Norwegian University of Life Sciences
Faculty of Biosciences
Department of Plant Sciences

Philosophiae Doctor (PhD)
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Detection and validation of disease resistance QTL in wheat

Deteksjon og validering av QTL for sykdomsresistens i hvete

Susanne S. Windju

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List of papers

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Theoretical and Applied Genetics, 130 (7): 1361-1374.

III **Identification of consistent loci for *Fusarium* head blight resistance in Northern European spring wheat through genome-wide association mapping.**

Susanne S. Windju, Tatiana Belova, Jon Arne Dieseth, Muath K. Alsheikh, Morten Lillemo

Manuscript.

Summary

Powdery mildew (PM), Fusarium head blight (FHB) and Septoria leaf blotch (SNB) are devastating wheat diseases. Breeding of disease resistant varieties is an economical and environmentally friendly approach and is given high priority in the Norwegian wheat breeding program at Graminor Breeding AS.

Resistance breeding is a challenging task. For some diseases, major resistance genes have been detected and utilised in breeding, but when commercial varieties carrying major genes are grown in large areas, the resistance can be overcome by the pathogen after a few years of cultivation. For these diseases, the search and utilisation of quantitative resistance genes is a more durable solution. For other diseases, the resistance mechanisms have been found to be mainly polygenic and quantitative. Utilisation of quantitative resistance genes, and combining several of these quantitative loci, is a solution for breeding of more resistance varieties against these diseases.

In this study, two SNP Chips were utilised; the Illumina 90K SNP Chip and the Affymetrix 35K SNP Chip. The recombinant inbred line (RIL) populations Shangahi3/Catbird (SHA3/CBRD) x Naxos and Soru#1 x Naxos, and a spring wheat association mapping panel consisting of 123 lines were genotyped with the Illumina 90K SNP Chip. An association mapping panel consisting of 299 spring wheat lines and RIL population Soru#1 x Naxos were genotyped with the Affymetrix 35K SNP Chip.

For the SHA3/CBRD x Naxos population, linkage maps containing both SNP, SSR and DArT markers were developed. For the Soru#1xNaxos population, two sets of linkage maps were developed; one set with Illumina 90K SNP markers and SSR markers, and a second set contained Illumina 90K and Affymetrix SNP markers in addition to SSR markers.

In **paper I**, we utilised the two RIL populations, SHA3/CBRD x Naxos and Soru#1 x Naxos, that both segregate for PM. These RIL populations had been evaluated for PM in several environments in Norway and China. The previous QTL mapping study had detected a major QTL for PM resistance on chromosome 1AS contributed by Naxos. That study was performed with SSR and DArT markers. With the saturation of the SHA3/CBRD x Naxos map with SNP markers and the SNP genotyping of Soru#1 x Naxos we could more precisely map and validate this 1AS QTL. Further work is now in progress to fine-map this 1AS QTL.

Paper II focused on SNB. We used the RIL population SHA3/CBRD which segregates for SNB. This population had previously been evaluated for adult plant resistance to SNB in field trials. Seedling resistance were tested in the greenhouse with inoculation of *P. nodorum* isolates and infiltrations with isolates and necrotrophic effectors. With the use of a more saturated marker map in the SHA3/CBRD x Naxos population we could map the *Snn3* locus on chromosome 5BS in SHA3/CBRD and detect QTL for sensitivity to SnTox3 in this locus both in adult plants and in seedlings.

In **paper III,** we utilised the 299 association mapping panel genotyped with the Affymetrix 35K SNP Chip. FHB was evaluated in several environments in spawn inoculated fields, and DON measurements was performed by GC-MC. Anther extrusion (AE), plant height (PH) and days to heading (DH) of the lines in the collection were also evaluated in field trials. Eight QTL were detected that were significant in three or more testing environments consistent for both FHB and DON. Of these eight QTL, seven coincided with AE. Evaluation of the mapping panel displayed a clear positive effect on resistance when combining several resistance alleles. The results also provided an overview of which of the detected QTL were present in different lines in the mapping panel and which QTL was less and more utilised in the different parts of the wheat collection.

Sammendrag

Meldugg (PM), Fusarium head blight (FHB) og hveteaksprikk (SNB) er svært skadelige sykdommer i hvete. Å foredle hvetesorter med resistens mot disse sykdommene er en økonomisk og miljøvennlig tilnærming, og er gitt høy prioritet i det norske hveteforedlingsprogrammet ved Graminor AS.

Å foredle sykdomsresistente sorter er utfordrende. For noen sykdommer har enkelte hovedgener blitt oppdaget og brukt i utviklingen av nye sorter. Når en sort med kun enkelt-gener mot en sykdom dyrkes kommersielt på store arealer blir denne resistensen ofte raskt brutt ned pga endring i patogenpopulasjonen. For slike sykdommer er det en bedre strategi å lete etter, og utnytte, flere gener med mindre resistens effekt enn hovedgenene som samlet vil kunne gi en god og mere varig resistens. For andre typer sykdommer er det ikke funnet hovedresistensgener, men kun gener med lavere grad av resistens enn hovedgenene og polygene resistensmekanismer hvor mange gener med liten grad av resistens samlet sett gir mer eller mindre resistente planter.

I dette prosjektet ble to ulike SNP Chiper med sekvenser fra referanse genomsekvensen til IWGSC (International Wheat Genome Sequencing Consortium) brukt; Illumina 90K SNP Chipen og Affymetrix 35K SNP Chipen. De to RIL populasjonene Shanghai3/Catbird (SHA3/CBRD) x Naxos og Soru#1xNaxos, og en assosiasjonskartleggings-kolleksjon bestående av 123 vårhvete- linjer ble genotypet med Illumina 90K SNP Chipen. En assosiasjons-kartleggingskolleksjon bestående av 299 vårhvete-linjer og RIL populasjonen Soru#1 x Naxos ble genotypet med Affymetrix 35K SNP Chipen.

For SHA3/CBRD x Naxos ble det laget koblingskart basert både på SNP, SSR og DArT markører. For Soru#1xNaxos ble det laget to ulike typer koblingskart; èn type koblingskart med Illumina 90K SNP markører og SSR markører, og en annen type med Illumina 90K SNP markører og Affymetrix 35K SNP markører i tillegg til SSR markører.

I **paper I** ble de to RIL populasjonene SHA3/CBRD x Naxos og Soru#1 x Naxos benyttet. Begge populasjonene spalter for PM, og hadde blitt evaluert for PM i flere miljøer i Norge og Kina. En tidligere QTL kartleggingsstudie hadde detektert et QTL for PM på brødhvetekromosom 1AS fra Naxos. Denne studien var utført med SSR og DArT markører. Med nye koblingskart med mange fler markører både i SHA3/CBRD x Naxos og Soru#1 x Naxos krysningene klarte vi å kartlegge dette 1AS QTLet mer presist og videre validere det. Videre arbeid er nå i gang for å finkartlegge dette 1AS QTLet

Paper II fokuserte på SNB. Vi brukte her RIL populasjonen SHA3/CBRD x Naxos, som spalter for SNB. Denne populasjonen hadde tidligere blitt evaluert for SNB i feltforsøk. Småplante-resistens ble testet i veksthus med inokulering av *P. nodorum* isolater og infiltrering med isolater og nekrotrofe effektorer. Ved å benytte det nye utviklede koblingskartet av SHA3/CBRD x Naxos med mange markører var det mulig å plassere *Snn3* locuset på kromosom 5BS og detektere QTL for mottakelighet til SnTox3 i *Snn3* locuset både på voksen- og småplantestadiet.

Paper III benyttet et panel bestående av 299 vårhvete linjer. Dette panelet ble genotypet med Affymetrix 35K SNP Chipen. FHB ble evaluert i flere miljøer i smittefelt og DON nivå ble målt med GC-MS. AE, PH og DH ble også evaluert i de ulike linjene i ulike felt. Åtte QTL ble avdekket som var signifikante i tre eller flere miljøer for både FHB og DON. Av disses åtte, hadde syv sammenfallende posisjon som detekterte QTL for AE. Evaluering av hvetekolleksjonen viste en klar positiv effekt av å kombinere flere av de detekterte QTLene i studien. Resultatene fra studien ga også en oversikt over hvilke linjer i kolleksjonen som inneholdt hvilke av de åtte konsistente QTLene og hvilke QTL som var høyt og lavt utnyttet i de ulike delene av hvetekolleksjonen.

Abbreviations

AE	Anther extrusion
APR	Adult plant resistance
CIMMYT	The International Maize and Wheat Improvement Centre
DArT	Diversity Array Technology
DH	Days to heading
DM	Days to maturity
DON	Deoxynivalenol
FHB	Fusarium head blight
KASP	Kompetitive Allele Specific PCR
LD	Linkage disequilibrium
MAS	Marker assisted selection
NIL	Near isogenic line
PH	Plant height
PM	Powdery mildew
QTL	Quantitative trait locus/loci
RIL	Recombinant inbred line
SNB	Septoria nodorum blotch
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
tDON	Transformed DON (log (DON level +1))

1. Introduction

1.1 Wheat origin

Western agriculture originated in the Fertile Crescent for about 12 000 to 9 500 years ago, when humans began the transition from hunter-gatherer to a society based on agriculture (Kilian et al., 2010; Salamini et al., 2002). The transition of wild to domesticated forms of crops changed three important morphological traits that made crops easier to harvest; seed size, ear rachis stiffness and the ease of which the seed is released from the glumes (Salamini et al., 2002).

The hexaploid bread wheat (2n = 6x = 42) has no wild hexaploid progenitor, it is an allohexaploid formed by three different wheat species (Fig. 1). Bread wheat possesses three sets of homologues chromosomes; AABBDD. The A genome originates from *Triticum uartu*, and a hybridizing event with the wild diploid B genome donor, belonging to *Aegilops*, formed the allotetraploid emmer wheat AABB; *Triticum turgidum*. This tetraploid emmer wheat evolved to the hexaploid bread wheat through an alloploidization event between *Triticum turgidum* (AABB) and the goat grass *Aegilpos tauschii* (DD) (Kilian et al., 2010; Marcussen et al., 2014) (Fig. 1).

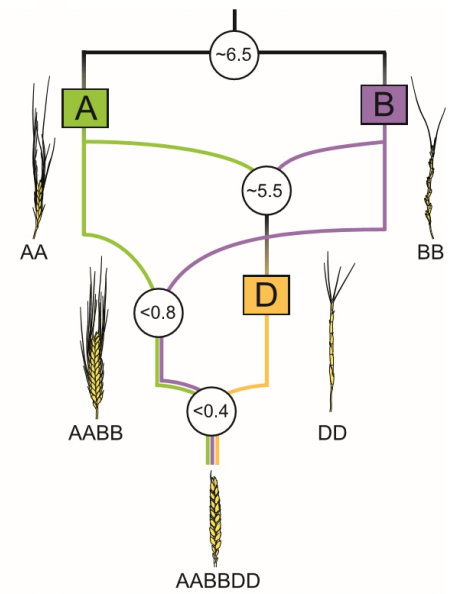
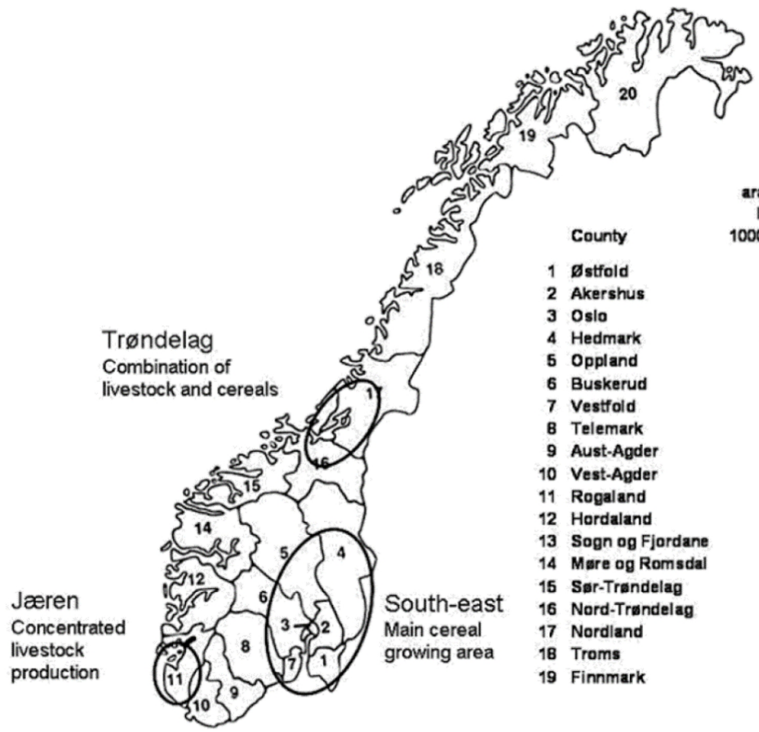


Figure 1. Origin of hexaploid wheat (2n = 6x = 42). Approximate dates for divergence and the three hybridization events are given in white circles in units of million years ago. The three diploid lineages are indicated with colour and labels (Marcussen et al., 2014).

1.2 Wheat in Norway

Norway stretches from 57 ˚ – 71˚ north, and represents the northern borders of the wheat growing areas. Only about 3 % of the total area of Norway is arable land, the rest includes mountains, forests, lakes and wetlands (Lillemo et al., 2011). In Norway, wheat is grown in the south, the main areas being the south-eastern part of the country (Fig. 2). Being in the marginal area of wheat cultivation, breeding for varieties able to grow in the Norwegian climate is highly important.



	County	arable land 1000 ha	% of wheat area
1	Østfold	77	36
2	Akershus	81	19
3	Oslo	-	-
4	Hedmark	109	11
5	Oppland	103	4
6	Buskerud	52	9
7	Vestfold	44	18
8	Telemark	26	2
9	Aust-Agder	12	-
10	Vest-Agder	20	-
11	Rogaland	97	-
12	Hordaland	47	-
13	Sogn og Fjordane	48	-
14	Møre og Romsdal	62	-
15	Sør-Trøndelag	76	-
16	Nord-Trøndelag	89	1
17	Nordland	58	-
18	Troms	27	-
19	Finnmark	11	-

Figure 2. Wheat growing areas in Norway (Lillemo et al., 2011).

The main breeding goals for Norwegian wheat are high yield and good quality, with good agronomic performance and disease resistance. Since the cultivation of wheat in Norway is at the northern limit of the crop, earliness is also a very important trait for the farmers to produce wheat of good quality (Lillemo et al., 2011).

In Norway, breeding for disease resistance is given a high priority to reduce the use of chemicals. The use of chemicals is expensive and a potential threat to the environment. In Norway there is also a political desire to reduce the pesticide use in agriculture, see Lillemo et al. (2011).

The most serious diseases in Norwegian wheat production, and therefore also the focus of disease resistance breeding in Norway are powdery mildew (PM), Septoria nodorum blotch (SNB), Fusarium head blight (FHB) and yellow rust (pers.com J.A, Dieseth) (Lillemo et al., 2011).

1.3 Sequencing of the hexaploid wheat genome

The International Wheat Genome Sequencing Consortium (IWGSC) (www.wheatgenome.org) was established in 2005 with the aim to sequence the hexaploid wheat genome (Gill et al., 2004; IWGSC, 2014). The different wheat chromosomes were analysed and sorted using flow cytometry (Doležel et al., 2011). Chromosomes were divided between different groups in the consortium and sequenced. Norway, represented by NMBU and Graminor Breeding Ltd, sequenced and mapped chromosome 7B (Belova et al., 2014; Belova et al., 2013). The IWGSC published the first whole-genome draft sequence of the hexaploid bread wheat genome in 2014 (IWGSC, 2014). This work was a milestone in facilitating the isolation of genes underlying agronomical important traits and improving the efficiency of wheat breeding (IWGSC, 2014). In 2017, NRGene accomplished the development of a high quality whole genome sequence assembly of the wheat genome that complemented the chromosome based draft sequence previously developed (http://www.nrgene.com/wheat-sequencing-consortium/). This accomplishment facilitated the development of more resources for the members of the IWGSC. A pangenome study, that reassembled and used the Chinese Spring wheat reference sequence, identified core and variable genes across 18 varieties of wheat (Montenegro et al., 2017). In this study, a pangenome of 140 500 ± 120 genes was predicted, with an average of 128 656 genes in each of the 18 varieties. In addition, the study identified 36 million intervarietal SNPs across the pangenome (Montenegro et al., 2017).

1.4 Molecular markers

Genetic markers and linkage maps have been used since the 1980's (Somers et al., 2004). PCR-based, multi-allelic markers like random amplified polymorphic DNA (RAPD) (Williams et al., 1990), amplified fragment length polymorphism (AFLP) (Vos et al., 1995), and simple sequence repeats (SSR) (Gupta et al., 2002; Pestsova et al., 2000; Röder et al., 1998), the array-

based methods diversity array technology (DArT) (Akbari et al., 2006; Jaccoud et al., 2001; Marone, Panio, et al., 2012) and single nucleotide polymorphism (SNP) have made it possible to use marker assisted selection (MAS) in breeding programs (Somers et al., 2004).

1.4.1 SSR markers

Microsatellite SSR markers are tandem repeats of DNA sequences of various length (1-6 bp), where the most abundant are the di-nucleotide repeats (Gupta et al., 1996). DNA sequences flanking the SSR tandem repeats are conserved, PCR primers can be developed and the SSR loci can be amplified (Gupta et al., 1996). The variation of the length of the SSR in different individuals results in a length polymorphism that can be detected after PCR with polyacrylamide gel electrophoresis, fluorescent capillary electrophoresis or high resolution melting curve (Paux et al., 2012). In wheat, SSR markers show a high level of polymorphism and have a high number of alleles at each locus, making them suitable for the study of genetic relationships among lines and varieties (Paux et al., 2012).

1.4.2 DArT markers

The DArT marker technology is a microarray technology for DNA polymorphism analysis not based on any prior sequence information (Jaccoud et al., 2001). DArT can simultaneously type several thousand loci in a single assay. The technique is hybridization-based, where a genomic representation (genomic library) is developed by DNA restriction enzyme digestion and ligation on arrays. The genotyping is based upon the presence versus absence of DNA fragments in the generated genomic representations (Akbari et al., 2006).

1.4.3 SNP markers

In recent years, the array-based, bi-allelic single nucleotide polymorphism (SNP) markers have become the marker-system of choice. SNP markers are used for studies regarding genetic variation, linkage mapping, population structure analysis, association genetics, map-based gene isolation and plant breeding (Ganal et al., 2009).

Development of genetic maps with thousands of SNP markers makes it possible to localize a quantitative trait loci (QTL) to a precise position and enables identification of tightly linked markers that only rarely show recombination with the respective trait (Ganal et al., 2012). Also, the development of linkage maps with thousands of SNP markers can facilitate the substitution of previously utilised SSR markers with SNP markers. SNP markers can be used effectively in MAS with the use of for example the KASP system (Semagn et al., 2014).

Illumina 90K SNP Chip

A genotyping array consisting of 81 587 SNP markers was developed and published in 2014 by Wang et al. (2014). The genotyping array was used to characterize genetic variation in allohexaploid and allotetraploid wheat. With the use of eight double haploid (DH) mapping populations, SNPs were ordered along the chromosomes and Wang et al. (2014) could genetically map 46 977 SNPs and develop a consensus map from this 90K SNP array.

Affymetrix 35K SNP Chip

Another wheat SNP genotyping project was developed by Allen et al. (2017). The aim of this project was to develop a set of informative markers useful for the wheat breeding community. A large collection of 819 571 (820K) previously characterized wheat markers (Winfield et al., 2016) were analysed. Allen et al. (2017) used the marker data from this 820K wheat array and identified 35 143 informative SNP markers potentially useful for the breeding community. This SNP array was named "The Wheat Breeder's array" due to its potential as a tool for breeding applications such as genome wide association studies (GWAS) and genomic selection (GS). A large global collection of hexaploid wheat varieties, two wheat DH mapping populations, two wheat RIL population and one wheat SSD population was genotyped with this "Wheat Breeder's assay". A screening displayed on average that 23 % of the SNPs on "The Wheat Breeder array" were predicted to be polymorphic SNPs between two random accessions (Allen et al., 2017).

The KASP genotyping platform

A wide range of SNP genotyping platforms have been developed and made available in recent years. Many of these are multi-plex chip based genotyping platforms that generate information on from several hundred to thousands of SNPs per run. The multiplex systems offer high throughput, but also require several thousand SNPs per assay. And once the SNPs are on the array, they are fixed, and can only be utilised for these SNPs (Burridge et al.; Semagn et al., 2014). For breeding purposes, where one often has a small number of markers to test in many samples, the uniplex SNP genotyping platform Kompetitive Allele Specific PCR (KASP) has been found to be highly suitable. The KASP system was developed by KBioscience, and is a homogeneous, fluorescence-based genotyping technology enabling the bi-allelic scoring of SNPs at specific loci (https://www.lgcgroup.com)(Semagn et al., 2014) (Fig. 3). In the KASP system, the genotyping can be carried out in 96, 384 and 1536-well-plates and is based on an allele extension and fluorescence resonance energy transfer (FRET) for signal generation (Kumpatla et al., 2012; Semagn et al., 2014). The result of the genotyping will be a fluorescent

signal from each DNA sample represented as an independent data point in a cluster plot (Fig. 3b). With a limited number of markers run routinely in a breeding program this system has proven to be flexible and efficient. When the marker sequence is known, primers can be developed and the breeding material tested. The system is highly flexible, one chooses markers and template for each run and it can be utilised for several crops.

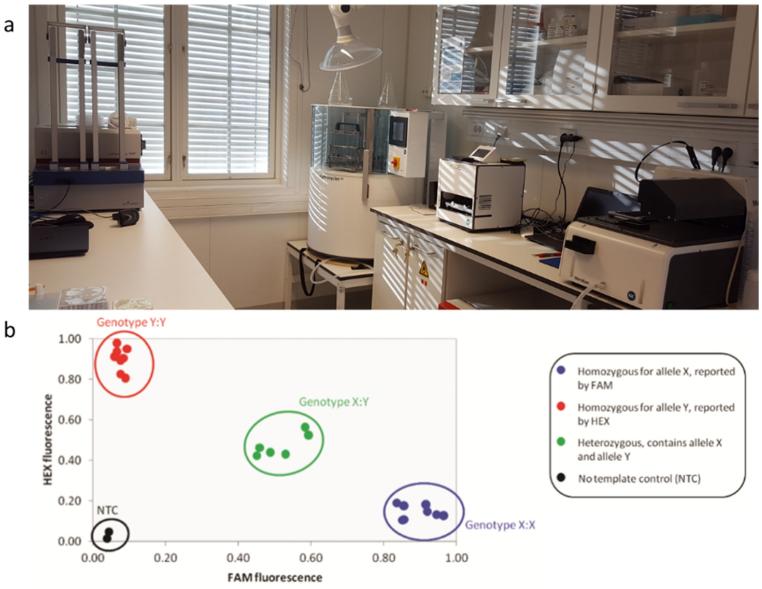


Figure 3. a) KASP genotyping platform at Bjørke research station (photo: Susanne S. Windju). b) Example of KASP genotyping result window (https://www.lgcgroup.com).

1.5 Linkage maps

Genetic linkage maps, based on recombination frequencies between polymorphic markers in a population was initially developed with a few RFLP markers (Botstein et al., 1980). Markers in the linkage maps must display clear segregation patterns and show polymorphism after genotyping to be informative in downstream mapping studies (Delourme et al., 2013). New sequencing and marker technology with SSR and SNP markers have made the development of linkage maps with thousands of polymorphic markers possible.

Consensus maps

With the development of high-density SSR and SNP linkage maps, consensus maps were also developed. Somers et al. (2004) published an SSR consensus map developed from four mapping populations; Synthetic/Opata (ITMI population), RL4452/AC Domain, Wuhan/Maringa and Superb/BW278. The three latter are all DH populations, while the ITMI population is an F_6 RIL

population. The fusion of SSR linkage maps from these four populations resulted in a consensus map consisting of 1 235 SSR markers. This consensus map was a great improvement from the single population maps previously published (Somers et al., 2004). Wang et al. (2014) utilized the Illumina 90K SNP Chip. They genotyped and screened eight DH mapping populations; BT-Schomburgk x AUS33384, Young x AUS33141, Chara x Glenlea, W7984 x Opata M85, Sundor x AUS30604, Westonia x Kauz, Avalon x Cadenza and Savannah x Rialto. From this genotyping and screening they developed a consensus map consisting of 46 977 SNP markers from the Illumina 90K SNP Chip (Akhunov et al., 2009; Wang et al., 2014). A consensus map of the durum wheat A and B genomes was published in 2012 (Marone, Laidò, et al., 2012). This map was developed by combining segregation data from six mapping populations; The RIL populations Creso x Pedroso, Ofanto x Capelli, Cirillo x Neodur, Ciccio x Svevo, Messapia x MG4343, and the F_2-F_3 family population 'Latino' x 'Primadur'. All lines were durum wheat, except the line MG4343 which is an accession of the *Triticm turgidum* sub-species *dicoccoides* (Marone, Laidò, et al., 2012). A consensus map developed by intergrating 13 datasets from biparental populations from durum wheat (*Triticum turgidum* ssp. *durum*), cultivated emmer (*T. turgidum* ssp. *dicoccum*) and wild emmer (*T. turgidum* ssp. *dicoccoides*) was published in 2015 (Maccaferri et al., 2015). This study aimed at developing a consensus map useful for both durum and bread wheat and harboured 30 144 markers (including 26 626 SNPs and 791 SSRs). Many of the mapped markers in the study were gene-derived markers, making them valuable for locus dissection. With such a high number of gene derived markers these maps can serve as a bridge between durum wheat and bread wheat (Maccaferri et al., 2015). A newly published consensus map of bread wheat was published by Wen et al. (2017). This map was based on four RIL populations from the crosses Duomai x Shi4185, Gacheng 8901 x Zhoumai 16, Linmai 2x Zhong 892 and Zhou8425 x Chinese spring. Each of these RIL populations were genotyped with the Illumina 90K SNP Chip and the final consensus map consisted of 29 692 SNP markers mapped to 21 chromosomes.

In development of linkage maps the high-density consensus maps serve as a good reference tool. The orientation of the chromosome arms in the map, and relative marker order can be evaluated. Consensus maps also enable comparison of markers and relative map-positions across QTL studies.

Integrated linkage maps

The consensus map developed by Maccaferri et al. (2015) contained both SSR and SNP markers. This type of "integrated" linkage maps with different marker types is highly valuable

when performing association mapping or QTL mapping. They can be used as a reference when comparing mapping results from studies utilizing different marker systems.

1.6 Mapping

1.6.1 QTL mapping

QTL mapping links phenotypic and genotypic data to explain the genetic basis of variation of complex traits aiming to locate the genes responsible and to explore their effect and interactions (Kearsey, 1998; Lander et al., 1989). QTL analysis depends on markers being in linkage disequilibrium, i.e. the non-random-association of alleles, with a QTL segregating for the trait of interest in a population. When this linkage disequilibrium is present, the marker locus and the QTL will not segregate independently and the difference in the marker genotypes will be linked and associated with the trait phenotype.

