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Philosophiae Doctor (PhD) Thesis 2011:66

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Partial resistance to Fusarium head blight and powdery mildew in wheat

Partiell resistens mot aksfusariose og mjøldogg i hvete

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List of papers

I. Lu, Q., Szabo-Hever, A., Bjørnstad, Å., Lillemo, M., Semagn, K., Mesterhazy, A., Ji, F., Shi, J. & Skinnes, H. (2011). Two major resistance quantitative trait loci are required to counteract the increased susceptibility to Fusarium head blight of the *Rht-D1b* dwarfing gene in wheat. *Crop Science*, 51 (6): 2430-2438.

II. Lu, Q.X., Lillemo, M., Skinnes, H., He, X.Y., Shi, J.R., Ji F., Bjørnstad, Å. The Fusarium head blight resistance in bread wheat line Shanghai-3/Catbird is under multigenic control and associated with anther extrusion. Manuscript.

III. Lu, Q.X., Lillemo, M., Skinnes, H., Ren,Y., Asad, M.A., Xia, X.C., Chen, X.M., Ji F., Shi, J.R., Bjørnstad, Å. Partial resistance to powdery mildew in German spring wheat Naxos is based on multiple genes with stable effects in diverse environments. *Theor. Appl. Genet*. Accepted.

Abstract

Both Fusarium head blight and powdery mildew are devastating diseases in wheat growing areas around the world. Breeding for resistance has been undergoing for many decades and mostly depends on phenotypic selection. Marker assisted selection (MAS) provides potential to accelerate the gain of selection. To exploit new resistance loci with closely linked makers is a key step to realize it. Our studies have mapped the resistance loci of promising sources of resistance and looked into the associations between FHB and morphological traits (plant height and anther extrusion).

The first two studies (**paper I** and **II**) confirmed that *Rht-D1b* and *Rht-B1b* both compromised FHB resistance after spawn/spray inoculation. In a DH population of Avle x Line685, the negative impact of *Rht-D1b* could be counteracted with a combination of *Fhb1* and the QTL on 5A, two frequently used resistance loci in breeding. In a RIL population from SHA3/CBRD x Naxos, *Rht-B1b* had less negative impact compared to *Rht-D1b* in DH population. The FHB resistance in SHA3/CBRD was found controlled by a major QTL on 2DLc and some minor QTL. The high anther extrusion (AE) alleles were always associated with FHB resistance after spawn/spray inoculation and considered as an escape of FHB at flowering time. We suggested that high AE could be used as visual assessment to screen large breeding populations at the early stage for FHB resistance.

In **paper III**, we worked on PM resistance and analyzed the genetic basis of a promising partial resistance cultivar Naxos in the same RIL population as **paper II**. This high level of partial resistance is controlled by two major QTL on 1AS and 2DL, and two minor ones on 2BL and 7DS. The QTL on 1AS coincided with *Pm3* and was effective in all environments, but Naxos doesn't have any known *Pm3* haplotype. Therefore, it is a race-non-specific QTL. We also looked into the relation between *Pm3* and *Pm8*, but the suppression of *Pm8* by *Pm3* was not observed at the adult plant stage.

Closely linked markers to the major QTL detected in our study could have potential for use in MAS. The closely linked markers near two coincident QTL on 2BL and 2DLc could improve both FHB and PM resistance.

Sammendrag

Aksfusariose og mjøldogg er to alvorlige sykdommer som angriper hvete og gjør skade i mange hvetedyrkingsområder rundt omkring i verden. Resistensforedling har foregått over mange tiår og er i stor grad basert på fenotypisk seleksjon. Markør-assistert seleksjon (MAS) gir muligheter til økt genetisk framgang. Å finne nye resistensgener og identifisere nært koblede markører er en viktig forutsetning for å nå dette potensialet. Våre studier har kartlagt resistensgenene til viktige resistenskilder og undersøkt sammenhenger mellom aksfusariose og morfologiske egenskaper som strålenge og støvknappfelling.

De to første studiene (**Paper I** og **II**) bekreftet at dverggenene *Rht-D1b* og *Rht-B1b* begge hadde negativ innvirkning på fusariumresistens etter kornsmitte eller dusjsmitting. I en dobbel haploid (DH)-populasjon av Avle x Line685, kunne den negative virkningen av *Rht-D1b* bli oppveid ved å kombinere *Fhb1* med resistens-QTL-et på 5A, to av de mest brukte resistensgenene i foredling. I en populasjon av rekombinante linjer (RIL) fra SHA3/CBRD x Naxos var den negative effekten av *Rht-B1b* mindre enn for *Rht-D1b* i DH-populasjonen. Fusariumresistensen i SHA3/CBRD viste seg å være kontrollert av et hoved-QTL på 2DLc og noen mindre QTL. Allelene for høy støvknappfelling (AE) var alltid assosiert med fusariumresistens etter kornsmitte eller dusjsmitting og kan betraktes som en mekanisme for å unngå fusarumangrep ved blomstring. Vi foreslår at visuell bedømming av støvknappfelling kan brukes som seleksjonskriterium til testing av store foredlingsmaterialer for fusariumresistens på tidlig stadium.

I den tredje studien (**Paper III**) analyserte vi det genetiske grunnlaget for mjøldoggresistens i den lovende resistenskilden Naxos ved bruk av samme RIL populasjon som i **paper II**. Den høye graden av partiell resistens var kontrollert av to hoved-QTL på kromosomene 1AS og 2DL, og to mindre QTL på 2BL og 7DS. QTL-et på 1AS var lokalisert i samme område som det rasespesifikke genet *Pm3* og viste effekt i alle miljø, men Naxos hadde ingen *Pm3*-haplotype. Dette er derfor et QTL for rase-uspesifikk resistens. Vi undersøkte også et mulig samspill mellom *Pm3* og *Pm8*, men fant ingen indikasjoner på at *Pm3*-allelet i SHA3/CBRD undertrykte resistensen til *Pm8* på voksenplantestadiet.

Nært koblede markører til de viktigste QTL-ene som er avdekket i denne avhandlingen kan ha potensial til bruk i MAS. De nært koblede markørene til to QTL sammenfallende QTL på 2BL og 2DLc kan brukes til å øke resistensen mot både aksfusariose og mjøldogg.

Abbreviations

AE	Anther extrusion
CIM	Composite interval mapping
CIMMYT	The International Maize and Wheat Improvement Center
CL	Cleistogamy
d°C	Day degrees
DArT	Diversity arrays technology
DH	Doubled haploid
DON	Deoxynivalenol
EST	Expressed sequence tag
FDK	Fusarium damaged kernel
FHB	Fusarium head blight
LOD	Logarithm of odds
MAS	Marker assisted selection
NB-LRR	Nucleotide binding leucine-rich repeat
NIL	Near isogenic line
NIV	Nivalenol
PM	Powdery mildew
QTL	Quantitative trait loci
Rht	Reduced height
RIL	Recombinant inbred line
SIM	Simple interval mapping
SSR	Simple sequence repeat
STS	Sequence tagged sites
ZEA	Zearalenone

1. Introduction

1.1 A brief history of wheat breeding

Since wheat was domesticated around 9,500-10,500 years ago (Tanno & Willcox 2006), "breeding" has been done by farmers from simply collecting wild plants for food to selecting those to be cultivated which began to guide the evolutionary process.

Modern breeding in wheat started in 17th century with making crosses with two plants carrying reciprocal characteristics and selecting fertile progenies with both characteristics. During the 19th-20th centuries, with the discovery of genetics and evolutionary theory, many modern technologies and methods were developed to facilitate the breeding (Xu 2010). The most notable renovation was "Green revolution" led by Norman Borlaug who developed new high-yield wheat varieties since 1940s. Combined with modern agricultural technologies, wheat production achieved significant increase from 2 to 6 metric tons per hectare in 40 years, while it took 1,000 years to increase from 0.5 to 2 metric tons per hectare in England (Hazell 2002). Such significant yield achievements worldwide helped the word out of starvation.

Both Fusarium head blight (FHB) and powdery mildew (PM) are devastating wheat diseases with great yield loss and quality reduction (FHB). Their epidemics can occur in wheat growing areas around the world (Bennett 1984; Lotterman 1998). In order to meet the increasing population in the world, disease resistance breeding is still a long term goal to guarantee the wheat production. Breeding for resistance has been undergoing for many decades and mostly depends on phenotypic selection.

In the 1980-1990s, DNA markers such as RFLP (restriction fragment length polymorphism), RAPD (randomly amplified polymorphic DNA), SSR (microsatellites) and SNP (single nucleotide polymorphism) have become a powerful tool and have been widely used in evaluation of germplasm, genetic mapping, marker assisted selection (MAS) and map-based cloning. QTL (Quantitative trait loci) mapping of disease resistance in wheat started in early 1990s (Ma et al. 1994; Williams et al. 1994). With molecular markers closely linked to a QTL, the QTL can be transferred into different

genetic backgrounds by MAS (Bai et al. 1999). Functional markers developed from the cloned genes are the "perfect marker" in MAS. With QTL mapping strategy, we can analyze genetic associations between markers and FHB/ PM resistance and find closely linked markers for MAS which is so called molecular breeding.

1.2 Fusarium head blight

Fusarium head blight (FHB), also known as scab, is a fungal disease that affects wheat, barley, oat and other cereals. It was first described in England by Smith in 1884 (Parry et al. 1995). From then on, outbreaks occurred in all wheat production regions over the world at various times (Lotterman 1998) and was considered as a main threat to wheat production. Epidemics of FHB are usually associated with warm, wet weather around flowering (Parry et al. 1995).

Besides yield loss, FHB also causes contaminations of mytoxins such as deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZEA) in infected kernels, which lower the grain quality and harm human beings and livestock. In addition, Fusarium infected seeds can also reduce the germination and seedling vigour. High transmission rates from infected seeds to seedling blight and crown rot were documented (Duthie & Hall 1987).

In recent years, the outbreak continued and has brought a great loss of both yield and quality. During the period 1998-2000, the economic losses caused by FHB were estimated at $2.7 billion in Midwestern United States (Nganje et al. 2002). In Manitoba (Canada) the economic losses to wheat producers reached US$300 million from 1993 to 1998 (Windels, 2000). In China, the estimate is that scab may affect up to 7 million ha, and 2.5 million tonnes of grain may be lost in epidemic years. Diseases related to fusarial mycotoxins in humans have been reported in China, India and Japan, whereas in animals diseases have been reported in numerous parts of the world (Dubin et al. 1996).

FHB has called for an increasing attention of international importance. The International Maize and Wheat Improvement Center (CIMMYT) has identified FHB as a major factor limiting wheat production in many parts of the world (Dubin et al. 1996). International symposiums on FHB as well as European Fusarium Seminar, National Fusarium Head

Blight Forum in US and Canadian Workshop on Fusarium Head Blight were held to seek the way to fight the disease. Integrated strategies combining cultural practice, seed treatment, crop rotation, fungicides and resistance cultivars have been recommended for FHB management (Bai & Shaner 2004; McMullen et al. 2008; Parry et al. 1995). Among these, breeding FHB-resistant varieties plays a key role as it is the most effective, economic, and environmental way.

1.2.1 Symptoms and causal agents

Symptoms

A typical symptom of FHB is premature bleaching of spikelets. First water-soaked brownish spots can be observed and eventually discoloration on the glumes. Under favorable conditions, the spikelets become pink or white, sometimes orange fruiting bodies will occur on the spikelets (Fig.1).

a b

Figure1. Symptoms of FHB. a) Infected heads (Wolf & Lipps 2003), b) Symptom in the fields

After harvest, the infected kernels (Fusarium damaged kernels, FDK) are shriveled, smaller in size and grayish or sometime pinkish in color compared to the healthy ones (Fig.2).

Figure 2. Heathly (left) and FHB infected wheat kernels (right). (Keller et al. 2011)

Main causal agents

FHB can be caused by several fungal species of *Fusarium*, but *F. graminearum* and *F. culmorum* are usually the most important (McMullen et al. 1997; Parry et al. 1995). *F. g* predominates in the warmer, humid areas of the world such as USA, Canada, China, Japan and Southern Europe (Stepien & Chelkowski 2010; Wagacha & Muthomi 2007). *F. c* predominates in the cooler areas such as north, central and west Europe and Canada (Demeke et al. 2005; Wagacha & Muthomi 2007). However, recently an increasing frenquency of *F.g* and decreasing of *F.c* were found in some areas such as the Nertherlands, Germany, Italy and Norway.

These two dominant species can produce trichothecene mycotoxins mainly DON, and NIV. *F. g* and *F. c* tend to produce either DON or NIV (Champeil et al. 2004). DON chemotypes are found worldwide but NIV chemotypes are restricted (Yoshida & Nakajima 2010), which is the reason why many researchers use DON content as a parameter to estimate the mytoxin content in the grains.

Fusarium pathogens are necrotroph or hemibiotroph (Bhadauria et al. 2009; Laluk & Mengiste 2010), because they first colonize on the living tissue and tend to kill the plant, eventually they live on the dead debris. They are not species specific and can affect a wide range of host (Goswami & Kistler 2004; Parry et al. 1995). After infection the

hyphae continue to grow in the host tissue, which is favored by the trichothecene secreted from hyphae (Kang & Buchenauer 2002) .

Many *Fusarium* species responsible for FHB can also cause seedling blight, crown rot and root rot (Li et al. 2010; Mudge et al. 2006; Parry et al. 1995). These less investigated diseases appear quantitative (Bovill et al. 2006) and are controlled by different host genes from FHB (Collard et al. 2005; Li et al. 2010), although they have common aetiology.

1.2.2 Disease cycles

The fungi survive and multiply on crop residues. Many plants in the grass family can be its reservoirs with respect of a wide host range of *Fusarium spp*. Long dispersal sources such as infected seed, infectious transplants and enriched spores in air (Maldonado-Ramirez et al. 2005) could also add to FHB infection. Ascospores produced by the sexual stage together with macroconidia produced by mycelia from the previous crop can both be effective as inoculum. During the anthesis, spores are dispersed by wind or rain splash onto the wheat spikes. Temperatures of 10° to 30°C and relative humidity above 95% for 40 to 60 hours are usually enough for macroconidia to successfully infect the spikes (Curtis et al. 2002). FHB depends on both weather conditions and inoculums from flowering through grain development. Under favorable conditions, it causes a great yield loss and DON contamination. Late infection also can cause DON contamination even without apparent symptoms (Yoshida & Nakajima 2010). After harvest, fungi bear fruit body on the crop residues and survive the winter. Mycelia can also survive during mild winter. In the next season, spores will develop as inoculum for a new cycle of FHB infection.

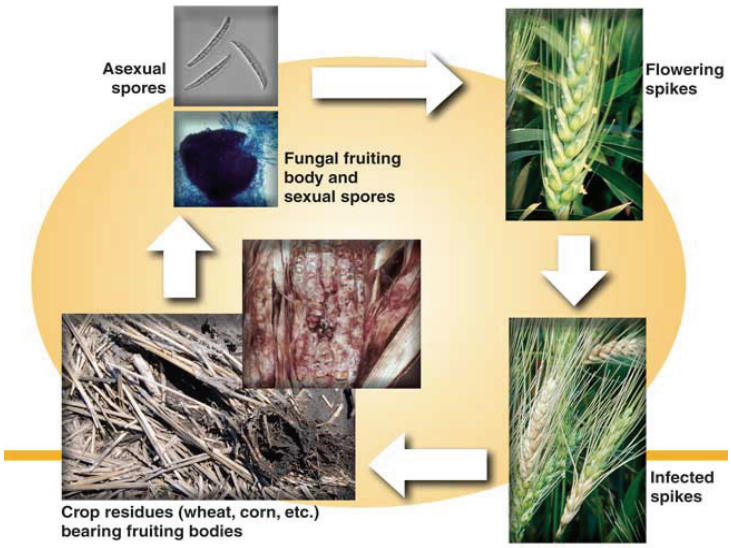


Figure 3. Disease cycle of Fusarium head blight (McMullen et al. 2008)

1.2.3 FHB resistance types and evaluation

FHB resistance

Resistance to FHB is a complex quantitative trait which is not species dependent and is controlled by a number of nonspecific genes (Singh et al. 1995; Snijders 1990; Van Eeuwijk et al. 1995). Host resistance has been described as five types (Mesterhazy et al. 1999) in Table 1.

Table 1. Resistance components and their evaluation parameters

Resistance components		Evaluation parameter
Type I	Resistance to invasion	FHB severity and incidence after spray/spawn inoculation
Type II	Resistance to fungal spread	FHB severity after point inoculation
Type III	Resistance to toxin accumulation	DON content in harvested grains
Type IV	Resistance to kernel infection	FDK percentage
Type V	Tolerance	Yield

Among these types, Type I and Type II resistance were first described by Schroeder and Christensen (1963) and have been extensively studied since they are relatively easier to evaluate visually.

Evaluation methods

Type II resistance is generally evaluated with point inoculation. However, Type I is more difficult to evaluate. With spray, spawn or natural infection, disease incidence is commonly used as a measurement (Buerstmayr et al. 2009). Alternatively, early scoring of disease severity (Yan et al. 2011) or using non-mycotoxin producing Fusarium (Gosman et al. 2010) were also documented. The disease severities after spray, spawn or natural infection commonly reflect Type I+II resistance.

Point inoculation, also named as single floret inoculation, is carried out at anthesis or early anthesis. A 10-15 ul drop of inoculum with concentration about $1x10^5$spores/ml is injected into a single floret in the middle of the wheat spike. Spray inoculation is carried out at anthesis with equally spraying inoculum of about $1x10^5$ spores/ml. All the heads should be sprayed evenly at all sides. Spawn inoculation can be carried out from around stem elongation to one week before anthesis. Infected grains are prepared and evenly applied in the field at a certain rate. In order to favor the conditions, mist irrigation is usually applied during anthesis.

Different starting points lead to the different resistance types. Infections usually occur at inner surfaces of the glumes (Kang & Buchenauer 2000). Even when glumes are inoculated from the outside with spray/spawn inoculation, the fungi must travel into the glumes to cause infections. Many factors during this trip could lead to the differences between spray/spawn inoculation and point inoculation.

1.2.4 QTL mapping of FHB resistance

QTL mapping studies have been performed to tag FHB resistance QTL and to identify useful makers for MAS. In recent years, many QTL of FHB resistance have been identified in different genetic populations (Buerstmayr et al. 2009). To date, resistance QTL have been mapped on all wheat chromosomes (Buerstmayr et al. 2009; Liu et al. 2009) and projected into clusters with meta-analysis (Fig.4).

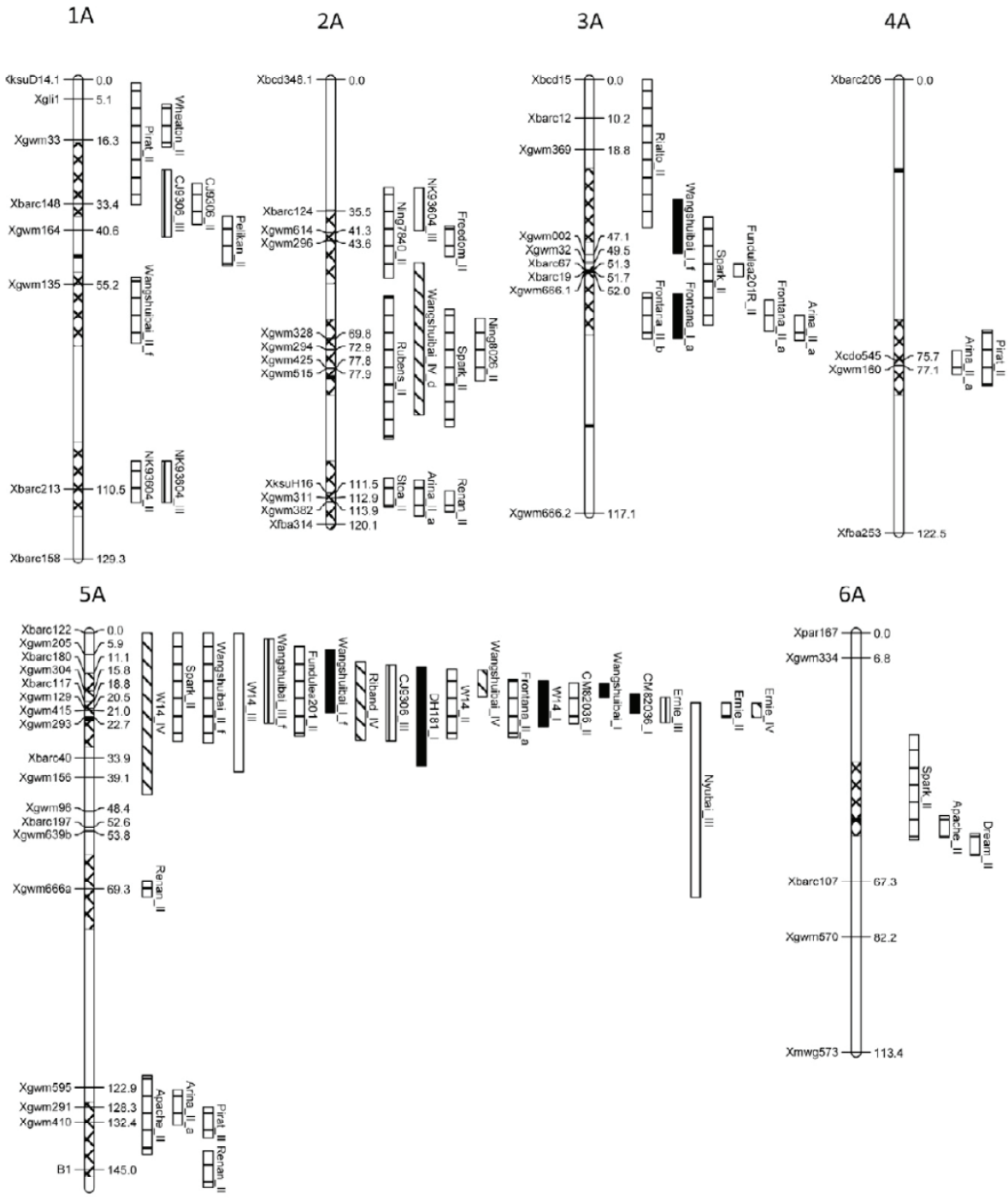


Figure 4. Chromosome locations of QTL associated with different types of Fusarium head blight (FHB) resistance in different resistant wheat sources. Roman numerals indicate the type of resistance, lowcase letters indicate the results from different experiments. (Liu et al. 2009)

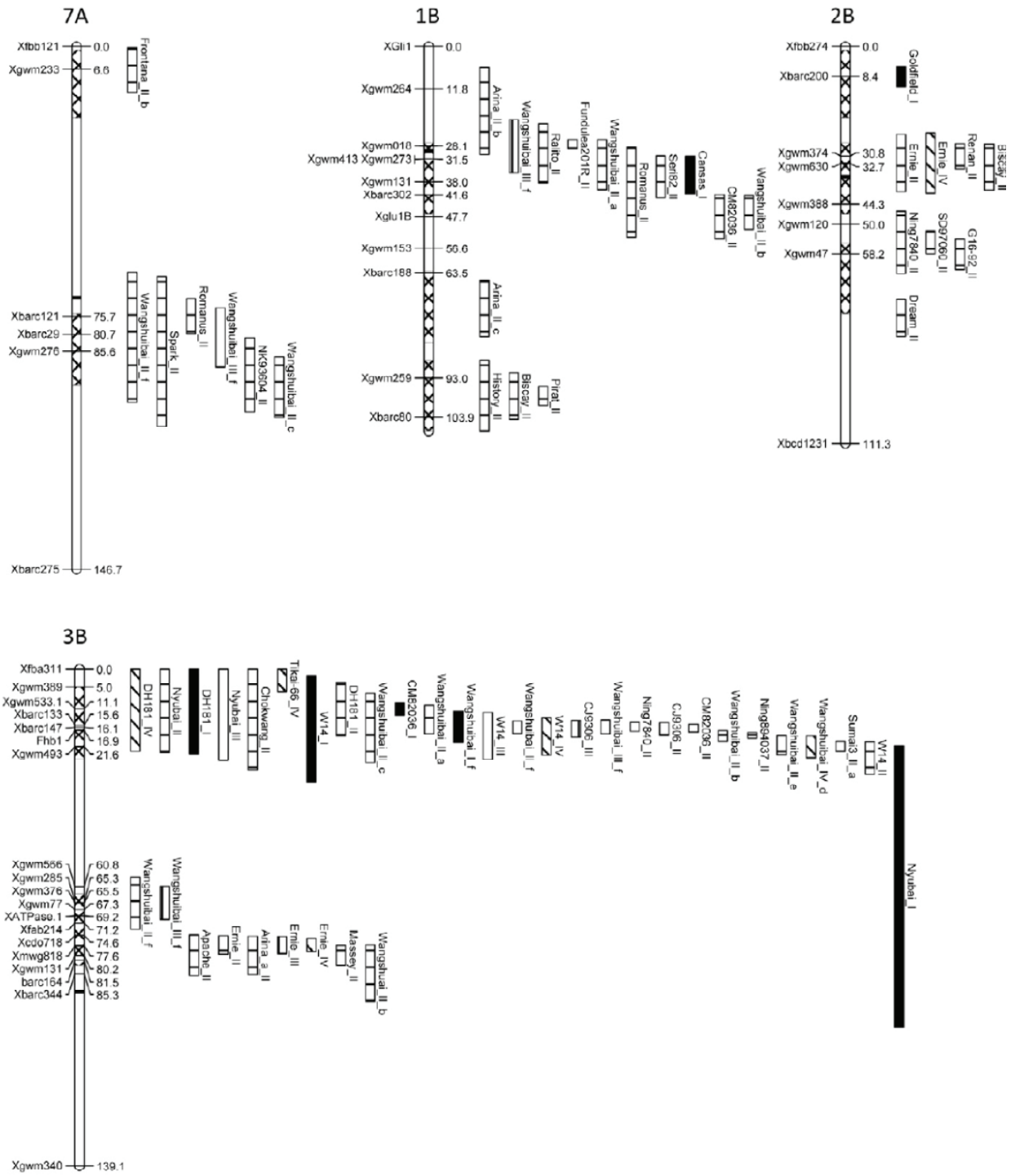
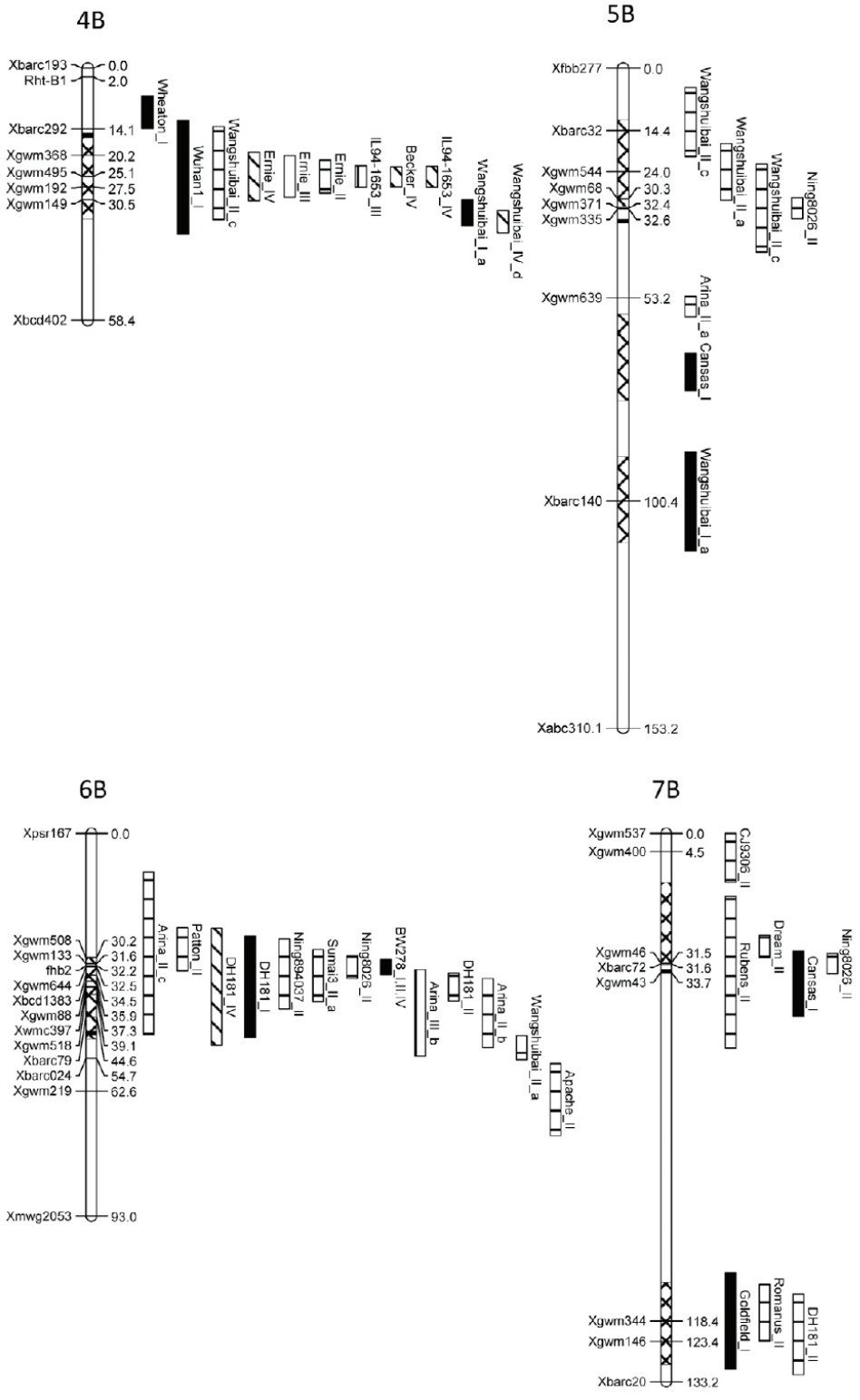


Figure 4. Continued.



