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Genetic characterization of Fusarium head blight resistance in durum wheat

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Genetic characterization of Fusarium head blight
resistance in durum wheat

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GENETIC CHARACTERIZATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN DURUM WHEAT

Dissertation

for obtaining a doctorate degree at

the University of Natural
Resources and Applied Life Sciences, Vienna

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the Université Blaise Pascal, Clermont-Ferrand

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UMR 1095 INRA-UBP Genetics, Diversity and Ecophysiology of Cereals,
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Abstract

Fusarium head blight (FHB) is a devastating disease affecting small-grain cereals worldwide causing yield and quality losses. FHB affects food safety due to the contamination of infected grains by mycotoxins. Host plant resistance is considered the most efficient and sustainable approach to contain FHB and mycotoxin contaminations. In durum wheat (*Triticum durum* Desf.) breeding for FHB resistance remains a challenge due to its extreme susceptibility and to lack of genetic variation available in the primary durum wheat gene pool. The primary goal of this thesis was to evaluate the effect of *Fhb1*, the major common wheat (*Triticum aestivum* L.) FHB resistance QTL, in elite durum wheat background. Three F7-RIL (recombinant inbred lines) mapping populations of about 100 lines were developed from crosses between the durum wheat experimental line DBC-480, harboring *Fhb1*, and the durum wheat cultivars Karur, Durobonus and SZD1029K. The RILs were tested under field conditions by artificial spray inoculation with *Fusarium culmorum* in three seasons. Morphological traits (flowering date, height) were also recorded to assess their influence on FHB infestation. Genotyping of the lines was performed with SSR and genotyping-by-sequencing (GBS) DArTseq markers. QTL analysis identified genomic regions associated with FHB resistance on chromosome arms 2BL, 3BS, 4AL, 4BS, 5AL and 6AS. DBC-480 contributed the resistant allele at all loci. *Fhb1* was detected in all three populations, demonstrating for the first time its successful deployment in durum wheat. The effect of *Fhb1* on FHB resistance in durum wheat was further verified by evaluating type 2 resistance in one of the three populations. Plant height had a strong influence in modulating FHB severity. Although the semi-dwarf allele *Rht-B1b* was associated with increased FHB susceptibility, its negative effect was efficiently counterbalanced in lines carrying *Fhb1*. Semi-dwarf lines with enhanced levels of resistance were selected and will assist the development of FHB resistant cultivars.

Key words: durum wheat, *Triticum durum*, Fusarium head blight, QTL, resistance, *Fhb1*

Résumé

Caractérisation des déterminants génétiques de la résistance à la fusariose chez le blé dur

La fusariose de l'épi est une maladie fongique qui touche toutes les cultures de céréales à paille à travers le monde entraînant des baisses de rendements et de la qualité des grains. La fusariose pose également un problème pour la sécurité alimentaire lié à la contamination des grains infectés par des mycotoxines. Le développement de variétés résistantes est considéré comme la méthode la plus efficace et la plus durable pour réduire les dommages causés par la maladie et pour limiter la contamination par les mycotoxines. L'amélioration de la résistance à la fusariose chez le blé dur (*Triticum durum* Desf.) demeure un défi du fait de son extrême sensibilité à la maladie et de la faible variabilité génétique disponible pour ce caractère. L'objectif principal de cette thèse a été d'évaluer l'effet de *Fhb1*, le QTL majeur de résistance à la fusariose chez le blé tendre (*Triticum aestivum* L.), au sein de fonds génétiques de blé dur élite. Pour cela, trois populations de cartographie, comprenant chacune environ 100 F7-RIL (lignées pures recombinantes ou « recombinant inbred lines »), ont été développées à partir de croisements entre la lignée expérimentale de blé dur DBC-480, portant une introgression de *Fhb1*, et les cultivars de blé dur Karur, Durobonus et SZD1029K. Les lignées ont été évaluées au champ, sur trois saisons, pour leur résistance globale à la fusariose après inoculation en spray de *Fusarium culmorum*. Des notations morphologiques (date de floraison, hauteur des plantes) ont également été réalisées afin d'évaluer leur influence sur l'infestation. Les lignées ont été génotypées à l'aide de marqueurs SSR et de marqueurs GBS (génotypage par séquençage ou « genotyping-by-sequencing ») développés par DArTseq. L'analyse de liaison a permis d'identifier des QTL de résistance sur les bras des chromosomes 2BL, 3BS, 4AL, 4BS, 5AL et 6AS. DBC-480 contribuait à l'allèle de résistance à tous ces loci. Le QTL sur 3BS a été détecté au sein des trois populations centré sur l'intervalle de *Fhb1*, confirmant, pour la première fois, son introgression dans le blé dur. L'évaluation de la résistance à la propagation après inoculation ponctuelle, réalisé au sein d'une des trois populations, a également permis de valider l'effet de *Fhb1* sur la résistance de type 2 chez le blé dur. La

hauteur des plantes influe fortement sur la résistance globale à la fusariose et, en particulier, l'allèle de nanisme *Rht-B1b* est associé à une plus grande sensibilité à la maladie dans les trois populations. Cependant, l'effet négatif de *Rht-B1b* sur la résistance est largement compensé dans les lignées possédant *Fhb1*. Des lignées semi-naines avec un meilleur niveau de résistance ont été sélectionnées et favoriseront le développement de cultivars de blé dur résistants à la fusariose.

Mots clés : blé dur, *Triticum durum*, fusariose de l'épi, QTL, résistance, *Fhb1*

Zusammenfassung

Ährenfusariose (FHB) wird durch die Pilze *Fusarium culmorum* und *Fusarium graminearum* verursacht und ist eine Pflanzenkrankheit, welche weltweit verheerende Ausmaße im Getreideanbau annehmen kann. Der Befall mit FHB hat erhebliche Ernteverluste sowie starke Qualitätseinbußen des Ernteguts zur Folge und verursacht somit massive wirtschaftliche Schäden. Durch die Anreicherung von Mykotoxinen, z.B. Deoxynivalenol (DON), im Erntegut befallener Pflanzen stellt FHB aber auch eine direkte Gefahr für Tier und Mensch dar. Kontaminierte Lebens- und Futtermittel sind für den Gebrauch nicht mehr geeignet, da mit ernsthaft negativen Auswirkungen auf die Gesundheit zu rechnen ist. Eine Schlüsselrolle im erfolgreichen Krankheitsmanagement von FHB spielt die Wirtsresistenz: Diese wird als die kostengünstigste und nachhaltigste Methode angesehen den Befall mit FHB und folglich auch die Mykotoxinverunreinigung zu minimieren. FHB Resistenz ist ein quantitativ vererbtes Merkmal und wird von sogenannten "quantitative trait loci" (QTL) gesteuert, welche gemeinsam die Resistenz bewerkstelligen. Die extreme Anfälligkeit von Durumweizen (*Triticum durum* Desf.) stellt in Kombination mit der fehlenden genetischen Variabilität betreffend FHB Resistenz im primären Genpool für die Züchtung resistenter Sorten eine große Herausforderung dar.

Im ersten Teil der vorliegenden Arbeit wurde zunächst eine Zusammenstellung von aktuell vorhandenem Wissen über die Genetik von FHB Resistenz in Durumweizen erarbeitet. Die genetischen Ressourcen, welche für die FHB Resistenzzüchtung bei Durumweizen zur Verfügung stehen wurden erhoben und umfassen sowohl eng verwandte als auch fremde Arten. Bis dato publizierte Ergebnisse von QTL Kartierungen in tetraploidem Weizen wurden zusammengefasst und miteinander verglichen: in Summe konnten 13 Resistenz QTL identifiziert werden, welche in unterschiedlichen Umwelten und unabhängigen Studien wiederholt aufgefunden wurden. Derartige QTL können als stabil angesehen werden und eignen sich daher besonders für Verwendung in Züchtungsprogrammen. Des Weiteren zeigte sich, dass die meisten Resistenz QTL in tetraploidem Weizen mit denen in hexaploidem Weizen (*Triticum aestivum* L.) überlappen – was auf eine gemeinsame genetische Basis der FHB Resistenz hindeutet.

Vorangegangene QTL Kartierungen in tetraploidem Weizen beschränkten sich darauf die genetische Architektur der Resistenz in exotischem Pflanzenmaterial wie Genbankeinträgen oder Landrassen zu untersuchen. Dementsprechend war das Hauptziel dieser Arbeit den Effekt von *Fhb1* - dem wichtigsten FHB Resistenz QTL in hexaploidem Weizen - in einem Durumweizen Elite Hintergrund zu untersuchen. Zu diesem Zweck wurden drei F7 Kartierungspopulationen entwickelt, welche jeweils etwa 100 rekombinante Inzuchtlinien umfassten. Diese Populationen basieren auf Kreuzungen zwischen der experimentellen Durumweizenlinie DBC-480, welche den QTL *Fhb1* trägt, und den europäischen Durumweizen Sorten Karur, Durobus und SZD1029K. Die Populationen wurden in Feldversuchen, welche mit *Fusarium culmorum* sprüh-inokuliert wurden, über 3 Jahre auf FHB Resistenz getestet. Zusätzlich zum FHB Befall wurden auch Wuchshöhe und das Blühdatum für alle Linien aufgezeichnet. Die Linien wurde mit SSR und genotyping-by-sequencing (GBS) DArTseq Markern genotypisiert. Mittels QTL Analyse konnten Regionen im Durumweizen Genom identifiziert werden, welche mit FHB Resistenz in Zusammenhang stehen und sich auf den Chromosomen Armen 2BL, 3BS, 4AL, 4BS, 5AL und 6AS befinden. Der Elter DBC-480 steuerte in allen Fällen das resistente Allel der identifizierten QTL bei. Wir konnten den QTL auf 3BS in allen drei Populationen innerhalb des *Fhb1* Konfidenzintervalls nachweisen und somit erstmalig die erfolgreiche Introgression von *Fhb1* in Durumweizen unter Beweis stellen. Der Effekt von *Fhb1* auf die FHB Resistenz in Durumweizen wurde durch die Evaluierung von Typ-2 Resistenz in einer der drei Populationen bestätigt. Es wurde ausserdem ein starker Einfluss der Wuchshöhe auf das Ausmaß des Befalls mit FHB beobachtet; im Speziellen konnte gezeigt werden, dass das Verzweigungsallel des Kurzstrohgens *Rht-B1* mit einer erhöhten Anfälligkeit für Ährenfusariose einhergeht. Die Kombination von *Fhb1* mit zusätzlichen Resistenz QTL konnte den negativen Effekt von *Rht-B1b* ausbalancieren und ermöglichte die Auswahl von kurzstrohigen Linien mit verbesserter FHB Resistenz. Das neue und einzigartige genetische Material, welches diese Arbeit hervorgebracht hat, ist von direkter Relevanz für die praktische Züchtung und wird die Entwicklung von FHB resistenten Durumweizensorten nachhaltig unterstützen.

Introduction

1. Origin of durum wheat

The evolutionary history of cultivated tetraploid wheat started during the development of agriculture about 10.000 years ago in the Fertile Crescent with the domestication of wild emmer (*Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf. $2n=4x=28$, genome AABB). Wild emmer is an allotetraploid species that arose between 0.25 and 1.3 million years ago from the natural hybridization of the wild diploid wheat *Triticum urartu* Thumanjan ex Gandilyan ($2n=2x=14$, genome AA) (Dvorák et al. 1993) and an unknown close relative of *Aegilops speltoides* Tausch ($2n=2x=14$, genome SS) which provided the B genome (Zohary and Feldman 1962; Kilian et al. 2007). The mutation that resulted in non-brittle rachis, allowing efficient harvest without spikes shattering at maturity, led to the first domesticated form of emmer (*Triticum dicoccum* (Schrank) Schübl., $2n=4x=28$, genome AABB) (Luo et al. 2007). Cultivated hulled emmer was an abundant grain crop until the Bronze Age and spread from the Levantine to the Mediterranean basin and Europe (Feldman 2001). This species is still grown today albeit on a small scale. The acquisition of the free-threshing trait in further domestication events allowed the emergence of cultivated types, such as durum wheat (*Triticum durum* Desfs, $2n=4x=28$, genome AABB), that gradually replaced emmer wheat. From the Hellenistic period onwards, durum wheat became a major cultivated crop around the Mediterranean (Zohary et al. 2012). Other free-threshing allotetraploid wheat includes Pollard wheat (*Triticum turgidum* L.), Polish wheat (*Triticum polonicum* L.), Khorasan wheat (*Triticum turanicum* Jakubz.) as well as Persian wheat (*Triticum carthlicum* Nevski). Common wheat (*Triticum aestivum* L., $2n=6x=42$, genome AABBDD) is a free-threshing allohexaploid wheat that emerged about 8000 years ago, and originated from the hybridization between an allotetraploid wheat of AB genome-composition and the diploid goat grass *Aegilops tauschii* Coss. which contributed the D genome (McFadden and Sears 1946; Petersen et al. 2006; Dvorak et al. 2012). In present times, common wheat dominates wheat production worldwide.

The wheat species form an allopolyploid series ($x=7$) at different ploidy levels and are largely interfertile. The different steps of domestication as well as the breeding history of durum wheat, marked by intense selection pressure in the last century, have contributed to the reduced level of genetic diversity of the current modern elite durum wheat germplasm (Thuillet et al. 2005). The diversity available in landraces as well as in wild and cultivated related species offers a useful reservoir of genetic variation for durum wheat improvement (Feuillet et al. 2008).

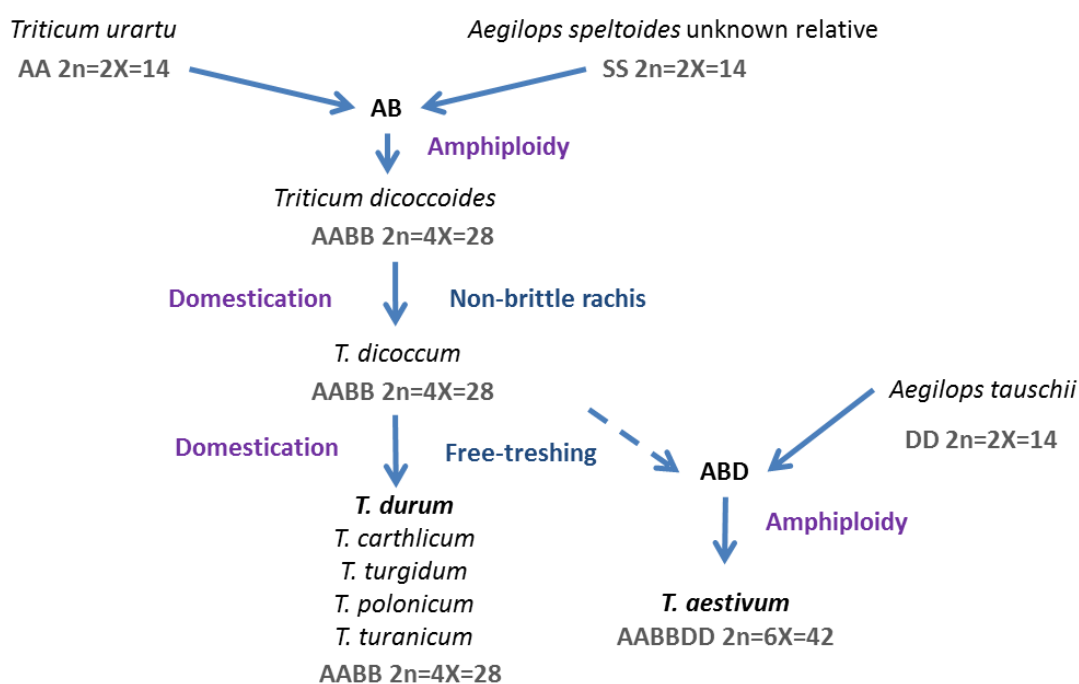


Figure 1: Phylogeny of wheat species

2. Economic importance of durum wheat

Wheat is one of the three most important staple foods worldwide together with maize and rice, providing an estimated 20% of all calories consumed by humans. Wheat cultivation occupies more arable land than any other crop and its worldwide production reached 730 million tons in 2015 (International Grains Council). Wheat production encompasses different species, of which common wheat represents the quasi-totality accounting for more than 90% of the total wheat grown worldwide. With a total of 39 million tons produced in 2015, durum wheat accounts for about 5% of the global wheat production. On a global scale, durum wheat can be considered a minor crop but represents the main cultivated crop and the primary staple food of the Mediterranean region where it is traditionally grown.

The major area of durum wheat cultivation is located around the Mediterranean basin, which includes South Europe, West Asia and North Africa, known as the SEWANA region. The European Union contributes to about 22% of the global durum wheat production (Italy, France, Greece and Spain being the major producers) and more than half of the world production is concentrated in the SEWANA region. Other main growing areas are the Northern Plains of the United-States and Canada as well as the desert areas between the United-States and Mexico. North America accounts for about 19% of the durum production but represents up to 80% of the durum wheat exports, directed primarily to the Mediterranean region. Cultivation of durum wheat is also found in Australia, Argentina, Central Europe and the Middle East.

Durum wheat is almost exclusively intended for human consumption making it an economically important crop. Grain quality is an important aspect for durum wheat processing and includes high amount of yellow pigment, protein content, gluten quality, vitreousness as well as a low proportion of diseased kernels (Troccoli et al. 2000). On the international market, high grain quality is sought after for production of premium pasta products. Durum wheat is also consumed in the form of couscous, bulgur and flat durum breads (Elias, 2005).

3. *Fusarium* head blight

Fusarium head blight (FHB) is a destructive disease of global importance, affecting durum and bread wheat in most regions where these cereals are cultivated (Parry et al., 1995). The disease was first described by W. G Smith in 1884 using the term “wheat scab” and was later renamed “*Fusarium* blight” by Atanasoff in 1920 (Stack 2003). The causal agents of FHB encompass more than 19 species from the *Microdochium* and the *Fusarium* genera. Among the species of the FHB complex, the most predominant ones are *Fusarium graminearum* [teleomorph: *Gibberella zeae*], *Fusarium culmorum* [teleomorph unknown], *Fusarium avenaceum* [teleomorph: *Gibberella avenacea*], *Fusarium poae* [teleomorph unknown], *Microdochium nivale* [teleomorph: *Monographella nivalis* var. *nivalis*], and *Microdochium majus* [teleomorph: *Monographella nivalis* var. *neglecta*], with their abundance depending on geographical location and climatic conditions, particularly temperature and moisture (Xu et al. 2005; Xu et al. 2008). *F. graminearum* is generally prevalent under warm and wet conditions, and is considered the most important species in North America, Central Europe and Australia. In Northwestern Europe, *F. culmorum* tends to dominate as it favors cooler and more humid conditions (Parry et al. 1995), although, in the last decade, shifts to a prevalence of *F. graminearum* has been observed in several regions (Waalwijk et al. 2003; Jennings et al. 2004). While these species appear to predominate, often two or more species coexist within the same field or even within the same plant (Xu et al. 2005; Siou et al. 2015). *F. graminearum* and *F. culmorum* show the highest level of pathogenicity of the different causal agents of FHB in wheat (Fernandez and Chen 2005). In addition, the isolates of these two species are all able to produce toxic secondary metabolites including deoxynivalenol (DON), nivalenol and zearalenone that accumulate in infected grains (Wegulo 2012). Due to their importance in both prevalence and disease incidence, *F. graminearum* and *F. culmorum* are used predominantly by breeders and researchers in artificially inoculated trials, with the majority of epidemiological researches focusing on *F. graminearum* solely.

Wheat is particularly susceptible to FHB at flowering time and infection may take place throughout the kernel development stage up to the soft dough stage. Outbreaks are favored by warm temperatures coupled with high humidity in the spring season. The risk of epidemics is further increased when natural inoculum is abundant. FHB pathogens are non-host specific

and can survive on many different plants, including cereals such as wheat, durum wheat, triticale, rye and maize, and notably may also live asymptomatic on grass hosts (Parry et al. 1995; Inch and Gilbert 2003). They overwinter in structures including sporodochia and perithecia on grass stuble, chaff and corn stalk residues left on the soil surface, as well as on infected grains. The conidia and ascospores produced by the fungi are dispersed by the wind and rain splash until they land on wheat heads serving as primary inoculum (Osborne and Stein 2007)

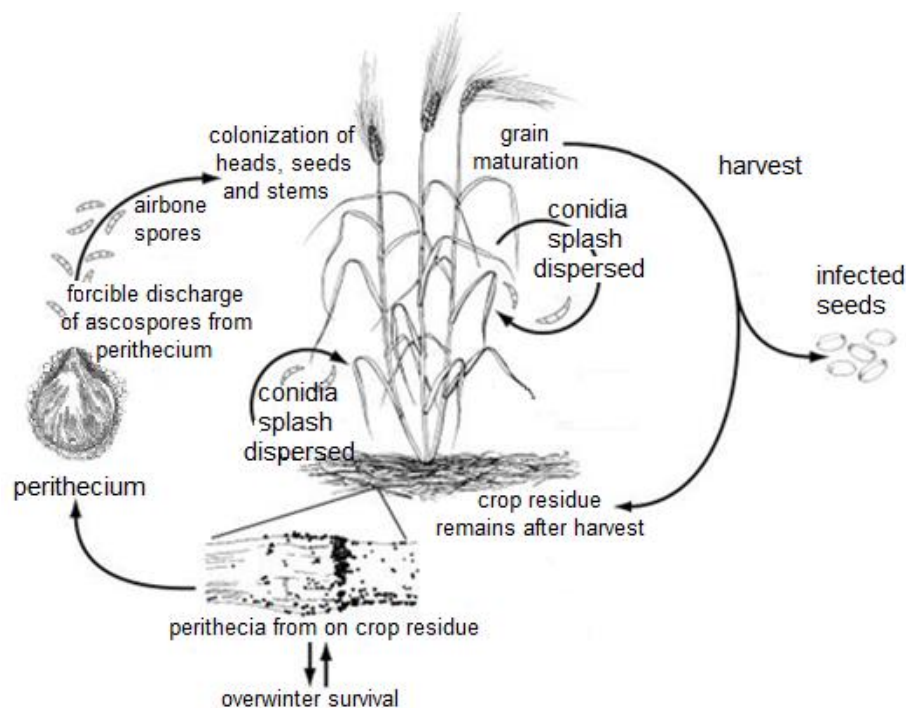


Figure 2: The life cycle of *Fusarium. graminearum* [teleomorph: *Gibberella zeae*] causing Fusarium head blight on wheat under field condition (modified from Trail 2009)

The infection starts when the spores germinate on the wheat heads and the fungal hyphae colonize the floral cavity. The fungus invades the floret via plant structure that are more prone to penetration including anthers, the surface of the glumes, via the lemma and palea, thin-walled cells at the base of the glumes and through natural openings such as stomates or wounds in the chaff (Walter et al. 2010). At the early stage of infection, the fungus establishes a brief biotrophic relationship with the host, while a shift to necrotrophic phase occurs at more advanced stages, causing host cell death (Brown et al. 2010; Kazan et al. 2012). The

trichothecene mycotoxins, notably DON, synthesized by the fungus at this stage, act as virulence factors contributing to cell damages (Desmond et al. 2008). The production of DON enables the spreading of the fungus through the rachis and the successful colonization of adjacent florets (Bai et al. 2002). The propagation in the rachis leads to vascular dysfunction associated with premature ripening of the spike above the point of infection, also termed as wilting (Goswami and Kistler 2004).



Figure 3: Durum wheat heads showing Fusarium head blight symptoms with premature bleached spikelets

The typical symptoms of the disease appear a few days after infection with necrotic lesions on the exterior surfaces of florets and glumes of brownish to dark color which evolve in bleaching of the spikelets. Several spikelets of an infected head may be affected. Over the time of infection, premature bleaching may progress throughout the entire head. Infected spikelets may fail to produce kernels, or may contain partially-filled kernels with an atrophied aspect contributing to a reduction on grain numbers and test weight (Parry et al. 1995; Bushnell et al. 2003). Colonization of developing kernels is also associated with mycotoxin accumulation. The affected grains are shriveled and wilted of white to tan color and are usually referred to as

Fusarium-diseased kernels (FDK). Besides, kernels contaminated late in development may appear healthy although they may contain toxins (Cowger et al. 2009; Siou et al. 2014).

The damages caused by FHB disease are multiple and result in both direct and indirect economic losses. In severe FHB epidemics, yields can be dramatically reduced and harvest containing damaged-kernels may be downgraded or discarded (McMullen et al. 1997). The disease affects also milling, baking and pasta-making properties due to the destruction of starch granules and storage protein in infected grains (Dexter et al. 1996; Nightingale et al. 1999). The main concern associated with FHB contamination is the accumulation of potent mycotoxins in affected samples. In wheat, the most frequently detected Fusarium mycotoxins are DON and its derivatives. DON acts as an inhibitor of protein synthesis and has been proved to be harmful for both human and livestock health when ingested (Pestka 2010). Many countries have established recommendations or regulations for maximum DON content in cereals and cereal products to insure public health safety. For instance, the European Community enacted a strict maximum level of DON in common wheat and durum wheat of 1.25 and 1.75 ppm, respectively (Official Journal of the European Communities 2007). Regulations also apply to products such as pasta (0.7 ppm), bread and bakery (0.5 ppm) and infant food (0.2 ppm). Similarly, the Food and Drug Administration of the USA has introduced, since 1993, advisory levels for DON of 1 ppm on finished wheat products intended for human consumption (Guidance for Industry and FDA 2010). Crops that exceeded acceptable DON levels may be devaluated or rejected contributing to further economic losses for wheat growers.

4. Management of Fusarium head blight

There are three main targets in FHB management: the reduction of the amount of primary inoculum, the prevention of inoculum dispersal and the limitation of infection when inoculum is present (Parry et al. 1995). To date no single solution is available for efficient FHB management and control of disease damages. Strategies available include:

Cultural practices: FHB pathogens over-winter on crop residues left on the soil surface after harvest. These infected plant debris represent the main source of primary inoculum for the following crop. FHB epidemics are therefore promoted by agronomic practices that leave high amount of debris on the soil surface and is further aggravated by slow degrading crop residues which provide a substrate for the pathogen for a longer time (Sutton et al. 1982; Pereyra and Dill-Macky 2005; Blandino et al. 2010). Dill-Macky and Jones (2000) showed that FHB damages were increased by crop rotations that involved consecutive cultivation of host plants – especially wheat after maize – and no or minimum tillage approaches, compared to crop sequences including non-host plants (i.e. wheat after soybean) and conventional tillage practice. Avoiding host plant rotations and burial of infested residues have thus been recommended as good practice to reduce FHB outbreaks. The main limitation of the use of deep plowing is its adverse impact on soil erosion and water conservation, rendering this control method environmentally costly. Current popular farming practices, including the adoption of minimum tillage and cereal rotation, support buildup of Fusarium inoculum and may have contributed to the increase in regional FHB epidemics (McMullen et al. 1997).

Chemical control: Chemical control has become available for wheat growers with fungicides of the demethylation inhibitor class (Paul et al. 2008; McMullen et al. 2012). Their application decreases FHB severity and DON contamination, especially when applied on cultivars showing some level of resistance (Wegulo et al. 2011; Willyerd et al. 2012). Many challenges are associated with the use of fungicide and include difficult proper application timing, variation in fungicide effectiveness and high economic costs (McMullen et al. 2012).

Disease monitoring: The development of FHB forecasting models that predict the risk of a FHB outbreak may help growers determine if fungicide deployment is justified.

Biological control: Microbial antagonists as biological control agents (BCA) have been investigated for FHB management but none are commercially available yet. BCA can reduce infection pressure when applied on crop residues by lowering over-winter survival of the inoculum and spore production potential (Inch and Gilbert 2007). BCA applied directly onto the spikes can contain FHB damages by slowing down disease progression (Palazzini et al. 2007; Xue et al. 2009). Notwithstanding, BCA have been shown to enhance the efficacy of DMI fungicides. They may be a mean to reduce the amount of chemical product needed to effectively control the disease and represent a promising tool in integrated management (Da Luz et al. 2003; McMullen et al. 2012).

Host resistance: The control methods mentioned above are only partially effective and, in the case of high disease pressure, they cannot provide sufficient protection to susceptible cultivars (Wegulo et al. 2011). Host resistance is not only regarded as the most effective and sustainable method for FHB control, but the deployment of resistant cultivars is also essential for efficient integrated management strategies (McMullen et al. 2008; Blandino et al. 2013). Since over two decades, breeding efforts have been put towards developing cultivars with improved FHB resistance (Anderson 2007).