QTL mapping is performed in families with known relatedness that differ regarding the trait of interest. Attempts are made to identify co-segregation of genetic markers and phenotypes within this family (Myles et al., 2009). In this approach mapping populations are developed and genotyped and phenotyped for traits of interest.

Recombinant Inbred Line (RIL) Populations

RIL is a population type useful in QTL mapping. A RIL population is a collection of lines derived from a cross of genetically divergent parents (Pollard, 2012). The RIL population is developed by crossing two inbred lines followed by repeated selfing or sibling mating to create new inbred lines whose genome will be a mosaic of the parental genomes (Broman, 2005; Pollard, 2012). QTL mapping is mostly based on biparental populations, and will only allow you to exploit the recombination events that happened during the development of the mapping population (Myles et al., 2009).

Double Haploid (DH) Populations

A second population type frequently used in mapping studies are DH populations. In double-haploid procedure, chromosomes of haploid plants are doubled to produce diploid plants. DH plants are normally homozygous at all loci and it is unnecessary to grow segregating generations (Sleper et al., 2006). The production of DHs is used to speed up the development of mapping populations. The DHs in plants are homozygous and can be achieved in one generation from hybrid plants. However, since only one generation of meiosis occurs, a DH population produced from F_1 will have less recombination than a RIL population. The production of DH includes two major steps; haploid induction and chromosome doubling (Niu et al., 2014). In wheat there

are two main approaches for developing haploids: androgenesis (anther or microspore culture) or gynogenesis (ovary or megaspore culture) (Niu et al., 2014).

Near Isogenic Line population (NIL)

To estimate more precisely and fine-map a detected QTL, NIL populations can be developed. This population differs only in the short chromosome segment harbouring the QTL. Because of the absence of any other segregating QTL in a NIL population, the target QTL is the major source for the variation and the QTL can be more precisely mapped (Salvi et al., 2005).

Multi-parent Advanced Generation InterCross (MAGIC) population

In traditional QTL mapping studies, bi-parental populations are developed. These populations have a narrow genetic base since it is only possible to detect the genomic regions different between the two founders. The MAGIC population is developed with several founder parents that are being intermated several generations prior to creating inbred lines. This leads to a diverse population where the lines are a fine-scale mosaic of the founder parents. MAGIC populations display a higher degree of polymorphism than the traditional biparental populations (Huang et al., 2015).

1.6.2 Association mapping

Association mapping (AM) is also called genome-wide association study (GWAS). AM involves searching for genotype and phenotype correlations in collections of breeding lines or varieties. AM has higher mapping resolution than QTL mapping, because it exploits all recombination events that has happened in the lines in the collection. The power of the association mapping is dependent on the degree of linkage disequilibrium (LD) in the collection (Myles et al., 2009). For this approach to be functional, the markers must be in strong LD with the QTL for the trait of interest, i.e. the marker alleles must be highly correlated with the trait of interest. In an AM study, false positive correlations, Type I errors, between markers and the trait might happen due to population structure in the collection (Crossa et al., 2007). This population structure comes from the breeding history of the lines in the collection and might be due to admixture, mating system, genetic drift or artificial or natural selection. An assessment of the population structure and including this into the association mapping approach is important to diminish or eliminate this Type I error, “false positive” marker-QTL interactions (Crossa et al., 2007). To overcome the problem of Type I errors, the structure and the relatedness of the lines in the population is considered. The assessment of the structure of the mapping population is performed assigning the lines in the population to different subpopulations (Q). And to determine the relatedness of the lines in the population, a kinship

matrix (K) is calculated. Association mapping can be performed using a Mixed-linear model (MLM). This model incorporates Q and K to account for the effect of population structure and relatedness in the association mapping. Type II error in GWAS is the possibility to not detect significant QTL for the trait of interest i.e. "false negative" error. This error might be causes by lower correlation between marker allele and QTL because of LD decay, or unbalanced design because of the presence of alleles at different frequencies which will eventually might lead to that the rarer alleles might not be detected (Breseghello et al., 2006; Carlson et al., 2004).

1.7 Genomic selection (GS)

GS utilises molecular markers, but where QTL and association analysis detect marker-trait associations, GS estimate the effect of all markers across the whole genome in the target population (Desta et al., 2014; Meuwissen et al., 2001). GS utilises a phenotyped and genotyped training population that is genetically similar the breeding population. With calculation of marker effects across the genome in the training population, a genomic estimated breeding value can be calculated of the breeding population (Desta et al., 2014). For highly complicated traits with high G x E effect, GS has been proposed to be a valuable method for breeders (Crossa et al., 2017).

1.8 Race specific and race-non-specific disease resistance

In general, there are two main types of plant stress factors; biotic and abiotic. The abiotic factors are the non-living factors in nature such as drought, sub-optimal temperatures and insufficient soil-fertility. The biotic stress factors are the living nature factors such as weeds, pathogens and pests (Niks et al., 2011).

Two different types of resistance to the biotic stress factors are documented; race-specific and race-non-specific resistance. The race specific resistance is also named vertical or seedling resistance and is a *qualitative* resistance. This resistance is mediated by major resistance genes with large effects (Bennett, 1984). The resistance mechanism in race-specific resistance is based on a gene-for gene model (Flor, 1955). For every gene in the plant that confers resistance, there is a corresponding gene in the pathogen that confers avirulence (Fig. 4). Race-specific resistance gives protection only against some isolates of the pathogen and are usually not effective against others. The resistance genes are vulnerable to changes in the pathogen population, and in the case of a change in the virulence the resistance can be broken and the plant will be susceptible (Hsam et al., 2002; McDonald et al., 2002). When a variety containing race-specific resistance is released and grown in large areas, a high selection pressure is put on

the pathogen to overcome the resistance, and the variety can become susceptible within a short period of time.

		Host Genotype	
		RR or Rr	rr
Pathogen Genotype	AA or Aa	Resistance	Disease
	aa	Disease	Disease

Figure 4. Gene-for-gene model (Flor, 1955).

A more durable resistance is the quantitative or partial race-non-specific resistance, also called adult plant-resistance (APR) (Gustafson et al., 1982). In this resistance mechanism several genes with major and minor effects work together to reduce the infection efficiency and retard growth and reproduction of the pathogen (Shaner, 1973). In the race-non-specific resistance there is no gene-for -gene relationship between host and pathogen, but rather several genes working through different mechanisms and together build a resistance towards all or most of the pathotypes, without creating complete immunity. This resistance will not display immunity to the pathogen, and there will be a reduced selection pressure on the pathogen; and thus reducing the risk of new virulent races (Li et al., 2014). With this pyramiding effect, the crop would not be overcome by the pathogen in a rapid manner as in the race-specific resistance and is therefore a more promising path to follow for development of resistant varieties. The race-non-specific resistance is not easy to detect by assessment of the breeding lines and varieties in the field; because it is often masked by the race-specific resistance genes. One promising way of detecting these quantitative resistance genes is the use of molecular markers and collections of known lines and mapping populations.

1.9 Powdery mildew (PM)

PM is one of the most common plant diseases, and affects a range of different plant species. The PM diseases of various crops and other plants are caused by many species and *formae speciales* of the fungi of the family *Erysiphaceae*, which is grouped into several genera (Agrios, 2005).

PM on wheat is caused by the biotrophic fungal pathogen *Blumeria graminis* f.sp. *tritici (Bgt)* and is considered one of the most devastating wheat diseases in many regions of the world with maritime and temperate climates. Crop practices like irrigation, use of semi-dwarf varieties, growth regulators and increased use of nitrogen fertilizers favour the development of the disease (Bennett, 1984). The PM pathogen seldom kills the host but uses the hosts' nutrients, reduces their photosynthesis, increases their respiration and transpiration, impairs growth and reduces yields (Agrios, 2005). It can cause significant yield losses ranging from 13- 34%, but if the disease attacks are severe to the flag leaf at the beginning of grain filling, the level of yield loss can reach up to 50% if not properly managed (Alam et al., 2013; Griffey et al., 1993).

1.9.1 Disease symptoms

Blumeria graminis produces mycelium that grows on the surface tissue of the plants, where it develops haustoria that penetrates the epidermal cells of the plant organs (Fig. 5a). The mycelia also produce conidiophores on the plant surface (Fig. 5b). The conidiophores produce conidia that are carried by air currents and starts a new infection when landing on a compatible host plant. Once the infection has begun, the mycelium continues to grow on the plant surface (Agrios, 2005).

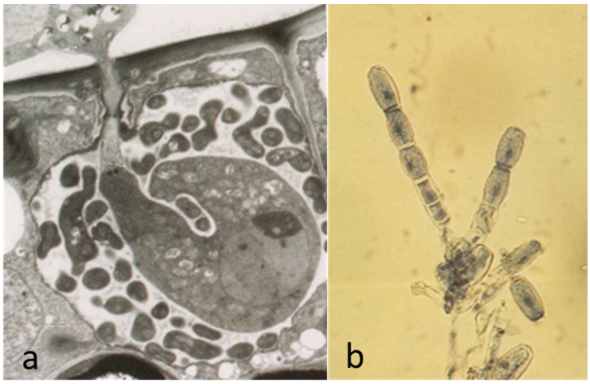


Figure 5. a) PM haustoria. b) PM conidiospores (Agrios, 2005).

PM appears as white and grey patches of mycelium on leaves, stems and heads of the wheat plants (Fig. 6). If the environment is threatening the survival of the host plant, the PM pathogen develops sexual ascospores, which develop in groups inside a closed ascocarp/ chasmothesium- a fungal fruiting body. The chasmothesia appear as black pinhead spherical containers in the mycelium and are usually produced at the end of the growing season after the fungus has reduced its conidial production (Mwale et al., 2014)

Figure 6. PM disease symptoms in field, on leaf and head. Photo: Susanne S. Windju.

1.9.2 Disease cycle

PM is wind-dispersed, it can infect fields distant from the field where the initial infection started. It survives as chasmothecia on winter wheat or plant debris waiting for the right conditions to be able to develop (Fig. 7) (Mwale et al., 2014). In areas where both spring and winter wheat are grown, the winter wheat can also serve as a "green bridge" between the growing seasons by allowing *B. graminis* to survive as mycelium on infected winter wheat seedlings.

In spring, when temperature and humidity rises, growth of the pathogen increases rapidly and infects the wheat leaves. The hyphae develop and produce the conidia which in turn are dispersed by wind and land on host plants (Fig. 7). Powdery mildew germinates best at a high relative humidity and the temperature range for germination is between 10-22 ˚C. Above 25 ˚C the disease development will rapidly decline (Te Beest et al., 2008). However, spores can be killed by free water.

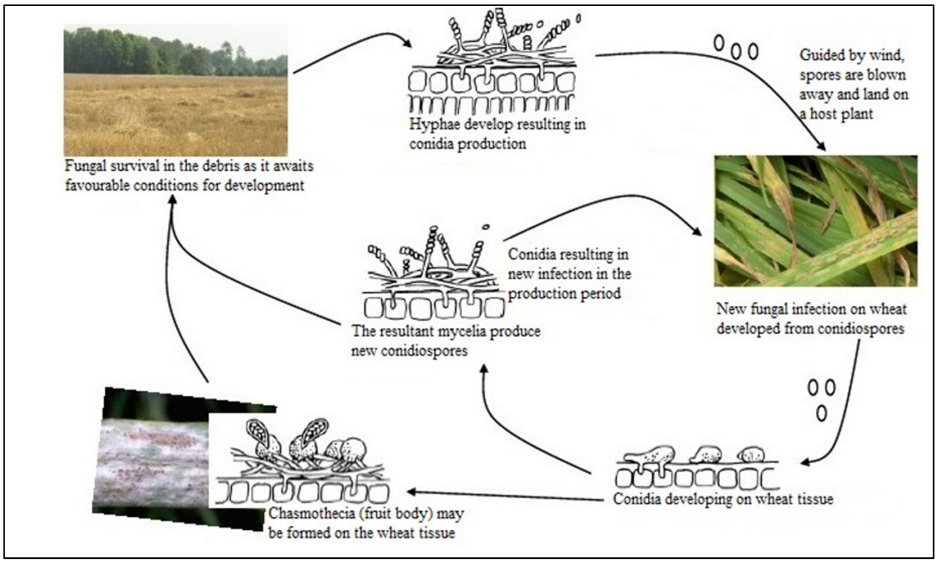


Figure 7. PM disease cycle (Mwale et al., 2014).

1.9.3 Field analysis

Due to its abundance in Norway, there is seldom need to artificially infect field trials with PM for disease evaluation. Sowing a known PM susceptible line as "spreader" among the other lines and varieties in the field, the disease attacks will be high enough under normal conditions to enable a good disease assessment. Powdery mildew disease assessment can be conducted in the fields, on the whole canopy as the percentage of leaf area infected, using a modified Cobb scale (0-100% infected leaf area) (Peterson et al., 1948). Disease assessment in research studies is usually conducted several times during the growing season, to be able to capture the variation among lines and varieties. In breeding programs, disease assessment is performed once during the field season, usually around two weeks after heading.

1.9.4 PM resistance genes

Nearly 77 *Pm* resistance genes have been catalogued at 49 loci, several of which have multiple alleles. The majority of these resistance genes are race-specific, and some have never been used in released varieties due to their short durability (Hao et al., 2015). Of these *Pm* resistance genes, only three (*Pm38, Pm39* and *Pm46*) show race-non-specific resistance to powdery mildew (Li et al., 2014). In addition to being race-non-specific, these three *Pm* loci also show pleiotropic effects to other diseases; leaf rust, stripe rust, stem rust and spot blotch (Herrera-Foessel et al., 2014; Li et al., 2014; Lillemo et al., 2008; Lillemo et al., 2013; Spielmeyer et al., 2005).

Cloned Pm resistance genes

Pm3

The *Pm3b* gene on chromosome 1AS was cloned by Yahiaoui et al. (2004). This study utilised bacterial artificial chromosome (BAC) libraries from two bread wheat relatives; the diploid *T.monococcum* and the tetraploid *T. durum*. These BAC libraries were used for physical mapping of the gene. Further analysis could reveal that the *Pm3b* gene was a member of the coiled-coil-nucleotide-binding site leucine-rich repeat (CC-NBS-LRR) type resistance genes. The *Pm3b* gene on chromosome 1AS, and subsequently the multi-allelic series *Pm3a-j* were isolated by PCR amplification and found to be true alleles of the same gene in a cluster of *Pm3*-like genes (Krattinger et al., 2016; Srichumpa et al., 2005; Yahiaoui et al., 2004). Bhullar et al. (2009) performed a study of the *Pm3* gene where they performed a screening of gene bank accession. They could molecularly identify and clone seven new alleles at the *Pm3* locus. In this study, it was reported that the main variability between the different alleles was in the LRR domain of the resistance gene.

Pm8

The race-specific- resistance gene *Pm8* was cloned by homology-based cloning and mapped to a gene-rich region at the distal end of chromosome arm 1RS. The *Pm8* gene has been found to be the rye ortholog of the *Pm3* wheat gene, the two genes share 81% sequence identity. The 1BL.1RS translocation containing the *Pm8* gene was extensively used after its development, and in the 1970's increasing virulence to *Pm8* was reported (Hurni et al., 2013).

Pm21

The race-specific resistance gene *Pm21* is located on the short arm of chromosome 6V in *Haynaldia villosa*. This gene was transferred to wheat as a 6VS/6AL translocation. The gene confers a broad-spectrum resistance to PM, and the resistance is likely caused by a serine and threonine protein kinase gene. The *Pm21* gene has been difficult to detect and map because the 6Vs chromosome of *Haynaldia villosa* does not pair and recombine with the 6AS chromosome (Cao et al., 2011).

Lr34/Yr18/Pm38/Sr57

The pleiotropic *Pm38* APR gene has been cloned (Krattinger et al., 2009). This gene confers resistance to leaf rust, yellow rust and stem rust in addition to powdery mildew. The *Lr34* gene is located on the short arm of chromosome 7D, between the markers *gwm1220* and *swm10* (Krattinger et al., 2009; Lillemo et al., 2008). The map-based cloning of the leaf rust resistance

gene *Lr34* revealed that this protein resembles adenosine triphosphate-binding cassette transporters of the pleiotropic drug resistance family (Krattinger et al., 2009).

Lr67/Yr46/ Sr55/Pm46/Ltn3

The cloning of the pleiotropic APR disease resistance gene *Pm46* was reported in 2015 (Moore et al., 2015). It has been found to have pleiotropic effect on stem rust, leaf rust, yellow rust and leaf tip necrosis and is designated *Lr67/Yr46/ Sr55/Pm46/Ltn3* (Herrera-Foessel et al., 2014; Lillemo et al., 2013; Moore et al., 2015). Moore et al. (2015) found the gene to encode a hexose transporter with two amino acid differences between the resistance and susceptible form of the gene.

1.9.5 APR QTL to PM

With novel marker and genotyping technologies, many QTL for APR to PM have been detected. One hundred and nineteen PM APR QTL have been mapped on the 21 wheat chromosomes. Many of these QTL are identical or closely mapped. These genes have ended up in diverse germplasm due to phenotypic selection in disease nurseries or under natural infection during plant breeding over many years. A review by Li et al. (2014) summarises some of the different APR QTL associated with powdery mildew that have been detected so far (Table 1).

Table 1. APR QTL to PM in wheat (copied from Li et al. (2014)).

QTL	Chromosome	Donor	Marker interval	*R2*†, %	Reference
QPm.osu-1A	1AS	2174	*Pm3a*	63.0‡	(Chen et al., 2009)
QPm.caas-1AS	1AS	Fukuho-komugi	*Xgdm33-Xpsp2999*	19.9–26.6	(Liang et al., 2006)
QPm.sfr-1A	1AL	Oberkulmer	*Xpsr1201b-Xpsr941*	7.7	(Keller et al., 1999)
QPm.crag-1A	1AL	RE714	*Xcdo572-Xbad442*	39.3–43.0 §	(Mingeot et al., 2002)
QPm.caas-1AL	1AL	Bainong 64	*Xbarc148-Xwmc550*	7.4–9.9	(Lan et al., 2009)
QPm.sfr-1B	1BS	Forno	*CD9b-Xpsr593a*	11.6	(Keller et al., 1999)
QPm.ttu-1B	1BS	*T. militinae*	*Xgwm3000*	4.0–5.0	(Jakobson et al., 2006)
QPm.vt-1BL	1BL	Massey	*Xgwm259-Xbarc80*	15.0–17.0	(Tucker et al., 2007)
QPm.vt-1B	1BL	USG3209	*WG241*	17	(Liu et al., 2001)
Yr29/Lr46/Pm39	1BL	Saar	*Xwmc719-Xhbe248*	7.3–35.9	(Lillemo et al., 2008)
QPm.osu-1B	1BL	2174	*WMC134*	14	(Chen et al., 2009)
Qaprpm.cgb-1B	1B	Hanxuan 10	*WMC269.2-CWM90*	4.8–20.3	(Huang et al., 2008)
QPm.inra-1D.1	1DS	RE9001	*Xgwm106*	12.6	(Bougot et al., 2006)
QPm.sfr-1D	1DL	Forno	*Xpsr168-Xglk558b*	9.5	(Keller et al., 1999)
QPm.sfr-2A	2AS	Oberkulmer	*Xpsr380-Xglk293b*	7.7	(Keller et al., 1999)
QPm.inra-2A	2AS	Courtot	*Xgwm275*	7.4	(Bougot et al., 2006)
QPm.crag-2A	2AL	RE714	*Pm4b-gbxG303*	22.7–33.6	(Mingeot et al., 2002)
QPm.ttu-2A	2AL	*T. militinae*	*Xgwm311-Xgwm382*	5	(Jakobson et al., 2006)
QPm.vt-2AL	2AL	Massey	*Xgwm304- Xgwm312*	29	(Liu et al., 2001)
QPm.vt-2A	2AL	USG3209	*Xgwm304-Xgwm294*	26.0–29.0	(Tucker et al., 2007)
QPm.crag-2B	2BS	Festin	*Xgwm148-gbxG553*	23.6–71.5	(Mingeot et al., 2002)
QPm.caas-2BS	2BS	Lumai 21	*Xbarc98-Xbarc1147*	10.6–20.6	(Lan et al., 2010)
QPm.umb-2BS	2BS	Folke	*wPt-9402*	3.9–13.0	(Lillemo et al., 2012)
QPm.umb-2BS	2BS	Folke	*Xgwm410b-Xgwm148*	8.0–10.2	(Lillemo et al., 2012)
CP5	2BS	Pedroso	*wPt-5513*	12.3	(Marone et al., 2013)
QPm.vt-2B	2BL	Massey	*WG338-Xgwm526a*	11	(Liu et al., 2001)

QPm.caas-2B	2BL	Fukuho-komugi	*Xgwm877-Xgwm47*	5.7–8.0	(Liang et al., 2006)
QPm.inra-2B	2BL	RE9001	*Xrtp114R-Xcfd267b*	10.3–36.3	(Bougot et al., 2006)
QPm.vt-2BL	2BL	USG3209	*Xgwm501-Xgwm191*	11.0–15.0	(Tucker et al., 2007)
QPm.caas-2BL	2BL	Lumai 21	*Xbarc1139-Xgwm47*	5.2–10.1	(Lan et al., 2010)
Qaprpm.cgb-2B	2B	Hanxuan 10	*Xwmc477-Xwmc272*	5.4	(Huang et al., 2008)
Qpm.caas-2DS	2DS	Libellula	*Xcfd51-Xcfd56*	2.3–3.4	(Asad et al., 2012)
QPm.inra-2D-a	2DS	RE9001	*Xgwm102*	19	(Bougot et al., 2006)
QPm.inra-2D-b	2DS	RE9001	*Xcfd2e*	16.5	(Bougot et al., 2006)
QPm.sfr-2D	2DL	Oberkulmer	*Xpsr932-Xpsr331a*	10	(Keller et al., 1999)
Qpm.ipk-2D	2DL	W7984	*Xglk558-XksuD23*	/¶	(Börner et al., 2002)
QPm.caas-2DL	2DL	Lumai 21	*Xwmc18-Xcfd233*	5.7–11.6	(Lan et al., 2010)
QPm.umb-2DL	2DL	Folke	*Xwmc167-Xgwm301*	4.3–9.5	(Lillemo et al., 2012)
QPm.sfr-3A	3AS	Forno	*Xpsr598-Xpsr570*	10.4	(Keller et al., 1999)
QPm.crag-3A	3AS	Festin	*Xpsr598-Xgwm5*	21.4–25.9	(Mingeot et al., 2002)
QPm.nuls-3AS	3AS	Saar	*Xstm844tcac-Xbarc310*	8.1–20.7	(Lillemo et al., 2008)
Qaprpm.cgb-3A	3A	Hanxuan 10	*Xwmc21-Xwmc505.2*	9.8	(Huang et al., 2008)
QPm.inra-3B	3BS	Courtot	*Xgwm389*	22.7	(Bougot et al., 2006)
QPm.osu-3B	3BS	2174	*WMS533*	10	(Chen et al., 2009)
QPm.caas-3B	3BS	Opata 85	*XksuG53-Xfba190*	7.3	(Huo et al., 2005)
CP2	3BS	Creso	*F103*	10.6	(Marone et al., 2013)
Qaprpm.cgb	3BL	Hanxuan 10	*Xgwm181-Xgwm340*	13.3	(Huang et al., 2008)
QPm.sfr-3D	3DS	Oberkulmer	*Xpsr1196a-Lrk10–6*	15.7	(Keller et al., 1999)
QPm.inra-3D	3DS	RE9001	*Xcfd152, Xgwm707*	9.3–15.2	(Bougot et al., 2006)
QPm.sfr-4A.1	4AL	Forno	*Xgwm111c-Xpsr934a*	14.7	(Keller et al., 1999)
QPm.sfr-4A.2	4AL	Forno	*Xmwg710b-Xglk128*	14.3	(Keller et al., 1999)
QPm.ttu-4A	4AL	*T. militinae*	*Xgwm232–Xgwm160*	35.0–54.0 #	(Jakobson et al., 2006)
QPm.inra-4A	4AL	RE714	*XgbxG036*	4.9–6.9	(Chantret et al., 2001)
QPm.crag-4A	4AL	RE714	*XgbxG036-XgbxG542*	22.3	(Mingeot et al., 2002)
QPm.inra	4AL	Courtot	*Xcfd71b*	8.9	(Bougot et al., 2006)
QPm.osu-4A	4AL	2174	*WMS160*	12	(Chen et al., 2009)
QPm.tut-4A	4A	8.1	*Xwmc232-Xrga3.1*	24–46	(Jakobson et al., 2012)
QPm.sfr-4B	4BL	Forno	*Xpsr593b-Xpsr1112*	7.5	(Keller et al., 1999)
QPm.ipk-4B	4BL	W7984	*Xcdo795-Xbcd1262*	/¶	(Börner et al., 2002)
QPm.caas-4BL	4BL	Oligoculm	*Xgwm375-Xgwm251 XwPt1505-*	5.9	(Liang et al., 2006)
QPm.nuls-4BL	4BL	Avocet	*Xgwm149*	21.0–40.2	(Lillemo et al., 2008)
QPm. Caas-4BL.1	4BL	Libellula	*Xgwm149-Xgwm495*	9.1–14.7	(Asad et al., 2012)
QPm.sfr-4D	4DL	Forno	*Xglk302b-Xpsr1101a*	14.4	(Keller et al., 1999)
qApr4D	4D	Yumai 57	*Xgwm194-Xcfa2173*	20	(Zhang et al., 2008)
QPm.caas-4DL	4DL	Bainong 64	*Xbarc200-Xwmc33*	15.2–22.7	(Lan et al., 2009)
QPm.sfr-5A.1	5AS	Oberkulmer	*Xpsr644a-Xpsr945a*	22.9	(Keller et al., 1999)
QPm.ttu-5A	5AS	*T. militinae*	*Xgwm186–Xgwm415*	4.0–6.0	(Jakobson et al., 2006)
QPm.sfr-5A.2	5AL	Oberkulmer	*Xpsr1194-Xpsr918b*	16.6	(Keller et al., 1999)
QPm.sfr-5A.3	5AL	Oberkulmer	*Xpsr911-Xpsr120a*	10.5	(Keller et al., 1999)
QPm.nuls-5A	5AL	Saar	*Xgwm617b-Xwmc327*	4.2–15.2	(Lillemo et al., 2008)
Qaprpm.cgb-5A	5A	Hanxuan 10	*P3616–185-P3616–195*	13.2	(Huang et al., 2008)
QPm.tut-5A	5A	8.1	*Xgwm666-Xcfd30-Xbarc319*	14–16	(Jakobson et al., 2012)
QPm.nau-5AL	5AL	TA2027	*Xcfd39/Xmag1491-Xmag1493*	59	(Jia et al., 2009)
QPm.umb-5AL	5AL	Folke	*wPt-2426*	4.0–9.7	(Lillemo et al., 2012)
QPm.umb-5BS	5BS	T2038	*wPt-1261*	3.1	(Lillemo et al., 2012)
QPm.umb-5BS	5BS	Folke	*Xbarc128a-Xgwm213*	8.1–12.9	(Lillemo et al., 2012)
QPm.ttu-5B	5BS	Tahti	*Xgwm133.mi6-Xgwm205.mi1*	4.0–6.0	(Jakobson et al., 2006)
QPm.nuls-5B	5BS	Saar	*Xbarc4-Xgwm274b*	9.7	(Lillemo et al., 2008)
QPm.sfr-5B	5BL	Oberkulmer	*Xpsr580b-Xpsr143*	12.6	(Keller et al., 1999)
QPm.inra-5B.2	5BL	Courtot	*Xgwm790b*	11.1	(Bougot et al., 2006)
Qaprpm.cgb-5B	5B	Lumai 14	*Xgwm213-Xgwm499*	19.8	(Huang et al., 2008)
QPm.inra-5D	5DS	RE9001	*cfd189*	9	(Bougot et al., 2006)
QPm.crag-5D.1	5DL	RE714	*Xgwm639a-Xgwm174*	30.2–38.9 §	(Mingeot et al., 2002)
QPm.crag-5D.2	5DL	RE714	*Xcfd8B9-Xcfd4A6*	24.0–37.8	(Mingeot et al., 2002)