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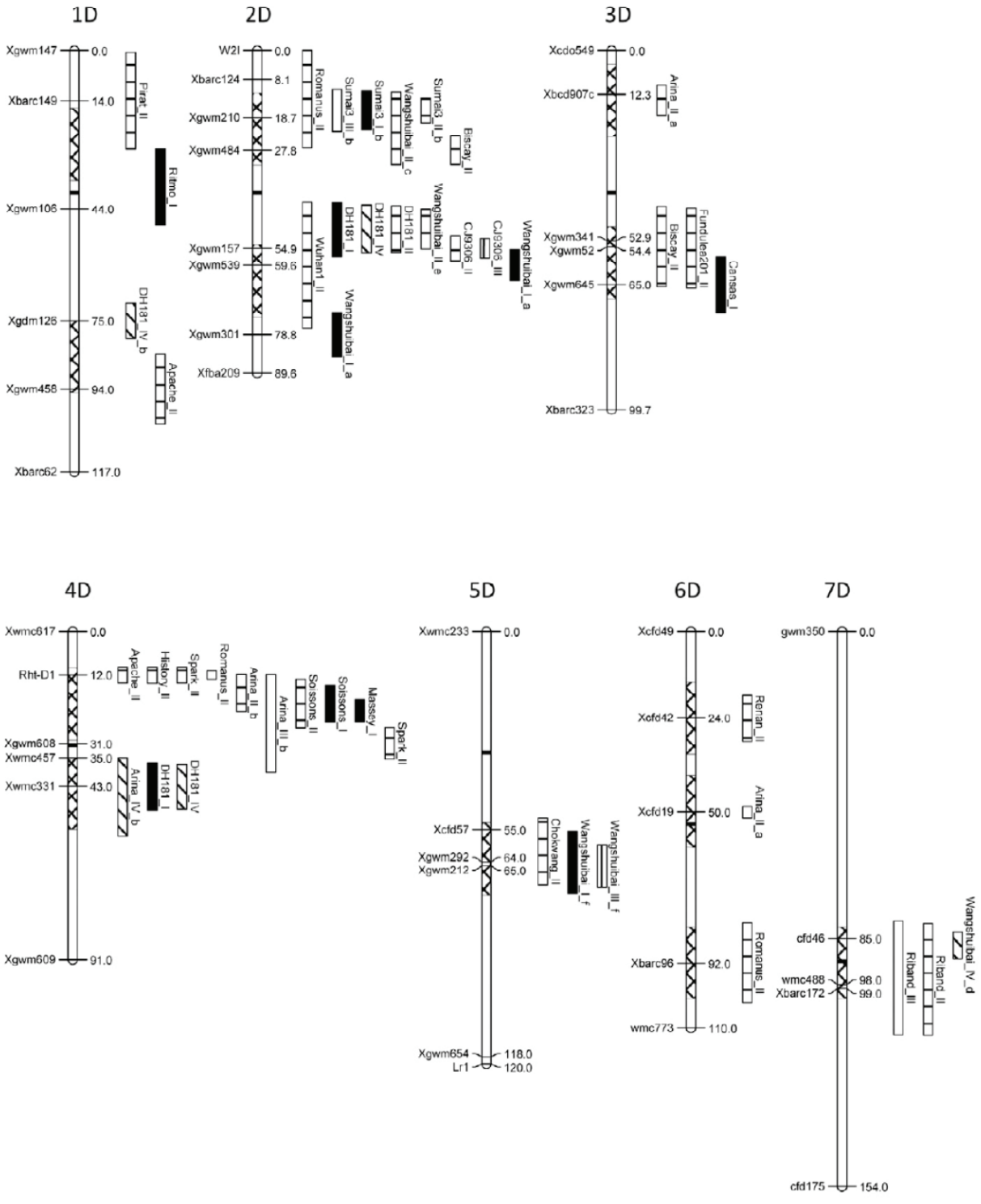

Figure 4. Continued.

Among 209 QTL from 46 unique lines, most of the identified QTL (130, 62%) confer Type II resistance, while 32 QTL for Type I, 25 QTL for Type III, and 22 QTL for Type IV were mapped (Liu et al. 2009). 12 QTL associated with FHB resistance have been confirmed in different populations (Table 2). More than 48% of reported QTL were derived from FHB resistance sources from Asia, which was due to the regular FHB epidemics and long tradition of resistance breeding in some Asian areas (Buerstmayr et al. 2009)

Table 2. Confirmed quantitative trait loci (QTL) for Fusarium head blight resistance in wheat based on a meta-analysis of QTL in 46 lines from 45 studies reported from 2001 to 2009.(Liu et al. 2009)

Chromosome	Type of resistance	Sources of resistance
3A	II	Frontana
5A	I,II, III	Sumai 3
5A	I	Wangshuibai
7A	II	Wangshuibai
1B	II	Wangshuibai
3BS	I,II, III,IV	Sumai 3
3BS	II	Wangshuibai
3BSc	II	Wangshuibai
5B	II	Wangshuibai
6BS	I,II, IV	Sumai 3
6B	II	Arina
2DL	II	Sumai 3

1.2.5 Some important loci for FHB resistance

Fhb1 on 3BS

The QTL on chromosome 3BS from Sumai 3 has been verified in different mapping populations. It explains 15–60% of the phenotypic variation for FHB in different backgrounds and mainly contributes Type II resistance (Anderson et al. 2001; Bai et al. 1999; Waldron et al. 1999) and also Type I resistance (Buerstmayr et al. 2003a; Buerstmayr et al. 2003b) and DON reduction (Jiang et al. 2007; Lemmens et al. 2005). In this QTL region, marker density has been increased by STS (sequence-tagged site) markers developed from wheat expressed sequence tags (ESTs) using the synteny between wheat and rice (Liu & Anderson 2003). This 3BS FHB resistance QTL has been fine mapped as the Mendelian locus *Fhb1* (Cuthbert et al. 2006; Liu & Anderson 2003; Liu et al. 2006), providing an accurate map location and tightly linked markers. Lemmens et al. (2005) found that wheat lines carrying *Fhb1* were able to convert DON into the less phytotoxic DON-3-O-glycoside and hypothesized that it either encodes a DON-glucosyltransferase or modulates the expression or activity of such an enzyme. DON was reported to play an important role in pathogenicity (Wagacha & Muthomi 2007) and inoculation with a *Fusarium* strain lacking trichothecene synthesis capacity resulted in that the disease symptom slowed down and even confined to the infected florets, which explained why *Fhb1* contributes to Type II resistance.

Fhb2 on 6BS

The resistance QTL on 6BS from Sumai 3 has smaller effect compared to *Fhb1* and mainly contributes to Type II resistance (Anderson et al. 2001; Cuthbert et al. 2007; Yang et al. 2003). Resistance QTL in the same cluster were also found in other Chinese resistance sources such as Wangshuibai, Ning8026 and Ning 894037 (Haberle et al. 2009; Lin et al. 2004; Shen et al. 2003), Swiss wheat Arina, US Patton and French Apache (Liu et al. 2009). Closely linked markers have been found via fine mapping with spray and point inoculation (Cuthbert et al. 2007).

Fhb3

Fhb3, associated with Type II resistance, was identified on T7AL.7Lr#1S translocation from *Leymus racemosus*. Homogenous lines of this translocation can significantly reduce severity by 15% compared to the heterozygotes. Three specific STS markers have been developed to identify this alien chromatin harboring *Fhb3*, and homogenous translocation can be achieved combined with seven SSR markers (Qi et al. 2008).

***Fhb4* on 4B, *Fhb5* on 5A**

The 4B and 5A resistance QTL from Wangshuibai contribute to Type I resistance, explaining 17.5% and 27% of the phenotypic variation (Lin et al. 2006). The 4B QTL has been designated as *Fhb4* and fine mapped into the 1.7-cM interval flanked by *Xhbg226* and *Xgwm149* (Xue et al. 2010b). The 5A QTL is designated as *Fhb5* and fine mapped into the 0.3-cM interval flanked by *Xgwm304* and *Xgwm415* (Xue et al. 2011)

2D

The 2DS QTL showed positive effect on either Type I or Type II resistance or both types in opposite parents of Sumai 3, Gamenya and highly resistant Nobeokabouzu-komugi (Handa et al. 2008), and also in resistant Wangshuibai (Jia et al. 2005) and European cultivar Romanus and Biscay (Holzapfel et al. 2008a) and also DON resistance in Maringa (Somers et al. 2003). With comparative genomics approach with rice, Handa et al. (2008) revealed that a putative gene for multidrug resistance-associated protein (MRP) is a possible candidate for the FHB resistance and/or DON accumulation controlling QTL on wheat chromosome 2DS and can be used as a molecular marker to eliminate the susceptible allele when the Chinese wheat variety Sumai 3 is used as a resistance source. However, this resistance QTL was reported negatively associated with *Rht8* (Mao et al. 2010; Somers et al. 2003).

The *Fhb1, 2, 3, 4, 5* and 2DS QTL have been fine mapped and their possible functions or candidate genes have been suggested. However, still no FHB resistance QTL has been cloned so far.

1.2.6 Associated traits

Morphological and developmental characters such as plant height (PH) and anther extrusion (AE) have been considered as factors influencing resistance to FHB.

***Rht* genes and FHB resistance**

Since the green revolution, *Rht* genes have been playing an important role in wheat breeding by reducing plant height to prevent lodging and increasing the yield potential. The effect of the genes was shown to increase partitioning of assimilates to yield (Curtis et al. 2002; Ellis et al. 2002). Among around 20 listed *Rht* genes (Ellis et al. 2005), the Norin 10 genes *Rht-D1b* and *Rht-B1b* (Gale & Youssefian 1985) are the most extensively used in CIMMYT breeding programmes and through which they spread worldwide to more than 70% of the world's semi-dwarf wheat crop (Hedden 2003). *Rht-B1* and *Rht-D1* are orthologues of maize dwarf-8 (*d8*) and the Arabidopsis Gibberellin Insensitive (*GAI*) gene (Peng et al. 1999). These genes have been isolated and encode GA-insensitive forms of a DELLA protein that functions as dominant and constitutively active repressors of stem growth (Hedden 2003; Peng et al. 1999).

Negative correlations between FHB resistance and plant height have been observed in many studies (Buerstmayr et al. 2000; Hilton et al. 1999; Mesterházy 1995; Steiner et al. 2004). The QTL mapping has verified that *Rht-B1b* and *Rht-D1b* coincide with major QTL for FHB susceptibility after spray inoculation (Draeger et al. 2007; Holzapfel et al. 2008b; Srinivasachary et al. 2008; Srinivasachary et al. 2009).

Near isogenic lines (NILs) studies showed that *Rht-D1b* increases susceptibility, whereas *Rht-B1b* may or may not do so, depending on genetic background and/or experimental conditions (Hilton et al. 1999; Miedaner & Voss 2008; Srinivasachary et al. 2009). The *Rht-D1b* increased the FHB severity by 52% in a Mercia background and 37.6% in a Maris Huntsman near isogenic background, while the increased susceptibility associated

with *Rht-B1b* was less (Miedaner & Voss 2008). Similar conclusions were arrived at by Srinivasachary et al. (2009) comparing the two genes using spray inoculation. With point inoculation, however, *Rht-B1b* was less affected than the tall control, while *Rht-D1b* was similar to the control. In general under high disease pressure these two alleles primarily decrease Type I resistance to different degrees and differentially affect Type II. Most authors suggested that this negative effect on FHB resistance is due to pleiotropy or linkage rather than plant height per se.

Recently, a QTL meta-analysis showed a negative association between PH and FHB resistance for both reported *Rht* genes (*Rht-B1b, Rht-D1b and Rht8*) and other PH QTL across studies (Mao et al. 2010). However, when the dwarf lines were raised to the same height level as wild type, this negative associations disappeared (Yan et al. 2011). Therefore, plant height per se still can't be ruled out.

Both *Rht-B1* and *Rht-D1* encode so-called DELLA proteins that are negative regulators of gibberellin (GA) signalling. In Arabidopsis, Cao et al. (2006) found that DELLA proteins are involved in the regulation of genes involved in response to disease and pathogens, toxin catabolism and multidrug transport and suggested that DELLA proteins might also mediate disease resistance. Nicholsen et al. (2008) showed that mutation of the DELLA genes enhanced the resistance to DON and consequently enhanced the Type II resistance in wheat and barley, while *Rht-D1b* did not conform to this model.

Anther extrusion and FHB resistance

Chasmogamy is a perquisite of AE. At the flowering stage, the florets of Chasmogamous plants are open and their anthers extrude and are exposed out of glumes (Fig. 5). This open flowering happens in the grasses when a small swollen structure (the lodicule) at the base of the carpel and stamens forces apart the palea and the lemma (the pair of bracts which encase the floret) (Nair et al. 2010). However in cleistogamy (CL), florets remain closed and anthers and pollen are not exposed out of the floret during flowering (Honda et al. 2005). Wheat is predominantly chasmogamous in flowering but the frequency of

chasmogamic flowers does vary from one accession to another (Chhabra & Sethi 1991) which leads to different magnitude of AE.

Figure 5. High anther extrusion (AE) and low AE spikes in wheat

Anther extrusion is a floral character influencing outcrossing and considered as a measurement of pollen-shedding capacity for restoration line in hybrid breeding as well as anther length and anther size (Beri & Anand 1971; Das 2006). AE due to its increasing outcrossing ability is also a concern for gene flow of transgenic plants (Honda et al. 2006; Oard et al. 2003; Ritala et al. 2002).

Anthers were considered as nutritious substrate for *Fusarium* and were observed initially infected after inoculation (Strange & Smith 1971; Strange et al. 1974). Kang & Buchenauer (2000) think the initial infection is not necessarily through anthers, but did observe that normally, hyphal density on anthers was higher than that on the inner surfaces of the lemma or palea. The retention of anthers inside the florets could be associated with high severity.

Recently significant negative correlations between AE and FHB/DON were observed in European wheat (Graham & Browne 2009; Skinnes et al. 2008). This correlation was

demonstrated in the Arina x NK93604 DH population, where coincident QTL of AE and FHB was found on chromosome 1B and closely linked on 7A (Skinnes et al. 2010).

1.2.7 Marker assisted selection for FHB resistance in breeding

With tightly linked makers of FHB resistance QTLs, MAS has been demonstrated potentially effective in selection. *Fhb1*, the most important FHB resistance locus in wheat breeding, has been introduced into many breeding populations worldwide using linked SSR markers or phenotypic selection (Buerstmayr et al. 2009). Anderson et al. (2007) demonstrated successful MAS for *Fhb1* which is the first one documented for use in practice.

Other QTL and their combinations were also tested for their use in MAS (Table 3). Miedaner et al. (2006) showed in MAS for FHB resistance, the combinations of 3B+5A+3A (3B and 5A from CM-82036, and 3A from Frontana) and 3B+5A provided highest resistance to FHB. McCartney et al. (2007) have evaluated introgression of FHB resistance (2D from Wuhan 1, 3BSc from Nyu Bai and Sumai 3, 5AS from Nyu Bai and Sumai 3) into elite Canadian spring wheat germplasm. Xue et al.(2010a) have demonstrated the feasibility of MAS to introduce *Fhb4, 5* and QTL on 2B and 3B from Wangshuibai into a susceptible Mianyang99-323 background NILs.

Base on linked markers, the QTL on 6AL and 7BS from Dream and the QTL on 2BL from G16-92 were successfully introduced into susceptible elite winter wheats Brando and LP235.1 (Wilde et al. 2008). The selection gain of severity reduction was also compared, which was higher for MAS with 2.5% per year, compared to 2.1% per year for phenotypic selection (Miedaner et al. 2009). The authors also suggested that additional phenotypic selection will further enhance the selection gain.

Fhb1 and *5A* QTL, two non-adapted QTL for Germany, were tested based on the closely linked markers in 10 environments (von der Ohe et al. 2010). Almost no side effect was found on agronomic and quality performance except little negative to yield which can be compensated by crossing with high yielding recurrent parent.

Table 3. Marker assisted selection for Fusarium head blight in wheat

Resistance	Chr.	Markers	FHB trait	Plant material	MAS with	References
Ning 7840	3BS	gwm389, gwm533, barc147	FHB spread	Ning7840 x Wheaton, Ning7840 X IL89-7978	6 markers	(Zhou et al. 2003)
DH181	3BS	gwm533–gwm493	FHB spread	DH181 x AC	8 markers	(Yang et al. 2003)
DH181	6B	gwm644	FHB spread	Foremost, 174 DH	8 markers	
93FHB21	3BS	gwm389–gwm493	FHB spread	AC Foremost x	8 markers	(Yang et al. 2003)
93FHB21	5A	gwm291	FHB spread	93FHB21 76 DH		
93FHB21	6B	gwm644	FHB spread			
Sumai 3	3BS	gwm493–gwm533	FHB spread	Sumai 3x Australian wheat,four crosses	2 markers	(Xie et al. 2007)
Wuhan 1	4B	wmc238, gwm149	FHB severity, DON content	3 backcross populations	15 SSR markers	(McCartney et al. 2007)
Wuhan 1	4B	wmc245, gwm608	FHB severity, DON content	involving:Nyu Bai, Wuhan 1 and Sumai3	15 SSR markers	
Nyu Bai or Sumai 3	3BSc	gwm566,wmc231, wmc625,wmc693, wmc307,wmc418	FHB severity, DON content		15 SSR markers	
Nyu Bai or Sumai 3	5AS	wmc705,gwm304, gwm154	FHB severity, DON content		15 SSR markers	
Sumai 3	3BS	gwm533,gwm493	FHB severity, DON content		15 SSR markers	
CM-82036	3BS	gwm389,gwm533, barc133	FHB severity, DON content	DH [CM-82036/Remus] /Nandu/2/DH[Frontana/Remus]/Munk	6 markers	(Miedaner et al. 2006; Wilde et al. 2007)
CM-82036	5A	gwm156, gwm304a	FHB severity, DON content		6 markers	
Frontana	3A	gwm720	FHB severity, DON content		6 markers	
Wangshuibai	2B	wmc474, wmc499	FHB severity FHB spread	BC population involving Wangshuibai	2 markers	(Xue et al. 2010a)
Wangshuibai	3B	gwm389,gwm533, barc147, gwm493	FHB spread		4 markers	

Wangshuibai	4B	barc20,gwm513, gwm192,gwm149, cfd22,wmc349	FHB severity		6 markers	
Wangshuibai	5A	barc180,barc117, gwm415,gwm304, mag3794	FHB severity		5 markers	
Dream	6AL	gwm82	FHB severity	DC [Dream/Lynx// Brando]/// [G16-92/Hussar// LP235.1]	1 marker	(Miedaner et al. 2009; Wilde et al. 2008)
Dream	7BS	gwm46	FHB severity		1 marker	
G16-92	2BL	gwm47	FHB severity		1 marker	
CM-82036	3BS	wms389, wms533, barc133	FHB severity	DH CM-82036/Remus //Opus	3 markers	(von der Ohe et al. 2010)
CM-82036	5A	wms304a,wms156	FHB severity	CM-82036/Remus //Anthus	2 markers	
Ning 7840	3BS	gwm533,cfd79		BC population involving Ning 7840	2 markers	(Kang et al. 2011)
Ning 7840	5A	gwm304, barc186			2 markers	
Ning 7840	2DL	gwm539, gwm608			2 markers	

1.3 Powdery mildew

Powdery mildew (PM) is one of the most important wheat diseases in many regions of the world with temperate climate. The disease robs the plant of nutrients and reduces the photosynthetic ability of the leaf. The significant yield losses can range from 5 to 34% (Conner et al. 2003; Griffey et al. 1993) and in certain instances as high as 45% (Hsam & Zeller 2002) .

Integrated managements for combating the disease such as using resistant varieties, crop rotation, destroying infected crop residues and volunteer cereals, balancing the fertilizer applications and using fungicides are available. Among these, breeding resistant cultivar is playing a key role and considered as most economic, environmental and benign way to control this disease.

Most identified PM resistance genes in wheat are race-specific. Though these genes have provided highly effective resistance, the rapid occurrence of corresponding virulence resulted in cultivars losing effective resistance within a short period of time. For instance in Norway, the spring wheat cultivars Bastian, Polkka, Brakar and Avle were all resistant at the time of release, but new virulences occurred before or shortly after their cultivation reached substantial areas (Lillemo et al. 2010b; Skinnes 2002). Race non-specific resistance genes may provide only partial resistance and small effect individually, but when pyramided and incorporated with other genes, highly effective and durable resistance can also be achieved. PM resistance breeding now increasingly focuses on identifying this partial resistance.

1.3.1 Symptoms and causal agent

Powdery mildew is recognized by small white to gray colonies of cottony mycelia (Fig. 6). These colonies usually occur on the upper and lower surfaces of the leaves. The white colonies are first observed on the lowest leaves of plants. Infection can move rapidly up the plant on leaves, sheaths, stems and heads under favorable conditions. As the plant matures, the white powdery colonies become grey-brown in color. Distinct round, black fruiting bodies (cleistothecia) then occur on the colonies.

a b

Figure 6. Symptom of powdery mildew. a. Colonies on a leaf (Lackermann et al. 2010); b. Symptom in the field.

The powdery mildew pathogen is an obligate biotroph. It has two significant differences compared to Fusarium. Though it can affect a wide range of plants such as wheat, barley, grape, melons, tree fruit and so on (Glawe 2008), most species show strict host specificity, in which a given species or race can infect only a narrow range of host plants. The *Blumeria graminis* (syn. *Erysiphe gramin*is) f. sp. *tritici* (*Bgt*) is the agent of wheat PM. Besides this, PM pathogen has its biotrophic nature and can only grow and reproduce on living tissues. The fungus penetrates into the epidermal cells, where it forms haustoria which absorbs nutrients from the living cells (Bélanger et al. 2002) .

1.3.2 Disease cycle

The causal fungus persists as fruiting bodies (cleistothecia) on crop residues, such as straw or stubble. Ascospores that have acquired a maturation period can be released in fall, winter or spring to serve as primary inoculum to infect wheat (Mashall 2009). In mild winter it can also persist as mycelia or conidia on infected volunteer plants or winter

wheat and then serve as inoculum for the coming season. Secondary disease cycles can be induced when sporulation on the plant surface ensues.

Powdery mildew can germinate at temperatures between 10 to 22°C. High relative humidity (>95%) but not free water favors the germination and infection. Disease development will decline rapidly with temperatures above 25°C (Te Beest et al. 2008). Vigorous growth and dense planting favor the disease by promoting high humidity. Powdery mildew also thrives in fields where high rates of nitrogen have been used which not only increases tiller formation, causing dense stands, but also increases the susceptibility of the crop.

When conditions are unfavourable for conidia production such as when plants start to mature, sexual reproduction occurs. PM persists as cleistothecia and waits for favorable conditions to start the next disease cycle.

1.3.3 PM resistance types

Resistance to powdery mildew is generally classified into race-specific and partial based on its inheritance and host specificity.

Race-specific resistance is qualitative and can provide complete or near immune protection to the specific races of pathogens, while it is not effective to others. This type of resistance is mediated by R genes and conforms to the gene-for-gene hypothesis described by Flor (1971). Incompatibility (i.e. resistance of the host) requires the simultaneous presence of the resistance allele in the host and the corresponding avirulence allele in the pathogen. With the co-evolution of host and pathogen, race-specific resistance can be overcome in a short period of time when virulence genes occur in the pathogen population (McDonald & Linde 2002).

Partial resistance is quantitative and controlled by many genes with major and minor effects. This resistance, also known as slow mildewing (Shaner & Finney 1977) and adult plant resistance (Johnson et al. 2003), can delay the progress of the disease and the spore production of the pathogen (Gustafson & Shaner 1982). It is general or horizontal and can

be effective to all races of the PM pathogen and considered to be more durable. Some components such as latent period, pustule production, intensity of sporulation (number of spores produced per lesion), pustule size or sporulation index (0-3 conidial chain density scale) have been considered for accurate evaluation of partial resistance. However, they could not be easily used by breeders and the field assessment is based on visual rating (Robe et al. 1996).

Recent reviews contribute to the understanding of the biotroph-host interaction. The arms race between pathogen and host follows a four-phase 'zig-zag' model (Jones & Dangl 2006; Poland et al. 2009). In phase I, the plant triggers immunity (basal resistance) when it perceives pathogen attack. In phase II, a successful pathogen secretes virulence effectors (specificity determinants) which trigger susceptibility. In phase III, the effectors are recognized by R gene encoded proteins (NB-LRR proteins) which results in resistance, usually hypersensitive cell death at the infection site. In phase IV, natural selection drives the pathogen to avoid recognition by shedding or diversifying the recognized effector gene or acquiring additional effectors. It results in new R specificities and phase I can be triggered again. This interaction results in either complete resistance or susceptibility. However, there are multiple genes involved in the resistance pathway, natural functional mutations could introduce quantitative variation to several or all of the phases involved in the 'zig-zag' model, adding shades of gray to the extremes of complete resistance and susceptibility (Poland et al. 2009). Those genes involved could be possible candidates for partial resistance genes.

1.3.4 Identified PM resistance genes

With the availability of molecular markers, the discovery of new powdery mildew resistance genes has greatly accelerated. So far, at least 43 loci for resistance to wheat powdery mildew (*Pm1–Pm45*) have been identified and assigned to specific chromosomes or chromosome arms (Blanco et al. 2008; Hua et al. 2009; Huang & Röder 2004; Lillemo et al. 2008; Liu et al. 2001; Ma et al. 2011; Miranda et al. 2006; Miranda et al. 2007; Xu et al. 2010; Zhu et al. 2005). And some temporary named loci such as *PmHNK* (Xu et al. 2010), *PmHNK54* (Xu et al. 2011), *Pm2026* (Xu et al. 2008) and

PmLK906 (Niu et al. 2008) have also been identified. At some loci, more than one allele have been identified such as *Pm1, 3, 4, 5* (Hao et al. 2008; Hsam et al. 1998; Hsam et al. 2001; Srichumpa et al. 2005). Most PM resistance loci are dominant, only *Pm5, 9, 26, 42,PmLK906* and *Pm2026* were documented as recessive (Hsam et al. 2001; Hua et al. 2009; Huang et al. 2003; Niu et al. 2008; Niu & He 2009; Rong et al. 2000). At least 27 genes of these loci were transferred from wild relatives of wheat (He et al. 2009).

1.3.5 Mapping for partial resistance

Most of these loci are race-specific and less durable. In contrast, partial resistance is considered to be non-race-specific and inherited quantitatively. It has been shown to be more durable (Kolmer 1996; Lillemo et al. 2008; Singh et al. 1998; William et al. 2003).

So far, with the favor of QTL mapping strategy many partial resistance loci have been identified in diverse resistance sources. The results of 12 studies on partial resistance mapping are summarized in Table 4, with phenotypic variation and position of the individual QTL, linked markers and the population types. Through the common markers, some QTL appear to be common. Massey and its derivative USG3209 have the same QTL on 1BL, 2AL and 2BL. RE9001, Massey, USG3209 and Lumai 21 appear to carry the same QTL on 2B. Saar has the same QTL on 1BL as Massey and USG3209. Additionally, Saar shares the 7DS QTL with Fukuho-komugi. Among all the QTL detected in these studies, the QTL on 1BL, 2AL, 2BL and 7DS were the most frequently detected and all have stable performance across environments and explained considerable proportions of the phenotypic variation. Corresponding to 7DS and 1BL QTL, *Pm38* and *Pm39* have been designated and showed to be co-localized with two leaf rust and yellow rust loci of partial resistance *Lr34*/*Yr18* and *Lr46/Yr29* (Lillemo et al. 2008; Singh et al. 2000; Spielmeyer et al. 2005).

