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Anther extrusion and plant height are associated with Type I resistance to Fusarium head blight resistance in bread wheat line ‘Shanghai-3/Catbird’

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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 anther extrusion (AE) and plant height (PH). 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 2DLc affecting FHB severity and DON after all inoculation methods. This QTL was also associated with AE and PH, with high AE and tall alleles contributed by SHA3/CBRD. Several QTL for AE and PH were detected, and low AE or reduced PH 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 AE or PH, while the QTL from Naxos were mostly not. After point inoculation, no other QTL for FHB traits was associated with AE or PH, 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.

Keywords Wheat Fusarium head blight Resistance Anther extrusion Plant height QTL mapping

Abbreviations Fusarium head blight (FHB) Anther extrusion (AE)
Plant height (PH) 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 (Buerstmayr et al. 2009; Liu et al. 2009). *Fhb5* on 5A (Buerstmayr et al. 2003b; Buerstmayr et al. 2003a; Steiner et al. 2004; Chen et al. 2006; Xue et al. 2011) and QTL on 3A (Steiner et al. 2004; Yu et al. 2008) contribute mainly Type I resistance and less Type II resistance. However, *Fhb1* on 3BS (Waldron et al. 1999; Anderson et al. 2001; Bai et al. 1999), *Fhb2* on 6B (Anderson et al. 2001; Yang et al. 2003; Cuthbert et al. 2007) and QTL on 2D (Jia et al. 2005; Lin et al. 2006; Yang et al. 2005) contribute mainly Type II resistance and less Type I resistance. 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).

Avoidance is conditioned by morphological and developmental characters such as anther extrusion (AE) and plant height (PH). 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* with two major components choline and betaine and the sites of initial infection after inoculation (Strange and Smith 1971; Strange et al. 1974). However, a later study indicated that endogenous compounds in floral parts may not be associated with wheat resistance to *F. graminearum* (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 in lines with a low AE, anthers trapped between glumes provide dead tissue readily colonized by *Fusarium*. Then active types of resistance are needed to reduce infection. Lines with high AE had much less chances to develop

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.

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/CBRD) 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 thus may add to the resistance diversity and elucidate the overlooked role of AE.

A recombinant inbred line (RIL) population was developed from SHA3/CBRD (high AE, *Rht-B1b*) and Naxos (low AE, *Rht-B1a*). 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 AE / PH .

Materials and methods

Plant materials

A RIL population of 181 F₆ 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, has high AE and carries the *Rht-B1b* allele. Naxos is susceptible to FHB in the field, has low AE and carries the *Rht-B1a* allele. It is a German spring variety with a high level of partial resistance to powdery mildew (Lillemo et al. 2010; Lu et al. 2012), and 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, Braunschweig, Germany, using a mixture of isolates of *F. graminearum* which were provided by the Norwegian Veterinary Institute, Oslo. Two isolates with low aggressivity (101177 and 101023) were used in 2008, these two plus 101118 and 101018 with somewhat higher aggressivity 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/m². 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 was 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 1x10⁵ 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 (Norwegian 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).

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 Skinnies et al. (2010). Plant height was measured in a separate experiment in 2008 and in the disease nurseries in 2009, 2010 and 2011.

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 late heading stage (before flowering), a single floret in the middle of the head was inoculated with about 20 μ l conidial suspension of 1×10^5 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 inoculated 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 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

After spawn inoculation, disease development in 2008 and 2011 varied widely with average severities of 4.6% and 26.9% in the RIL population. Significant variation was

observed within the population in each year, but with large GxE (genotype x year) interactions which led to a heritability of only 0.14 (Table 1). Transgressive segregants were observed, and the distribution was skewed towards low mean severity of two years (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, 0.70 for DON content and 0.60 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 GxE interactions were significant, FHB severities were still correlated with each other across years for the same inoculation methods (Table 2), as was 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 spray 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. Naxos always had much higher severity than SHA3/CBRD in high or moderate disease pressures after spawn and spray inoculation (Fig. 1), but clearly less FDK and DON content in 2010 and similar DON content in 2009. This indicates that the two parents carry different resistance types: SHA3/CBRD has more resistance to infection (Type I and/or Type II) than Naxos, the latter more resistance to DON content and FDK (Type III and Type IV), which was later confirmed in the QTL analysis.

The RIL population showed wide and significant variation in AE and PH (Fig. 1, Table 1). Transgressive segregation was apparent towards both sides for AE and PH.

Despite significant GxE interactions the heritabilities were still high, 0.80 and 0.93 for AE and PH respectively. Both AE and PH were negatively correlated with FHB severity both within and across years after spawn and spray inoculation. The negative correlations of FHB with AE ($r = -0.45$ to -0.64) were of same magnitude as with PH ($r = -0.37$ to -0.53), except severity in 2009 after spray inoculation (Table 3) in which high disease pressure and shorter plant height occurred. 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 combined impact of AE and PH on FHB severities after the three inoculation methods is 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

Resistance to FHB severity in SHA3/CBRD was controlled by one major QTL on 2DLc and four minor QTL on 1AL, 3DL, 5AL, 6AS and 7AL (Table 4, Fig. 3). 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 1AL,

3DL, 5AL and 7AL 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 1BS, 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 1BS and 2DL were only detected in 2008.