QpmVpn.inra-5D	5DL	Courtot	*Xcfd8*	11	(Bougot et al., 2006)
QPm.inra-5D.1	5DL	RE714	*Xcfd26*	28.1–37.7	(Chantret et al., 2001)
QPm.inra-5D.2	5DL	RE714	*XgbxG083c*	37.7	(Chantret et al., 2001)
QPm.caas-5D	5D	W7984	*Xmwg922-Xbcd1103*	5.9	(Huo et al., 2005)
qApr5D	5D	Yumai 57	*Xwmc215-Xgdm63*	1.3	(Zhang et al., 2008)
CP1	6AS	Pedroso	*MAG1200b*	12.6	(Marone et al., 2013)
QPm.inra-6A	6AL	RE714	*MIRE(Xgwm427)*	8.8–13.4	(Chantret et al., 2001)
QPm.crag-6A	6AL	RE714	*MIRE*	19.8–53.9 ††	(Mingeot et al., 2002)
QPm.sfr-6B	6BS	Forno	*Xpsr167b-Xpsr964*	8.7	(Keller et al., 1999)
QPm.umb-6BS	6BS	Folke	*wPt-6437-Xwmc494*	6.5–10.3	(Lillemo et al., 2012)
QPm.caas-6BS	6BS	Bainong 64	*Xbarc79-Xgwm518*	10.3–16.0	(Lan et al., 2009)
Qaprpm.cgb-6B	6B	Hanxuan 10	*Xgwm193-P3470–210*	21	(Huang et al., 2008)
QPm.caas-6BL.1	6BL	Huixianhong	*Xgwm219-Xbarc24*	2.5–5.2	(Asad et al., 2012)
QPm.caas-6BL.2	6BL	Huixianhong	*Xbarc24-Xbarc345*	0.5–1.9	(Asad et al., 2012)
CP3	6BL	Pedroso	*Xgwm219-Xgwm889*	14.8–18.5	(Marone et al., 2013)
CP4	6BL	Pedroso	*wPt-5270*	13.4	(Marone et al., 2013)
QPm.osu-6D	6DS	2174	*BARC196*	5	(Chen et al., 2009)
QPm.inra-7A	7AS	RE714	*Xfba069-Xgwm344*	2.9–6.4	(Chantret et al., 2001)
QPm.caas-7A	7AS	Bainong 64	*Xbarc127-Xbarc174*	6.3–7.1	(Lan et al., 2009)
Qaprpm.cgb-7A	7A	Hanxuan 10	*CWM462.2-Xgwm635.2*	8	(Huang et al., 2008)
QPm.tut-7A	7A	8.1	*Xgwm635-Xbarc70-Waxy*	9–28	(Jakobson et al., 2012)
QPm.umb-7AL	7AL	T2038	*Xgwm428-Xcfa2040*	6.4–13.0	(Lillemo et al., 2012)
QPm.sfr-7B.1	7BL	Forno	*Xpsr593c-Xpsr129c*	11.3	(Keller et al., 1999)
QPm.sfr-7B.2	7BL	Forno	*Xglk750-Xmwg710a*	31.8	(Keller et al., 1999)
QPm.crag-7B	7BL	RE714	*XpdaC01-XgbxR035b*	22.8–33.5	(Mingeot et al., 2002)
QPm.inra-7B	7BL	RE714	*Xgwm577*	1.7	(Chantret et al., 2001)
QPm.nuls-7BL	7BL	Saar	*Xwmc581- XwPt8007*	4.9	(Lillemo et al., 2008)
Qaprpm.cgb-7B	7B	Lumai 14	*Xwmc273-Xwmc276*	12.6	(Huang et al., 2008)
QPm.caas-7DS	7DS	Libellula	*XcsLV34-Xgwm295*	7.6–13.8	(Asad et al., 2012)
QPm.ipk-7D	7DS	Optata	*Xwg834-Xbcd1872*	/¶	(Börner et al., 2002)
QPm.caas-7DS	7DS	Fukuho-komugi	*Ltn-Xgwm295.1*	12	(Liang et al., 2006)
Yr18/Lr34/Pm38	7DS	Saar	*Xgwm1220-Xswm10*	19.0–56.5	(Lillemo et al., 2008)
QPm.inra-7D.1	7DS	Courtot	*Xgpw1106*	10.6	(Bougot et al., 2006)
Qaprpm.cgb-7D	7D	Hanxuan 10	*Xwmc436-Xgwm44*	3.8–4.6	(Huang et al., 2008)
QPm. Caas-7D	7D	Opata 85	*Xwg834-Xbcd1438*	29.6	(Huo et al., 2005)

† R2, percentage of variance explained by the QTL

‡ Residual effect of major resistance gene Pm3a

§ QTL detected by the software Mapmaker QTL

/¶, R2 of this QTL is unknown

QTL detected by the software Map Manager QTX Version b16

†† QTL was attributed to the residual effect of MIRE

1.10 Fusarium head blight (FHB)

FHB is a devastating fungal disease in cereals caused by several different *Fusarium* and *Microdochium* species. In Norway, *Fusarium graminearum* is the most prevailing species of FHB (Hofgaard et al., 2016). The disease affects wheat, barley, oat, rye, triticale, maize and many grass species.

F. graminearum causes severe yield loss because of either failed kernel development or because the infected kernels are shrivelled and light in test weight (McMullen et al., 2012). In addition

to yield loss, *F. graminearum* is also the causal agent for the development of the mycotoxin deoxynivalenol (DON). DON causes feed refusal and poor feed weight in animals, and it may also cause immunological problems in humans (Hofgaard et al., 2016; McMullen et al., 2012). Because of the harmful effect, the European Commission has set regulations to levels of DON accepted for human consumption. These levels range from 200 microgram/kg for processed cereal based foods and foods for infants and young children, to 750 microgram/kg for cereals intended for direct human consumption and cereal flour. For unprocessed cereal the maximum DON level has been set to 1250 microgram/kg (EC, 2006).

1.10.1 Disease symptoms

The disease symptoms on wheat in the field are several. Brown dark necrotic lesions form on the exterior surface of florets and glume, these symptoms being called scab even though it is not related to other scab diseases. The peduncles below the inflorescence become discoloured brown, purple, pink (Goswami et al., 2004). The tissue within the inflorescence will become white, pale and sometimes pink. The grains will also be discoloured, bleached and pink, and after some time the grain will look shrivelled and dry when compared to healthy non-infected grain (Fig. 9). Sometimes several inflorescences will be killed by the fungus, and sometimes the entire spike will die from the infection (Fig. 9).

Figure 8. Disease symptoms FHB in field, in spikes (a, b) and grain (c). Photo: Susanne S. Windju.

1.10.2 Disease cycle

The primary inoculum for FHB comes from infected plant debris where the fungus overwinters. In summer, parallel to the wheat flowering, ascospores of *F. graminearum* are released due to warm humid weather and these spores are dispersed by wind, rain or insects and land on the flowering spike (Goswami et al., 2004; Trail, 2009). The fungus develops hyphae that grow towards natural openings in the floret, e.g. the stomata openings (Fig. 10). Once the fungus is inside the floret, the anthers, stigma and lodicules are most easily colonized. The fungus has a short biotrophic phase before it switches to a necrotrophic phase and eventually kills the plant tissue. Entire florets might be killed due to FHB infection (Goswami et al., 2004).

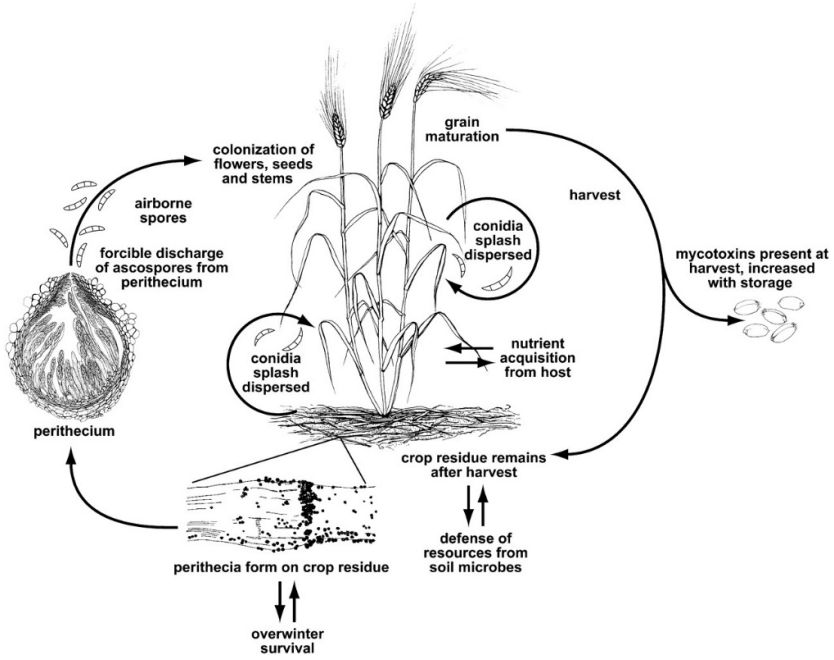


Figure 9. *Fusarium graminearum* disease cycle in wheat (Trail, 2009).

1.10.3 Resistance mechanisms

Resistance to FHB has been divided into passive and active resistance mechanisms.

The passive resistance mechanisms are divided into 4 different mechanisms: Type I: plant height; Type II: presence of awns increase disease severity while absence decreases disease severity; Type III: spike density within the head; and Type IV: escape, flowering in boot stage (Mesterházy, 1995).

The active resistance mechanisms are further divided into 5 different mechanisms (Table 2); Type I: Resistance to initial infection; Type II: Resistance to fungal spread (Schroeder et al., 1963); Type III: Resistance to toxin accumulation; Type IV: Resistance to kernel infection; and Type V: Tolerance (Mesterházy, 1995; Miller et al., 1985). Often, when FHB is discussed, the resistance Type I and Type II are the main resistance types.

Both the passive resistance and the active resistance mechanisms are under quantitative control and highly influenced by the environment.

Table 2. Active FHB resistance types and evaluation method.

Type	Component	Evaluation parameter	Reference
Type I	Resistance to initial infection	FHB severity after spray/spawn inoculation	(Schroeder et al., 1963)
Type II	Resistance to fungal spread	FHB severity after point inoculation, late evaluation in field	(Schroeder et al., 1963)
Type III	Resistance to toxin accumulation	DON content	(Mesterházy, 1995; Miller et al., 1985)
Type IV	Resistance to kernel infection	Fusarium damaged kernel percentage	(Mesterházy, 1995; Miller et al., 1985)
Type V	Tolerance	Yield	(Mesterházy, 1995; Miller et al., 1985)

1.10.4 Disease assessment

Disease assessment is often done in nurseries where the plant material is artificially infected with FHB. To ensure high disease pressure, mist irrigation can be applied during the flowering stage. In this way one can optimize conditions to capture resistance QTL in the varieties and lines tested (Fig. 11). In field trials, visual scorings of FHB are performed in the nurseries selecting a representative number of heads and counting number of spikelets infected by FHB and divide this by the total number of spikelets giving a percentage of infected spikelets in each plot. FHB disease pressure is influenced by inoculum, resistance level of the wheat line and highly on the climatic conditions (Snijders, 2004). Due to the high environmental factor, disease assessments in several environments are important to be able to detect consistent resistance. Both anther extrusion (AE) (Buerstmayr et al., 2015; Lu et al., 2013; Skinnes et al., 2010), plant height (PH) (He, Singh, et al., 2016; Lu et al., 2013; Mao et al., 2010; Srinivasachary et al., 2009; Srinivasachary et al., 2008) and days to heading (DH) (Buerstmayr et al., 2012; Emrich et al., 2008; He, Lillemo, et al., 2016; Schmolke et al., 2005) have been reported to be associated with FHB.

Figure 10. Top pictures: Oat grain infected with FHB, used to infect the disease nursery field. Middle: Bags containing oat spawn inoculum. Bottom picture: Disease nursery infected with FHB infected oat kernels at Staur research farm with mist irrigation system. Photo: Susanne S. Windju.

1.10.5 FHB resistance

Numerous studies have been performed in mapping populations to detect possible QTL associated with FHB resistance. A meta-analysis performed by Liu et al. (2009) clustered 249 FHB resistance QTL from 46 unique lines from 45 studies. They classified 209 QTL for FHB resistance Type I, II, III and IV in 43 clusters on 21 chromosomes (Table 3). Many of these QTL overlap and might possibly be the same. Most of these resistance QTL have been detected in Asian sources (Liu et al., 2009). Several FHB resistance genes have been mapped, *Fhb2* from the resistance source Sumai 3 has been mapped to chromosome 6BS (Cuthbert et al., 2007). *Fhb4,* a major QTL, was fine-mapped by Xue et al. (2010), and mapped to chromosome 4B with the markers *Xhbg226* and *Xgwm149* flanking the QTL. Another major QTL for FHB resistance, *Fhb5*, was fine-mapped between the SSR markers *Xgwm304* and *Xgwm405* to the centromeric region of chromosome 5A (Xue et al., 2011).

Table 3. FHB resistance QTL identified from various wheat sources from Asia, Europe and America. Table copied and modified from Liu et al. (2009).

Chrom.	Sources	Resistance types	Linked markers	Population	References
1A	History	II	XP68M51.352	RIL	(Holzapfel et al., 2008)
1A	CJ9306	II	Xwmc024-Xbarc148	RIL	(Jiang, Dong, et al., 2007)
1A	NK93604	II	XwPt-5577-Xbarc213	DH	(Semagn et al., 2007)
1A	CJ9306	III	Xbarc148-Xwmc24	RIL	(Jiang, Shi, et al., 2007)
1A	NK93604	III	XwPt-5577-Xbarc213	DH	(Semagn et al., 2007)
1A	Pelikan	II	Xgwm164-Xbarc28	RIL	(Häberle et al., 2009)
1A	Pirat	II	Xwmc818	RIL	(Holzapfel et al., 2008)
1A	Wangshuibai_f	II	XpAG-mTCGA338	RIL	(Yu et al., 2007)
1A	Wheaton	III	XpACTG-mTGC521	RIL	(Yu et al., 2007)
1B	Cansas	I	XE38M52.378-Xgwm131	RIL	(Klahr et al., 2007)
1B	CM82036	II	XgluB1	DH	(Buerstmayr et al., 2002)
1B	Arina_b	II	XS16M14.400	DH	(Draeger et al., 2007)
1B	Romanus	II	XP70M56.308	RIL	(Holzapfel et al., 2008)
1B	Wangshuibai_a	II	Xgwm018-Xbarc181	RIL	(Lin et al., 2004)
1B	Seri8	II	Xe38m50_10	RIL	(Mardi et al., 2006)
1B	Arina_c	II	XP43/M62-400-XwPt-3475	DH	(Semagn et al., 2007)
1B	Fundulea201	II	Xbarc8	RIL	(Shen, Ittu, et al., 2003)
1B	Rialto	II	XwPt-0705	DH	(Srinivasachary et al., 2008)
1B	Wangshuibai_b	II	Xwms759	RIL	(Zhou et al., 2004)
1B	History	II	XP64M51.190	RIL	(Holzapfel et al., 2008)
1B	Biscay	II	XP64M51.190	RIL	(Holzapfel et al., 2008)
1B	Pirat	II	XP64M51.190	RIL	(Holzapfel et al., 2008)
1B	Wangshuibai_f	III	Xwms759	RIL	(Yu et al., 2007)
1D	Ritmo	I	XS16M22.162-Xwhs2001	RIL	(Klahr et al., 2007)
1D	Apache	II	Xgwm458	RIL	(Holzapfrl et al., 2008)
1D	DH181	IV	Xgdm126	DH	(Yang, Gilbert, et al., 2005)
1D	Pirat	II	Xbarc149	RIL	(Holzapfel et al., 2008)
2A	Stoa	II	XksuH16	RIL	(Anderson et al., 2001)
2A	Renan	II	Xgwm382c	RIL	(Gervais et al., 2003)
2A	Rubens	II	Xgwm425	RIL	(Holzapfel et al., 2008)
2A	Arina_a	II	Xcfa2086-Xgwm311	RIL	(Paillard et al., 2004)
2A	Spark	II	Xgwm515	DH	(Srinivasachary et al., 2008)
2A	Ning7840	II	Xgwm614	RIL	(Zhou et al., 2002)

2A	NK93604	III	XwPt-6148-Xbarc124.1	DH	(Semagn et al., 2007)
2A	Wangshuibai_d	IV	Xzmh302-Xgwm328	RIL	(Li et al., 2008)
2A	Freedom	II	Xgwm296	RIL	(Gupta et al., 2000; Gupta et al., 2001)
2A	Ning8026	II	Xgwm294-Xwmc170	RIL	(Häberle et al., 2009)
2B	Goldfield	I	Xbarc200-Xgwm210	RIL	(Gilsinger et al., 2005)
2B	Frontana_a	I	XS13M25	DH	(Steiner et al., 2004)
2B	Arina_b	II	Xbarc28	DH	(Draeger et al., 2007)
2B	Renan	II	Xgwm374	RIL	(Gervais et al., 2003)
2B	Biscay	II	XP68M53.119	RIL	(Holzapfel et al., 2008)
2B	Ernie	II	Xgwm271	RIL	(Abate et al., 2008; Liu et al., 2007)
2B	SD97060	II	XwPt-3132	RIL	(Malla et al., 2008a)
2B	Tokai-66	II	XwPt-1903, wPt-3334	RIL	(Malla et al., 2008b)
2B	G16-92	II	Xgwm047	RIL	(Schmolke et al., 2008)
2B	Dream	II	XP74M53.272-XS25M12.206	RIL	(Schmolke et al., 2005)
2B	Frontana_a	II	XS13M25	DH	(Steiner et al., 2004)
2B	Ning7840	II	Xgwm120-Xbarc101	RIL	(Zhou et al., 2002)
2B	Ernie	IV	Xgwm271	RIL	(Liu et al., 2007a; Abate et al. 2008)
2B	History	II	XP68M52.318	RIL	(Holzapfel et al., 2008)
2D	Sumai3	I	Xgwm261	DH	(Handa et al., 2008)
2D	Wangshuibai_a	I	Xwmc181-Xaf12	RIL	(Lin et al., 2006)
2D	Wangshuibai_a	I	Xwmc445-Xgwm311	RIL	(Lin et al., 2006)
2D	DH181	I	Xwmc144-Xgwm539	DH	(Yang, Gilbert, et al., 2005)
2D	Sumai3	II	Xgwm261	DH	(Handa et al., 2008)
2D	Biscay	II	Xgwm484	RIL	(Holzapfel et al., 2008)
2D	Wangshuibai_c	II	Xgwm261- Xgwm484	DH	(Jia et al., 2005)
2D	CJ9306	II	Xgwm157-Xwmc243a	RIL	(Jiang, Shi, et al., 2007)
2D	Wangshuibai_e	II	Xgwm539-XS15M24	RIL	(Mardi et al., 2005)
2D	Alondra	II	Xgwm296-Xgwm261	RIL	(Shen, Ittu, et al., 2003)
2D	Wuhan1	II	Xgwm539	DH	(Somers et al., 2003)
2D	DH181	II	Xwmc144-Xgwm539	DH	(Yang, Gilbert, et al., 2005)
2D	Sumai3	III	Xgwm261	DH	(Handa et al., 2008)
2D	CJ9306	III	Xgwm157-Xwmc243a	RIL	(Jiang, Shi, et al., 2007)
2D	DH181	IV	Xwmc144-Xgwm539	DH	(Yang, Gilbert, et al., 2005)
2D	Romanus	II	Xcfd56	RIL	(Holzapfel et al., 2008)

3A	Frontana_a	I	Xdupw227-Xgwm720	DH	(Steiner et al., 2004)
3A	Frontana_b	II	Xgwm720-Xgwm1121	RIL	(Mardi et al., 2006)
3A	Arina_a	II	Xwmc264-Xgwm155	RIL	(Paillard et al. 2004)
3A	Fundulea201	II	Xgwm674	RIL	(Shen et al., 2003)
3A	Rialto	II	XwPt-7992	DH	(Srinivasachary et al., 2008)
3A	Spark	II	Xbarc19	DH	(Srinivasachary et al., 2008)
3A	Rialto	II	Xwmc11	DH	(Srinivasachary et al., 2008)
3A	Spark	II	Xgwm497	DH	(Srinivasachary et al., 2008)
3A	Frontana_a	II	Xdupw227-Xgwm720	DH	(Steiner et al., 2004)
3A	Tokai-66	III	XwPt-0398	RIL	(Malla et al., 2008b)
3A	Wangshuibai_f	I	XpCGA-mGTG352	RIL	(Yu et al., 2007)
3B	CM82036	I	Xgwm533-Xgwm493	DH	(Buerstmayr et al., 2002; Buerstmayr et al., 2003)
3B	W14	I	Xbarc133-Xgwm493	DH	(Chen et al., 2006)
3B	Ritmo	I	XE35M39.107-XE38M52.441	RIL	(Klahr et al., 2007)
3B	Nyubai	I	Xgwm566	DH	(Somers et al., 2003)
3B	DH181	I	Xgwm533	DH	(Yang, Gilbert, et al., 2005)
3B	DH181	I	Xwmc612	DH	(Yang, Gilbert, et al., 2005)
3B	Sumai 3/ND2603	II	Xgwm493-Xgwm533	RIL	(Anderson et al., 2001)
3B	CM82036	II	Xgwm533-Xgwm493	DH	(Buerstmayr et al., 2002; Buerstmayr et al., 2003)
3B	W14	II	Xgwm493-Xgwm533B	DH	(Chen et al., 2006)
3B	Sumai3	II	XSTS3B80-XSTS3B142		(Cuthbert et al., 2006)
3B	Renan	II	Xgwm383b	RIL	(Gervais et al., 2003)
3B	Apache	II	XP74M55.203	RIL	(Holzapfel et al., 2008)
3B	Wangshuibai_c	II	Xgwm533- Xgwm493	DH	(Jia et al., 2005)
3B	CJ9306	II	Xgwm533b-Xgwm493	RIL	(Jiang, Shi, et al., 2007)
3B	Wangshuibai_a	II	Xgwm533-Xbarc147	RIL	(Lin et al., 2004)
3B	Sumai3	II	XSTS3B-80-STS3B-206		(Liu, Zhang, et al., 2006)
3B	Ernie	II	Xwmc1	RIL	(Abate et al., 2008; Liu et al., 2007)
3B	Wangshuibai_e	II	Xgwm533-XS18M12	RIL	(Mardi et al., 2005)
3B	Arina_a	II	Xcfa2134b-Xgwm131b	RIL	(Paillard et al. 2004)
3B	Ning894037	II	Xbarc133-Xgwm493	RIL	(Shen, Zhou, et al., 2003)

3B	Nyubai	II	Xgwm533	DH	(Somers et al., 2003)
3B	Chokwang	II	Xgwm533	RIL	(Yang, Bai, et al., 2005)
3B	DH181	II	Xgwm533	DH	(Yang, Gilbert, et al., 2005)
3B	Ning7840	II	Xgwm533-Xbarc147	RIL	(Zhou et al., 2002)
3B	Wangshuibai_b	II	Xgwm533, Xbarc147	RIL	(Zhou et al., 2004)
3B	Wangshuibai_b	II	Xbarc344	RIL	(Zhou et al., 2004)
3B	W14	III	Xbarc133-Xgwm493	DH	(Chen et al., 2006)
3B	CJ9306	III	Xgwm533b-Xgwm493	RIL	(Jiang, Shi, et al., 2007)
3B	Ernie	III	Xwmc1	RIL	(Abate et al., 2008; Liu et al., 2007)
3B	SD97060	III	XwPt-9032	RIL	(Malla et al., 2008a)
3B	Nyubai	III	Xgwm533	DH	(Somers et al., 2003)
3B	W14	IV	Xbarc133-Xgwm493	DH	(Chen et al., 2006)
3B	Wangshuibai_d	IV	Xgwm533.1-Xwmc11	RIL	(Li et al., 2008)
3B	Ernie	IV	Xwmc1	RIL	(Abate et al., 2008; Liu et al., 2007)
3B	Tokai-66	IV	XwPt-2757-XwPt-1081	RIL	(Malla et al., 2008b)
3B	DH181	IV	Xgwm533	DH	(Yang, Gilbert, et al., 2005)
3B	DH181	IV	Xwmc527	DH	(Yang, Gilbert, et al., 2005)
3B	Wangshuibai_f	I	Xbarc147	RIL	(Yu et al., 2007)
3B	Massey	II	Xbarc164	RIL	(Liu et al., 2009)
3B	Wangshuibai_f	II	Xbarc147	RIL	(Yu et al., 2007)
3B	Wangshuibai_f	II	Xgwm376	RIL	(Yu et al., 2007)
3B	Wangshuibai_f	III	Xbarc147	RIL	(Yu et al., 2007)
3B	Wangshuibai_f	III	Xgwm376	RIL	(Yu et al., 2007)
3D	Cansas	I	XE33M57.457-Xgwm645	RIL	(Klahr et al., 2007)
3D	Biscay	II	Xgwm52	RIL	(Holzapfel et al., 2008)
3D	Arina_a	II	Xbcd907c-Xgwm161	RIL	(Paillard et al. 2004)
3D	Fundulea201	II	Xgwm341	RIL	(Shen, Ittu, et al., 2003)
3D	Romanus	II	XP64M52.257	RIL	(Holzapfel et al., 2008)
3D	Wangshuibai_f	II	XpCAT-mTGCG188	RIL	(Yu et al., 2007)
4A	Arina_a	II	Xcdo545-Xgwm160	RIL	(Paillard et al. 2004)
4A	Pirat	II	XP75M53.254	RIL	(Holzapfel et al., 2008)
4A	Apache	II	XP74M52.646	RIL	(Holzapfel et al., 2008)
4B	Wangshuibai_a	I	Xwmc349-Xgwm149	RIL	(Lin et al., 2006)
4B	Wuhan1	I	Xwmc238	DH	(Somers et al., 2003)
4B	Frontana_a	I	XS13M25_9	DH	(Steiner et al., 2004)