At present, a combination of strategies including cultural practices, fungicide application and disease monitoring as well as cultivation of resistant varieties is most promising to reduce the risk of FHB epidemics (Gilbert and Haber 2013; Wegulo et al. 2015).

5. Breeding for *Fusarium* head blight resistance

FHB resistance in cereals is a quantitative trait governed by numerous genes (Buerstmayr et al. 2009). Resistance can be classified into two major components: resistance to initial infection (type 1) and resistance to spread of the disease within the spike (type 2) (Schroeder and Christensen 1963). Additional components of resistance have later been described and include resistance to toxin accumulation, resistance to kernel infection and tolerance (Mesterhazy 1995). Expression of FHB resistance is modulated by environmental factors, especially temperature and rainfall during the establishment of the infection, leading to significant genotype-by-environment interactions (Campbell and Lipps 1998; Miedaner et al. 2001). Accurate estimation of the level of resistance requires multiple independent phenotyping experiments, replicated over years and/or locations, to avoid confounding effects caused by the environment.

Historically, phenotypic selection has been applied for FHB improvement, evaluating the disease in breeding populations after artificial inoculation at anthesis (Bai and Shaner 1994). This approach has yielded cultivars with enhanced resistance but suffers from many drawbacks: it is laborious, time-consuming and costly. More importantly, the development of cultivars combining enhanced FHB resistance with stable grain yield and quality performances remained challenging for breeders (Rudd et al. 2001; Bai and Shaner 2004).

Since the 1990's and the emergence of molecular markers, QTL (quantitative trait loci) mapping has become the method of choice to dissect the genetic basis of FHB resistance. Due to its worldwide high economic value, most of the QTL mapping projects performed to date have been focusing on common wheat. Buerstmayr et al. (2009) summarized the findings of 52 QTL mapping studies and, among them, only four were performed with tetraploid wheat. In total, more than 100 FHB-resistance QTL have been identified and 22 genomic regions were detected in at least two common wheat populations. Consistent and reliable QTL were further confirmed by meta-QTL analyses which pointed out 19 confirmed QTL (Liu et al. 2009; Löffler et al. 2009). These QTL are expressed consistently across different genetic backgrounds and environments and are therefore potentially useful for breeders.

The first major resistance QTL for FHB resistance in common wheat was identified from the resistant Asian common wheat cultivar Sumai-3 and mapped to the short arm of the chromosome 3B (Waldron et al. 1999). This QTL, formally designated as *Fhb1*, is well validated and has been precisely mapped: it is flanked by markers *Xgwm533*, *Xbarc133* and *Xgwm493* (Cuthbert et al. 2006) and highly diagnostic marker such as *Xumn10* are available (Liu et al. 2008; Schweiger et al. 2016). *Fhb1* contributes mainly to type 2 resistance and provides some level of type 1 resistance (Anderson et al. 2001; Buerstmayr et al. 2003). Another well-characterized major QTL derived from Sumai-3 has been mapped on the chromosome 5A (*Qfhs.ifa-5A*), associated primarily to type 2 resistance (Buerstmayr et al. 2002; Buerstmayr et al. 2003). Due to their large and stable effect on FHB resistance, these two QTL are promising targets for marker-assisted selection (MAS). Other well-validated and precisely mapped QTL are *Fhb2* descending from Sumai-3 and located on the chromosome arm 6BS (Cuthbert et al. 2007; Dhokane et al. 2016) as well as *Fhb4* and *Fhb5*, identified in the Asian common wheat cultivar Wangshuibai and mapped on the chromosome 4B and 5A respectively (Xue et al. 2010; Xue et al. 2011).

Although only a few of the many QTL reported for FHB resistance have been validated and deployed in commercial breeding programs, MAS for large effect QTL coupled with phenotypic selection to capture undiscovered small effect QTL is considered to be an efficient strategy for FHB resistance breeding (Agostinelli et al. 2012). Since the last decade, MAS for *Fhb1* has been widely implemented in North American common wheat breeding program (Anderson 2007). MAS can be applied for stacking resistance QTL: FHB resistance is mainly affected by additive effects and pyramiding several QTL using marker-based introgression has been shown to be effective in enhancing FHB resistance and reducing DON contamination in elite common wheat (Miedaner et al. 2006). Marker-based backcrossing has also proven efficient to rapidly transfer QTL of exotic resistance sources, such as Sumai-3, into locally adapted backgrounds by avoiding or reducing negative linkage drag. For example, the QTL *Fhb1* and *Qfhs.ifa-5A* were successfully introduced in European elite common wheat background without systematic deleterious yield and quality penalties (von der Ohe et al. 2010; Salameh et al. 2011). In recent years, several resistant cultivars carrying *Fhb1* have been released from North American breeding programs (e.g. Mergoum et al. 2008; Anderson et al.

2015) and in Europe, the variety Jaceo, registered in France in 2012 (Syngenta Seeds), is the first variety carrying *Fhb1* commercially available.

In comparison to common wheat, progress for improving FHB resistance in durum wheat has been slow. Durum wheat has been early reported to be more susceptible to FHB than common wheat (Atanasoff 1920; Christensen et al. 1929). Current durum wheat cultivars are generally highly susceptible and the few improved varieties released recently by North American breeding program fail to show levels of resistance equivalent to the ones achieved in common wheat (Zhang et al. 2014). Breeders are still lacking efficient resistance source and research has been targeted at broadening the genetic basis for FHB resistance by introgressing resistance from related species. To date, only resistance from tetraploid wheat has been exploited and, to our best knowledge, durum wheat did not yet benefit from the numerous QTL detected in common wheat. Our work constitutes the first characterization of FHB resistance derived from hexaploid common wheat into elite durum wheat background.

6. Objectives of the thesis

The main objective of the project was to gain knowledge on the genetics of FHB resistance in durum wheat and to provide breeders with novel germplasm which will support efforts in enhancing durum wheat resistance against FHB.

The first target was to provide a rich source of information on the QTL associated with FHB resistance in tetraploid wheat. I summarized the current status of FHB improvement in durum wheat and provided an overview of the resistance QTL that have been identified for enhancing FHB resistance (**Publication 1: Current knowledge on resistance to Fusarium head blight in tetraploid wheat**).

The project aimed at broadening the genetic basis for FHB resistance in durum wheat by exploiting resistance source derived from common wheat. I evaluated the effect of the major common wheat resistance QTL *Fhb1* in the background of three European elite durum wheat cultivars. The association of FHB resistance with the agronomic traits plant height and flowering date was also examined (**Publication 2: QTL mapping of Fusarium head blight resistance in three related durum wheat populations**).

Publication 1

Current knowledge on resistance to Fusarium head blight in tetraploid wheat

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Current knowledge on resistance to Fusarium head blight in tetraploid wheat

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Abstract Fusarium head blight (FHB) is a serious threat worldwide due to its dramatic consequences and effects on small grain cereal production such as yield and quality losses and most importantly mycotoxin contamination. Durum wheat (*Triticum durum* Desf.) is particularly susceptible to FHB. Enhancing resistance has proven difficult due to the narrow genetic variation for this trait in the durum wheat gene pool. Broadening the genetic basis by incorporating resistance alleles from wild and cultivated relatives is a promising approach for durum resistance breeding. This review summarizes the current information on sources available for FHB resistance improvement in durum wheat which include wild and cultivated tetraploid wheat, hexaploid wheat and alien species.

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The genetic basis of FHB resistance of a few tetraploid sources in the *T. durum* background has been dissected by QTL mapping. So far, thirteen QTL with small to moderate effects have repeatedly been detected on 11 chromosomes with alleles improving FHB resistance deriving from relatives and durum wheat itself. Notably, the QTL found in tetraploid wheat populations largely overlap with the QTL identified in hexaploid wheat suggesting a common genetic basis of FHB resistance. FHB resistance breeding by allele introgression into durum wheat is feasible, and QTL pyramiding appears a practicable strategy for durum resistance breeding.

Keywords Review · *Triticum durum* · Fusarium head blight resistance · QTL

Introduction

Fusarium head blight (FHB) is a persistent threat for wheat growers almost worldwide. A range of Fusarium species has the ability to cause FHB, and their distribution differs depending on agro-ecological conditions. The most pre-eminent species worldwide are *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwein.) Petch], predominant under temperate climate conditions, and *Fusarium culmorum* (W.G. Smith) Sacc., found more frequently in cooler environments (Parry et al. 1995; Xu and Nicholson 2009). FHB is responsible for considerable

economic losses in terms of yield drop and quality deterioration of the harvest. The major concern is the contamination of the crop with mycotoxins such as deoxynivalenol (DON), nivalenol and zearalenone. For more background on *Fusarium* as a cereal pathogen and strategies for disease control refer to Parry et al. (1995), Leonard and Bushnell (2003), Bai and Shaner (2004), Buerstmayr et al. (2009), McMullen et al. (2012), Gilbert and Haber (2013).

Mycotoxin contamination of the grains is of particular concern for durum wheat (*Triticum durum* Desf.) which is used predominantly for semolina and pasta intended for direct human consumption. To ensure food safety, maximum limits for the most prevalent mycotoxins in cereals and cereal products have been established in many countries. The European Commission set a maximum tolerated level of 1.75 ppm for DON in unprocessed durum wheat and of 0.75 ppm in pasta [Commission Regulation (EC) No. 1126/2007]. Similarly, the Food and Drug Administration of the USA indicated an advisory level of 1 ppm on finished wheat products for consumption by humans (Guidance for Industry and FDA 2010).

Several factors have deepened the risks of FHB epidemics in wheat. Changes in agricultural practices towards reduced or minimum tillage and a shorter or missing crop rotation between small grains and maize produce a surplus of *Fusarium* inoculum for subsequent reinfection. Weather patterns characterized by frequent rainfall and high humidity periods promote *Fusarium* infestations especially around anthesis when wheat is the most susceptible (Osborne and Stein 2007). The growing demand for pasta products has led to an increase in durum wheat production zones. In Europe, the durum wheat growing area has expanded from the traditional Mediterranean basin with warm and dry conditions to more humid regions with climatic conditions conducive for the disease in Central and Western Europe. Cultivation of winter durum, favoured for its higher yield potential, is more likely exposed to *Fusarium* as its earlier flowering coincides more often with rainfall and wet conditions (Miedaner and Longin 2014). In the upcoming years, durum and bread wheat (*Triticum aestivum* L.) production is expected to face an increasing pressure of FHB as climate change models forecast a greater risk of contamination associated with a greater prevalence of the highly toxigenic and aggressive *F. graminearum* (Chakraborty and Newton 2011).

Managing FHB remains difficult, cultural practices and chemical treatment offer only limited efficacy to reduce damages. Breeding resistant cultivars is pivotal and considered the most effective and sustainable strategy to control the disease. While the development of resistant cultivars has been under intensive research in hexaploid bread wheat particularly during the past decade, durum wheat, which is considered a minor crop, has received significantly less attention. Resistant durum wheat cultivars are essentially lacking, and there is an urgent need to identify appropriate resistance sources and understand the genetic basis of FHB resistance in durum wheat. This review aims to summarize the recent advances of breeding for FHB resistance in durum wheat.

Components of FHB resistance and their phenotyping methods

Resistance to FHB in wheat is polygenic and of quantitative nature (Buerstmayr et al. 2009). The complexity of the resistance reaction renders its evaluation and subsequent resistance breeding non-trivial and laborious. Environmental factors significantly influence FHB response by modulating disease infection and development (Huhn et al. 2012; Miedaner and Longin 2014). Accurate evaluations of FHB resistance need to be repeated over environments (years and/or locations). Different phenotypic expressions of the resistance have been described (Schroeder and Christensen 1963; Mesterhazy 1995): Type 1 (resistance to initial infection) and Type 2 (resistance to fungal spread within the spike) are the two components that contribute to “field resistance” (Yan et al. 2011) and are usually evaluated in QTL mapping studies.

Different inoculation procedures allow testing for specific resistance components. Single-floret inoculation and measure of speed and/or amount of spread of the symptoms along the ear are typically performed to assess Type 2 resistance. Spray inoculation allows evaluating Type 1 resistance by measuring disease incidence (percentage of ears presenting FHB symptoms). Type 1 resistance is considered difficult to assess, and studies using spray inoculation focus usually on evaluating disease severity (percentage of diseased spikelets per unit area) which encompasses Type 1 and Type 2 resistance (Buerstmayr et al. 2012a). While spray inoculation is considered to

mimic natural infection conditions, subsequent FHB evaluations may suffer from environmental effects and are therefore prone to genotype-by-environment interactions (Fuentes et al. 2005; Ma et al. 2006). Most studies performed in tetraploid wheat have relied on evaluating Type 2 resistance which is less biased by environmental variations as the experiments are usually set up in the greenhouse offering better-controlled conditions and avoid disease escape because the inoculum is placed directly into the florets.

Further components of resistance have been described notably the resistance to DON accumulation in infected grains also termed Type 3 resistance (Miller et al. 1985; Mesterhazy et al. 1999). Evaluation of resistance to DON accumulation is done post-harvest, and mycotoxin content is usually determined using analytical tools including chromatographic and immunochemical methods (Krska et al. 2008; Berthiller et al. 2013). DON is considered a virulence factor of several *Fusarium* spp. and has been shown to play a role in the spread of FHB through the spike (Bai et al. 2001). Resistance to toxin accumulation has been demonstrated to play a major role in FHB resistance mechanism in wheat (Lemmens et al. 2005) and, as the other components of resistance, is influenced by environmental conditions (Wegulo 2012; Ovando-Martinez et al. 2013). Although reducing DON concentration in grains is a target for breeders, direct selection for Type 3 resistance remains impractical due to its phenotyping costs (Sneller et al. 2012).

FHB resistance has frequently been found associated with plant morphological and developmental traits, especially plant height, spike architecture, anther extrusion and flowering date, acting mainly as passive resistance factors (Mesterhazy 1995). The phenotype of a characteristic durum spike differs considerably from the spike of hexaploid bread wheat. The typically compact spike phenotype of durum wheat in combination with the tendency to retain anthers inside the florets may to some extent aggravate disease development.

Genetic resources for improvement of FHB resistance in durum wheat

The generally higher susceptibility of durum wheat in contrast to bread wheat has been noted early on (Atanasoff 1920), and still today, most durum wheat

cultivars are susceptible (Clarke et al. 2010; Miedaner and Longin 2014). A prerequisite for resistance breeding is the availability of resistance donors. Unfortunately, variation for this trait is narrow in the species *T. durum*. Efforts to identify durum wheat accessions with enhanced resistance have met with limited success. Elias et al. (2005) screened large collections including several thousand durum wheat accessions and failed to identify resistant lines. A later evaluation of accessions from CIMMYT and ICAR-DIA identified only five Tunisian durum wheat landraces with moderate Type 2 resistance (Elias et al. 2005; Huhn et al. 2012). Talas et al. (2011) evaluated Syrian landraces and reported a significant variation for FHB resistance after spray inoculation. The authors identified four accessions with stable resistance as promising candidates for durum wheat breeding.

The reason for the scarcity of FHB-resistant sources in durum wheat has not yet been clearly elucidated. A possible explanation could be that current durum wheat, mostly descending from germplasm cultivated in the warm and summer-dry Mediterranean basin, has not been exposed to relevant disease pressure (Ban et al. 2005). Furthermore, breeding investment in durum wheat has been lower than in bread wheat. As a result, modern durum cultivars are developed by a limited number of durum breeding programs, which may have led to a narrower genetic base compared to hexaploid wheat (Oliver et al. 2008). Several sources suggest that the expression of FHB resistance in durum wheat is compromised by the presence of susceptibility factors and/or suppressor genes in its genome (Stack et al. 2002; Kishii et al. 2005; Garvin et al. 2009; Ghavami et al. 2011). To face the lack of resistance sources, breeders have targeted their research at broadening the genetic basis available for durum wheat improvement by searching resistance donors in wild and cultivated relatives.

Hexaploid bread wheat has benefited from intensive research in the last decade, and resistance sources have been identified originating from diverse regions of the world. Among others, the Chinese wheat cultivar Sumai-3 and its derivatives provide among the highest levels of resistance. Major resistance QTL from Sumai-3-derived populations have been consistently identified, namely *Fhb1* and *Fhb2* on chromosome 3B (Waldron et al. 1999; Anderson et al. 2001; Liu et al. 2006) and 6B (Waldron et al. 1999; Shen

et al. 2003; Cuthbert et al. 2007), respectively, which contribute to Type 2 resistance, and *Qfh.ifa-5A* on chromosome 5A (Buerstmayr et al. 2002, 2003a) which provides mainly Type 1 resistance and to a lesser extent Type 2 resistance.

Attempts to transfer resistance QTL identified in *T. aestivum* into *T. durum* are impeded by differences in ploidy levels and were of limited success so far though some progress has been made. The use of hexaploid wheat as a resistance donor is not straight forward. Even though most of the resistance QTL reported in hexaploid wheat are located on the A and B genome (Buerstmayr et al. 2009), the role of the D-genome, which is absent in tetraploid wheat, may play a role in boosting FHB resistance (Fakhfakh et al. 2011). Despite that, recent own preliminary results indicate successful transfer of resistance QTL *Fhb1* through recurrent back-crossing from Sumai-3 into durum wheat. The introgressed QTL in tetraploid background provides stable and enhanced resistance in field nursery experiments. In contrast, efforts to introgress 5A resistance QTL of Sumai-3 have been hindered by the reduced spike fertility of the durum wheat back-cross lines (own unpublished data). To our knowledge, no successful transfer of *Fhb2* in tetraploid wheat has been reported.

Resistance that is not confounded by differences in ploidy has been searched in tetraploid relatives of durum wheat. Wild relatives have developed wide genetic adaptation to biotic stresses and offer a large reservoir of genes for durum wheat breeding. Genetic variation in resistance exists in tetraploid wheat subspecies of the primary gene pool of durum wheat. Various studies identified moderately FHB-resistant accessions in wild emmer wheat, *T. dicoccoides*, cultivated emmer wheat, *T. dicoccum*, and Persian wheat, *T. carthlicum*.

The direct progenitor of durum wheat *T. dicoccoides* has been extensively studied. Miller et al. (1998) screened 290 accessions of *T. dicoccoides* of the USDA National Small Grains Collections for reaction to FHB and identified several accessions with Type 2 resistance. Buerstmayr et al. (2003b) evaluated 151 lines originating mainly from Israel and pointed out eight accessions with enhanced resistance. A large set of germplasm comprising 416 *T. dicoccoides* accessions of diverse origins was evaluated by Oliver et al. (2007). The authors noted a broad variation for FHB response with several accessions exhibiting high

resistance. Oliver et al. (2008) screened 376 different tetraploid wheat species accessions and successfully identified 16 *T. carthlicum* and four *T. dicoccum* with a medium to high level of resistance.

Although these tetraploid species may offer useful sources of resistance for durum wheat improvement, they have received little attention so far in breeding. Up to now, none of the resistant accessions are comparable to Sumai-3 and the use of wild relatives is associated with several difficulties due to their exotic nature that impairs their immediate incorporation in elite breeding programs. To reduce the amount of undesirable linkage drag associated with the introgression of wild alleles, the transfer of resistance from *T. dicoccoides* accessions to durum wheat has been performed using chromosome substitutions, which yielded experimental lines with increased resistance. Stack et al. (2002) evaluated disomic substitution lines of the moderately resistant *T. dicoccoides* accession Israel A in the genetic background of the *T. durum* cultivar Langdon. The line carrying chromosome 3A of *T. dicoccoides* showed consistently increased FHB resistance. Similarly, substitution lines harbouring chromosome 7A from the *T. dicoccoides* accession, PI478742, showed consistently enhanced resistance (Kumar et al. 2007).

Recent improvements in chromosome engineering and manipulation permit the incorporation of FHB resistance from more distantly related species of the secondary or tertiary gene pool of durum wheat. Some species from the genera *Leymus*, *Roegneria* or *Thinopyrum* grow in regions with climates favourable to FHB, and some lines have been found with very high to complete resistance (Cai et al. 2005). They represent a valuable gene pool for FHB improvement.

A FHB resistance locus has been identified on the chromosome 1E of the perennial diploid wild grass *Thinopyrum elongatum*. Disomic substitution and addition lines of chromosome 1E in the background of *T. durum* have been successfully developed which express improved resistance (Jauhar 2014). Similarly, another resistance locus from chromosome 7E of *T. elongatum* has been incorporated in bread wheat using substitution and translocation lines (Fu et al. 2012). The method is potentially applicable to durum wheat. The use of alien sources is impeded by their negative agronomic traits, and detailed mapping of the desired locus is underway to efficiently use *T. elongatum* in FHB resistance improvement (Chen et al. 2013).

Table 1 Information on mapping populations and the phenotyping methods performed for mapping Fusarium resistance QTL in tetraploid wheat

Plant material		Phenotyping			References
Parents	Population	Number of exp. ^a	IM ^b	Iso ^c	
Langdon(Dic-3A) × Langdon	83 RIL	2 GH	SFI	Fg	Otto et al. (2002)
Langdon(Dic-3A) × Langdon	83 RIL	2 GH	SFI	Fg	Chen et al. (2007)
Langdon(Dic-7A) × Langdon	118 RIL	3 GH	SFI	Fg	Kumar et al. (2007)
Langdon(Dic-2A) × Langdon	63 RIL	3 GH	SFI	Fg	Garvin et al. (2009)
Strongfield × Blackbird	85 DH	1 GH	SFI	Fg	Somers et al. (2006)
Mt. Hermon#22 × Helidur	140 BC ₁ F ₅	1 GH, 2 Field	SFI	Fc	Gladysz et al. (2007)
Tun34 × Lebsock	169 BC ₁ F ₆	2 GH	SFI	Fg	Ghavami et al. (2011)
Multi-parental association mapping population	171 BC ₁ F ₇ and 169 BC ₁ F ₆	2 GH	SFI	Fg	Ghavami et al. (2011)
Td161 × Floradur	129 BC ₁ F ₄	4 Field	SPI	Fg, Fc	Buerstmayr et al. (2012b)
Td161 × DS-131621	134 BC ₁ F ₄	4 Field	SPI	Fg, Fc	Buerstmayr et al. (2012b)
Td161 × Helidur	126 BC ₁ F ₄	4 Field	SPI	Fg, Fc	Buerstmayr et al. (2012b)
BGRC3487/2 × DT735	160 BC ₁ RIL	4 Field	SPI	Fg, Fc	Ruan et al. (2012)
Mt. Gerizim#36 × Helidur	103 BC ₁ F ₆	4 GH	SFI	Fc	Buerstmayr et al. (2013)

^a Number of experiments: *GH* greenhouse experiment, *Field* field experiment

^b Inoculation method: *SFI* single-floret inoculation, *SPI* spray inoculation

^c Isolate used for inoculation: *Fg* *F. graminearum*, *Fc* *F. culmorum*

Successful use and incorporation of the resistance loci into adapted cultivars would greatly benefit from precise mapping, to allow targeted QTL selection and at the same time avoid undesired alleles in order to limit unwanted linkage drag.

QTL mapping for FHB resistance in tetraploid wheat

While in hexaploid wheat numerous QTL studies have been performed and more than 100 QTL have been reported (reviewed by Buerstmayr et al. 2009), tetraploid wheat has received less attention. To date, QTL mapping studies have been performed dissecting the genetics of only a few tetraploid resistance sources of *T. dicoccoides*, *T. dicoccum*, *T. carthlicum* and *T. durum* landraces. We summarize here the QTL reported in crosses with *T. durum* that have been repeatedly identified in different environments or in independent studies. Details on the mapping population and the inoculation methods performed for each mapping study are presented in Table 1. Information on the relevant QTL detected in tetraploid wheat is

given in Table 2. Their estimated positions as well as the position of *Fhb1* and *Fhb2* hexaploid QTL are indicated by vertical bars on the linkage maps shown in Fig. 1. Linkage maps were drawn with MapChart v2.2 (Voorrips 2002) based on Somers et al. (2004) consensus map available online on GrainGenes database (<http://wheat.pw.usda.gov>). The map for chromosome 5B relies on the publication by Ghavami et al. (2011) as we were not able to anchor the markers provided by the mapping study to the consensus map. For reading ease, only selected markers are displayed in Fig. 1. More detailed information including all available markers with their positions as well as comparison with Marone et al. (2012) consensus map is included in Online Resource 1. The linkage groups shown in Online Resource 1 focus only on the markers located near the QTL. Position of the markers and the genetic distances are kept as in the original consensus maps. Homologous loci used for map comparison are displayed in red. For complete map information, the readers are invited to refer to the original publications.