Resistance after spray inoculation

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 (Table 4, Fig. 3). 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 1BS, 2DL and 5DL 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 chromosome 5A and accounted for over 10% of the phenotypic variation for both traits. The other one was located on 2AS 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 7AL 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 1AL, 2BL, 3AS

and 5BL, while SHA3/CBRD contributed two minor QTL for DON content on 2DLc and 6ASc (Table 4, Fig. 3).

Resistance after point inoculation

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 1DS, 2AL and 2BL (Table 5, Fig. 3). 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 consistent 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 the QTL on 2DLc and 2BL were effective but with variable phenotypic contributions. The 2DLc QTL mainly reduced severity after spawn and spray inoculation, less after point inoculation. The 2BL QTL added resistance to severity after point inoculation and a minor effect on resistance to DON content after spray inoculation.

QTL mapping of AE and PH

AE was mainly controlled by a major QTL on 4BS at the *Rht-B1* locus which explained 10% 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 all other AE enhancing alleles at three minor QTL on 2DLc, 3DL and 7AL (Table 6, Fig. 3).

For PH, three significant QTL were detected on 4BS, 4AL, 1BL and four putative QTL on 2DLc, 5AL, 6ASc and 6AS (Table 6, Fig. 3). As expected, the major QTL on 4BS coincided with the *Rht-B1* locus and accounted for 40% of the phenotypic variation. Another major QTL on 4AL explained 12% of the phenotypic variation. Naxos contributed the tallness alleles at *Rht-B1* and on 1BL, while SHA3/CBRD contributed the rest.

Association between FHB and AE/ PH

Generally, AE and PH were more associated with FHB severity than with other FHB traits (Table 3 and 4). 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. The contour plots well illustrate the relationship of increasing AE and PH with reduced FHB severity which was observed for both spawn and spray inoculation, but not for point inoculation. Such negative associations between AE/PH and severity were confirmed by the QTL analysis: at each of the coincident QTL, both low AE and reduced PH increased the FHB severity.

After spawn and spray inoculation, six of seven QTL with resistance from SHA3/CBRD to FHB severity were associated with AE or PH, meanwhile only two of five QTL with severity resistance from Naxos were associated with these 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 2DLc resistance QTL was associated with both AE and PH, while the 4AL and 3DL QTL were associated respectively with PH and 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 is commonly used for evaluation of Type II resistance. Although spawn and spray inoculations were performed in Norway and point inoculation in China, we have good reasons to believe that 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. 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; 2010). 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.

Lack of correlation between point and spray/spawn inoculation data was observed, except for a weak correlation between severity after point and spawn inoculation (Table 2). One reason for the discrepancy might be the lack of *Fhb1* in this population, which has a big effect on both Type I and Type II resistance. However, in the present study only two common QTL were detected with small effects for both types of resistance.

Both the continuous distribution of AE and the QTL analysis confirmed that several factors are involved in the inheritance of AE. This supports the results by Skinnes et al. (2010). The broad sense heritability of 0.80 for AE 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-B1* 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 7AL 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 2AS 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 they are under different genetic control. A meta-analysis with 163 studies showed generally high positive correlations among FHB traits (Paul et al. 2005), but with some exceptions (Wiśniewska et al. 2004; Mesterhazy et al. 1999) showing little or no correlations. In the present study, such exceptions were also observed: the lack of relationship between severity and DON content, but moderate correlation of FDK with DON content. Moreover, the QTL analysis showed clearly that SHA3/CBRD carries resistance QTL mostly to severity, while Naxos mostly to FDK and DON content. These all emphasized the importance of pyramiding different resistance components in improving the overall FHB resistance level.

AE and FHB

The negative correlations between AE and FHB severity observed after spawn/spray inoculation ($r = -0.45$ to -0.64) agree with Skinnies et al. (2008; 2010). The correlations between AE and FHB severity from different years clearly indicate they are genetic and do not differ much from those seen between FHB scores in different years. Moreover the QTL analysis confirmed that all AE QTL coincided with FHB severity. Most of the AE QTL detected in the present study are different from those reported by Skinnies et al. (2010), only the 7AL QTL was located in a similar region. This supports that AE is controlled in a quantitative manner. The high heritabilities indicate that it can be easily selected for in breeding programmes. Also the relationship between floral opening, AE and FHB may be complex. Kubo et al. (2010) found that cleistogamous RILs were less infected after spray inoculation than chasmogamous ones, with no difference after point inoculation. However, even incomplete flower closure was able to prevent infection, corresponding to our results with regard to AE. 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). Despite this complexity, the anthers did add risk to FHB infection. After point

inoculation in both resistant Sumai-3 and susceptible Zhemai-1, the emasculated florets were less infected than those with anther retained florets (Liang et al. 1981). Cleaning up the anthers on Yangmai-1 every day during flowering led to the control efficiency by 40% (Liu et al. 1985). 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. 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). Hence AE is an avoidance mechanism.