4B	Wheaton	I	Xwmc47	RIL	(Yu et al., 2007)
4B	Stoa	II	Xwg909	RIL	(Anderson et al., 2001)
4B	Wangshuibai_c	II	Xgwm368- Xgwm149	DH	(Jia et al., 2005)
4B	Ernie	II	Xgwm495	RIL	(Abate et al.,2008; Liu et al., 2007)
4B	Chokwang	II	Xbarc1096	RIL	(Yang, Bai, et al., 2005)
4B	IL94-1653	III	Xgwm495	RIL	(Bonin et al., 2009)
4B	Ernie	III	Xgwm495	RIL	(Abate et al., 2008; Liu et al., 2007)
4B	IL94-1653	IV	Xgwm495	RIL	(Bonin et al., 2009)
4B	Wangshuibai_d	IV	Xgwm149-Xwmc349	RIL	(Li et al., 2008)
4B	Becker	IV	Xwpt1708	RIL	(Liu et al. 2009)
4B	Ernie	IV	Xgwm495	RIL	(Abate et al., 2008; Liu et al., 2007)
4B	Pirat	II	Xgwm375	RIL	(Holzapfel et al., 2008)
4D	Massey	I	Rht2	RIL	(Liu et al. 2009)
4D	Soissons	I	Rht-D1	DH	(Srinivasachary et al., 2009)
4D	DH181	I	Xwmc331	DH	(Yang, Gilbert, et al., 2005)
4D	Arina_b	II	Rht-D1	DH	(Draeger et al., 2007)
4D	Spark	II	Xpsp3103	DH	(Srinivasachary et al., 2008)
4D	Spark	II	XRht-D1b	DH	(Srinivasachary et al., 2008)
4D	Spark	II	Xgwm265	DH	(Srinivasachary et al., 2008)
4D	Soissons	II	Rht-D1	DH	(Srinivasachary et al., 2009)
4D	Arina_b	III	Rht-D1	DH	(Draeger et al., 2007)
4D	Arina_b	IV	Xwmc457	DH	(Draeger et al., 2007)
4D	DH181	IV	Xwmc331	DH	(Yang, Gilbert, et al., 2005)
4D	History	II	Rht-D1	RIL	(Holzapfel et al., 2008)
4D	Apache	II	Rht-D1	RIL	(Holzapfel et al., 2008)
4D	Romanus	II	Rht-D1	RIL	(Holzapfel et al., 2008)
4D	Apache	II	Xgwm265	RIL	(Holzapfel et al., 2008)
5A	CM82036	I	Xgwm304-Xgwm156	DH	(Buerstmayr et al., 2002; Buerstmayr et al., 2003)
5A	W14	I	Xbarc117- Xbarc186	DH	(Chen et al., 2006)
5A	Wangshuibai_a	I	Xgwm304-Xbarc56	RIL	(Lin et al., 2006)
5A	DH181	I	Xgwm293	DH	(Yang, Gilbert, et al., 2005)

5A	CM82036	II	Xgwm304-Xgwm156	DH	(Buerstmayr et al., 2002; Buerstmayr et al., 2003)
5A	W14	II	Xbarc117- Xbarc186	DH	(Chen et al., 2006)
5A	Renan	II		RIL	(Gervais et al., 2003)
5A	Renan	II	Xgwm639b	RIL	(Gervais et al., 2003)
5A	Renan	II	B1	RIL	(Gervais et al., 2003)
5A	Ernie	II	Xgwm415	RIL	(Abate et al., 2008; Liu et al., 2007)
5A	Arina_a	II	Xgwm291-Xglk348c	RIL	(Paillard et al. 2004)
5A	Fundulea201	II	Xgwm304	RIL	(Shen, Ittu, et al., 2003)
5A	Spark	II	Xgwm443	DH	(Srinivasachary et al., 2008)
5A	Frontana_a	II	Xgwm129-Xbarc197	DH	(Steiner et al., 2004)
5A	W14	III	Xbarc117- Xbarc186	DH	(Chen et al., 2006)
5A	CJ9306	III	Xgwm425-Xbarc186	RIL	(Jiang, Shi, et al., 2007)
5A	Ernie	III	Xgwm415	RIL	(Abate et al., 2008; Liu et al., 2007)
5A	Nyubai	III	Xgwm96	DH	(Somers et al., 2003)
5A	W14	IV	Xbarc117- Xbarc186	DH	(Chen et al., 2006)
5A	Riband	IV	XS18M24.330	DH	(Draeger et al., 2007)
5A	Wangshuibai_d	IV	Xmag694-Xgwm304	RIL	(Li et al., 2007)
5A	Ernie	IV	Xgwm415	RIL	(Abate et al., 2008; Liu et al., 2007)
5A	Wangshuibai_f	I	Xbarc180	RIL	(Yu et al., 2007)
5A	Apache	II	Xwmc410	RIL	(Holzapfel et al., 2008)
5A	Pirat	II	Xgwm410	RIL	(Holzapfel et al., 2008)
5A	Wangshuibai_f	II	Xbarc180	RIL	(Yu et al., 2007)
5A	Wangshuibai_f	III	Xbarc180	RIL	(Yu et al., 2007)
5B	Cansas	I	XE35M52.331-XS25M20.245	RIL	(Klahr et al., 2007)
5B	Wangshuibai_a	I	Xgwm408-Xbarc140	RIL	(Lin et al., 2006)
5B	Wangshuibai_c	II	Xgwm443-Xbarc32	DH	(Jia et al., 2005)
5B	Wangshuibai_c	II	Xgwm335- Xgwm371	DH	(Jia et al., 2005)
5B	Wangshuibai_a	II	Xwmc113-Xgwm544	RIL	(Lin et al., 2004)
5B	Arina_a	II	Xgwm639a-Xpsr120a	RIL	(Paillard et al. 2004)
5B	Ning8026	II	Xgwm335	RIL	(Häberle et al., 2009)
5B	Apache	II	Xp70M58.189	RIL	(Holzapfel et al., 2008)
5D	Chokwang	II	Xbarc239	RIL	(Yang, Bai, et al., 2005)
5D	Wangshuibai_f	I	Xgwm292	RIL	(Yu et al., 2007)
5D	Wangshuibai_f	II	Xgwm97	RIL	(Yu et al., 2007)
5D	Wangshuibai_f	III	Xgwm212	RIL	(Yu et al., 2007)

6A	Apache	II	XP76M47.189	RIL	(Holzapfel et al., 2008)
6A	Dream	II	Xbarc107	RIL	(Häberle et al., 2007; Schmolke et al., 2005)
6A	Spark	II	XwPt-8833	DH	(Srinivasachary et al., 2008)
6B	BW278	I	Xgwm133-Xgwm644	RIL	(Cuthbert et al., 2007)
6B	Frontana_a	I	XS23M14_4	DH	(Steiner et al., 2004)
6B	DH181	I	Xwmc397	DH	(Yang, Gilbert, et al., 2005)
6B	Sumai 3/ND2603	II	Xbarc101/Xbcd1383	RIL	(Anderson et al., 2001)
6B	Patton	II	Xwmc494	RIL	(Bonin et al., 2009)
6B	BW278	II	Xgwm133-Xgwm644	RIL	(Cuthbert et al., 2007)
6B	Arina_b	II	Xpsp3131	DH	(Draeger et al., 2007)
6B	Wangshuibai_a	II	Xwmc539-Xbarc024	RIL	(Lin et al., 2004)
6B	Arina_c	II	XP46/M62-107-XP45/M60-265	DH	(Semagn et al., 2007)
6B	Ning894037	II	Xgwm88-Xgwm644	RIL	(Shen, Ittu, et al., 2003)
6B	Frontana_a	II	XS23M14_4	DH	(Steiner et al., 2004)
6B	DH181	II	Xwmc397	DH	(Yang, Gilbert, et al., 2005)
6B	Arina_b	III	Xpsp3131	DH	(Draeger et al., 2007)
6B	BW278	IV	Xgwm133-Xgwm644	RIL	(Cuthbert et al., 2007)
6B	DH181	IV	Xwmc397	DH	(Yang, Gilbert, et al., 2005)
6B	Ning8026	II	XP75M60.563-Xgwm644	RIL	(Haberle et al., 2009)
6B	Apache	II	XP63M47.204	RIL	(Holzapfel et al., 2008)
6B	Rubens	II	XP77M53.178	RIL	(Holzapfel et al., 2008)
6D	Renan	II	Xcfd0042	RIL	(Gervais et al., 2003)
6D	Arina_a	II	Xpsr915-Xcfd19a	RIL	(Paillard et al. 2004)
6D	Romanus	II	Xbarc96	RIL	Holzapfrl et al., 2008
7A	Ritmo	I	XS23M21.271-XS18M22.369	RIL	(Klahr et al., 2007)
7A	Arina_b	II	XS14M14.225	DH	(Draeger et al., 2007)
7A	Romanus	II	XP66M55.351	RIL	(Holzapfel et al., 2008)
7A	Wangshuibai_c	II	Xgwm276- Xgwm282	DH	(Jia et al., 2005)
7A	Frontana_b	II	XE77M47-Xgwm233	RIL	(Mardi et al., 2006)
7A	NK93604	II	Xgwm276-XDuPw226	DH	(Semagn et al., 2007)
7A	Spark	II	XwPt-7299	DH	(Srinivasachary et al., 2008)
7A	Spark	II	Xpsp3050(2)	DH	(Srinivasachary et al., 2008)
7A	Wangshuibai_b	II	Xwms1083	RIL	(Zhou et al., 2004)

7A	Arina_b	IV	XS14M14.225	DH	(Draeger et al., 2007)
7A	Ning8026	II	XP78M51.104_XP66M49.105	RIL	(Haberle et al., 2009)
7A	Romanus	II	Xgwm344	RIL	(Holzapfel et al., 2008)
7A	Wangshuibai_f	II	Xwms1083	RIL	(Yu et al., 2007)
7A	Wangshuibai_f	III	Xwms1083	RIL	(Yu et al., 2007)
7B	Goldfield	I	Xgwm344	RIL	(Gilsinger et al., 2005)
7B	Cansas	I	Xgwm46-XE42M58.394	RIL	(Klahr et al., 2007)
7B	Rubens	II	Xgwm43	RIL	(Holzapfel et al., 2008)
7B	CJ9306	II	Xgwm400-Xgwm573	RIL	(Jiang, Dong, et al., 2007)
7B	Dream	II	Xgwm46	RIL	(Häberle et al., 2007; Schmolke et al., 2005)
7B	DH181	II	Xwmc526	DH	(Yang, Gilbert, et al., 2005)
7B	Ning8026	II	Xbarc72	RIL	(Häberle et al., 2009)
7B	Romanus	II	XP70M61.206	RIL	(Holzapfel et al., 2008)
7B	History	II	XP66M53.115	RIL	(Holzapfel et al., 2008)
7D	Riband	II	Xwmc488	DH	(Draeger et al., 2007)
7D	Riband	III	Xwmc488a	DH	(Draeger et al., 2007)
7D	Wangshuibai_d	IV	Xwmc405-Xcfd14	RIL	(Li et al., 2008)

Fhb1

To date, *Fhb1* is the only FHB resistance gene cloned. The gene at *Fhb1* locus was found to be a pore-forming toxin-like (PFT) gene encoding a chimeric lectin (Rawat et al., 2016). The heterogeneous group of plant lectin proteins has been reported to play a role in plant defence against insects, nematodes, bacteria and fungi (Lannoo et al., 2014; Peumans et al., 1995).

1.10.6 Associated traits

Anther extrusion (AE)

AE have been reported to be negatively correlated with FHB. (He, Singh, et al., 2016; Lu et al., 2013; Skinnes et al., 2010). Anthers not fully extruded are major targets during the initial stages of FHB infection, where the they provide dead tissue readily colonized by *Fusarium* (Skinnes et al., 2010) (Fig. 12). A study performed by Skinnes et al. (2010) assessed AE in a DH population Arina x NK93604. Their results suggested involvement of several genes for AE, each with moderate to small effects. In this study, AE was consistently negative correlated to both FHB and DON, and in the QTL analysis, one AE QTL coincided with QTL for FHB. A QTL mapping study on AE and FHB was later performed by Lu et al. (2013) in the RIL

population SHA3/CBRD x Naxos. FHB was assessed in field trials after spawn, spray and point inoculation. In this study, the negative correlation, the continuous distribution of AE and the QTL analysis supported the findings from Skinnes et al. (2010); that AE is controlled by several genes. In the QTL mapping performed by Lu et al. (2013), five AE QTL coincided with QTL for FHB severity after spray and spawn inoculation. Interestingly, one of the detected AE QTL was located at the *Rht-B1* locus on 4BS. The results from Lu et al. (2013) indicated a pleiotropic effect of anther extrusion on Type I FHB resistance. Lu et al. (2013) suggested that many QTL previously reported for FHB resistance might be caused by AE. This was further supported by Buerstmayr et al. (2015), in a QTL study of anther retention and FHB in the Arina x Capo RIL population. They found two of three anther retention QTL to coincide with FHB QTL. Kubo et al. (2010) detected that closed flowering of the wheat plants and full anther retention decreased FHB infection. On a follow-up of these findings, Kubo et al. (2013) investigated the differences in AE level and the effect this had on resistance to FHB. They discovered that lines with partially extruded anthers had severe FHB symptoms compared to lines that had fully extruded anthers. They concluded that partially extruded anthers remained between the glumes was a source of FHB infection. Both closed flowering and rapid anther extrusion and ejection might prevent FHB infection. While cleistogamous flowering reduces the chance of infection, full anther extrusion would decrease favourable conditions for growth of the fungus (Buerstmayr et al., 2015).

Figure 11. Anther extrusion in wheat. Arrows indicating anthers not extruded, but trapped between glumes. Photo: Susanne S. Windju.

Plant height (PH)

The dwarfing genes *Rht-B1b (Rht1)*, *Rht-D1b (Rht2)* and *Rht8* on wheat chromosomes 4B, 4D and 2D respectively have been shown to be negatively correlated with both Type I and Type II FHB resistance (He, Singh, et al., 2016; Lu et al., 2013; Mao et al., 2010; Srinivasachary et al., 2009; Srinivasachary et al., 2008). A FHB QTL mapping study performed by Srinivasachary et al. (2008) detected one FHB QTL that was coincident with the *Rht-D1b* locus on 4D. This QTL accounted for up to 51% of the phenotypic variance and it was demonstrated that the *Rht-D1b* allele was associated with reduced Type I resistance, while being ineffective against Type II resistance. In this study, it was suggested that the observed enhanced susceptibility associated with *Rht-D1b* was not influenced by the height of the plant *per se*, because there were several other QTL for height segregating in the population in the study that did no coincide with FHB QTL in the QTL mapping (Srinivasachary et al., 2008). A second study by Srinivasachary et al. (2009) demonstrated that *Rht-D1b* and *Rht-B1b* differ in their influence on resistance to FHB. Both *Rht-B1b* and *Rht-D1B* were shown to decrease Type I resistance, but only *Rht-B1b* increased Type II resistance in their study. A QTL meta-analysis performed by Mao et al. (2010) comparing PH QTL with FHB QTL found several locations on the map where QTL for PH and FHB coincided. In their study *Rht-B1*, *Rht-D1* and *Rht8* all coincided with QTL for FHB.

Studies report about a complicated relationship between PH and FHB. The correlations have been suggested to be due to morphological escape (Buerstmayr et al., 2002; Mesterházy, 1995) or result from linkage or pleiotropy (Srinivasachary et al., 2009). Later QTL mapping studies have further detected and validated the association between FHB and the *Rht-B1* and *Rht-D1*, in addition AE has also been suggested to be associated with PH. A QTL mapping study by He, Singh, et al. (2016) found that the two dwarfing genes *Rht-B1b* and *Rht-D1b* both reduced AE in the populations tested. Based on their findings, He, Singh, et al. (2016) suggested that the two dwarfing genes lead to low AE, which in turn causes increased Type I FHB susceptibility.

Days to heading (DH)

Wheat lines are at its most susceptible to FHB during early flowering stage. Days to heading (DH) has been reported associated with FHB, with later flowering being associated with reduced FHB severity (Buerstmayr et al., 2012; Emrich et al., 2008; He, Lillemo, et al., 2016; Schmolke et al., 2005). A QTL on 7BS for FHB have been found to coincide with a QTL for DH (Buerstmayr et al., 2012; Schmolke et al., 2005)

1.11 Septoria nodorum leaf blotch (SNB)

Some of the most widespread leaf-spotting pathogens world-wide are the fungal species belonging to *Septoria*. (Quaedvlieg W et al., 2013). Septoria nodorum leaf and glume blotch are caused by *Parastagonospora nodorum* (*P. nodorum*).

P. nodorum is an important necrotrophic pathogen of wheat in some of the major wheat producing regions of the world (Francki, 2013; Solomon et al., 2006) and is the major leaf blotch pathogen on spring wheat in Norway (Ruud et al., 2017). *P. nodorum* infects both leaves, stems and glumes through openings like the stomata or directly through epidermal tissues, causing both leaf and glume blotch. The main hosts are bread wheat (*Triticum aestivum*), durum wheat (*Triticum durum*) and triticale. *P. nodorum* has been reported in other cereals and wild grasses as well (Francki, 2013; Solomon et al., 2006). Infection of *P. nodorum* occurs in wet and humid climate, over a wide temperature range. It causes grain yield reduction due to the decreased photosynthetic capacity of heads, peduncle and flag leaf, and has the most devastating effect during infection at the heading stage of the host (Francki, 2013). On cereals, the diagnostic symptoms of the disease are red-brown chlorotic lesions with a yellow halo on lower leaves of plants that later develop brown specks of pycnidia (Fig. 13) (Agrios, 2005; Francki, 2013).

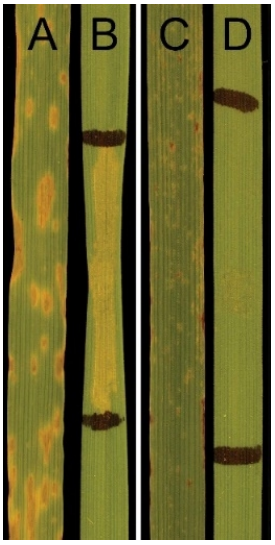


Figure 12. SNB susceptible, toxin-sensitive line (A and B) and SNB resistant, toxin-insensitive line (C and D) (Friesen et al., 2010).

1.11.1 Disease cycle

P. nodorum overwinters as mycelium or pycnidia in infected seed and on plant debris. The fungus has a mixed reproduction system with asexual pycnidiospores and sexual ascospores. The ascospores are released from the pseudothecia and are wind dispersed through spore-clouds causing epidemics (Francki, 2013). The pycnidiospores produce long, one-to several-celled conidia in spherical pycnidia. The conidia are released from the pycnidia that have been wetted by rain, dew or irrigation. The conidia is spread by rain, wind-blown rain, rain splash-dispersal or contact with animal etc. (Agrios, 2005; Bhathal et al., 2003) (Fig.14).

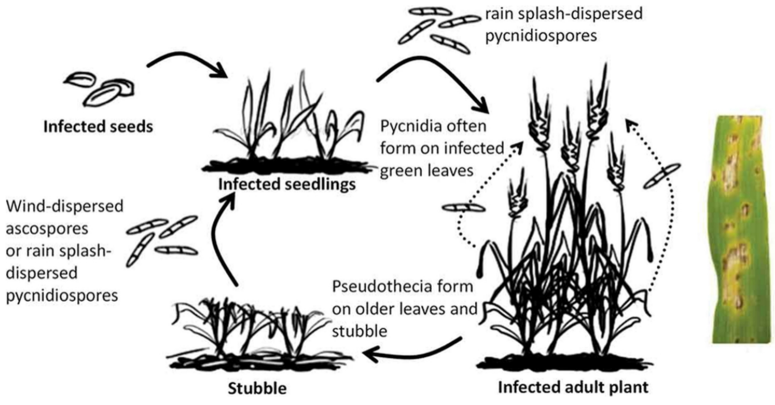


Figure 13. *P. nodorum* life cycle (Chooi et al., 2014).

1.11.2 Resistance

Morphological traits like PH, DH and maturity are associated with the development of SNB (Francki, 2013). The genetic control of SNB resistance is polygenic with significant G x E interactions (Francki, 2013; Friesen et al., 2008). In recent years, the underlying mechanism of SNB have been investigated and the presence of multiple host-specific toxins demonstrated (Friesen et al., 2008). When infecting host tissue, *P. nodorum* secretes effector proteins that interact with the host to initiate disease. This mechanism is inverse to the gene-for gene-model proposed by Flor (1955) (Oliver et al., 2010). In this inverse gene -for-gene model, a specific host-toxin interaction relies on the recognition of the toxin (pathogen necrotrophic effectors, NE) by a host sensitivity locus (*Snn*), which again leads to toxin sensitivity and disease susceptibility. The absence of the toxin or the dominant host gene results in an incompatible or resistance response (Friesen et al., 2008). So far, eight NE (SnToxA, SnTox1, SnTox2, SnTox3, SnTox4, SnTox5, SnTox6 and SnTox7) and nine corresponding host *Snn* genes (*Tsn1*, *Snn1*, *Snn2*, *Snn3-5B*, *Snn3-5D*, *Snn4*, *Snn5*, *Snn6* and *Snn7*) have been characterized (Abeysekara et al., 2009; Friesen et al., 2007; Friesen et al., 2006; Gao et al., 2015; Liu, Friesen, et al., 2006; Shi et al., 2015).

Two of the host sensitivity *Snn* genes have been cloned, *Tsn1* and *Snn1*. Both these sensitivity genes encode proteins associated with resistance to biotrophic pathogens, indicating that the necrotrophic pathogens utilise the biotrophic resistance pathways when they infect host cells (Faris et al., 2010; Shi et al., 2016). QTL for resistance have been detected on several chromosomes and a review published by Francki (2013) summarized APR QTL for *S. nodorum* resistance in seedlings, flag leaf, and glume in bread and durum wheat (Table 4).

Table 4. QTL for *S.nodorum* resistance in seedlings, flag leaf and glume in bread and durum wheat. Table copied and modified from Francki (2013).

Population	Plant tissue	Inoculation*	QTL and chromosome	Marker interval	Percent total variation**	Allele	Reference
T. aestivum Liwilla × Begra, DH (n = 111)	Seedling	Mixed isolate (15), CE	QSnl.ihar-2B	gwm501–gwm410	16	Liwilla	(Czembor et al., 2003)
			QSnl.ihar-5B	barc32–gwm499	3	Liwilla	
			QSnl.ihar-5D	gwm205–gwm212	37	Liwilla	
T. aestivum W7984 × Opata85	Seedling (3 leaf)	Single isolate (Sn2000), Greenhouse	1B	mwg938–snn1	27–58	Opata85	(Liu et al., 2004)
			4B	cdo1312	6–9	W7984	
T. aestivum Alba × Begra, DH (n = 131)	Seedling (5 leaf)	Mixed isolate (15), CE	QSnl.ihar-6A	gwm570–mwg934	36	Alba	(Arseniuk et al., 2004)
Triticum turgidium L. LDN × LDN (Dic-5B), RIL (n = 133)	Seedling (2 leaf)	Single isolate (Sn2000), Greenhouse	5B	bcd9–fbb237	37	LDN (Dic-5B)	(Gonzalez-Hernandez et al., 2008)
T. aestivum BR34 × Grandin, RIL (n = 118)	Seedling (2–3 leaf)	Single isolate (BBCSn5), Greenhouse	QSnb.fcu-2DS	gwm614–cfd53	24	BR34	(Friesen et al., 2009)
			QSnb.fcu-5AL	barc151–fcp13	11	BR34	
			QSnb.fcu-5BL	barc1116–barc43	37	BR34	
T. aestivum Forno × Oberkulmer, RIL (n = 226)	Flag Leaf	Mixed isolate (40), Field	QSnl.eth-2D	psr932–psr331a	2	Oberkulmer	(Aguilar et al., 2005)
			QSnl.eth-4B	glk348–psr921	17	Oberkulmer	
			QSnl.eth-7B	mwg710a–glk576	12	Forno	

T. aestivum WAWHT2074 × 6HRWSN125	Flag Leaf	Mixed isolate (6–10), Field	QSnl.daw-2D	cfd11–gwm30	8–17	6HRWSN125	(Shankar et al., 2008)
T. aestivum BR34 × Grandin, RIL (n = 118)	Flag Leaf	Single isolate (BBSSn5), Field	QSnb.fcu-1BS	fcp267–barc240	1	BR34	(Friesen et al., 2009)
			QSnb.fcu-2DS	gwm614–cfd53	12–15	BR34	
			QSnb.fcu-5AL	barc151–fcp13	12–18	BR34	
			QSnb.fcu-5BL	barc1116–barc43	11–18	BR34	
T. aestivum P92201D5 × P91193D1, RIL (n = 254)	Flag Leaf	Mixed isolate (10), Field	QSnl.daw-2A	gwm614a–wPt7056	11–21	P92201D5	(Francki et al., 2011)
T. aestivum EGA Blanco × Millewa, DH (n = 235)	Flag Leaf	Mixed isolate (6–10), Field	QSnl.daw-1B	wPt8949–wPt2575	15–16	EGA Blanco	(Francki et al., 2011)
			QSnl.daw-5B	wPt3457–wPt0935	8–16	EGA Blanco	
T. aestivum Arina × Forna	Glume	Natural infection, Field	QSng.sfr-3B	gwm389–cfd79c	12–24	Arina	(Schnurbusch et al., 2003)
			QSng.sfr-4B	gwm165–glk335	7–22	Forno	
T. aestivum P92201D5 × P91193D1, RIL (n = 254)	Glume	Natural infection and mixed isolate (10), Field and Greenhouse	QSng.pur-2DL.1	gwm526a–cfd50b	12–38	P91193D1	(Uphaus et al., 2007)
			QSng.pur-2DL.2	cfd50c–wPt9848	5–6	P92201D5	
T. aestivum WAWHT2074 ×	Glume	Mixed isolate (6–10), Field	QSng.daw-4B	Rht1–gwm495	8–19	6HRWSN125	(Shankar et al., 2008)

* Single isolate designation or total number of isolates used in mixed inoculation are shown in parentheses.

** Field evaluation for at least two environments with minimum and maximum percentage or total phenotypic variation shown for each QTL

2. The thesis

2.1 Background and main objectives

Breeding of disease resistant wheat varieties is an environmental friendly and economical approach. Major race-specific resistance genes have been detected and utilised for several traits, but when a variety is grown commercially on large areas, changes in the pathogen population can easily overcome this resistance. To be able to find race-non-specific resistance genes within lines and varieties which also contain major genes is challenging because the major genes might make the detection of minor genes difficult. The use of genetic markers and different population types and collections is a valuable tool to search for and detect QTL containing race-non-specific resistance. For some diseases, the resistance is mainly partial, consisting of several genes working to maintain resistance. The use of molecular markers to detect this partial quantitative resistance is highly valuable. For molecular markers to be effective in the search for QTL associated with disease resistance, the linkage maps developed need to span parts of the genome where the resistance lies. And the markers need to be in linkage with the trait of interest, to be used as markers for the trait in subsequent generations.