Most of the studies evaluated Type 2 resistance by single-floret inoculation under greenhouse conditions, and only two studies reviewed herein assessed FHB

Table 2 List of QTL in tetraploid wheat. Information on location and associated markers, magnitude of QTL effect on the Fusarium-related trait evaluated, parent contributing to the resistant allele as well as association with other traits and co-localization with major hexaploid wheat QTL are included

Source of resistance	QTL location		Markers, marker interval	PV% ^a	Analysed trait	Comments	References ^b
	Chromosome	Location					
<i>T. durum</i> Langdon	2A		<i>Xwmc296-Xgwm445</i>	21–26	FHB spread	Associated with FHB susceptibility	[4] Garvin et al. (2009)
<i>T. durum</i> Helidur	2B		<i>Xgwm374-Xbarc128</i>	5.9	FHB spread		[6] Gladysz et al. (2007)
<i>T. durum</i> Strongfield	2BL		<i>Xwmc474-Xwmc175</i>	26	FHB spread		[5] Somers et al. (2006)
<i>T. dicoccoides</i> Israel A	3AS		<i>Xgwm2</i>	37–41	FHB spread	Also known as <i>Qfhs.ndsu-3AS</i>	[1] Otto et al. (2002)
<i>T. dicoccoides</i> Israel A	3AS		<i>Xgwm2</i>	38–42	FHB spread	Refined map from [1]	[2] Chen et al. (2007)
<i>T. dicoccoides</i> Mt. Hermon#22	3AS		<i>Xgwm2</i>	10.3	FHB spread		[6] Gladysz et al. (2007)
<i>T. dicoccoides</i> Mt. Gerizim#36	3AS		<i>Xgwm779-Xgwm1121</i>	17–22	FHB spread		[10] Buerstmayr et al. (2013)
Tunisian <i>T. durum</i>	3BS		<i>tPt-6487</i>	– ^d	FHB spread		[7] Ghavami et al. (2011)
<i>T. durum</i> Floradur	3BS		<i>Xbarc133</i>	5.3	FHB severity	Colocalize with <i>Fhb1</i>	[8] Buerstmayr et al. (2012b)
<i>T. dicoccum</i> BGRC3487/2	3BS		<i>wPt-6239-wPt-8592</i>	16.0	FHB index ^c	Colocalize with <i>Fhb1</i>	[9] Ruan et al. (2012)
<i>T. dicoccum</i> BGRC3487/2	3BS		<i>wPt-6239-wPt-8592</i>	15.8	FHB severity		[9] Ruan et al. (2012)
<i>T. dicoccum</i> BGRC3487/2	3BS		<i>wPt-6239-wPt-8592</i>	8.7	FHB incidence		[9] Ruan et al. (2012)
<i>T. durum</i> Helidur	4A		<i>Xgwm375-Xs24m17-6</i>	6.8	FHB spread		[6] Gladysz et al. (2007)
<i>T. dicoccum</i> -161	4BS		<i>Rht-B1</i>	4.9	FHB severity	Association with plant height	[8] Buerstmayr et al. (2012b)
<i>T. dicoccum</i> -161	4BS		<i>Rht-B1</i>	3.1	FHB severity	Association with plant height	[8] Buerstmayr et al. (2012b)
<i>T. dicoccum</i> -161	4BS		<i>Rht-B1</i>	18	FHB severity	Association with plant height	[8] Buerstmayr et al. (2012b)
<i>T. durum</i> Lebsock	5BL		<i>wPt-0054-wPt-7400</i>	11.8–14.7	FHB spread		[7] Ghavami et al. (2011)
<i>T. durum</i> Lebsock	5BL		<i>wPt-0054</i>	– ^d	FHB spread		[7] Ghavami et al. (2011)
<i>T. dicoccum</i> -161	6AL		<i>Xgwm356</i>	4.0	FHB severity		[8] Buerstmayr et al. (2012b)
<i>T. carthlicum</i> Blackbird	6BS		<i>Xgwm518-Xbarc125</i>	23	FHB spread	Colocalize with <i>Fhb2</i>	[5] Somers et al. (2006)
<i>T. dicoccum</i> -161	6BS		<i>Xwmc398-Xgwm816</i>	4.8	FHB severity	Colocalize with <i>Fhb2</i>	[8] Buerstmayr et al. (2012b)
<i>T. dicoccoides</i> Mt. Gerizim#36	6B		<i>Xs23m17-5-Xgwm626</i>	19–22	FHB spread		[10] Buerstmayr et al. (2013)
<i>T. dicoccoides</i> PI478742	7AL		<i>Xksuim176-Xbarc121</i>	19	FHB spread		[3] Kumar et al. (2007)
<i>T. dicoccum</i> -161	7BS		<i>Xs24m12-f6h5</i>	3.3	FHB severity	Association with flowering date	[8] Buerstmayr et al. (2012b)

^a Percentage of phenotypic variance explained by the QTL

^b Numbers in square brackets for cross reference with Fig. 1

^c FHB index: calculated by multiplying the disease incidence by the disease severity

^d Not estimated

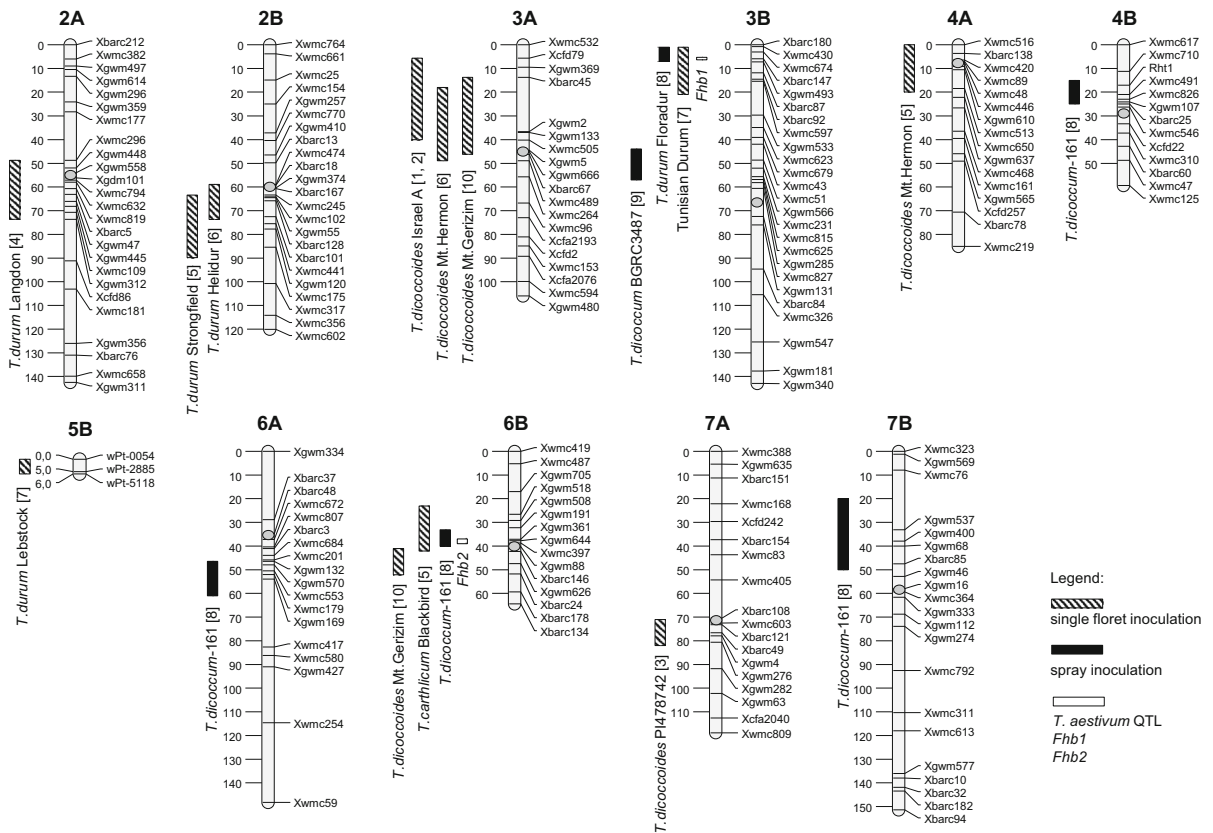


Fig. 1 Location of FHB resistance QTL on durum wheat chromosomes projected on the consensus wheat map by Somers et al. (2004). For readability, only selected markers are shown. Names of the lines contributing the resistant alleles are

severity after spray inoculation in field trials. The majority of these mapping studies relied on relatively small population sizes which may impair QTL detection while at the same time tend to over-estimate QTL effects. Primarily, small to moderate effect QTL have been identified on altogether 11 chromosomes, namely 2A, 2B, 3A, 3B, 4A, 4B, 5B, 6A, 6B, 7A and 7B. Several QTL have been detected in independent studies, such as QTL on chromosomes 2B, 3A, 3B and 6B, which we consider therefore independently validated. The QTL on 3A near *Xgwm2* was reported in three unrelated populations using different *T. dicoccoides* accessions as resistance source (Otto et al. 2002; Gladysz et al. 2007; Chen et al. 2007; Buerstmayr et al. 2013). The three *T. dicoccoides* lines are likely to possess the same resistance allele at this locus. Allelic variation for FHB resistance in tetraploid wheat appears for QTL on 6B detected in two

indicated, and numbers in brackets refer to the publication in which the QTL was reported (for cross reference see Table 1). Approximate QTL positions are designated by vertical bars. Patterns of bars indicate the inoculation method performed

mapping populations using *T. dicoccum* (Buerstmayr et al. 2012b) and *T. carthilicum* (Somers et al. 2006) accessions as resistant donors. These two diverse tetraploid species probably carry different resistant alleles at the 6B locus.

Most of the QTL regions identified in tetraploid wheat have previously been reported in hexaploid wheat suggesting a common genetic base of resistance. Tetraploid resistance QTL on 2A, 2B, 3A, 3B, 4A, 4B, 6B, 7A, 7B overlap with QTL found in diverse hexaploid-resistant sources comprising spring and winter wheat material of various origins including Asia, Europe and North America [see review by Buerstmayr et al. (2009)]. Notably, QTL on 3B (Ghavami et al. 2011; Buerstmayr et al. 2012b) and on 6B (Somers et al. 2006; Buerstmayr et al. 2012b) coincide with the major resistance QTL *Fhb1* (Liu et al. 2006) and *Fhb2*

(Cuthbert et al. 2007), respectively, which both originate from Sumai-3.

Allele comparisons using SSR markers linked to *Fhb1* and *Fhb2* carried out by Buerstmayr et al. (2012b) revealed different haplotypes for the tetraploid and the hexaploid lines indicating genetic diversity at these loci and different resistance alleles in bread wheat and in tetraploid wheat. The existence of resistance-improving alleles at these loci in tetraploid wheat may eliminate the need to transfer resistance from hexaploid Asian sources into durum wheat.

In hexaploid wheat, *Fhb1* has been described to govern the detoxification of DON into the conjugate deoxynivalenol-3-glucoside (DON-3G) (Lemmens et al. 2005). Wheat lines harbouring *Fhb1* showed lower FHB symptoms associated with lower amount of total mycotoxins and an increased ability to glycosylate DON, represented by high DON-3G/DON ratio. In durum wheat, variation in response to DON accumulation was recently pointed out. Dall'Asta et al. (2013) detected for the first time the presence of DON-3G in DON contaminated durum wheat samples. Detoxification of DON and accumulation of DON-3G appear also to be a resistance mechanism towards Fusarium infection in durum wheat. Less susceptible lines showed a higher DON-3G/DON ratio and thus an increased DON conversion capacity (Dall'Asta et al. 2013; Cirlini et al. 2014). The genetic basis of DON accumulation resistance in durum wheat has yet to be investigated.

The mapping studies carried out in tetraploid wheat have focused mostly on crosses of *T. durum* with a resistant relative. Most resistance-improving alleles are derived from the tetraploid wheat relative, but *T. durum* contributed resistance-improving alleles on 2B (Somers et al. 2006; Gladysz et al. 2007), 3B (Ghavami et al. 2011; Buerstmayr et al. 2012b) and 5B (Ghavami et al. 2011). Despite its general high susceptibility, durum wheat possesses a certain level of FHB resistance.

Transgressive segregation for FHB resistance was observed when crossing moderately resistant or moderately susceptible lines, and in several mapping populations, a few lines developed lower disease severity than the resistant parent (Somers et al. 2006; Ghavami et al. 2011; Ruan et al. 2012). Taking advantage of transgressive segregation is a great opportunity to yield lines with improved resistance. In hexaploid wheat, the cultivar Sumai-3 was selected from a cross of two moderately resistant parents and its

outstanding resistance can be explained by a combination of FHB-improving alleles at different loci predominantly acting in an additive manner (Bai et al. 2003; Schweiger et al. 2013).

Epistatic interactions and the existence of susceptibility factors, which modulate FHB resistance in tetraploid wheat, have been reported. A QTL was found on chromosome 2A of *T. dicoccoides* that increased FHB susceptibility when transferred into durum wheat. This locus may act as a suppressor of resistance and potentially acts epistatic in reducing the effect of FHB resistance QTL (Garvin et al. 2009). Ghavami et al. (2011) also suspected the existence of a QTL which mitigates FHB response in the same chromosomal region of 2A in Tunisian durum wheat. Epistatic interactions have also been described by Somers et al. (2006) in a *T. durum* × *T. carthlicum* population with QTL on 2A and 5A that influenced the FHB resistance QTL on 6B. These findings emphasize the need to select against genetic factors that increase FHB susceptibility or that may suppress resistance.

Of the tetraploid FHB resistance mapping studies performed to date, only two take in consideration morphological and developmental traits in their analyses (Buerstmayr et al. 2012b, 2013). It is well accepted that these traits are involved in passive resistance modulating FHB response in wheat. While morphological differences have less impact on disease development in greenhouse experiments using point inoculation, they may have a great effect in field experiments. Thus, considering these plant traits in the analysis of FHB resistance is important.

In the study performed by Buerstmayr et al. (2012b), it appeared that developmental and morphological traits influence FHB severity in tetraploid wheat in the same way as in hexaploid wheat. The large effect resistance QTL on 4B mapped at the position of semi-dwarfing gene *Rht-B1* and a QTL on 7B overlapped with QTL for flowering date. Coincidences of resistance QTL with *Rht* genes are common in hexaploid wheat. It remains unclear whether or not these associations are due to pleiotropic effects, close linkage with resistance genes or mainly an effect of plant height per se. Resistance QTL associated with plant height may have an adverse impact on the agronomic performances of lines and are thus inappropriate for elite breeding programs.

Mapping of QTL underlying important morphological and developmental characteristics needs to be

performed upon mapping of resistance QTL as they may have an adverse impact on the agronomic performances of lines. QTL that are not associated with agronomically undesirable traits are to be favoured for efficient improvement of FHB resistance in elite breeding programs. Newly released high-density consensus maps of durum wheat with larger numbers of markers and providing a better bridge between tetraploid and hexaploid wheat (Maccaferri et al. 2014a, b) will facilitate a more accurate genetic dissection of concurrent QTL effects.

Conclusion

FHB is a serious concern in durum wheat, and there is an urgent need to develop resistant cultivars. The deployment of relatives of durum wheat will enable to expand the genetic diversity for this trait, but their efficient use in elite cultivar breeding is hampered by linkage drag with unfavourable agronomic characteristics. Back-crossing strategies should allow transfer of desired alleles into regionally adapted elite germplasm. Several stable QTL have been detected and genetically mapped. Similarities at the genetic level are observed between tetraploid wheat and hexaploid wheat with most of their resistance QTL overlapping. QTL mapping studies also pointed out that susceptible *T. durum* possesses some resistance-improving alleles.

Combining resistance alleles through advanced phenotypic selection, marker-assisted selection (MAS) for validated resistance QTL, or genomic selection associated with a selection against suspected susceptibility factors and/or resistance suppressors is a promising approach to enhance FHB resistance in durum wheat breeding programs. Recurrent selection strategies should be employed to rapidly accumulate medium to small effect resistance alleles in breeding populations.

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Publication 2

QTL mapping of Fusarium head blight resistance in three related durum wheat populations

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ABSTRACT

Durum wheat (*Triticum durum* Desf.) is particularly susceptible to Fusarium head blight (FHB) and breeding for resistance is hampered by limited genetic variation within this species. To date, resistant sources are mainly available in a few wild relative tetraploid wheat accessions. In this study, the effect of the well-known hexaploid wheat (*Triticum aestivum* L.) quantitative trait locus (QTL) *Fhb1* was assessed for the first time in durum wheat. Three F7-RIL mapping populations of about 100 lines were developed from crosses between the durum wheat experimental line DBC-480, which carries an *Fhb1* introgression from Sumai-3, and the European *T. durum* cultivars Karur, Durobonus and SZD1029K. The RILs were evaluated in field experiments for FHB resistance in three seasons using spray inoculation and genotyped with SSR as well as genotyping-by-sequencing markers. QTL associated with FHB resistance were identified on chromosome arms 2BL, 3BS, 4AL, 4BS, 5AL and 6AS at which the resistant parent DBC-480 contributed the positive alleles. The QTL on 3BS was detected in all three populations centered at the *Fhb1* interval. The *Rht-B1* locus governing plant height was found to have a strong effect in modulating FHB severity in all populations. The negative effect of the semi-dwarf allele *Rht-B1b* on FHB resistance was compensated by combining with *Fhb1* and additional resistance QTL. The successful deployment of *Fhb1* in *T. durum* was further substantiated by assessing type 2 resistance in one population. The efficient introgression of *Fhb1* represents a significant step forward for enhancing FHB resistance in durum wheat.

KEY MESSAGE

The QTL *Fhb1* was successfully introgressed and validated in three durum wheat populations. The novel germplasm and the QTL detected will support improvement of Fusarium resistance in durum wheat.

KEY WORDS

Tetraploid wheat; durum wheat; Fusarium head blight; resistance breeding; QTL; *Fhb1*

INTRODUCTION

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum* and *Fusarium culmorum*, is one of the major fungal diseases affecting wheat production almost worldwide (Parry et al. 1995). The direct consequences of FHB are yield losses and seed quality reductions in both common wheat (*Triticum aestivum*) and durum wheat (*Triticum durum*) (McMullen et al. 2012). Highly problematic is the contamination of infected grains with Fusarium mycotoxins rendering harvests unfit for food and feed (Pestka 2010; Covarelli et al. 2014). Mycotoxin contamination is of particular concern in durum wheat as it is mainly utilized for human consumption. Host plant resistance is considered pivotal for an integrated plant protection strategy to control and reduce FHB damages (Gilbert and Haber 2013).

FHB is a complex disease and its response shows polygenic inheritance modulated by environmental factors with significant genotype-by-environment interactions (Buerstmayr et al. 2009; Löffler et al. 2009). Several components of resistance have been defined (Schroeder and Christensen 1963; Mesterházy 1995) among which resistance to initial infection (type 1) and resistance to fungal spread within infected spikes (type 2) are commonly accepted and have been widely investigated in QTL mapping studies. Under field conditions, overall FHB resistance is usually assessed through scoring of disease severity after spray inoculation and is considered to reflect the genotypic response during natural epidemics. Both active and passive mechanisms influence FHB resistance (Mesterházy 1995). The latter include morphological and developmental features which affect primary fungal infection and/or disease development through disease escape mechanisms. Plant height is one of the foremost morphological traits affecting FHB response and the widely deployed Norin 10 semi-dwarfing *Rht* alleles, namely *Rht-B1b* and *Rht-D1b*, have been found associated with increased FHB severity under field conditions in common wheat (Hilton et al. 1999; Miedaner and Voss 2008; Voss et al. 2008) and in durum wheat (Buerstmayr et al. 2012). Their effect on FHB development may be imputed to plant height *per se* and differences in canopy structure (Yan et al. 2011) as well as to pleiotropic physiological effects of the *Rht* genes and/or presence of tightly linked genes (Srinivasachary et al. 2009; Saville et al. 2012).

Compared to common wheat limited efforts have been dedicated to improve FHB resistance in durum wheat (Buerstmayr et al. 2009). Most current durum wheat cultivars are highly

susceptible and breeding progress is hampered by the narrow genetic variation for FHB resistance in durum wheat elite germplasm. Extensive screening of large germplasm collections identified only few durum landraces with improved levels of resistance (Elias et al. 2005; Talas et al. 2011; Huhn et al. 2012). Alternative sources of resistance have been screened in the related tetraploid species of *Triticum turgidum* to identify resistance donors for breeding (Buerstmayr et al. 2003b; Oliver et al. 2007; Oliver et al. 2008). A relatively small number of QTL mapping studies aimed at dissecting the genetic architecture of FHB resistance in tetraploid wheat to date, which have been recently reviewed by Prat et al. (2014). QTL descending from *Triticum dicoccoides* accessions Israel A, PI478742, Mt. Hermon#22, and Mt. Gerizim#36 have been identified on chromosomes 3A (Otto et al. 2002; Gladysz et al. 2007; Chen et al. 2007; Buerstmayr et al. 2013; Zhu et al. 2016b), 4A (Gladysz et al. 2007), 6B (Buerstmayr et al. 2013) and 7A (Kumar et al. 2007). *Triticum dicoccum* accessions PI 41025, Td-161 and BGRC3487 provided resistance QTL on 3A, 3B, 4B, 5A, 6A, 6B, 7A and 7B (Buerstmayr et al. 2012; Ruan et al. 2012; Zhang et al. 2014). *Triticum carthlicum* accession Blackbird contributed one resistance QTL mapping to 6B (Somers et al. 2006). The dissection of the genetic architecture of resistant *T. durum* Tunisian landraces by association mapping located a QTL on 3B (Ghavami et al. 2011). In mapping studies based on crosses between susceptible durum cultivars and other resistant sources, a few resistance-conferring QTL alleles were contributed by the susceptible durum parents, notably those on chromosomes 2A, 2B, 3B and 5B from cultivars Ben (Zhang et al. 2014), Strongfield (Somers et al. 2006), Floradur (Buerstmayr et al. 2012), and Lebsock (Ghavami et al. 2011), respectively.

The QTL detected in tetraploid wheat have failed so far to provide similarly high levels of resistance like *Fhb1*, the major resistance QTL identified in common wheat cultivar Sumai-3 (Waldron et al. 1999; Anderson et al. 2001). Near diagnostic markers for *Fhb1* are available (Liu et al. 2008; Schweiger et al. 2016) and have been successfully implemented into applied wheat breeding using marker assisted selection (Anderson et al. 2007; Wilde et al. 2007; Salameh et al. 2011). Notwithstanding, the consequence of transferring this major QTL into tetraploid wheat has not been communicated until now. Here we report on the effect of *Fhb1* in three biparental populations that have been generated by crossing line DBC-480, a FHB resistant experimental durum line possessing the *Fhb1* allele from Sumai-3, with a modern

European durum breeding line and two current durum cultivars. We also show the association of plant height with FHB resistance, and more specifically the effect of *Rht-B1* and its interaction with *Fhb1* on disease severity in durum wheat.

MATERIALS AND METHODS

Plant materials

Three mapping populations comprising 111, 100 and 100 F₇ RILs were developed by single seed descent from crosses of the tetraploid resistant line DBC-480 to the susceptible European *T. durum* cultivars Karur (KD) and Durobonus (DD) and the advanced breeding line SZD1029K (SD), respectively. Karur and Durobonus are registered varieties bred by RAGT, France (registered 2002) and Saatzucht-Donau, Austria (registered 2004), respectively. The breeding line SZD1029K was provided by Saatzucht-Donau for this study. Experimental line DBC-480 was developed at IFA-Tulln, Austria by four generations of marker-assisted backcrossing of the highly resistant *T. aestivum* cultivar Sumai-3 into the background of the Austrian *T. durum* variety Semperdur and subjected to rigorous phenotypic selection for improved FHB resistance in field trials (details not shown). The presence of the resistant allele at *Fhb1* was verified using the SSR markers *Xgwm389*, *Xgwm533* and *Xgwm493*. Karur, Durobonus and SZD1029K possess the semi-dwarfing allele *Rht-B1b*, while DBC-480 is a tall line that harbours the *Rht-B1a* wild type allele.

FHB resistance phenotyping

The three mapping populations along with their parental lines were evaluated in multiple field experiments at IFA-Tulln, Austria (16°04,16'E, 48°19,08'N, 177 m above sea level) in 2013, 2014 and 2015. Experiments were laid out as randomized complete block designs with two blocks in 2013 and three blocks in 2014 and 2015. Plots consisted of single rows in 2013 and double rows of 1 m length at 17 cm spacing in 2014 and 2015. Sowing of the individual blocks was performed in early spring and staggered one to two weeks apart leading to slightly different flowering dates between the blocks. Management of the field trials was conducted following good agronomical practice as described in Buerstmayr et al. (2002). At anthesis,

trials were spray-inoculated using a motor-driven backpack sprayer in the late afternoons with the virulent DON-producing *F. culmorum* isolate Fc91015 at a conidial concentration of $2.5 \times 10^4 \text{ ml}^{-1}$. Inoculum suspension was prepared by utilizing the protocol described in Buerstmayr et al. (2000). Aliquots of conidia stock solutions were stored at $-30 \text{ }^\circ\text{C}$ then thawed at $37 \text{ }^\circ\text{C}$ and diluted with deionized water to achieve the desired final spore concentration just prior to inoculation. Inoculations were performed within each block on all plots, starting when 50% of the plants in the earliest plot of a block reached anthesis. Inoculations were repeated at 2-day intervals and ended two days after the last plot of the block flowered, resulting in up to 6 inoculum applications per block. At each inoculation cycle about 100 ml.m^{-2} of conidial suspension was sprayed onto the durum wheat heads. The crop canopy was kept moist by mist-irrigating during 20 h after inoculations to facilitate spore germination and infection. FHB severity was visually estimated as the percentage of infected spikelets within each plot on days 14, 18, 22 and 26 after anthesis. In 2013, scoring was performed at two time points: 18 and 26 days after anthesis. The area under the disease progress curve (AUDPC) was calculated and used as an integrated measure of overall disease severity as described by Buerstmayr et al. (2000). At all experimental plots, plant height (PH) was measured in centimetre and flowering date was recorded and converted into number of days after May 1st. The KD population and the respective parental lines Karur and DBC-480 were also tested in the greenhouse for FHB spread within the spike (type 2 resistance) using single-floret inoculation in three unreplicated greenhouse trials at Florimond-Desprez (France) in winter 2015 (GH1) and at IFA-Tulln in summer 2016 (GH2 and GH3). Seeds were germinated in multi-trays and subjected to a cold treatment at $5 \text{ }^\circ\text{C}$ for one week. Ten seedlings per line were planted in 7.5-liter pots (23.5 cm diameter, 23 cm height) filled with a standard potting mix consisting of 70 % recycled compost, 28 % peat, and 2 % silica sand. Pots were designated as experimental units and arranged in a randomized design. The temperature in the greenhouse was maintained at $22/18 \text{ }^\circ\text{C}$ (day/night) with a 16-hour photoperiod. Management of the greenhouse trial was essentially as described by Buerstmayr et al. (2013). Inoculations were performed at anthesis by pipetting $10 \text{ } \mu\text{l}$ of conidia suspension between the lemma and palea of the four outer florets of two central spikelets per spike using the same inoculum preparation and concentration as for the field experiments. High humidity was ensured to promote fungal infection by covering the spikes with translucent polyethylene bags for 24 h. Type 2 resistance

was assessed as the percentage of infected spikelets per spike (PIS) measured at 24 days post inoculation by counting the number of infected spikelets and the total number of spikelets per spike. On average, eight spikes per genotype were inoculated in each experiment. Plant height was recorded at each greenhouse pot.

Phenotypic data analysis

Statistical analysis were performed in R 3.1.3 (R development core team, 2016) using the lme4 package for mixed model analysis (Bates et al. 2015). For each trait under investigation, a linear mixed model was fitted for each population with all three experiments combined:

(1) $P_{ijk} = \mu + G_i + E_j + E_j(R_k) + G_i \times E_i + e_{ijk}$, where P_{ijk} is the phenotypic value, μ is the population mean, G_i is the effect of i^{th} genotype, E_j the effect of j^{th} experiment, $E_j(R_k)$ the effect of the k^{th} replicate within the j^{th} experiment, $G_i \times E_i$ the ij^{th} effect of the genotype-by-experiment interaction and e_{ijk} designated the residual. The genotype effect was treated as fixed and all other terms as random effects. For single experiments a reduced linear mixed model was fitted: (2) $P_{ik} = \mu + G_i + R_k + e_{ik}$, where P_{ik} is the phenotypic value, μ is the population mean, G_i is the effect of i^{th} genotype, R_k the effect of k^{th} replicate (block) and e_{ik} the residual. The genotype effect was again treated as fixed and the replication as random effect.

Fixed and random effects of the models were tested one by one using the likelihood ratio test (LRT). Best linear unbiased estimates (BLUES) of each genotype were computed for the different phenotyped traits according to model (1) for the analysis across experiments and according to model (2) for an analysis within individual experiments. BLUES calculated across experiments are also referred to as overall means. Broad sense heritability (H^2) was estimated using variance components determined by the restricted maximum likelihood (REML) method setting all effects as random and based on the equation $H^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_{G \times E}^2 / m + \sigma_e^2 / p)$ where σ_G^2 denotes the genotypic variance, $\sigma_{G \times E}^2$ the genotype-by-experiment interaction variance, σ_e^2 the error variance, m the number of experiments and p the total number of replications across experiments (Holland et al. 2003).

Marker data and genetic map construction

Genomic DNA was extracted from fresh leaves of 10 pooled plants of each line using a simplified CTAB-based procedure modified from Saghai-Marroof et al. (1984). High density genotyping of all individuals was performed using genotyping-by-sequencing (GBS) with the DArTseq platform (DArT PL, Canberra, Australia). Markers identified by the DArTseq assay include SNPs as well as presence-absence variations (PAV) (Li et al. 2015). Markers were filtered based on a reproducibility $\geq 95\%$. Furthermore, PAV with $\geq 10\%$ missing data and SNP markers with $\geq 10\%$ missing data or heterozygotes were removed for each population separately. Markers showing significant ($p < 0.01$) segregation distortion were also discarded. Finally, a total of 7965, 4150 and 6235 high quality polymorphic DArTseq markers were available for mapping in the KD, DD and SD populations, respectively. All lines were genotyped with the two selected markers *Xbarc147* (Song et al. 2005) and *Xumn10* (Liu et al. 2008) that are known to be linked with *Fhb1* as well as with allele-specific markers for *Rht-B1* (Ellis et al. 2002).

Linkage map construction

Linkage maps for each population were constructed using the MSTmap algorithm (Wu et al. 2008) included in the R package ASMap v0.4 (Taylor and Butler 2015). The objective function was set to minimize the sum of recombination events between markers for map construction. In a first step, robust linkage groups were constructed using a p-value threshold set to 1×10^{-8} , and the assignment of the linkage groups to chromosome was performed by comparing the location of markers to the wheat DArTseq consensus map provided by DArT PL (A. Kilian, Diversity Arrays Technologies, personal communication, 2016). In a second step, genotypic data were pooled on a chromosome basis and regrouped at a less stringent threshold using a p-value of 1×10^{-6} . Distances were calculated with the Kosambi mapping function. Genetic maps were drawn on MapChart software (Voorrips 2002) and collinearity among the individual maps was checked.

QTL mapping

Quantitative trait loci analysis was performed for each trait with the BLUES calculated for each individual experiment and across experiments using the R-package R/qtl (Broman et al. 2003). Missing genotypic information was imputed using the multiple imputation method of Sen and Churchill (2001). Main effect QTL were detected by performing interval mapping and composite interval mapping via Haley-Knott regression. For composite interval mapping the number of marker covariates was selected by a forward approach, while setting a window size of 10 cM. LOD significance threshold for type I error rate $\alpha=0.05$ were obtained for each trait and experiment based on a 1000 permutations test. Significant QTL were subsequently fitted using a multiple QTL model. The existence of further QTL and the presence of QTL-by-QTL interaction were tested using the *addqtl* and *addint* functions, respectively. The final multiple QTL model was fitted against the null model by ANOVA and the percentage of phenotypic variance explained by each QTL, the additive effects as well as LOD scores were estimated. Confidence intervals were defined for each QTL by calculating a 1.5-LOD support interval.

