dit gen heeft een specifieke gen-om-gen interactie met *Stb6,* het eerste gekloneerde en wijdverspreide resistentiegen tegen *Z. tritici.* Een nog belangrijkere ontdekking betreft het nieuwe epidemiologische exclusieve vaderlijk-ouderschapsmodel (EPP). Dit laat zien dat waardplantresistentie weliswaar de ontwikkeling van biomassa van avirulente stammen verhindert, maar seksuele voortplanting niet tegengaat. Avirulentiegenen worden daardoor gehandhaafd in natuurlijke populaties waardoor de levensduur van resistente tarwerassen wordt verlengd. Het EPP-model bevestigt vele waarnemingen in landbouwkundige en natuurlijke omgevingen en is daarom waarschijnlijk van toepassing op vele andere pathosystemen.

Hoofdstuk 3 ontvouwt de genetische basis van resistentie tegen *Z. tritici* in de emmertarwe (*Triticum dicoccum*) accessie PI41025. Karteringspopulaties ontwikkeld uit kruisingen tussen PI41025 en het hedendaagse ras "Ben" zijn gebruikt om deze resistentie te ontrafelen. Dit heeft geresulteerd in een eerste QTL die verantwoordelijk is voor een breedwerkende resistentie tegen *Z. tritici* in durumtarwe. De QTL is gekarteerd op chromosoom 3AL, is afkomstig uit PI41025 en heeft als aanduidig *Stb22q* gekregen. Daarnaast is een ander nieuw locus geïdentificeerd op chromosoom 5A van cv. Ben dat verantwoordelijk is voor een isolaat-specifieke en dus beperkte resistentie.