Table 4. Summary of partial resistance mapping of powdery mildew in wheat

Resistance	Source	Variation %	Chr.	Markers	Population	Reference
Forno	Swiss	7.7	1A	psr1201b-psr941	Forno X Oberkulmer RIL	(Keller et al. 1999)
Forno		9.5	1D	psr168-glk558b		
Forno		10	2D	psr932-psr331a		
Forno		15.7	3D	psr1196a-Lrk10-6		
Forno		22.9	5A	psr644a-psr945a		
Forno		10.5	5A	psr911-psr120a		
Forno		16.6	5A	psr1194-psr918b		
Forno		12.6	5B	psr580b-psr143		
RE714	French	16.8–25.3	5D	gwm174	RE714xHardi F2:3	(Chantret et al. 2000)
RE714		28.1–37.7	5D	cfd26	RE714xHardi F2:3	(Chantret et al. 2001)
RE714		4.9-6.9	4A	gbxG036	RE714xHardi DH	
RE714		33.5–37.9	5D	gbxG083c	RE714xHardi DH	
RE714		12.2	6A	MlRE	RE714xHardi DH &F2:3	
RE714		8.8–13.4	6A	gwm427	RE714xHardi F2:3	
RE714		6.4	7A	gwm344	RE714xHardi F2:3	
RE714		1.7	7B	gwm577	RE714xHardi F2:3	
RE714		39.3–43.0	1A	cdo572b-bcd442	RE714xFestin DH	(Mingeot et al. 2002)
RE714		22.7–39.2	2A	Pm4b-gbxG303	RE714xFestin DH	
RE714		22.2–54.3	5D	gwm639a-gwm174	RE714xFestin DH, RE714xHardi DH	
RE714		19.8–53.9	6A	MlRE	RE714xFestin DH, RE714xHardi DH	
RE714		22.8–33.5	7B	pdaC01-gbxR035b	RE714xFestin DH,	
RE714		22.3	4A	gbxG036-gbxG542	RE714xHardi DH	
RE9001	France	12.6	1D	gwm106	RE9001 x Courtot RIL	(Bougot et al. 2006)
RE9001		10.3-36.6	2B	gwm877a-gwm47		
RE9001		19.0	2D	gwm102		
RE9001		16.5	2D	cfd2e		
RE9001		9.3-15.2	3D	cfd152-gwm707		
RE9001		9.0	5D	cfd189		
Massey	US	17	1B	gwm259-wg241	Becker x Massey F2:3	(Liu et al. 2001)
Massey		29	2A	gwm304a-gwm312		
Massey		11	2B	wg338-gwm526a		
Massey		15-17	1B	wg47	Becker x Massey F2:3	(Tucker et al. 2007)

Resistance	Source	Variation %	Chr.	Markers	Population	Reference
Massey		26-29	2A	gwm304		
Massey		11-15	2B	gwm501-gwm191		
USG3209	US	13	1BL	barc80	USG 3209 x Jaypee RIL	(Tucker et al. 2007)
USG3209		59-69	2AL	gwm304		
USG3209		22-48	2BL	gwm47		
Saar	CIMMYT	9.1-35.9	1BL	wmc719-hbe248	Saar x Avocet-YrA RIL	(Lillemo et al. 2008)
Saar		8.7-20.7	3AS	stm844tcac-barc310		
Saar		4.2-15.2	5AL	gwm617b-wmc327		
Saar		4.5-9.7	5BS	barc4-gwm274b		
Saar		19-56.5	7DS	gwm1220-swm10		
Fukuho-komugi	Japan	19.5-26.6	1AS	gdm33-psp2999	Fukuho-komugi x Oligoculm DH	(Liang et al. 2006)
Fukuho-komugi		5.7-8.0	2BL	gwm877.1-wmc435.1		
Fukuho-komugi		9.8-12.0	7DS	Ltn-gwm295.1		
Bainong64	China	7.4-9.9	1A	barc148-wmc550	Bainong64 x Jingshuang16 DH	(Lan et al. 2009)
Bainong64		15.2-22.7	4DL	barc200-gwm165		
Bainong64		9.0-13.2	6BS	barc79-gwm518		
Bainong64		6.3-7.1	7A	barc127-barc174		
Lumai21	China	10.6-20.6	2BS	barc98-barc1147	Lumai21xJingshuang16 F2:3	(Lan et al. 2010)
Lumai21		5.2-10.1	2BL	Xbarc1139-Xgwm47		
Lumai21		5.7-11.6	2DL	Xwmc18-Xcfd233		

1.3.6 Cloned PM genes

So far, two powdery mildew resistance genes have been cloned via map-based strategy, one race-specific gene *Pm3* on 1AS and one partial resistance gene *Lr34/Yr18/Pm38* on 7DS with additional resistances to leaf rust and yellow rust.

Pm3

One of the *Pm3* alleles, *Pm3b*, was first isolated from hexaploid (*Triticum aestivum*) bread wheat genome (Yahiaoui et al. 2004). The candidate gene is a member of the coiled-coil nucleotide binding site leucine-rich repeat (NBS-LRR) type of disease resistance genes. The haplotype analysis at the *Pm3* locus of lines carrying the 10 different *Pm3* resistance specificities led to the cloning of *Pm3* allelic series of *Pm3a, Pm3d* and *Pm3f* (Srichumpa et al. 2005). The other three different alleles of *Pm3c*, *Pm3e* and *Pm3g* were later isolated based on PCR (Yahiaoui et al. 2006). With the fact that the susceptible *Pm3* allele (*Pm3CS*) from the landrace Chinese Spring and the European cultivar Kanzler showed 97–99% sequence identity to the *Pm3* resistance alleles, the authors suggested that *Pm3CS* was probably the origin of *Pm3* resistance alleles. Functional markers for seven *Pm3* alleles (*Pm3a–Pm3g*) have been developed and proved highly diagnostic for specific *Pm3* resistance alleles in a wide range of varieties and breeding lines (Tommasini et al. 2006). Recently, seven new *Pm3* resistance alleles were isolated by the large screening of global gene bank accessions (Bhullar et al. 2009).

The specificities of different alleles revealed (Brunner et al. 2010) that *Pm3a* has a resistance spectrum that completely contains that of *Pm3f*, so is the *Pm3b* and *Pm3c* gene pair. The resistance specificity was determined by variable residues of the N-terminal and a single residue in the C-terminal LRR motifs which is the main determinant of allele specificity. Based on this information, the authors constructed a chimeric *Pm3* gene by intragenic allele pyramiding of *Pm3d* and *Pm3e* that showed the combined resistance specificity and, thus, a broader recognition spectrum compared to the parental alleles.

Lr34/Yr18/Pm38

Lr34 is a gene for durable partial resistance to leaf rust (*Puccinia triticina*) (Singh 1992) and was mapped to 7DS by Dyck (1987). One yellow rust (*P. striiformis f. sp. tritici*) locus *Yr18* was found to be associated with leaf rust (Mcintosh 1992; Singh 1992). Subsequently, association was found between PM resistance and *Lr34/Yr18* (Singh et al. 2000; Spielmeyer et al. 2005). Lillemo et al. (2008) demonstrated that this locus

significantly decreased three disease severities simultaneously and designated it as *Lr34/Yr18/Pm38*.

Fine mapping and physical mapping narrowed this locus into a genetic interval of less than 0.5 cM (Spielmeyer et al. 2008). Recently, *Lr34/Yr18/Pm38* was cloned and shown to encode a putative ABC (adenosine triphosphate–binding cassette) transporter (Krattinger et al. 2009). Alleles of this gene conferring resistance or susceptibility differ by three genetic polymorphisms, one SNP in intron 4, one 3bp deletion in extron 11 and one SNP in extron 12. The two extron differences affect the first transmembrane domain connecting the two nucleotide binding domains and may alter the structure and substrate specificity of the transporter. The authors also suggested that *Lr34* may be involved in leaf senescence or play a more direct role in resistance by exporting metabolites that affect fungal growth.

1.3.7 Marker assisted selection for PM resistance in breeding

Race-specific resistance genes usually provide complete protection and are frequently used in wheat breeding (Hsam & Zeller 2002). However, the durability of such resistance is usually very short and breeding durable PM resistance is a major task for wheat breeders. Durable resistance could be achieved by pyramiding different PM resistance genes into one cultivar, employing partial resistance loci or combining both types of resistance. Molecular markers which are closely linked with resistance genes could facilitate the selection for corresponding resistance genes, especially for partial resistance. Because of some reasons, field selection for partial resistance is difficult. First, the effect of partial resistance would be masked when race-specific resistance is present in the plant. Second, when the matching virulence is low in the pathogen population, race-specific resistance may provide some partial resistance phenotype in the field (Lillemo et al. 2010a; Yu et al. 2001).

Some examples have shown that marker assisted selection is effective for pyramiding either race-specific resistance genes or partial resistance loci. With the assistance of molecular markers, the powdery mildew resistance genes *Pm1, Pm4a* and *Pm21* have been successfully introgressed in a pair-wise manner into an elite wheat cultivar Yang158 (Liu et al. 2000). It was demonstrated that with marker assisted selection, RILs combining 1B, 2A and 2B QTL resistance showed greatest resistance compared to other RILs in the population from USG3209 /Jaypee (Tucker et al. 2006)

These closely linked markers for resistance loci are also useful in map based cloning, understanding the mechanism of resistance and in development of functional markers which could provide more accuracy for the prediction of resistance gene in the plants. The map based cloning of *Pm3* (Yahiaoui et al. 2004) and *Lr34/Yr18/Pm38* (Spielmeyer et al. 2008) have been achieved through these markers and functional markers now are available for *Pm3*, both general and allele specific (Tommasini et al. 2006) and for *Lr34/Yr18/Pm38* (Lagudah et al. 2009). The functional markers for *Pm3* have been used in diversity studies (Bhullar et al. 2010) and evolution studies (Cloutier et al. 2010; Yahiaoui et al. 2009).

So far, there are only a few studies on partial resistance and partial resistance loci have been identified in over 9 varieties or breeding lines (Table 4). In combating powdery mildew, it is essential to continue searching for new effective resistance genes to enrich the resistance gene pool.

2. Main results

Paper I Two major resistance QTL are required to counteract the increased susceptibility to Fusarium head blight of the *Rht-D1b* dwarfing gene in wheat

In the DH population from the cross between the Swedish cv. Avle (susceptible spring type, *Rht-D1a*) and breeding Line 685 (resistant winter type, *Rht-D1b*), the *Rht-D1* locus on 4D explained up to 38% of the phenotypic variation and was the most important QTL for FHB severity after spray inoculation, but it did not show any effect after point inoculation. *Fhb1* on 3BS contributed resistance after both inoculation methods, but was relatively more important under point inoculation. Comparison of phenotypic effects of different allele combinations revealed that a combination of both *Fhb1* and the QTL on 5A was required to counteract the increased susceptibility conferred by *Rht-D1b*. Although breeding of FHB resistant cultivars with this dwarfing allele is possible, it requires the pyramiding of several resistance QTL to achieve adequate levels of resistance.

Paper II The Fusarium head blight resistance in bread wheat line Shanghai-3/Catbird is under multigenic control and associated with anther extrusion

In a recombinant inbred lines (RIL) population of Naxos x SHA3/CBRD, FHB traits were negatively correlated with both plant height (PH) and anther extrusion (AE) after spray/spawn inoculation. The QTL analysis showed that the *Rht-B1b* dwarfing allele co-localized with a QTL for low AE and increased susceptibility after spawn/spray inoculation. In general, SHA3/CBRD contributed most of the favourable alleles for reduced head blight severity after spray inoculation, Naxos contributed more favourable alleles for reduction in FDK and DON and to severity resistance after point inoculation. SHA3/CBRD contributed a major resistance QTL close to the centromere on 2DL affecting FHB severity and DON. This QTL was also associated with PH and AE, with favourable alleles contributed by SHA3/CBRD. Several QTL for PH and AE were detected, and reduced PH or low AE were always associated with increased susceptibility after spawn/spray inoculation. Most other minor FHB QTL from SHA3/CBRD were

associated with AE, while QTL from Naxos were mostly not. While after point inoculation, except the 2DL QTL which was common across all inoculation methods, no other QTL for FHB traits was associated with PH or AE. Marker-assisted selection based on the 2DLc QTL from SHA3/CBRD combined with phenotypic selection for AE is recommended for resistance breeding based on this valuable source of resistance.

Paper III Partial resistance to powdery mildew in German spring wheat Naxos is based on multiple genes with stable effects in diverse environments

In the same population as Paper II, we identified that partial resistance to powdery mildew in German spring wheat Naxos is controlled by two major QTL on 1AS and 2DL, and two minor ones on 2BL and 7DS. The major QTL on 1AS with resistance from Naxos was detected close to the *Pm3* locus in all environments, and explained up to 38% of the phenotypic variation. QTL with resistance from SHA3/CBRD were detected on 1RS, 2DLc, 6BL and 7AL. The QTL on the 1B/1R translocation showed highly variable effects across environments corresponding to known virulence differences against *Pm8*. SHA3/CBRD was shown to possess the *Pm3* haplotype on 1AS, but none of the known *Pm3a-g* alleles. The RIL population did not provide any evidence to suggest that the *Pm3* allele of SHA3/CBRD acted as a suppressor of *Pm8*.

3. Discussion

3.1 Breeding FHB resistance in the light of dwarfing genes and AE.

3.1.1 Is this negative association general?

The negative association between plant height and FHB resistance has been widely observed, especially *Rht-B1, Rht-D1* and *Rht8* (Draeger et al. 2007; Miedaner & Voss 2008; Srinivasachary et al. 2008; Srinivasachary et al. 2009; Voss et al. 2008). Moreover, plant height QTL on 3A and 7A were identified with negative effect on FHB resistance (Mao et al. 2010). In **paper II**, we found that most resistance QTL from SHA3/CBRD coincided with plant height. It appears that this negative association is general. Also when the NILs were raised to the same height, the negative effect of dwarf genes disappeared (Yan et al. 2011) and the authors suggested that this negative effect might only be because of plant height *per se*. In **paper II**, we found it appeared general that most of FHB resistance QTL were associated with PH. In contrast, a plant height QTL on 2B coincided with FHB resistance QTL but conferred resistance (Draeger et al. 2007). At the same time, *Rht-B1b* and *RhtD-1b* have different effects on Type II resistance. Nicholson et al. (2008) are working on this via *Arabidopsis thaliana* to understand the mechanism. For other coincidence, all the mapping populations used to investigate this association were all preliminary with hundreds lines and the resolution of maps were usually low. And we know that genes are clustered in certain regions on wheat chromosomes. Under this circumstance, it might give false positive results about associations. With the development of markers and fine mapping, the shade would be lighted in future.

3.1.2 Pyramiding more resistance loci or use other *Rht* genes?

In **paper I**, we found that at least *Fhb1* and the 5A QTL are required to counteract the negative effect on FHB resistance from *Rht-D1b*. Still, more resistance factors would be required to achieve desired levels of resistance. Dwarf plant is desired to achieve better yield and prevent lodging. Though we still don't know if this negative association is general, at least we know they influence FHB resistance differently. *Rht-B1b* has less

effect on FHB resistance compared to *Rht-D1b* (Srinivasachary et al. 2009). Moreover, *Rht-B1b* increased Type II resistance, while *Rht-D1b* had no effect. It can therefore be concluded that *Rht-B1b*, at least under moderate disease pressure, can be used to achieve the desired plant height with less compromising effect on FHB resistance than *Rht-D1b* (Miedaner & Voss 2008; Srinivasachary et al. 2009). The *Rht8c* dwarfing allele commonly used in southern European breeding programs is also associated with less negative impact on FHB resistance and could be considered as well (Miedaner & Voss 2008).

3.1.3 AE, can we use it as a criterion in field selection?

In **paper II**, we found that AE was positively associated with FHB resistance which was confirmed by QTL results that six out of seven AE QTL coincided with FHB severity.

In barley, cleistogamous cultivars exhibited greater resistance than open flowering type (Yoshida et al. 2005), but they could be infected later when the anthers were forced out of the floret by the growing caryopsis (Yoshida et al. 2007). Skinnes et al. (2010) suggested that anthers retained and trapped between glumes provide a substrate for saprophytes like *Fusarium* and subsequent infection of living tissues occur under conducive conditions. Hyphal density on anthers was observed higher than that on the inner surfaces of glumes (Kang & Buchenauer 2000). Hence AE is considered as an avoidance mechanism.

Both **paper II** and Skinnes et al. (2010) showed that lines with high AE tend to have low severities. The high heritability of AE indicates a strong selection response over years (Skinnes et al. 2010) and the author also stated that AE was easier to monitor than flower opening *per se*. We both recommend that an early screening of AE could reduce the FHB severity. But differently, in **paper II** we didn't find any negative correlation between DON content and AE. One possible reason could be that late infection occurred more frequently in the present study, and AE only has effect on infection at the time of anthesis.

3.2 General implications for partial resistance to FHB and PM

In our study, the RIL population of SHA3/CBRD x Naxos was used both in powdery mildew and Fusarium head blight study. Although these two diseases did not have apparent correlations, we did find coincident QTL both for FHB resistance in **paper II** and PM resistance in **paper III** on 2DLc, 2BL and 7A. Among them, the resistances at two QTL were from the same parents, the 2BL QTL was from Naxos and 2DLc from SHA3/CBRD. Markers close to these QTL could have potential to improve the resistance level both to FHB and PM when Naxos or SHA3/CBRD is used as resistance sources. In this population, some RILs integrating both resistances could be used as breeding lines for cultivar development.

MAS did provide higher gain per year than phenotypic selection, while phenotypic selection still has its advantages. Miedaner et al. (2009) observed that lines in the MAS group were little taller than that in its counter group and the authors suggested additional phenotypic selection can enhance the selection gain. In **Paper I** the resistant parent Line685 which was developed from field selection still achieved high level of FHB resistance even in the background where *Rht-D1b* conferred a big negative effect. This also indicates the advantage of phenotypic selection which can efficiently incorporate both major QTL and many minor loci besides what we detected here. Therefore, MAS combined with additional phenotypic selection is recommended in partial resistance breeding in wheat.

4 Conclusion

- A combination of both *Fhb1* and the QTL on 5A was required to counteract the increased susceptibility conferred by *Rht-D1b*. Although breeding of FHB resistant cultivars with this dwarfing allele is possible, it requires the pyramiding of several resistance QTL to achieve adequate levels of resistance.

- Most FHB QTL were associated with high AE. AE could be used as a visual assessment for FHB resistance in screening a big breeding population at early stage in field.

- A major QTL on 2DLc contributed resistance to PM and different types of resistance to FHB, when SHA3/CBRD is used as a resistance sources. Selection based on this QTL would improve both disease resistances.

- Most PH QTL compromised FHB resistance, the negative association between them and FHB appears to be general.

- The PM resistance in German spring wheat Naxos is controlled by two major QTL on 1AS and 2DL, and two minor ones on 2BL and 7DS. The 1AS QTL with resistance from Naxos was detected close to the *Pm3* locus and likely contributed partial resistance.

5 Future perspective

Stable QTL, closely linked markers (functional makers are even better) and the efficiency compared to the phenotypic selection are important key factors to achieve successful MAS in breeding. Some major QTL for resistance have been identified across different environments and with different evaluation methods (FHB). Our further work would involve following aspects.

1. To verify them in different population, make sure those QTL are real and not false positives. To evaluate the selection efficiency of MAS in different populations compared to the phenotypic selection.

2. To enrich the QTL regions and find new closer markers for MAS which could provide better prediction for resistance loci and accordingly will improve the selection efficiency. With undergoing of map-based cloning of resistance QTL, functional markers would be perfect for use in MAS.

3. To continue searching for new resistance sources. The diversity of resistance always needs to be expanded to catch up with the co-evolution of pathogen and plant.

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Paper I

RESEARCH

Two Major Resistance Quantitative Trait Loci are Required to Counteract the Increased Susceptibility to Fusarium Head Blight of the *Rht-D1b* Dwarfing Gene in Wheat

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ABSTRACT

Fusarium head blight (FHB) is a destructive wheat (*Triticum aestivum* L.) disease of global importance. The widely used dwarfing allele *Rht-D1b* has recently been shown to compromise FHB resistance. The objectives of this study were to investigate the impact of this dwarfing allele in a segregating population with major resistance quantitative trait loci (QTL) derived from 'Sumai-3' and Nobeokabozu, and to determine how many resistance QTL are needed to counteract its negative effect. Fusarium head blight resistance was evaluated in four field trials with spray inoculation and two field trials with point inoculation in a double-haploid (DH) population from a cross between the Swedish cv. Avle (susceptible spring type; wild-type allele *Rht-D1a*) and Line 685 (resistant winter type; semi-dwarf allele *Rht-D1b*). The *Rht-D1* locus explained up to 38% of the phenotypic variation and was the most important QTL for FHB severity under spray inoculation but did not show any effect after point inoculation. *Fhb1* on 3BS was detected with both inoculation methods but was relatively more important after point inoculation. Another two QTL on 5A and 2BL were detected after spray inoculation and a QTL on 2D after point inoculation. Comparison of phenotypic effects of different allele combinations revealed that a combination of both *Fhb1* and the 5A QTL was required to counteract the increased susceptibility of *Rht-D1b*. Although breeding of FHB resistant cultivars with this dwarfing allele is possible, it requires the pyramiding of several resistant QTL to achieve adequate levels of resistance.

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Abbreviations: CIM, composite interval mapping; d°C, day degrees; DArT, diversity array technologies; DH, double haploid; FHB, Fusarium head blight; LOD, logarithm of the odds; MAS, marker-assisted selection; QTL, quantitative trait locus/loci; SIM, simple interval mapping; SSR, simple sequence repeat.

Fusarium head blight (FHB), also known as scab, is a destructive disease of wheat (*Triticum aestivum* L.) in many regions around the world. It can be caused by several species of *Fusarium*, but *F. graminearum* (Schwabe) [teleomorph: *Gibberella zeae* (Schwein.) Petch] and *F. culmorum* (W.G. Sm.) Sacc. are usually the most important (McMullen et al., 1997). It causes accumulation of mycotoxins such as deoxynivalenol, nivalenol, and zearalenone in infected kernels, which is a threat to human beings and livestock. In Manitoba (Canada) the economic losses to wheat producers reached US$300 million from 1993 to 1998 (Windels, 2000). Moister and warmer weather in combination with agronomic practices such as reduced tillage, the lack of adequate crop rotation, and cultivation of susceptible cultivars all contribute to epidemics (Beyer et al., 2006; Champeil et al., 2004; Dill-Macky and Jones, 2000; Edwards, 2004). Breeding FHB-resistant varieties is considered the most effective, economic, and environmental way to control this disease.

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Resistance to FHB in wheat is a complex quantitative trait. Five types of host resistance have been described (Mesterhazy et al., 1999), among which Type I (resistance to invasion) and Type II (resistance to fungal spread) were first described by Schroeder and Christensen (1963) and have been extensively studied because of their relatively easy visual evaluation. Point inoculation of single florets in the spike is commonly used to evaluate Type II resistance, while spray inoculation reflects a combination of Type I and Type II resistance. Fusarium head blight symptoms are highly influenced by environmental conditions and accurate phenotypic evaluation in multiple environments is necessary to get reliable results.

Resistance breeding has progressed slowly due to the complex genetics and difficulties of large-scale and labor-costing phenotyping. In recent years, many quantitative trait loci (QTL) of FHB resistance have been identified in different populations (Buerstmayr et al., 2009; Liu et al., 2009). The most prominent QTL for FHB resistance have been associated with specific types of resistance: Type II resistance on chromosome 3BS (Anderson et al., 2001; Bai et al., 1999; Waldron et al., 1999) and 6B (Anderson et al., 2001; Cuthbert et al., 2007; Yang et al., 2003) and Type I resistance on 3A (Steiner et al., 2004; Yu et al., 2008) and 5A (Buerstmayr et al., 2003a, b; Chen et al., 2006; Steiner et al., 2004). McCartney et al. (2007) demonstrated that marker-assisted selection (MAS) can be an efficient strategy for introgressing FHB resistance into adapted elite varieties.

The realization that resistance may be compromised by dwarfing genes calls for special attention in breeding. The 'Norin 10' genes *Rht-B1b* and *Rht-D1b* (Gale and Youssefian, 1985) have been widely used in modern wheat breeding since the Green Revolution to prevent lodging and increase the yield potential. These giberellic acid–insensitive alleles are probably present in more than 90% of the world's semidwarf wheat crop (Worland et al., 1998). Results from several mapping populations have recently indicated that *Rht-D1b* coincides with a major QTL for FHB susceptibility when spray inoculation is used (Draeger et al., 2007; Holzapfel et al., 2008; Srinivasachary et al., 2008, 2009). These results have been confirmed in experiments with near-isogenic lines showing that *Rht-D1b* increases susceptibility after spray inoculation, whereas *Rht-B1b* may or may not do so, depending on genetic background and/or experimental conditions (Hilton et al., 1999; Miedaner and Voss, 2008; Srinivasachary et al., 2009). *Rht-D1b* increased FHB severity by 52% in a 'Mercia' background and 38% in a 'Maris Huntsman' background, while *Rht-B1b* was less associated with increased susceptibility (Miedaner and Voss, 2008). Similar conclusions were arrived at by Srinivasachary et al. (2009) comparing the two genes using spray inoculation. With point inoculation, however, *Rht-B1b* was less affected than the tall control, while *Rht-D1b* was similar to the control. The implication is that under high disease pressure these two alleles primarily decrease Type I resistance to different degrees and differentially affect Type II. It has been proposed that the increased FHB susceptibility of *Rht-D1b* is due to pleiotropy or closely linked genes than plant height per se (Holzapfel et al., 2008; Miedaner and Voss, 2008; Srinivasachary et al., 2009). Yan et al. (2011), however, showed that there were no negative effects on Type I resistance when *Rht-B1b* and *Rht-D1b* near-isogenic lines were raised to the same heights at their tall counterparts, while the same dwarfing genes were associated with increased Type II resistance after point inoculation. This indicates the effect of plant height per se, which probably mediated by microclimatic effects in the canopy. Important questions are: Can the negative effect of *Rht-D1b* be compensated for by resistance breeding, and in that case, how many resistance genes are required to counteract it? Alternatively, can other dwarfing genes be used to achieve the desired plant height with less negative impact on FHB? The effect of *Rht-D1b* has so far only been assessed in genetic backgrounds of European winter wheat with relatively moderate levels of resistance. The objectives of this study were to investigate the impact of this dwarfing allele in a segregating population with major resistance QTL derived from 'Sumai-3' and Nobeokabozu commonly used for MAS and to determine how many resistance QTL are needed to counteract its negative effect.

MATERIALS AND METHODS

Plant Materials

An F_2–derived double-haploid (DH) population of 171 lines was developed from the cross between Line 685 and 'Avle' using the wheat × maize system (Laurie and Bennett, 1988). Strong winter types were excluded as the population was developed under normal greenhouse conditions. Thirty-four intermediate types appeared in the field at Ås, Norway, and could not be tested here for FHB resistance. 'Avle' is a susceptible spring wheat cultivar with the pedigree TW232-62/'Kadett'//'Nemares' from the Swedish breeding company Lantmännen SW Seed Ltd. Line 685 is a resistant winter wheat line from the cross 'Sagvari'/Nobeokabozu//Mini Mano/'Sumai-3' developed by the Cereal Research Institute, Szeged, Hungary.

Field Experiments

Norway

Spray inoculation evaluation was performed at Vollebekk Research Farm in Ås, Norway, over 2 yr (2004 and 2005). The 137 spring types of the DH population were planted in May in hill plots, 40 by 45 cm apart in three replicates following a randomized complete block design. Propiconazole plus fenpropidin were applied at rates of 125 and 450 g ha^{-1}, respectively, 1 wk before anthesis to control other disturbing pathogens without affecting FHB. A bundle of about 10 to 15 heads per plot were inoculated with hand sprayers at full flowering by spraying 10 to 15 mL of a conidial suspension at 1×10^5 spores ml^{-1} of *F. culmorum*. The inoculum consisted of a mixture of five isolates and was produced as described by Semagn et al. (2007). Inoculated heads

were covered with a transparent polyethylene bag as described by Mesterhazy (1995) for 2 to 3 d (45 day degrees [d°C]). The proportion of infected spikelets per bundle was estimated visually using a linear scale from 0 to 100%. Fusarium head blight severity was scored three times each year on the basis of constant temperature sums after inoculation: 267, 385, and 502 d°C in 2004 and 240, 295, and 348 d°C in 2005. The mean FHB severity of the three scores in each year was used for further analysis. Plant height was scored in 2004 and 2008.