RESULTS

Trait variations and correlations

Evaluation of FHB severity was performed on three biparental populations in artificially inoculated field trials to investigate the relevant factors that play a role in reducing FHB disease under natural conditions. In all experiments, the common resistant parent DBC-480 was significantly less diseased than the susceptible parents. The average AUDPC value across experiments of the resistant parental line DBC-480 was 125 (corresponding to an average of 12% symptomatic spikelets 26 days after flowering) while Karur, Durobonus and SZD1029K had approximately 5-fold higher AUDPC values (Table 1). Large variation was observed within each population (Fig.1), but also between populations where the average FHB severity was lowest in the KD population and highest in the SD population. Transgressive segregation was observed in all populations and some lines showed lower disease symptoms than the resistant parent DBC-480, although these differences were not statistically significant. Disease pressure between experiments was comparable in 2013 and 2014 while the experiment of

2015 showed overall higher symptoms. AUDPC broad sense heritability for means across experiments were high and within the same range for the three populations ($0.74 < H^2 < 0.89$) as in all cases, genotypic variances were higher than variances due to genotype x experiment interaction and residual error (Online Resource 1). Significant genotypic effects for all traits were revealed by ANOVA.

To evaluate specific type 2 component of resistance as conferred by *Fhb1*, the percentage of infected spikelets per spike (PIS) was measured in three glasshouse experiments for the KD population. Heritability for PIS was moderate $H^2 = 0.51$ although ANOVA showed significant genotype effects for the 111 RILs (Table 1, Online Resource 1). Some of the RILs showed transgressive segregation for type 2 resistance although only significant for higher susceptibility. As expected, DBC-480 showed less disease symptoms than Karur with an average of 18.3% and 38.4% PIS, respectively (Fig.1). Weak but significant correlation was observed between means of FHB spread and FHB severity ($r = 0.2, p < 0.05$).

Variation for plant height was apparent in the three populations (Fig.1). The susceptible parents Karur, Durobonus and SZD1029K were 38-49 cm shorter than the resistant donor DBC-480. PH showed a bi-modal frequency distribution in the KD and DD populations while a tri-modal distribution was displayed in the SD population (Fig.1). FHB severity was negatively correlated with PH within all populations (Table 2), accordingly shorter plants showed higher FHB severity. On the contrary, FHB spread after point inoculation (PIS) showed no significant correlation with PH. Distribution of date of anthesis showed continuous variation in all populations although no significant difference in flowering date was observable among the parents. FHB severity and flowering date were significantly positively correlated in the SD population across experiments, while a weak negative correlation and no evidence for significant correlation were observed in the KD and DD populations, respectively (Table 2). Within individual experiments however, correlation analysis showed no significant association between disease severity and date of anthesis in 2013 and 2015 for the KD population in 2013 and 2014 for the DD population and in 2014 for the SD population (Online Resource 2).

Table 1 Means of parents and mean, minimum and maximum values of populations, least significant differences at $\alpha < 0.05$ ($LSD_{0.05}$) and broad-sense heritability coefficient (H^2) or repeatability of analyzed traits in field and greenhouse (GH) experiments

	Parents				Population					
	DBC-480	Karur	Durobonus	SZD1029K	KD					
					Mean	Min	Max	$LSD_{0.05}$	H^2	
FHB severity (AUDPC)										
overall mean	125	642	693	845	360	65	816	117	0.89	
2013	126	668	601	874	272	23	830	258	0.66 ^c	
2014	54	457	567	519	289	16	781	175	0.87 ^c	
2015	200	805	917	1130	516	94	1128	185	0.92 ^c	
FHB spread (PIS ^b)	18.3	38.4	-	-	30.2	11.6	62.6	25.4	0.51	
Flowering date ^a	40.3	40.1	40.1	40.1	39.6	37.6	41.9	1.1	0.67	
Plant height (cm) in field	110	73	67	61	97	67	126	5	0.98	
Plant height (cm) in GH	119	71	-	-	105	65	143	12	0.97	
Population										
	DD					SD				
	Mean	Min	Max	$LSD_{0.05}$	H^2	Mean	Min	Max	$LSD_{0.05}$	H^2
FHB severity (AUDPC)										
overall mean	451	110	1152	142	0.78	667	131	1237	136	0.74
2013	292	47	699	210	0.61 ^c	401	47	915	249	0.69 ^c
2014	240	32	768	151	0.84 ^c	328	28	903	165	0.86 ^c
2015	791	201	1718	302	0.93 ^c	1257	251	2031	264	0.96 ^c
FHB spread (PIS ^b)	-	-	-	-	-	-	-	-	-	-
Flowering date ^a	39.3	36.5	41.7	1.2	0.76	41	38.3	43.3	1.1	0.81
Plant height (cm) in field	95	68	121	6	0.97	85	54	123	5	0.99
Plant height (cm) in GH	-	-	-	-	-	-	-	-	-	-

^aNumber of days from May 1st to anthesis

^bPercent infected spikelets

^cRepeatability

Table 2 Pearson correlation coefficients between FHB severity (AUDPC), plant height (cm) and flowering date (days after May 1st) for the overall means

	FHB severity (AUDPC)		
	KD	DD	SD
Plant height	- 0.82 ***	- 0.67 ***	-0.85 ***
Flowering date	-0.20 *	0.15 n.s.	0.43 ***

* $p < 0.05$
 *** $p < 0.001$
 n.s. non significant

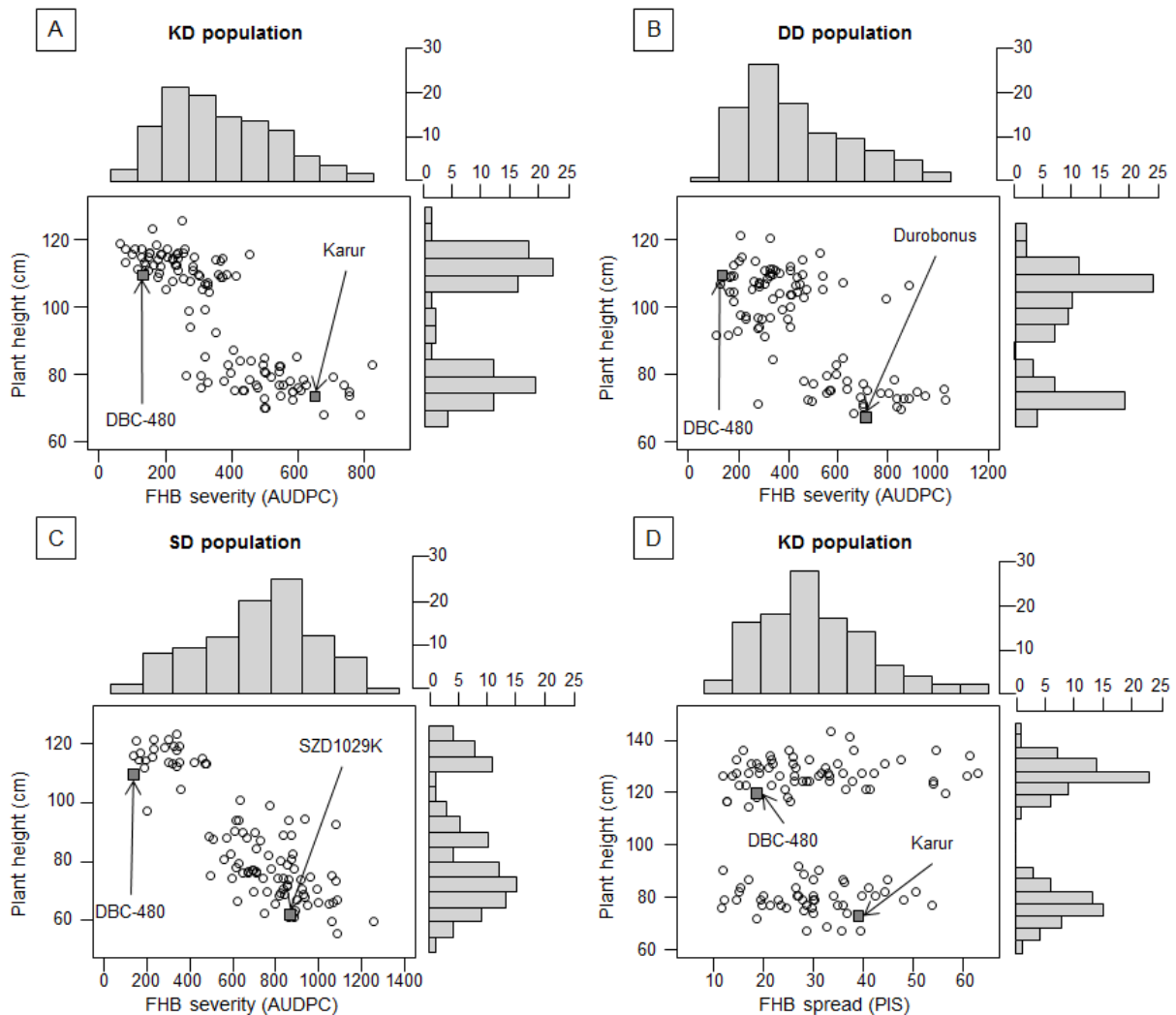


Fig. 1 Scatterplots and marginal histogram of frequency distribution of overall means for FHB severity (AUDPC) against plant height (cm) measured in the field trials for each population (A, B, C) and for FHB spread (PIS) against plant height (cm) measured in the greenhouse trials for KD population (D). Parents are indicated by arrows

QTL analysis

Generation of linkage maps

7975, 4153 and 6242 polymorphic markers were generated from DARTseq and SSR marker data for the KD, DD and SD populations, respectively. Of these markers, 1064 were common across all three populations. The number of markers within maps for the KD, DD and SD populations was reduced to 1609, 1052 and 1006 unique loci, respectively. Total map lengths were 2806, 1781 and 2219 cM with an average marker distance of 1.9, 1.7 and 2.5 cM for the KD, DD and SD populations, respectively. Each linkage group could be unambiguously assigned to a chromosome based on the wheat DARTseq consensus map. Alignment to the consensus map showed low-coverage regions for the DD and SD populations on chromosomes 1A and 3A and on chromosomes 5A and 7A for the DD population. Despite that, all chromosomes were represented (Online Resource 3).

QTL analysis for FHB severity

QTL analysis conducted in individual populations identified a total of 6 genomic regions associated with FHB severity on chromosomes arms 2BL, 3BS, 4AL, 4BS, 5AL and 6AS (Table 3). The resistant parent DBC-480 contributed the resistance improving alleles at all loci. Linkage groups and confidence intervals of QTL are shown in Fig. 2. For reading ease, only selected markers at about 5 cM distances are displayed, while more detailed information including all mapped markers with their positions can be found in Online Resource 4. The two genomic regions on 3BS and 4BS were found repeatedly associated with FHB resistance at the same location in all three populations. The major QTL on 4BS co-localized with the *Rht-B1* locus, which explained 64%, 38% and 19% of the total phenotypic variance in the KD, DD and SD population, respectively. On chromosome 3BS, the QTL mapped to marker positions *Xbarc147* and *Xumn10*, which signposts the position of the introgressed *Fhb1* and was detected for the analysis across experiments in all populations. The 3BS QTL was found consistently in all experiments for the KD population, while it was significant in two out of three experiments for the DD population and in one experiment for the SD population. The effects of the contrasting alleles at the *Fhb1* and *Rht-B1* loci, as well as the effect of allelic

combinations at these loci for the overall mean FHB severity are illustrated for each population in Online Resource 5. Analysis revealed further QTL specific to individual populations. Two major QTL were detected in the SD population on 4AL and 6AS explaining 19% and 25% of the total phenotypic variation. Both QTL overlapped with QTL associated with plant height and flowering date. In the DD population a QTL on 5AL was found in the analysis across experiments where it contributed to 6% of the phenotypic variation and had a stronger effect in 2013 explaining 15% of the phenotypic variation while it was not significant in 2014 and 2015. A small effect QTL was detected on 2BL in the KD population which contributed to 4% of the phenotypic variance and was significant in 2014 and for the across experiments analysis. There was no evidence for epistatic QTL interactions in any of the analyses; QTL for FHB severity acted thus in an additive manner.

Table 3 Locations and estimates of QTL for FHB severity (AUDPC) using multiple QTL mapping

Population	Chr.	Closest marker	Overall mean		2013		2014		2015		
			Add ^a	%PV ^b	LOD ^c	%PV ^b	LOD ^c	%PV ^b	LOD	%PV ^b	LOD ^c
KD	2BL	1072874	37	4.3	4.8	-	-	7.2	5.5	-	-
DD	3BS	4410793	86	14.0	8.7	-	-	16.0	4.7	12.2	7.6
KD	3BS	Xbarc147	60	11.1	10.8	14.1	5.5	12.8	9.0	6.1	5.6
SD	3BS	Xbarc147	60	5.0	3.3	-	-	8.1	3.8	-	-
SD	4AL	4541598	123	18.8	10.4	-	-	15.4	6.7	14.4	6.6
DD	4BS	RhtB1	156	38.4	18.6	18.8	6.0	26.0	7.1	55.5	23.1
KD	4BS	RhtB1	140	64.2	35.0	29.1	10.2	47.6	23.7	69.0	33.2
SD	4BS	RhtB1	126	19.4	10.7	-	-	10.3	4.7	16.0	7.2
DD	5AL	1111359	59	6.2	4.3	15.0	5.0	-	-	-	-
SD	6AS	4008755	139	24.9	12.9	28.0	6.9	24.6	9.8	25.4	10.6

^a Positive additive effects denote trait-increasing effect of the DBC-480 allele; additive effects were estimated as half the difference between phenotype averages for the homozygote

^b Percentage of phenotypic variance explained by the QTL

^c LOD (logarithm of the odds) above LOD threshold at the 0.05 level of probability obtained through a 1,000-iteration permutation test

QTL analysis for FHB spread

QTL detection for FHB spread in the KD population identified a QTL on 3BS which peaked at the SSR marker *Xbarc147* and was thus located in the same region as FHB severity 3BS QTL, matching likewise with the *Fhb1* locus. The resistance-conferring allele was derived from DBC-480. The QTL was consistently detected in all three individual experiments and for the analysis across all experiments in which it explained 33% of the phenotypic variation (Table 4). Two additional QTL were detected on 2A and 4AL at which the allele of the durum cultivar Karur conferred resistance. These QTL were found in single greenhouse experiments only and, contrary to the QTL at the *Fhb1* locus, were not considered as stable.

Table 4 Locations and estimates of QTL for FHB spread (percent of infected spikelets PIS) using multiple QTL mapping

Experiment	Chr.	Closest marker	Add ^a	%PV ^b	LOD ^c
GH1	2A	1698827	-4.9	13.5	4.7
GH1	3BS	Xbarc147	14.6	33.6	10.2
GH2	3BS	1032004	4.9	14.6	3.8
GH3	3BS	Xbarc147	4.7	10.3	3.0
overall mean	3BS	Xbarc147	7.4	33.3	9.7
GH3	4AL	1235993	-5.2	12.6	3.6

^a Positive additive effects denote trait-increasing effect of the DBC-480 allele; additive effects were estimated as half the difference between phenotype averages for the homozygote

^b Percentage of phenotypic variance explained by the QTL

^c LOD (logarithm of the odds) above LOD threshold at the 0.05 level of probability obtained through a 1,000-iteration permutation test

QTL analysis for plant height and flowering date

QTL for plant height were detected on 4AL, 4BS and 6AS to which DBC-480 alleles contributed to increased height (Table 5). The *Rht-B1* locus on 4BS was significant in all populations and explained 95%, 81% and 37% of the variation for PH in the KD, DD and SD population. In the SD population two further QTL were associated with PH on 4AL and 6AS. The main effects were 11% and 27% for 4AL and 6AS, while epistatic interaction was evident

for both loci with the *Rht-B1* locus. The percentage of variation explained by the interaction of 4ALx4BS and 6ASx4BS were low compared to the main effects of each locus contributing to 4% and 7% of the phenotypic variance. QTL detection for flowering date revealed significant QTL on 1BL, 2BS, 4AL, 5AL, 6AS and 6BS (Table 5). The DBC-480 allele contributed to a delayed flowering of RILs carrying it on QTL 2BS, 4AL, 5AL, 6AS and 6BS. The QTL on 2BS was found at the same position in all three populations and mapped at a location distal from the QTL for FHB severity identified in the KD population on 2BL. This QTL had the strongest effect in the DD population where it explained 44% of the phenotypic variation. In the SD population, co-localization of QTL for FHB severity, PH and date of anthesis was found on 4AL and 6AS and thus these loci appeared to have pleiotropic effects.

Table 5 Locations and estimates of QTL for plant height (cm) and flowering date (days after May 1st) using multiple QTL mapping

Trait	Population	chr	Closest marker	Add ^a	%PV ^b	LOD ^c
Plant height	SD	4AL	4541598	-5.6	10.8	22.7
	KD	4BS	RhtB1	-17.4	95.4	74.4
	DD	4BS	RhtB1	-14.9	81.4	36.2
	SD	4BS	RhtB1	-11.0	37.0	45.8
	SD	6AS	4008755	-9.0	27.2	38.6
	SD	4BSx4AL		3.6	3.6	9.5
	SD	4BSx6AS		4.5	7.3	16.3
Flowering date	DD	1BL	4009852	-0.3	6.9	3.6
	KD	2BS	1238155	0.3	11.6	5.0
	DD	2BS	4404789	0.8	44.3	16.8
	SD	2BS	988615	0.4	10.8	3.9
	SD	4AL	4541598	0.5	16.6	5.7
	KD	5AL	1148774	-0.6	37.8	13.6
	SD	6AS	4008755	0.5	20.4	6.8
	DD	6BS	1077913	0.3	7.1	3.7

^a Positive additive effects denote trait-increasing effect of the DBC-480 allele; additive effects were estimated as half the difference between phenotype averages for the homozygote

^b Percentage of phenotypic variance explained by the QTL

^c LOD (logarithm of the odds) above LOD threshold at the 0.05 level of probability obtained through a 1,000-iteration permutation test

Association between FHB resistance QTL and plant height QTL

The target genotype for a durum wheat breeder is a semi-dwarf plant type with improved FHB resistance. To investigate the feasibility of such an ideotype in our populations, we compared the effects of the detected FHB resistance QTL. In the SD population the three major resistance QTL mapping to *Rht-B1*, 4AL and 6AS overlapped with QTL for PH. The effect of *Fhb1* - the only QTL not associated with PH in this population - does not efficiently counteract the increased susceptibility associated with the short-stemmed allele at the *Rht-B1*, 4AL and 6AS loci. In the KD and DD populations only the *Rht-B1* locus contributed to both FHB severity and PH, while two other QTL, including *Fhb1*, were not associated with this morphological trait. To investigate the effects of allele combinations at the FHB resistance loci on FHB severity and PH, the RILs of each population were first classified in subgroups according to their allele status at the detected resistance QTL as illustrated in Fig 3. The resistance level and average height were then compared among the different subgroups. In both populations, lines carrying the dwarfing allele *Rht-B1b* were significantly shorter and more susceptible than the ones harboring the wild type allele *Rht-B1a*. Plant height on average was reduced by 31% and 25% in the KD and DD *Rht-B1b* subpopulations, respectively, but the level of disease symptoms relative to *Rht-B1a* were about two-fold increased. In the KD population, lines carrying both resistance QTL at *Fhb1* and 2BL loci in combination with *Rht-B1b* had between 22% to 38% less disease severity than the dwarf lines carrying one or no resistance QTL while showing equivalent levels of resistance as lines carrying *Rht-B1a* with no supplementary resistance QTL. In the DD population, the FHB resistance levels of dwarf lines carrying positive alleles at *Fhb1* and 5AL were not significantly different from any *Rht-B1a* subpopulation while being 53% less diseased than dwarf lines with no resistance QTL. Taken together, our findings demonstrate that the combination of 3BS+2BL QTL in the KD population and 3BS+5AL in the DD population efficiently offset the negative effect of *Rht-B1b* on FHB resistance.

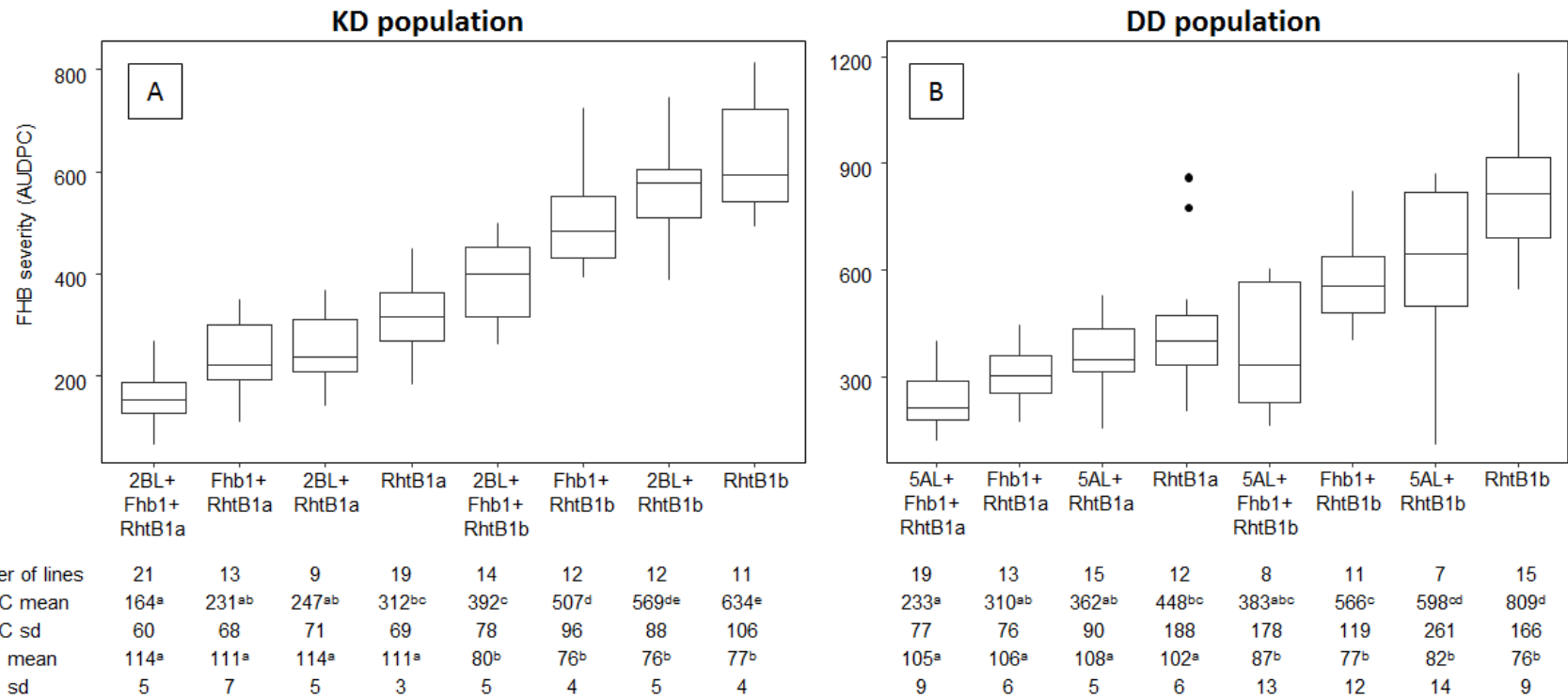


Fig. 3 Boxplot distributions of RILs according to their allele combinations at the FHB resistance loci for the KD (A) and DD (B) populations for overall mean FHB severity (AUDPC). Medians are indicated by solid lines, points represent outliers. For each subgroup, the number of lines, mean values and standard deviations of FHB severity (AUDPC) and plant height (cm) are indicated. Values followed by different letters are significantly different ($p < 0.05$) based on Tukey test

DISCUSSION

FHB resistance has become a priority in durum wheat breeding programs in the last decades. The limited sources of resistance available in durum wheat have urged breeders in incorporating FHB resistance from related species. In this research work, resistance derived from common wheat was investigated for the first time in the genetic background of durum wheat. The resistant line DBC-480, which carries the major common wheat resistance QTL *Fhb1*, presented enhanced resistance in field and greenhouse experiments after artificial inoculation. The use of three different F7-RIL populations allowed the dissection of the genetic basis of FHB resistance and to concomitantly validate the effects of the detected QTL in the different elite durum backgrounds.

Quantitative variation for FHB symptoms was evident in all three populations and for both inoculation techniques. FHB severity and FHB spread were significantly but weakly correlated. This low correlation between the two FHB related traits may be explained by the different mechanisms of infection accounted for by the two inoculation methods. FHB severity assessed after spray inoculation in the field accounts for both resistance to primary infection and subsequent spread of the symptoms within the heads. This measure evaluates thus a combination of type 1 and type 2 resistance under conditions that mimic natural epidemics, while single floret inoculation estimates solely type 2 component of resistance. Reports have shown that type 1 and type 2 resistance vary independently among cultivars (Schroeder and Christensen 1963) and are likely controlled by different genes (Buerstmayr et al. 2003a). Additionally, a high negative correlation between plant height and FHB resistance was evident in the field trials, while no association between these traits was observed in the greenhouse experiments. The discrepancy of plant height influencing FHB response between the two inoculation methods may also contribute to this low correlation as several reports have pointed out type 2 resistance to be less affected by plant height than type 1 resistance (Steiner et al. 2004; Srinivasachary et al. 2008; Srinivasachary et al. 2009; Lu et al. 2011).

Genetic architecture of FHB resistance

The genetic architecture of FHB resistance in our populations appears to be quantitative and oligo- to polygenic. A total of six QTL located on chromosome arms 2BL, 3BS, 4AL, 4BS,

5AL and 6AS were repeatedly associated with enhanced resistance and DBC-480 contributed the favorable alleles at all loci. Genotyping of the populations was performed using GBS DArTseq marker technology supplemented with DNA markers specific to *Fhb1* and *Rht-B1*. Comparisons of QTL positions were performed based on the consensus wheat map provided by DArT PL (A. Kilian, Diversity Arrays Technologies, personal communication, 2016), which includes DArTseq GBS, DArT and SSR markers, and consensus maps published by Somers et al. (2004) and Marone et al. (2012). It appears that the genomic regions found to be responsible for FHB resistance in our study coincide with locations where QTL have already been identified in common wheat.

The QTL on 3BS mapped at the *Fhb1* locus near *Xbarc147* and *Xumn10* was found repeatedly in all populations. The effect of *Fhb1* on FHB severity varied, and depending on the durum genetic background and the individual experiments explained between 5%-16% of the phenotypic variance. In the different populations, the *Fhb1* resistance allele reduced FHB severity symptoms on average by 30% in the KD and 36% in the DD populations, while in the SD population the resistance was only increased by 6%. The discrepancies observed among the KD and DD populations on one side, and the SD population on the other side, may be due to differences in their respective resistance genetic architecture. In the KD and DD populations only one further major QTL affecting FHB severity was detected and similar effects for *Fhb1* were observed, while in the SD population the relative effect of *Fhb1* may be diminished by the presence of three further major QTL. When evaluating FHB spread after single floret inoculation the *Fhb1* locus had a large effect explaining 33% of the total phenotypic variance. Our study demonstrates that in durum wheat *Fhb1* is effective in providing type 2 resistance in a similar way as established in common wheat where *Fhb1* improves mainly type 2 resistance and to a lesser extent type 1 resistance (Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Buerstmayr et al. 2003a; Cuthbert et al. 2006). *Fhb1* is a well characterized QTL descending from the Asian cultivar Sumai-3 which has been found in numerous QTL studies (Buerstmayr et al. 2009). In tetraploid wheat a resistance QTL has also been found in proximity of the *Fhb1* genomic region in the durum cultivar Floradur (Buerstmayr et al., 2012) and in Tunisian durum landraces (Ghavami et al., 2011). However, haplotype comparison using SSR markers by Buerstmayr et al. (2012) revealed different alleles for Sumai-3 and Floradur at the *Fhb1* locus indicating thus the existence of different QTL alleles at this locus.