Most of the resistance QTL to severity from SHA3/CBRD coincided with AE QTL and is likely due to such avoidance. It points out the possibility that several of the FHB resistance QTL reported previously in numerous papers which could actually be caused by AE. If so, instead of MAS for such avoidance QTL, visual selection for AE could be more cost-effective with its high heritability. Also based on these results we recommend AE to be included in FHB resistance studies in order to clarify whether reduced FHB severity is due to avoidance or active resistance.

PH and FHB

Significant negative correlations were observed between PH and FHB severity ($r = -0.37$ to -0.53) after spawn/spray inoculation. The exception was in 2009, 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 varied 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.

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 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. 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”. Conversely, although Naxos has *Rht-B1a* it was the most susceptible parent. This shows that there is ample scope for gene combination for PH and maintain resistance to FHB, especially if high AE is also actively pursued.

Significant association between AE and PH were found with moderate correlation coefficient ($r=0.43$) and two common QTL were detected for the two traits. At these coincident QTL, both low AE and reduced PH conferred increased susceptibility. Despite some common genetic control, much of the variability in AE and PH is controlled by independent genetic factors. Hence, developing FHB resistant cultivars with high AE and short straw is possible in breeding.

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. Most resistance QTL from SHA3/CBRD coincided

with AE QTL, indicating that its resistance is mostly due to avoidance. With high heritability, phenotypic selection for AE combined with the marker-assisted selection based on the 2DLc QTL is recommended for resistance breeding when SHA3/CBRD is used as resistance donor. Naxos, although it had higher severity in the field, still provided three major resistance QTL for FDK and DON content. These components different from SHA3/CBRD could also be combined. The RILs with integrated resistance components from both parents could be valuable breeding lines for further application.

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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	0.14
	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	0.70
	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	0.60
	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 x Year	166	142.15	0.54	0.9999	
	Rep (Year)	1	940.65	3.61	0.0594	
	Error	154	260.83			
Anther extrusion	Genotype	167	13.11	5.09	<.0001	0.80
	Year	1	25.65	9.96	0.0019	
	Genotype x year	166	2.58	2.17	<.0001	
	Rep (year)	2	0.7	0.59	0.5539	
	Error	316	1.19			
Plant height	Genotype	167	613.06	14.97	<.0001	0.93
	Year	3	9766.09	238.54	<.0001	
	Genotype x Year	486	40.94	2.39	<.0001	
	Rep (Year)	4	425.96	24.88	<.0001	
	Error	599	17.12			

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					
Point	DON10	0.02	-0.00	0.13	0.06	0.45***	0.65***				
	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

*** $P < 0.0001$, ** $P < 0.001$

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

		Anther extrusion	Plant height
Spawn	FHB08	-0.47***	-0.47***
	FHB11	-0.47***	-0.44***
	FHB mean	-0.51***	-0.48***
Spray	FHB09	-0.45***	-0.16
	FHB10	-0.64***	-0.53***
	FHB mean	-0.56***	-0.37***
	FDK10	-0.28**	0.10
	DON09	-0.08	0.01
	DON10	-0.10	0.11
Point	FHB09	-0.15	0.09
	FHB10	-0.25*	-0.01
	FHB mean	-0.24*	0.04
	DON09	-0.08	0.05

*** $P < 0.0001$, ** $P < 0.001$, * $P < 0.05$

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.

QTL	Marker interval	Spawn inoculation			Spray inoculation			FDK	DON	Resistance source ^a	Associations ^b		
		FHB severity	FHB severity	FHB severity	FHB severity	FHB severity	FHB severity						
		2008	2011	mean	2009	2010	mean	2010	2009	2010	mean		
1AL.1	wPt-8797-wPt-7030								1.3	2.6	2.0	N	
1BS	gwm550-wmc619	5.7			3.5	4.1	4.7					N	
2AS	gwm636-barc124							9.3	7.3	1.8	4.1	N	
2BL	wmc441-gwm1267b								5.2		2.8	N	
2DL	gwm265-mag3616	3.1			2.0	2.9	2.0					N	
3AS	wmc489b-wmc695b									4.4	2.2	N	
4BS	<i>Rht-B1</i> -gwm368	11.2	1.6	3.4	4.3	10.8	6.5					N	AE PH
5AS	wmc489d-wPt-8226							11.5	7.1	14	10.8	N	
5BL	barc275-barc232	7.0		6.1					2.9		1.8	N	AE
5DL	gwm174-wPt-1400					3.3	3.5					N	
7AL.1	wmc603-barc292								16.2	7.8	11.5	N	
1AL.2	wPt-8016-wPt-2847		8.3	9.3								S	
2DLc	wmc18-wmc41	7.5	22.3	24.3	2.0	12.4	5.0		7.4			S	AE PH
3DL	cf9-barc323	3.8		1.7	2.6	0.9	2.0					S	AE
4AL	gwm160-wPt-5172					10.5						S	PH
5AL	gwm617-gwm291		5.7	6.8	2.4	6.5	3.6					S	PH
6AS	wPt-0832-wPt-6904	7.1			5.3		4.2					S	PH
6ASc	barc37-wmc748a								2.6		2.1	S	PH
7AL.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		

^aN=Naxos, S=SHA3/CBRD

^b AE= anther extrusion, PH= plant height

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.