Hungary

A total of 167 DH lines (spring and winter) were tested for FHB resistance in Hungary (Cereal Research Non-Profit Limited Company, Szeged, Hungary) in 2006 and 2008. The nurseries were sown in October using 170 cm rows at 18 cm distance with three rows per plot and one replicate per line. Four groups of 15 to 25 heads per plot were sprayed from all sides using about 15 to 20 mL of conidial suspensions of 0.7 to 4×10^5 spores ml^{-1} and covered by polyethylene bags that were removed after 48 h. Two of the groups were sprayed with a single *F. culmorum* isolate and the other two with a single isolate of *F. graminearum*. The isolates were tested for aggressiveness as described by Mesterhazy (1985). Two inoculations were made at full flowering every year. The percentage of infected spikelets was recorded 10 d after inoculation and repeated every 3 or 4 d as long as the control heads were green. The mean of the FHB severity scores in each year was used for further analysis.

China

Point inoculations were performed to evaluate Type II resistance at the Jiangsu Academy of Agricultural Sciences, Nanjing, China, for 2 yr (2007 and 2008). All 171 lines were sown in late October in 150 cm rows at 33 cm distance in one randomized replicate each year. Macroconidia were produced in mungbean extraction liquid medium as described by Shi et al. (2008). An aggressive *F. graminearum* strain F0613 was used both in 2007 and 2008. At the heading stage, a single floret in the middle of each of 20 heads per row was inoculated with about 20 μL conidial suspension of 1×10^5 spores ml^{-1}. Twenty days after inoculation the number of infected spikelets and the total number of spikelets per head were counted and the percentage of infected spikelets calculated for each head. The mean FHB severity of all 20 heads was calculated and used for further analysis.

Genetic Map

A total of 127 polymorphic simple sequence repeat (SSR) markers covering all the chromosomes were selected from consensus maps (Somers et al., 2004; GrainGenes: USDA-ARS, 1993) and used for initial genotyping of the DH population. Diversity array technologies (DArT) markers and then more SSR markers from 3BS and 5A QTL regions were supplemented. In addition, umn10, which is a highly diagnostic marker of *Fhb1* (Liu et al., 2008), and a functional marker of the dwarfing locus *Rht-D1* (Ellis et al., 2002) were also genotyped. After initial QTL detection, the genetic map was refined with more SSR markers in detected QTL regions. The genotypic data of 166 lines including 170 DArT and 166 SSR loci were finally used to construct a genetic linkage map with the software JoinMap v. 3.0 (Van Ooijen and Voorrips, 2001). Map distances were based on the Kosambi function, and consensus map information was used to assign linkage groups to chromosomes.

Statistical Analysis and Quantitative Trait Loci Detection

The phenotypic data was analyzed using the SAS software package (SAS Institute, 2004). The distribution of each trait in each year and location was tested for normality using PROC UNIVARIATE and Pearson correlation coefficients were calculated using PROC CORR. Analysis of variance was performed using the PROC GLM. Histograms and scatterplots were created in Minitab (Minitab, 2007) and Sigmaplot (Systat Software, 2006).

Quantitative trait loci analysis was performed with PLABQTL v. 1.2 (Utz and Melchinger, 1996). Simple interval mapping (SIM) was conducted first to detect the major QTL for FHB. The markers most closely linked to each QTL across environments were then used as cofactors in composite interval mapping (CIM). Significant QTL in single environments and for the overall mean were decided based on 1000 permutations and fivefold cross validation for each phenotypic trait. The logarithm of the odds (LOD) threshold was set at 2.9 after permutation. Quantitative trait loci reaching this level in one environment were also reported for other environments if they showed significant effects in multiple regression. Genetic map drawing and QTL marking were conducted by the software MapChart v.2.1 (Voorrips, 2002).

RESULTS

Phenotypic Evaluation

Histograms of mean FHB severity in different locations are shown in Fig. 1. Distributions of all the traits in each environment were close to normal except for FHB severity in Hungary, which was skewed toward low infection levels in both years. Fusarium head blight severity ranged from almost 0 to over 50% in Norway and Hungary and to over 90% in China. In all environments there was a continuous variation among the lines with transgressions mostly toward higher susceptibility.

Plant height showed highly significant negative correlations with FHB severity after spray inoculation (Table 1; Fig. 2). These correlations were greatly reduced or absent in *Rht-D1a* and *Rht-D1b* subpopulations ($r = -0.03$ to -0.38). Fusarium head blight severity and plant height were always uncorrelated under point inoculation in China.

Map Construction and Quantitative Trait Loci Mapping of Fusarium Head Blight Resistance

From the total of 336 polymorphic marker loci 277 loci were assembled into 51 linkage groups. The genetic map spanned a total of 1076 cM and represented all chromosomes except 3D.

Quantitative trait loci for FHB severity were detected on 4D, 3BS, and 5A by SIM in most environments. Composite interval mapping was run with the consistent QTL from SIM as cofactors (Table 2; Fig. 3). Five QTL for resistance were identified with favorable alleles either from the resistant Line 685 or susceptible 'Avle'. The most important

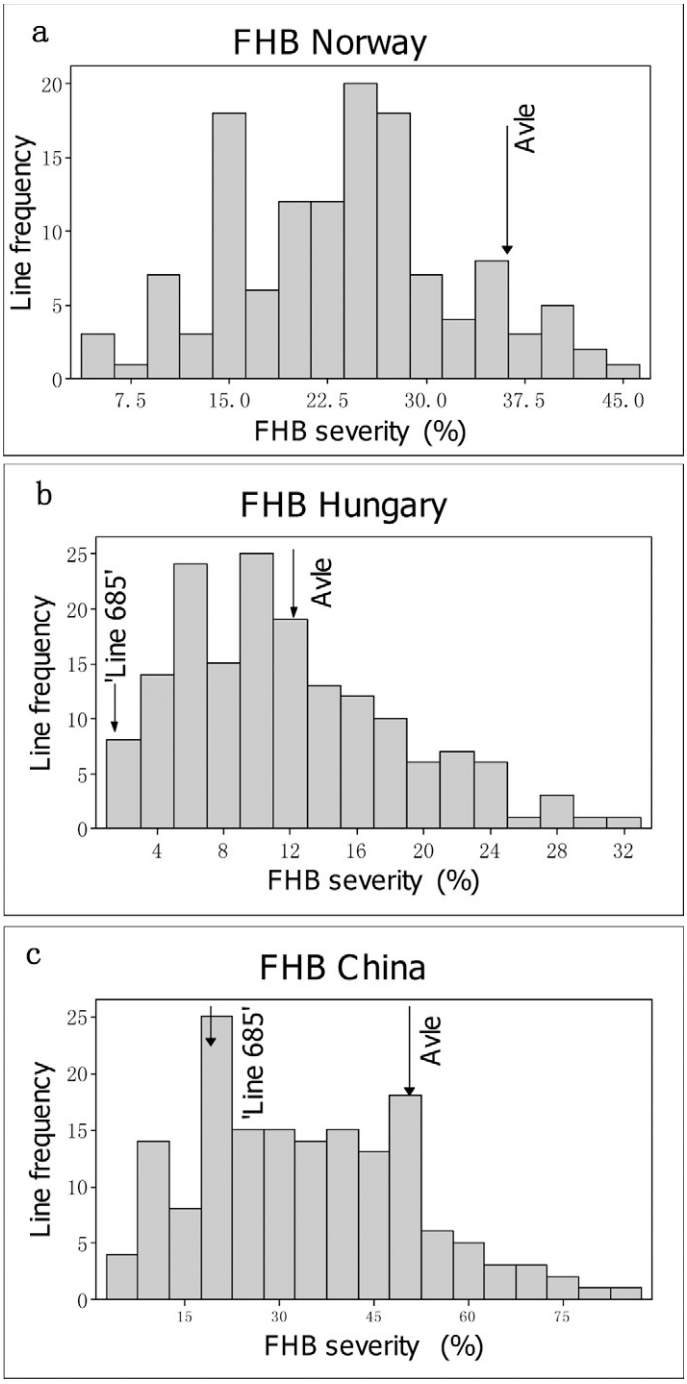

Figure 1. Frequency distributions of Fusarium head blight (FHB) in the Line 685 × 'Avle' double-haploid (DH) population. (a) FHB severity mean in Norway in 2004 and 2005; (b) FHB severity mean in Hungary in 2006 and 2008; c) FHB severity mean in China in 2007 and 2008.

QTL for FHB severity after spray inoculation in Norway and Hungary mapped to 4D between *XwPt-5809* and *Rht-D1*. It explained from 10 to 38% of the phenotypic variation and was consistently detected in each of the five cross validation splits. This QTL coincided with a major plant height QTL. In contrast, no QTL for FHB severity was detected at this position using point inoculation in China (Table 2).

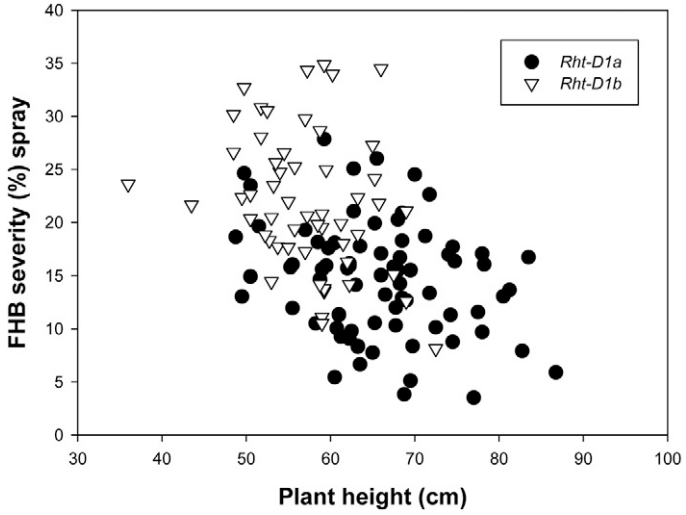

Figure 2. The relationship between plant height and Fusarium head blight (FHB) severity after spray inoculation (mean across both years in Norway and Hungary) in the Line 685 × 'Avle' double-haploid (DH) population. Each DH line was plotted based on the *Rht-D1* status: *Rht-D1a*, wild tall allele; *Rht-D1b*, semidwarf allele.

Table 1. Pearson correlation coefficients between plant height mean and Fusarium head blight severity in different environments for the Line 685 × 'Avle' double-haploid population.

Environment	Whole population	Subpopulations	
		Rht-D1a	*Rht-D1b*
Norway spray inoculation 2004	–0.16*	–0.03	–0.14
Norway spray inoculation 2005	–0.52***	–0.35*	–0.38*
Hungary spray inoculation 2006	–0.39***	–0.32*	–0.18
Hungary spray inoculation 2008	–0.36***	–0.18	–0.10
China point inoculation 2007	0.02	–0.001	–0.02
China point inoculation 2008	–0.04	–0.04	0.11

*Significant at 0.05 level.
***Significant at 0.001 level.

A QTL at the *Fhb1* locus near *Xumn10* and *Xbarc147* on 3BS was detected using both inoculation methods and resistance was contributed by Line 685. Its impact varied strongly: while accounting for 14% of the phenotypic variation in mean FHB severity after point inoculation in China, it explained on average less than 7% after spray inoculation in Norway and Hungary.

The frequently detected QTL on chromosome 5A was of greater magnitude than the 3BS QTL after spray inoculation when considering the mean data across 2 yr in Norway and Hungary. The resistance at this locus was derived from Line 685. It explained almost 17% of the phenotypic variation in Norway in 2005 but only around 5% in Hungary and was not detected after point inoculation in China. Though the intervals differed slightly between Norway 2004 (*Xbarc056–Xbarc40*) and Norway 2005 and Hungary (*Xgwm156–Xbarc141*), they were considered the same QTL because of overlapping confidence intervals.

In Hungary another well cross-validated QTL was detected on 2B near *Xgwm382b* and *Xbarc122*. The

Table 2. List of quantitative trait loci (QTL) for plant height and Fusarium head blight (FHB) severity detected by composite interval mapping with fivefold cross validation in the Line 685 × 'Avle' double-haploid population. The percentage of explained phenotypic variation (R^2) in the multiple regression models is shown. QTL that were detected with a logarithm of the odds score above 2.9 determined by 1000 permutation tests are underlined. Other putative QTLs are also listed if they showed significant contribution in the multiple regression model.

QTL location	Plant height	FHB Norway spray inoculation				FHB Hungary spray inoculation				FHB China point inoculation				Resistance source
		2004	2005	Mean	5 splits	2006	2008	Mean	5 splits	2007	2008	Mean	5 splits	
2BL			7.0			15.3	6.2	8.2	4					Avle
2D										9.7		6.5	1	Line 685
3BS		14.8		6.3	2		5.5	6.5	1	10.2	10.9	13.6	5	Line 685
4D	24.4	9.8	38.2	28.1	5	16.1	35.8	30.5	5					Avle
5A		8.4	16.6	13.6	4	4.9	5.2	6.7	1					Line 685
Total				34.9				35.1				18.0		

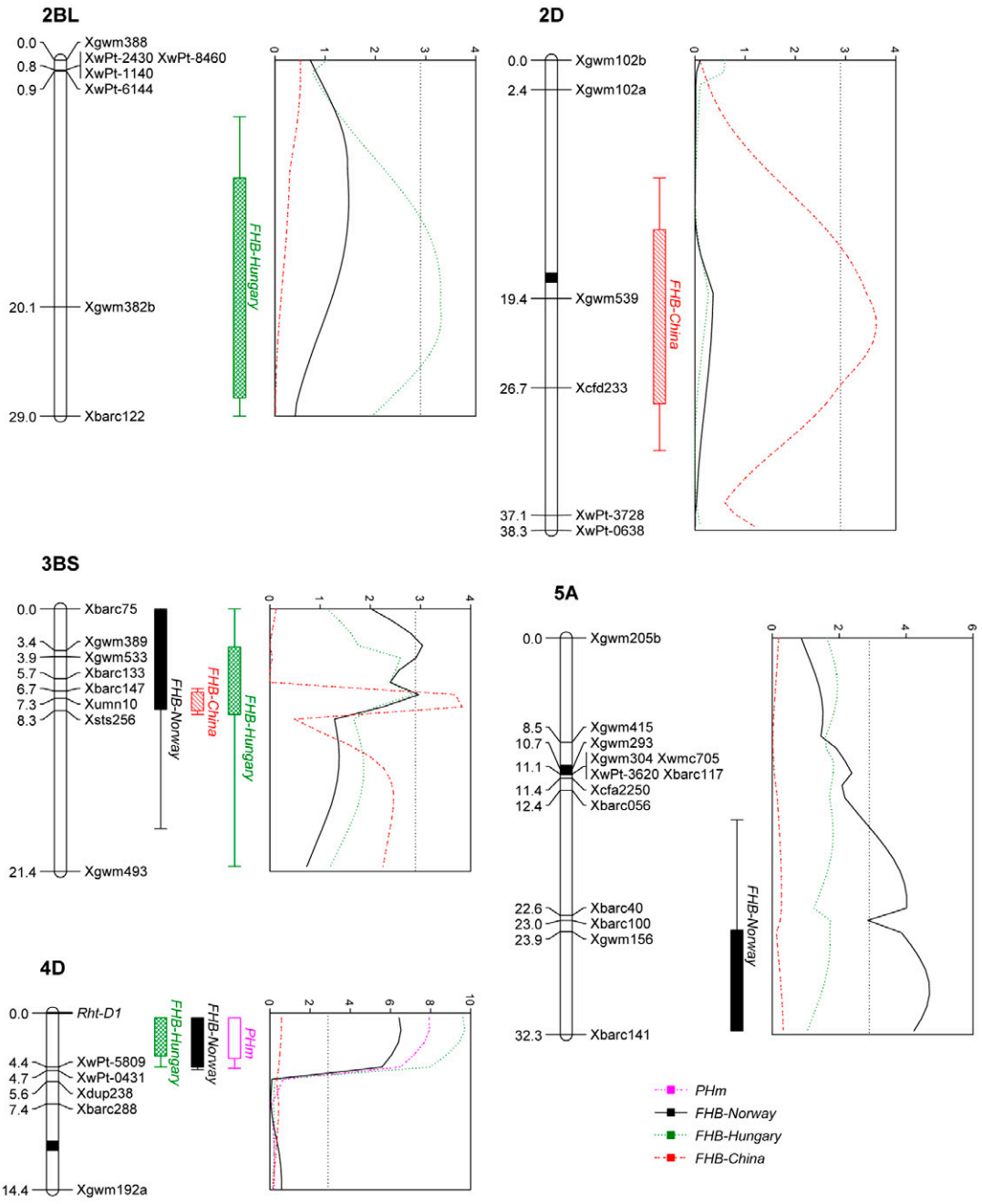

Figure 3. Linkage groups with significant quantitative trait loci (QTL) with corresponding logarithm of the odds (LOD) curves obtained from composite interval mapping (CIM). Genetic distances are shown in centimorgans to the left of the chromosomes. A threshold of 2.9 is indicated by a dashed vertical line in the LOD graphs. The approximate positions of centromeres are indicated by solid squares. QTL positions in each location were all based on the mean severity over years. FHB, Fusarium head blight; PHm, plant height mean.

Table 3. Phenotypic effects of alleles affecting plant height and Fusarium head blight (FHB) in different environments.

	Rht-D1†			3BS‡			5AL§		
	a	b	Difference	–	+	Difference	–	+	Difference
Plant height	66.0	57.1	–8.9***	63.4	60.5	–2.9	61.5	64.5	3.0
FHB spray inoculation Norway	20.3	28.2	7.9***	25.1	21.0	–4.1**	25.2	20.1	–5.1*
FHB spray inoculation Hungary	9.1	15.6	6.5***	12.7	9.9	–2.8**	12.5	10.4	–2.1
FHB point inoculation China	33.3	36.0	2.7	38.9	25.6	–13.3***	36.3	30.5	–5.8
Number of lines	96	67		109	52		114	48	

*Significant at 0.05 level.

**Significant at 0.01 level.

***Significant at 0.001 level.

†a and b mean *Rht-D1a* and *Rht-D1b* allele, respectively.

‡Alleles at the 3BS quantitative trait loci (QTL) are based on *Xumn10*.

§Alleles at the 5AL QTL are based on the flanking markers *Xbarc141* and *Xgwm156*.

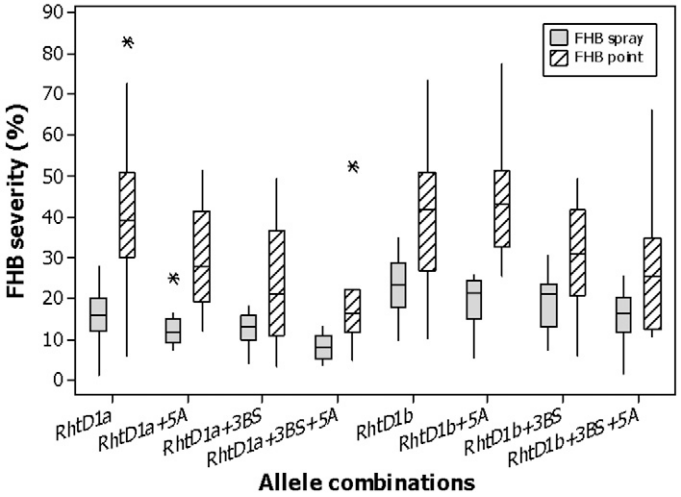

Figure 4. Effects of *Rht-D1*, 3BS and 5A allele combinations on Fusarium head blight (FHB) severity after spray and point inoculation in the Line 685 × 'Avle' double-haploid population. "FHB spray" is overall mean severity after spray inoculation. "FHB point" is overall mean severity after point inoculation. The different allele combinations were determined by the flanking markers *Xbarc141* and *Xgwm156* for the 5A quantitative trait locus (QTL) and *Xumn10* for the 3BS QTL.

resistance at this QTL was contributed by the susceptible parent Avle and explained from 6 to 15% of the phenotypic variation, that is, more than the 3BS QTL.

In China in 2007 a resistance QTL was detected on 2D near the marker loci *Xcfd233* and *Xgwm539*. The resistance was derived from Line 685 and explained 7% of the phenotypic variation.

The Relative Magnitudes of *Rht-D1* and Major Fusarium Head Blight Resistance Quantitative Trait Loci

The *Rht-D1*, 3BS, and 5A alleles were used to classify the DH lines into subpopulations (Table 3). Plant height on average was reduced by 13% in the *Rht-D1b* subpopulation, but the FHB susceptibility relative to *Rht-D1a* significantly increased by 39% in Norway and 71% in Hungary. After spray inoculation both in Norway and in Hungary, the 3BS and 5A resistance QTL alleles on average balanced the negative effect of *Rht-D1b* (exceeding it in Norway but not in Hungary). After point inoculation in China, the 3BS resistance had a predominating effect, while the *Rht-D1* locus and the 5A resistance QTL showed no significant effect. Box plots of DH lines with all triple locus combinations are shown in Fig. 4.

Days to flowering was also classified based on *Rht-D1* and was unaffected (data not shown). *Rht-D1* explained 24% of the phenotypic variation for plant height and was the only QTL detected for this trait. Still, the range and distribution in plant height exceeded that expected from segregation at a single locus with an average additive effect of 4.5 cm.

DISCUSSION

Effects of *Rht-D1b* and Fusarium Head Blight Resistance Quantitative Trait Loci after Spray Inoculation and Point Inoculation

The results of the present study confirmed earlier reports that the *Rht-D1b* allele compromises FHB resistance after spray inoculation (Draeger et al., 2007; Holzapfel et al., 2008; Miedaner and Voss, 2008; Srinivasachary et al., 2008, 2009). These earlier reports were all based on experiments with near-isogenic lines or mapping populations in European winter wheat with moderate levels of resistance. This is the first time the effect of *Rht-D1b* has been assessed in a mapping population segregating for strong FHB resistance loci such as *Fhb1* and the Sumai-3 derived resistance QTL on 5A. Even in such a genetic background, the *Rht-D1* locus explained up to 38% of the phenotypic variation and was by far the most important QTL affecting FHB resistance. That the significant negative correlation (r = –0.16 to –0.52) between plant height and FHB severity after spray inoculation were reduced (r = –0.03 to –0.38) in *Rht-D1* subpopulations agrees with previous findings (Draeger et al., 2007; Voss et al., 2008). After point inoculation, on the other hand, the FHB severity was more dependent on the resistance QTL and the effect of the dwarfing gene was negligible. *Rht-D1b*

does, in other words, compromise the resistance to initial infection (Type I) but not the resistance to spread within the spike (Type II).

That the *Fhb1* locus on 3BS mostly gives Type II resistance is well established (Anderson et al., 2001; Buerstmayr et al., 2003b; Jiang et al., 2007; Lin et al., 2004; Waldron et al., 1999; Zhou et al., 2002, 2004), although it also may give a weak Type I effect (Buerstmayr et al., 2003b; Jia et al., 2005; Yang et al., 2005). This QTL was detected in all environments in our study but most consistently in China where point inoculation was used.

The QTL on 5A was only detected in the spray inoculation trials. This is consistent with previous studies showing that the 5A QTL contributes more to Type I than to Type II resistance (Anderson et al., 2001; Bai et al., 1999; Buerstmayr et al., 2002, 2003b; Chen et al., 2006; Yang et al., 2005).

The resistance QTL on 2D contributed by Line 685 was only detected after point inoculation and accordingly contributes to Type II resistance as previously found in 'Sumai-3' derivatives (Jiang et al., 2007; Yang et al., 2005), and this QTL maps in the 2DL cluster (Liu et al., 2009) not the 2DS QTL associated with *Rht8* (Handa et al., 2008). The 2B QTL only detected in Hungary belongs to the same cluster as those detected in 'Ning 7840' (Zhou et al., 2002) and G16-92 (Schmolke et al., 2008).

Implications for Resistance Breeding

The increased susceptibility to FHB associated with *Rht-D1b* poses a major challenge to wheat breeding as this is considered a highly favorable allele for improved yield and less lodging under intense cultivation practices of modern agriculture.

In this study we have shown that a combination of both *Fhb1* and the 5A QTL, two of the strongest QTL known for FHB resistance, was required just to balance the negative effect of *Rht-D1b*. Still, more resistance factors would be required to achieve desired levels of resistance comparable to widely used sources such as 'Sumai-3' or Nobeokabozu. Line 685 used in the present study is actually such a breeding line with high levels of resistance accumulated from both 'Sumai-3' and Nobeokabozu into an *Rht-D1b* background of European winter wheat. This was achieved through phenotypic selection in field trials under artificial inoculation and several cycles of crossing and selection. The line clearly must have accumulated more genes for resistance than those detected by the QTL mapping. Epistatic effects may also have contributed to this high level of resistance.

The other widely used semidwarfing allele, *Rht-B1b* from Norin 10, seems to have less compromising effects on FHB resistance while giving the same height reductions as *Rht-D1b*. This was shown in a mapping population segregating for both *Rht-B1b* and *Rht-D1b*; a major QTL for FHB susceptibility was detected at the *Rht-D1* locus while *Rht-B1* showed no similar effect (Srinivasachary et al., 2009). This was followed up by inoculation experiments with near-isogenic lines showing that both semidwarfing alleles significantly decreased Type I resistance, but while *Rht-D1b* had no effect on Type II resistance, *Rht-B1b* significantly increased it. It can therefore be concluded that *Rht-B1b*, at least under moderate disease pressure, can be used to achieve the desired plant height with less compromising effect on FHB resistance than *Rht-D1b* (Miedaner and Voss, 2008; Srinivasachary et al., 2009). The *Rht8c* dwarfing allele commonly used in southern European breeding programs is also associated with less negative impact on FHB resistance and could be considered as well (Miedaner and Voss, 2008).

In conclusion, our study confirmed the negative effect of *Rht-D1b* on FHB resistance under spray inoculation and demonstrated that pyramiding of at least two resistance genes with strong effects (*Fhb1* and 5A QTL) was necessary to balance it. In contrast, it had no negative effect under point inoculation.

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Paper II

The *Fusarium* head blight resistance in bread wheat line 'Shanghai-3/Catbird' is under multigenic control and associated with anther extrusion

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Abstract

Fusarium head blight (FHB) is a destructive wheat disease of global importance. Resistance breeding depends heavily on the *Fhb1* gene. The CIMMYT line Shanghai-3/Catbird (SHA3/CBRD) is a promising source without this gene. A recombinant inbred line (RIL) population from the cross of SHA3/CBRD with the German spring wheat cv. Naxos was evaluated for FHB resistance and related traits in field trials using spray and spawn inoculation in Norway and point inoculation in China. After spray and spawn inoculation, FHB severities were negatively correlated with both plant height (PH) and anther extrusion (AE). The QTL analysis showed that the *Rht-B1b* dwarfing allele co-localized with a QTL for low AE and increased susceptibility after spawn and spray inoculation. In general, SHA3/CBRD contributed most of the favorable alleles for resistance to severity after spray and spawn inoculation, while Naxos contributed more favorable alleles for reduction in FDK and DON content and resistance to severity after point inoculation. SHA3/CBRD contributed a major resistance QTL close to the centromere on 2DL affecting FHB severity and DON after all inoculation methods. This QTL was also associated with PH and AE, with tall and high AE alleles contributed by SHA3/CBRD. Several QTL for PH and AE were detected, and reduced PH or low AE were always associated with increased susceptibility after spawn and spray inoculation.

Most of the other minor FHB resistance QTL from SHA3/CBRD were associated with PH or AE, while the QTL from Naxos were mostly not. After point inoculation, no other QTL for FHB traits was associated with PH or AE, except the 2DLc QTL which was common across all inoculation methods. Marker-assisted selection based on the 2DLc QTL from SHA3/CBRD combined with phenotypic selection for AE is recommended for resistance breeding based on this valuable source of resistance.

Abbreviations Fusarium head blight (FHB) Plant height (PH) Anther extrusion (AE) Fusarium damaged kernels (FDK) Deoxynivalenol (DON) Confidence interval (CI) Diversity arrays technology (DArT) Day degrees (d°C) Composite interval mapping (CIM) Simple interval mapping (SIM)

Introduction

Fusarium head blight (FHB), also known as scab, is a destructive disease of wheat (*Triticum aestivum* L.) in many regions around the world. *F. graminearum* and *F. culmorum* are usually the most important agents (McMullen et al. 1997). It causes high yield loss and grains contaminated by mycotoxins such as deoxynivalenol (DON), nivalenol and zearalenon. Moister and warmer weather in combination with agronomic practices like reduced tillage, the lack of adequate crop rotation and cultivation of susceptible cultivars all contribute to epidemics (Champeil et al. 2004; Dill-Macky and Jones 2000; Beyer et al. 2006; Edwards 2004). Breeding FHB-resistant varieties is considered the most effective, economic and environmental way to control this disease.