The aims of this project were to utilise two SNP Chips; The 90K Illumina SNP Chip (Wang et al., 2014) and the 35K Affymetrix SNP Chip (Allen et al., 2017) to detect and validate disease resistance QTL against powdery mildew, Fusarium head blight and leaf blotch in wheat.

Two RIL populations; SHA3/CBRD x Naxos and Soru#1 x Naxos were genotyped with the 90K Illumina SNP Chip (Wang et al., 2014). These two populations had also been evaluated for PM in field trials in Norway and China for several years. The SHA3/CBRD x Naxos population was also evaluated for SNB in both field and greenhouse experiments in Norway. With the genotypic and phenotypic data, the aim with these two RIL populations were to detect, narrow down and validate QTL for PM and SNB.

A panel of 123 spring wheat accessions were genotyped with the 90K Illumina SNP Chip (Wang et al., 2014). An association mapping panel of 299 spring wheat accessions was genotyped with the 35K Affymetrix SNP Chip (Allen et al., 2017). For the 299 spring wheat association mapping panel, FHB disease assessments were performed at two locations in Norway over four years in addition to DON level measurements and AE, DH and PH evaluation. An association study was performed with the aim to detect QTL effective for both FHB resistance and DON.

2.2 Materials and methods

Materials and methods are thoroughly described in each paper. This section will provide an overview of the plant material used, the field and green house disease assessments and experiments, the development of linkage maps of the association mapping panel and the RIL populations and an overview of the QTL mapping and association mapping performed.

2.2.1 Plant material

Recombinant inbred line populations

Two inbred line (RIL) populations developed in generation F_6 by single seed descent were used in this project. One RIL population was Shanghai3/Catbird (SHA3/CBRD) x Naxos, which consisted of 177 lines. The second RIL population was a population consisting of 131 lines from the cross Soru#1 x Naxos. Naxos exhibits high level of partial resistance to PM at the adult plant stage (Lu et al., 2012) and is susceptible to SNB (Lu et al., 2012; Lu et al., 2014). SHA3/CBRD is a breeding line developed at CIMMYT. SHA3/CBRD has shown to be moderately susceptible to PM (Lu et al. 2012) and highly resistant to SNB (Lu et al., 2014). Soru#1 is a synthetic hexaploid derived wheat line (AABBDD). This line is highly susceptible to PM.

Association mapping panel

The extended association mapping panel in the study was a collection of 299 hexaploid spring wheat accessions. The collection was a diverse panel with accessions mostly from Norway and Sweden, but also consisting of lines from Australia, Brazil, Canada, China, CIMMYT, Czech Republic, Denmark, Finland, France, Germany, Netherlands, Poland. Russia, Slovakia, South Africa, Switzerland, United Kingdom and USA.

Field evaluation

PM

SHA3/CBRD x Naxos

For the SHA3/CBRD x Naxos population, PM data from Lu et al. (2012) were utilized. In that study, PM resistance was evaluated at two locations in Norway in 2008, 2009 and 2010; Vollebekk research farm at the Norwegian University of Life Sciences (Ås) and Staur research farm (Hamar). In addition, the population was evaluated for PM resistance at three locations in China; In 2009 in Nanjing, in 2010 in Beijing and Anyang.

Soru#1 x Naxos

For the Soru#1 x Naxos population, PM disease severity was evaluated at Vollebekk research farm in 2012, 2013 and 2016, and Staur research farm in 2013.

PM disease severity was assessed on the whole canopy basis as the percentage of leaf area infected, using a modified Cobb scale (0 to 100% infected leaf area) (Peterson et al., 1948). The disease severity was scored two to three times during the field season.

Septoria nodorum blotch

SHA3/CBRD x Naxos

For the SNB evaluation in SHA3/CBRD x Naxos, data from Lu et al. (2014) was used. The SHA3/CBRD x Naxos RILs had been evaluated for SNB at Vollebekk research farm in 2010, 2011, 2012 and 2013. The testing had been performed in naturally infected field trials, and mist-irrigated to promote leaf blotch epidemics and avoid competing disease such as powdery mildew (Lu et al., 2014).

Leaf blotch severity was assessed visually as the percentage of diseased leaf area based on the whole canopy (Lu et al., 2014).

FHB

FHB was evaluated in the association mapping panel. The mapping panel was tested at two locations in Norway; Vollebekk research farm in 2013, 2014, 2015 and Staur research farm in 2015. To ensure good FHB disease pressure, spawn inoculation of the fields was performed. Grain spawn, oat kernels infected with *F. graminearum*, was prepared and distributed as described by Lu et al. (2013). A mist irrigation was applied to ensure high disease pressure after spawn inoculation and during the flowering stage. FHB disease assessments were performed at the beginning of maturity, when the stems of the plants in the individual plots were just starting to turn yellow, and the spikes were still green. The level of DON in each harvested field plot was evaluated by GC-MS at the University of Minnesota DON testing lab (Mirocha et al., 1998).

Days to heading, plant height and anther extrusion

Days to heading (DH) and plant height (PH) were scored in the association mapping panel, in the same field as the FHB evaluation. DH was scored in every testing environment when 50% of the spikes in the plot had emerged. Plant height (PH) was evaluated at Vollebekk in 2013 and 2014, and at Staur in 2015.

Anther extrusion (AE) was evaluated in the association mapping panel, in different hill plot nurseries adjacent to the Fusarium disease assessment field, at Vollebekk in 2014 and 2015, and at Staur in 2014 and 2015.

Green house experiments

Septoria nodorum blotch

Seedling inoculation

The four SNB isolates Sn4, NOR4, 201593 and 201618 were used in the seedling inoculation.14-day-old plants of the SHA3/CBRD x Naxos RIL population were spray inoculated. After seven days, the second leaf of each plant in the accessions was evaluated for disease reactions on a scale from 0 – 5 (Liu et al., 2004), where 0 is highly resistant and 5 is highly susceptible.

Infiltrations

Liquid cultures of the same four isolates as used for seedling inoculations were produced as described in Friesen et al. (2012). In addition, purified toxins of SnToxA, SnTox1 and SnTox3, produced in *Pichia pastoris* were used. 12-14 day old seedlings were infiltrated with filter sterilized culture filtrates or purified toxins. After 3-5 days the reactions were scored according to a 0-3 scale where 0 is insensitive and 3 is complete sensitivity (necrosis with tissue collapse) (Friesen et al., 2012).

Development of linkage maps

SHA3/CBRD x Naxos and Soru#1 x Naxos

Illumina 90K wheat SNP Chip

The Soru#1 x Naxos and SHA3/CBRD x Naxos RIL populations were genotyped with the Illumina 90K wheat SNP Chip (Wang et al., 2014). Analysis and scoring of the genotyping results for each population were performed manually for every SNP marker using Genome Studio Genotyping Module v1.0 software from Illumina.

In both populations, SNP markers scored as polymorphic were used for constructing linkage groups and genetic linkage maps. The markers were sorted in linkage groups with MSTmap (Wu et al., 2008). The linkage groups were assigned to chromosomes based on the best BLASTn hit from a comparison of SNP-flanking sequences with the Chinese Spring chromosome survey sequences (http://wheat-urgi.versailles.inra.fr/Seq-Repository). Previously developed SSR and DArT marker data in the SHA3/CBRD x Naxos population (Lu et al., 2012)

were added to the SNP marker data. Genotyped SSR markers from the Soru#1 x Naxos population were further added to the SNP marker data of this population.

For both populations, markers belonging to linkage groups that were assigned to the same chromosomes based on the BLASTn search were loaded into JoinMap v. 4.0 v. 4.0 (Van Ooijen, 2006) and the linkage maps were refined using the maximum likelihood mapping algorithm. The genetic distances between markers were calculated by converting recombination fractions into map distances (cM) based on the Kizami mapping function with minimum LOD score of 3.0 (Kosambi, 1943). To develop maps with many shared markers between the two populations, shared markers between the two populations were discovered and the function "fixed order" was utilized in JoinMap v. 4.0.

Affymetrix 35K SNP chip

The Soru#1 x Naxos population was also genotyped with the Affymetrix 35K SNP chip (Allen et al., 2017). An integrated linkage map of the Soru#1 x Naxos population was developed in JoinMap v. 4.0. with the aim of placing as many of the polymorphic markers from both genotyping (Illumina 90K SNP Chip (Wang et al., 2014) and Affymetrix 35K SNP Chip (Allen et al., 2017)) into one linkage map of Soru#1 x Naxos. This could later serve as a tool when performing and investigating QTL mapping in the Soru#1 x Naxos population.

Association mapping panel

Illumina 90K SNP Chip

A collection of 123 spring wheat lines was genotyped with the Illumina 90K SNP Chip (Akhunov et al., 2009). Analysis and scoring of the genotyping results for each population were performed manually for every SNP marker using Genome Studio Genotyping Module v1.0 software from Illumina. Positional information was assigned using the consensus 90K SNP map (Wang et al., 2014).

Affymetrix 35K SNP chip

An extended collection of 299 spring wheat lines was genotyped with the Affymetrix 35K SNP chip (Allen et al., 2017). Markers were filtered based on the presence in more than 90% of wheat lines and MAF > =5%. Positional information was assigned using the consensus 35K SNP map (Allen et al., 2017). In total 14095 markers were remained for the association mapping.

QTL mapping PM

Interval mapping (IM) and multiple QTL mapping (MQM) were performed on both SHA3/CBRD x Naxos and Soru#1 x Naxos to detect QTL using MapQTL6 software (Van Ooijen, 2011). Interval mapping (IM) was conducted to detect potential major QTL for powdery mildew resistance. The LOD profiles from interval mapping were observed, and markers closely linked to each QTL that showed effects in several environments were used as cofactors in the multiple QTL mapping (MQM). The LOD significance threshold level of powdery mildew was determined to be 3.2 for SHA3/CBRD x Naxos and 3.4 for Soru#1 x Naxos after a permutation test with 1000 permutations, and was used for QTL declaration. QTL effects were estimated as the proportion of phenotypic variance (R^2) explained by each QTL. Genetic maps and LOD curves were drawn in the software MapChart, v.2.1 (Voorrips, 2002).

QTL mapping SNB

QTL mapping was performed using the software MapQTL6 (Van Ooijen, 2006). Multiple QTL mapping (MQM) was used, based on cofactors for major QTL detected with an initial interval mapping (IM). The LOD significance threshold was set to 3.0. The software MapChart, v2.2 was used to draw the genetic maps and LOD curves. For analysis of field resistance, the confounding traits plant height (PH), heading date and maturity were used as covariates to disease score in MapQTL6 as described by Lu et al. (2014).

Association analysis

Association analysis was performed on the 299 spring wheat lines in the extended spring wheat collection using the software TASSEL v5.0 (Bradbury et al., 2007). Association analysis was performed for the traits AE, FHB corrected for PH and DH and log-transformed DON (log (DON level +1)) corrected for PH and DH. The analyses were performed on the least square means of the data for all traits. Same analyses were performed on single years (2013 Vollebekk, 2014 Vollebekk, 2015 Vollebekk and 2015 Staur) for FHB and DON. Association analyses were performed on two sets of the spring wheat panel; on the total wheat collection of 299 lines/varieties and on a subset of the panel containing only European lines (237 lines/varieties).

Several statistical methods were tested in TASSEL v5.0 for association mapping. The method chosen for the association analysis was a mixed linear model (MLM) with kinship matrix from Tassel (K) and Q-matrix obtained from Structure (Q), (MLM + K +Q).

Unmapped markers were placed on the fictitious “chromosome 22” in the initial association analysis. After an initial association analysis, markers associated with AE, FHB or DON were

detected. These markers were added to the rest of the marker set and analysed in Haploview. High LD between these added markers and markers in the linkage maps suggested their position, and they were placed in the map. An additional association analysis was performed with these added markers positioned in the linkage map.

2.3 Main results

2.3.1 SNP marker scoring and development of linkage maps

After genotyping of the two QTL mapping populations SHA3/CBRD x Naxos and Soru#1xNaxos, and the spring wheat collection with the Illumina 90K SNP Chip (Wang et al., 2014), the lines in the populations had to be scored as polymorphic or monomorphic manually for each marker because the hexaploid nature of wheat hampered the correct cluster calls for many markers. For this scoring, the software Genome Studio Genotyping Module v1.0 from Illumina (Illumina, 2008) was utilised. Every marker had to be scored and edited manually by visual inspection in the Genome Studio Genotyping Module v1.0 (Illumina, 2008) (Fig. 15). When Genome Studio had clustered the marker wrong, a manual editing was performed.

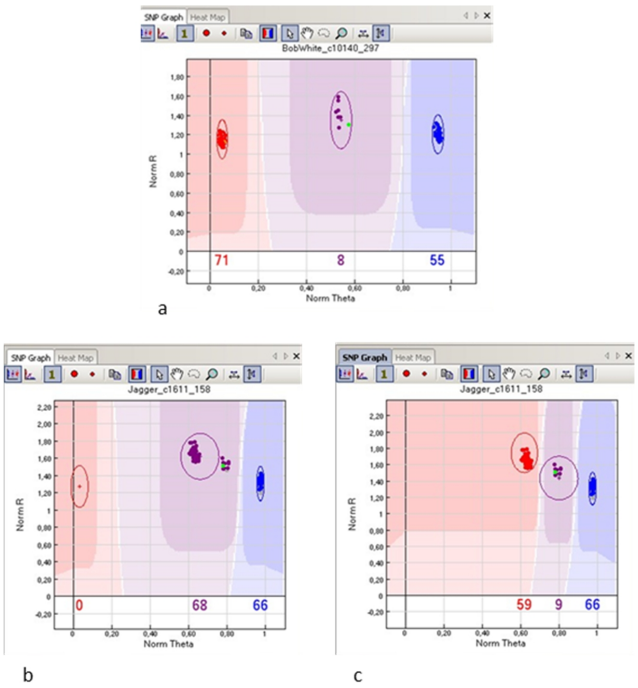


Figure 14. Clustering in the Genome Studio Genotyping Module v1.0. a) Correct clustering, no need to manually edit. b) Incorrect clustering, manual editing needed. c) Clustering pattern in b) after manual editing.

After manually clustering, 9230 SNP markers were scored as polymorphic in SHA3/CBRD x Naxos. In Soru#1 x Naxos, 10 500 SNP markers were scored as polymorphic. In the association mapping panel consisting of 123 lines, 21 566 SNP markers were scored as polymorphic and placed on a map covering all 21 chromosomes.

Before linkage maps of the markers in the two QTL mapping populations could be developed in JoinMap v. 4.0 (Ooijen, 2006), the markers had to be assigned to linkage groups in MST map. JoinMap v. 4.0 was not able to sort the large number of markers in the two RIL populations. The 9230 markers scored as polymorphic in the SHA3/CBRD x Naxos population were assigned to 45 linkage groups in MST map, while the 10 500 polymorphic markers in the Soru#1 x Naxos population were sorted into 83 linkage groups. After the MST map sorting, the linkage groups were assigned to chromosomes based on the best BLASTn hit from a comparison of SNP-flanking sequences with the Chinese Spring chromosome survey sequences (http://wheat-urgi.versailles.inra.fr/Seq-Repository). These linkage groups were then loaded into JoinMap v. 4.0 for linkage map construction.

For the SHA3/CBRD x Naxos population, 566 SSR, DArT and gene specific markers were added to the marker set before the development of linkage maps in JoinMap v. 4.0 (Ooijen, 2006). After redundant markers and markers causing disorder in the map had been removed, the linkage map of SHA3/CBRD x Naxos consisted of 3512 SNP markers and 224 SSR and DArT markers, spanning 3130 cM, covering all chromosomes. In the Soru#1 x Naxos population, 50 SSR markers were added to the dataset. Of the 10 500 polymorphic markers in Soru#1 x Naxos, 4113 were discarded due to poor quality. In total 6387 SNP markers and 50 SNP markers were loaded into JoinMap v. 4.0 (Ooijen, 2006) for map construction for the Soru#1 x Naxos population. Out of these markers, 2788 SNP markers and 36 SSR markers were included in the linkage map, spanning 3031 cM, covering all chromosomes.

Soru#1xNaxos 35K SNP Chip analysis and linkage map development

Before development of the Soru#1xNaxos SNP map after genotyping with the Affymetrix 35K SNP Chip, and finally the integrated map of this mapping population, the polymorphic markers from the Affymetrix 35K SNP Chip genotyping was sorted into linkage groups and assigned to chromosomes based on the best BLASTn hit from a comparison of SNP-flanking sequences with the Chinese Spring chromosome survey sequences (http://wheat-urgi.versailles.inra.fr/Seq-Repository). Most of the linkage groups could be assigned to chromosome and even to chromosome arm. A total of 5452 SNP markers were loaded into JoinMap v. 4.0 for map construction of the Soru#1 x Naxos population. The final map after refining and deleting redundant markers contained 3428 markers, spanning 6085 cM (Map in Appendix). The linkage map developed after the genotyping of Soru#1 x Naxos with the 35K Affymetrix Chip contained over 600 more markers than the maps developed after the Illumina 90K genotyping of this population. The map of Soru#1 x Naxos after the Affymetrix genotyping

was more fractioned than the map developed after the Illumina 90K SNP genotyping, with the final map consisting of 75 linkage groups.

The two linkage maps developed of Soru#1 x Naxos after the 90K SNP Chip and 35K SNP Chip genotyping, were merged in JoinMap v. 4.0 to develop an integrated linkage map containing markers from both SNP Chips. After redundant markers and markers giving suspect linkage were filtered out, the map contained 5225 markers and consisted of 48 linkage groups (Map in Appendix). Since the map of Soru#1 x Naxos developed after the 90K SNP Chip genotyping also had SSR markers, the final integrated map contained both SNP and SSR markers.

Spring wheat panel genotyped with Illumina 90K SNP Chip and Affymetrix 35K SNP Chip. The 123 spring wheat lines genotyped with the Illumina 90K SNP Chip were a subset of the lines in a larger collection of spring wheat (299 lines) also genotyped with the Affymetrix 35K SNP Chip. From the Illumina 90K genotyping, 21 568 SNP markers were positioned in the map, covering all chromosomes. From the Affymetrix 35K SNP Chip 14 090 SNP markers were positioned on the map, covering all chromosomes. The Affymetrix 35K SNP Chip genotyping results displayed a much higher frequency (frequency of mapped markers: 0.4) of polymorphic and mapped markers than the Illumina 90K SNP Chip genotyping (frequency of mapped markers: 0.26). The Affymetrix 35K SNP Chip is a collection of SNP markers selected from a large 820 000 SNP array (Allen et al., 2017). This suggests that the prior evaluation and validation of markers before the development of the Affymetrix 35K SNP Chip made it a user-friendly SNP Chip, with a higher proportion of polymorphic markers.

2.3.2 Paper I: Mapping and validation of powdery mildew resistance loci from spring wheat cv. Naxos with SNP markers

The QTL mapping with linkage maps containing both SSR, DArT and SNP markers revealed 12 QTL for PM resistance in the SHA3/CBRD x Naxos population. Eight of these with Naxos as the source for the resistant allele (Windju et al., 2017).

A major PM QTL, with contribution from Naxos, had been detected by Lu et al. (2012) on 1AS in SHA3/CBRD x Naxos. This study had been performed with 564 SSR and DArT markers. With the refined linkage maps with 3512 SNP markers, and 224 SSR and DArT markers, this SHA3/CBRD x Naxos 1AS QTL was validated and more precisely mapped. The QTL could explain up to 14.4 % of the phenotypic variance. This 1AS QTL was also validated

further in the Soru#1xNaxos population based on flanking markers in both these RIL populations (Fig. 16).

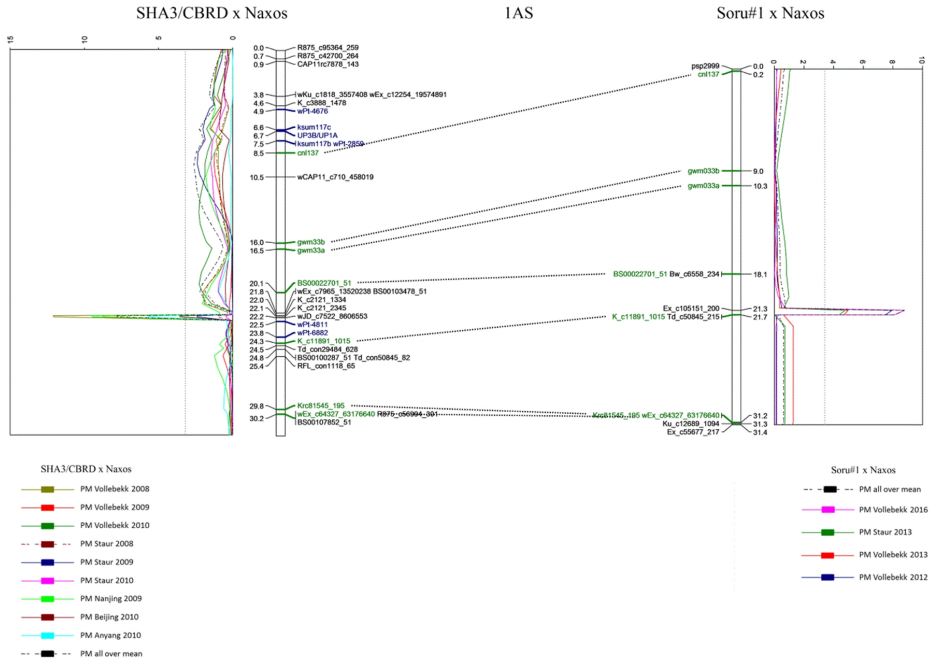


Figure 15. Segment of chromosome 1AS with major QTL with resistance from Naxos in the two RIL populations SHA3/CBRD x Naxos and Soru#1 x Naxos. The corresponding LOD curve was obtained from MQM. Genetic distances are shown in centimorgans (cM) to the left of the chromosome in SHA3/CBRD x Naxos and right of the chromosome in Soru#1 x Naxos. A threshold of 3.2 is indicated as a dashed line in the LOD graph of SHA3/CBRD x Naxos and a threshold of 3.4 is indicated as a dashed line in the LOD graph of Soru#1 x Naxos. Common markers between the two populations are marked in green, and markers also mapped in the QTL area by Lu et al., (2012) marked in blue. Dotted lines show the position of the common markers between the two populations.

A PM QTL, from Naxos, detected by Lu et al. (2012) in SHA3/CBRD x Naxos on chromosome 2BL was also detected in our QTL mapping on SHA3/CBRD x Naxos, and validated based on markers flanking the QTL. In our mapping study, this QTL could explain up to 13.4% of the phenotypic variation.

A PM QTL, with contribution from Naxos, detected on 2AL in SHA3/CBRD x Naxos, could be verified in the Soru#1xNaxos QTL mapping by flanking markers in the two populations

(Fig. 17). In our study, this QTL could explain up to 4.7% of the phenotypic variance in SHA3/CBRD x Naxos and 9.7 % of the phenotypic variance in Soru#1 x Naxos.

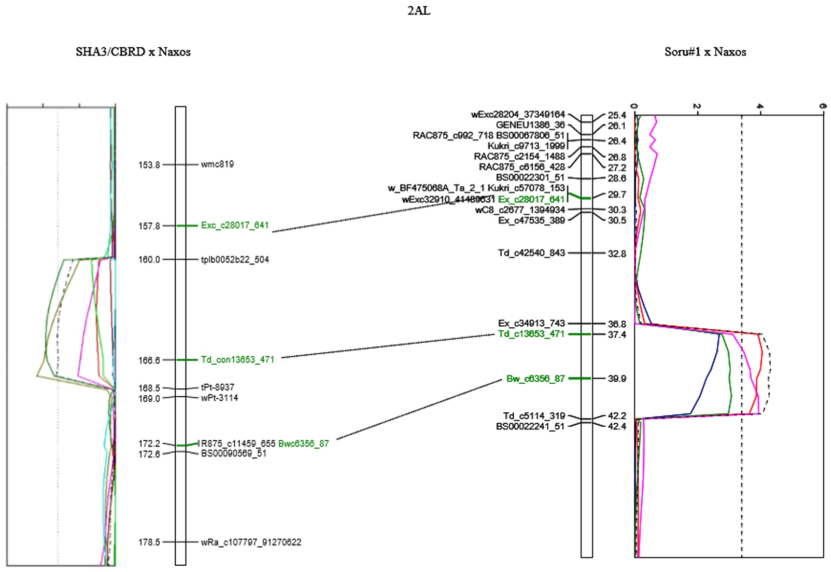


Figure 16. Segment of chromosome 2AL with major QTL with resistance from Naxos in the two RIL populations SHA3/CBRD x Naxos and Soru#1 x Naxos. The corresponding LOD curve was obtained from MQM. Genetic distances are shown in centimorgans (cM) to the left of the chromosome. A threshold of 3.2 is indicated as a dashed line in the LOD graph of SHA3/CBRD x Naxos and a threshold of 3.4 is indicated as a dashed line in the LOD graph of Soru#1 x Naxos. Common markers between the two populations are marked in green, dotted lines show the position of the common markers between the two populations.

2.3.3 Paper II: Mapping of SnTox3-*Snn3* as a major determinant of field susceptibility to *Septoria nodorum* leaf blotch in the SHA3/CBRD x Naxos population

The developed integrated linkage map of SHA3/CBRD x Naxos with previously mapped SSR markers (Lu et al., 2014) and SNP markers from the Illumina 90K array (Wang et al., 2014) gave a better mapping resolution for mapping SNB sensitivity loci (Fig. 18) (Ruud et al., 2017).

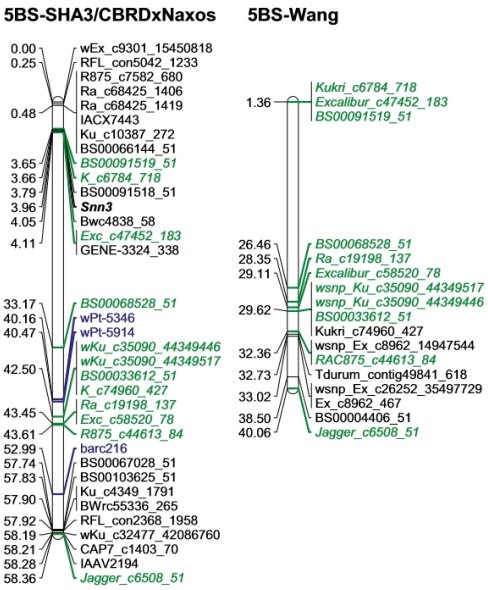


Figure 17. Mapping of the Snn3 locus on chromosome 5BS in SHA3/CBRD × Naxos based on segregation of SnTox3sensitivity. Right region of 5BS in the Wang et al. (2014) consensus map covered by polymorphic SNPs in SHA3/ CBRD × Naxos. Common markers are indicated in italics.

For SHA3/CBRD x Naxos seedlings inoculated with the SnTox3-positive, SnTox1 negative isolate 201593, the sensitivity QTL *Snn3* mapped to 5BS could explain up to 51.8% of the phenotypic variation. The 5BS QTL was also highly significant with inoculation of the two isolates Sn4 and NOR4 (SnTox1- positive, SnTox3-supressed).