QTL	Marker interval	FHB severity			DON	Resistance source ^a	Associations ^b
		2009	2010	mean	2009		
1DS	wmc432-barc152	3.7				N	
2AL	gwm328-gdm93		3.9	4.4		N	
2BL	wmc441-mag548a	2.8	8.9	9.4		N	
2DS	gwm296-wPt-11625	8.9	4.8	10.3	4.1	N	
2DLc	wmc18-wmc41		4.2			S	AE PH
4DL	barc98-cfd71	4.6	5.3	5.9	3.8	S	
Total R^2		20.7	26.4	26.6	8.5		

^aN=Naxos, S=SHA3/CBRD

^bAE= anther extrusion, PH= plant height

Table 6 QTL for AE mean (anther extrusion) and PH mean (plant height) 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 ($2 < \text{LOD} < 3$) are also listed if they showed significant contribution in the multiple regression model or their confidence interval (CI) were overlapping with FHB QTL.

QTL	Marker interval	Anther extrusion	Plant height	High AE /tallness ^a	Association ^b
1BL	gwm268-barc188		4.2	N	
2DLc	wmc18-gwm539	5.9	3.9	S	FHBs FHBp
3DL	cf9-barc323	4.3		S	FHBs
4AL	barc78-wPt2794		12.0	S	FHBs
4BS	<i>Rht-B1</i> -gwm368	10.1	39.7	N	FHBs
5AL	gwm617-gwm291		0.3	S	FHBs
5BL	wmc75-barc275	6.1		N	FHBs
6AS	wPt-0832-wPt-2153		3.1	S	FHBs
6ASc	wPt-0902-barc146		3.1	S	
7AL	barc121-wPt-8399	6.8		S	FHBs
Total R^2		31.7	55.3		

^aN=Naxos, S=SHA3/CBRD

^bFHBs 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
<i>Rht-B1b</i>	65	68.1	18.9	41.1	20.3
<i>Rht-B1a</i>	101	79.8	13.8	33.9	22.9
Difference		-11.7***	5.1*	7.2***	-2.6

*** $P < 0.0001$, ** $P < 0.05$

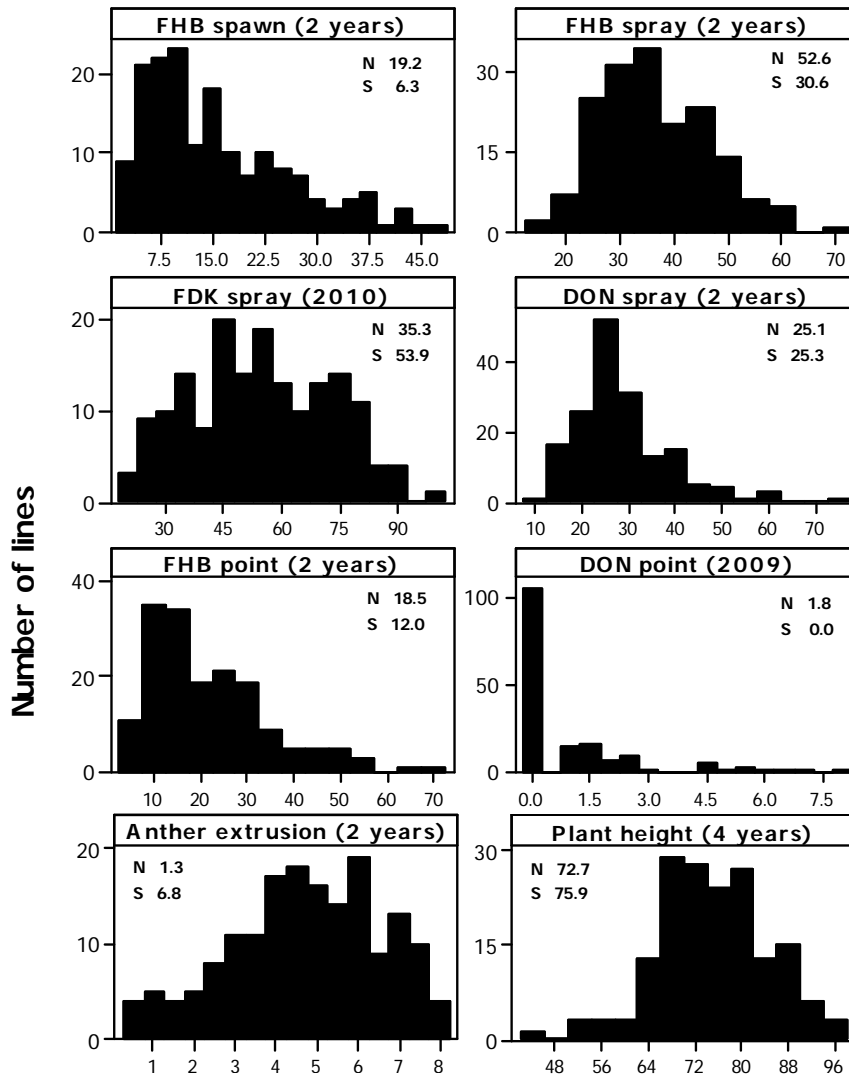


Fig.1 Frequency distribution of FHB and associated traits in the SHA3/CBRD x Naxos RIL population based on the mean data except FDK after spray inoculation and DON content after point inoculation which only have one year data. Inoculation methods were marked behind the trait name. N= Naxos, S= SHA3/CBRD