Resistance to FHB in wheat is a complex quantitative trait where five types of parameters have been discerned (Mesterhazy et al. 1999): Type I (resistance to invasion), Type II (resistance to fungal spread), Type III (resistance to toxin accumulation), Type IV (resistance to kernel infection), and Type V (tolerance). Generally, point inoculation of single florets is used to evaluate Type II resistance, while disease assessment following spray inoculation or grain spawn inoculation reflects a combination of both Type I and Type II resistance. In recent years, numerous QTL analyses of FHB resistance have been

reported. The most prominent QTL for FHB resistance have been associated with specific types of resistance: Type II resistance on chromosome 3BS (*Fhb1*) (Waldron et al. 1999; Anderson et al. 2001; Bai et al. 1999), 6B (*Fhb2*) (Anderson et al. 2001; Yang et al. 2003; Cuthbert et al. 2007) and 2D (Jia et al. 2005; Lin et al. 2006; Yang et al. 2005); and Type I resistance on 3A (Steiner et al. 2004; Yu et al. 2008), and 5A (*Fhb5*) (Buerstmayr et al. 2003b; Buerstmayr et al. 2003a; Steiner et al. 2004; Chen et al. 2006; Xue et al. 2011). The *Fhb1* explains 15–60% of the phenotypic variation for FHB in different backgrounds and has made the Chinese cultivar Sumai-3 the most popular source of resistance through derivatives like DH181 (Yang et al. 2005), CJ9306 (Jiang et al. 2007b; Jiang et al. 2007a), Ning 7840 (Zhou et al. 2002), CM-82036 (Buerstmayr et al. 2002; Buerstmayr et al. 2003a) and Line 685 (Lu et al. 2011).

Morphological and developmental characters such as plant height (PH) and anther extrusion (AE) have long been considered as factors influencing resistance to FHB.

Negative correlations between FHB resistance and PH are commonly observed (Hilton et al. 1999; Buerstmayr et al. 2000; Somers et al. 2003), and QTL mapping has recently verified that the Norin 10 genes *Rht-D1b* and *Rht-B1b* (Gale and Youssefian 1985) coincide with major QTL for FHB susceptibility after spray inoculation (Holzapfel et al. 2008; Draeger et al. 2007; Srinivasachary et al. 2008; Srinivasachary et al. 2009). Studies with near-isogenic lines showed that both dwarfing alleles compromise Type I resistance under high disease pressure, but to different degrees (Srinivasachary et al. 2009; Hilton et al. 1999; Miedaner and Voss 2008). However, *Rht-B1b* conferred Type II FHB resistance, whereas *Rht-D1b* showed no effect (Srinivasachary et al. 2009). A QTL meta-analysis showed a negative association between PH and FHB resistance for both reported *Rht* genes and other PH QTL (Mao et al. 2010). A recent study reported that these negative associations disappeared when the dwarf lines were raised to the same height level as wild type (Yan et al. 2011). This indicates that the PH effect might be mediated through a canopy architecture favoring disease development.

The role of AE in FHB etiology has been discussed since mentioned by Percival et al. (1921). Fifty years later it was claimed that anthers were a nutritious substrate for *Fusarium* and were the sites of initial infection after inoculation (Strange and Smith 1971;

Strange et al. 1974), but later studies did not confirm this relationship (Engle et al. 2004). Recently significant negative correlations between AE and FHB/DON were demonstrated in the Arina x NK93604 DH population, where coincident QTL of AE and FHB was found on chromosome 1B, and closely linked QTL for the two traits on 7A (Skinnes et al. 2010). From the phenotypic distribution the authors suggested that lines with a high AE had much less chances to develop FHB, while lines with low AE needed active types of resistance to reduce infection.

Resistance breeding efforts around the world depend heavily on Sumai-3 and its derivatives with the *Fhb1* gene. As shown by Lu et al. (2011) this gene is not enough to counteract the negative impact of *Rht-D1b*. Hence, there is a need to broaden resistance diversity. Shanghai-3/Catbird (SHA3/CRBD) showed moderate resistance to FHB in the field and a haplotype analysis demonstrated the absence of *Fhb1*. It also has a high AE and carries the dwarfing gene *Rht-B1b* and is hence suitable for a comprehensive QTL analysis. The genetic analysis of this non-Sumai 3 resistance source could contribute to the resistance diversity and elucidate little investigated traits like AE.

A recombinant inbred line (RIL) population was developed from SHA3/CRBD (*Rht-B1b*, high AE) and Naxos (*Rht-B1a*, low AE). The objectives were to 1) detect QTL for FHB resistance in a non-*Fhb1* germplasm, 2) assess their effects across environments and inoculation methods, and 3) investigate associations between FHB traits and PH/AE.

Materials and methods

Plant materials

A RIL population of 181 F_6 lines was developed by single seed descent from the cross SHA3/CBRD x Naxos. SHA3/CBRD is a spring type breeding line from CIMMYT with the pedigree 'Shanghai-3//Chuanmai 18/Bagula' and selection history "-0SHG-6GH-0FGR-0FGR-0Y". It is moderately resistant to FHB and carries the *Rht-B1b* allele. Naxos, a German spring variety with a high level of partial resistance to powdery mildew (Lillemo et al. 2010; Lu et al. 2012), is susceptible to FHB in the field. It was developed by Strube GmbH & Co.KG from the cross 'Tordo/St.Mir808-Bastion//Minaret'.

Molecular marker analysis

Genomic DNA of the parents and recombinant inbred lines was extracted from young leaves with the DNeasy Plant DNA extraction kit (QIAGEN). Microsatellite (SSR) analysis was performed with fluorescently labeled primers and PCR products were separated by capillary electrophoresis on an ABI 3730 Gene Analyzer. PCR was conducted as described by Semagn et al. (2006). DArT markers were analyzed by Triticarte Pty. Ltd. (Canberra, Australia; http://www.triticarte.com.au) as described by Akbari et al. (2006).

Field trials

Norway

Spawn inoculation

In 2008 and 2011, the RIL population was grown in hillplots, 40x45 cm apart in three replications with an alpha-lattice design. Grain spawn (infected oat kernels) was prepared based on a protocol from Dr. Bernd Rodemann, Julius Kühn Institute, Braunchweig, Germany, using a mixture of isolates of *F. graminearum* which were provided by the Norwegian Veterinary Institute, Oslo. Two islolates with low agressivity (101177 and 101023) were used in 2008, these two plus 101118 and 101018 with somewhat higher agressivity were used in 2011. 'Belinda' oat was soaked overnight (12h) in water and autoclaved for 60 min at 121˚C. Each isolate was cultivated 7 days in liquid culture (1g oat flour in 100 ml ionized water), and then mixed with the sterile oat kernels. After cultivation for 3-4 weeks at room temperature/ambient light until abundant development of mycelium, the infected oats were kept on trolleys at room temperature/ambient light, with depth 3-4 cm, and sparsely irrigated at daily intervals with water to stimulate the development of perithecia. After 3 weeks, the infected oats were then mixed and distributed in the field experiment at Zadoks stage 32-33 with a density of 10 g/m^2. Mist irrigation (9 min/hour) was applied in the evenings for two hours/day after spawn application and 3-4 hours/day during the flowering stage (for optimal germination of ascospores). A bundle of 10-15 heads was scored and the percentage of infected spikelets determined on a linear scale from 0 to 100%. Scorings (twice in 2008 at about one week

interval and once in 2011) were carried out based on the symptom development of the susceptible control. The maximum severity was used for further analysis.

Spray inoculation

In 2009 and 2010, the RILs were grown in 75x200 cm plots, with 15 cm between rows and 30 cm between plots in two replications with alpha-lattice design. The central two rows of each plot were inoculated at full flowering by spraying about 70 ml of a macro-conidial suspension at $1x10^5$ spores/ml of *F. culmorum* with a backpack sprayer. The inoculation was repeated after 2-3 days at 45 day degrees (d°C) interval. Inoculum was prepared as described by Semagn et al. (2007).A mixture of five isolates (no. 7, 8, 9, 200–104, 33–3) from BIOFORSK Crop Research Institute, Ås, were used. Mist irrigation was applied (9 min/hour) in the evening (7 pm to 10 pm) to provide humid conditions for infection at night until one week after the last inoculation. Two bundles of about 20 inoculated heads per plot were scored as percentage of infected spikelets with a linear scale from 0 to 100%. Two scorings were carried out on the basis of constant temperature sums after inoculation (217, 335 d°C in 2009, and 240, 440 d°C in 2010). The average of the two observations was used for further analysis.

Fusarium damaged kernels (FDK) of samples from 2010 was visually estimated by comparing with prepared standards according to Jones & Mirocha (1999) with minor modifications. The standards were prepared by mixing healthy and damaged kernels from the RILs to create ratios equivalent to 0, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, 98 and 100% on a 400-kernel basis, which just covered the bottom of a standard petri dish. DON content of samples after spray inoculation in 2009 and 2010 were determined by GC-MS at University of Minnesota (Fuentes et al. 2005; Mirocha et al. 1998).

Plant height was measured in a separate experiment in 2008 and in the disease nurseries in 2009, 2010 and 2011. AE was observed in 2009 and 2010 in separate, but adjacent experiments, avoiding the confounding effect of mist irrigation. AE was estimated visually based on a linear scale from 0 (no anther extrusion) to 9 (100% extruded anthers) as described by Skinnes et al. (2010).

China

Point inoculation

Point inoculations were carried out at the Jiangsu Academy of Agricultural Sciences, Nanjing, China, for two years. All RILs were sown in late October in 150 cm rows at 33 cm distance, in one randomized replication in 2009 and two replications with a randomized block design in 2010. Macroconidia were produced in mungbean extraction liquid medium as described by Shi et al. (2008). The same aggressive *F. graminearum* strain was used both in 2009 and 2010. Disease evaluation was carried out as described by Lu et al. (2011): At the heading stage, a single floret in the middle of the head was inoculated with about 20 µl conidial suspension of $1x10^5$ spores/ml and 15 heads were inoculated per row. 20 days after inoculation, the percentage of infected spikelets was calculated for each inoculated head. The mean FHB severity of 15 heads was calculated and used for further analysis. The DON content of samples from 2009 was determined by Enzyme-linked immunosorbent assay (ELISA) (Ji et al. 2011; Li et al. 2007).

Statistical analysis

Analyses of variance were performed using the PROC GLM procedure in SAS (SAS Institute Inc.,Version 9.1). Heritability (broad sense) was estimated from the ANOVA information using the formula $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_E^2 / r)$ within a year and the formula $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{g\times y}^2 / y + \sigma_E^2 / ry)$ across years, where σ_g^2 is genetic variance, $\sigma_{g\times y}^2$ is genotypes-by-years interaction, σ_E^2 is error variance, y is number of years, and r is number of replicates. The Pearson correlation coefficients were calculated using the PROC CORR procedure of SAS. FHB traits used for QTL analysis were estimated with LSmeans from mixed model in SAS with inoculation date considered as a random factor.

Genetic map construction and QTL analysis

Initially, 283 polymorphic DArT markers covering all the chromosomes were used for preliminary mapping of the RIL population. Based on the SSR consensus map of Somers et al. (2004), the gap regions were supplemented with 105 polymorphic SSR markers. The initial QTL detection was conducted and the genetic map was refined with more SSR markers in the detected QTL regions both for powdery mildew (Lu et al. 2012) and FHB

traits. The final genotypic data of 181 lines including 283 DArT and 271 SSR loci were used to construct a genetic linkage map with the software JoinMap v. 3.0 (Van Ooijen and Voorrips 2001). Map distances were based on the Kosambi function with minimum LOD score 2. Consensus map information was used to assign linkage groups to chromosomes.

QTL analysis was performed with PLABQTL v. 1.2 (Utz and Melchinger 1996). Simple interval mapping (SIM) was conducted first to detect the major QTL for FHB. The markers most closely linked to each QTL across environments were then used as cofactors in composite interval mapping (CIM). The LOD threshold was set at 3.0 after 1000 permutations. QTL reaching this level in one environment either in SIM or CIM were also reported for other environments even though their LOD scores were under threshold. For studying the relationships between FHB and the associated traits AE and PH, putative QTL for AE and PH were also listed if they coincided with FHB traits. QTL with overlapping confidence interval (CI) were considered as common. Genetic map drawing and QTL marking were conducted by the software MapChart v.2.1 (Voorrips 2002).

Results

Phenotypic analysis

We believed that our experiments reflected more inoculation methods rather than the locations where they were conducted. Therefore, in the following context only different methods are considered instead of locations. This issue will also be discussed later in the discussion section.

After spawn inoculation, disease development in 2008 and 2011 varied largely with average severities of 4.6% and 26.9% in the RIL population. Significant variation was observed within the population in each year, while it was masked by the large GxE interaction across years which led to a lower heritability of 0.41 (Table 1). Significant transgressions were observed and the distribution was skewed towards low infections (Fig 1).

After spray inoculation, FHB severity developed well with average severities of 43.7% and 29.7% respectively in RILs in 2009 and 2010. FDK and DON content also showed wide variation, which followed similar patterns as their corresponding severities (Fig.1). These significant variations in the FHB traits were confirmed in ANOVA analysis (Table 1). Moderate heritabilities were observed: 0.57 for FHB severity, 0.60 for DON content and 0.70 for FDK.

After point inoculation, there were marked year effects, with a high severity in 2010, but low severity and DON content in 2009 due to unfavorable conditions (Fig.1). For the latter reason, the heritability for FHB across years was only 0.57 (Table 1). A higher correlation was observed between DON content and FHB severity after point inoculation (r=0.57) than those after spray inoculation (r=0.06 and r=0.20) (Table 2).

Although significant genotype x year interactions were noted, FHB severities were still significantly correlated with each other across years for the same inoculation methods (Table 2), similar to DON content. FDK was more highly correlated with DON than FHB severity (r= 0.45 and 0.23 respectively in 2010). In general, FHB severities after spawn and pray inoculation were more correlated with each other than with severities after point inoculation (Table 2). However, weak correlations (r= 0.25 and 0.31) were observed between severity after spawn inoculation in 2011 and severities after point inoculation. Additionally, Naxos always had much higher severity than SHA3/CRBD in high or moderate disease pressures after spawn and spray inoculation (Fig. 1). However, Naxos had clearly less FDK and DON content than SHA3/CBRD in 2010 and similar DON content in 2009. This indicated that the two parents carried different resistance types: SHA3/CBRD more severity resistance than Naxos, the latter more FDK and DON content resistance, which was later confirmed in the QTL analysis.

The RIL population showed wide and significant variation in PH and AE (Fig. 1, Table 1). Transgressive segregation was apparent towards both sides for PH and AE. Despite the significant genotype x year interactions the heritabilities were still considerably high, 0.93 and 0.80 for PH and AE respectively. Both PH and AE were negatively correlated with FHB severity both within and across years after spawn and spray inoculation. The

negative correlations of FHB with PH (r= -0.26 to -0.54) were of same magnitude as with AE (r= -0.29 to -0.67) except severity in 2009 after spray inoculation (Table 3) which experienced high disease pressure and shorter plant height. However, PH was independent of other FHB traits, while AE was weakly correlated with FDK after spray inoculation as well as severity after point inoculation. The general relationships are well visualized in the contour plots in Fig. 2.

QTL mapping for FHB traits

From the total of 554 polymorphic marker loci, 422 loci were assembled into 29 linkage groups. The genetic map spanned a total of 2192.3 cM and represented all chromosomes.

QTL mapping for FHB was first conducted with both simple interval mapping (SIM) and composite interval mapping (CIM). The QTL regions consistent across multiple environments with low resolution or partial peaks were then supplemented with more SSR markers based on consensus maps (Somers et al., 2004; GrainGenes: http://wheat.pw.usda.gov/GG2/index.shtml). The final QTL results verified with cross validation are presented in Table 4 and 5 and Fig. 3.

FHB resistance components in both parents were controlled by few major and many minor QTL. SHA3/CBRD contributed more QTL for FHB severity after spawn and spray inoculation, Naxos contributed more QTL for resistance to FDK and DON accumulation after spray inoculation and for FHB severity after point inoculation.

Resistance after spawn inoculation (Table 4, Fig. 3)

Resistance to FHB severity in SHA3/CBRD was controlled by one major QTL on 2DLc and four minor QTL on 1A, 3DL, 5AL, 6AS and 7A. The major QTL on 2DLc was located on the long arm of 2D close to the marker *Xgwm539* near the centromere, explaining 8-24% of the phenotypic variation. The QTL on 1A, 3DL, 5AL and 7A were detected both based on the mean data and in single environments and accounted for 2-9% of the phenotypic variation, whereas the minor QTL on 6AS was only detected in 2008.

Resistance to FHB severity in Naxos was controlled by a major QTL on 4BS at the *Rht-B1* locus and three minor QTL on 1B, 2DL and 5BL. The *Rht-B1* locus explained 11% of the phenotypic variation for FHB severity in 2008, while its impact was less in 2011 and with the mean data. The minor QTL on 1B and 2DL were only detected in 2008.

Resistance after spray inoculation (Table 4, Fig. 3)

Resistance to FHB severity in SHA3/CBRD was controlled by a major QTL on 2DLc and four minor QTL on 3DL, 4AL, 5AL and 6AS. The major and consistent QTL on 2DLc close to *Xgwm539* explained 2-12% of the phenotypic variation in FHB severity, and was the only QTL that also contributed to reduced DON content. The QTL on 3DL, 5AL and 6AS were detected across environments and accounted for 1-7% of the phenotypic variation. A QTL on 4AL that accounted for 11% of the phenotypic variation in FHB severity was only detected in 2010. Naxos contributed a major QTL for severity resistance at the *Rht-B1* locus on 4BS, accounting for 4-11% of the phenotypic variation, and three minor QTL on 1B, 2DL and 5D for severity resistance.

Apart from the QTL on 2DLc that had resistance contributed by SHA3/CBRD, all the other important alleles for reduction in FDK and DON content were contributed by Naxos. Two QTL were responsible for both traits, and the most important one mapped close to *Xgwm156* on the short arm of 5A and accounted for over 10% of the phenotypic variation for both traits. The other one was located on 2A close to *Xbarc124*. It accounted for 9% of phenotypic variation of FDK reduction, while it had much less effect on DON accumulation in the same experiment. For DON content, another major QTL was mapped on 7A near the centromere close to the marker *Xwmc603*. It explained 8-16% of the phenotypic variation and was stable across two years. Naxos contributed these major QTL and four minor QTL on 1A, 2BL, 3AS and 5BL, while SHA3/CBRD contributed two minor QTL for DON content on 2DLc and 6ASc.

Resistance after point inoculation (Table 5, Fig. 3)

FHB severity in Naxos was controlled by a major QTL on 2DS, accounting for up to 10% of the phenotypic variation and three minor QTL on 1D, 2A and 2BL. The QTL on 2BL

coincided with the one detected for reduction in DON content after spray inoculation (Table 4). SHA3/CBRD contributed two minor QTL, of which only the one on 4DL was consisted across the two environments. The other QTL on 2DLc was only detected in 2010, and coincided with the QTL detected after spawn and spray inoculation. For DON content, only two minor QTL were detected, and these coincided with the FHB severity QTL on 2DS and 4DL with resistance from Naxos and SHA3/CBRD, respectively.

Across all inoculation methods, only two QTL on 2DLc and 2BL were effective with diverse phenotypic contribution. The 2DLc QTL mainly contributed to the reduction of severity and was more effective after spawn and spray inoculation than after point inoculation. The 2BL QTL showed resistance to severity after point inoculation and a small effect on resistance to DON content after spray inoculation.

QTL mapping of PH and AE

For PH, five significant QTL were detected on 4BS, 4AL, 1B, 6AS, 2DLc and two putative QTL on 5A and 6ASc (Table 6, Fig. 3). The major QTL on 4BS coincided with the *Rht-B1* locus and accounted for 27-46% of the phenotypic variation across three environments. Another major QTL on 4A explained 8-13% of the phenotypic variation. Naxos contributed the tallness allele at *Rht-B1* and the QTL on 1B, while SHA3/CBRD contributed for the rest.

AE was mainly controlled by a major QTL on 4BS at the *Rht-B1* locus and explained 5-11% of the phenotypic variation. Surprisingly the Naxos allele conditioned high AE at this 4BS QTL and a putative QTL on 5BL, although it phenotypically had the lowest AE of the parents. SHA3/CBRD contributed the rest of the alleles that enhanced AE at three minor QTL on 2DLc, 3D and 7A and two putative QTL on 4AL and 5AL (Table 6, Fig. 3).

Association between FHB and AE/ PH

Generally, QTL for PH and AE were more associated with QTL for FHB severity than other FHB traits. This association was only observed after spawn and spray inoculation.

In the following, attention will only be paid to FHB severity since the associations with FDK and DON are more likely a consequence of severity.

After spawn and spray inoculation, six of seven QTL with resistance from SHA3/CBRD to FHB severity were associated with PH or AE, meanwhile two of five QTL with severity resistance from Naxos were associated with other traits. The major QTL for increased susceptibility on 4BS was associated with a major QTL for reduced height and low AE. RILs carrying *Rht-B1b* were more susceptible than their counterparts both after spawn and spray inoculation, while they were slightly more resistant after point inoculation (Table 7, Fig. 4). The 4A resistance QTL detected in 2010 was associated with a minor AE QTL detected in the adjacent experiment and a major PH QTL. The 2DLc and 3D QTL were only associated with AE. The 6ASc QTL was associated with PH, while independent of AE. At these loci, both low AE and reduced PH increased the FHB severity.

After point inoculation, associations with related traits were only found at the common QTL on 2DLc, which contributed severity resistance after all inoculation methods.

Discussion

Phenotypic evaluation

Spawn and spray inoculation mimic the situation under natural infection (Buerstmayr et al. 2009) and reflect both Type I and Type II resistance (Schroeder and Christensen 1963), while point inoculation was used for evaluation of Type II resistance. Both spawn and spray inoculation were performed in Norway, and point inoculation in China. We do believe the results reflect techniques rather than environments. First, the results conform well to the recent review by Liu et al. (2009) which shows a remarkable consistency of QTL across studies (genotypes and environments). Second, we have found the same pattern in a different population (Lu et al. 2011). Third, despite significant GxE interactions, FHB traits were well correlated with each other across years. This indicates that the correlations are genetic, since GxE does not contribute to correlations across environments. As pointed out by Aastveit and Aastveit (1993) the magnitude of the

correlation depends only on linkage distance and phase, not on GxE. For FHB traits and AE this corresponds well with the results obtained by Skinnes et al. (2008). Fourth, for powdery mildew in this (Lu et al. 2012) and other (Lillemo et al. 2008) populations we found high consistency of partial resistance QTL between these environments, while race specific genes show strong interactions. Since FHB QTL are partial and additive in nature, our conclusions could make sense.

In contrast to other QTL mapping studies with different inoculation methods, a striking observation was the lack of correlation between point and spray inoculation data. The spawn inoculation data was also mostly uncorrelated with point inoculation data, except for a weak correlation with severity after point inoculation for the spawn data in 2011 (Table 2). One reason might be the lack of *Fhb1*, a common gene in many publications which has big effect on both Type I and Type II resistance. However, in the present study only two common QTL were detected with less effect for both types of resistance.

Both the continuous distribution of AE and QTL analysis showed that several factors were involved in the inheritance of AE, which supports the results by Skinnes et al. (2010). The broad sense heritability of 0.80 across two years also agrees with previous reports (Skinnes et al. 2010; Singh et al. 2001).

FHB QTL mapping

Among the 23 QTL for FHB resistance detected in the current study, 5 QTL had effect on different types of resistance to FHB. The map location and resistance feature of important QTL were compared with the meta-analysis by Liu et al (2009).

The 2DLc QTL for severity and DON, belonging to the 2DL cluster near the centromere, contributed different types of resistance as in Wangshuibai (Lin et al. 2006; Mardi et al. 2005) and Sumai-3 derivatives (Jiang et al. 2007a; Yang et al. 2005; Jiang et al. 2007b).

The 4BS QTL for FHB severity at the *Rht-B1b* locus conforms to the studies reviewed above (Srinivasachary et al. 2009), but the effect of the dwarfing gene appears weaker in

this study. However, it does not impact DON. Additionally the coinciding QTL for AE is new. This locus also coincided with a major QTL for ear compactness (data not shown since no other QTL were associated with FHB in this study). The coincidence between PH and ear compactness seems due to pleiotropy, whereas the effect on AE may be so, given that shorter internodes between floral phytomers may affect flower opening and/or duration.

The 5AS QTL corresponds to the 5AS cluster because of its overlapping CI. This QTL, recently named *Fhb5* (Xue et al. 2011), has provided different resistance types in Wangshuibai and W14 (Liu et al. 2009), whereas only resistance to FDK and DON content were detected in the present study. It could be due to genotype differences, environmental factors and power of QTL detection.

The QTL on 7A close to *Xwmc603* belongs to the 7AL cluster. In Wangshuibai, this QTL contributed similar effect on Type II and DON content (Yu et al. 2008) and showed stronger effect on FDK and DON content than *Fhb1* in CS-Sumai 3-7ADSL after point inoculation (Jayatilake et al. 2011). A considerable effect for DON was observed after spray inoculation in the present study although the LOD curve had a below-threshold peak for severity (Fig. 3). The minor effect on resistance to severity after spawn inoculation was from the opposite parent and its non-overlapping CI indicates that they are closely linked QTL.

The 4AL QTL only detected in 2010 with major effect belongs to the Pirate and Arina cluster of Type II resistance (Liu et al. 2009). However, in the present study this QTL was detected only after spray inoculation, which reflects a combination of both Type I and Type II resistance. The 2A QTL belonging to the 2AS cluster contributed reduction in FDK and resistance to DON content, while the meta-analysis only reported Type II resistance in Ning7840 and Freedom and resistance to DON content in NK93604 (Liu et al. 2009). The 6AS QTL may be a novel minor QTL where no cluster and recent published QTL has been found. Other minor QTL all belong to known QTL clusters in the wheat genome, although they not always contributed to the same type of resistance as reported by Liu et al (2009).

Associations among traits

FHB traits

Our results underline the importance of including different resistance parameters beyond FHB severity, namely DON content and FDK, since these components are not always correlated with each other. A meta-analysis with 163 studies showed generally high positive correlations among FHB traits (Paul et al. 2005), but there were some exceptions (Wiśniewska et al. 2004; Mesterhazy et al. 1999) showing little or no correlations. The lack of relationship between severity and DON content, but moderate correlation of FDK with DON content in the present study underlines this point.

PH and FHB

Significant negative correlations were observed between PH and FHB severity (r= -0.26 to -0.54) after spawn/spray inoculation except in 2009. In this environment, plants were shorter and the differences in PH were less, possibly due to a slight drought stress at the time of stem elongation. Negative correlations are in agreement with previous studies that vary from weak to moderate coefficients (Srinivasachary et al. 2009; Buerstmayr et al. 2000; Steiner et al. 2004; Srinivasachary et al. 2008; Voss et al. 2008). In the present study the PH effects were pervasive, since five out of seven QTL for PH coincided with FHB severity QTL, not only *Rht-B1*. It supports that not only the *Rht* genes but also other PH QTL are associated with FHB (Draeger et al. 2007; Holzapfel et al. 2008; Srinivasachary et al. 2008; Srinivasachary et al. 2009; Mao et al. 2010).

Some researchers suggested this could more likely result from pleiotropic effects or genetic linkage (Draeger et al. 2007; Srinivasachary et al. 2009), while PH *per se* still can't be ruled out with the fact that negative associations disappeared when the dwarf lines were raised to the same height level as wild type (Yan et al. 2011). Our results that multiple PH QTL affect FHB severity points to a general effect.

AE and FHB

The negative correlations between AE and FHB severity observed after spawn/spray inoculation (r= -0.29 to -0.67) agreed with Skinnes et al. (2008; 2010). Moreover the QTL analysis confirmed that all AE QTL coincided with FHB severity. Most of AE QTL detected in the present study are different from those reported by Skinnes et al. (2010), only the 7AL QTL was located in a similar region. This again supports that AE inherits in a quantitative manner. Also the relationship with FHB may be complex. In a RIL population from parents with similar Type I resistance, cleistogamous lines (no AE) had greater resistance to FHB (Kubo et al. 2010). The narrow flower opening and short duration also reduced the risk of FHB infection (Gilsinger et al. 2005). In barley, cleistogamous cultivars exhibited greater resistance than the open flowering type (Yoshida et al. 2005), but they could be infected later when the anthers were forced out by the growing caryopsis (Yoshida et al. 2007). Skinnes et al. (2010) suggested that anthers retained and trapped between glumes provide a substrate for saprophytes like *Fusarium* and that subsequent infection of living tissues can occur under conducive conditions. Hence AE is an avoidance mechanism. This corresponds with microscopic observations showing that when anthers were retained in the florets, the hyphal density on anthers was higher than that on the inner surfaces of glumes (Kang and Buchenauer 2000).