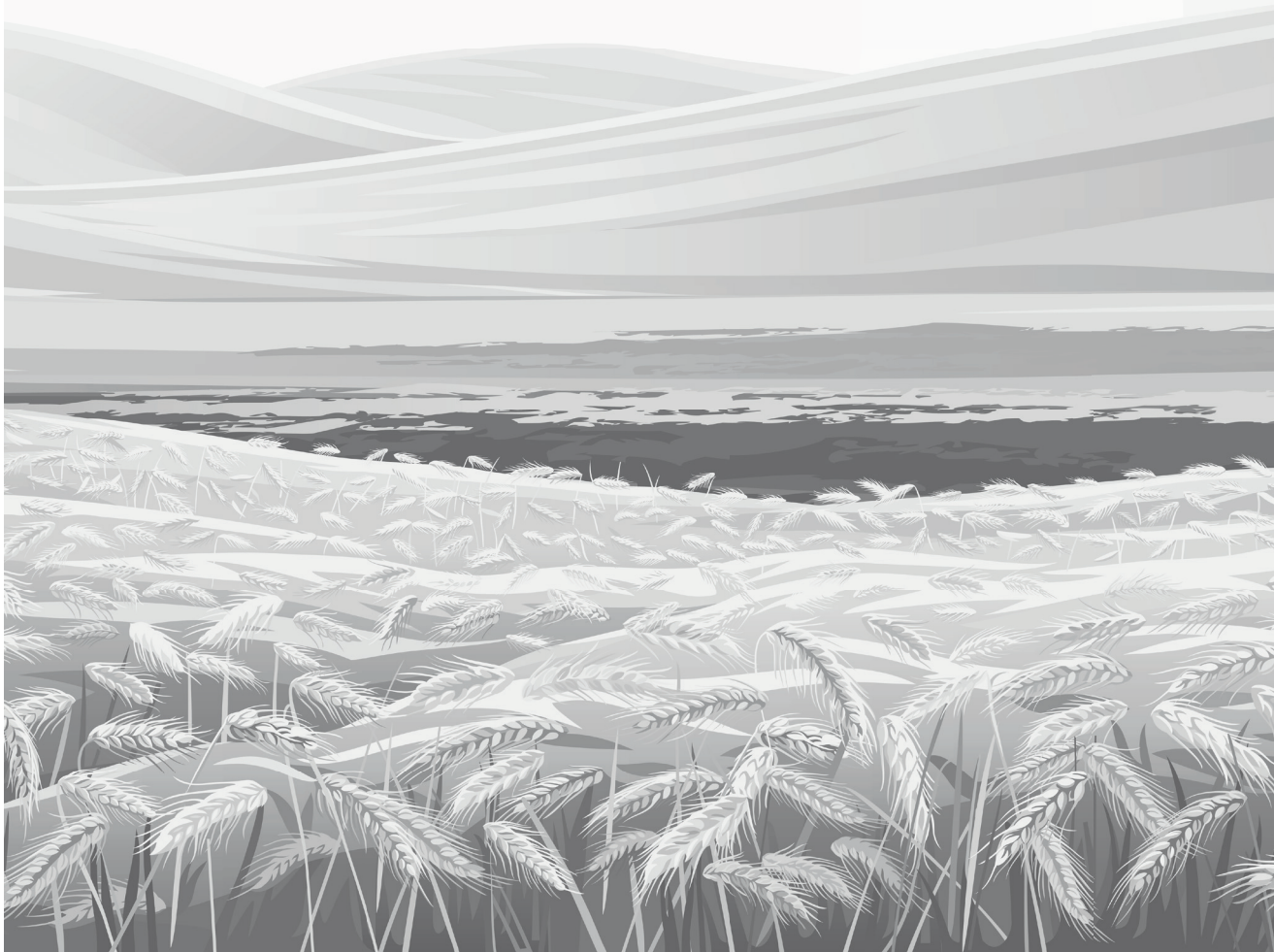
Hoofdstuk 4 neemt de lezer mee naar recentere tijden en focust op de resistentie tegen *Z. tritici* in een reeks Tunesische durumtarwe landrassen. Het uitstekende landras 'Agili39' is gekruist met het hedendaagse ras "Khiar" dat zeer vatbaar is voor *Z. tritici.* De analyse van de resistentie in de genegereerde recombinante inteeltpopulatie heeft laten zien dat de breed-spectrum resistentie in 'Agili39' het gevolg is van een natuurlijke stapeling van QTLs met een beperkt kwantiatief effect. Desalniettemin vertoonden de QTLs op chromosomen 2BL en 2BS een groter efect op de resistentie van 'Agili39'. De laatste QTL was uitsluitend betrokken bij volwassenplantresistentie, terwijl de andere QTL op dezelfde plaats werd gekarteerd als *Stb9,* een resistentiegen met een zeer beperkte werkzaamheid tegen *Z. tritici* in broodtarwe, maar dat cruciaal blijkt te zijn in 'Agili39'.

Hoofdstuk 5 voert de lezer naar de huidige tijd door STB resistentie te onderzoeken in hedendaagse dutumtarwerassen die door boeren worden geprefereerd omdat ze een hoog

opbrengstpotentieel hebben. Recombinante inteeltpopulaties werden ontwikkeld uit kruisingen tussen cvs. Simeto en Levante en cvs. Kofa en Svevo die vervolgens werden getest met vier *Z. tritici* isolaten onder kasomstandigheden en met één isolaat in het veld. De analyse van de gegenereerde data laat zien dat STB resistentie in deze rassen het gevolg is van de synergie tussen meerdere QTLs met een klein effect die op meerdere locaties in het genoom werden gelocaliseerd en gezamenlijk een acceptabel resistentieniveau bewerkstelligen.

Hoofdstuk 6 is het afsluitend gedeelte van het proefschrift en omvat de algemene discussie die de resultaten in een breder perspectief en overkoepelende context plaatst. Het nieuw ontdekte epidemiologische model is van toepassing op zowel durum- als boordtarwe. Samen met de nieuw ontdekte *Stb* genen en QTLs zal dit leiden tot effectievere (durum) tarweveredelingproagramma's die gericht zijn op resistentie tegen *Z. tritici.*