With the improved map resolution and reanalysis of the data, 11 significant QTL were detected for APR. The most significant APR QTL, explaining most of the phenotypic variation in any environment, was the QTL on 5BS at the *Snn3* locus (Fig. 19). This host-sensitivity gene could explain up to 24% of the phenotypic variance.

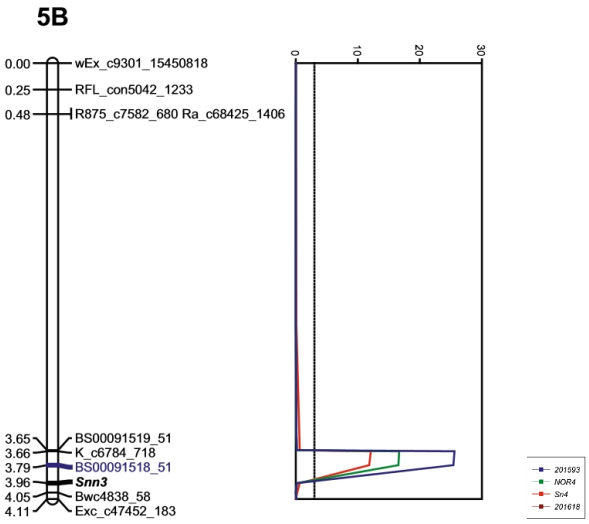


Figure 18. QTL on 5B after inoculation with NOR4, Sn4 and 201593. Genetic distances are shown in centimorgans to the left of the chromosomes. A threshold of 3.0 is indicated by a dashed vertical line in the LOD graphs. The maps are drawn in Mapchart v.2.2.

The SNPs co-segregating with *Snn3* could be matched to genes on scaffold TGACv1_scaffold_423631_5BS. Some of these genes have the characteristics of R-genes, like coiled-coil, leucine-rich-repeats and nucleotide binding sites domains.

Previous mapping studies performed for leaf blotch by Lu et al. (2014), did not detect the 5BS QTL at the *Snn3* locus. The previous SSR and DArT linkage maps did not cover this part of the 5BS chromosome. The saturated map of SHA3/CBRD x Naxos with SNP markers spanned more of the genome and could detect areas of chromosome 5B that were previously uncovered by markers.

2.3.4 Paper III: Identification of consistent loci for *Fusarium* head blight resistance in Northern European spring wheat through genome-wide association mapping

Structure analysis of the 299 spring wheat lines in the collection revealed the most sensible subdivision of the collection to be three subpopulations. This divided the lines into one subpopulation with Norwegian and Nordic lines (104 lines), the second subpopulation to consisting of Nordic and European lines (133 lines) and the third subpopulation to consisting of mostly exotic non-European lines (62 lines) (Table 5)

Table 5. Subdivision of the 299 spring wheat lines in the association mapping panel.

K=3					
Origin	**Nr.**	**Origin**	**Nr.**	**Origin**	**Nr.**
Subpop. 1		**Subpop. 2**		**Subpop. 3**	
Finland	2	CIMMYT	1	Australia	5
Norway	100	Finland	2	Brazil	1
Sweden	2	Germany	5	Canada	1
		Norway	82	China	7
		Poland	2	CIMMYT	36
		Slovakia	1	Czech. Republic	1
		Sweden	38	Finland	2
		Switzerland	1	Norway	4
		UK	1	South Africa	1
				UK	1
				USA	3

Association mapping was performed on two subsets of the wheat panel; on the entire set of lines in the collection, and in a subset consisting of the lines in sub-population 1 and sub-population 2 from Table 5 (The CIMMYT line from subpop.2 was also excluded). The subpopulation consisting of subpopulation 1 and subpopulation 2 was named European sub-population.

The association mapping was performed for FHB corrected for PH and DH and transformed DON corrected for PH and DH. This was done to avoid any confounding effects of PH and DH on FHB and DON. Further results and discussion of FHB and transformed DON corrected for DH and PH will be named FHB and DON. Analysing the results from the association mapping, QTL detected below the defined critical p-value (0.1 percentile) for each trait in three or more environments were considered significant and consistent. Association mapping was also performed for AE in the wheat collection.

The GWAS results revealed eight QTL mapped to seven chromosomes, that were significant in more than three environments for both DON and FHB (Table 6). Of these eight QTL, seven coincided with QTL for AE (Table 6).

Table 6. QTL significant in three or more environments for both FHB corrected for PH and DH and tDON corrected for PH and DH.

Chr.	cM	Significant traits total population			Significant traits European sub-population		
		FHB	DON	AE	FHB	DON	AE
1A	49-59	2013, 2014, 2015Vb, 2015St	Mean, 2013, 2014, 2015Vb, 2015St	-	2015Vb	2015Vb, 2015St	Mean
1A	74-77	Mean, 2013, 2014, 2015St	Mean, 2013, 2014, 2015Vb	Mean	Mean, 2013, 2014, 2015Vb, 2015St	Mean, 2014Vb	Mean
2B	102-105	Mean, 2013, 2014, 2015Vb, 2015St	Mean, 2013, 2015Vb, 2015St	Mean	Mean, 2013, 2014, 2015Vb, 2015St	Mean, 2013, 2014, 2015Vb, 2015St	Mean
3B	85-90	Mean, 2013, 2015Vb, 2015St	Mean, 2013, 2014, 2015Vb, 2015St	Mean	Mean, 2013, 2015Vb, 2015St	Mean, 2013, 2014, 2015Vb, 2015St	Mean
4A	73-78	Mean,2013, 2014, 2015St	Mean, 2013, 2014, 2015St	-	2013, 2014,2015St	Mean	-
5A	69-82	Mean, 2013,2014,2015Vb, 2015St	Mean, 2013,2014,2015Vb, 2015St	Mean	2013, 2014, 2015Vb	Mean, 2013, 2014	-
7A	25-38	Mean, 2013,2014,2015Vb, 2015St	Mean, 2013,2014,2015Vb, 2015St	Mean	Mean, 2013, 2014, 2015Vb, 2015St	Mean, 2013, 2014, 2015Vb, 2015St	-
7B	27-33	2014Vb, 2015St	2013,2014,2015Vb, 2015St	Mean	2014, 2015Vb, 2015St	Mean, 2013, 2014, 2015Vb, 2015St	Mean

For FHB and DON, the number of resistance alleles in the different lines in the population had an effect for the resistance. An elevated resistance was observed in the lines containing six of the resistance alleles compared to the lines containing one resistance allele (Fig. 20, left figure). The same effect was observed for DON. A markedly enhanced resistance was observed in the lines displaying six resistance alleles for DON compared to the lines containing zero or one of the resistance alleles (Fig. 20, right figure). More resistance alleles present in the lines gave in general better resistance. A plateau seemed to be reached at the accumulation of six resistance alleles for both FHB and DON (Fig. 20) in the material tested.

Different lines and varieties in the mapping panel, together with resistance alleles and frequencies are listed in Supplementary material of **Paper III**.

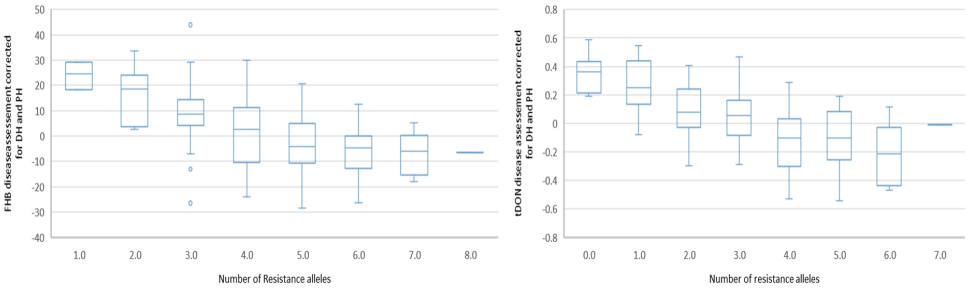


Figure 19. Effect of number of resistance alleles for; left figure: FHB corrected for DH and PH and right figure: transformed DON corrected for DH and PH.

The frequency of the resistance alleles of the eight consistently detected QTL were different in the population. The allele frequency of the 1AS resistance allele was the highest for both the FHB allele and DON allele, with a frequency of 0.9. The 7BS allele frequency was the lowest in the population, with a frequency of 0.11 for both FHB and DON (Allele frequency table in Appendix). This overview of which line in the wheat panel contains which resistance allele detected can be valuable when searching for new crossing parents.

2.4 Discussion

2.4.1 The RIL populations SHA3/CBRD x Naxos and Soru#1 x Naxos

Paper I and **II** made use of RIL populations to detect QTL for the trait of interest. The RIL populations SHA3/CBRD x Naxos utilised in the QTL mapping for PM and SNB showed segregation for both SNB and PM. For the RIL population SHA3/CBRD x Naxos, SHA3/CBRD is moderately susceptible to PM and highly resistant to SNB, while Naxos show high levels of APR to PM and is susceptible to SNB. The second RIL population utilised in **Paper I**, also show transgressive segregation to PM since Soru#1 is a line highly susceptible to PM. The development of RIL populations to generation F_6 takes time, but if the founder parents used for the RIL population differ for several traits they are valuable populations that can be utilised for several mapping studies as shown in this PhD thesis.

2.4.2 Linkage maps

SHA3/CBRD x Naxos and Soru#1 x Naxos Illumina 90K SNP Chip Linkage maps

Detection of QTL in both QTL mapping studies and GWAS, relies on the map resolution. In both **Paper I** and **II**, the higher resolution obtained due to more saturated markers maps after the Illumina 90K SNP Chip genotyping facilitated the detection of new QTL for the traits investigated. In **Paper II**, when also including the smaller linkage groups being deleted in the initial map-development in JoinMap v. 4.0, the 5BS *Snn3* locus was detected. These small linkage groups might not be in linkage with other parts of the map, and be difficult to map, but still they can segregate for QTL and hence be valuable in mapping studies.

The development of the linkage maps after Illumina 90K SNP Chip genotyping of the two RIL populations SHA3/CBRD x Naxos and Soru#1 x Naxos was performed in JoinMap v. 4.0. This was done after sorting the polymorphic SNP markers in linkage groups in MST map and assigning the linkage groups to chromosomes. The number of markers and linkage groups loaded into JoinMap v. 4.0 for map construction were 9230 SNP markers and 566 SSR and DArT markers sorted in 45 linkage groups for SHA3/CBRD x Naxos and 6387 SNP and 36 SSR markers divided into 83 linkage groups for Soru#1 x Naxos. When developing the linkage maps in JoinMap v. 4.0, the addition of SSR markers and the many minor linkage groups made the development of these maps challenging. In addition, to try to preserve as many SSR and DArT markers as possible the function “fixed order” was utilised in JoinMap v. 4.0. This function allows for a predefined “backbone” map to be constant and set the base for the rest of the maps. With these different considerations, many SNP markers and minor linkage groups were removed from the final map. It is possible that there are also interesting QTL within these

linkage groups not mapped in the final map, and that there could be QTL we have missed because of this.

Soru#1 x Naxos Affymetrix 35K SNP Chip linkage map

When developing the Soru#1 x Naxos linkage map after genotyping with the Affymetrix 35K SNP Chip a total of 3428 SNP markers were mapped in 75 linkage groups. This final linkage map was 6085 cM. In the final map of Soru#1 x Naxos after Affymetrix 35K SNP Chip genotyping, there are many linkage maps, some smaller than others. In the development of these maps it was difficult to join several of the linkage groups and the decision was made to try to keep as many as possible of them. This would possible lead to less extent of suspect linkages in JoinMap v. 4.0 during the construction of the map, and perhaps we were able to retain more markers in the map by doing this. The downstream applications with the Soru#1xNaxos map developed after the Affymetrix 35K SNP Chip genotyping would need more attention perhaps, since one must take into consideration the many map-fractions. But this map could also reveal interesting QTL in some of the minor linkage groups. With the experience of the detection of *Snn3* sensitivity locus in **Paper II**, where the *Snn3* locus on 5B was detected in a minor linkage group initially removed from the map, the preservation of minor linkage groups can be highly useful.

2.4.3 GWAS FHB and DON

In **Paper III,** the results from the association mapping revealed many QTL for both FHB and DON in the different environments tested. For a trait that is highly influenced by the environment, the interesting QTL will be the ones that are consistently significant in several environments. The detection of the eight QTL consistently significant more than three environments and the identification of the different lines and their combination of QTL could make a base for choosing crossing parents and screening of offspring. In the spring wheat genotyping panel genotyped with the Affymetrix 35K SNP Chip, 14095 SNP markers were mapped in the map of this collection based on consensus map. Many of these markers mapped to the same position in the map. And they were all utilised in the association mapping. In the association mapping results, where we detected the eight consistent QTL, there were hardly ever the same SNP markers turning up as the most significant for DON and FHB, only their position in the map was consistent. This makes the application of the SNP in MAS more challenging. A careful evaluation and validation must be done before using these SNP markers in resistance- breeding.

Type 2 error in GWAS

Several well-known FHB and DON disease resistance lines were included in the association mapping panel. Sumai 3, which inhabits the *Fhb1* gene is one of the lines in the panel. The *Fhb1* is cloned and mapped to 3BS (Rawat et al., 2016). Of the QTL detected as significant and consistent in three or more environments, none seemed to be mapped to 3BS. We detected one QTL on 3B, but probably not in the 3BS area. One disadvantage of GWAS is that the rare alleles in the collection tested will not be significant and not be detected in the association mapping. The *Fhb1* is not widely used in the Nordic breeding material and is not present in more than a few lines in the association mapping panel (personal communication with Graminor wheat breeder John Arne Dieseth). This might be one of the reasons for the lack of a significant and consistent QTL on 3BS in the study. One can suspect that there are other resistance alleles we were unable to detect in the FHB and DON association mapping due to the resistance allele being too rare. To be able to capture more of the minor QTL effects, genomic selection (GS) could serve as an option for breeding for more FHB and DON resistant varieties.

2.4.4 Confounding effects

In **Paper III**, the raw phenotypic data were regressed with data for DH and PH as factors for correction. FHB and DON were slightly positively correlated to DH, the correlation was not significant for FHB. Other studies have pointed to a negative correlation between these two traits (Emrich et al., 2008). PH was negatively correlated to both DON and FHB in our data, supporting several other studies (Kubo et al., 2013; Lu et al., 2013; Mao et al., 2010). In **Paper II**, the field data used were obtained from a previous study by Lu et al. (2014). SNB field assessment data used DM, DH and PH as covariates in regression to avoid these confounding effects in the QTL mapping study. SNB had shown to be significantly negative correlation to all three traits in this study.

When the aim of the studies in **Paper II** and **III** was to detect QTL for resistance to SNB, FHB and DON it was important to correct for PH and DH. We need DH to be as low as possible in Norway due to the short growing period and we don't want the taller plant because of the risk of lodging. The regression with these traits as cofactors eliminate their effect in the mapping study, making sure the QTL detected are not QTL for more days to heading or taller plants. In **Paper III**, a third trait correlated to DON and FHB, AE, was not used a factor for correction in the regression. In the GWAS several FHB and DON QTL detected in the study coincided with QTL for AE. In the field data used in **Paper II**, the data are also regressed with DM as covariate. DM is a trait, like DH, that need to be low in Norway due to the short field season. In **Paper I**,

the PM field assessment data were used without regression. Correlation between PM and PH and DH were only slightly negative and not significant. These traits will most likely not contribute to detecting a "false" PM QTL in the mapping.

Of the eight QTL for both FHB and DON detected in **Paper III**, seven coincided with QTL detected for AE. The correlation between AE and DON was -0.441 and between AE and FHB -0.519 in the study. High anther extrusion has been proposed to be a valuable escape trait to avoid FHB infection. Many studies have reported the correlation between AE and FHB and DON, and detected QTL coinciding for AE and FHB (Buerstmayr et al., 2015; He, Lillemo, et al., 2016; He, Singh, et al., 2016; Kubo et al., 2013; Lu et al., 2013; Skinnes et al., 2010). Even though high AE has been proposed to be valuable for disease escape, it has been shown that also lines with high AE get FHB and DON infection (Lu et al., 2013; Skinnes et al., 2010). Since AE and FHB and DON were negatively correlated, when we choose a line with high AE we indirectly choose a line with low FHB and DON. The trait AE was therefore not corrected for in a regression before performing the GWAS in **Paper III**, because QTL for AE can aid the development of more FHB and DON resistant varieties.

2.4.5 Biparental QTL mapping and GWAS in wheat collections

Biparental QTL mapping and GWAS based on collections of lines and varieties are the two mapping strategies utilised in this PhD project.

In the QTL mapping studies, the aim was to detect and validate QTL for resistance and sensitivity we suspected to be present in the lines, and that we needed to more precisely map and elucidate. Both in Paper **I** and **II**, QTL mapping could detect the QTL we were searching for. The development of the linkage maps was very laborious work, but we could develop maps where most markers had a unique position in the map. When the QTL mapping detected a consistent QTL, the same marker was present as significant in all environments. This makes downstream application of interesting QTL easier; it could be possible to test markers associated with the QTL in MAS. This was not the case for the GWAS in **Paper III**; After genotyping of the spring wheat core collection with the Affymetrix 35K SNP Chip, the way forward for developing a map to use in GWAS, was to utilise published consensus maps. With this technique, and with the high number of markers, many markers were mapped to the same map position. Subsequently, in the GWAS performed on data from several environments and two traits, the consistent QTL did hardly ever have the same SNP markers as the most significant. The reason for this might possible be because the different lines in the spring wheat collection had not been tested in every environment. A future MAS in resistance breeding

would possible be more challenging, because much attention need to be given to selection and validation of markers.

In the QTL mapping studies (**Paper I** and **II**) we did only reveal and map the QTL present in one population developed by two parents. In the GWAS (**Paper III**) we detected several QTL in a wide collection of lines and varieties. The two techniques QTL mapping and GWAS are both highly useful, each for its own purpose. Now the wheat breeder at Graminor may choose the best crossing parents in the spring wheat collection, used in **Paper III,** for development of more FHB and DON resistant varieties. For PM and SNB, we now have several QTL with associated markers interesting for breeding and further study.

2.5 Further work

2.5.1 Fine mapping of PM QTL on 1AS

A RIL population developed from the cross Avocet x Naxos was genotyped with forty-three markers closely linked to the QTL for PM resistance detected in Soru#1 x Naxos. A PM QTL mapping performed on Avocet x Naxos using these 43 SNP markers displayed a QTL on 1AS.

In the Avocet x Naxos RIL population, one F_6 family (AxN-39) was detected that segregated at the 1AS PM QTL. Near-isogenic lines (NIL) segregating only in the 1AS QTL area have been produced from this AxN-39 family. In Vollebekk research farm field testing of this NIL population in 2016, the resistance allele at the 1AS QTL showed more than 50% reduction in powdery mildew severity.

To further narrow down the QTL area, the plants in the genotyped AxN-39 NIL population that showed different recombination events in the 1AS QTL area were selfed. These different NILs represent different breakpoints within the 1AS QTL area. When sowing, and performing a PM disease assessment in the 2017 field season, the QTL was further narrowed down and we are one step closer of being able to elucidate the gene within this resistance and perhaps understand its mechanism.

2.5.2 Association mapping panel

The structure analysis and the map of the 299 spring wheat lines in the association mapping panel will be utilised as a framework in several studies. There are other phenotypic data from several environments on PM, SNB and yellow rust that will be the base for association analysis.

2.5.3 Soru#1 x Naxos integrated maps

A yellow rust QTL mapping study has been performed at CIMMYT in Mexico on the Soru#1 x Naxos mapping population. The developed integrated maps of Soru#1 x Naxos with both Illumina 90K SNP Chip (Wang et al., 2014) and Affymetrix 35K SNP Chip (Allen et al., 2017) have been utilised in this study. A manuscript is in progress from this work.

2.5.4 Wheat project: "Expanding the technology base for Norwegian wheat breeding: genomic tools for breeding of high quality bread wheat (EXPAND).

The currently funded wheat project "Expanding the technology base for Norwegian wheat breeding: genomic tools for breeding of high quality bread wheat (EXPAND)" started in 2016. This project focuses on pre-harvest sprouting (PHS). PHS is one of the most important quality-degrading factors of wheat in Norway. The aim of this project is to develop and implement selection methodologies to improve PHS resistance. Seed dormancy has been evaluated in the

spring wheat panel. With the use of the structure results and map of this panel, an association mapping will be performed to possibly detect QTL for seed dormancy PHS resistance in the wheat collection.

Also as a part of this projects, the linkage maps developed of SHA3/CBRD x Naxos and Soru#1 x Naxos will be utilised to map QTL for seed dormancy and PHS resistance.

2.6 Wheat breeding

In plant breeding, crossing of lines and selection of progenies to develop varieties is based on information of lines tested. Phenotypic, disease and quality assessments are evaluated and used as selection criteria. With the use of molecular markers in breeding it is possible to obtain more precise information of the genetics of the lines, and both selection of crossing parents and selection of progenies can be more precise and effective.

The PM QTL mapping study validated a QTL on 1AS, and narrowed it down. It would be possible to use Naxos as a crossing parent and select offspring by MAS to incorporate this 1AS QTL into the breeding germplasm to obtain better PM resistance.

For SNB, it would be interesting to validate the markers detected as significant in the SHA3/CBRD x Naxos population associated to the *Snn3* locus. And possibly to further test the effect of the elimination of this *Snn3* sensitivity locus in diverse germplasm.

The FHB and DON association mapping study revealed eight QTL for resistance and which of the lines in the panel contained which resistance allele. This information can serve as a reference when selecting future crossing parents to obtain better FHB and DON resistant varieties, and MAS could aid the selection of lines with beneficial QTL combinations. Selecting lines with high AE would also indirectly lead to selection of lines with better FHB and DON resistance, these traits are correlated and several of their QTL coincide.

2.7 Conclusions

- **Paper I**: Genotyping of the two RIL populations SHA3/CBRD x Naxos and Soru#1xNaxos with the Illumina 90K SNP Chip (Wang et al., 2014) and the development of high-density linkage maps with incorporated SSR, DArT and gene-specific markers made it possible to detect QTL for PM and to validate and more precisely map the 1AS PM QTL previously detected by Lu et al. (2012)
- A Near-Isogenic-Line population (AxN-39) has been developed that show heterozygous genotyping results in the PM 1AS QTL area detected in **Paper I**.
- **Paper II**: The re-mapping of QTL for field-resistance with linkage maps developed after genotyping with the Illumina 90K SNP Chip in the RIL population SHA3/CBRD x Naxos, detected a SnTox3-*Snn3* interaction on 5B explaining 24% of the phenotypic variation in the field and 51% of the variation in seedling inoculation.
- **Paper III:** The association mapping of the spring wheat panel of 299 lines revealed eight QTL on seven chromosomes that were significant in more than three environments and for both FHB and DON. An enhanced resistance to both FHB and DON was achieved when stacking several of the resistance alleles detected in the study.
- **Paper III**: Of the eight QTL detected in the association mapping, seven coincided with QTL for AE.
- The necessity to develop wheat varieties with improved disease resistance to suit the Norwegian (and Nordic) climate is the business initiative for Graminor AS to collaborate in this project. This project presented great potential to generate significant amount of novel and applicable results. The research in this project has contributed to a better understanding and knowledge on wheat diseases and the use of molecular markers in wheat breeding program. The success in this project was mainly related to the identification of closely linked markers to genes affecting the disease resistances in wheat. The molecular markers identified in this study will be validated and included in the pipe-line for wheat marker-assisted selection. The project has given information about markers and genotypes that can aid in the development of more disease resistant varieties at Graminor breeding. The outcome of this project has the potential to improve selection efficiency, giving Graminor an advantage in the market over competitors by providing wheat varieties with better disease resistance in a timely fashion. In addition, these results will supplement the ongoing biotechnological development at Graminor and they will impact future breeding strategies in Norway.

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Paper I

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Mapping and validation of powdery mildew resistance loci from spring wheat cv. Naxos with SNP markers

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Abstract Powdery mildew, caused by *Blumeria graminis* f.sp. *tritici*, is a major wheat disease in maritime and temperate climates. Breeding for race-non-specific or partial resistance is a cost-effective and environmentally friendly disease control strategy. The German spring wheat cultivar Naxos has proven to be a good source for partial resistance to powdery mildew. The objectives of the present study were to map the resistance loci in Naxos with use of high-density SNP markers in the Shanghai3/Catbird x Naxos inbred line population and validate the results in a different genetic background; Soru#1 x Naxos. Both populations were genotyped with the Illumina iSelect 90K wheat chip, and integrated linkage maps developed by inclusion of previously genotyped SSR and DArT markers. With the new linkage maps, we detected a total of 12 QTL for powdery mildew resistance in Shanghai3/Catbird x Naxos, of which eight were derived from Naxos. Previously reported QTL on chromosome arms 1AS and 2BL were more precisely mapped and the SNP markers enabled discovery of new QTL on 1AL, 2AL, 5AS and 5AL. In the Soru#1 x Naxos population, four QTL for powdery mildew resistance were detected, of which three had resistance from Naxos. This mapping verified the 1AS and 2AL QTL detected in Shanghai3/Catbird x Naxos, and identified a new QTL from Naxos on 2BL. In conclusion, the improved linkage maps with SNP markers enabled discovery of new resistance QTL and more precise mapping of previously known QTL. Moreover, the results were validated in an independent genetic background.

Keywords Wheat · Illumina 90K SNP chip · Powdery mildew · QTL mapping

Key message:
QTL for powdery mildew resistance were discovered and validated in two wheat recombinant inbred line populations utilizing maps with SNP, SSR and DArT markers.

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Introduction

Powdery mildew (PM), caused by the biotrophic fungal pathogen *Blumeria graminis* f. sp. *tritici (Bgt)*, is one of the devastating diseases of wheat in areas with maritime and temperate climates (Bennett 1984). It can cause significant yield losses ranging from 13 to 34%, but if the disease attacks are severe to the flag leaf during the heading and grain filling stage, losses can reach 50% (Alam et al. 2013; Griffey et al. 1993). Powdery mildew infection and disease development

have been favoured due to widespread use of irrigation, semi-dwarf cultivars and nitrogen fertilizers (Bennett 1984; Selter et al. 2014). Chemicals are extensively used to control the disease and maintain high yields when susceptible cultivars are grown. Breeding of resistant cultivars is a more economical and environmentally safe disease control strategy (Petersen et al. 2014; Worthington et al. 2014).

Two main types of resistance to powdery mildew are generally recognized: race-specific and race-non-specific. Race-specific resistance is qualitative, and also called vertical, or seedling resistance and mediated by single major race-specific (*Pm*) genes of relatively large effects (Bennett 1984). This type of resistance works through recognition of the pathogen in a gene-for-gene relationship (Flor 1955). Race-specific resistance gives complete protection to specific races of pathogens and are, usually, effective only against some isolates of powdery mildew, but ineffective to others (McDonald and Linde 2002). Due to the high vulnerability to genetic changes in the pathogen, new virulent races can quickly evolve to overcome single race-specific resistance genes, resulting in short durability of resistance (Hsam et al. 2002; McDonald and Linde 2002).