Breeding implications for Fusarium resistance

Significant correlations among FHB traits are not always the case. Therefore, active resistance mechanisms against FHB, FDK and DON content should be considered in conjunction with morphological avoidance in the breeding strategies. The big dilemma is how to mitigate the negative effects of the dwarfing genes. Although both *Rht-B1b* and *Rht-D1b* result in very similar height reductions, it is apparent that the former has a less negative effect on FHB resistance (Miedaner and Voss 2008) and is therefore a favorable choice for FHB resistance breeding in environments where short straw is required. The seemingly unavoidable negative effect of the dwarfing gene should be compensated for by other active and passive resistance mechanisms. That the most resistant parent in our mapping population carried *Rht-B1b*, but in combination with the tall alleles of most

other minor PH QTL underlines the merit of the "tall dwarf approach" in wheat breeding and that there is ample scope for this if high AE is also actively pursued.

The two parents in our mapping population contributed different types of resistance that could preferably be combined to produce cultivars with high levels of multiple components of FHB resistance.

The marker-assisted selection based on the 2DLc QTL combined with phenotypic selection for AE is recommended for resistance breeding based on this valuable source of resistance. Naxos contributed three major QTL for resistance to FDK and DON which are all independent of AE. These components different from SHA3/CBRD could be combined into one variety. The RILs with integrated resistance components from both parents could be valuable breeding lines for further application.

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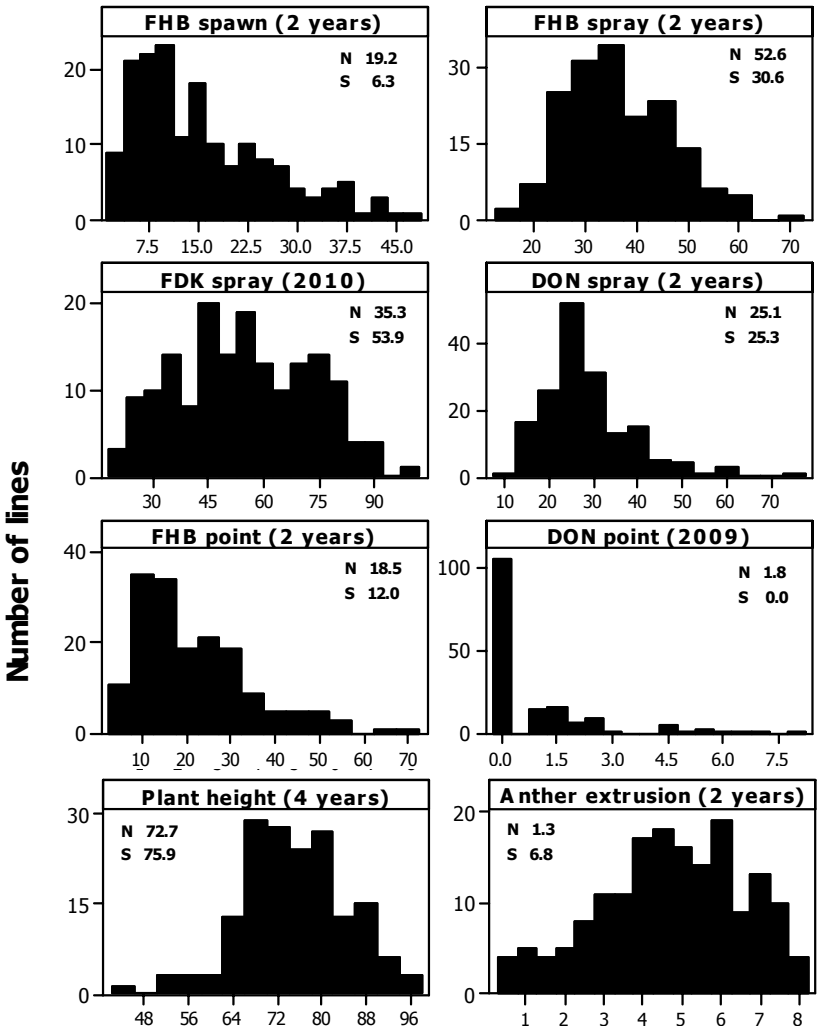


Fig.1 Frequency distribution of FHB and associated traits in the SHA3/CBRD x Naxos RIL population based on the mean data except FDK after spray inoculation and DON content after point inoculation which only have one year data. Inoculation methods were marked behind the trait name. N= Naxos, S= SHA3/CBRD

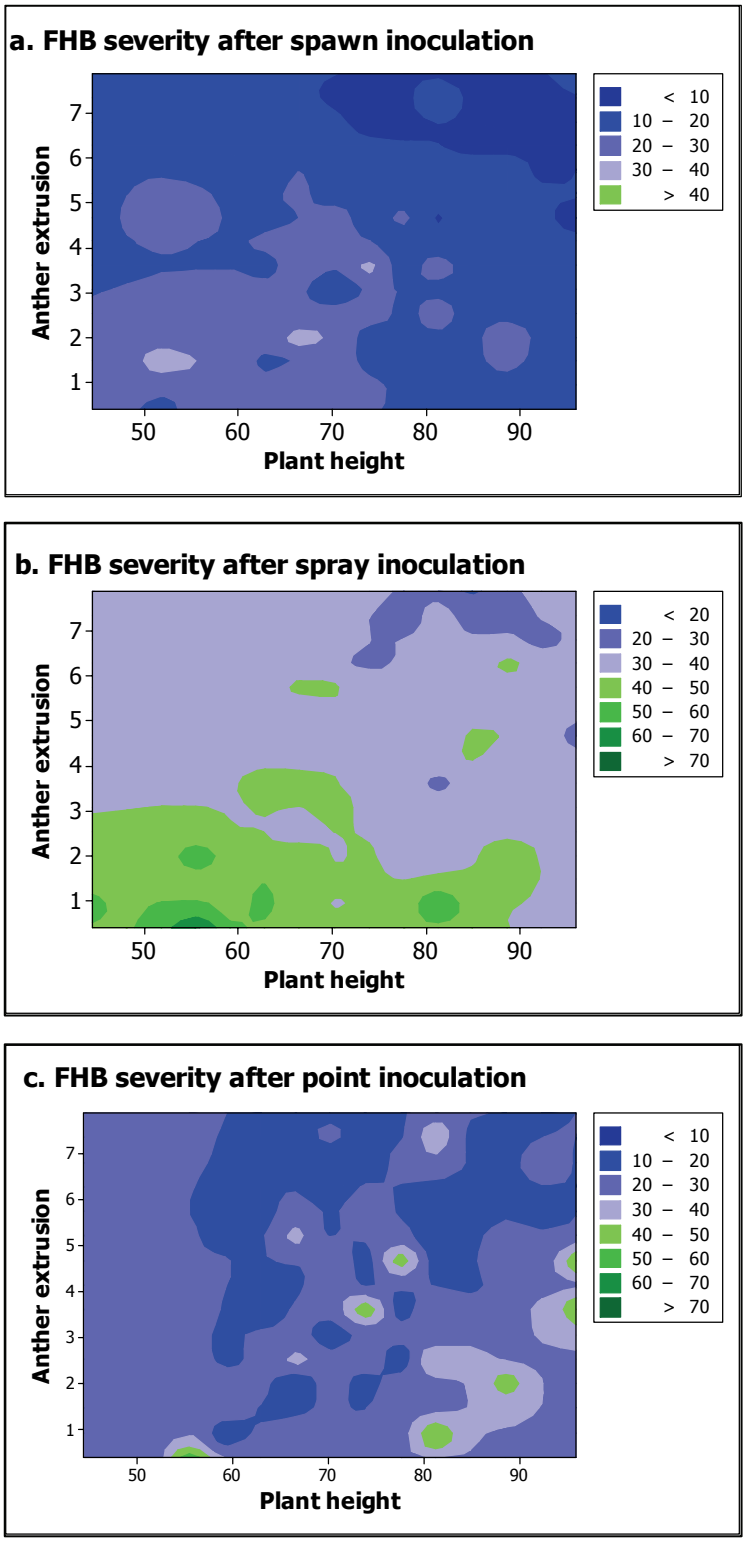
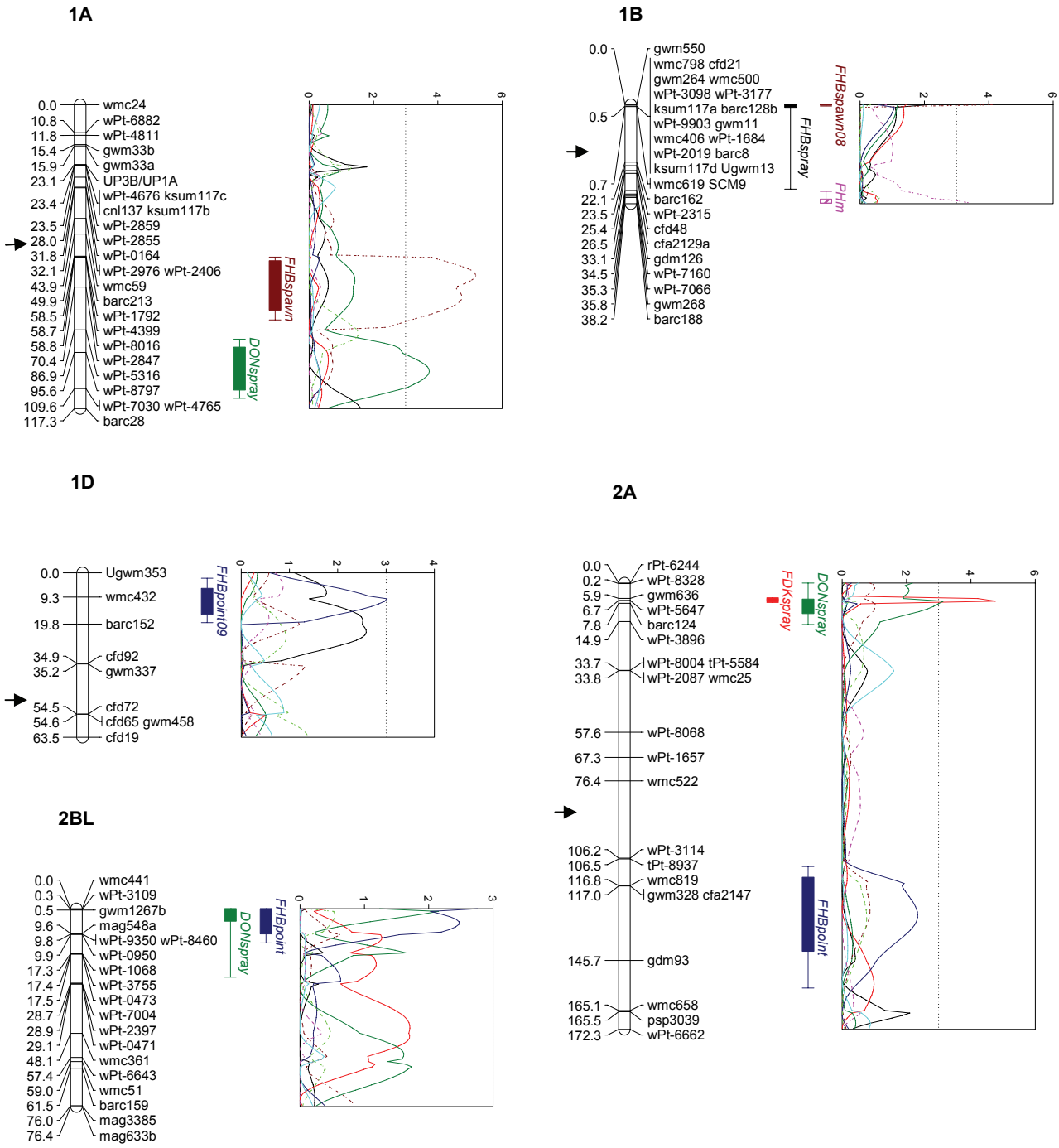


Fig 2. Contour plots of plant height and anther extrusion vs. a) FHB severity after spawn inoculation, b) FHB severity after spray inoculation, c) FHB severity after point inoculation. All the traits are plotted based on the mean data.


1A
0.0 wmc24
10.8 wPt-6882
11.8 wPt-4811
15.4 gwm33b
15.9 gwm33a
23.1 UP3B/UP1A
23.4 wPt-4676 ksum117c
cnl137 ksum117b
23.5 wPt-2859
28.0 wPt-2855
31.8 wPt-0164
32.1 wPt-2976 wPt-2406
43.9 wmc59
49.9 barc213
58.5 wPt-1792
58.7 wPt-4399
58.8 wPt-8016
70.4 wPt-2847
86.9 wPt-5316
95.6 wPt-8797
109.6 wPt-7030 wPt-4765
117.3 barc28
FHBspawn
DONspray
1B
0.0 gwm550
wmc798 cfd21
gwm264 wmc500
wPt-3098 wPt-3177
0.5 ksum117a barc128b
wPt-9903 gwm11
wmc406 wPt-1684
wPt-2019 barc8
ksum117d Ugwm13
0.7 wmc619 SCM9
22.1 barc162
23.5 wPt-2315
25.4 cfd48
26.5 cfa2129a
33.1 gdm126
34.5 wPt-7160
35.3 wPt-7066
35.8 gwm268
38.2 barc188
FHBspray
FHBspawn08
PHm
1D
0.0 Ugwm353
9.3 wmc432
19.8 barc152
34.9 cfd92
35.2 gwm337
54.5 cfd72
54.6 cfd65 gwm458
63.5 cfd19
FHBpoint09
2BL
0.0 wmc441
0.3 wPt-3109
0.5 gwm1267b
9.6 mag548a
9.8 wPt-9350 wPt-8460
9.9 wPt-0950
17.3 wPt-1068
17.4 wPt-3755
17.5 wPt-0473
28.7 wPt-7004
28.9 wPt-2397
29.1 wPt-0471
48.1 wmc361
57.4 wPt-6643
59.0 wmc51
61.5 barc159
76.0 mag3385
76.4 mag633b
DONspray
FHBpoint
2A
0.0 rPt-6244
0.2 wPt-8328
5.9 gwm636
6.7 wPt-5647
7.8 barc124
14.9 wPt-3896
33.7 wPt-8004 tPt-5584
33.8 wPt-2087 wmc25
57.6 wPt-8068
67.3 wPt-1657
76.4 wmc522
106.2 wPt-3114
106.5 tPt-8937
116.8 wmc819
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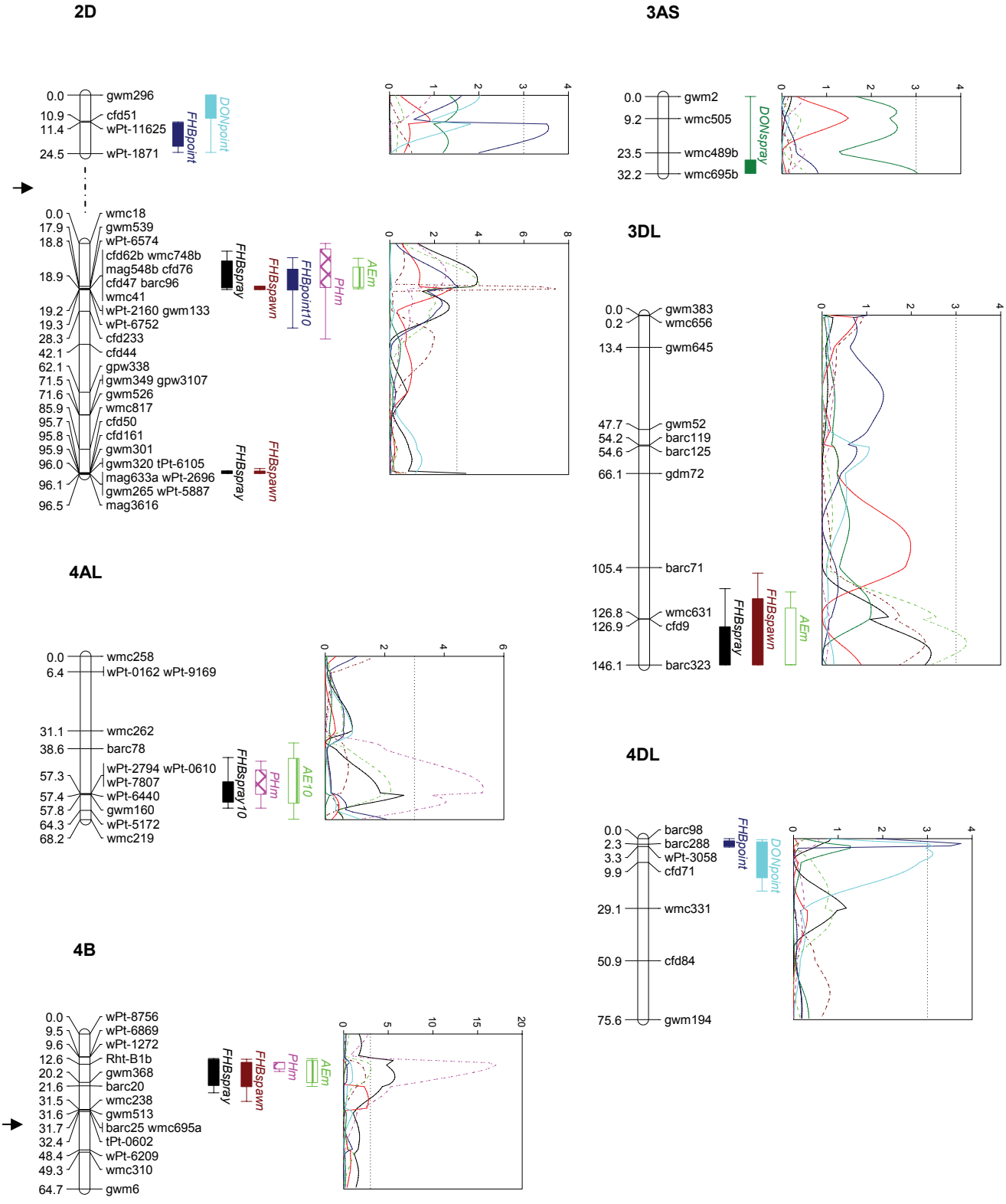
FDKspray
DONspray
FHBpoint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24.5 wPt-1871
FHBpoint
DONpoint
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17.9 gwm539
18.8 wPt-6574
cfd62b wmc748b
mag548b cfd76
18.9 cfd47 barc96
wmc41
19.2 wPt-2160 gwm133
19.3 wPt-6752
28.3 cfd233
42.1 cfd44
62.1 gpw338
71.5 gwm349 gpw3107
71.6 gwm526
85.9 wmc817
95.7 cfd50
95.8 cfd161
95.9 gwm301
96.0 gwm320 tPt-6105
96.1 mag633a wPt-2696
gwm265 wPt-5887
96.5 mag3616
FHBspray
FHBspawn
FHBpoint10
PHm
AEm
FHBspray
FHBspawn
3AS
0.0 gwm2
9.2 wmc505
23.5 wmc489b
32.2 wmc695b
DONspray
3DL
0.0 gwm383
0.2 wmc656
13.4 gwm645
47.7 gwm52
54.2 barc119
54.6 barc125
66.1 gdm72
105.4 barc71
126.8 wmc631
126.9 cfd9
146.1 barc323
FHBspray
FHBspawn
AEm
4AL
0.0 wmc258
6.4 wPt-0162 wPt-9169
31.1 wmc262
38.6 barc78
57.3 wPt-2794 wPt-0610
wPt-7807
57.4 wPt-6440
57.8 gwm160
64.3 wPt-5172
68.2 wmc219
FHBspray10
PHm
AE10
4DL
0.0 barc98
2.3 barc288
3.3 wPt-3058
9.9 cfd71
29.1 wmc331
50.9 cfd84
75.6 gwm194
FHBpoint
DONpoint
4B
0.0 wPt-8756
9.5 wPt-6869
9.6 wPt-1272
12.6 Rht-B1b
20.2 gwm368
21.6 barc20
31.5 wmc238
31.6 gwm513
31.7 barc25 wmc695a
32.4 tPt-0602
48.4 wPt-6209
49.3 wmc310
64.7 gwm6
FHBspray
FHBspawn
PHm
AEm

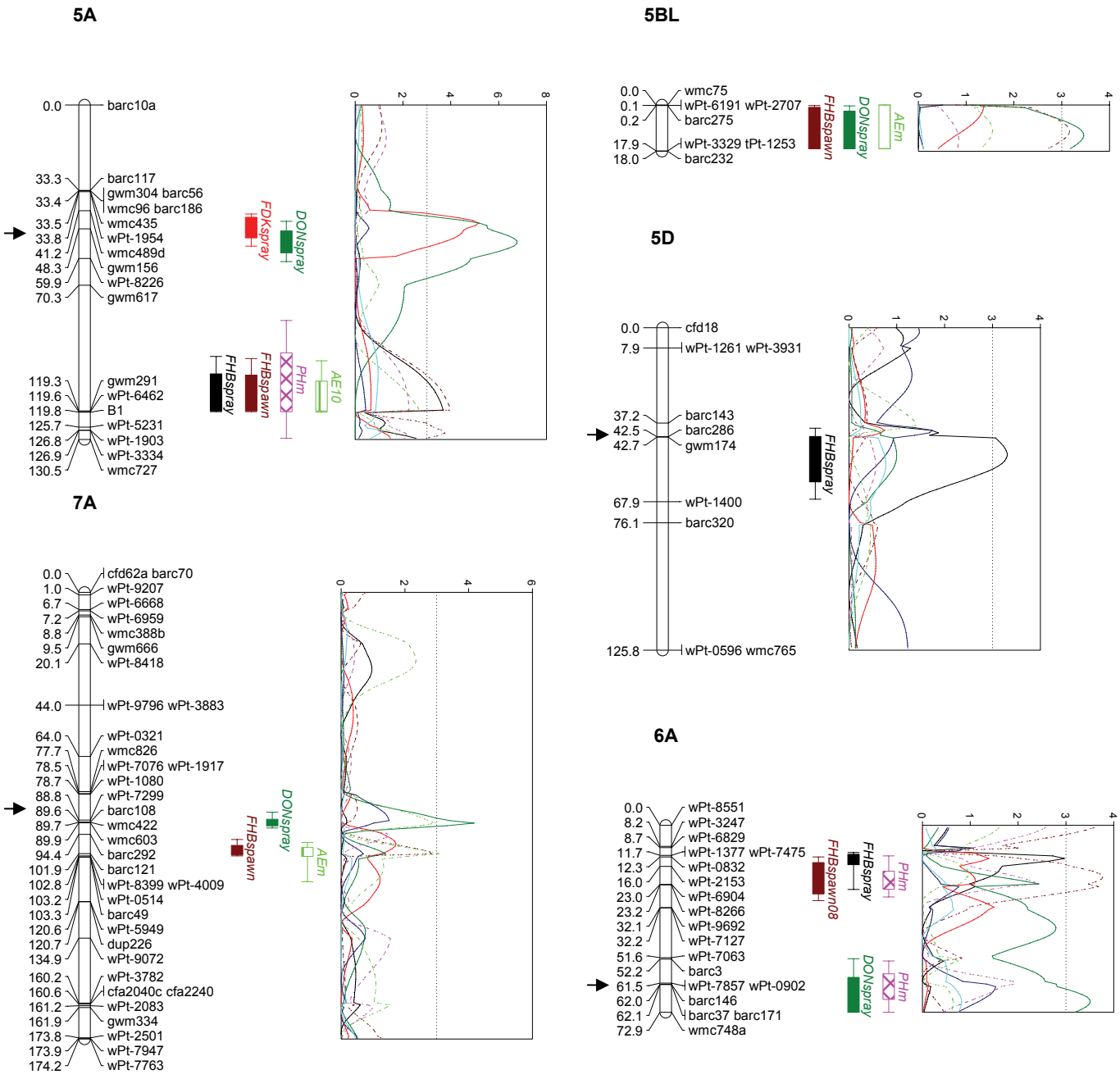

Fig. 3 Chromosomes with QTL from mean data with corresponding LOD curves. If there was no QTL detected based on the mean, the environment with significant QTL effect was marked instead with the year behind the QTL name. Genetic distances are shown in centimorgans to the left of the chromosomes. A threshold of 3.0 is indicated by a dashed vertical line in the LOD graphs. The proximate positions of centromeres are indicated by arrows.

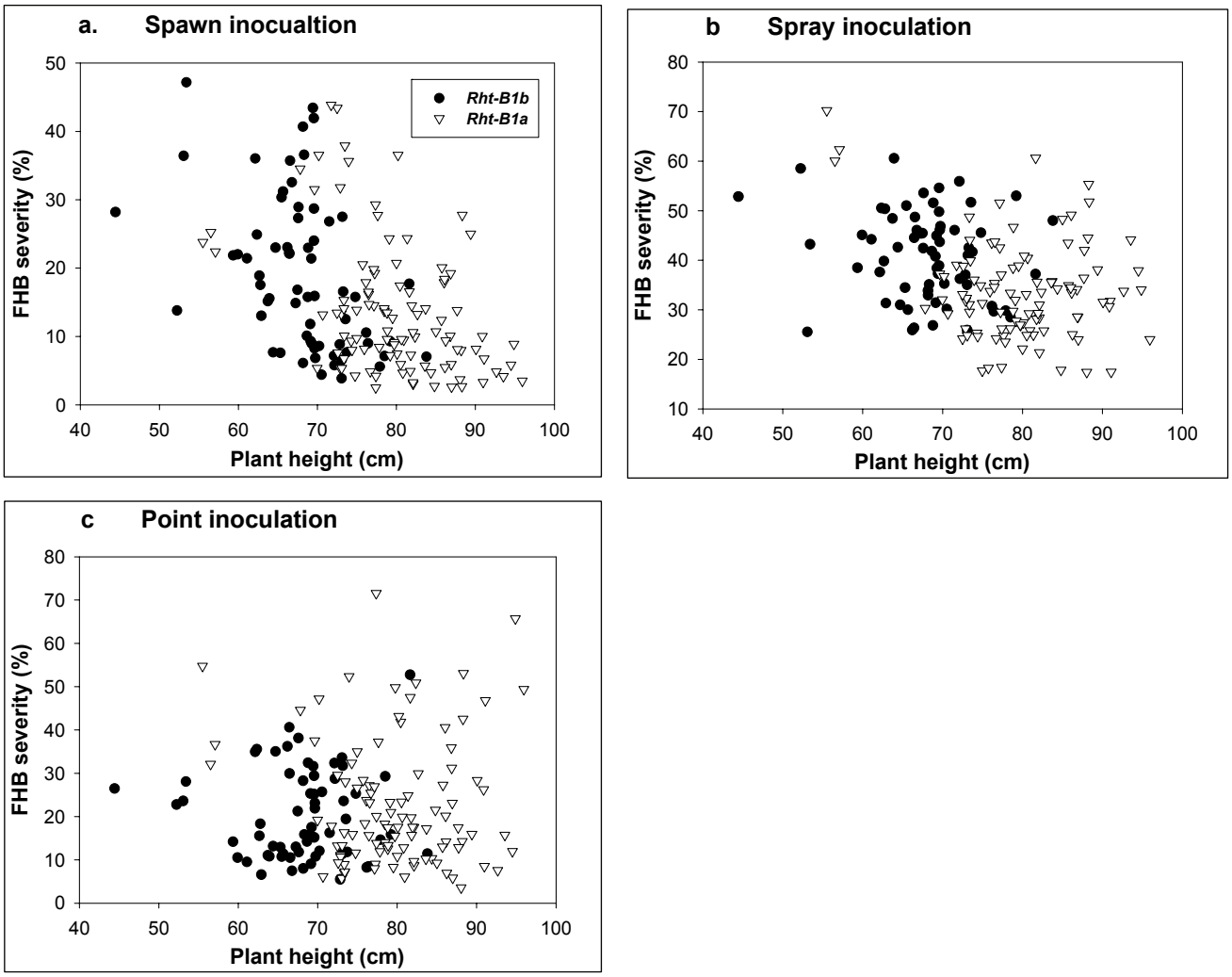

Fig 4. The relationship between plant height and Fusarium head blight (FHB) severity after a) spawn inoculation, b) spray inoculation and c) point inoculation in the SHA3/CBRD x Naxos recombinant inbred line (RIL) population. Each DH line was plotted with mean data and grouped based on the *Rht-B1* status: *Rht-B1a*, wild tall allele; *Rht-B1b*, semidwarf allele. Combining most of other short plant height alleles, three lines with *Rht-B1a* are extremely short compare to other RILs.