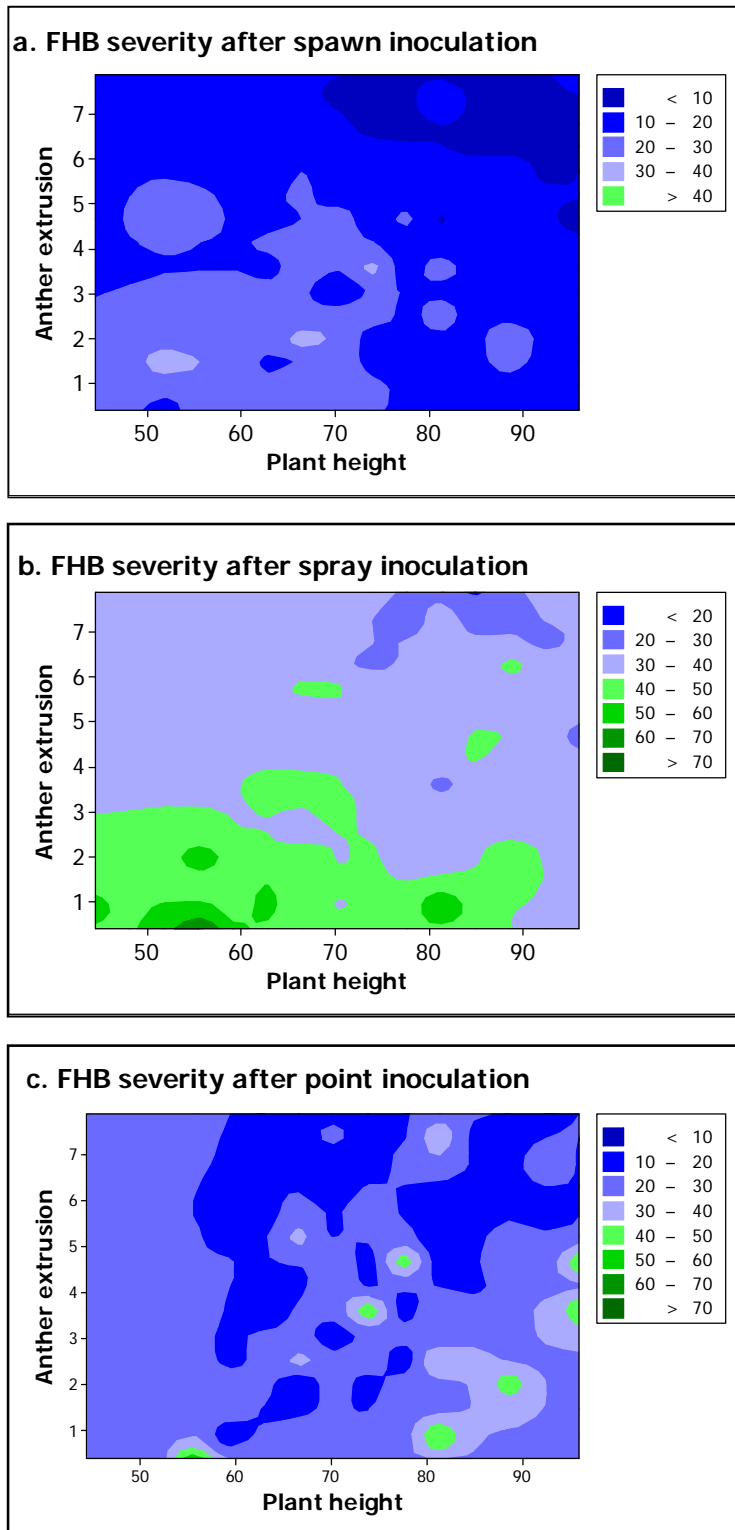
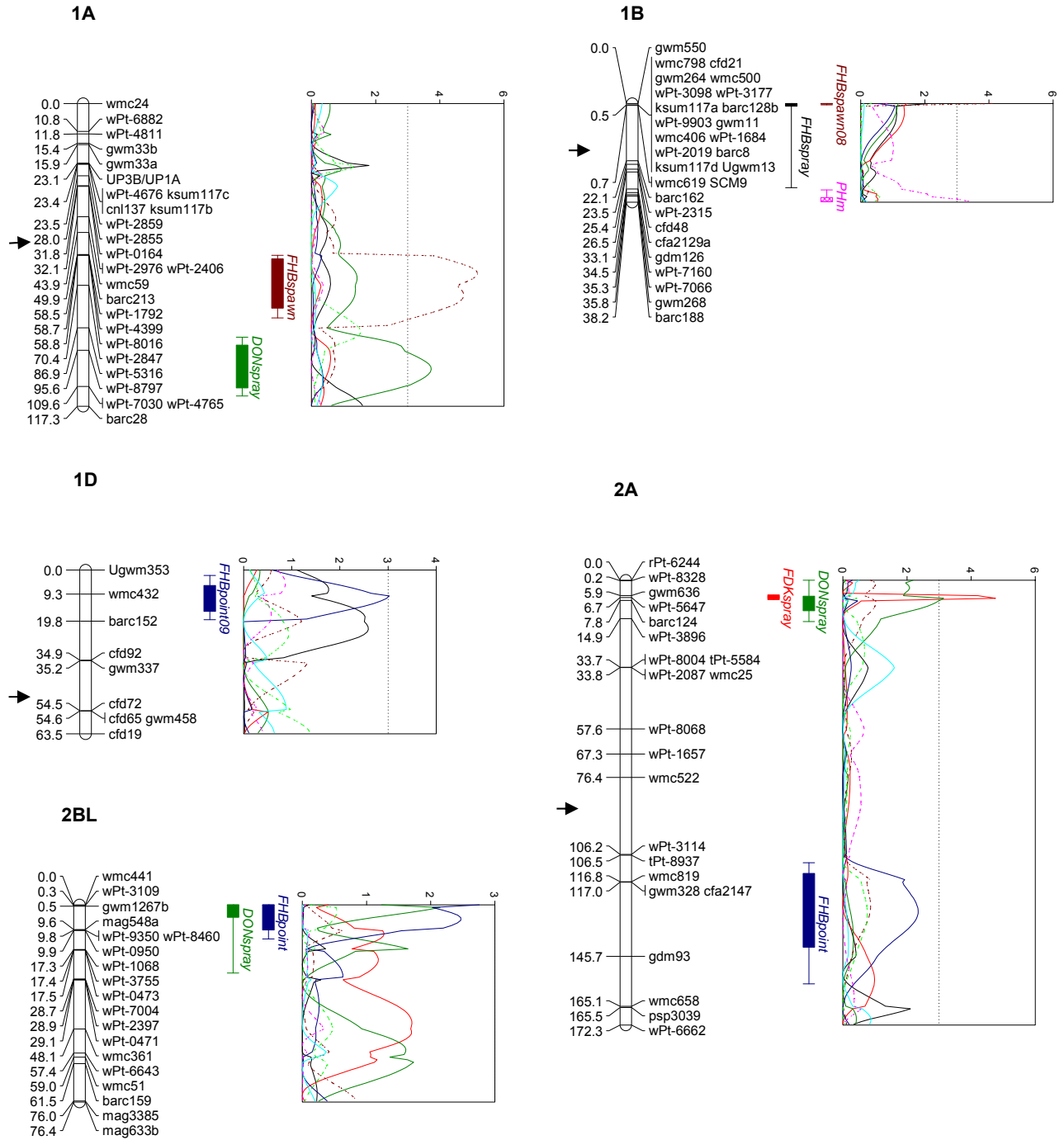