We report here the first successful deployment of *Fhb1* in durum wheat which marks a significant step forward in durum wheat breeding towards improving FHB resistance. Common wheat represents a useful reservoir of resistance for durum wheat as most of the QTL that have been identified are located on the A and B genomes (Buerstmayr et al. 2009; Liu et al. 2009; Löffler et al. 2009). The difficulties pointed out in previous studies when transferring resistance QTL from common wheat into durum wheat may be attributed to complex interactions of genes among the A, B and D genomes as emphasized in a recent study by Zhu et al. (2016a). In our study no epistatic interactions that may modulate the effect of *Fhb1* were detected. The absence of the D genome in durum wheat, hypothesized to carry factors that enhance resistance (Fakhfakh et al. 2011), has been speculated one of the limiting factors for effective deployment of resistance from hexaploid wheat. Our results show that the presence of the D genome appears to be not required for efficient expression of *Fhb1* in durum wheat. Comparing the effect of *Fhb1* in durum wheat with previous studies is not trivial due to wide range of phenotypic variances reported for this QTL in common wheat (Buerstmayr et al. 2009). In studies evaluating type 2 resistance in common wheat, R^2 values for *Fhb1* ranged between 11% (Yang et al. 2005) and 60% (Bai et al. 1999) while in spray inoculated field trials Buerstmayr et al. (2003a) reported an R^2 of 29%. The resistance improving effect of *Fhb1* in our durum wheat populations is in a similar range as reported in a series of near isogenic lines in common wheat by Pumphrey et al. (2007) who found an average reduction of disease severity by 23%, though varying from 0 to 70%. Differences in population size, genetic background, inoculation techniques and environments in which the evaluations were performed can all be reasons for these variations. While in the literature, there are speculations that durum wheat may carry or lack certain genetic factors that modulate the resistance improving effect of *Fhb1* (Rudd et al. 2001), we find no evidence to support this hypothesis.

A major QTL on 4BS associated with FHB severity was found in all three populations with effects of different magnitude. The location of the QTL coincided with the *Rht-B1* gene. The QTL was responsible for the greatest amount of variation for resistance in the KD and DD populations while in the SD population the QTL had a major effect but was not the greatest contributor to FHB resistance. As mentioned previously, the discrepancy of effects observed is certainly due to differences in genetic backgrounds. In all cases, the reduced height allele *Rht-B1b* accounted to higher disease severity. Such associations of the semi-dwarf *Rht-B1b* allele

with increased FHB severity have been previously reported in hexaploid wheat (Hilton et al. 1999; Srinivasachary et al. 2009). Supporting results have also been found in three durum wheat backcross populations from crosses of the tall and FHB resistant donor *T. dicoccum*-line 161 to the semi-dwarf durum wheat lines Helidur, Floradur and DS-131621. The resistance QTL at the *Rht-B1* locus was the most important QTL affecting FHB resistance after spray inoculation and in all three populations, plants carrying the *Rht-B1b* allele showed higher FHB severity scores (Buerstmayr et al. 2012).

In the SD population two further major QTL were detected on 4AL and 6AS. Both resistance QTL overlapped with QTL for flowering date and plant height. QTL have been already identified on 4A and 6A in tetraploid wheat, however they appear to be located on different chromosome arms and do not match the position of the QTL identified in our study. Gladysz et al. (2007) identified a QTL for type 2 resistance derived from the resistant *T. dicoccoides* accession Mt.Hermon#22 near *Xgwm610* which mapped on the short arm of chromosome 4A while Buerstmayr et al. (2012) found a small effect QTL for FHB severity derived from *T.dicoccum*-line 161 in a cross with line DS-131621 near *Xgwm356* on 6AL. Meanwhile, several mapping projects performed in hexaploid wheat identified QTL in the same region of 4AL in the U.S winter wheat Heyne (Zhang et al. 2012) and in the Swiss winter wheat Arina (Paillard et al. 2004; Buerstmayr and Buerstmayr 2015). No coinciding QTL for flowering date or plant height were reported but an overlap with QTL for anther retention was found in the Arina/Capo population (Buerstmayr and Buerstmayr, 2015). The QTL on 6AS mapped to a similar position as the type 2 resistance QTL identified in hexaploid wheat in the ND2603/Butte86 population derived from the resistant line ND2603 (Sumai 3/Wheaton) (Anderson et al. 2001).

In the KD population a minor effect QTL was found on 2BL at the proximity of the centromere. This region has been reported to carry resistance QTL in two unrelated tetraploid wheat populations where the susceptible durum wheat parents Strongfield (Somers et al. 2006) and Helidur (Gladysz et al. 2007) contributed the resistance improving allele. These QTL were detected after point inoculation providing type 2 resistance while the QTL identified in our study was found after spray inoculation.

Another QTL for FHB severity was identified on 5AL in the DD population, which had a major effect in 2013 but remained undetected in 2014 and 2015. In the DD population very

few GBS markers were polymorphic on chromosome 5A making exact positioning of the QTL difficult. In order to improve map density 21 SSR markers were additionally screened, yet none was found to be polymorphic suggesting close genetic relatedness of the parental lines for this genomic region. Comparison with previous studies is therefore difficult but map comparison suggests that the QTL does not map to the same region as the major hexaploid wheat QTL on 5A *Qfhs.ifa-5A* derived from Sumai-3 which is located close to the centromere (Buerstmayr et al. 2003a).

Association of QTL for FHB resistance, flowering date and plant height

In our study 6 QTL were found associated with flowering date. Co-localization of QTL for flowering date and FHB resistance was evident for the SD population on 4AL and 6AS. The two QTL exert a strong effect on both traits for which a positive correlation was observed. In the KD and DD populations weak and non-significant correlations were found, and when individual experiments were analyzed separately, the correlations varied greatly. No general pattern was evident for the association between earliness and the level of FHB symptoms in these two populations. This non-dependency may be attributed to the absence of overlapping QTL for these two traits, while environment-specific factors around flowering and inoculation may account for variability in the correlations observed in individual experiments.

In contrast, plant height was significantly negatively correlated with FHB severity in all three populations, which is in agreement with previous findings (Talas et al. 2011; Buerstmayr et al. 2012; Miedaner and Longin 2014). All PH QTL identified in this study coincided with QTL for FHB severity on chromosomes 4AL, 4BS and 6AS. Co-localization of PH and FHB severity QTL is a common feature in wheat and supported by meta-QTL analysis (Mao et al. 2010). The mechanisms of association between the two traits are complex and may be attributed to effects of height differences *per se* and/or to pleiotropic effects of the dwarfing genes or tightly linked genes that increase FHB susceptibility. The mutant allele *Rht-B1b*, as well as its homoeologous allele *Rht-D1b* on chromosome 4D, encode single nucleotide polymorphism (SNP) mutations in the DELLA domain that create a premature stop codon that is responsible for reduced sensitivity to the phytohormone gibberellin leading to shorter plant height (Peng et al. 1999; Hedden and Sponsel 2015). DELLA proteins have been shown to be

associated with abiotic and biotic stress tolerance (Achard and Genschik 2009) and in the case of FHB, a DELLA protein mutation may have physiological effects linked to changes in cell death response (Saville et al. 2012). Alternatively to these genetic effects, differences in microclimatic conditions around the heads of tall and dwarf genotypes have been considered to play a significant role particularly under field conditions, with short plants being exposed to higher infection pressure than tall plants (Yan et al. 2011). The *Rht-B1b* allele is also known to have pleiotropic effects on different morphological and structural traits including reduced peduncle length and increased cell density which may also affect response to FHB. In common wheat *Rht-B1b* and *Rht-D1b* were found associated with reduced anther extrusion, which was supposed to partly explain their association with higher FHB susceptibility (Buerstmayr and Buerstmayr 2016). In the case of our study, all features may be important. The strong effect of the QTL on 4BS, coinciding with the reduced height gene *Rht-B1*, on PH and FHB severity was evident in all populations. The *Rht-B1* locus explained 95%, 81% and 37% of the variation for PH and 64%, 38% and 19% of the variation for FHB severity in the KD, DD and SD populations, respectively. In the SD population the two additional QTL on 4AL and 6AS exerted at the same time strong effect on plant height and FHB resistance. The QTL on 4AL mapped to a similar region as a QTL associated with PH in hexaploid wheat corresponding to a kaurenic acid oxidase (KAO) gene (Khlestkina et al. 2010; Zanke et al. 2014), while reduced height genes have been reported on 6AS in durum wheat (Haque et al. 2011). These genes are gibberellin-sensitive and not comparable to *Rht-B1* in that aspect. In agreement with Yan et al. (2011), we hypothesize that considering the large variation for plant height with differences of about 60 cm between the shortest and the tallest plants, part of the apparent negative correlation may be attributed to plant height *per se*. Even under spray-inoculation and mist-irrigation, heads of short plants tend to remain more humid and therefore under more severe infection pressure than heads of tall plants. This is in agreement with Buerstmayr et al. (2012) who evaluated FHB severity of three durum wheat populations with similarly large variation for plant height as observed in our present study and argued for a probable disease escape of tall lines despite controlled mist-irrigation after spray-inoculation.

Perspective for durum wheat breeding and conclusion

Obviously in our populations plant height had a strong influence on modulating FHB disease response. The increased FHB susceptibility associated with medium to short height plant and with *Rht-B1b* is challenging for durum wheat breeders. *Rht-B1b* confers beneficial attributes linked to higher yield and harvest index concomitant to the desired reduced plant height, thereby limiting lodging, in plant production systems with modern agronomic practices (Royo et al. 2007; Subira et al. 2016). We show that the successful deployment of *Fhb1* in combination with minor effect QTL enabled the discovery and the selection of semi-dwarf lines with upsurge levels of resistance. These results are in agreement with a previous report in hexaploid wheat where pyramiding two resistance QTL balanced the negative effect of the semi-dwarf allele *Rht-D1b* to achieve improved levels of resistance in semi-dwarf wheat (Lu et al. 2011). The progeny lines of the KD and DD populations carrying favorable allele combinations at the *Rht-B1* and *Fhb1* loci, and additional FHB resistance alleles on 2BL or 5AL provide unique and novel resources for durum wheat breeding. The introgression of *Fhb1* by recurrent backcrossing into durum wheat to develop the resistant experimental line DBC-480 and its crossing to elite durum cultivars enabled the development of novel FHB resistant breeding lines that are agronomically close to modern European germplasm. These novel improved lines are thus readily incorporable into practical durum wheat breeding programs for enhancing FHB resistance.

AUTHORS CONTRIBUTIONS

NP performed the study and drafted the manuscript. CG, UP and EW helped collecting phenotypic data and genotyping of markers. BS carried out the initial crosses and developed the plant material used in this study. TL, OR and HB initiated the study and obtained funding.

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Conclusion

Fusarium head blight is considered one of the most important disease affecting durum wheat and common wheat production because of its damaging effect on grain production, including yield and quality losses, its deleterious effect on food safety and the intensifying disease pressure reported in cropping regions in the last few decades. The cultivation of resistant cultivars is pivotal to efficiently control FHB and breeding for increased resistance has consequently become an important objective. Common wheat has benefited from great research efforts and improved cultivars have been identified. In comparison, durum wheat, considered a minor crop on a global scale, has received little attention and the need to develop cultivars with enhanced resistance is pressing.

The identification and understanding of genetic factors underlying FHB resistance in durum wheat is a prerequisite for successful resistance breeding. The review paper, set up in the course of this work, represents the first summary focusing specifically on the results from QTL mapping investigations performed in tetraploid wheat. This paper provides the durum wheat breeding and research community an extensive overview for future investigations.

Only a limited number of QTL mapping have been performed so far to dissect the genetic basis of resistance in tetraploid wheat and have widely relied on the use of wild and cultivated relative resistant sources. Stable QTL of small to moderate effect have been identified and, most interestingly for durum wheat breeders, resistance-conferring QTL have been also detected in the susceptible durum wheat cultivars. A persistent finding of QTL mapping investigations is that most of the QTL identified in tetraploid wheat coincide with QTL detected in common wheat, thereby suggesting similarities at the genetic level for FHB resistance.

The aim of this work was to evaluate and validate for the first time the effect of *Fhb1* - from the Asian common wheat Sumai-3 – in durum wheat background. The transfer of resistance from common wheat to durum wheat is obviously not straightforward as no successful deployment had been reported until now. Recurrent backcrossing of Sumai-3 into durum

wheat and thorough phenotypic selection allowed the identification of the improved tetraploid experimental lines DBC-480 carrying an *Fhb1* introgression. DBC-480 was crossed with three elite durum wheat cultivars and *Fhb1* was detected in all three mapping populations, explaining between 5%-16% of the phenotypic variance for FHB severity depending on the genetic background. Plant height heavily influenced FHB resistance in these populations, and the semi-dwarf allele *Rht-B1b* was associated with increased FHB severity. Nonetheless, the negative effect of *Rht-B1b* on FHB resistance was largely compensated in lines carrying also *Fhb1*. Lines with moderate height and enhanced levels of resistance have been selected and provide new resources for breeding durum wheat cultivars with improved resistance.

The use of non-adapted resistance donors in breeding programs may be hindered by the presence of linkage drag associated with deleterious agronomic characteristics or negative quality traits. The lines developed in our study are agronomically close to modern durum wheat germplasm, representing a great asset for their implementation in breeding programs.

In durum wheat the genetic architecture of FHB resistance shows mainly additive inheritance, although some epistatic interactions and the presence of genetic factors that increase susceptibility have been reported in previous studies. The use of MAS, for pyramiding resistance QTL and recurrent selection approach appear promising for rapidly developing durum wheat cultivars with enhanced FHB resistance.

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Appendices

Publication 1 – Supplementary material

Online Resource 1: Comparison of Somers et al. (2004) and Marone et al. (2012) consensus map near the approximate QTL positions which are indicated by vertical bars. For readability only selected markers are shown. The complete maps with all mapped markers can be obtained from the original publications. Names of the lines contributing the resistant alleles are mentioned and numbers in brackets refer to the publication in which the QTL was reported (for cross reference see Table 1). Homologous loci between the maps of the same chromosome are colored in red.

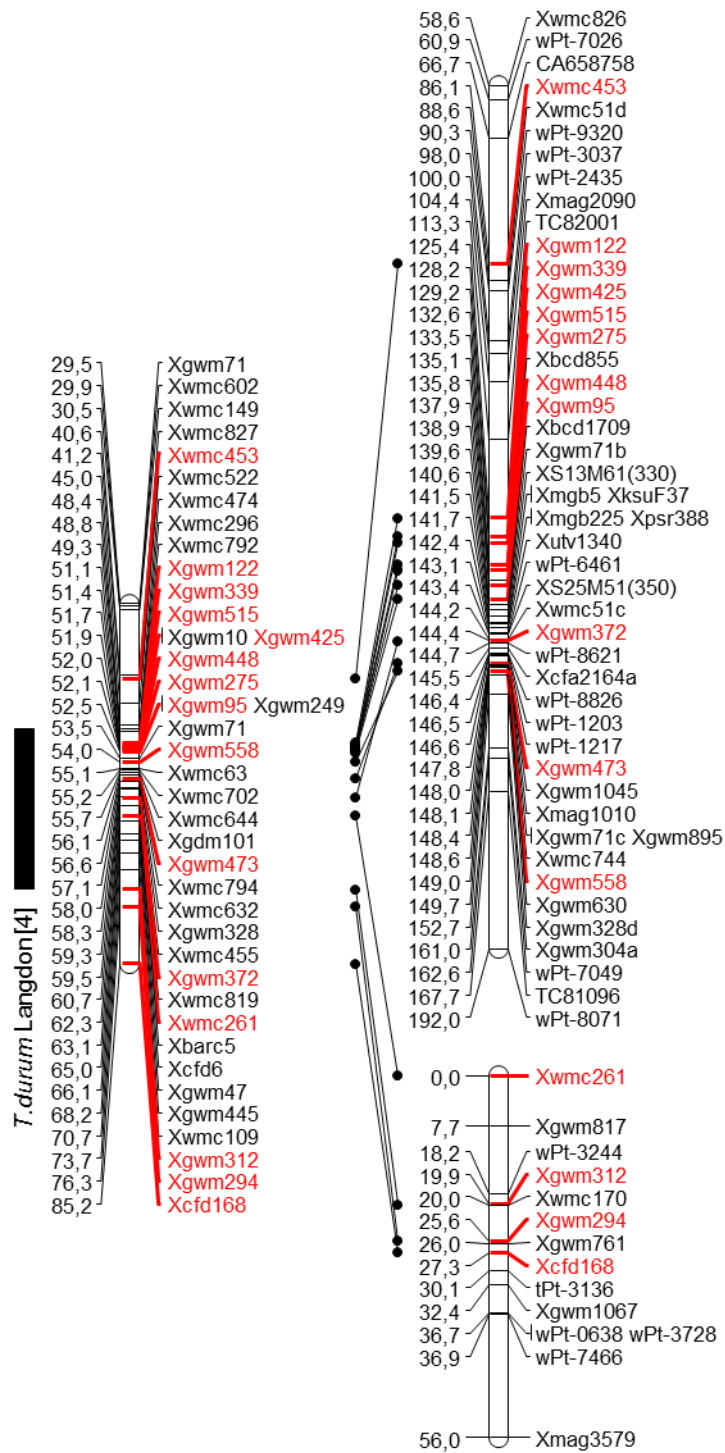
(a) Linkage map based on Somers et al. (2004)

(b) Linkage map based on Marone et al. (2012)

(c) Linkage map based on Ghavami et al. (2011)

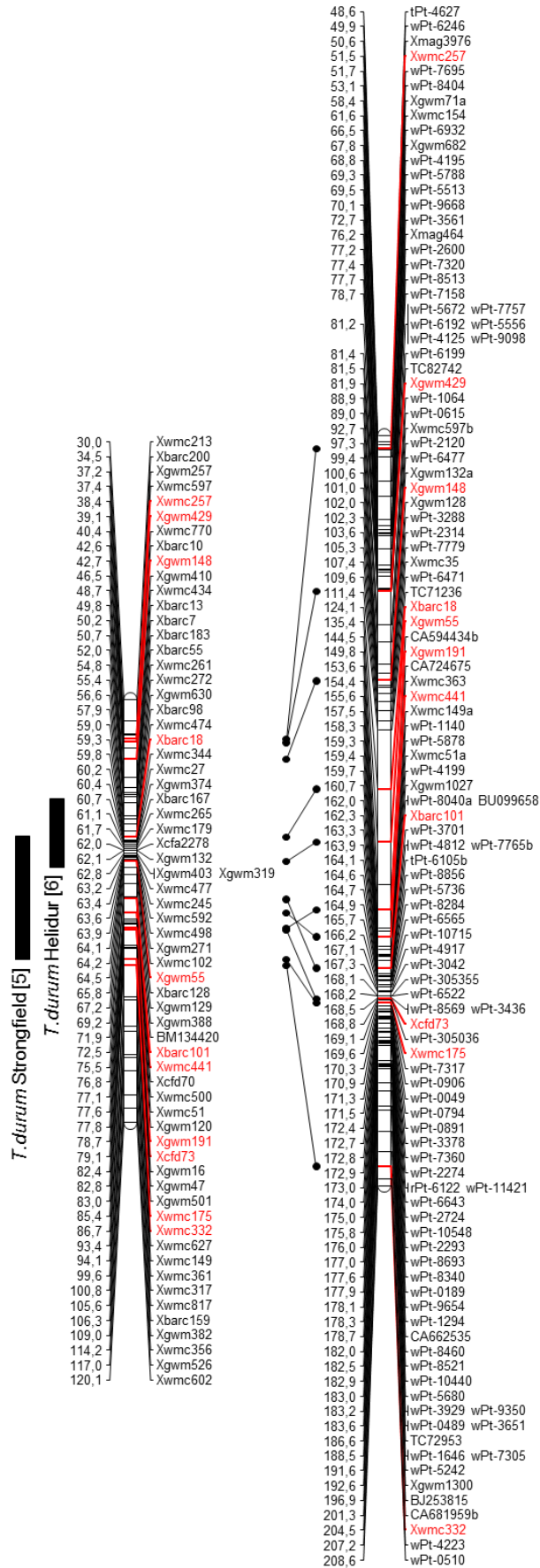
2A (a)

2A (b)

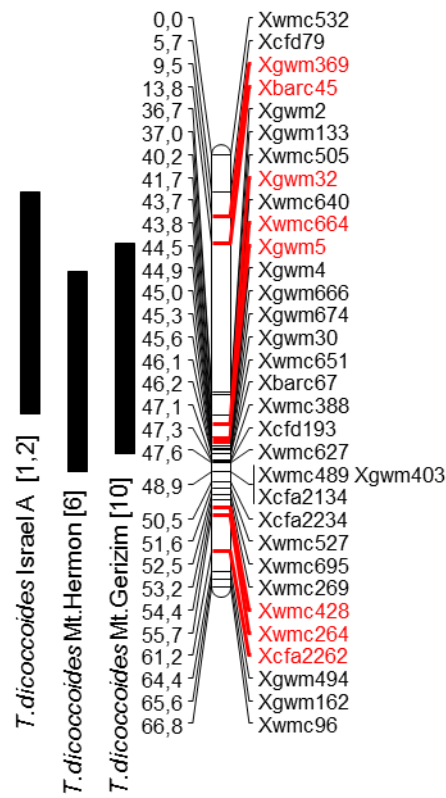


2B (a)

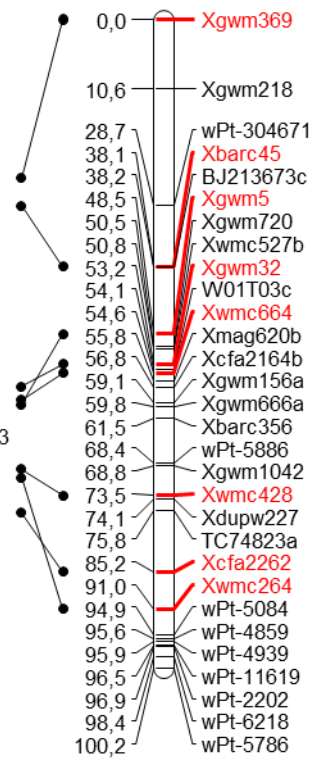
2B (b)



3A (a)

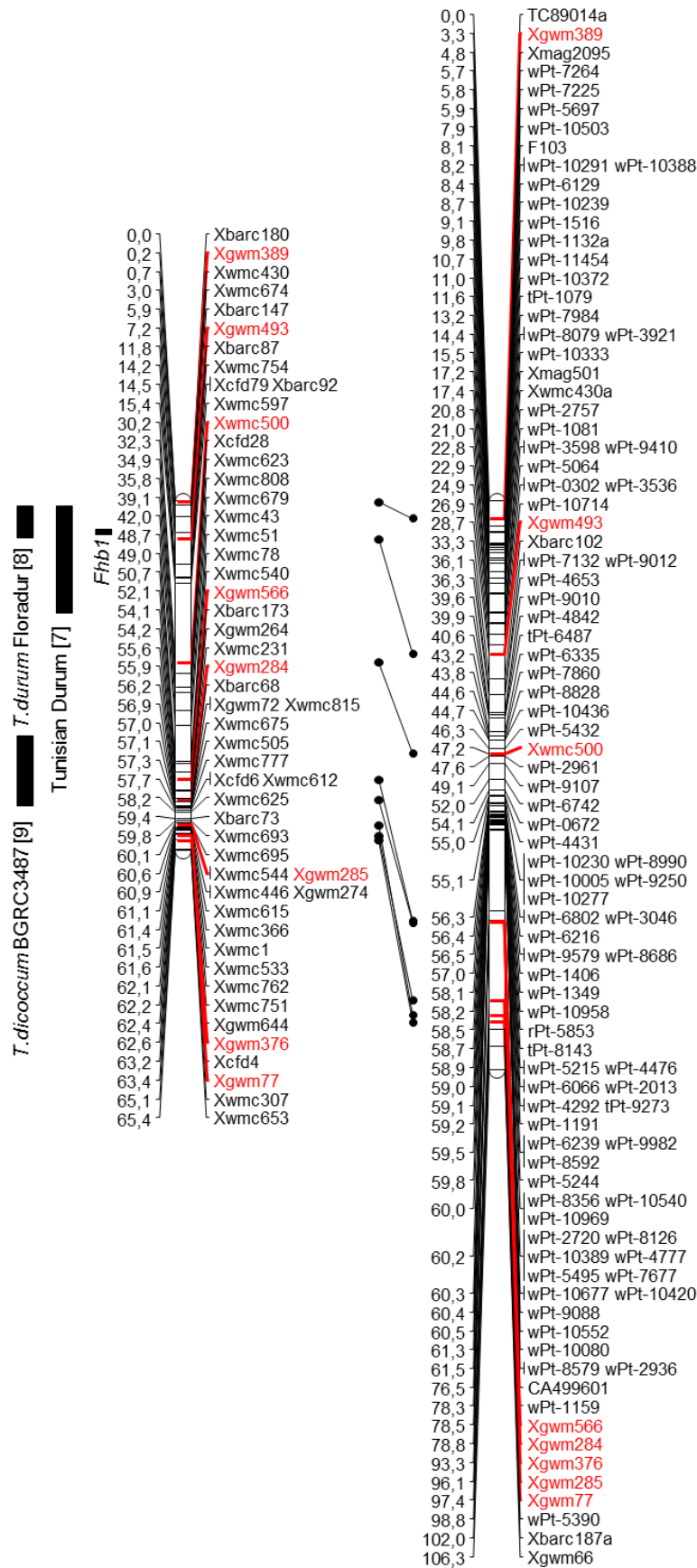


3A (b)



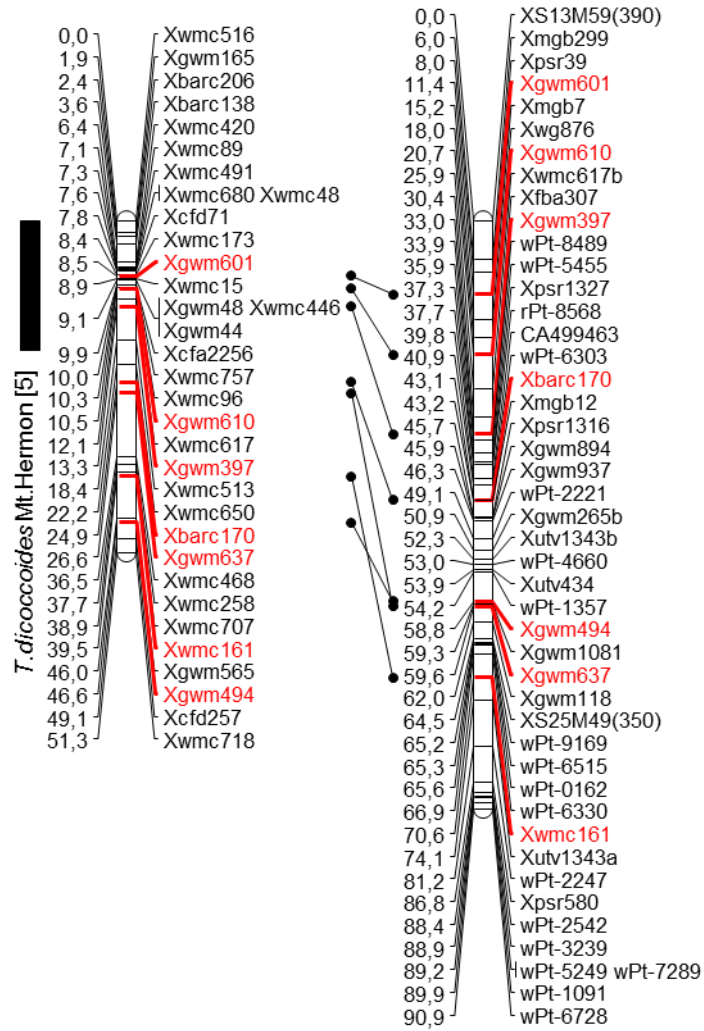
3B (a)

3B (b)