Résumé



Résumé

Le blé dur (*Triticum durum*) est l'une des cultures les plus importantes du bassin méditerranéen qui englobe l'Europe du Sud et l'Afrique du Nord, des grandes plaines du Nord des États-Unis et de l'Asie du Sud. Cette culture, qui représente une importance économique et diététique notamment pour les petites exploitations des zones marginales du Nord-Afrique, a amplement contribué à la diversité génétique contemporaine du blé tendre. Toutefois, et malgré son statut de culture de base, principalement en Afrique du Nord et en Europe du Sud, une vulnérabilité du blé dur aux maladies fongiques a été généralement reconnue et fréquemment signalée. Parmi celle-ci, la septoriose ou septoria tritici blotch (STB) qui représente la principale maladie foliaire du blé en Europe causée par *Zymoseptoria tritici* (*Z. tritici*). Cependant, la communauté scientifique a accordé très peu d'attention au blé dur et s'est plutôt focalisé sur le blé tendre. Par conséquent, nos connaissances concernant la base génétique de la résistance au STB dans le blé tétraploïde sont très restraintes. Dans le blé tendre, 21 gènes majeurs (*Stb)* et plusieurs loci quantitatifs (QTLs) ont été identifiés et intensivement déployés dans des programmes de sélection, tandis qu'aucun gène *Stb* n'a été reconnu ou cartographié dans les blés tétraploïdes encore non-prospectés. L'une des raisons multiples qui a entravé l'étude de la résistance du blé dur à *Z. tritici*, est la spécialisation physiologique des isolats *Z. tritici* sur blé dur ou tendre. Cette dichotomie constitue un obstacle majeur à l'amélioration de la résistance du blé dur à *Z. tritici,* vu que les isolats bien caractérisés de *Z. tritici* et qui sont virulents sur blé tendre ne peuvent pas être utilisés dans les essais de phénotypage du blé dur (et vice versa). En outre, la spécificité sur l'une ou l'autre des formes de blé a été longtemps investiguée, et a entravé les stratégies de sélection efficaces. Ainsi, déchiffrer la génétique de l'interaction blé-*Z. tritici*, en particulier pour les blés tétraploïdes, contribue considérablement à améliorer notre compréhension de ce pathosystème important, et par conséquent, à déployer des stratégies de sélection plus efficaces dans cette importante culture de base.

Le chapitre 1 est une introduction de la thèse qui donne un aperçu historique sur l'émergence des formes actuelles de blé dur et leurs vulnérabilités à *Z. tritici*. Ce pathogène fongique a évolué en étroite association avec le blé, déployant ainsi un arsenal de gènes effecteurs, et un style de vie très stratégique qui a généré une diversité abondante. Par conséquent, *Z. tritici* a évolué en tant que pathogène majeur du blé. Le chapitre se termine par un aperçu de la thèse.

Le chapitre 2 décrit le clonage et l'analyse fonctionnelle du premier gène effecteur de *Z. tritici*, AvrStb6, qui interagit selon le modèle gène-pour-gène avec le premier gène de résistance majeur cloné et largement répandu *Stb6.* Une découverte encore plus importante représente le nouveau modèle épidémiologique de la parentalité paternelle exclusive (exclusive paternal parenthood or EPP). Ceci montre que la résistance de l'hôte empêche en effet le développement de la biomasse des souches (a)virulentes, mais ne peut pas arrêter la reproduction sexuée. Ainsi, les gènes d'avirulence des parents (a)virulents sont maintenus dans les populations naturelles, ce qui prolonge la durabilité des cultivars de blé résistants. Le modèle EPP confirme de nombreuses observations dans les milieux agricoles et naturels et est donc très probablement applicable à de nombreux autres pathosystèmes.

Le chapitre 3 dévoile la base génétique de la résistance à *Z. tritici* dans l'accession sauvage de l'Emmer cultivé (*Triticum dicoccum*) PI41025. La population recombinante entre le PI41025 et le cv contemporain. Ben a été utilisée pour étudier la résistance à *Z. tritici* dans l'ancienne accession. Cette analyse a permi l'identification du premier QTL majeur conférant une résistance à large spectre à *Z. tritici* dans le blé dur. Le QTL a été cartographié sur le chromosome 3AL, et a été dérivé de PI41025, désigné par *Stb22q*. En outre, un autre nouveau locus a été cartographié sur le chromosome 5A de cv. Ben. Ce locus fournit une résistance spécifique à l'isolat, donc avec une efficacité limitée.