Table 1 Analysis of variance for Fusarium head blight and associated traits and their heritabilities in the SHA3/CBRD x Naxos RIL population

Traits	Source	DF	Mean Square	F-Value	P-Value	Heritability
FHB spawn	Genotype	167	523.01	1.16	0.1697	0.41
	Year	1	98635.43	219.03	<.0001	
	Genotype x Year	166	450.33	6.76	<.0001	
	Rep (year)	3	860.11	12.92	<.0001	
	Error	489	66.59			
FHB spray	Genotype	167	343.49	2.26	<.0001	0.57
	Year	1	60339.53	397.28	<.0001	
	Genotype x Year	153	151.88	3.30	<.0001	
	Rep (year)	2	824.37	17.93	<.0001	
	Error	272	45.97			
DON spray	Genotype	167	362.19	3.36	<.0001	0.60
	Year	1	33772.18	313.45	<.0001	
	Genotype x Year	153	107.74	4.25	<.0001	
	Rep (year)	2	367.42	14.51	<.0001	
	Error	267	25.32			
FDK spray (2010)	Genotype	166	665.67	2.49	<.0001	0.70
	Rep	1	4809.70	18.00	<.0001	
	Error	155	267.28			
FHB point	Genotype	167	491.09	3.45	<.0001	0.57
	Year	1	22821.78	160.55	<.0001	
	Genotype*Year	166	142.15	0.54	0.9999	
	Rep (Year)	1	940.65	3.61	0.0594	
	Error	154	260.83			
Plant height	Genotype	167	613.06	14.97	<.0001	0.93
	Year	3	9766.09	238.54	<.0001	
	Genotpe *Year	486	40.94	2.39	<.0001	
	Rep (Year)	4	425.96	24.88	<.0001	
	Error	599	17.12			
Anther extrusion	Genotpe	167	13.11	5.09	<.0001	0.80
	Year	1	25.65	9.96	0.0019	
	Genotpe *year	166	2.58	2.17	<.0001	
	Rep (year)	2	0.7	0.59	0.5539	
	Error	316	1.19			

Table 2 Pearson correlation coefficients among FHB traits in the SHA3/CBRD X Naxos RIL population

		Spawn		Spray					Point		
		FHB08	FHB11	FHB09	FHB10	FDK10	DON09	DON10	FHB09	FHB10	DON 09
Spawn	FHB08	1									
	FHB11	0.29**									
Spray	FHB09	0.44***	0.13								
	FHB10	0.55***	0.33***	0.56***							
	FDK10	0.07	-0.11	0.20	0.23						
	DON09	0.29**	0.04	0.20	0.17	0.18					
	DON10	0.02	-0.00	0.13	0.06	0.45***	0.65***				
Point	FHB09	-0.04	0.25**	0.12	-0.04	-0.03	-0.07	0.03			
	FHB10	0.07	0.31***	0.10	0.11	-0.08	-0.09	-0.06	0.52***		
	DON09	-0.01	0.12	0.04	0.06	0.01	0.02	0.02	0.57***	0.33***	1

*** 0.0001,

**0.001

Table 3 Pearson correlation coefficients between FHB traits and Plant height/anther extrusion in the SHA3/CBRD X Naxos RIL population

		PH08	PH09	PH10	PH11	PH mean	AE09	AE10	AE mean
Spawn	FHB08	-0.41***	-0.41***	-0.47***	-0.46***	-0.47***	-0.37***	-0.49***	-0.47***
	FHB11	-0.48***	-0.32***	-0.40***	-0.42***	-0.44***	-0.47***	-0.38***	-0.47***
	FHB mean	-0.51***	-0.34***	-0.43***	-0.46***	-0.48***	-0.50***	-0.43***	-0.51***
Spray	FHB09	-0.08	-0.15	-0.18	-0.18	-0.16	-0.29**	-0.54***	-0.45***
	FHB10	-0.43***	-0.44***	-0.52***	-0.54***	-0.53***	-0.51***	-0.67***	-0.64***
	FHB mean	-0.26**	-0.28**	-0.39***	-0.39***	-0.37***	-0.39***	-0.65***	-0.56***
	FDK10	0.16	0.12	0.07	0.02	0.10	-0.22	-0.30***	-0.28**
	DON09	0.03	0.01	-0.00	-0.30	0.01	-0.06	-0.08	-0.08
	DON10	0.10	0.09	0.12	0.06	0.11	-0.11	-0.08	-0.10
Point	FHB09	0.03	0.14	0.10	0.06	0.09	-0.17	-0.11	-0.15
	FHB10	-0.08	0.05	0.01	-0.01	-0.01	-0.26	-0.19	-0.25*
	FHB mean	-0.04	0.10	0.05	0.03	0.04	-0.25	-0.18	-0.24*
	DON09	0.00	0.10	0.05	0.04	0.05	-0.08	-0.09	-0.08

*** 0.0001, ** 0.001, * 0.05

Table 4 QTL for FHB traits after spray and grain spawn inoculation in the SHA3/CBRD x Naxos RIL population and their association with other traits. The percentage of explained phenotypic variation (R^2) in the multiple regression models is shown. QTL detected with a LOD score above 3.0 are bolded. Other putative QTL are also listed if they showed significant contribution in the multiple regression model.

		Spawn inoculation			Spray inoculation								
		FHB			FHB			FDK	DON			Resistance	
QTL	Marker interval	2008	2011	mean	2009	2010	mean	2010	2009	2010	mean	source[1]	Associations
1A.1	wPt-8797-wPt-7030								**1.3**	**2.6**	**2**	N	
1B	gwm550-wmc619	**5.7**			**3.5**	**4.1**	4.7					N	
2A	gwm636-barc124							**9.3**	**7.3**	1.8	**4.1**	N	
2BL	wmc441-gwm1267b								**5.2**		2.8	N	
2DL	gwm265-mag3616	**3.1**			2	**2.9**	**2**					N	
3AS	wmc489b-wmc695b									**4.4**	**2.2**	N	
4BS	Rht-B1-gwm368	**11.2**	1.6	3.4	**4.3**	**10.8**	**6.5**					N	PH AE
5AS	wmc489d-wPt-8226							**11.5**	**7.1**	**14**	**10.8**	N	
5BL	barc275-barc232	**7**		**6.1**					**2.9**		**1.8**	N	AE
5D	gwm174-wPt-1400					3.3	**3.5**					N	
7A.1	wmc603-barc292								**16.2**	**7.8**	**11.5**	N	
1A.2	wPt-8016-wPt-2847		**8.3**	**9.3**								S	
2DLc	wmc18-wmc41	**7.5**	**22.3**	**24.3**	2	**12.4**	**5**		**7.4**			S	PH AE
3DL	cfd9-barc323	**3.8**		**1.7**	**2.6**	0.9	2					S	AE
4AL	gwm160-wpt-5172					**10.5**						S	PH AE
5AL	gwm617-gwm291		**5.7**	**6.8**	2.4	**6.5**	**3.6**					S	PH AE
6AS	wPt-0832-wPt-6904	**7.1**			**5.3**		**4.2**					S	PH
6ASc	barc37-wmc748a								**2.6**		**2.1**	S	PH
7A.2	barc121-wPt-8399		**1.8**	2.8								S	AE
Total R^2		43	36.9	45.3	30.8	46.4	39.8	23.2	44.5	32.9	42.7		

[1]N=Naxos, S=SHA3/CBRD

[2]Naxos contributed to reduction of FDK at this locus. PH= plant height, AE= anther extrusion.

Table 5 QTL for FHB traits after point inoculation in the SHA3/CBRD x Naxos RIL population and their association with other traits. The percentage of explained phenotypic variation (R^2) in the multiple regression models is shown. QTL detected with a LOD score above 3.0 are bolded. Other putative QTLs are also listed if they showed significant contribution in the multiple regression model.

		FHB			DON	Resistance	
QTL	Marker interval	2009	2010	mean	2009	source[1]	Associations
1D	wmc432-barc152	**3.7**				N	
2A	gwm328-gdm93		**3.9**	4.4		N	
2BL	wmc441-gwm1267b	2.8	**8.9**	9.4		N	
2DS	gwm296-wPt-11625	**8.9**	**4.8**	**10.3**	4.1	N	
2DLc	gwm539-wmc41		**4.2**			S	PH AE
4DL	barc98-cfd71	**4.6**	5.3	**5.9**	**3.8**	S	
Total R2		20.7	26.4	26.6	8.5		

[1]N=Naxos, S=SHA3/CBRD

Table 6 QTL for other traits in the SHA3/CBRD x Naxos RIL population and their association with FHB severity. The percentage of explained phenotypic variation (R^2) in the multiple regression models is shown. QTL detected with a LOD score above 3.0 are bolded. Other putative QTLs (2<LOD<3) are also listed if they showed significant contribution in the multiple regression model or their confidence interval (CI) were overlapping with FHB QTL.

Traits	QTL	Marker interval	2008	2009	2010	2011	Mean		Association*
Plant height								Tall	
	1B	gwm268-barc188		**7.9**	**6.3**		**4.4**	N	
	2DLc	wmc18-gwm539	4.7			**3.9**	3.2	S	FHBs FHBp
	4AL	barc78-wPt-2794	**12.8**	**10.5**	**8.1**	**8.6**	**12.5**	S	FHBs
	4BS	Rht-B1-gwm368	**26.5**	**38.7**	**45.8**	**30.3**	**39.6**	N	FHBs
	5AL	gwm617-gwm291		2.7	0.5	1.4	0.5	S	FHBs
	6AS	wPt-0832-wPt-2153		1.2	**4.7**			S	FHBs
	6ASc	wPt-0902-barc146		5.2	2.7	4.8	4.4	S	
	Total R^2		35.9	55.1	57.5	42.4	53.9		
Anther extrusion								High AE	
	2DLc	wmc18-gwm539		**5**	**4.4**		**5.9**	S	FHBs FHBp
	3DL	cfd9-barc323		3.6	**3.9**		**4.3**	S	FHBs
	4AL	barc78-wPt2794			8.3			S	FHBs
	4BS	Rht-B1-gwm368		5.1	**10.9**		**10.1**	N	FHBs
	5AL	gwm617-gwm291			2.7			S	FHBs
	5BL	wmc75-barc275			4.5		6.1	N	FHBs
	7A	barc121-wPt-8399		**7.9**	6.3		**6.8**	S	FHBs
	Total R^2			22.7	38.6		31.7		

* FHBs FHB severity after spray or grain spawn inoculation, FHBp FHB severity after point inoculation.

Table 7 Phenotypic effects of different *Rht-B1* alleles affecting plant height and Fusarium head blight (FHB) after different inoculation methods.

	Number of lines	Plant height (cm)	FHB severity (%)		
			Spawn	Spray	Point
Rht-B1b	65	68.1	18.9	41.1	20.3
Rht-B1a	101	79.8	13.8	33.9	22.9
Difference		-11.7***	5.1*	7.2***	-2.6

*** 0.0001, level

*0.05 level

Paper III

Partial resistance to powdery mildew in German spring wheat 'Naxos' is based on multiple genes with stable effects in diverse environments

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Abstract

Powdery mildew is one of the most important wheat diseases in temperate regions of the world. Resistance breeding is considered to be an economical and environmentally benign way to control this disease. The German spring wheat cv. 'Naxos' exhibits high levels of partial and race non-specific resistance to powdery mildew in the field and is a valuable source in resistance breeding. The main objective of the present study was to map the genetic factors behind the resistance in Naxos, based on a population of recombinant inbred lines (RIL) from a cross with the susceptible CIMMYT breeding line SHA3/CBRD. Powdery mildew severity was evaluated in six field trials in Norway and four field trials in China. A major QTL with resistance from Naxos was detected close to the *Pm3* locus on 1AS in all environments, and explained up to 36% of the phenotypic variation. Naxos was shown to carry another major QTL on 2DL and minor ones on 2BL and 7DS. QTL with resistance from SHA3/CBRD were detected on 1RS, 2DLc, 6BL and 7AL. The QTL on the 1B/1R translocation showed highly variable effects across environments corresponding to known virulence differences against *Pm8*. SHA3/CBRD was shown to possess the *Pm3* haplotype on 1AS, but none of the known *Pm3a-g* alleles. The RIL population did not provide any evidence to suggest that the *Pm3* allele of SHA3/CBRD acted as a suppressor of *Pm8*.

Keywords: wheat *Triticum aestivum* L. powdery mildew *Blumeria graminis* f. sp. *tritici* partial resistance QTL mapping

Introduction

Powdery mildew (PM), caused by *Blumeria graminis* (syn. *Erysiphe graminis*) f. sp. *tritici* (*Bgt*), is one of the most important wheat diseases in many regions of the world with temperate climate. It can cause significant yield losses, in the range of 5-34% (Griffey et al. 1993; Conner et al. 2003). Fungicides are extensively applied to prevent epidemics and maintain high yields when susceptible cultivars are grown.

Breeding for resistance is a more economical and environmentally benign way to control this disease and could reduce the dependence on fungicides. So far, at least 43 loci for resistance to wheat powdery mildew (*Pm1–Pm45*) have been identified and assigned to specific chromosomes or chromosome arms (Hua et al. 2009; Huang and Röder 2004; Zhu et al. 2005; Miranda et al. 2006; Liu et al. 2001; Blanco et al. 2008; Miranda et al. 2007; Xu et al. 2010; Lillemo et al. 2008; Ma et al. 2011). However, most of them are race-specific and likely of short durability (McDonald and Linde 2002; Skinnes 2002). Most race-specific resistance genes tend to lose their effect within a few years when cultivars are widely grown, due to the occurrence of matching virulence in the pathogen population.

Partial or race non-specific resistance is inherited in a quantitative manner and has been shown to be more durable. Breeding cultivars with partial resistance is therefore a more sustainable way to control this disease. Identification of germplasm with partial resistance is, however, hampered by symptom similarity with incomplete effects of race-specific resistance (Lillemo et al. 2010a). Breeding for resistance can therefore be greatly enhanced by the use of molecular markers.

Several major QTL for partial resistance have been identified in the North American winter wheats ‘Massey’ (Liu et al. 2001) and ‘USG3209’ (Tucker et al. 2007), the Swiss winter wheat ‘Forno’ (Keller et al. 1999), the French winter wheats RE714 (Mingeot et al. 2002; Chantret et al. 2000) and RE9001 (Bougot et al. 2006), the Japanese wheat ‘Fukuho-komugi’ (Liang et al. 2006), in the CIMMYT spring wheats ‘Opata 85’, W7984 (Börner et al. 2002) and Saar (Lillemo et al. 2008), and in the Chinese cultivars ‘Lumai

21' (Lan et al. 2010) and 'Bainong 64' (Lan et al. 2009). The QTL on 1BL, 2BL and 7DS were the most frequently detected. These QTL all have stable performance across environments and explained considerable proportions of the phenotypic variance. Recently, two leaf rust and yellow rust loci of partial resistance, *Lr34/Yr18* and *Lr46/Yr29* have been demonstrated to be associated with partial PM resistance (Spielmeyer et al. 2005; Lillemo et al. 2008; Singh et al. 2000) and given the gene designations *Pm38* and *Pm39*, respectively (Lillemo et al. 2008). Both genes are present in diverse germplasm and have contributed to effective partial resistance over several decades (Lillemo et al. 2008; William et al. 2003; Kolmer 1996; Singh et al. 1998).

The German spring wheat cv. 'Naxos' exhibits high levels of partial resistance to powdery mildew in the field. The race non-specificity of its resistance was recently confirmed in a seedling test with differential isolates (Lillemo et al. 2010b). It is a promising source of resistance, but little is known about the genetic basis of its resistance. The objectives of this study were: 1) to detect QTL responsible for partial resistance in a population of recombinant inbred lines (RIL) from a cross with Shanghai3/Catbird (SHA3/CBRD); 2) to assess the stability of detected QTL across different environments; and 3) to identify closely linked markers for resistance breeding.

Materials and methods

Plant materials

A RIL population of 181 F_6 lines was developed by single seed descent from the cross SHA3/CBRD x Naxos. Naxos was developed by Strube GmbH & Co.KG from the cross 'Tordo/St.Mir808-Bastion//Minaret'. It does not show any race-specific resistance at the seedling stage but high levels of partial resistance to powdery mildew at the adult plant stage (Lillemo et al. 2010b). SHA3/CBRD is a breeding line from CIMMYT with the pedigree 'Shanghai 3//Chuanmai 18/Bagula' and selection history "-0SHG-6GH-0FGR-0FGR-0Y". It is moderately susceptible to powdery mildew, carrying *Pm8* on the 1B/1R translocation and the *Pm3* haplotype on chromosome 1AS based on the UP3B/UP1A primers, but none of the *Pm3a-g* alleles based on allele-specific PCR markers (Tommasini et al. 2006). The spring wheat cultivar 'Prins' (NGB 6688) and its near-

isogenic line (NIL) with *Pm8* (Weique/8*Prins, NGB 6099) were obtained from the Nordic Genetic Resource Center (Alnarp, Sweden).

Field trials

Norway

Powdery mildew resistance was evaluated over three years (2008, 2009 and 2010) at two locations in southeastern Norway that represent powdery mildew populations with different virulence composition (Skinnes 2002): Vollebekk Research Farm at the Norwegian University of Life Sciences, Aas (59°N, 90 m above sea level) and Staur research farm close to Hamar (60°N, 153 m above sea level). Both locations experienced severe natural powdery mildew epidemics during experimental seasons. Field trials were carried out in a randomized complete block design with two replications. The RIL population, parents and *Pm8* NILs were planted as hillplots to provide favorable conditions for disease development. The planting was delayed compared with the normal planting to ensure sufficient natural mildew inoculum, and the highly susceptible line Avocet-S was planted as spreader rows on each side of the experiments. Disease severity was scored on penultimate leaves as the percentage of infected leaf area at three times during the disease development with about one week intervals (Lillemo et al. 2008). The area under the disease progress curve (AUDPC) was calculated according to Bjarko and Line (1988). Heading date was recorded every year in the same trials at Aas. In 2008 heading date in a separate Fusarium head blight (FHB) experiment was also scored. The days to heading (DH) were calculated for further analysis.

China

Powdery mildew severity was evaluated during cropping seasons 2010 and 2011 at Chinese Academy of Agricultural Sciences (CAAS), Beijing (39^{o}N, 43.5 m above sea level) and in 2010 at CAAS Cotton Research Institute, Anyang, Henan province (36^{o}N, 70-80 m above sea level). The field trials were laid out in randomized complete blocks with three replications. Fifty seeds of each genotype were sown together in a circle (diameter, 8 cm) and circle to circle distance was maintained by 0.4 m in a row of 2 m length, and row to row distance was 30 cm apart to produce a conducive environment for

disease development. The susceptible cultivar 'Jingshuang16' was planted as a check at each tenth row and around the experimental plot to ensure plenty of powdery mildew inoculum in spring. Artificial inoculation was carried out using a highly virulent *Blumeria graminis* f. sp. *tritici* isolate E20 prior to stem elongation in Beijing. Disease severity was scored first time five weeks after inoculation based on percentage of leaf area covered by powdery mildew on penultimate leaves (F-1 leaf) and then one week later when disease appeared at its maximum level around May 20. In Anyang, powdery mildew disease severity was evaluated once under natural inoculum conditions when the infection level on the susceptible check Jingshuang16 reached its maximum around the third week of May.

Another powdery mildew testing in China was carried out at Jiangsu Academy of Agricultural Sciences (JAAS), Nanjing (32^{o}N, 15 m above sea level), Jiangsu province in 2009. The field trial was laid out in a randomized complete block design with two replications, and sown in late October in 150 cm rows at 33 cm distance. On the bilateral of experiment blocks, Sumai 3, a highly susceptible local cultivar, was planted as spreader rows. PM inoculum was prepared from the pathogen mixture collected from the local area in the previous season, and inoculated onto the Sumai 3 spreader rows in the spring when plants returned green. Disease severity was scored on penultimate leaves as the percentage of infected leaf area at the stage of flowering.

Statistical analysis

The distribution of each trait in each year and location was tested for normality using the PROC UNIVARIATE procedure of the SAS software package (SAS Institute Inc.,Version 9.1). Analysis of variance was performed using the PROC GLM procedure of SAS. Heritability was estimated from the ANOVA information using the formula $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_E^2 / r)$ within a year and the formula $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{gxy}^2 / y + \sigma_E^2 / ry)$ across years, where σ_g^2 is genetic variance, σ_{gxy}^2 is genotypes-by-years interaction, σ_E^2 is error variance, y is number of experiments, r is number of replications. The Pearson correlation coefficients were calculated using the PROC CORR procedure of SAS.

Genetic map construction and QTL analysis

Initially, 283 polymorphic DArT markers covering all the chromosomes were used for mapping of the RIL population. Based on the SSR consensus map of Somers et al. (2004), the gap regions were supplemented with 105 polymorphic SSR markers. After initial QTL detection, the genetic map was refined with more SSR markers in the QTL regions. In addition, SCM9 for detection of the 1B/1R wheat-rye translocation (Schneider and Molnár-Láng 2009) and the *Pm3* haplotype specific marker UP3B/UP1A (Tommasini et al. 2006; Yahiaoui et al. 2006) were also included. The genotypic data of 181 lines including 283 DArT and 271 SSR loci were finally used to construct a genetic linkage map with the software JoinMap v. 3.0 (Van Ooijen and Voorrips 2001). Consensus map information from Graingenes (http://wheat.pw.usda.gov/GG2/index.shtml) was used to assign linkage groups to chromosomes.

QTL analysis was performed with PLABQTL v. 1.2 (Utz and Melchinger 1996). Simple interval mapping (SIM) was conducted first to detect the major QTL for PM. The markers most closely linked to each QTL across environments were then used as cofactors in composite interval mapping (CIM). Significant QTL in single environments and for the overall mean were set at 3.0 LOD threshold based on 1000 permutations for each phenotypic trait. QTL reaching this level in one environment were also reported for other environments even though their LOD scores were lower. Genetic map drawing and QTL marking were conducted by the software MapChart v.2.1 (Voorrips 2002).

AUDPC was calculated and used together with maximum PM severity for correlation analysis and QTL mapping. The results were consistent with those for PM severity. Hence, all the tables and figures only present the results from PM severity.

Results

Phenotypic evaluation

Histograms of mean PM severity of the 181 RILs in each environment are shown in Fig. 1. Powdery mildew developed well in Norway both in 2008 and 2010 with some extreme

lines exceeding 90% disease severity. The 2009 season in Norway experienced less favorable conditions, and the powdery mildew symptoms ceased to develop due to a leaf blotch epidemic promoted by rainy weather. The distributions were therefore skewed towards low severity. In China, a broad variation was observed in Nanjing in 2009 with maximum severity around 80%. PM epidemics at Anyang and Beijing developed less with maximum severities around 15%.

Despite unevenly developed symptoms, the correlation coefficients among PM scores remained highly significant across environments, ranging from 0.35 to 0.87 (Table 1). The heritabilities across environments were 0.92 in Norway and 0.60 in China (Table 2).

Continuous variation with transgressive segregation was present in all environments (Fig. 1), which indicates that both parents carry resistance genes to powdery mildew. This was confirmed later in the QTL mapping. ANOVA analysis confirmed significant variation among RILs both within and across locations (Table 2).

PM mapping

From the total of 554 polymorphic marker loci, 422 loci were assembled into 29 linkage groups. The genetic map spanned a total of 2192.3 cM and represented all chromosomes.

QTL for PM severity were detected on 1AS and 2DL in most environments with simple interval mapping (SIM). These two consistent QTL were used as cofactors in composite interval mapping (CIM). QTL regions with lower resolution or partial peaks were then supplemented with more SSR markers based on consensus maps (Somers et al., 2004; GrainGenes: http://wheat.pw.usda.gov/GG2/index.shtml). The final QTL analysis from CIM is presented in Table 3 and Fig. 2. Eight QTL for resistance were identified across ten environments. Two major QTL with resistance from Naxos were detected on 1AS and 2DL in most environments, while two other minor QTL with resistance from Naxos were detected on 2BL and 7DS. SHA3/CBRD contributed a total of four QTL on 1RS, 2DLc (2DL near centromere), 6BL and 7AL, which showed more consistent effects in Norway.

The most frequently detected major QTL was mapped on 1AS about 6 cM from the *Pm3* locus (Fig.2, Table 3). It explained from 15 to 36% of the phenotypic variation across all

environments with the resistance contributed by Naxos, which does not have the *Pm3* haplotype based on the functional marker UP3B/UP1A. Another major QTL mapped to the distal region of 2DL in the interval *Xwmc817-Xcfd50*. This QTL was highly consistent across eight environments and explained from 7 to 22% of the phenotypic variation. The third consistent QTL with favorable allele from Naxos was detected near *Xwmc463* and *Xwmc438* on 7DS, and explained up to 12% of the phenotypic variation. The QTL on 2BL with resistance from Naxos was detected at both locations in Norway in 2008 and 2010, but not in the 2009 season which had less disease development. The QTL was located near *Xwmc441* and explained up to 12% of the phenotypic variation in single environments.

The QTL on 1RS was detected in five of the six environments in Norway (except Hamar 2009) with the explained phenotypic variation ranging from 6 to 13%, however it was less effective in Nanjing and showed no effect in Beijing and Anyang. The 2DLc QTL with resistance from SHA3/CBRD was detected in Norway in 2008 and 2010 and in Anyang. It mapped near the centromere close to *Xgwm539* and explained from 6 to 13% of the phenotypic variation in detectable environments. The other two minor QTL close to the centromeres on 6BL and 7AL with resistance from SHA3/CBRD were only detected in Norway.

Effect of race-specific alleles

Pm8 was more effective in Norway than in China (Fig. 3). In Norway, RILs with *Pm8* significantly decreased PM severity by 25 to 60% compared with its counterpart. In China, *Pm8* decreased PM severity by 22% in Nanjing while the effect was negligible in Beijing and Anyang. The effect of *Pm8* was stronger in the Prins NILs than in the RILs (Fig. 3), but followed the same pattern of environmental variation as in the RILs.

Pm3 haplotype analysis identified the *Pm3*-specific 900 bp product in SHA3/CBRD, while only the 1.1 kb homoeologue on 1B was amplified in Naxos. None of the *Pm3a-g* allele specific primers (Tommasini et al. 2006) amplified any product from SHA3/CBRD, indicating that the line has an unknown allele at this locus. RILs were classified into 4 groups based on the *Pm3* haplotype analysis (Fig. 4). This could also be

considered a combination analysis of *Pm3* and *Pm8* since RILs carrying the 1B/1R translocation had the 1B homoeologue replaced by *Pm8*. A similar pattern was observed in all five locations. The combination of *Pm8* from SHA3/CBRD and the 1AS QTL from Naxos showed the highest resistance to PM, while the combination of the 1B homoeologue from Naxos and the *Pm3* haplotype from SHA3/CBRD always provided the highest susceptibility. Whether combined with *Pm8* or the 1B homoeologue of *Pm3*, the 1AS QTL from Naxos always provided better resistance than the *Pm3* haplotype from SHA3/CBRD. The *Pm3* haplotype of SHA3/CBRD increased the susceptibility of RILs by different degrees ranging from 20 to 114% compared with the 1AS QTL from Naxos.

Discussion

Naxos has a long record of highly effective adult plant resistance to powdery mildew, and the lack of any race specific resistance at the seedling stage (Lillemo et al 2010) suggested that the resistance should be polygenic. This was confirmed both by the phenotypic distributions and the QTL mapping in the present study.

Comparison with other reports

Previous studies have already detected resistance QTL to powdery mildew at the *Pm3* locus on 1AS (Bougot et al. 2006; Liang et al. 2006; Mohler et al. 2011). At this locus, Liang et al. (2006) detected a QTL with resistance from Fukuho-komugi which was derived from the *Pm3a* carrier Norin 29 and suggested that the adult plant resistance might be a residual effect of *Pm3a*. Bougot et al. (2006) mapped a QTL for powdery mildew resistance at the vernalized seedling stage with resistance conferred by the *Pm3g* carrier RE9001. Recently, *Pm3e* conferred resistance at both seedling and adult plant stages in the German winter wheat cultivar Cortez (Mohler et al. 2011). Interestingly, in contrast to the previous studies where the favorable alleles were derived from parents carrying race specific *Pm3* alleles, Naxos carries no race specific resistance (Lillemo et al. 2010b) and apparently lacks the *Pm3* gene based on the UP3B/UP1A marker. The QTL with resistance from Naxos is therefore most likely due to a gene for race non-specific resistance located in the same area as the *Pm3* gene on 1AS.