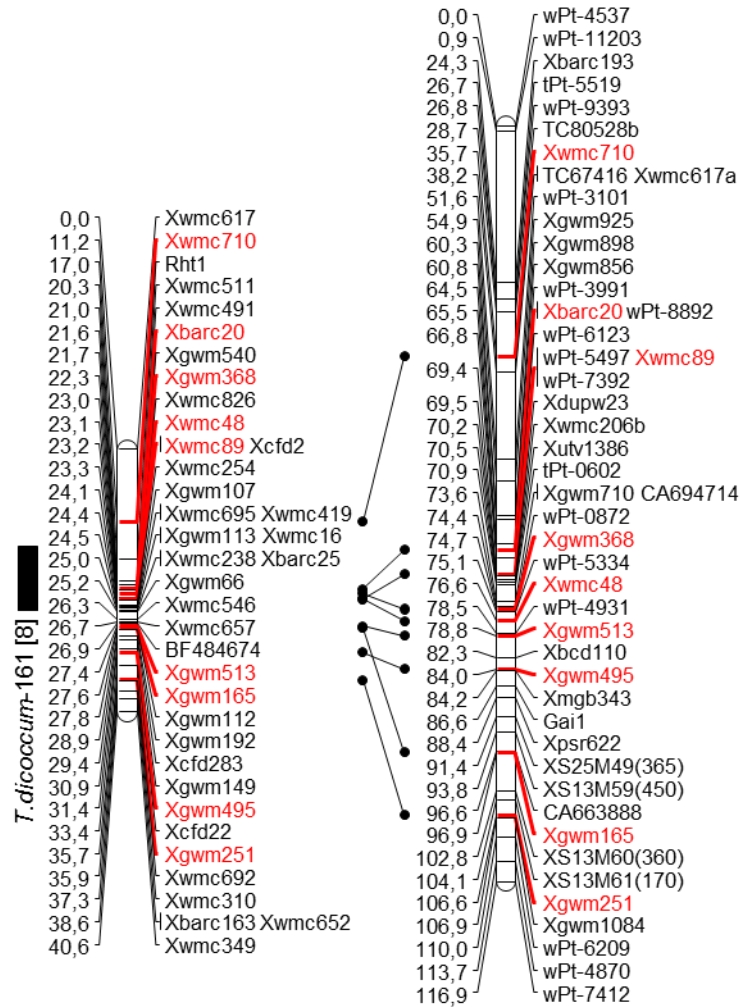
4A (a)

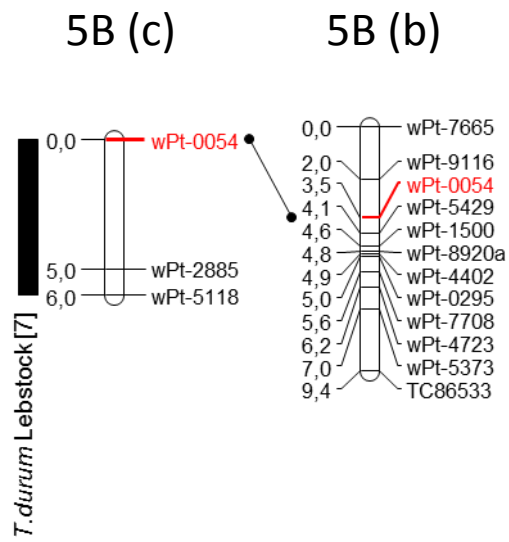
4A (b)



4B (a)

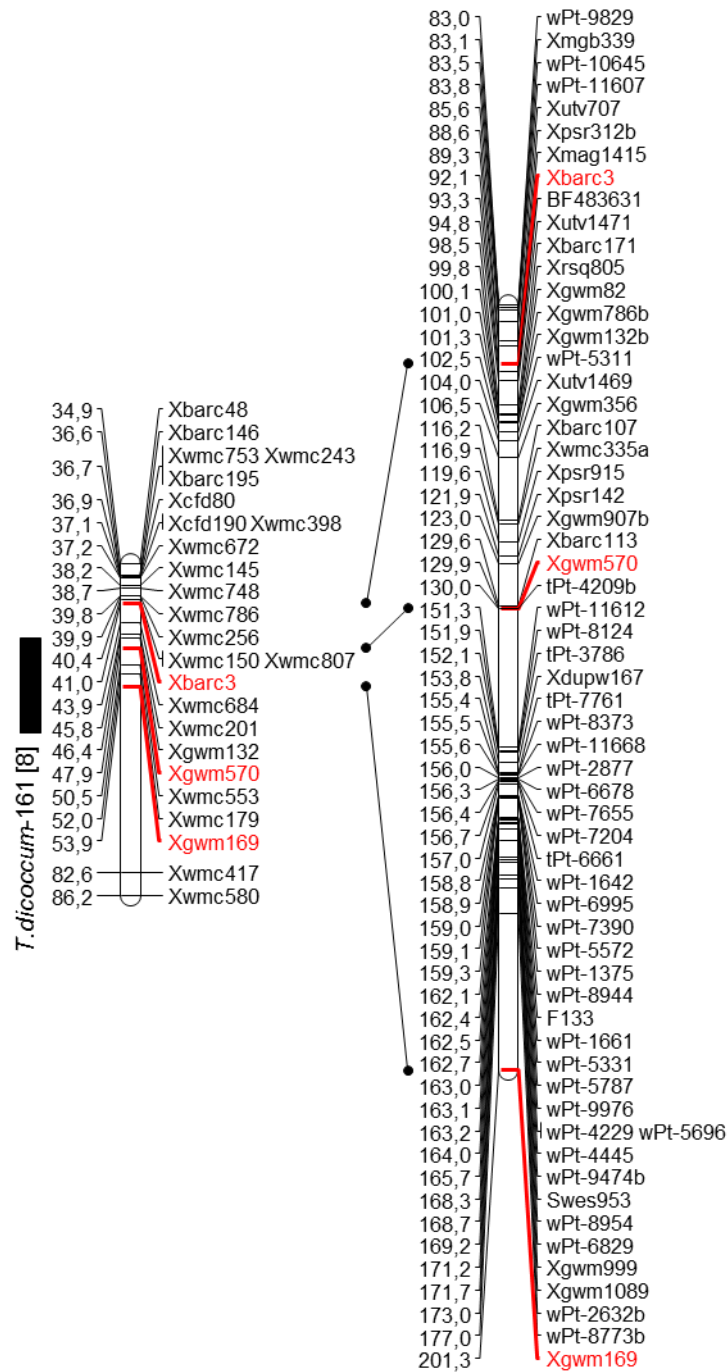
4B (b)





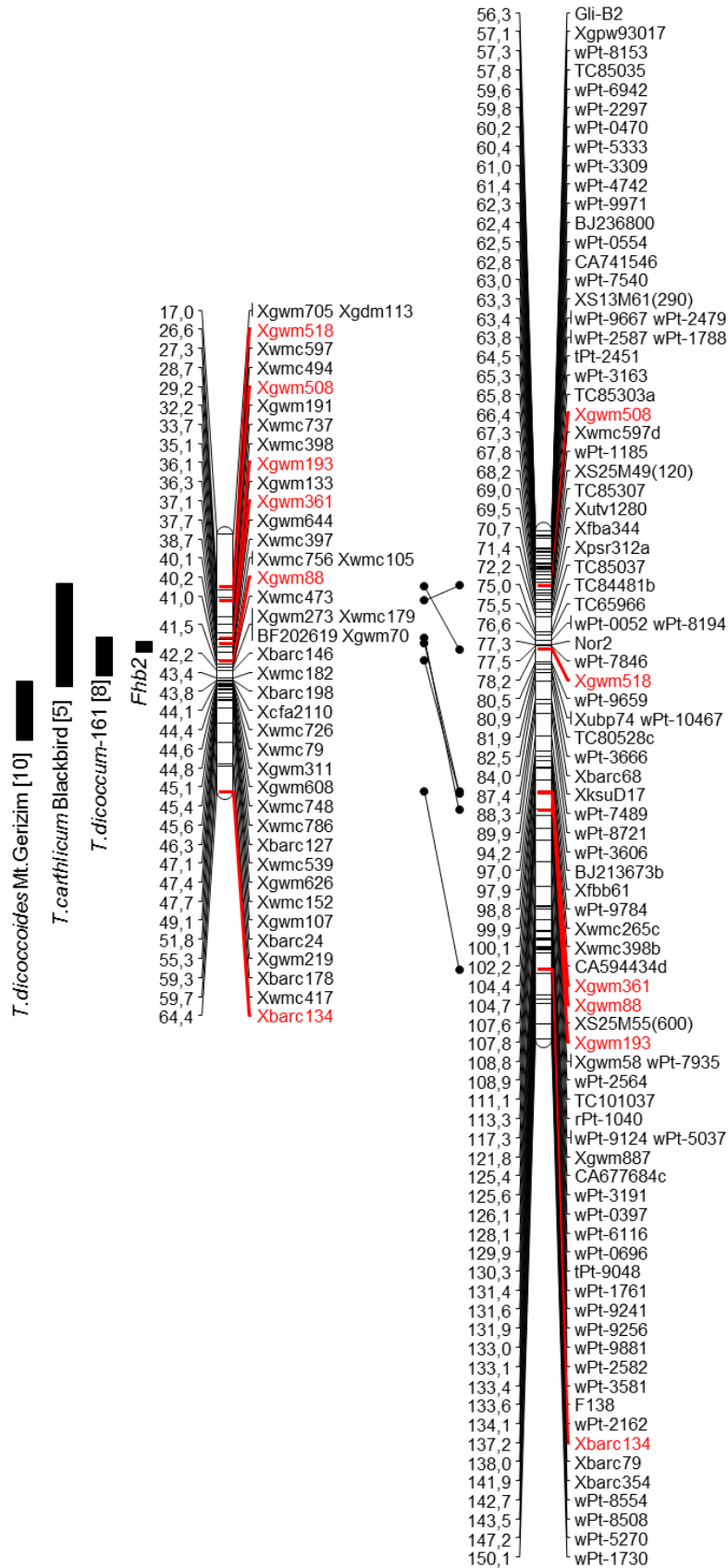
6A (a)

6A (b)



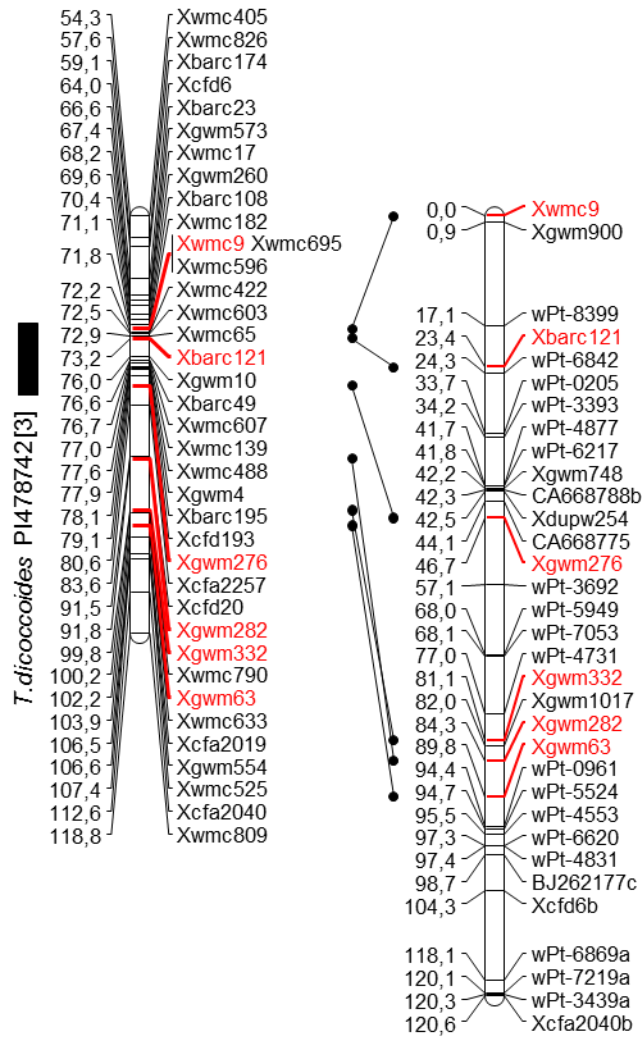
6B (a)

6B (b)



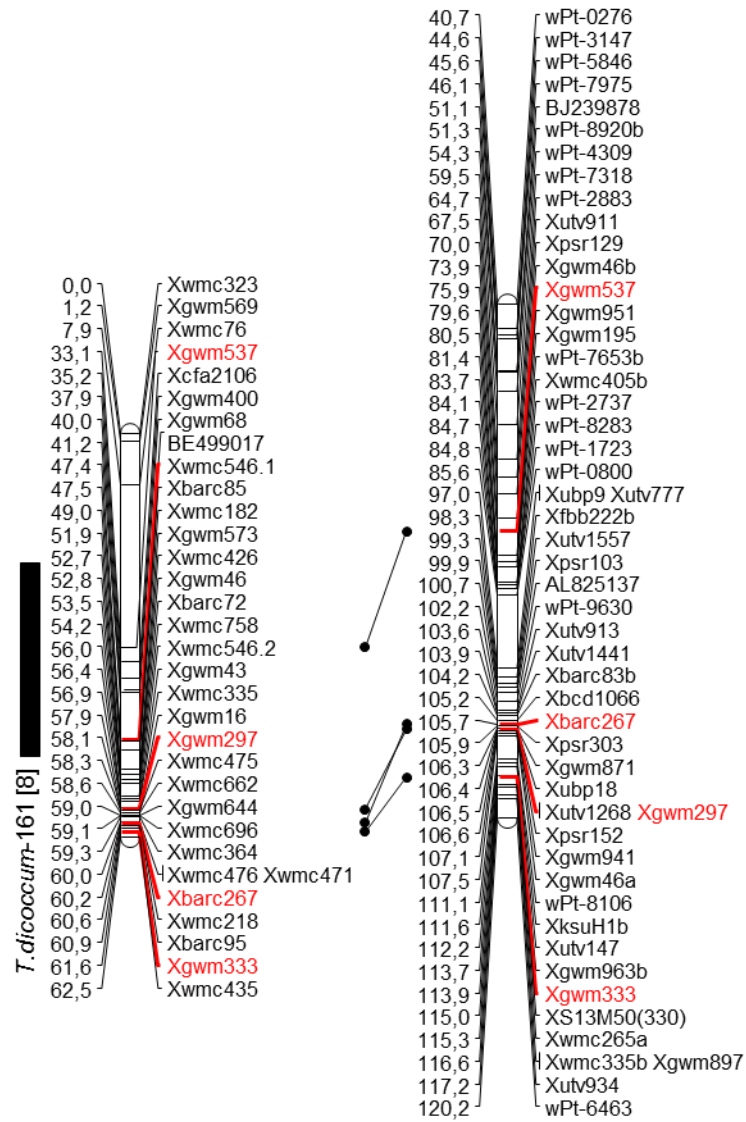
7A (a)

7A (b)



7B (a)

7B (b)



Publication 2 – Supplementary material

Online Resource 1_A: Variance component estimates of genotype $\sigma^2_{\text{Genotype}}$, year σ^2_{Year} , block within year $\sigma^2_{\text{Block within Year}}$, genotype \times year $\sigma^2_{\text{Genotype} \times \text{Year}}$ and the residual effects σ^2_{error} for FHB severity (AUDPC), plant height (cm) and flowering date (days after May 1st) across three experiments for the KD, DD and SD populations

Online Resource 1_B: Variance component estimates of genotype $\sigma^2_{\text{Genotype}}$, Experiment σ^2_{Exp} , and the residual effects σ^2_{error} for FHB spread (percent infected spikelets PIS) and plant height (cm) in the KD population across three unreplicated experiments

Online Resource 2: Pearson correlation coefficients between FHB severity, FHB spread, plant height and flowering date in individual experiments for the KD, DD and SD populations

Online Resource 3: Marker distribution in the KD, DD and SD populations

Online Resource 4: Chromosome locations and positions of markers in linkage map of the KD, DD and SD populations

Online Resource 1_A Variance component estimates of genotype $\sigma^2_{\text{Genotype}}$, Year σ^2_{Year} , block within year $\sigma^2_{\text{Block within Year}}$, genotype x year $\sigma^2_{\text{Genotype} \times \text{Year}}$ and the residual effects σ^2_{error} for FHB severity (AUDPC), plant height (cm) and flowering date (days after May 1st) across three experiments for the KD, DD and SD populations

Karur x DBC-480 (KD) population

Trait	Variance component				
	$\sigma^2_{\text{Genotype}}$	σ^2_{Year}	$\sigma^2_{\text{Block within Year}}$	$\sigma^2_{\text{Genotype} \times \text{Year}}$	σ^2_{error}
FHB severity	28156.91 ***	17626.10	1939.89	5176.38	13615.78
Plant height	321.42 ***	139.98	2.71	12.33	25.13
Flowering date	0.58 ***	55.52	1.47	0.45	1.11

*** p<0.001

Durobonus x DBC-480 (DD) population

Trait	Variance component				
	$\sigma^2_{\text{Genotype}}$	σ^2_{Year}	$\sigma^2_{\text{Block within Year}}$	$\sigma^2_{\text{Genotype} \times \text{Year}}$	σ^2_{error}
FHB severity	43582.79 ***	88140.07	8915.16	28239.04	22843.86
Plant height	252.07 ***	109.17	3.35	12.57	31.58
Flowering date	0.92 ***	52.45	3.24	0.41	1.27

*** p<0.001

SZD1029K x DBC-480 (SD) population

Trait	Variance component				
	$\sigma^2_{\text{Genotype}}$	σ^2_{Year}	$\sigma^2_{\text{Block within Year}}$	$\sigma^2_{\text{Genotype} \times \text{Year}}$	σ^2_{error}
FHB severity	52400.75 ***	261620.52	7280.05	46452.49	20393.17
Plant height	386.28 ***	84.10	8.06	6.83	23.87
Flowering date	1.06 ***	42.72	1.85	0.33	1.11

*** p<0.001

Online Resource 1_B Variance component estimates of genotype $\sigma^2_{\text{Genotype}}$, Experiment σ^2_{Exp} and the residual effects σ^2_{error} for FHB spread (percent infected spikelets PIS) and plant height (cm) in the KD population across three unreplicated experiments

Karur x DBC-480 (KD) population

Trait	Variance component		
	$\sigma^2_{\text{Genotype}}$	σ^2_{Exp}	σ^2_{error}
FHB spread	84.10 ***	43.17	247.26
Plant height	609.11 ***	15.66	55.74

*** p<0.001

Online Resource 2 Pearson correlation coefficients between FHB severity, FHB spread, plant height and flowering date in individual experiments for the KD, DD and SD populations

Karur x DBC-480 (KD) population

FHB severity (AUDPC)			
	2013	2014	2015
Plant height	-0.65***	-0.71***	-0.85***
Flowering date	0.16 ns	-0.27 ***	-0.05 ns

** p< 0.01
 *** p< 0.001
 n.s non significant

Durobonus x DBC-480 (DD) population

FHB severity (AUDPC)			
	2013	2014	2015
Plant height	-0.55***	-0.51***	-0.65***
Flowering date	0.04 ns	-0.19 ns	0.43 ***

** p< 0.01
 *** p< 0.001
 n.s non significant

SZD1029K x DBC-480 (SD) population

FHB severity (AUDPC)			
	2013	2014	2015
Plant height	-0.63***	-0.7***	-0.86***
Flowering date	0.33**	0.11 ns	0.66***

** p< 0.01
 *** p< 0.001
 n.s non significant

Online Resource 3 Marker distribution in the KD, DD and SD populations

Karur x DBC-480 (KD) population						Durobonus x DBC-480 (DD) population						SZD1029K x DBC-480 (SD) population					
Chr.	LG ^a	Number of markers	UP ^b	av. dist. (cM) ^c	Total cM mapped	Chr.	LG ^a	Number of markers	UP ^b	av. dist. (cM) ^c	Total cM mapped	Chr.	LG ^a	Number of markers	UP ^b	av. dist. (cM) ^c	Total cM mapped
1A	2	318	93	1.8	187.6	1A	3	91	35	1.1	47.3	1A	2	129	20	2.8	41.5
1B	2	490	117	1.6	188.9	1B	2	431	121	1.6	227.1	1B	1	292	42	2.7	111.5
2A	3	897	135	1.3	207.4	2A	2	309	68	1.6	108.7	2A	2	619	69	1.9	125.5
2B	3	692	160	1.3	202.8	2B	3	935	233	1.4	340.3	2B	2	609	43	3.1	125.4
3A	3	307	75	2.3	157.6	3A	1	91	13	1.1	12.6	3A	2	60	22	2.9	55.4
3B	4	856	127	1.6	185.4	3B	2	200	91	2	163.6	3B	3	623	113	1.8	207.5
4A	2	686	140	1.8	219.8	4A	1	61	27	2.8	72	4A	3	728	125	2.1	226.5
4B	1	589	154	1.4	207.7	4B	1	201	49	1.6	77.2	4B	1	351	93	1.9	178.7
5A	2	218	100	2.4	221.3	5A	3	65	21	1.6	32.1	5A	2	148	53	3.1	136.4
5B	3	412	118	1.9	225.5	5B	2	141	41	2	75.5	5B	3	412	77	2.5	189.5
6A	1	705	84	2.7	221.5	6A	1	626	133	2	266.4	6A	2	492	65	2.3	166
6B	1	711	96	1.8	174.9	6B	1	541	137	1.8	244.9	6B	1	714	116	1.9	216.8
7A	2	318	61	2.3	169.7	7A	2	24	15	2.4	36.3	7A	2	322	50	4	191.1
7B	2	776	149	3	236.5	7B	2	437	68	1.5	77.8	7B	2	743	118	2.2	252.5
Total	31	7975	1609	1.9	2806.6	Total	26	4153	1052	1.7	1781.8	Total	28	6242	1006	2.5	2224.3

^a Number of linkage groups in each chromosome

^b Number of unique positions

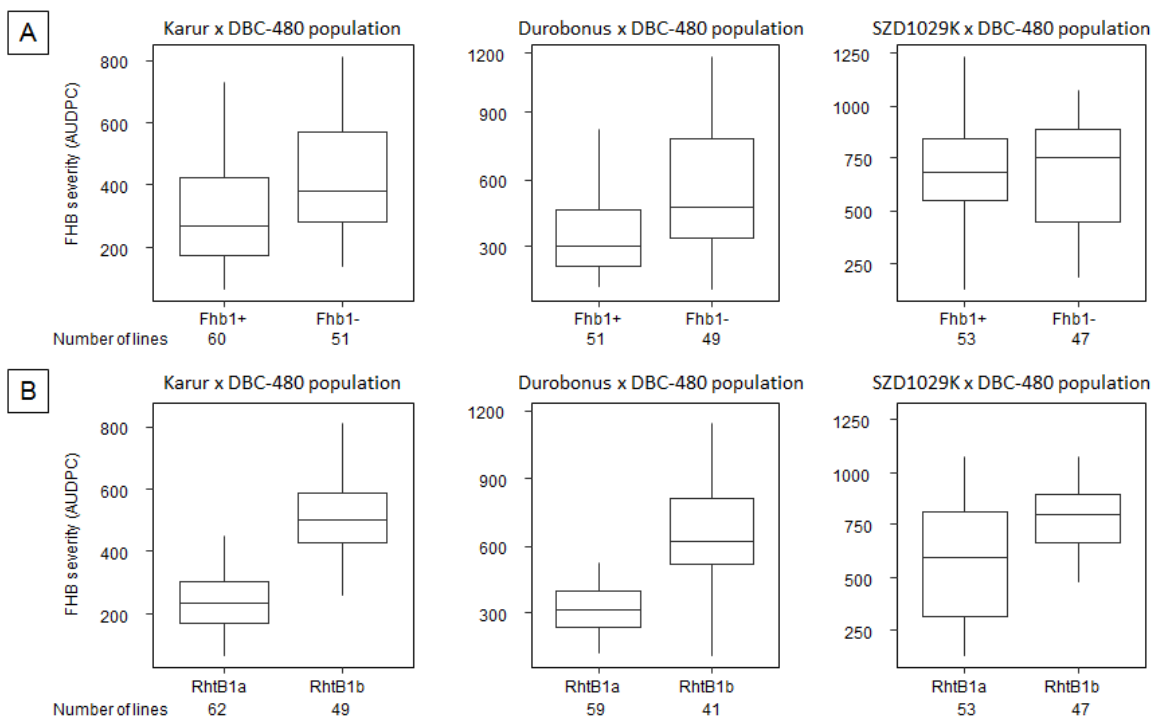
^c average distance between adjacent markers

Online Resource 4: Chromosome locations and positions of markers in linkage map of the KD, DD and SD populations

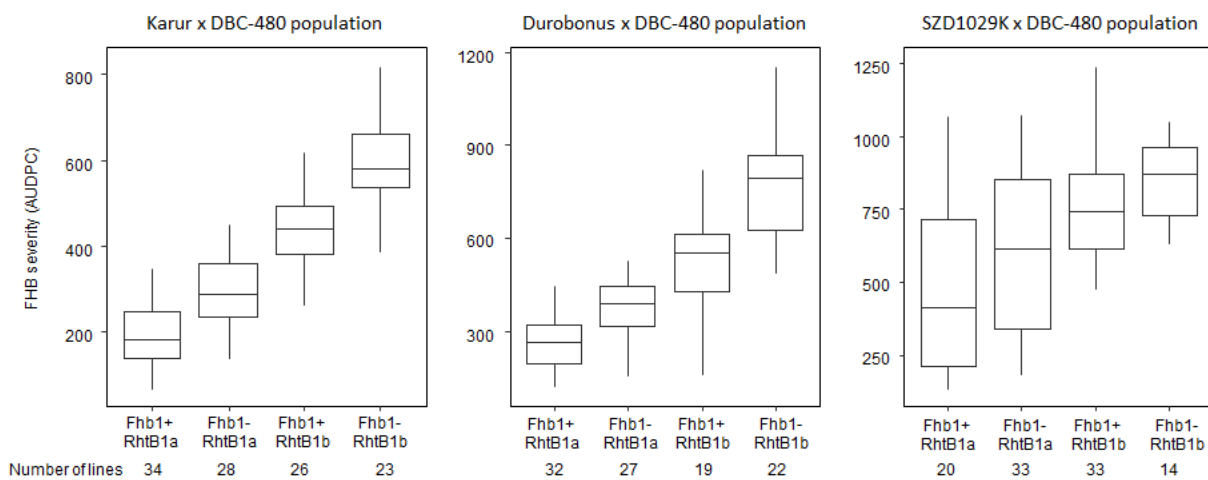
Refer to online publication for visualization of the table

Theoretical and Applied Genetics <http://link.springer.com/journal/122>

Online Resource 5_A Boxplot distributions of RILs according to their allele status at *Fhb1* (A) and *Rht-B1* (B) loci for overall mean FHB severity (AUDPC) for each population. Medians are indicated by solid lines. For each group, the number of lines is indicated



Online Resource 5_B Boxplot distributions of RILs according to their allele combinations at *Fhb1* and *Rht-B1* loci for overall mean FHB severity (AUDPC) for each population. Medians are indicated by solid lines. For each subgroup, the number of lines is indicated



Résumé

Caractérisation des déterminants génétiques de la résistance à la fusariose chez le blé dur

Importance économique du blé dur

Le blé est une des principales denrées alimentaires dans le monde, représentant environ 20% des apports caloriques totaux consommés par les humains. Le blé est la première céréale en termes de surfaces agricoles cultivées, devant le riz et le maïs, avec une production mondiale atteignant en 2015 plus de 730 millions de tonnes (International Grains Council). La culture de blé englobe différentes espèces parmi lesquelles le blé tendre hexaploïde (*Triticum aestivum* L.) qui en constitue la quasi-totalité et qui représente plus de 90% du blé cultivé à travers le monde. Le blé dur tétraploïde (*Triticum durum* Desf.), avec un total de 39 millions de tonnes produites en 2015, représente quant à lui, environ 5% de la production globale de blé. Le blé dur est considéré comme une culture secondaire à l'échelle mondiale. Il est toutefois la culture principale et la denrée alimentaire de base du bassin méditerranéen où il est cultivé traditionnellement. Le blé dur est presque exclusivement utilisé pour l'alimentation humaine et constitue, de fait, une culture d'importance économique majeure. La qualité du grain est un aspect important pour la transformation du blé dur. La qualité du grain inclut une teneur élevée en pigments jaunes et en protéines, une bonne qualité du gluten, la vitrosité et une faible proportion de grains malades (Troccoli et al. 2000). Sur le marché mondial, les grains de haute qualité sont recherchés pour la fabrication de pâtes de qualité supérieure. Le blé dur est également consommé sous la forme de coucous, de boulgour et de pain plats (Elias 1995).