Le chapitre 4 interpelle l'attention du lecteur à des temps plus modernes en focalisant sur la résistance à *Z. tritici* dans une série de variétés locales de blé dur tunisien. L'accession rarissime 'Agili39' a été croisée au cv contemporain à haut rendement. Khiar, très sensible à *Z. tritici*. Les analyses de la résistance dans la population recombinante générée ont révélé que la résistance à large spectre de 'Agili39' résulte du pyramidage naturel de plusieurs QTLs à effet mineur. Néanmoins, les QTLs identifiés sur les régions chromosomiques 2BL et 2BS ont un effet majeur sur la résistance de 'Agili39' à *Z. tritici*. Le QTL 2BS est exclusivement associé à la résistance des plantes adultes, tandis que le QTL 2BL co-segrège avec le gène majeur *Stb9* qui a une très faible efficacité dans le blé tendre, mais est crucial dans 'Agili 39'.

Le chapitre 5 amène le lecteur à l'époque actuelle en étudiant la résistance au STB chez les cultivars contemporains de blé dur, et qui sont préférés par les agriculteurs en raison de leur potentiel de rendement élevé. Des populations recombinantes ont été ensuite générées entre les cvs. Simeto / Levante et cvs. Kofa / Svevo et ont été testées avec quatre isolats de *Z. tritici* sous conditions contrôlées et un seul isolat sous conditions naturelles. Les analyses des données phénotypiques générées ont montré que la résistance au STB chez ces cultivars

résulte de l'effet synergique de plusieurs QTLs à effet mineur sur plusieurs nouveaux sites génomiques qui, conjointement, fournissent un niveau acceptable de résistance au STB.

Le chapitre 6, la dernière partie de cette thèse, est une discussion générale qui place les résultats obtenus en perspective réelle et place toutes les données générées dans un contexte plus général. Le modèle épidémiologique nouvellement élucidé s'applique au blé tendre, ainsi qu'au blé dur. Ce nouveau modèle joint aux gènes *Stb* et aux QTLs récemment découverts, mènera à des programmes d'amélioration du blé à la résistance à *Z. tritici* plus efficaces.

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To all of you who greatly contributed to this work and success, thank you a lot, merci beaucoup, شكرا جزيلا, heel erg bedankt, grazie mille and muchas gracias. I know that my life will not be the same anymore. You will all echo in my heart forever and ever.

Curriculum Vitae

Lamia Aouini was born on January 2nd, 1983 in Tunis, Tunisia. She received her academic high school degree in Mathematics in 2002 in Bizerte, moved to Tunis to follow engineering studies in Agronomy at the National Agronomic Institute of Tunisia (INAT) and obtained her BSc degree in 2007. She was then admitted to the Biotechnology and Plant breeding MSc program at INAT in 2010. During her MSc thesis project, she worked at the Department of Genetics and Plant Breeding under the supervision of Prof. Walid Hamada. Her MSc project focused on the identification of sources of resistance to *Zymoseptoria tritici* in a TILLING population of durum wheat. Resistant lines were eventually identified and subsequently deployed in breeding programs aiming for resistance to *Z. tritici* in durum wheat. In 2011, she was awarded a Monsanto's Beachell-Borlaug International scholarship resulting from a collaborative project proposal between Prof. Gert Kema at Wageningen University and Research and Prof. Roberto Tuberosa at the University of Bologna as well as a Graduate Research Training Program's scholarship (GRTP) from the International Center for Agricultural Research in the Dry Areas (ICARDA). She then moved to Wageningen University and Research for her PhD in Plant Breeding at the Department of Bio-interactions and Plant Health (BIOINT) of Plant Research International (now Wageningen Plant Research) under the supervision of Prof. Gert Kema and Prof. Richard Visser. In 2013, she conducted part of her PhD research at the University of Bologna under the supervision of Prof. Roberto Tuberosa and Dr. Marco Maccaferri. During her PhD, she was also involved in several additional research projects and supervised BSc and MSc students She was also appointed as a non-permanent assistant in 2015 at BIOINT, which enabled her to complete her PhD research. Since 2016 she is a member of the precision phenotyping platform for septoria tritici blotch (STB) in Tunisia and was appointed as Laboratory manager and coordinator from June until December 2016. During that time she supervised and coordinated all STB research activities at the laboratory of ICARDA in Tunis. She is currently employed as an assistant at INAT in the team of Prof. Dr. Sonia Hamza.