Keller et al. (1999) reported a QTL for PM resistance from the winter wheat cultivar Forno on 2DL in the marker interval *Xpsr932-Xpsr331a*, explaining 8-12% of the phenotypic variation. Börner et al. (2002) detected a minor QTL on 2DL in the ITMI population with resistance from the synthetic wheat W7984. Additionally, a partial QTL near marker *Xgwm301* was also detected in the Swedish winter wheat cultivar Folke (Lillemo et al. submitted). In the present study, this locus explained up to 22% of the phenotypic variation, much higher than that in other genetic and environmental backgrounds.

Börner et al. (2002) reported a 7DS QTL in W7984 at the adult plant stage. Subsequently, partial resistance QTL to PM were detected in the same region in Fukuho-komugi and Saar (Liang et al. 2006; Lillemo et al. 2008) and identified as *Lr34/Yr18/Pm38*. The 7DS QTL in the present study mapped to the same region based on the consensus map, but the functional marker *cssfr5* (Lagudah et al. 2009) amplified the 523 bp fragment specific for the non-*Lr34* allele in both Naxos and SHA3/CBRD (data not shown). The LOD curves for this QTL (Fig. 2) did, however, not peak at the *Xgwm1220* and *swm10* loci that are known to delineate the *Lr34* locus (Lillemo et al. 2008; Bossolini et al. 2006), but closer to *Xwmc438* and *Xwmc463* located 18 cM more distally on 7DS. Both parents showed expression of leaf tip necrosis (LTN), which is often used as a phenotypic marker for *Lr34* (Singh 1992), but this trait is also associated with *Lr46*. We could therefore not use LTN to sort out this anomaly. Additionally, *Lr34/Yr18/Pm38* usually shows stronger and more stable resistance across environments than the 7DS QTL detected in the present study (Spielmeyer et al. 2005; Lillemo et al. 2008). We therefore conclude that the resistance from Naxos at this locus is likely different from *Lr34/Yr18/Pm38*, but this warrants further study.

The 2BL QTL from Naxos was located in the same region as PM resistance QTL from Massey (Liu et al. 2001), the progeny line USG3209 (Tucker et al. 2007), Chinese cv. Lumai 21 (Lan et al. 2010) and Japanese cv. Fukuho-Komugi (Liang et al. 2006). This QTL was not detected in China, which might result from the insufficient symptom development and lower heritabilities in the Chinese disease nurseries. This QTL was

relatively stable and detectable across environments in Norway, but showed consistently less effect than the 1AS and 2DL QTL.

The resistance QTL on 1B was located to the 1B/1R translocation of SHA3/CBRD based on the rye specific markers SCM9 (Saal and Wricke 1999) and NOR (Koebner 1995). Since *Pm8* is known to be located on this translocation, we conclude that this QTL was caused by *Pm8*.

The 6BL QTL near *Xwmc539* in this study is located more than 40 cM away from the previously reported PM resistance QTL on this chromosome in Bainong 64 (Lan et al. 2009) and Folke (Lillemo et al. submitted) based on the consensus map, and must therefore be a different locus. Interestingly, based on another marker *Xbarc354*, the QTL in Naxos is located close to the high-temperature adult plant yellow rust resistance gene *Yr36* originating from wild emmer wheat (Uauy et al. 2005), and recently cloned and found to be absent from modern wheat varieties (Fu et al. 2009). Hence, this 6BL QTL is likely caused by a novel and previously uncharacterized gene for partial and race non-specific resistance to powdery mildew.

The 2DLc QTL with resistance from SHA3/CBRD mapped in the same region as *Pm43* originating from *Thinopyrum intermedium* (He et al. 2009) and an adult plant resistance (APR) QTL from Lumai 21 (Lan et al. 2010). It is more likely to share the same resistance basis with the latter rather than *Pm43,* which is derived from a wheat relative. The 7AL QTL was located close to *Xbarc108* near the centromere similar to the QTL detected in RE714 (Chantret et al. 2001). Besides, a putative QTL on 7AL close to *Xgwm334* in this study (Fig. 2) mapped to the same position as similar QTL reported by Chantret et al. (2001) and Lillemo et al. (submitted).

Effect of race specific genes

Pm8 was more effective in Norway, while negligible in China. This could be attributed to the differences in the composition of PM pathogen populations in Norway and China. Besides, the trials in Beijing were inoculated with strain E20, which is virulent to *Pm8* (Wang et al. 2005). The 1B/1R translocation with *Pm8* derived from 'Petkus' rye has been widely used in Chinese breeding programs since the 1970s (He et al. 2001). Almost

half of the Chinese varieties carry the 1B/1R translocation (Yu 2000; Wang et al. 2005), and the frequency of matching virulence in the pathogen populations had already reached over 90% in the late 1990s (Duan and Sheng 1998). Nevertheless, *Pm8* still had a weak effect in Nanjing which might be due to the lower frequency of 1B/1R cultivars in its belonging wheat production region (20%) compared to the area around Anyang (42%) (Zhou et al. 2004).

In Norway, however, *Pm8* is rare and not present in any of the current spring and winter wheat cultivars (Lillemo et al. 2010b). Additionally, only 1% of Nordic wheat accessions carry *Pm8* (Hysing et al. 2007). This indicates a much weaker selection pressure for *Pm8* virulence in the local pathogen populations in Norway and neighbouring countries, and accordingly *Pm8* was more effective.

Although PCR amplification with the UP3B/UP1A primers indicated the presence of a *Pm3* gene in SHA3/CBRD, the identity is unknown as the allele-specific markers did not detect any of the previously characterized *Pm3a-g* alleles. Moreover, no conclusions can be made as to whether SHA3/CBRD carries a functional allele of this gene since the resistance of the line has not been tested with differential isolates. In the QTL analysis, the effect of the *Pm3* allele can only be compared with the corresponding 1AS QTL for partial resistance located at the same position in Naxos. It indicated that the Naxos QTL provided much stronger effect on PM resistance than the *Pm3* allele from SHA3/CBRD.

Pm8 was suppressed at the seedling stage in certain 1B/1R translocation lines (Ren et al. 1997; Hanusova et al. 1996). The suppressor of *Pm8* has been mapped to the short arm of chromosome 1A, and McIntosh et al. (2011) recently demonstrated that *Pm3* could act as a suppressor of the *Pm8* resistance. Both SHA3/CBRD and its parent Catbird carry the *Pm3* haplotype and *Pm8* based on diagnostic markers, but Catbird did not express the *Pm8* resistance at the seedling stage (Lillemo et al. 2010b). If the *Pm3* allele of SHA3/CBRD functioned as a suppressor of *Pm8* in our mapping population, we would not have expected to see any effect of *Pm8* in the background of *Pm3*. However, the opposite was observed, with *Pm8* providing additional resistance in the backgrounds of both *Pm3* and the 1AS QTL from Naxos. As McIntosh et al (2011) suggested that

translation of *Pm3* is necessary for *Pm8* suppression, we may conclude that the *Pm3* allele of SHA3/CBRD is likely not functional.

Implications for breeding

In this study, genetic analysis showed that the high level of partial resistance to powdery mildew in Naxos was mainly governed by two major QTL on 1AS and 2DL, and minor QTL on 2BL and 7DS. Markers closely linked to these loci have potential to improve the selection for PM resistance in breeding populations generated from this highly valuable source of resistance.

Several other promising sources of partial resistance have also been identified and demonstrated their effectiveness under highly conducive environments for powdery mildew epidemics (Lillemo et al. 2010a; Lillemo et al. 2010b). Markers are already available for some of them such as Saar (Lillemo et al. 2008), Folke (Lillemo et al. submitted), Massey and USG 3209 (Liu et al. 2001; Tucker et al. 2007) and RE714 (Muranty et al. 2009). The QTL reported here for Naxos and their corresponding closely linked molecular markers might serve to diversify the genetic basis of partial and potentially durable resistance to powdery mildew and accelerate the breeding process.

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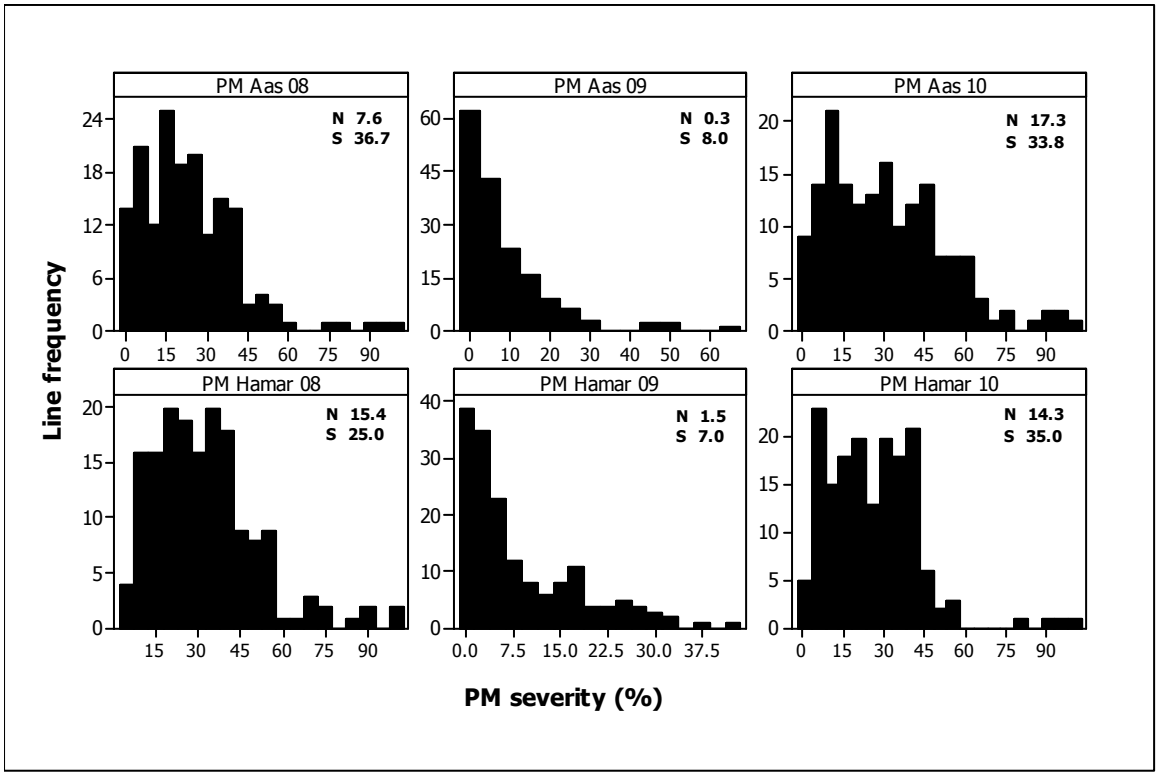
a.



b.

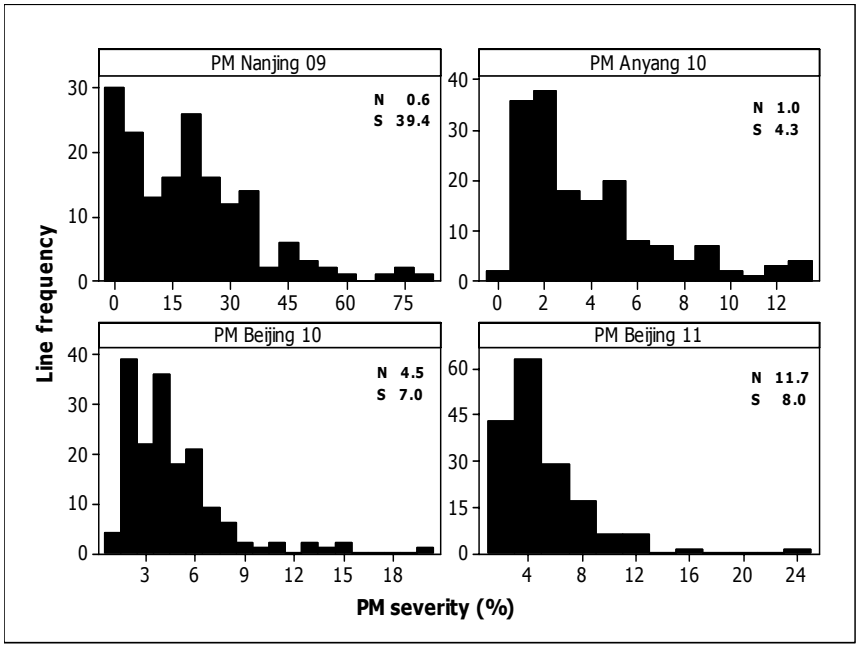
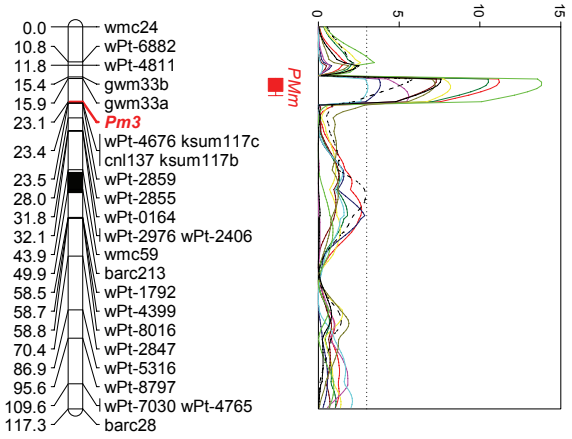
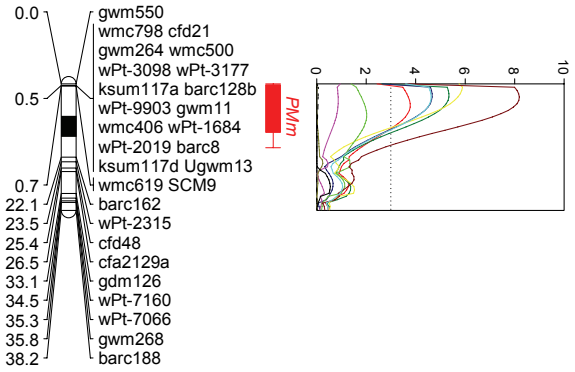


Fig.1 Frequency distribution of powdery mildew (PM) severity in the SHA3/CBRD x Naxos RIL population in a) Norway and b) China. N= mean severity of Naxos, S= mean severity of SHA3/CBRD

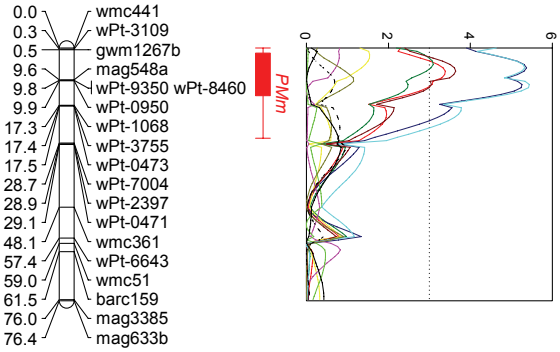
1A



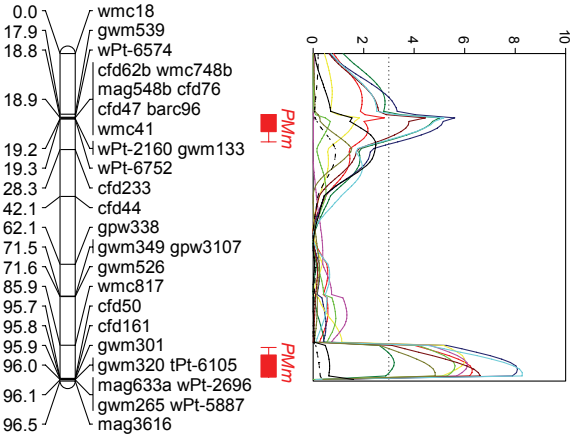
1BS/1RS.1BL



2BL



2DL



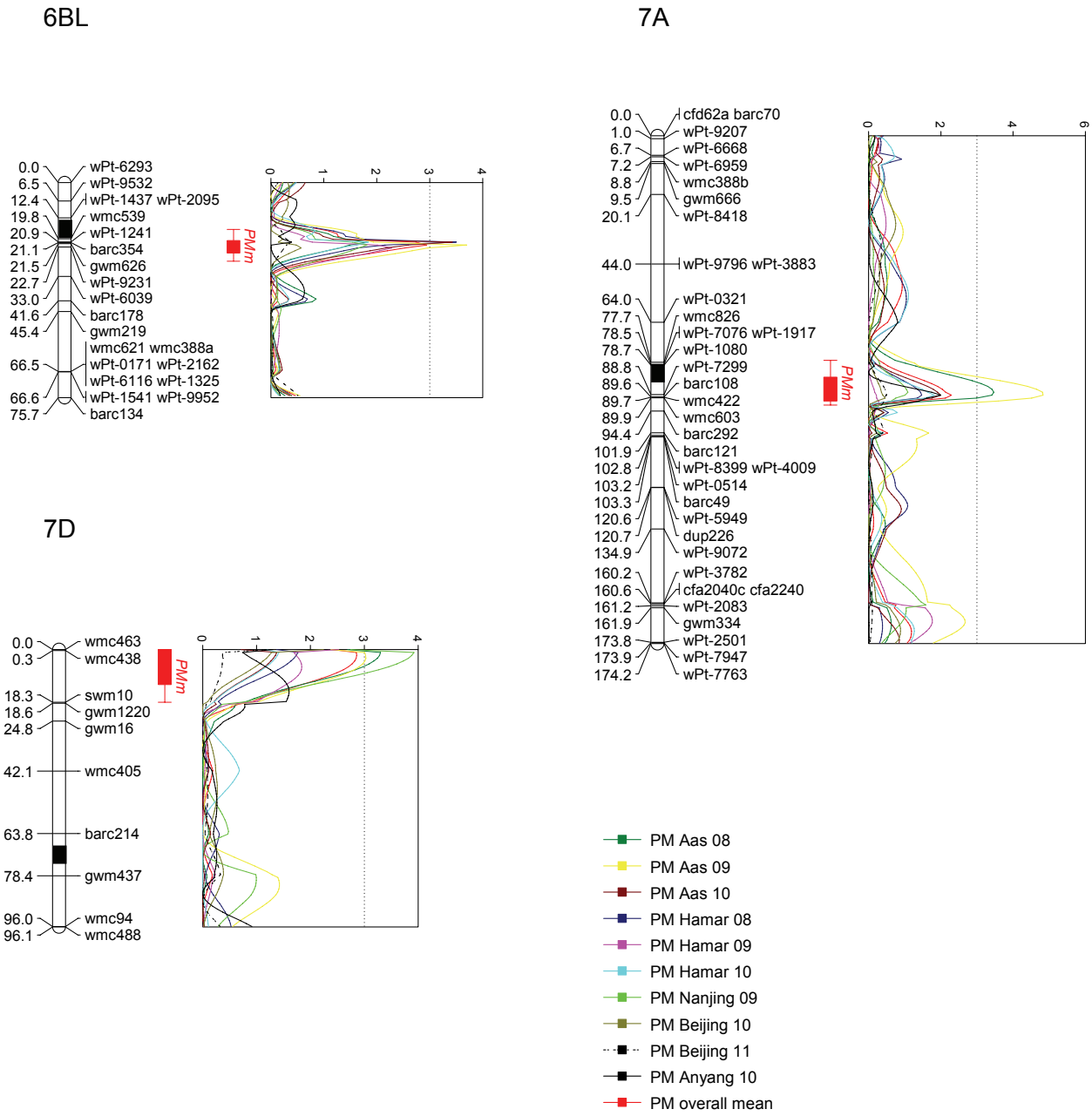


Fig.2 Chromosomes with significant QTL, with corresponding LOD curves obtained from CIM. Genetic distances are shown in centimorgans to the left of the chromosomes. A threshold of 3.0 is indicated by a dashed vertical line in the LOD graphs. The approximate positions of centromeres are indicated by solid squares.

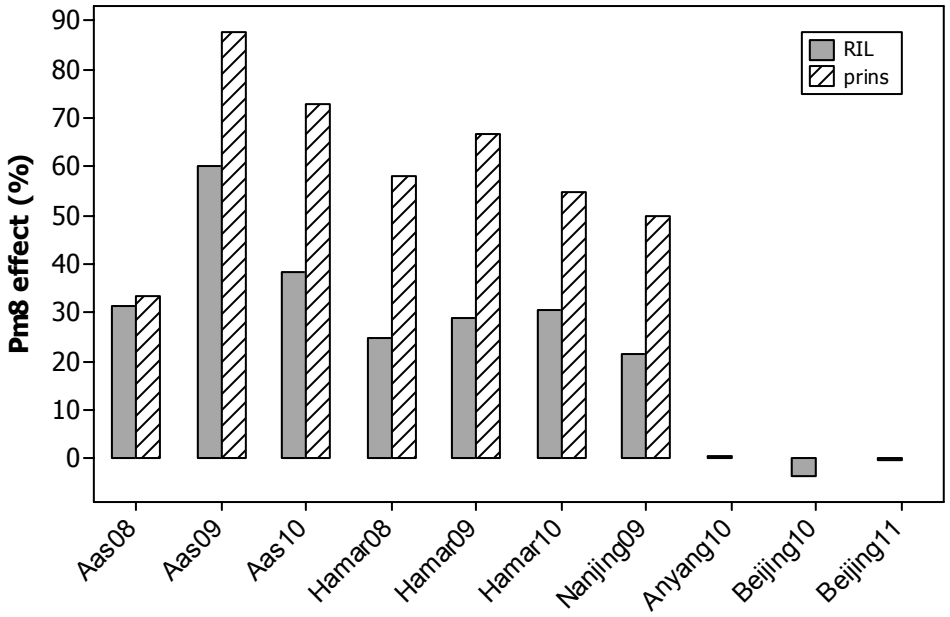


Fig. 3 The effect of *Pm8* in RIL and Prins NIL background in each environment. The effect was charted in terms of relative reduction in PM severity compared with its counterparts.

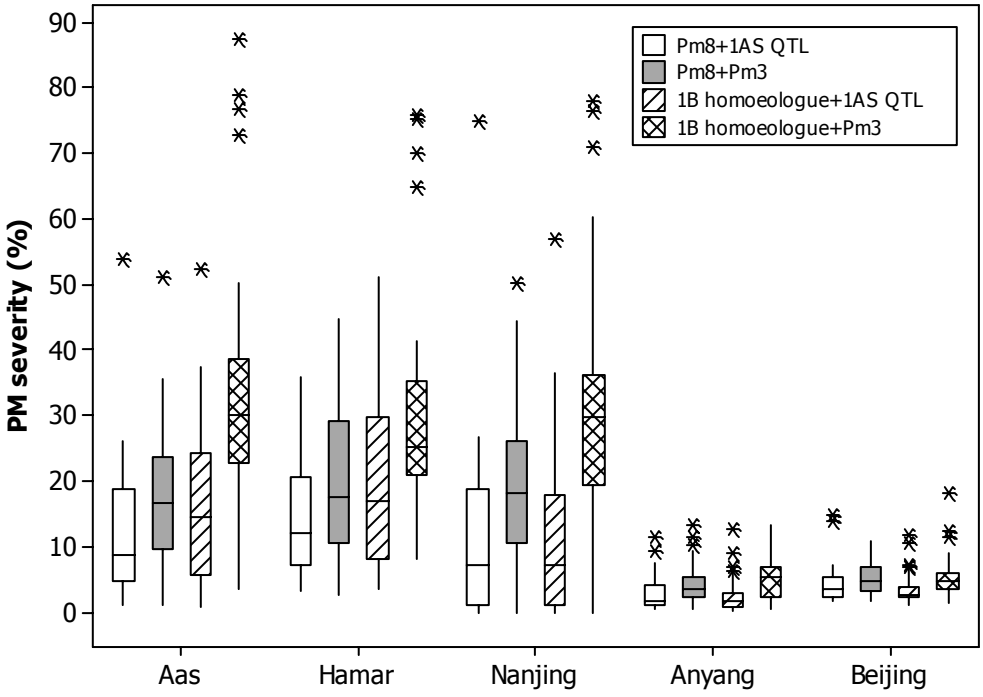


Fig. 4 Boxplot of *Pm3* haplotypes in the RIL population. *Pm3* allele determination was based on the functional marker UP3B/UP1A. Additionally, 1RS specific marker SCM 9 was used to differentiate the 1B homoeologue of *Pm3* from 1RS. *Pm3* homoeologue on 1B and 1AS QTL are from Naxos, while *Pm3* and *Pm8* are from SHA3/CBRD.

Table 1 Pearson correlation coefficients among powdery mildew severities for individual environments, mean days to heading, and heritability estimated for each environment of the SHA3/CBRD x Naxos RIL population.

	AaS 08	Aas 09	Aas 10	Hamar 08	Hamar 09	Hamar 10	Nanjing 09	Anyang 10	Beijing 10	Beijing 11
Aas 09	0.82***									
Aas 10	0.81***	0.74***								
Hamar 08	0.81***	0.67***	0.80***							
Hamar 09	0.51***	0.63***	0.57***	0.48***						
Hamar 10	0.81***	0.74***	0.83***	0.87***	0.58***					
Nanjing 09	0.71***	0.77***	0.65***	0.60***	0.62***	0.59***				
Anyang 10	0.55***	0.50***	0.44***	0.44***	0.42***	0.44***	0.51***			
Beijing 10	0.58***	0.57***	0.57***	0.54***	0.56***	0.55***	0.64***	0.59***		
Beijing 11	0.45***	0.43***	0.41***	0.38***	0.36***	0.35***	0.49***	0.58***	0.66***	
Mean days to heading	-0.09	0.01	-0.04	-0.07	0.39**	-0.06	0.18	0.09	0.12	0.10
Heritability	0.94	0.95	0.89	0.93	0.87	0.94	0.7	0.79	0.77	0.83

0.0001 level ***, 0.001 level **

Table 2 Analysis of variance for powdery mildew severity in the SHA3/CBRD x Naxos RIL population

	Source	DF	Mean Square	F Value	P	Heritability
Norway	Genotype	167	2295.01	13.14	<.0001	0.92
6 exp.	Experiment	5	36091.62	206.63	<.0001	
	Genotype*Experiment	832	174.66	3.55	<.0001	
	Rep(Experiment)	6	212.83	4.33	0.0003	
	Error	966	49.14			
China	Genotype	167	322.60	2.43	<.0001	0.60
4 exp.	Experiment	3	19873.06	149.79	<.0001	
	Genotype*Experiment	495	132.67	4.41	<.0001	
	Rep(Experiment)	7	327.18	10.87	<.0001	
	Error	1092	30.11			
Total	Genotype	167	2250.50	10.54	<.0001	0.91
10 exp.	Experiment	9	45879.74	214.83	<.0001	
	Genotype*Experiment	1494	213.56	5.47	<.0001	
	Rep(Experiment)	13	274.40	7.03	<.0001	
	Error	2058	39.04			

Table 3 QTL results from composite interval mapping (CIM) in the SHA3/CBRD x Naxos RIL population, showing the percentage of explained phenotypic variation (R^2) of individual QTL effect in multiple regression and for the whole model. The table indicates all the QTL that were detected with a minimum LOD score of 3 (highlighted in bold) in at least one environment. Other QTL are also listed if they were detected above the threshold in other environments and showed significant contribution in multiple regression.

	PM severity in Hamar				PM severity in Aas				PM severity in China				overall	Resistance
QTL	08	09	10	Mean	08	09	10	Mean	Nanjing09	Anyang10	Beijing10	Bejing11	mean	source
1AS	**15.2**	**19.2**	**15.4**	**20**	**33.7**	**25.7**	**26.6**	**36.4**	**36**	**20.1**	**20.8**	**16.5**	**37.5**	Naxos
1RS	**6.0**		**9.3**	**6.4**	**9**	**7.1**	**13.4**	**12.1**	3.8				**1.5**	SHA3/CBRD
2BL	**9.7**		**12.2**	**10.4**	**5.1**		**4.1**	**3.8**					**6**	Naxos
2DLc	**12.6**		**12.9**	**9.5**	**8.2**		**12**	**10**		**6.4**			**5.7**	SHA3/CBRD
2DL	**19.8**	**16.8**	**22.2**	**23.2**	**6.7**	**17.1**	**16.3**	**15.8**	**15.4**		**13.1**		**15.1**	Naxos
6BL	**5.8**	6.6		3.7		**6.4**	**5.4**	**4.7**					8.1	SHA3/CBRD
7AL					**4.4**	**10.1**	1.5	**4**					2.1	SHA3/CBRD
7DS	1	6.5		2.8	**6.5**	**11.3**		**4.9**	**12.2**		**5.1**		5.8	Naxos
Total R^2				49.5				55.7					57.4	