La fusariose de l'épi

La fusariose de l'épi est une des principales maladies fongiques qui affectent la production de blé à travers le monde (Parry et al. 1995). La fusariose est responsable non seulement de pertes

économiques directes dues à des baisses de rendements et de qualité des grains infectés mais également responsable de pertes indirectes en affectant la qualité sanitaire des récoltes en raison de la présence de mycotoxines dans les grains. Cette maladie est provoquée par un complexe de champignons pathogènes, du genre *Fusarium* et *Microdochium*, qui regroupe plus de 19 espèces capables d'induire la fusariose. La fréquence à laquelle ces espèces sont retrouvées dépend à la fois de la localisation géographique et des conditions climatiques locales, principalement la température et l'humidité (Xu et al. 2005; Xu et al. 2008). *Fusarium graminearum* [téléomorphe: *Gibberella zae*], prédomine généralement sous des conditions humides et chaudes et est l'espèce la plus importante en Amérique du Nord, en Europe Centrale et en Australie, alors que *Fusarium culmorum* [téléomorphe inconnu] est retrouvé plus fréquemment en Europe de l'Ouest (Parry et al. 1995). Parmi les différents agents causant la fusariose, ces deux espèces sont hautement pathogènes et leurs isolats sont tous susceptibles de produire des métabolites secondaires toxiques, notamment le déoxynivalénol (DON), qui s'accumulent dans les grains infectés. Du fait de leur importance sur l'incidence de la maladie, *F. graminearum* et *F. culmorum*, sont principalement utilisés par les sélectionneurs et les chercheurs lors des essais par inoculations artificielles, avec toutefois la majorité des études épidémiologiques se concentrant sur *F. graminearum* uniquement.

Le blé est particulièrement sensible à la maladie lors de la floraison. L'infection peut avoir lieu tout au long du développement du grain jusqu'au stade de grain pâteux. Les épidémies de fusariose sont favorisées par une humidité élevée et des précipitations importantes au cours du printemps. Le risque d'infestation est d'autant plus élevé qu'on se retrouve face à une forte présence d'inoculum naturel. Les agents pathogènes de la fusariose sont non-hôtes spécifiques et peuvent contaminer un grand nombre de plantes, notamment les céréales comme le blé tendre, le blé dur, le triticale, le seigle ou le maïs (Parry et al. 1995; Inch and Gilbert 2003). Les symptômes de la maladie apparaissent quelques jours après l'infection, caractérisés par des liaisons nécrotiques sur l'extérieur de la surface des glumes et l'apparition d'épillets prématurément échaudés. Plusieurs épillets d'un même épi peuvent être affectés, la maladie pouvant progresser jusqu'à l'échaudage complet de l'épi. Au sein des épillets infectés, les grains peuvent être absents ou présenter un aspect atrophié entraînant une baisse de rendement liés à une réduction du nombre de grains et du poids spécifique (Parry et al. 1995 ; Bushnell et al. 2003). La contamination des grains en développement peut également être associée à l'accumulation de mycotoxines. Les grains fusariés (« Fusarium-diseased kernels » ou FDK) ont une apparence

typique : mince, ridée et rugueuse, de couleur blanchâtre à brunâtre. Les grains contaminés plus tardivement peuvent présenter une apparence normale bien que contenant des mycotoxines (Cowger et al. 2009; Siou et al. 2014).

Les dommages causés par la fusariose de l'épi sont multiples. Lors de fortes épidémies, les rendements peuvent être considérablement réduits et les lots contenant des grains fusariés susceptibles d'être déclassés ou éliminés (McMullen et al. 1997). La maladie a des effets néfastes sur les propriétés de la mouture et de la fabrication des pâtes du fait de la détérioration de la teneur en protéines et de la force du gluten des grains fusariés (Dexter et al. 1996; Nightingale et al. 1999). La principale préoccupation associée à la fusariose est la présence de mycotoxines dont l'exposition, par ingestion de grains infectés, provoque de graves mycotoxicoses représentant un risque sanitaire. Ce problème est d'autant plus inquiétant dans la production du blé dur qu'elle est essentiellement utilisée pour l'alimentation humaine. Le déoxynivalénol (DON) et ses dérivés, sont les mycotoxines les plus fréquemment rencontrées lors d'infections par *Fusarium*. Le DON perturbe le fonctionnement des cellules eucaryotes et induit notamment une inhibition de la synthèse protéique (Pestka 2010). Des recommandations ou régulations ont ainsi été mises en place par plusieurs Etats afin de limiter la teneur totale en DON des produits céréaliers bruts destinés à l'alimentation humaine et animale. La Commission Européenne, par exemple, a mis en place une réglementation stricte de la teneur maximale en DON avec un seuil de 1.25 ppm pour le blé tendre et de 1.75 ppm pour le blé dur (Official Journal of the European Communities 2007). La réglementation s'applique aussi aux produits transformés tels que les pâtes (0.7 ppm), le pain et produits de boulangerie (0.5 ppm) et les aliments pour nourrissons (0.2 ppm). De même, l'Agence américaine des produits alimentaires et médicamenteux (Food and Drug Administration, FDA) des Etats-Unis a introduit depuis 1993 une recommandation du seuil maximal de DON à 1 ppm pour les produits céréaliers destinés à l'alimentation humaine (Guidance for Industry and FDA 2010). Les lots dont la teneur en DON excède les limites acceptables sont susceptibles d'être dévalués ou refusés contribuant ainsi à des pertes économiques supplémentaires pour les producteurs de blés.

Ces dernières décennies, plusieurs facteurs ont accentué le risque d'épidémie de fusariose. L'adoption de pratiques culturales sans labour ou avec labours restreints, alliées à la présence de plantes hôtes sensibles, telles que le maïs, dans les rotations culturales, favorise le développement de l'inoculum primaire, augmentant ainsi les risques d'infections pour la culture suivante (McMullen et al. 1997). Les conditions climatiques caractérisées par des pluies fréquentes et une

forte hygrométrie autour de la période de floraison du blé, sont favorables à l'initiation et au développement de la fusariose (Osborne and Stein 2007). La demande croissante en pâtes alimentaires a engendré une expansion de la zone de culture du blé dur vers des régions où les conditions climatiques sont propices au développement de la maladie. En Europe, la production de blé dur s'est étendue des régions chaudes et sèches du pourtour Méditerranéen vers des régions plus humides d'Europe centrale et occidentale. La culture de blé dur d'hiver, possédant un potentiel de rendement plus élevé, est également d'avantage exposée à un risque d'infection du fait de sa date de floraison plus précoce coïncidant souvent avec une hygrométrie élevée (Miedaner and Longin 2014).

La résistance à la fusariose

L'utilisation de variétés résistantes est considérée comme essentielle en matière de lutte intégrée contre la fusariose et comme le levier le plus efficace et le plus économique pour contrôler et réduire les dommages liés à la maladie (Gilbert and Haber 2013). La résistance à la fusariose est de nature quantitative et polygénique, contrôlée par de nombreux loci à caractères quantitatifs (QTL ou « quantitative trait loci »). L'expression de la résistance à la fusariose est modulée par les facteurs environnementaux, en particulier la température et l'hygrométrie lors de l'établissement de l'infection, impliquant des interactions génotype x environnement (Campbell and Lipps 1998; Miedaner et al. 2001). L'estimation précise du niveau de résistance requiert donc une évaluation au sein d'essais indépendants, répétés sur plusieurs années et/ou plusieurs lieux, afin de réduire la variabilité associée aux facteurs environnementaux.

Plusieurs types de résistance à la fusariose ont été décrits et sont communément acceptés et étudiés lors des analyses de QTL de résistance (Schroeder and Christensen 1963; Mesterhazy 1995) parmi lesquels la résistance à l'infection initiale (pénétration), dite de type 1, ainsi que la résistance à la propagation du pathogène dans l'épi, dite de type 2. Des procédures d'inoculations spécifiques permettent d'évaluer ces différents types de résistance. La résistance de type 2 est généralement estimée après inoculation ponctuelle en évaluant la vitesse de progression des symptômes ou le nombre d'épillets infectés le long de l'épi. L'inoculation par spray permet d'évaluer la résistance de type 1 en mesurant l'incidence de la maladie (nombre d'épis présentant des symptômes). L'évaluation de la résistance de type 1 est difficile à réaliser et de fait, les essais utilisant l'inoculation par spray mesurent le plus souvent la sévérité de la maladie (pourcentage

d'épillets infectés par unité de surface) qui englobe les résistances de type 1 et 2 (Buerstmayr et al. 2012a).

En parallèle des mécanismes actifs de résistance, des mécanismes dits passifs modulent également la réponse à la fusariose (Mesterhazy 1995). Ces mécanismes incluent des caractéristiques morphologiques ou développementales qui affectent notamment la capacité du pathogène à pénétrer dans l'épi et le développement de la maladie par des phénomènes d'évitement. La taille des plantes est considérée comme un des facteurs morphologiques le plus important lié à la résistance à la fusariose. Les allèles de nanismes, *Rht-B1b* et *Rht-D1b*, sont associés à une plus grande sensibilité à la maladie chez le blé dur tétraploïde (Buerstmayr et al. 2012b) et le blé tendre hexaploïde (Hilton et al. 1999; Miedaner and Voss 2008; Voss et al. 2008). Cet effet sur la résistance à la fusariose peut être expliqué par la hauteur des plantes *per se* et par les différences de structure de canopée favorisant le développement du pathogène (Yan et al. 2011), aussi bien que par des effets pléiotropiques des gènes *Rht* et/ou par la présence de gènes liés (Srinivasachary et al. 2009; Saville et al. 2012).

Plus d'une centaine de QTL de résistance à la fusariose chez le blé tendre ont été décrits dans la littérature (Buerstmayr et al. 2009; Liu et al. 2009; Löffler et al. 2009). Le premier QTL à effet majeur à avoir été identifié chez le blé tendre provient de la variété asiatique Sumai-3 et est localisé sur le bras court du chromosome 3B (Waldron et al. 1999). Ce QTL, appelé *Fhb1*, est particulièrement bien validé et utilisé comme source de résistance dans les programmes de sélection. *Fhb1* est précisément cartographié. Il est flanqué par les marqueurs *Xgwm533*, *Xbarc133* and *Xgwm493* (Cuthbert et al. 2006) et des marqueurs diagnostics tels que *Xumn10* sont disponibles (Liu et al. 2008; Schweiger et al. 2016). *Fhb1* contribue principalement à la résistance de type 2 et, dans un moindre mesure, à la résistance de type 1 (Anderson et al. 2001; Buerstmayr et al. 2003a).

La plupart des études de détection de QTL de résistance à la fusariose ont été menées chez le blé tendre du fait de son importance économique au niveau mondial. Parmi les 52 analyses de QTL répertoriées par Buerstmayr et al. (2009), seules quatre ont été réalisées sur le blé tétraploïde. Les efforts consacrés à l'amélioration de la résistance du blé dur à la fusariose sont limités et les progrès, en comparaison avec le blé tendre, sont lents.

Dans une première partie de la thèse, les ressources génétiques disponibles pour l'amélioration de la résistance à la fusariose ainsi que les QTL détectés à ce jour chez le blé tétraploïde ont été

résumées. Ce travail a fait l'objet d'une publication dans le journal *Molecular Breeding* sous la forme d'une revue scientifique (Prat et al. 2014).

Ressources génétiques pour l'amélioration de la résistance à la fusariose chez le blé dur

Le blé dur a depuis longtemps été signalé comme plus sensible à la fusariose que le blé tendre (Atanasoff 1920; Christensen et al. 1929). La plupart des cultivars de blé dur actuels présentent une haute sensibilité à la maladie. Les quelques variétés avec un niveau de résistance amélioré, issues des programmes de sélection Nord-Américains, ne présentent pas des niveaux de résistance équivalents à ceux atteints chez le blé tendre (Zhang et al. 2014). Jusqu'à présent, les progrès pour améliorer la résistance à la fusariose ont été freinés par la faible variation génétique disponible pour ce caractère au sein du germoplasme de blé dur. Le criblage d'importantes collections n'a permis l'identification que de seulement quelques variétés locales, ou landraces, avec un meilleur niveau de résistance à la fusariose (Elias et al. 2005; Talas et al. 2011; Huhn et al. 2012).

La raison pour laquelle il existe peu de sources de résistance à la fusariose chez le blé dur n'a pas encore clairement été élucidée. Une des explications avancée indique que les cultivars de blé dur actuels, issus principalement de germoplasme cultivé dans les conditions chaudes et sèches du bassin méditerranéen, n'aurait pas été exposés à des conditions de fortes pressions de la maladie (Ban et al. 2005). En outre, les investissements dans les programmes de sélection de blé dur ont été plus faibles comparé au blé tendre. Par conséquent, les variétés modernes de blé dur ont été développées à partir d'un nombre restreint de programmes de sélection, ce qui a pu mener à une base génétique plus étroite par rapport au blé tendre (Oliver et al. 2008). Enfin, différents auteurs ont suggéré l'existence de facteurs de sensibilité et/ou la présence de gènes suppresseurs dans le génome du blé dur qui affaiblirait sa résistance à la fusariose (Stack et al. 2002; Ban et al. 2005; Garvin et al. 2009; Ghavami et al. 2011). Pour faire face à ce déficit de sources de résistance, les sélectionneurs ont ciblé leurs recherches vers le criblage de matériel résistant au sein d'espèces apparentées, sauvages et cultivées, afin d'élargir la base génétique utilisable pour l'amélioration de la résistance du blé dur à la fusariose.

Le blé tendre hexaploïde a bénéficié d'intenses efforts de recherche ce qui a permis l'identification de sources de résistances issues de diverses régions du monde. Parmi celles-ci, la

variété de blé tendre chinois Sumai-3 confère un des plus hauts niveaux de résistance. Les tentatives de transfert de QTL de résistance identifié chez le blé tendre hexaploïde vers le blé dur tétraploïde sont gênées par les différences de niveau de ploïdie et ont eu, jusque-là, un succès limité. L'utilisation du blé tendre comme source de résistance pour le blé dur n'est pas simple. Bien que la plupart des QTL de résistance identifiés chez le blé tendre sont localisés sur les génomes A et B (Buerstmayr et al. 2009), le rôle du génome D, absent chez le blé dur, est susceptible de jouer un rôle déterminant dans la stimulation de la résistance à la fusariose (Fakhfakh et al. 2011).

Afin de pallier aux différences de ploïdie, des sources de résistance ont été criblées chez les autres espèces de blé tétraploïdes. Les espèces sauvages apparentées ont développé une large adaptation génétique aux stress biotiques et offrent un important réservoir de gènes pour l'amélioration du blé dur. Différentes études ont identifié des accessions modérément résistantes à la fusariose chez les blés tétraploïdes *T. dicocoides*, *T. dicocum* et *T. carthlicum*.

L'ancêtre du blé dur, *T. dicocoides*, a été intensément étudié. Miller et al. (1998) ont criblé 290 accessions de *T. dicocoides* issus de la collection de l'USDA pour leur réponse à la fusariose et ont identifié plusieurs accessions présentant des bons niveaux de résistance de type 2. (Buerstmayr et al. 2003b) ont évalué 151 lignées, originaires principalement d'Israël, et ont identifié huit accessions avec un bon niveau de résistance. De même, une large collection, comprenant 416 accessions de *T. dicocoides* d'origines diverses, a été évaluée par Oliver et al. (2007). Une large variation pour la réponse à la fusariose a été signalée par les auteurs, avec plusieurs accessions possédant un haut niveau de résistance. Enfin, Oliver et al. (2008) ont criblé 376 accessions de différentes espèces de blé tétraploïde et ont identifié 16 *T. carthlicum* et quatre *T. dicocum* avec des niveaux de résistance à la fusariose modérés à hautement résistant. Ces accessions de blé tétraploïde sont des sources de résistance intéressantes pour l'amélioration du blé dur. Elles ont été jusqu'à présent peu utilisées dans les programmes de sélection. Aucune de ces accessions n'est comparable à Sumai-3 en termes de niveaux de résistance. De plus, l'incorporation d'espèces sauvages apparentées dans les programmes de sélection de blé dur élite pose des difficultés du fait de leurs caractéristiques exotiques. Pour réduire le transfert d'allèles défavorables lors de l'introgession d'un allèle favorable (linkage drag), fréquemment rencontré lors de l'utilisation de matériel sauvage ou exotique, des techniques de substitution chromosomiques ont été utilisées pour transférer la résistance de *T. dicocoides* vers le blé dur. Stack et al. (2002) ont ainsi évalué des lignées de substitution disomiques de l'accession,

modérément résistante, de *T. dicoccoides* Israel-A dans le fond génétique du cultivar de blé dur Langdon. Les lignées de substitution portant le chromosome 3A de *T. dicoccoides* présentaient des meilleurs niveaux de résistance. De la même façon, les lignées de substitution portant le chromosome 7A de l'accèsion de *T. dicoccoides* PI478742, présentaient également une meilleure résistance à la fusariose.

Les progrès réalisés en ingénierie et manipulation chromosomique ont permis l'utilisation d'espèces plus éloignées, appartenant aux pools secondaires et tertiaire du blé dur. Certaines de ces espèces, du genre *Leymus*, *Roegneria* ou *Thinopyrum*, sont issues de régions dont le climat est favorable à la fusariose et certaines lignées possèdent une très haute, voire une complète, résistance à la maladie (Cai et al. 2005). Elles représentent un précieux réservoir de gènes pour l'amélioration de la résistance à la fusariose. Un locus associé à la résistance a été identifié sur le chromosome 7E de *T. elongatum* et a été incorporé chez le blé tendre par l'utilisation de lignée de substitution et de translocation (Fu et al. 2012). Cette méthode est potentiellement utilisable également dans un fond génétique de blé dur. L'utilisation de ces espèces éloignées est toutefois entravée par leurs caractéristiques agronomiques défavorables. Une cartographie fine du locus à intégrer est en cours afin d'utiliser de manière efficace *T. elongatum* pour l'amélioration de la résistance à la fusariose (Chen et al. 2013). La cartographie précise des locus de résistance de ces espèces éloignées permettra l'utilisation et l'incorporation des locus associés à la résistance dans des cultivars adaptés, tout en réduisant le transfert non désiré d'allèles défavorables.

Détection de QTL de résistance à la fusariose chez le blé tétraploïde

Jusqu'à présent, seules quelques sources de résistances tétraploïdes de *T. dicoccoides*, *T. dicoccum*, *T. carthlicum* et de landraces de *T. durum* ont été analysées par cartographie de QTL. La plupart des études réalisées se sont intéressées à la résistance de type 2 après inoculation ponctuelle mesurée en serre et seulement deux études ont évalué la résistance globale de la sévérité à la maladie après inoculation en spray dans les champs. La majorité de ces analyses de QTL ont été faites sur des populations de taille limitée, susceptibles de réduire la capacité à détecter les QTL tout en surestimant leurs effets. Des QTL à effets faibles à modérés ont été principalement identifiés, localisés sur les chromosomes 2A, 2B, 3A, 3B, 4A, 4B, 5B, 6A, 6B, 7A et 7B. Plusieurs QTL ont été détectés dans des études indépendantes, tels que les QTL sur les chromosomes 2B, 3A, 3B et 6B, que l'on considère comme validées de manière indépendante. Le

QTL sur 3A, à proximité du marqueur *Xgwm2*, a été signalé au sein de trois populations non-apparentées avec différentes accessions de *T. dicoccoides* comme source de résistance (Otto et al. 2002; Gladysz et al. 2007; Chen et al. 2007; Buerstmayr et al. 2013). Ces trois accessions de *T. dicoccoides* portent probablement le même allèle de résistance à ce locus. Des variations alléliques peuvent être notées chez le blé tétraploïde au niveau du QTL détecté sur le chromosome 6B au sein de deux populations utilisant les accessions de *T. dicoccum* (Buerstmayr et al. 2012b) et *T. carthlicum* (Somers et al. 2006) comme sources de résistance. Ces deux différentes espèces tétraploïdes portent probablement des allèles différents au niveau du locus 6B. La plupart des régions portant des QTL identifiés chez le blé tétraploïde correspondent à des QTL identifiés chez le blé tendre, suggérant une base génétique commune pour la résistance à la fusariose. Les QTL localisés sur les chromosomes 2A, 2B, 3A, 3B, 4A, 4B, 6B, 7A et 7B coïncident avec des QTL issus de différentes sources de résistances de blé tendre d'hiver et de printemps originaires d'Asie, d'Europe et d'Amérique du Nord [voir la revue publiée par Buerstmayr et al. (2009)]. Les QTL identifiés sur le chromosome 3B (Ghavami et al. 2011; Buerstmayr et al. 2012b) et 6B (Somers et al. 2006; Buerstmayr et al. 2012b), notamment, coïncident respectivement avec les QTL majeurs de résistance *Fhb1* (Liu et al. 2008) et *Fhb2* (Cuthbert et al. 2007) issus tous deux de Sumai-3. Toutefois, la comparaison des allèles aux marqueurs associés à *Fhb1* et *Fhb2* effectuée par Buerstmayr et al. (2012b), a révélé différents haplotypes pour les lignées tétraploïdes et hexaploïdes. Ceci suggère une diversité génétique à ces loci et l'existence de différents allèles de résistance chez le blé dur et le blé tendre.

Les analyses de QTL réalisées chez le blé tétraploïde se sont principalement intéressées à des croisements du blé dur sensible avec une source de résistance apparentée. La plupart des allèles de résistance dérivent des blés tétraploïdes apparentés. Toutefois, les allèles de blé dur sont également associés à la résistance à la fusariose au niveau des QTL sur les chromosomes 2B (Somers et al. 2006; Gladysz et al. 2007), 3B (Ghavami et al. 2011; Buerstmayr et al. 2012b) et 5B (Ghavami et al. 2011). En dépit de sa sensibilité, le blé dur possède tout de même un certain niveau de résistance.

Des phénomènes de ségrégation transgressive pour la résistance à la fusariose ont été observés lors de croisement de lignées modérément sensibles ou modérément résistantes et, au sein de plusieurs populations, quelques lignées montrent moins de symptôme de fusariose que le parent résistant (Somers et al. 2006; Ghavami et al. 2011; Ruan et al. 2012). Tirer parti de la ségrégation transgressive est prometteur pour le développement de lignées avec un meilleur niveau de

résistance. Chez le blé tendre, Sumai-3 a été sélectionné à partir d'un croisement de deux parents modérément résistants. Son niveau exceptionnel de résistance peut s'expliquer par la combinaison d'allèles de résistance à différents loci, agissant principalement de manière additive (Bai et al. 2003; Schweiger et al. 2013).

L'existence d'interactions épistatiques et de facteurs de sensibilité qui modulent la résistance à la fusariose chez le blé tétraploïde a été signalée. Un QTL a été détecté sur le chromosome 2A de *T. dicoccoides* qui augmente la sensibilité à la fusariose quand il est transféré vers le blé dur. Ce locus pourrait agir comme un suppresseur de résistance et agir potentiellement de manière épistatique, réduisant l'effet d'autres QTL de résistance (Garvin et al. 2009). Ghavami et al. (2011) ont également soupçonné l'existence de QTL qui module la réponse à la fusariose au niveau de la même région du chromosome 2A de blés durs tunisiens. L'existence d'interactions épistatiques a également été décrite par Somers et al. (2006) dans une population issue d'un croisement de blé dur avec *T. carthlicum*. Les QTL détectés sur les chromosomes 2A et 5A influencent l'effet du QTL de résistance sur le chromosome 6B. Il apparaît ainsi important de contre-sélectionner ces facteurs génétiques qui augmentent la sensibilité ou qui diminuent la résistance à la fusariose.

Parmi les différentes analyses de QTL de résistance à la fusariose réalisées sur le blé tétraploïde, seules deux d'entre elles tiennent compte des caractères morphologiques et développementaux (Buerstmayr et al. 2012b; Buerstmayr et al. 2013). Il est généralement admis que ces caractères sont impliqués dans les mécanismes de résistance passifs. Bien que les caractères morphologiques aient un impact moins important sur le développement de la maladie après une inoculation ponctuelle, ils sont susceptibles d'avoir un effet important lors des évaluations en champs. Il est donc important de prendre ces effets en considération lors des études de résistance à la fusariose. Dans l'étude menée par Buerstmayr et al. (2012b), il apparaît que ces caractères influencent la résistance à la fusariose du blé dur de la même façon que chez le blé tendre. Un QTL avec un fort effet sur la résistance a été détecté sur le chromosome 4B à la position du gène de nanisme *Rht-B1* et un QTL détecté sur le chromosome 7B coïncide avec un QTL de précocité. Chez le blé tendre, il est commun que des QTL de résistances coïncident avec les gènes *Rht*. Il n'est pas encore clairement établi si cette association est due à des effets pléiotropiques, à l'existence de gènes liés ou bien si elle est la conséquence de la hauteur des plantes *per se*. Les QTL de résistance associés à la hauteur des plantes peuvent avoir un impact négatif sur les performances agronomiques des lignées et sont donc inappropriés pour les programmes de

sélection élités. La cartographie de QTL de caractères agronomiques importants doit ainsi être réalisée en concomitance avec la cartographie de QTL de résistance à la fusariose. Les QTL qui ne sont pas associés à des caractéristiques agronomiques indésirables sont à favoriser pour une amélioration efficace de la résistance à la fusariose dans les programmes de sélection.

Jusqu'à présent, seules les sources de résistance issues de blés tétraploïdes ont été exploitées pour l'amélioration de la résistance du blé dur. Les QTL de résistance détectés ne confèrent pas des niveaux de résistance équivalents à *Fhb1*, le QTL de résistance majeur identifié chez le cultivar de blé tendre Sumai-3 (Waldron et al. 1999; Anderson et al. 2001). Le blé dur n'a pas encore bénéficié des nombreux QTL détectés chez le blé tendre et les conséquences du transfert de *Fhb1* du blé tendre vers le blé dur n'ont pas été communiquées à ce jour.

La deuxième partie de cette thèse rend compte de l'effet de *Fhb1* au sein de trois populations biparentales développées par croisement de DBC-480 avec trois blés durs élités européens. DBC-480 est une lignée expérimentale de blé dur, résistante à la fusariose, qui possède l'allèle de résistance de *Fhb1* dérivé de Sumai-3. L'association de la résistance à la fusariose avec la hauteur des plantes est également décrite et, en particulier, l'effet de *Rht-B1* et son interaction avec *Fhb1* sur la sévérité de la maladie est examiné. Cette étude a fait l'objet d'une publication dans le journal Theoretical and Applied Genetics (Prat et al. 2016).

Détection de QTL de résistance à la fusariose au sein de trois populations apparentées de blé dur

MATERIELS ET METHODES

Matériel végétal

Le matériel végétal utilisé dans cette étude consiste en trois populations biparentales contenant 111, 100 et 100 F_7 RIL (lignées pures recombinantes ou « recombinant inbred lines) issues, respectivement, de croisement de la lignée expérimentale de blé dur, résistante à la fusariose, DBC-480, avec les cultivars sensibles de blé dur Karur (KD), Durobonus (DD) et SZD1029K (SD). Karur et Durobonus sont des variétés inscrites au catalogue et sélectionnées respectivement par RAGT (inscription en 2002) et Saatzucht-Donau (inscription en 2004). SZD1029K est une lignée issue des programmes de sélection de Saatzucht-Donau. La lignée expérimentale tétraploïde DBC-480 a été développée à l'IFA-Tulln en Autriche par quatre générations de

rétrocroisement assisté par marqueurs de la variété de blé tendre Sumai-3 avec la variété de blé dur autrichienne Semperdur puis soumise à une sélection phénotypique rigoureuse en champ pour sa résistance à la fusariose. L'introgession de *Fhb1* a été vérifiée en utilisant les marqueurs SSR *Xgwm389*, *Xgwm533* et *Xgwm493*. Karur, Durobonus et SZD1029K possèdent l'allèle de nanisme *Rht-B1b* ; au contraire de DBC-480 qui est une lignée haute, possède l'allèle sauvage *Rht-B1a*.

Phénotypage de la résistance à la fusariose

Les trois populations biparentales, ainsi que les lignées parentales, ont été évaluées au sein de plusieurs essais en champs en 2013, 2014 et 2015, à l'IFA-Tulln en Autriche. Les essais ont été disposés en blocs complets randomisés avec deux blocs en 2013 et trois blocs en 2014 et 2015. Les essais ont été inoculés en spray, lors de la floraison, par une suspension d'un isolat de *Fusarium culmorum* Fc91015, à une concentration de $2.5 \times 10^4 \text{ ml}^{-1}$. Afin de faciliter la germination des spores et le développement de la maladie, la canopée a été maintenue humide par brumisation durant 20h après chaque cycle d'inoculation. La sévérité de la maladie a été estimée visuellement comme le pourcentage d'épillets infectés au sein de chaque plot à 14, 18, 22 et 26 jours après la date de floraison. En 2013, la notation a été réalisée à 18 et 26 jours après la floraison. L'aire sous la courbe de développement de la maladie (AUDPC ou « area under disease progress curve ») a été calculée et utilisée comme mesure intégrée de la résistance à la sévérité de la maladie. La taille des plantes a également été mesurée en centimètre et la date de floraison notée et convertie en nombre de jours après le 1^{er} mai.