List of publications

Aouini L, Faris J, Grootens RJF, Visser RGF, Xu SS, Kema GHJ. Broad spectrum resistance to *Zymoseptoria tritici* in the tetraploid emmer wheat accession PI41025. Submitted for publication.

Ferjaoui S*, **Aouini L***, Slimane RB, Ammar K, Dreisigacker S, Ben M'Barek S, Schouten HJ, Visser RGF, Kema GHJ, Hamza S. Deciphering durable resistance to *Zymoseptoria tritici* in the Tunisian durum wheat landrace 'Agili 39'. Submitted for publication

Aouini L*; Maccaferri M*; Frascaroli E; Prodi A.; Tuberosa R.; Stefanelli S; Corneti S; Mantovani P; Massi A; Tabib Ghaffary SM; Verstappen ECP; Visser RGF.; Kema GHJ. Resistance to *Zymoseptoria tritici* in elite durum wheat is defined by major and minor, breeding-exploitable quantitative trait loci. Submitted for publication

Kema GHJ*, Mirzadi Gohari A*, **Aouini L***, Gibriel HAY, Ware SB, Van den Bosch F, Manning-Smith R, Alonso-Chavez V, Helps J, Ben M'Barek S, Mehrabi R, Diaz-Trujillo C, Zamani E, Schouten HJ, Van der Lee TAJ, Waalwijk C, De Waard MA, De Wit PJGM, Verstappen ECP, Thomma BPHJ, Meijer HJG and Seidl MF (2018). Stress and sexual reproduction affect the dynamics of the wheat pathogen effector AvrStb6 and strobilurin resistance. Nature Genetics, in press, doi:10.1038/s41588-018-0052-9

Omrane S, Audéon C, Ignace A, Duplaix C, **Aouini L**, Kema GHJ, Walker AS, Fillinger S (2017). Plasticity of the MFS1 promoter leads to multidrug resistance in the wheat pathogen *Zymoseptoria tritici*. mSphere2: e00393-17

Meamiche Neddaf H; **Aouini L**; Bouznad Z, and Kema GHJ. (2017). Equal distribution of mating type alleles and the presence of strobilurin resistance in Algerian *Zymoseptoria tritici* field populations. Plant Disease 101 (4):544-549

* Equal contribution

Education Statement of the Graduate School Experimental Plant Sciences

Issued to: **Lamia Aouini**

Date: **17 April 2018**

Group: **Laboratorium of Plant Breeding**

University: **Wageningen University & Research**

1) Start-up phase	*date*
► **First presentation of your project**	
Title: Co-evolutionary genetics of Zymosptoria tritici and durum wheat	05 Jul 2012
► **Writing or rewriting a project proposal**	
Title: Co-evolutionary genetics of Zymosptoria tritici and durum wheat	Feb 2012
► **Writing a review or book chapter**	
► **MSc courses**	
► **Laboratory use of isotopes**	
Subtotal Start-up Phase	*3.5 credits**

2) Scientific Exposure	*date*
► **EPS PhD student days**	
EPS PhD student day, Amsterdam, NL	30 Nov 2012
EPS PhD student day, Leiden, NL	29 Nov 2013
► **EPS theme symposia**	
EPS theme 2 symposium 'Interactions between Plants and Biotic Agents', together with Willie Commelin Scholten Day, Utrecht, NL	24 Jan 2013
EPS theme 2 symposium 'Interactions between Plants and Biotic Agents', together with Willie Commelin Scholten Day, Amsterdam, NL	25 Feb 2014
► **National meetings (e.g. Lunteren days) and other National Platforms**	
► **Seminars (series), workshops and symposia**	
EPS Mini-Symposium : Plant Breeding in the genomics era, Wageningen, NL	25 Nov 2011
Conference Next Generation Breeding Generation, Wageningen University & Research	11-14 Nov 2012
How to Write a world-Class paper	17 Oct 2013
► **International symposia and congresses**	
Mycosphaerella graminicola Workshop - INRA-Grignon - France	19-20 Jul 2012
World Food Prize	15-19, Oct 2012