Race-non-specific resistance is quantitative or partial (Hautea et al. 1987) and controlled by several genes with major or minor effects. This type of resistance is also known as 'adult-plant resistance' (APR) (Gustafson and Shaner 1982) or 'slow mildewing' (Roberts and Caldwell 1970) as several resistance genes work together to reduce the infection efficiency and retard growth and reproduction of the pathogen, especially in adult plants (Shaner 1973). Breeding of wheat cultivars with partial resistance to powdery mildew has been suggested as a more promising and sustainable strategy to control this disease. It may be difficult to identify and select for partial resistance in the field, especially in the presence of race-specific resistance genes that mask the effect of race-non-specific resistance during field selection (Keller et al. 1999; Lillemo et al. 2010). In such situations, molecular markers offer the opportunity to select directly for the presence of genes for partial resistance, once they have been mapped and validated.

Since the first dominant resistance gene *Pm1* was described in 1953 (Pugsley and Carter 1953), more than 77 powdery mildew resistance genes or alleles at 49 loci (*Pm1-Pm54)* have been catalogued and assigned to specific chromosomes and chromosome arms in common wheat (Hao et al. 2015; Ma et al. 2015). Lillemo et al. (2008) described two race-non-specific genes, *Pm38 and Pm39*, which exhibit strong partial resistance to powdery mildew, and are pleiotropic to the rust resistance genes *Lr34/Yr18 and Lr46/Yr29*, respectively. Furthermore, the race-non-specific powdery mildew resistance gene *Pm46* was found to be pleiotropic to the rust resistance locus *Lr67/Yr46* (Herrera-Foessel et al. 2014; Moore et al. 2015).

During the past two decades, different types of molecular markers have been used to localize powdery mildew resistance genes in the wheat genome (Li et al. 2014). More recently, single nucleotide polymorphisms (SNPs) have gained preference in genetic mapping studies. These markers are abundant, co-dominant and evenly distributed across the genome. SNPs can be generated in a high-throughput and cost-effective manner, which makes them an ideal marker system (Colasuonno et al. 2014; Gupta et al. 2008). In recent years, high-density 9K SNP chip and 90K SNP chip platforms have been developed for wheat (Cavanagh et al. 2013; Wang et al. 2014). These high-density genotyping arrays further enhance the development of SNP marker resources for wheat breeding and improve the construction of high-resolution genetic maps for understanding complex traits.

The spring wheat cultivar Naxos shows highly effective partial resistance to powdery mildew in the field while being susceptible at the seedling stage. With its adult plant resistance, this cultivar is therefore a valuable source for partial and more durable resistance against powdery mildew (Lillemo et al. 2010; Lu et al. 2012).

A QTL mapping study using SSR and DArT markers in the mapping population Shanghai3/Catbird x Naxos performed by Lu et al. (2012) revealed several important powdery mildew resistant QTL from Naxos. A highly significant QTL originating from Naxos was detected on chromosome 1AS, in the same area as the *Pm3* locus. Naxos is, however, known to not carry any race-specific gene for resistance to powdery mildew. Moreover, it lacks the *Pm3* gene based on the UP3B/UP1A and Pm3MF/Pm3ER1 markers (Lu et al. 2012; Tommasini et al. 2006). The QTL with resistance from Naxos revealed on 1AS is therefore likely due to a gene for race-non-specific resistance. This QTL was reported to explain up to 35% of the phenotypic variance in some environments in the study. Another major QTL from Naxos was detected on 2DL and two minor QTL on

2BL and 7DS. In addition, QTL with resistance from Shanghai3/Catbird were detected on chromosome arms 1RS, 2DLc, 6BL and 7AL by Lu et al. (2012).

The main objectives of the present study were to detect new QTL for powdery mildew resistance derived from the resistance source Naxos, and to confirm and more precisely map powdery mildew resistance QTL previously detected in the study performed by Lu et al. (2012). In addition, the study aimed at validating the detected QTL in another genetic background and to identify tightly linked SNPs for powdery mildew resistance breeding. By utilizing the Illumina iSelect 90K wheat SNP chip (Wang et al. 2014), marker maps with SNP markers were developed and incorporated with previously genotyped SSR, DArT and gene-specific markers, to achieve high-density marker maps for QTL—and associated SNP—detection.

Materials and methods

Plant material

Two populations of recombinant inbred lines (RIL) in generation F_6 developed by single seed descent were utilized in the study: 177 lines from the cross Shanghai3/Catbird (SHA3/CBRD) x Naxos and 131 lines from the cross Soru#1 x Naxos. Naxos is a German spring wheat developed by Strube GmbH & Co.KG from the cross 'Tordo/St. Mir808-Bastion/Minaret'. Naxos exhibits high levels of partial resistance to powdery mildew at the adult plant stage (Lu et al. 2012). SHA3/CBRD is a breeding line developed at CIMMYT with the pedigree "Shanghai3//Chuanmai 18/Bagula" and selection history "-0SHG-6GH-0FGR-0FGR-0Y". SHA3/CBRD has been shown to be moderately susceptible to powdery mildew, carrying the *Pm8* resistance allele on 1RS and the *Pm3* haplotype on 1AS, but none of the *Pm3a-g* alleles (Lu et al. 2012; Tommasini et al. 2006). Soru#1 is a synthetic hexaploid derived wheat line (AABBDD). It was developed by CIMMYT from the cross 'SABUF/5/BCN/4/RABI//GS/CRA/3/*Ae. tauschii* (190)'. It is highly susceptible to powdery mildew.

Seedlings were grown in the greenhouse in Ås, Norway and genomic DNA was extracted from fresh young leaves of parents, F_6 RILs, and controls using the DNeasy plant DNA extraction kit (Qiagen).

Field evaluation

SHA3/CBRD x Naxos

For the SHA3/CBRD x Naxos population, powdery mildew disease resistance data from Lu et al. (2012) was utilized. The evaluation of powdery mildew resistance had been performed at two locations in Norway in 2008, 2009 and 2010; Vollebekk research farm at the Norwegian University of Life Sciences, Ås (59°N, 90 m above sea level) and Staur research farm close to Hamar (60°N, 153 m above sea level). Both locations experience natural epidemics of powdery mildew, but are characterized by different *Bgt* virulence compositions (Skinnes 2002). In addition, the population had been evaluated for powdery mildew resistance at three locations in China; In 2009 at Jiangsu Academy of Agricultural Sciences (JAAS), Nanjing (32°N, 15 m above sea level), Jiangsu province and in 2010 at the Chinese Academy of Agricultural sciences (CAAS), Beijing (39°N, 43.5 m above sea level), and at CAAS Cotton Research Institute, Anyang (36°N, 70–80 above sea level), Henan province.

Soru#1 x Naxos

For the Soru#1 x Naxos population, powdery mildew disease severity was evaluated at Vollebekk research farm in 2012, 2013 and 2016, and at Staur research farm in 2013.

Field trials were conducted in hill plots using an alpha lattice block design (12 plots per block) with two replications in each experiment. Lines were planted with 50 cm between plots and 40 cm between each row. The susceptible line Avocet-S was planted as spreader rows surrounding the nurseries, and the moderately resistant cv. Bastian was planted as a barrier next to the experimental plots.

Powdery mildew disease severity was assessed on the whole canopy basis as the percentage of leaf area infected, using a modified Cobb scale (0 to 100% infected leaf area) (Peterson et al. 1948). The disease severity was scored two to three times with the first score being made when the most susceptible lines had reached about 50–70% severity, and then repeated at weekly intervals until the powdery mildew ceased to develop on the most susceptible lines.

Statistical analysis

For the Soru#1 x Naxos population, phenotypic data of powdery mildew severity was analysed using the software MINITAB 17 (Minitab 2010). Average percentage of powdery mildew from different dates was calculated. Analysis of variance (ANOVA) was conducted using general linear model (GLM) to determine differences in mildew scores among the F_6 lines, and heritability of the phenotypic traits for single environments (h^2) was calculated in Agrobase Generation II from Agronomix Software Inc.

Linkage mapping

The Soru#1 x Naxos and SHA3/CBRD x Naxos populations were genotyped with the Illumina iSelect 90K wheat SNP chip (Wang et al. 2014). Analysis and scoring of the genotype results for each population was performed manually for every SNP marker with the software Genome Studio Genotyping Module v1.0 from Illumina.

In both populations, markers scored as polymorphic were used for constructing linkage groups and genetic linkage maps. The markers were sorted in linkage groups with MST map (Wu et al. 2008). The linkage groups were assigned to chromosomes based on the best BLASTn hit from a comparison of SNP-flanking sequences with the Chinese Spring chromosome survey sequences (http://wheat-urgi.versailles.inra.fr/Seq-Repository). Previously developed SSR and DArT marker data in the SHA3/CBRD x Naxos population (Lu et al. 2012) were added to the SNP marker data. Genotyped SSR marker data from the Soru#1 x Naxos population was added to the SNP marker data of this population.

For both populations, markers belonging to linkage groups assigned to the same chromosomes based on the BLASTn search were loaded into Join Map v. 4.0 (Van Ooijen 2006) and the linkage groups were refined using the maximum likelihood mapping algorithm. The genetic distances between markers were calculated by converting recombination fractions into map distances (cM) based on the Kosambi mapping function with minimum LOD score of 3.0 (Kosambi 1943). To develop maps with many shared markers between the two populations, shared markers between the two populations were found and the function "fixed order" was utilized in JoinMap. Some of these common markers were removed from the maps during the refining, but many remained.

QTL mapping

Interval mapping (IM) and multiple QTL mapping (MQM) were performed on both populations using the software MapQTL6 (van Ooijen 2011) to detect QTL. Interval mapping (IM) was conducted to detect possible major QTL for powdery mildew resistance. The LOD profiles from interval mapping were observed, and markers closely linked to each QTL that showed effects in several environments were used as cofactors in multiple QTL mapping (MQM). The LOD significance threshold level of powdery mildew was set to 3.2 for SHA3/CBRD x Naxos and 3.4 for Soru#1 x Naxos after a permutation test with 1000 permutations, and was used for declaration of a QTL. QTL reaching this level in one environment either in IM or MQM were also reported for other environments even though their LOD scores were under the threshold. QTL effects were estimated as the proportion of phenotypic variance (R^2) explained by each QTL. Genetic maps and LOD curves were drawn in the software MapChart, v.2.1 (Voorrips 2002).

Results

Phenotypic analysis

Powdery mildew severity histograms show continuous distributions with transgressive segregation in all testing environments. The 2012 season in Vollebekk experienced less favourable conditions for powdery mildew development than the 2013 and 2016 seasons. Maximum powdery mildew severity from Soru#1 x Naxos RILs in Vollebekk 2012 was approximately 60%, while it exceeded 75% in all other environments. Histograms of mean powdery mildew severity of the Soru#1 x Naxos RILs from Staur 2013 and Vollebekk in 2012, 2013 and 2016 are shown in Fig. S1.

Correlation and heritability

In Soru#1 x Naxos, the powdery mildew severity for the years 2012, 2013 and 2016 in both locations were all significantly ($p < 0.001$) correlated. Days to heading (DH) showed a weak negative correlation with PM in all testing environments, but the relationship was significant only for Vollebekk 2013. The PM heritability (h^2) calculated from the ANOVA table was high for all

testing environments with h^2 estimates 0.68 for Vollebekk 2012, 0.86 for Vollebekk 2013, 0.85 for Vollebekk 2016 and 0.84 for Staur 2013 (Table S1).

QTL analysis: SHA3/CBRD x Naxos

Of the 81,587 SNP chip from Illumina, 9230 SNP markers were scored as polymorphic in the SHA3/CBRD x Naxos population using the Genome Studio software. The SNP markers were sorted into 45 linkage groups and based on BLASTn searches the linkage groups were assigned to chromosomes. In addition, the 566 SSR, DArT and gene-specific markers genotyped by Lu et al. (2012) were added to the marker set. Out of the 9230 SNP dataset, 3512 SNPs were placed on the map after removing redundant markers, and of the 566 SSR and DArT marker dataset, 224 SSR, DArT and gene-specific markers were placed on the map. The map spanned 3130 cM, covering all 21 chromosomes.

In QTL IM analysis, QTL for powdery mildew resistance were detected on chromosomes and chromosome arms 1A, 1RS, 2A, 2B, 2D and 3D in several environments. SNP markers in close proximity to detected QTL were chosen as cofactors in the MQM analysis. After MQM analysis major QTL were detected on 1AS, 1AL, 2BL, 2DS and 2DL (Table 1). Minor QTL were detected on 1AL, 1RS, 2AL, 5AS and 7DS and two minor QTL on 5AL (Table 2). Of the QTL detected, the QTL on 1AS, 2AL, 2BL, 2DL, 5AS, 7DS and one of the two detected on 1AL and 5AL had resistance from Naxos, while the rest had resistance from SHA3/CBRD (Table 1).

QTL with resistance from Naxos

A major QTL mapped on 1AS with resistance from Naxos was consistent across all environments except Staur 2009 and 2010. The QTL was flanked by markers *wPt-4811* and *wsnp_JD-c7522_8606553*, and explained 0.5–14.4% of the phenotypic variation (Table 1, Fig. 1a).

On the long arm of chromosome 1A, a new major QTL with resistance from Naxos was detected. This QTL was consistent across all environments except Staur 2009, Nanjing 2009, Beijing 2010 and Anyang 2010. This QTL explained 0.6–10.3% of the phenotypic variation (Table 1). Several SNP markers mapped close to the QTL, the markers *Excalibur_c50394_199* and *wsnp_Ex_c22284_31478675* flanking one side of the QTL and the marker *cfa2129b* flanking the other side of the QTL (Fig. 1b).

A new QTL with resistance from Naxos was also detected on 2AL. This QTL had a LOD score above 3.2 in Staur 2008 and in Vollebekk 2008 and 2010. This QTL explained 0–4.7% of the phenotypic variation (Table 1). Only few markers mapped in this QTL region, but the SNP marker *Tdurum_contig13653_471* mapped in the QTL area, and the two DArT markers *tPt_8937* and *wPt_3114* mapped 1–1.5 cM away from the QTL peak (Fig. S2a; Fig. 3).

As in the study by Lu et al. (2012), a major QTL was discovered on chromosome 2BL. The QTL was consistent across all environments except Staur 2008, Vollebekk 2010 and Anyang 2010 in the present study. This QTL explained 2.9–13.4% of the phenotypic variation (Table 1). The QTL was difficult to map precisely, it consisted of several linked QTL with different LOD scores over the testing environments. In the overall mean data of the QTL, the SNP markers *Excalibur_rep_c67411_210*, *Excalibur_c69493_1208* and *Tdurum_contig42095_3235* mapped closest to the QTL peak with the highest LOD score.

Two new QTL with resistance from Naxos were mapped on chromosome 5A, one on the short arm of the chromosome and one on the long arm. The 5AS QTL had a LOD score above 3.2 in the overall mean data. This QTL explained 0.3–2.8% of the phenotypic variation (Table 1). The QTL had several SNP markers surrounding it, of which *Excalibur_c4964_275* and *BS00067096_51* mapped closest to the QTL (Fig. S2b). The QTL on 5AL had a LOD score above 3.2 at Staur in 2008. This QTL explained 0.0–4.8% of the phenotypic variation (Table 1). The two SNP markers *BS00021669_51* and *BS0002215_51* mapped closest to the peak of the QTL (Fig. S2c).

The two QTL previously detected by Lu et al. (2012) on 2DL and 7DS were also detected in the study. No additional markers were mapped in the area of the QTL, only SSR and DArT markers previously mapped by Lu et al. (2012). The 2DL QTL explained 3.6–17.3% of the phenotypic variation and had a LOD score above 3.2 in all testing environments except Vollebekk 2008, Nanjing 2009 and Anyang 2010 (Table 2). As in the study by Lu et al. (2012), the SSR markers *gwm320* and *mag3616* mapped in the QTL area (Fig. 1d). The 7DS QTL was significant only in Nanjing 2009, and it explained 9.7% of the phenotypic variation this year and testing environment (Table 1). The SSR marker *wmc438* mapped in the QTL area as in the study by Lu et al. (2012) (Fig. S2d).

Table 1 Results of QTL mapping in the RIL population SHA3/CBRD x Naxos. Results from MQM mapping, showing the percentage of explained phenotypic variance

		PM severity Vollebekk			PM severity Staur			PM severity China			Overall mean	
QTL	Markers in close proximity	PM08v	PM09v	PM10v	PM08s	PM09s	PM10s	PM09nj	PM10bj	PM10ay	PMallm	Source
IAS	wJD_c7522_8606553, K_c2121_2345	*13.7*	*11.7*	*4.1*	1.3	4.8	0.5	*14.4*	*6.7*	*12.5*	*8.1*	Naxos
IAL	Exc_50394_199, wEx_c22284_31478675,cfa2129b	*5.8*	*4.1*	*10.3*	*5.7*	3.4	*7.9*	1.4	4.8	0.6	*7.4*	Naxos
IAL	Bw_c6664_644, wJD_c6664_7807201	*5.2*	3.2	*5.5*	*7.1*	0.1	*5.6*	1.2	5.1	0.3	*5.2*	SHA3/CBRD
IRS	K_c24684_134, RFL_con5444_1284	2.0	*6.5*	*4.0*	1.1	1.2	2.2	1.6	1.5	1.4	2.8	SHA3/CBRD
2AL	Td_con13653_471	*4.3*	1.2	*4.7*	*4.6*	0	2.5	1.0	0.3	0.3	3.2	Naxos
2BL	R875_c66657_91	*6.5*	*7.9*	1.9	1.8	*10.3*	*7.4*	*7.2*	*7.6*	1.4	*4.7*	Naxos
2BL	Td_con42095_3235, Exc_c7446634	*6.2*	*10.9*	2.9	*5.7*	*12*	*13.4*	*7.0*	*9.6*	3.1	*8.6*	Naxos
2DS	IAAV8527, wBQ161779D_Ta_2_1	*10.5*	4.5	*7.9*	*8.3*	0.1	*12.6*	2.5	0.7	2.9	*8.9*	SHA3/CBRD
2DL	gwm 320, mag3616	5.9	*9.8*	*15.3*	*16.7*	*13.2*	*17.3*	6.4	*10.6*	3.6	*15.5*	Naxos
5AS	Exc_c4964_275, BS00067096_51	1.9	2.7	1.6	1.1	0.8	2.8	3.1	0.8	0.3	*2.7*	Naxos
5AL	BS00022215_51, BS00021669_51	0.9	0.5	1.7	*4.8*	1.9	2.8	0.8	0.4	0	2.2	Naxos
5AL	R875_c13931_205, IAAV8669	2.4	0	0.4	1.9	*16.3*	0.8	0.4	0.8	0	0.1	SHA3/CBRD
7DS	wmc438	6.0	7.4	2.7	3.8	6.8	3.5	*9.7*	3.9	2.2	7.0	Naxos

QTL with LOD score above 3.2 highlighted in italics

Table 2 Results of QTL mapping in the RIL population Soru#1 x Naxos. Results from MQM mapping, showing the percentage of explained phenotypic variance

		PM severity Vollebekk			PM severity Staur	Overall mean	
Chrom	Markers in close proximity	PMv12	PMv13	PMv16	PMs13	PMallm	Source
1AS	Ex_c105151_200, K_c11891_1015	*18.7*	*11.0*	*20.1*	*9.8*	*17.6*	Naxos
2AL	Bw_c6356_87, Td_c13653_471	6.1	*9.7*	*8.6*	6.8	*8.8*	Naxos
2BL	wExrc73919_71799491, wExc51661_55531646	1.9	*6.9*	3.9	*15.8*	*7.8*	Naxos
3AS	BS00022524_51, wExrc67635_66291944	*7.8*	6.5	5.9	5.9	*8.3*	Soru#1

QTL with LOD score above 3.2 highlighted in italics

QTL with resistance from SHA3/CBRD

QTL originating from SHA3/CBRD were detected on four chromosomes.

A QTL on chromosome 1AL with resistance from SHA3/CBRD had a LOD score above 3.2 in Norway, at Vollebekk and Staur in 2008 and 2010. This QTL explained from 0.1 to 7.1% of the phenotypic variance (Table 1; Fig. S3a).

A second QTL derived from SHA3/CBRD was detected on 1RS. This QTL had a LOD score above 3.2 in Vollebekk 2009 and 2010 and explained 1.2–6.5% of the phenotypic variation (Table 1; Fig. S3b).

The major QTL on 2DS with SHA3/CBRD as the resistance source was like the QTL on 1AL only significant in Norway, with a LOD score above 3.2 at Vollebekk in 2008 and 2010 and Staur in 2010. This QTL explained 0.1–12.6% of the phenotypic variation (Table 1; Fig. S3c).

A fourth QTL detected with resistance from SHA3/CBRD was located on 5AL. This QTL was highly significant in Staur 2009 with an explained phenotypic variation of 16.3%, but had a LOD score below 3.2 in all other testing environments (Table 1; Fig. S3d).

QTL analysis: Soru#1 x Naxos

A total of 10,500 SNPs were polymorphic in the Soru#1 x Naxos population. By MST mapping these markers were assembled into 83 linkage groups assigned to chromosomes based on a BLASTn search. In addition to the SNP markers, 50 SSR markers were added to the dataset. Of the 10,500 polymorphic SNPs, 4113 were discarded due to poor quality. In total, 6387 SNP markers and 50 SSR markers were loaded into JoinMap v. 4.0 for map construction. Out of these markers, 2788 SNP markers and 36 SSR markers were included in maps spanning 3031 cM, covering all chromosomes.

In QTL Interval Mapping (IM), QTL for PM resistance were detected on chromosome 1AS, 2AL, 2BL and 3AS in most environments. Markers in close proximity to the detected QTL in IM were chosen as cofactors in Multiple-QTL model (MQM) mapping. After final MQM mapping, four major QTL were identified on chromosomes 1AS, 2AL, 2BL and 3AS. The major QTL on 1AS, 2AL and 2BL had resistance from Naxos, the major QTL on 3AS was contributed by Soru#1 (Table 2).

Major QTL with resistance from Naxos

The major QTL detected on chromosome 1AS was consistent across all environments. It explained 9.8–20.1% of the phenotypic variation (Table 2). There were not many markers mapped to the QTL region of this chromosome, but three SNP markers mapped in close proximity to the QTL; *Excalibur_c105151_200*, *Kukri_c11891_1015* and *Tdurum_contig50845_25*. This QTL mapped in the same area as the 1AS QTL in SHA3/CBRD x Naxos population. The SNP markers *BS00022701_51*, *Kukri_c11891_1015*, *Kukri_rep_c81545_195* and *wsnp_Ex_c64327_63176640*, and SSR markers *cnl137*, *gwm33b* and *gwm33a* mapped in the QTL area in both populations, verifying that the 1AS QTL is the same (Fig. 2).

The major QTL on 2AL had a LOD score of 3.07 at Staur 2013 and a LOD score above 3.4 at Vollebekk 2013 and 2016. This QTL explained from 6.1 to 9.7% of the phenotypic variation (Table 2). The two SNP markers *Bobwhite_c6356_87* and *Tdurum_contig13653_4712* mapped in close proximity of the QTL on this chromosome. The QTL on 2AL in Soru#1 x Naxos mapped in

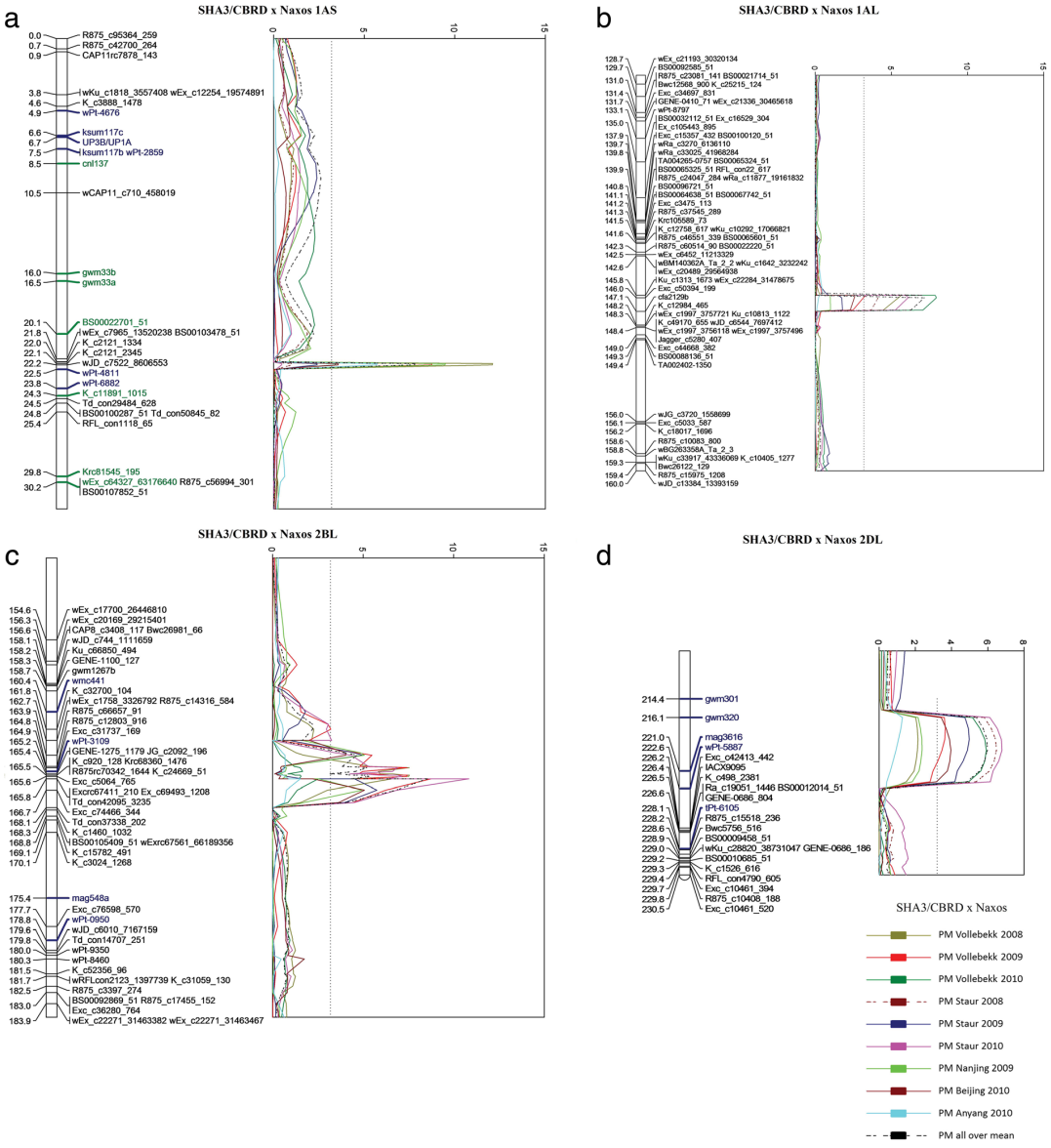

Fig. 1 Segments of chromosomes with resistance QTL derived from Naxos in the SHA3/CBRD x Naxos population. The corresponding LOD curves were obtained from MQM. Genetic distances are shown in centimorgans (cM) to the left of the chromosomes. A threshold of 3.2 is indicated as a *dashed line* in the LOD graphs. DArT and SSR markers also mapped in the study by Lu et al. (2012) marked in *blue*. **a** Chromosome 1AS. **b** Chromosome 1AL. **c** Chromosome 2BL. **d** Chromosome 2DL

the same region as the 2AL QTL in SHA3/CBRD x Naxos. This QTL on 2AL could be verified by the SNP markers *Ex_c28017_641*, *Td_con13653_471* and *Bwc17403_635*, which mapped in the same area around the QTL both in Soru#1 x Naxos and SHA3/CBRD x Naxos (Fig. 3).