La population KD et les parents Karur et DBC-480 ont également été testés en serre pour la résistance à la propagation dans l'épi, ou résistance de type 2, après inoculation ponctuelle au sein de trois essais non-répliqués à Florimond-Desprez, France, au cours de l'hiver 2015 (GH1) et à l'IFA-Tulln, Autriche, en été 2016 (GH2 et GH3). L'inoculation ponctuelle a été réalisée à la floraison en pipetant 10 μl d'inoculum de *F. culmorum* Fc91015, à la même concentration que pour les essais inoculés en spray, entre les glumelles et les paléas de quatre épillets situés au centre de l'épi. Pour favoriser l'infection par le pathogène, les épis ont été couverts par des sacs transparents de polyéthylène durant 24h après l'inoculation. La résistance de type 2 a été mesurée par le pourcentage d'épillets infectés (PIS) 24 jours après inoculation en comptant le nombre d'épillets infectés et le nombre total d'épillets pour chaque épi. En moyenne, huit épis par

génotype ont été inoculés pour chacun des essais. Pour chaque lignée, la hauteur des plantes a également été mesurée.

Analyse statistiques des données phénotypiques

L'analyse des données a été réalisée sous R 3.1.3 (R development core team, 2016) en utilisant le paquet lme4 pour l'analyse des modèles mixtes (Bates et al. 2015). Pour chacune des populations, deux modèles ont été ajustés afin d'obtenir, pour chacun des caractères étudiés, le meilleur estimé linéaire non biaisé (BLUES ou « best linear unbiased estimates ») lors de l'analyse conjointe des essais (modèle 1) et au sein de chaque essai analysé individuellement (modèle 2).

Modèle 1 : $P_{ijk} = \mu + G_i + E_j + E_j(R_k) + G_i \times E_i + e_{ijk}$ où P_{ijk} est la valeur phénotypique, μ est la moyenne de la population, G_i est l'effet du $i^{\text{ème}}$ génotype, E_j est l'effet du $j^{\text{ème}}$ essai, $E_j(R_k)$ est l'effet de la $k^{\text{ème}}$ répétition au sein du $j^{\text{ème}}$ essai j , $G_i \times E_i$ est le $ij^{\text{ème}}$ effet de l'interaction génotype-par-essai et e_{ijk} la résiduelle. L'effet du génotype a été considéré comme fixe et tous les autres termes comme aléatoires.

Modèle 2 : $P_{ijk} = \mu + G_i + R_k + e_{ik}$ où P_{ijk} est la valeur phénotypique, μ est la moyenne de la population, G_i est l'effet du $i^{\text{ème}}$ génotype, R_k est l'effet de la $k^{\text{ème}}$ répétition et e_{ik} la résiduelle. L'effet du génotype a été également considéré comme fixe et la répétition comme aléatoire.

L'héritabilité au sens large de chaque caractère a été calculée selon l'équation $H^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_{G \times E}^2 / m + \sigma_e^2 / p)$ où σ_G^2 est la variance génotypique, $\sigma_{G \times E}^2$ est la variance de l'interaction génotype-par-environnement, σ_e^2 la variance de la résiduelle, m est le nombre d'essais et p le nombre total de répétition dans les différents essais (Holland et al. 2003).

Génotypage de la population et construction des cartes de liaison

Le génotypage de la population a été réalisée à l'aide de marqueurs GBS (génotypage-par-séquençage ou « genotyping-by-sequencing ») développés par DArTseq. Un total de 7965, 4150 and 6235 marqueurs polymorphiques de haute qualité ont été utilisés pour la construction des cartes de liaison pour, respectivement, les populations KD, DD et SD. Le matériel végétal a de plus été génotypé avec les marqueurs moléculaires *Xbarc147* (Song et al. 2005) et *Xumn10* (Liu et al. 2008), associés à *Fhb1* ; et des marqueurs spécifiques pour les allèles de *Rht-B1* (Ellis et al. 2002). Les cartes de liaison ont été construites individuellement pour chacune des populations en utilisant l'algorithme MSTmap (Wu et al. 2008) inclus dans le paquet ASMap v0.4 (Taylor and Butler 2015) sous R. Une fois les cartes individuelles obtenues, les groupes de liaison ont été

assignés en comparant la localisation des marqueurs à la carte consensus blé DArTseq fournie par DArT PL (A. Kilian, Diversity Arrays Technologies, communication personnelle, 2016). Les cartes ont ensuite été tracées grâce au logiciel de visualisation MapChart (Voorrips 2002).

Détection de QTL

L'analyse de QTL a été réalisée pour chaque caractère en utilisant les BLUES calculés au sein de chaque essai et pour les essais étudiés de manière conjointe, en utilisant le paquet R/qtl sous R (Broman et al. 2003). Les données manquantes ont été imputées en utilisant la méthode d'imputation de Sen and Churchill (2001). Les QTL ont été détectés via la régression de Haley-Knott en utilisant une analyse par intervalle et une analyse par intervalle composite. Le seuil de LOD pour l'existence de QTL a été calculé pour une erreur de type I à $\alpha=0.05$ pour chaque caractère et pour chaque essai après un test basé sur 1000 permutations. Les QTL significatifs ont été intégrés dans un modèle QTL multiple. L'existence de QTL additionnels et la présence d'interaction entre QTL a ensuite été testée. Le modèle final établi a été comparé par ANOVA au modèle nul et le pourcentage de la variation phénotypique expliquée par chaque QTL, les effets additifs ainsi que les valeurs de LOD ont été estimés. Un intervalle de confiance a été défini pour chaque QTL en prenant pour chaque extrémité le LOD score maximum - 1.5.

RESULTATS

Variation et corrélations des caractères étudiés

L'évaluation du niveau d'infection par la fusariose a été mise en place sur trois populations biparentales inoculées artificiellement au champ. Cela a permis de déterminer les facteurs ayant un rôle significatif dans la diminution des effets de la maladie en conditions naturelles. Au cours de toutes ces expérimentations, le parent commun résistant, DBC-480, s'est avéré être significativement moins malade que les parents sensibles. La valeur d'AUDPC moyenne déterminée pour la lignée résistante DBC-480 a été de 125 (soit environ 12% d'épillets infectés, 26 jours après l'inoculation). Tandis que pour Karur, Durobonus, et SZD1029K, elle a été approximativement 5 fois plus élevée. Une grande diversité dans le niveau d'infection a pu être observée au sein de chacune des populations évaluées et entre les différentes populations. Le niveau d'infection moyen étant en effet le plus bas pour la population KD et le plus élevé pour la population SD. Des phénomènes de transgressions ont pu être observés au sein de chacune des populations. Les différences observées entre les lignées présentant moins de symptômes que le

parent résistant DBC-480 et le parent lui-même, n'ont, cependant, pas été significatives. La pression de la maladie entre les expérimentations a été comparable, sauf en 2015 où davantage de symptômes ont été observés. L'héritabilité au sens large, calculée pour l'AUDPC est élevée au sein des trois populations (comprise entre 0,74 et 0,89). Dans chaque cas, la variance génotypique s'est avérée être plus élevée que les variances dues à l'interaction génotype x environnement et à la résiduelle. Un effet significatif du facteur génotype a également été mis en évidence par ANOVA pour tous les caractères étudiés.

Afin d'évaluer la résistance de type 2 conférée par *Fhb1*, le pourcentage d'épillet infectés par épis (PIS) a été mesuré lors de trois essais en serre pour la population KD. Une héritabilité moyenne ($H^2=0,51$) a été déterminée, bien que l'ANOVA ait montré un effet génotype significatif pour les 111 RILs évaluées. Des phénomènes de transgressions ont également été observés pour la résistance de type 2 bien que, de nouveau, les différences n'ont été significatives que pour les lignées très sensibles. Le parent DBC-480 a présenté moins de symptômes que le parent Karur avec des PIS respectifs de 18,3% et 38,4%. Des corrélations faibles mais néanmoins significatives ont pu être relevées entre la propagation et la sévérité ($r=0,2$, $p<0,05$).

Une importante variation pour la hauteur des plantes (PH) a pu être observée dans les trois populations. Les parents sensibles Karur, Durobonus et SZD1029K ont été de 38 à 49 cm plus petits que le parent résistant DBC-480. Le caractère PH a présenté une distribution bimodale dans les populations KD et DD, et trimodale pour SD. Pour toutes les populations, la sévérité a été négativement corrélée à la hauteur des plantes, les individus les plus petits étant en moyenne plus sensibles à la fusariose. A l'inverse, aucune corrélation significative n'a pu être démontrée entre la résistance à la propagation de la fusariose après inoculation ponctuelle et la hauteur des plantes. La période de floraison a présenté une distribution continue au sein des trois populations et cela bien qu'aucune différence significative n'ait pu être établie entre les parents. La sévérité de la maladie et la date de floraison ont été positivement corrélées pour la population SD, faiblement et négativement corrélées pour KD et non corrélées pour DD. Néanmoins, au sein des essais pris individuellement, aucune corrélation entre ces deux caractères n'a été observée en 2013 et 2015 pour la population KD, en 2013 et 2014 pour la population DD et en 2014 pour la population SD.

Analyse QTL

Élaboration des cartes de liaison

Un total de 7975, 4153, et 6242 marqueurs polymorphiques ont été respectivement générés pour les populations KD, DD, et SD. Parmi ces marqueurs, 1064 étaient communs aux trois populations. Le nombre final de marqueurs sélectionnés pour établir les cartes de liaison a été de 1609, 1052, 1006 et la longueur totale des cartes de liaison établies a été de 2806, 1781, et 2219 cM avec une distance inter-marqueur moyenne de 1.9, 1.7, 2.5 cM pour, respectivement, les populations KD, DD et SD. Chaque groupe de liaison a pu être assigné sans ambiguïté à un chromosome grâce à la carte consensus blé DArTseq. L'alignement avec la carte consensus a mis en évidence une faible couverture pour les populations DD et SD sur les chromosomes 1A et 3A et pour les chromosomes 5A et 7A pour la population DD. Malgré cela, tous les chromosomes ont été représentés.

Analyse de QTL pour la sévérité de la fusariose

Les analyses QTL menées indépendamment sur les trois populations ont permis d'identifier six régions chromosomiques associées à la résistance à la fusariose sur les bras 2BL, 3BS, 4AL, 4BS, 5AL, et 6AS. Le parent résistant DBC-480 confère l'allèle de résistance à tous les loci détectés. Les deux régions chromosomiques sur 3BS et 4BS sont associées à la résistance à la fusariose au sein des trois populations. Le QTL majeur sur 4BS co-localise avec le locus *Rht-B1*. Il explique 64%, 38% et 19% de la variance phénotypique totale au sein des populations KD, DD et SD. Le QTL identifié sur 3BS est lié aux marqueurs *Xbarc147* et *Xumn10*, marqueurs qui signalent également le QTL de résistance introgressé *Fhb1*. Ce QTL présente un effet significatif pour la population KD lors de tous les essais, mais seulement pour deux essais sur trois pour la population DD et un essai sur trois pour la population SD. L'analyse révèle la présence d'autres QTL spécifiques à chacune des populations. Deux QTL majeurs ont été détectés chez SD sur les bras chromosomiques 4AL et le 6AS expliquant 19% et 25% de la variation phénotypique totale. Ces deux QTL coïncident avec des QTL associés à la hauteur des plantes et à la date de floraison. Dans la population DD, un QTL a été identifié sur 5AL lors de l'analyse conjointe des essais, où il explique 6% de la variation phénotypique. Ce QTL présente, en fait, un effet fort en 2013, où il explique 15% de cette variation, alors qu'il n'est pas détecté en 2014 et 2015. Dans la population KD, un QTL à effet faible a pu être détecté sur 2BL. Il explique 4% de la variance phénotypique, et est significatif en 2014 et lors de l'analyse conjointe de tous les essais. Aucune interaction

épistatique n'a pu être mise en évidence au cours de ce travail. Les QTL associés à la sévérité de la fusariose agissent donc principalement de manière additive.

Analyse de QTL pour la propagation dans l'épi

La détection de QTL menée dans la population KD a permis d'identifier un QTL sur le 3BS lié aux marqueurs *Xbarc147* et *Xumn10*. Il se trouve donc dans la même zone que le QTL de sévérité présenté précédemment et que *Fhb1*. L'allèle de résistance provient également du parent DBC-480. Ce QTL a été détecté systématiquement sur les trois essais réalisés en serre et également lors de l'analyse conjointe des essais. Il explique 33% de la variation phénotypique. Deux autres QTL ont été détectés sur le 2A et le 4AL mais seulement au sein d'un seul essai. Dans ces cas ci, l'allèle de résistance provenait du cultivar Karur.

Analyse QTL pour la taille des plantes et la date de floraison

Des QTL influant sur le caractère taille des plantes ont été détectés sur 4AL, 4BS, et 6AS. L'allèle permettant l'augmentation de la taille provient à chaque fois du parent DBC-480. Le locus *Rht-B1b* a été identifié au sein des trois populations et explique respectivement 95%, 81% et 37% de la variation pour PH dans les populations KD, DD et SD. Dans la population SD, deux autres QTL sont associés à la hauteur des plantes sur 4AL et 6AS expliquant 11% et 27% de la variation. Ces QTL exercent un effet épistatique sur le locus *Rht-B1* bien que considéré comme faible comparé à leurs effets additifs. L'analyse de QTL pour la date de floraison a permis la détection de QTL sur les chromosomes 1BL, 2BS, 4AL, 5AL, 6AS et 6BS. L'allèle de DBC-480 confère une précocité plus tardive pour les QTL sur 2BS, 4AL, 5AL, 6AS et 6BS. Le QTL sur 2BS a été identifié sur la même région dans les trois populations et est localisé dans une région distale du QTL associé à la sévérité de la maladie identifié sur 2BL dans la population KD. Dans la population SD, les QTL associés à la sévérité de la maladie, la hauteur des plantes et la date de floraison, coïncident sur 4AL et 6AS. Ces loci semblent posséder des effets pléiotropiques.

Co-localisation entre les QTL de taille et ceux de résistance

L'idéotype recherché par le sélectionneur de blé dur est une variété semi-naine avec une résistance améliorée à la fusariose. Pour vérifier la faisabilité d'un tel idéotype au sein des populations étudiées, les effets des QTL de résistance détectés ont été comparés. Dans la population SD, trois QTL majeurs de résistance ont coïncidé avec des QTL associés à la hauteur des plantes. L'effet de *Fhb1*, le seul QTL qui ne soit pas associé à la hauteur dans cette

population, n'a pas contrebalancé l'effet négatif des allèles de nanismes aux loci *Rht-B1*, 4AL et 6AS, qui confèrent une plus grande sensibilité à la fusariose. Dans les populations KD et DD, trois QTL, dont *Fhb1*, sont associés à la résistance à la maladie et, parmi eux, seul le locus *Rht-B1* est associé à la fois à la résistance et à la hauteur des plantes. Les individus possédant l'allèle de nanisme *Rht-B1b* ont été significativement plus petits et plus sensibles que les individus possédant l'allèle sauvage *Rht-B1a*. La hauteur des plantes, pour les individus portant l'allèle *Rht-B1b*, était réduite de 31% et 25% en moyenne pour les populations KD et DD respectivement, et les niveaux de sévérité de la maladie étaient multipliés par deux en comparaison aux individus portant l'allèle *Rht-B1a*. Toutefois, dans la population KD, les individus portant les allèles de résistance sur *Fhb1* et 2BL en combinaison avec *Rht-B1b* ont présenté des niveaux de sévérité réduits de 38% comparé aux lignées naines ne portant aucun QTL de résistance tout en montrant un niveau de résistance équivalent aux lignées portant l'allèle *Rht-B1a* et aucun QTL de résistance. Dans la population DD, les individus portant les allèles de résistance sur *Fhb1* et 5AL en combinaison avec *Rht-B1b* ont vu les niveaux de sévérité réduits de 53% par rapport aux lignées naines sans QTL de résistance. De même, leur niveau de résistance n'a statistiquement pas été différent de celui des lignées portant l'allèle *Rht-B1a*. Ces résultats montrent que, dans ces populations, la combinaison des allèles de résistance aux différents QTL compense efficacement l'effet négatif de *Rht-B1b* sur la résistance à la fusariose.

DISCUSSION

L'amélioration de la résistance du blé dur à la fusariose est devenue ces dernières années une priorité dans les programmes de sélection. Le nombre limité de sources de résistance disponible chez le blé dur a poussé les sélectionneurs à exploiter la résistance dérivée d'espèces apparentées. Dans cette étude, le blé tendre a été utilisé pour la première fois comme source de résistance dans un fond génétique de blé dur. La lignée tétraploïde résistante DBC-480, qui porte le QTL majeur de résistance *Fhb1*, présentait un bon niveau de résistance après inoculation artificielle dans les champs et en serre. L'utilisation de trois différentes populations biparentales a permis de disséquer les bases génétiques de la résistance à la fusariose tout en validant les effets des QTL détectés au sein de différents fonds génétiques de blé dur élite.

Architecture génétique de la résistance à la fusariose

L'architecture génétique de la résistance à la fusariose dans les trois populations est quantitative et de nature oligo- à polygénique. Au total six QTL associés à la résistance à la fusariose ont été détectés sur les bras chromosomiques 2BL, 3BS, 4AL, 4BS, 5AL et 6AS. L'allèle de résistance dérive de DBC-480 à tous ces loci. La comparaison de la position des QTL identifiés avec la carte consensus fournie par DArT PL (A. Kilian, Diversity Arrays Technologies, communication personnelle, 2016) et les cartes génétiques publiées par Somers et al. (2004) et Marone et al. (2012), montre que les QTL détectés dans cette étude coïncident avec des régions où des QTL ont préalablement été identifiés chez le blé tendre et le blé dur.

Le QTL détecté sur 3BS, à proximité des marqueurs *Xbarc147* et *Xumn10*, correspond au locus de *Fhb1*. Des variations de l'effet de *Fhb1* sur la sévérité de la maladie ont été observées et expliquent entre 5% et 16% de la variance phénotypique en fonction du fond génétique de blé dur et de l'essai considéré. L'allèle de résistance à *Fhb1* est associé à une réduction des symptômes de sévérité de la maladie de 30% en moyenne dans la population KD et de 36% dans la population DD alors que dans la population SD cette réduction est de 6% seulement. Cette disparité, observée entre les populations KD et DD d'un côté et la population SD de l'autre, peuvent être attribuées à leurs différentes architectures génétiques pour la résistance à la maladie. Dans les populations KD et DD, seulement un autre QTL majeur de résistance associé à la sévérité de la maladie a été détecté. Des effets similaires pour *Fhb1* ont été observés. En revanche, pour la population SD, l'effet relatif de *Fhb1* a été probablement diminué par la présence de trois autres QTL majeurs. L'effet de *Fhb1* observé dans les populations de blé dur étudiées est analogue aux effets observés dans une série de lignées quasi-isogéniques de blé tendre par Pumphrey et al. (2007) où la sévérité de la maladie était réduite en moyenne de 23% et variant entre 0 et 70%. Lors de l'évaluation de la propagation de la maladie après inoculation ponctuelle, *Fhb1* a présenté un large effet, expliquant 33% de la variation phénotypique. Ce résultat montre que, chez le blé dur, *Fhb1* contribue principalement à la résistance de type 2. Cela est en accord avec ce qui a été précédemment établi chez le blé tendre où *Fhb1* contribue principalement à la résistance de type 2 et, dans une moindre mesure, à la résistance de type 1 (Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Buerstmayr et al. 2003a; Cuthbert et al. 2006). Cette étude valide pour la première fois l'effet de *Fhb1* dans un fond génétique de blé dur et marque une avancée significative pour l'amélioration de la résistance à la fusariose. Le blé tendre représente une ressource importante et utile pour l'amélioration du blé

dur face à cette maladie car la plupart de ses QTL de résistance sont localisés sur les génomes A et B, communs au blé dur (Buerstmayr et al. 2009; Liu et al. 2009; Löffler et al. 2009).

Un QTL majeur de résistance sur 4BS, associé à la sévérité de la maladie, a été détecté au sein des trois populations étudiées. La localisation de ce QTL coïncide avec le gène *Rht-B1*. L'allèle de nanisme *Rht-B1b* est associé, dans les trois populations, à une plus grande sévérité de la maladie. Cette association a déjà été rapportée précédemment chez le blé tendre (Hilton et al. 1999; Srinivasachary et al. 2009). Des résultats équivalents ont également été trouvés dans trois populations de blé dur issues du rétrocroisement de l'accession résistante *T. dicoccum*-161 avec les cultivars semi-nains de blé durs Helidur, Floradur et DS-131621. Le QTL de résistance localisé au niveau du locus de *Rht-B1* expliquait la plus grande partie de la variation phénotypique pour la sévérité de la maladie mesurée après inoculation en spray. De plus, dans ces trois populations, les individus possédant l'allèle *Rht-B1b* présentaient, aussi, un niveau plus élevé de symptômes (Buerstmayr et al. 2012b).

Au sein de la population SD, deux autres QTL majeurs ont été identifiés sur 4AL et 6AS. Ces deux QTL de résistance coïncident avec des QTL associés à la date de floraison et à la taille. Des QTL de résistance ont précédemment été détectés sur les chromosomes 4A et 6A chez le blé tétraploïde. Ils ont cependant été cartographiés sur des bras chromosomiques différents et leur localisation ne correspond pas à celle des QTL détectés dans cette étude. Plusieurs QTL de blé tendre ont, en revanche, été cartographiés dans la même région de 4AL chez les cultivars de blé tendre d'hiver Heyne (Zhang et al. 2012) et Arina (Paillard et al. 2004; Buerstmayr and Buerstmayr 2015). Le QTL sur 6AS est localisé dans la même région que le QTL associé à la résistance de type 2 chez la lignée résistante de blé tendre ND2603 (Sumai 3/Wheaton) (Anderson et al. 2001).

Un QTL à effet mineur a été cartographié dans la population KD sur 2BL à proximité du centromère. Des QTL de résistance ont été identifiés dans cette région dans deux populations de blé tétraploïde. Pour ces QTL, l'allèle de résistance dérive des parents de blé dur sensibles à la fusariose, Strongfield (Somers et al. 2006) et Helidur (Gladysz et al. 2007).

Dans la population DD, un QTL a été identifié sur 5AL. Un effet majeur de ce QTL a été détecté en 2013 alors qu'il n'a eu aucun effet en 2014 et 2015. Il semble que ce QTL n'est pas localisé dans la même région que le QTL majeur de résistance du blé tendre *Qfhs.ifa-5A* issu de Sumai-3 (Buerstmayr et al. 2003a).

Association des QTL pour la résistance à la fusariose, la date de floraison et la hauteur des plantes

Dans cette étude, parmi les six QTL associés à la date de floraison, deux d'entre eux coïncident avec des QTL de résistance sur 4AL et 6AS dans la population SD. Ces deux QTL ont un effet fort sur ces deux caractères et une corrélation positive a été observée entre la date de floraison et la sévérité de la maladie dans cette population. Dans les populations KD et DD, une corrélation faible ou non significative a été trouvée entre les deux caractères et lorsque les essais sont analysés séparément, les corrélations varient considérablement. Ces résultats ne laissent apparaître aucune tendance générale quant à l'association de ces caractères. Cela peut-être expliqué par l'absence de QTL coïncidant pour ces caractères dans les populations KD et DD. La variabilité des corrélations observées reflète certainement la variation des conditions environnementales locales au moment de la floraison et de l'inoculation, influençant le développement de la maladie.

Au contraire, la hauteur des plantes est négativement et significativement corrélée à la sévérité de la maladie au sein des trois populations. Cela est en accord avec les résultats observés précédemment dans d'autres populations de blé dur (Talas et al. 2011; Buerstmayr et al. 2012b; Miedaner and Longin 2014). Tous les QTL de hauteur des plantes identifiés dans cette étude coïncident avec des QTL associés à la sévérité de la maladie sur les QTL 4AL, 4BS et 6AS. La coïncidence de QTL de hauteur de la plante et de sévérité est couramment observée chez le blé et est appuyée par les résultats de méta-analyse de QTL (Mao et al. 2010). Les mécanismes sous-jacents à l'association de ces deux caractères sont complexes et peuvent être attribués aux effets de différence de hauteur des plantes *per se* et/ou à des effets pléiotropiques des gènes de nanismes ou à l'existence de gènes liés qui augmentent la sensibilité à la fusariose. Dans cette étude, en raison de la grande variation de hauteur des plantes observées, avec des différences d'environ 60 cm entre les plantes les plus petites et les plus hautes, une partie de cette association peut être attribuée à la hauteur des plantes *per se*. En dépit d'une inoculation en spray des épis et en assurant une brumisation homogène, les épis des plantes semi-naines ont tendance à rester plus humides et donc sous des conditions de pression d'infection plus fortes, que les épis des plantes hautes. Cela est en accord avec les conclusions tirées par Buerstmayr et al. (2012b) lors de l'évaluation de la sévérité de la fusariose de trois populations de blé tétraploïdes présentant des variations pour la hauteur des plantes similaires à celles observées dans cette étude. Les auteurs

supposent un évitement de l'infection pour les plantes les plus hautes, et ce, malgré la mise en place d'un système de brumisation contrôlé après inoculation en spray.

Perspectives pour l'amélioration du blé dur pour la résistance à la fusariose et conclusion

Il apparaît clairement que, dans ces populations, la hauteur des plantes exerce une forte influence en modulant la réponse à la fusariose. La plus grande sensibilité à la fusariose observée chez les plantes semi-naines, et associée à *Rht-B1b*, est un défi pour les sélectionneurs. *Rht-B1b* confère des caractéristiques agronomiques bénéfiques, liées à un rendement et un indice de récolte plus important, tout en permettant l'obtention de plantes semi-naines qui réduisent le risque de verse dans les systèmes de production agricoles modernes pratiques (Royo et al. 2007; Subira et al. 2016). Cette étude montre que la présence de *Fhb1*, associé à un QTL mineur de résistance, permet la sélection de lignées semi-naines possédant un meilleur niveau de résistance à la fusariose. Ces résultats sont en accord avec les résultats obtenus chez le blé tendre où la combinaison de deux QTL contrebalance l'effet négatif de l'allèle de nanisme *Rht-D1b* et permet l'amélioration de la résistance des lignées semi-naines (Lu et al. 2011). Les lignées des populations KD et DD possédant la combinaison d'allèles favorables aux loci *Rht-B1* et *Fhb1*, ainsi que l'allèle de résistance sur 2BL ou 5AL, représentent une ressource nouvelle et unique pour l'amélioration de la résistance du blé dur à la fusariose. Le matériel végétal développé dans cette étude est proche agronomiquement du germoplasme moderne européen. Les lignées améliorées sélectionnées sont ainsi facilement incorporables dans les programmes de sélection de blé dur en vue d'améliorer la résistance à la fusariose.

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Abbreviations

ANOVA	Analysis of variance
BLUES	Best linear unbiased estimator
DD	Durobonus x DBC-480
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
FDK	Fusarium-damaged kernel
FHB	Fusarium head blight
GBS	Genotyping-by-sequencing
GH	Greenhouse
KD	Karur x DBC-480
LOD	Logarithm of odds
LSD	Least significant difference
MAS	Marker-assisted selection
PAV	Presence/absence variation
PH	Plant height
PIS	Percentage infected spikelets
QTL	Quantitative trait loci
RIL	Recombinant inbred line
SD	SZD1029K x DBC-480
SEWANA	South Europe, West Asia and North Africa
SNP	Single nucleotide polymorphism
SSR marker	Single sequence repeat or microsatellite marker

Declaration

Ich erkläre eidesstattlich, dass ich die Arbeit selbständig angefertigt, keine anderen als die angegebenen Hilfsmittel benutzt und alle aus ungedruckten Quellen, gedruckter Literatur oder aus dem Internet im Wortlaut oder im wesentlichen Inhalt übernommenen Formulierungen und Konzepte gemäß den Richtlinien wissenschaftlicher Arbeiten zitiert, durch Fußnoten gekennzeichnet bzw. mit genauer Quellenangabe kenntlich gemacht habe.

Wien, August 2016