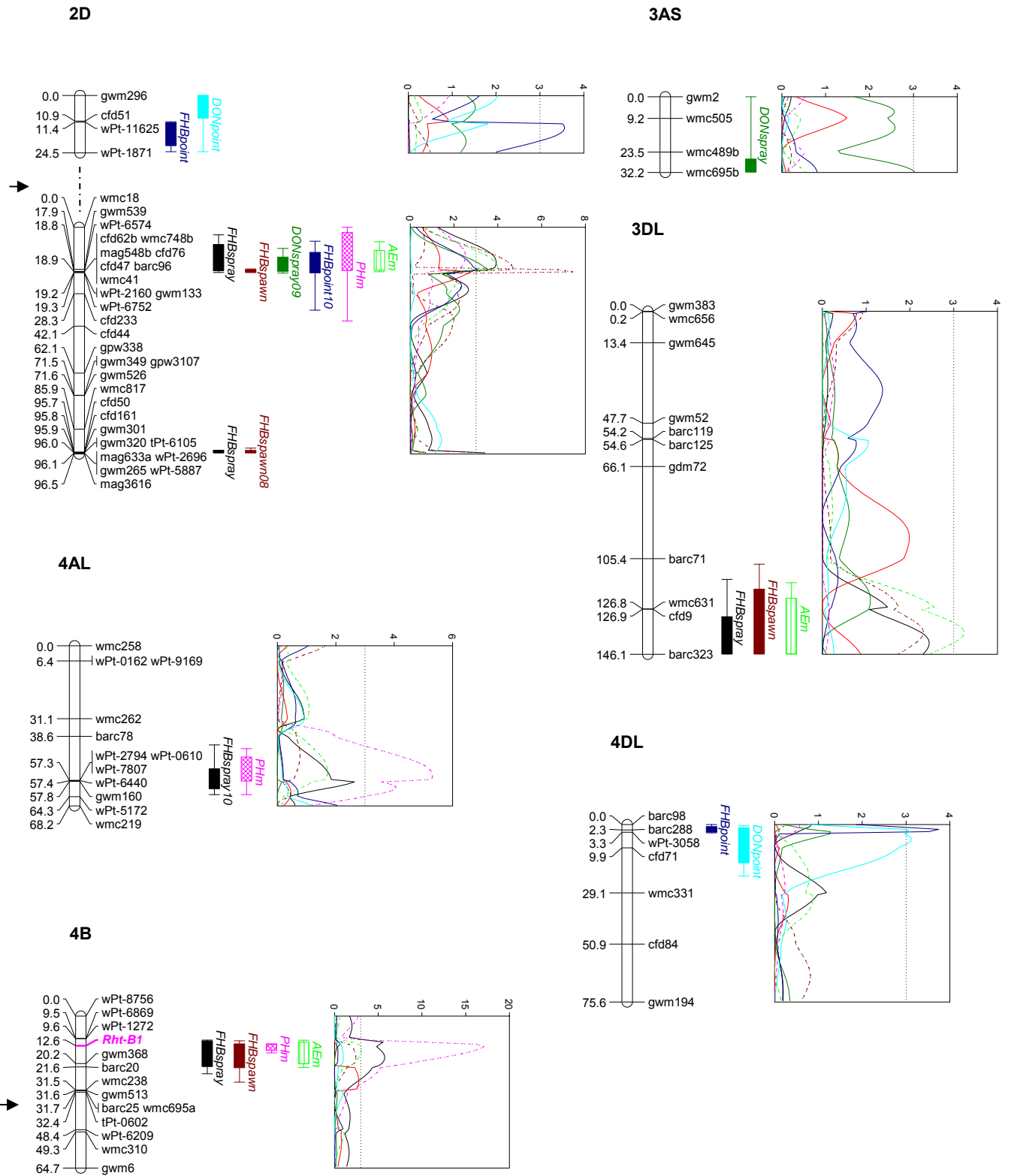