Mycosphearella graminicola Workshop- Rothamsted Research center-UK	05-06 May 2013
Annual Monsanto meeting in CYMMIT- Mexico	24-28 Mar 2014
6th European Plant Science Retreat for PhD stuents-Amsterdam	01-04 Jul 2014
Mycosphaerella graminicola Workshop-Exeter-UK	11-12 Sep 2014
COST Action SUSTAIN workshop on "Pathogen-informed crop improvement	08-10 Apr 2015
Septoria precision Phenotyping Platform, Tunisia	20-28 Apr 2015
Congress From Seed to Pasta, Bologna Italy	31 May-04 Jun 2015
APS Annual meeting, Pasedena , California	01-05 Aug 2015
9th International Symposium on Septoria Diseases of Cereals- Paris, France	07-09 Apr 2016
2nd Regional Workshop on STB management, Tunisia	05-07 May 2017
► **Seminar plus**	
► **Presentations**	
Talk: Mycosphaerella graminicola Workshop- INRA-Grignon- France	19-20 Jul 2012
Poster: Annual meeting of the MMBI shoolars of Monsanto	16 Oct 2012
Talk: Annual meeting of the MMBI Schoolars of Monsanto	16 Oct 2012
Talk: Mycosphaerella graminicola Workshop- Rothamsted Research Center- UK	. 05 May 2013
Talk: Mycosphaerella graminicola Workshop- Exeter-UK	11 Sep 2014
Talk: Septoria precision Phenotyping Platform, Tunisia	20-28 Apr 2015
Poster: APS Annual meeting, Pasedena , California, USA	01-05 Aug 2015
Talk: 9th International Symposium on Septoria Diseases of Cereals- Paris, France	09 Apr 2016
Talk: 2nd Regional Workshop on STB management, Tunisia	05 May 2017
► **IAB interview**	
Meeting with a member of the International Advisory Board of EPS	03 Dec 2014
► **Excursions**	
Bioplante Field visit, breeding for resistance to Mycosphaerella graminicola, Florimond Deprez Capelle-en-Pevele, Lille, France.	15 May 2012
Subtotal Scientific Exposure	*25.7 credits**

3) In-Depth Studies	*date*
► **EPS courses or other PhD courses**	
PhD Autumn School 'Host-Microbes Interactomics, Wageningen, NL	Nov 1-3, 2011
Course 'Linkage mapping and QTL analysis', Wageningen, NL	March 20-27, 2012
Course 'Mixed model based genetic analysis in GenStat:from QTL mapping and association mapping to genomic prediction', Wageningen, NL	02-04 Sep 2013
Course 'Basic Statistics', Wageningen, NL	26-27 May, 04-06 Jun 2014

► **Journal club** Participant in litterature discussion group	2011- 2015
► **Individual research training** training for 5 months at University of Bologna-Italy	13 Feb-17 Jul 2013

Subtotal In-Depth Studies *10.2 credits**

4) Personal development	*date*
► **Skill training courses**	
Leadership course, Tero international, Inc., USA.	11-15 Oct 2012
Course 'Project and Time Managment', Wageningen, NL	Mar- Apr 2014
Course 'Preparation for IELTS', Wageningen, NL	18-29 Aug 2014
Course 'Information Literacy PhD including EndNote Introduction', Wageningen, NL	02-03 Dec 2014
Course 'Scientific writing', Wageningen, NL	Feb-Apr 2015
Course 'Introduction to R for statistical analysis', Wageningen, NL	08-09 May 2017
► **Organisation of PhD students day, course or conference**	
► **Membership of Board, Committee or PhD council**	

Subtotal Personal Development *6.0 credits**

TOTAL NUMBER OF CREDIT POINTS*	**45,4**

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.*