Fig. 2 Contour plots of plant height and anther extrusion vs. a) FHB severity after spawn inoculation, b) FHB severity after spray inoculation, c) FHB severity after point inoculation. All the traits are plotted based on the mean data.





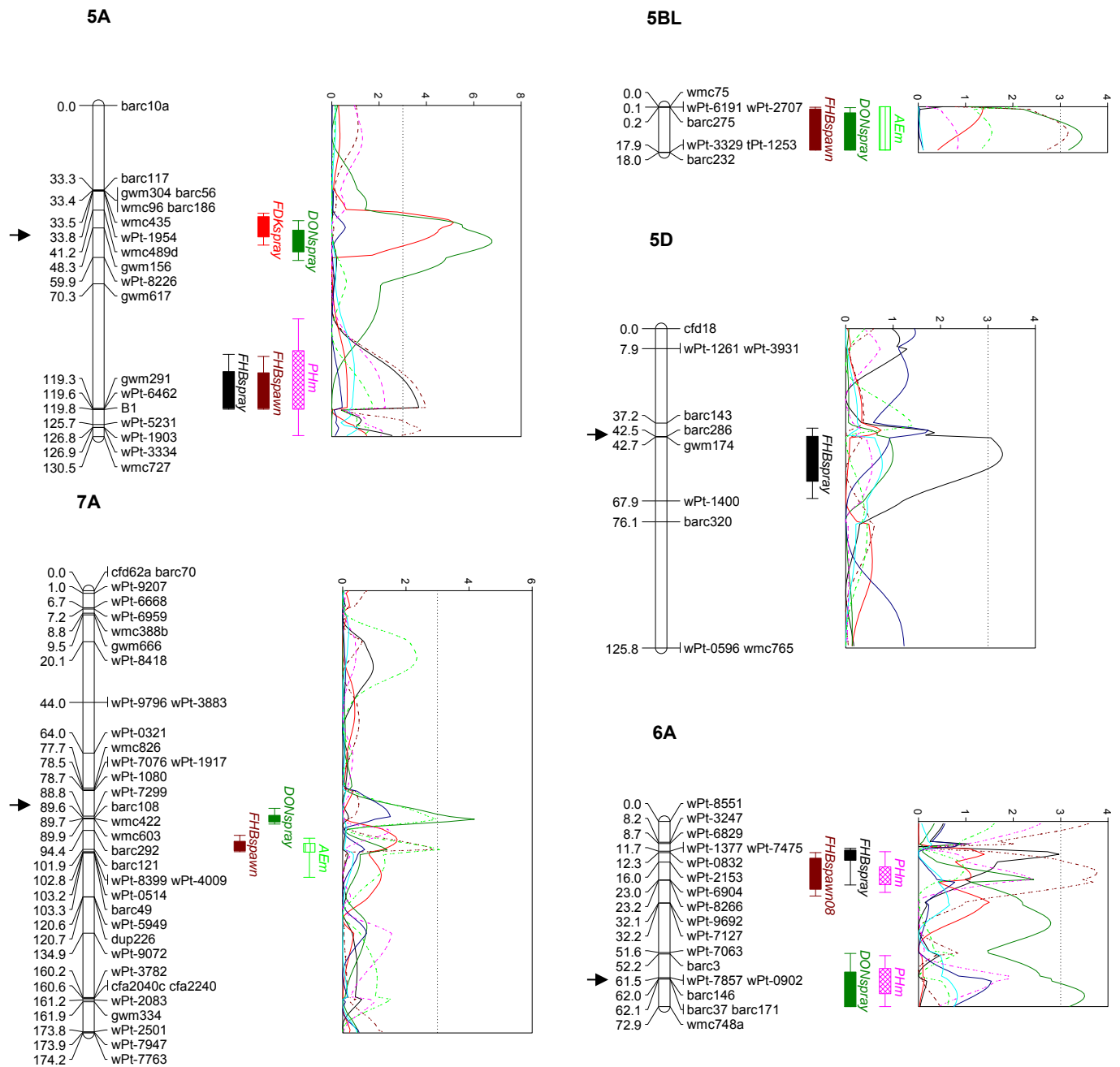
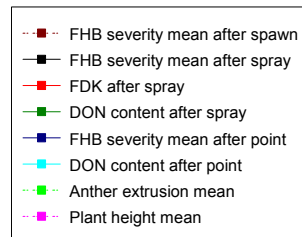


Fig. 3 Chromosomes with QTL from mean data with corresponding LOD curves. If there was no QTL detected based on the mean, the environment with significant QTL effect was marked instead with the year behind the QTL name. Genetic distances are shown in centimorgans to the left of the chromosomes. A threshold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



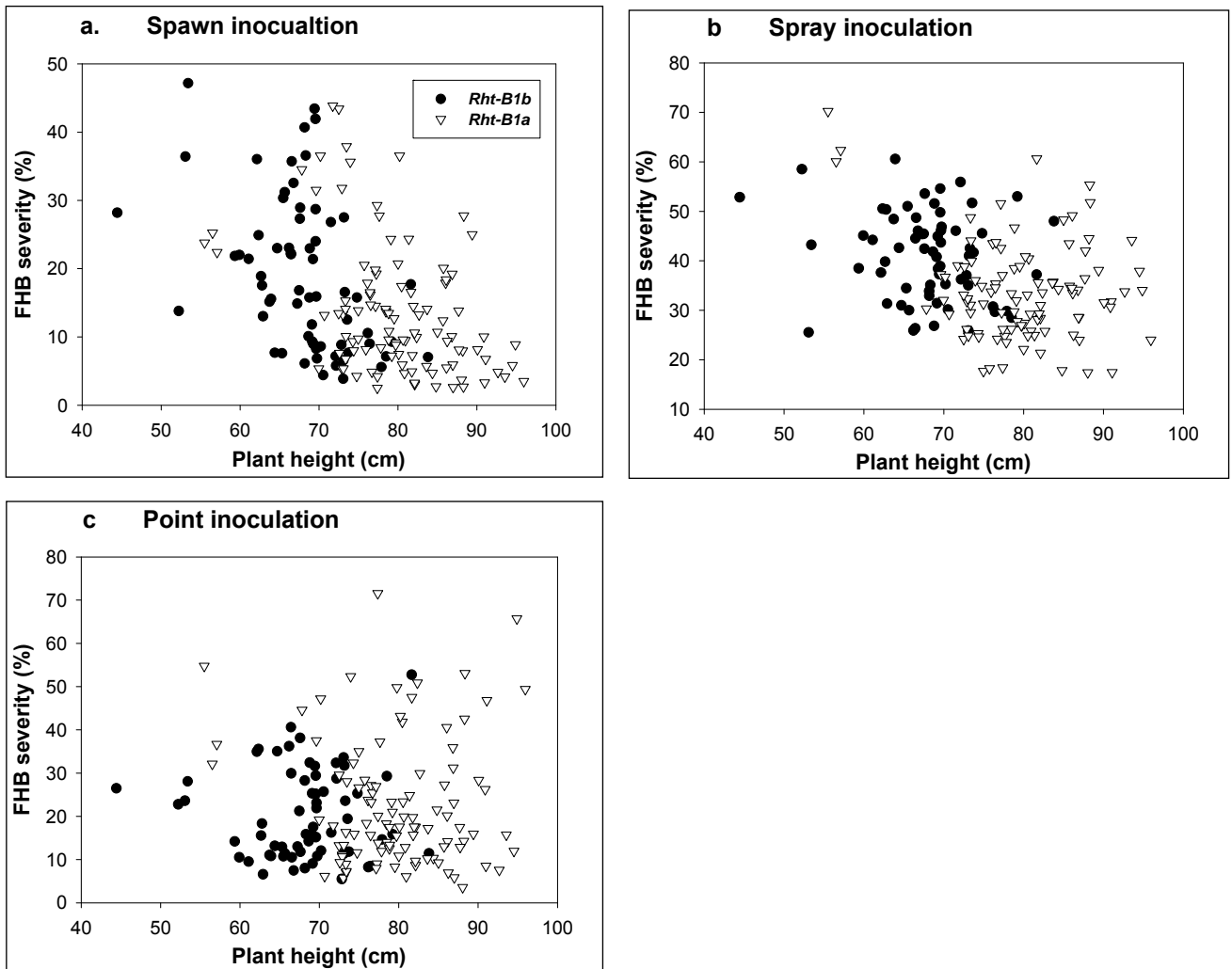


Fig. 4 The relationship between plant height and Fusarium head blight (FHB) severity after a) spawn inoculation, b) spray inoculation and c) point inoculation in the SHA3/CBRD x Naxos recombinant inbred line (RIL) population. Each DH line was plotted with mean data and grouped based on the *Rht-B1* status: *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 compared to other RILs.