A third major QTL with resistance from Naxos was located on 2BL and had a LOD score above 3.4 at Staur

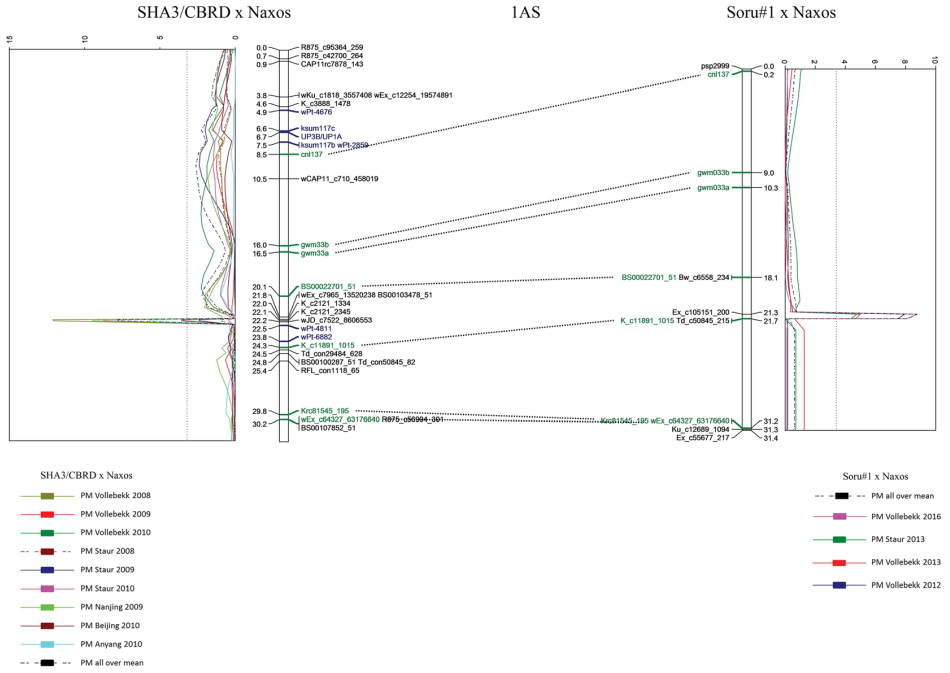

Fig. 2 Segment of chromosome 1AS with major QTL with resistance from Naxos in the two RIL populations SHA3/CBRD x Naxos and Soru#1 x Naxos. The corresponding LOD curves were obtained from MQM. Genetic distances are shown in centimorgans (cM) to the left of the chromosome in SHA3/CBRD x Naxos and right of the chromosome in Soru#1 x Naxos. A threshold of 3.2 is indicated as a *dashed line* in the LOD graph of SHA3/CBRD x Naxos and a threshold of 3.4 is indicated as a *dashed line* in the LOD graph of Soru#1 x Naxos. Common markers between the two populations are marked in *green*, and markers also mapped in the QTL area by Lu et al. (2012) marked in *blue*. *Dotted lines* show the position of the common markers between the two populations

and Vollebekk in 2013. This QTL explained 1.9–15.8% of the phenotypic variation (Table 2). In this part of the 2BL chromosome many markers were mapped, giving a narrow QTL peak with several flanking SNP markers. The two SNP markers most closely linked to the QTL were *wExrc73919_71799491* and *wExc51661_55531646* (Fig. S4). The QTL on 2BL in Soru#1 x Naxos was mapped approximately 30 cM away from the QTL on 2BL in the SHA3/CBRD x Naxos population, and had different markers in the QTL area.

Major QTL with resistance from Soru#1

The last major QTL detected in the Soru#1 x Naxos was located on the short arm of chromosome 3A and had resistance from Soru#1. This QTL had a LOD score above 3.4 at Vollebekk in 2012, and LOD scores above 2.6 the other years and explained 5.9–7.8% of the phenotypic variation (Table 2; Fig. S5).

Discussion

Naxos has previously been demonstrated to be a good source for partial resistance to powdery mildew by Lillemo et al. (2010) and Lu et al. (2012). With the use of the two RIL populations SHA3/CBRD x Naxos and Soru#1 x Naxos, and development of high-density marker maps with both SNP, SSR, DArT and gene-specific markers, we have been able to detect more QTL with resistance from Naxos, and in addition validate QTL on chromosome arms 1AS and 2BL previously detected by Lu et al. (2012).

Phenotypic evaluation Soru#1 x Naxos

For the Soru#1 x Naxos population, powdery mildew severity histograms show continuous distributions with transgressive segregation in all testing environments indicating that both parents carry resistance genes to powdery mildew. This was further demonstrated in the

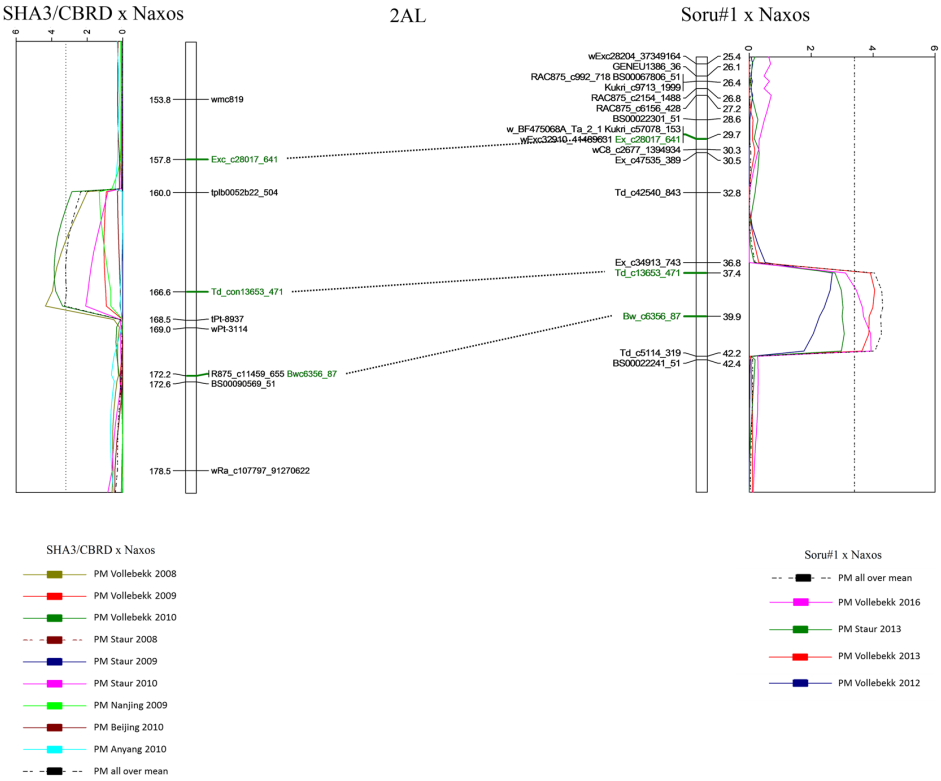

Fig. 3 Segment of chromosome 2AL with major QTL with resistance from Naxos in the two RIL populations SHA3/CBRD x Naxos and Soru#1 x Naxos. The corresponding LOD curves were obtained from MQM. Genetic distances are shown in centimorgans (cM) to the left of the chromosome. A threshold of 3.2 is indicated as a *dashed line* in the LOD graph of SHA3/CBRD x Naxos and a threshold of 3.4 is indicated as a *dashed line* in the LOD graph of Soru#1 x Naxos. Common markers between the two populations are marked in *green*, *dotted lines* show the position of the common markers between the two populations

QTL analysis, where QTL for powdery mildew resistance came from both parents.

Correlation and heritability

Correlation between days to heading (DH) and powdery mildew severity was negative in all testing environments. Lines heading early are exposed to powdery mildew infection over a longer time period compared to lines heading later, resulting in a higher infection rate in these "earlier" lines and a negative correlation with the DH. The heritability estimates for the Soru#1 x Naxos population for powdery mildew were high in all environments, indicating that genetic factors play an important role in this disease.

High-density molecular marker maps with SSR, DArT and SNP markers

Previous reports using SSR, DArT and RFLP markers have conducted mapping with a few hundred polymorphic markers placed on map (Asad et al. 2014; Lu et al. 2012; Somers et al. 2004). The 90K SNP chip from Illumina has enabled the development of marker maps with a higher number of markers, increasing the probability of detecting, and linking a marker to a QTL of interest (Wang et al. 2014). Development of mapmaking programs alongside with the development of high-density SNP chips also make it feasible to construct integrated maps with different marker types, giving an even better map resolution and making it possible to exchange previously used SSR, DArT and RFLP

markers with SNP markers. The SNP markers are easy to utilize in genotyping with today's technology platforms, e.g. KASP (Semagn et al. 2014).

The QTL mapping study in SHA3/CBRD x Naxos by Lu et al. (2012) revealed QTL for powdery mildew resistance on chromosome arms 1AS, 1RS, 2BL, 2DLc, 2DL, 6BL, 7AL and 7DS, where the QTL on 1AS, 2BL, 2DL and 7DS originated from the resistance source Naxos. In the present study, with the use of the 90K SNP chip in addition to the previously used SSR, DArT and gene-specific markers, additional QTL were detected on 1AL, 2AL, 5AS and 5AL with Naxos as resistance source and from SHA3/CBRD additional QTL on 1AL and 3BS were detected. The QTL on 1AS and 2BL with Naxos as resistance source detected by Lu et al. (2012) were validated in this study. The QTL on 2DL and 7DS detected in the study were the same QTL as detected by Lu et al. (2012), but no SNP markers mapped close to the QTL on these two chromosomes in the present study.

Comparison with previous reports

Chromosome arm 1AS harbours the major resistance gene *Pm3*, encoding seven alleles conferring resistance to different races of *Blumera graminis* f.sp. *tritici* (Tommasini et al. 2006). The resistance on 1AS from Naxos was suggested to be race-non-specific by Lu et al. (2012) where it was demonstrated that Naxos lacks the *Pm3* gene based on *UP3B/UP1A* and *Pm3MF/Pm3ER1* primers. In the present study, the 1AS QTL was more precisely mapped with the addition of SNP markers to the map. Moreover, it was also validated in the Soru#1xNaxos population where it was significant in all years and testing locations. The QTL explained a high proportion of the phenotypic variance in both populations, pointing to the 1AS QTL as an important APR QTL for PM resistance.

Two QTL were detected on chromosome arm 1AL in SHA3/CBRD x Naxos, one originating from Naxos. Several other studies have detected QTL for race-non-specific resistance in the centromeric and *cfa2129* marker area of 1AL.This includes QTL detected in winter wheat line RE714 (Mingeot et al. 2002), the spelt variety Oberkulmer (Keller et al. 1999) and Chinese winter wheat Bainong 64 (Lan et al. 2009). Based on mapping data in the above mentioned studies, it is likely that the QTL from Naxos detected in SHA3/CBRD x Naxos is the same QTL, suggesting that there is an APR QTL at this locus effective in several environments and genetic backgrounds.

The 2AL QTL derived from Naxos detected in both SHA3/CBRD x Naxos and Soru#1 x Naxos was flanked by several common markers in the two populations, strongly indicating that this QTL is the same in both populations. Powdery mildew QTL on 2AL has also been detected in the winter wheat cultivar Massey by Liu et al. (2001) and in a derivative of Massey, USG3209, by Tucker et al. (2007). Comparison of the integrated maps in the present study and the mapping data from Liu et al. (2001) and Tucker et al. (2007) suggests the 2AL QTL derived from Naxos to be the same 2AL QTL as detected in Massey and USG3209.

There have been several reports of a QTL for PM resistance on 2BL. Both in the winter wheat cv. Massey (Liu et al. 2001), the Japanese wheat cultivar Fukuho-komugi (Liang et al. 2006), in the line RE9001 (Bougot et al. 2006), in the line USG3209 (Tucker et al. 2007) and in the Chinese wheat cultivar Lumai 21 (Lan et al. 2010). Lan et al. (2010) suggested this 2BL QTL in Massey, Fukuho-komugi, RE9001, USG3209 and Lumai 21 to be at the same or closely linked loci according to their position and flanking markers in the wheat SSR consensus map (Somers et al. 2004). In the present study, a QTL on 2BL from Naxos was detected in both SHA3/CBRD x Naxos and Soru#1 x Naxos. In the SHA3/CBRD x Naxos population, this 2BL QTL was verified to be the same as the QTL detected by Lu et al. (2012) in Naxos due to the common markers flanking the QTL in both studies. In addition, based on mapping data and detection of common markers, the QTL from Naxos in SHA3/CBRD x Naxos is possibly also the same as the QTL detected in Massey, Fukuho-komugi, RE9001, USG3209 and Lumai 21. A 2BL QTL with resistance from Naxos was also detected in the Soru#1 x Naxos population in the present study. When investigating the mapping data for both populations, it is likely that this 2BL QTL is not the same as the QTL detected with resistance from Naxos in the SHA3/CBRD x Naxos population. The QTL on 2BL detected in Soru#1 x Naxos is possibly a newly discovered QTL, but this needs further investigation.

A 5AS QTL significant in Nanjing 2009 was detected in the present study. In the area of the detected QTL several minor QTL were also detected, these might be linked to the major QTL. A 5AS QTL for APR have also been reported in *Triticum militinae* (Jakobson et al. 2006), and in the Swiss spelt variety Oberkulmer (Keller

et al. 1999). The mapping data suggests the QTL detected in the studies by Keller et al. (1999) and Jakobson et al. (2006) to be the same QTL and in addition to be closely linked to the QTL detected in SHA3/CBRD x Naxos with resistance from Naxos in our study.

The major QTL detected on 5AL with resistance from Naxos was significant at Staur in 2008. There have been several reports of QTL for APR to PM on 5AL; the CIMMYT bread wheat line Saar (Lillemo et al. 2008), the Swedish winter wheat cultivar Folke (Lillemo et al. 2012), the Swiss spelt variety Oberkulmer (Keller et al. 1999) and the DH line 8.1.8.1 x T, made from a cross of the spring wheat cv. Tahti with tetraploid *T. militinae* (Jakobson et al. 2012). Studies of the maps and markers in these different lines and cultivars and comparisons with the wheat composite map (graingenes.com; http://wheat.pw.usda.gov/GG3/) suggest the 5AL QTL found in Saar, Folke, Oberkulmer, 8.1.8.1 x T and Naxos to be the same or closely linked QTL, but this needs further study.

In conclusion, high-density marker maps with SSR, DArT and SNP markers were developed in the two RIL populations SHA3/CBRD x Naxos and Soru#1 x Naxos. With these new high-density maps, 12 QTL were detected in the SHA3/CBRD x Naxos population, eight of them with Naxos as the resistance source. Of these, the QTL on 1AS and 2BL are verified as the same QTL as detected by Lu et al. (2012). These two QTL are now more precisely mapped, and the identification of closely linked SNP markers will greatly facilitate the use of these QTL for resistance breeding. The QTL on 1AS was in addition further confirmed in the Soru#1 x Naxos population. The new QTL detected in SHA3/CBRD x Naxos on chromosome 2AL, possibly the same QTL that has previously been reported in Massey and USG3209, was also verified in the Soru#1 x Naxos population, making also this QTL highly interesting for resistance breeding.

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Authors' contributions Susanne Windju analysed and scored the SNP markers from the Illumina 90K SNP chip in Genome Studio, performed linkage mapping in Join Map and QTL mapping in MapQTL, analysed field data in the Soru#1 x Naxos population, wrote the manuscript.

Keshav Malla performed field work, data collection and analysis.

Tatiana Belova performed linkage mapping in MST map, assigned markers to chromosomes.

Robert C. Wilson supervised the work, reviewed the manuscript.

Jon Arne Dieseth executed the field design and experiment, reviewed the manuscript.

Muath Alsheikh obtained funding, supervised the work, edited the manuscript.

Morten Lillemo obtained funding, designed and led the project, designed and performed fieldwork, supervised the work, edited the manuscript.

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Paper II

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ORIGINAL ARTICLE

Mapping of SnTox3–*Snn3* as a major determinant of field susceptibility to Septoria nodorum leaf blotch in the SHA3/CBRD × Naxos population

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Abstract

Key message **The effect of the SnTox3–*Snn3* interaction was documented for the first time under natural infection at the adult plant stage in the field. Co-segregating SNP markers were identified.**

Abstract *Parastagonospora nodorum* is a necrotrophic pathogen of wheat, causing Septoria nodorum blotch (SNB) affecting both the leaf and glume. *P. nodorum* is the major leaf blotch pathogen on spring wheat in Norway. Resistance to the disease is quantitative, but several host-specific interactions between necrotrophic effectors (NEs) and host sensitivity (*Snn*) genes have been identified, playing a major role at the seedling stage. However, the effect of these interactions in the field under natural infection has not been investigated. In the present study, we saturated the genetic map of the recombinant inbred (RI) population SHA3/CBRD × Naxos using the Illumina 90 K SNP chip. The population had previously been evaluated for segregation of SNB susceptibility in field trials. Here, we infiltrated the population with the purified NEs SnToxA, SnTox1 and SnTox3, and mapped the *Snn3* locus on 5BS based on sensitivity segregation and SNP marker data. We also conducted inoculation and culture filtrate (CF) infiltration experiments on the population with four selected *P. nodorum* isolates from Norway and North America. Remapping of quantitative trait loci (QTL) for field resistance showed that the SnTox3–*Snn3* interaction could explain 24% of the phenotypic variation in the field, and more than 51% of the variation in seedling inoculations. To our knowledge, this is the first time the effect of this interaction has been documented at the adult plant stage under natural infection in the field.

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Introduction

Parastagonospora (syn. *Septoria*, syn. ana *Stagonospora*) *nodorum* (Berk.) (Quaedvlieg et al. 2013) [teleomorph:*Phaeosphaeria* (syn. *Leptosphaeria*) *nodorum* (Müll), Hedjar.] is the causal agent of Septoria nodorum leaf and glume blotch (SNB), a disease that can cause yield losses of up to 31% (Bhathal et al. 2003). The main hosts of *P. nodorum* are bread wheat (*Triticum aestivum*), durum wheat (*T. durum*) and triticale but also other cereals and a range of wild grasses. The pathogen is common in major geographical regions where wheat is grown, including the USA, Australia and Europe (Solomon et al. 2006; Francki 2013), particularly in rainy climates, and is the major leaf blotch pathogen in Norwegian spring wheat.

QTL for flag leaf resistance have consistently been detected on chromosomes 1A, 1B, 2A, 2D, 3AS, 3B, 5A, 5B, 7A and 7B (Aguilar et al. 2005; Shankar et al. 2008; Friesen et al. 2009; Francki et al. 2011; Lu and Lillemo

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2014). Most of the QTL explain less than 20% of the phenotypic variation, as reviewed by Francki (2013).

Lately, it has been shown that host-specific interactions play an important role in this pathosystem, at least at the seedling stage (Oliver and Solomon 2010). The necrotroph and the host interact in an inverse gene-for-gene manner based on necrotrophic effectors (NEs) and corresponding sensitivity loci (*Snn*) in the host (Friesen and Faris 2012). The effect of each SnTox-*Snn*-interaction is incomplete and usually additive in nature (Friesen and Faris 2010). However, epistatic interactions are also involved, affecting toxin expression, host gene action and cross talk between pathways (Friesen et al. 2008b). At least eight NE (SnToxA, SnTox1, SnTox2, SnTox3, SnTox4, SnTox5, SnTox6 and SnTox7) and nine corresponding *Snn* genes (*Tsn1, Snn1, Snn2, Snn3-5B, Snn3-5D, Snn4, Snn5, Snn6 and Snn7*) have been characterized (Friesen et al. 2006, 2007; Liu et al. 2006, 2009; Abeysekara et al. 2009; Gao et al. 2015; Shi et al. 2015). *SnToxA, SnTox1* and *SnTox3* have been cloned into *Pichia pastoris*, and the purified effectors are being used for seedling screenings (Friesen et al. 2006; Liu et al. 2009, 2012). In Australia, screenings with NEs have been implemented in wheat breeding programs (Tan et al. 2014). Two of the sensitivity genes have been cloned. *Tsn1* encodes a protein with N-terminal nucleotide binding site, leucine-rich repeats (NBS-LRR) and a C-terminal serine/threonine protein kinase (S/TPK) (Faris et al. 2010)—representing a minor class of the classical NBS-LRR resistance genes typically conferring race-specific resistance to biotrophs. The recent positional cloning of *Snn1* identified a wall-associated kinase class of receptor, which is also associated with resistance to biotrophic pathogens (Shi et al. 2016b), supporting the hypothesis that the necrotrophic pathogens hijack biotrophic resistance pathways.

SnTox3–*Snn3* was the fourth NE–*Snn* interaction to be identified (Friesen et al. 2008a) and *SnTox3* the second necrotrophic effector from *P. nodorum* to be cloned (Liu et al. 2009). The gene encodes for a 693 bp small secreted protein with no known homology to other proteins (Liu et al. 2009), and at least 11 haplotypes are known (McDonald et al. 2013). The SnTox3–*Snn3* interaction was first described by Friesen et al. (2008b), and the sensitivity locus mapped to the distal end of 5BS, with *cfd20* as the closest marker, but almost 30 cM from the next linked markers. In the BR34 × Grandin population the interaction explained up to 17% of the phenotypic variation in disease after inoculation at the seedling stage. Recently, a saturated map covering the *Snn3-B1* region was also published, delineating the gene to a 1.5 cM interval (Shi et al. 2016a). At least two NB-LRR-like genes were linked to markers (*fcp652* and *fcp665, fcp666*) within this interval.

The SnTox3–*Snn3* interaction has been reported to be significant only in the presence of incompatible SnTox2–*Snn2* interaction, the SnToxA-*Tsn1* interaction is epistatic to SnTox3–*Snn3* (Friesen et al. 2008b; Cockram et al. 2015) and SnTox1 can suppress the expression of SnTox3 (Phan et al. 2016). A low, but significant negative correlation between sensitivity to SnTox3 and lower disease resistance ratings in Australian wheat cultivars has been reported (Waters et al. 2011; Francki 2013), indicating, but not confirming, that the interaction probably is significant in disease development also in the field.

Leaf infiltrations with single effectors have uncovered gene-for-gene-interactions, but the interactions are not always additive and the relative importance of each effector in a mixed natural pathogen population might change over time. Thus, it is necessary to investigate the relationships further. One study showed the significant effect of the SnToxA–*Tsn1* and SnTox2–*Snn2* interactions on adult plants in the field after inoculation with a single isolate (Friesen et al. 2009). An experimental design with naturally infected plants better explains the relationship between the natural pathogen population and the host. However, such a study is more complex and one can run the risk of not finding consistent effects across years due to fluctuations in the pathogen populations.

The damaging effect of SNB is largest in moist periods when the pathogen infects the flag and sub-ultimate leaf during grain filling (Francki 2013) and the milk stage in particular (Bhathal et al. 2003). Evaluation and genetic analysis of adult plants under field conditions are therefore of great importance, but also challenging. Considerable genotype × environment (G × E) interaction is expected, and many QTL have been detected in only one environment. To be relevant for breeders, the QTL should be consistent in several environments (Francki 2013).

Breeders usually rely on natural infection in the field for evaluation of leaf blotch resistance (Cowger and Murphy 2007). Fraser et al. (2003) suggested that promotion of infection by natural inoculum, by overhead irrigation and/or inoculation with naturally infected straw gives a better estimate of host resistance under natural epidemics than inoculation of the nurseries with selected isolates.

The recombinant inbred line (RIL) population SHA3/CBRD × Naxos was previously analyzed for leaf blotch susceptibility (Lu and Lillemo 2014). Screenings with the cloned effectors showed that it most likely segregated for *Snn3*, but the sensitivity locus did not map to any linkage group, the population was monomorphic to linked markers *cfd20* and *gwm234*, and the effect of the interaction in the field could not be verified. To improve the map resolution, SHA3/CBRD × Naxos was genotyped with the Illumina iSelect 90 K wheat SNP Chip (Wang et al. 2014) and QTL mapping was performed again on the field data. The population was also inoculated and infiltrated at the seedling stage with four *P. nodorum* isolates with different

effector profiles (Table 1). This mapping revealed that the SnTox3–*Snn3* interaction indeed could explain a major proportion of the variation in resistance between genotypes. To our knowledge, this is the first time the effect of SnTox3 has been mapped under natural infection in the field.

The objectives of this study were to (1) perform new and more precise QTL mapping of the field data with high-density SNP marker maps and (2) investigate to what degree these field QTL can be explained by seedling reactions to single isolates and infiltration with purified effectors.

Materials and methods

Plant material and foregoing field study

The development and field evaluation of Shanghai3/Catbird (SHA3/CBRD) × Naxos are described by Lu and Lillemo (2014). Briefly, it is an F_6-derived RIL population that segregates for SNB resistance in the field. The CIMMYT line SHA3/CBRD is highly resistant while the German spring wheat parent Naxos is susceptible. The main conclusion from Lu and Lillemo (2014) was that the field resistance was based on many minor effect genes. Although the population segregated for SnTox3 sensitivity, the position or any clear effect of the interaction in the field could not be mapped or verified in the study, which used a set of 564 SSR and DArT markers.

Linkage mapping

A total of 166 individuals from the SHA3/CBRD × Naxos RIL population were genotyped with the Illumina iSelect 90 K wheat SNP Chip (Wang et al. 2014). Analyzing and scoring of the genotype results were performed manually for every SNP marker with the software Genome Studio Genotyping Module v1.0 from Illumina.

Markers scored as polymorphic were used for constructing linkage groups and genetic linkage maps. The markers were sorted in linkage groups with MSTmap (Wu et al. 2008). The linkage groups were assigned to chromosomes based on the best BLASTn hit from a comparison of SNP-flanking sequences with the Chinese Spring chromosome survey sequences (http://wheat-urgi.versailles.inra.fr/Seq-Repository). Previously developed SSR and DArT marker data in the population (Lu et al. 2012) were added to the SNP marker data.

Markers belonging to linkage groups assigned to the same chromosomes based on the BLASTn search were loaded into Join Map v. 4.0 (Van Ooijen 2006), and the linkage groups were refined using the maximum likelihood mapping algorithm. The genetic distances between markers were calculated by converting recombination fractions into map distances (cM) based on the Kosambi mapping function with minimum LOD score of 3.0 (Kosambi 1943).

QTL analysis

QTL analysis was performed using the software MapQTL6 (van Ooijen 2011). Multiple QTL mapping (MQM) was used, based on cofactors for major QTL initially detected with interval mapping (IM). The LOD significance threshold was set to 3.0. The software MapChart, v.2.2 was used to draw the genetic maps and LOD curves. For analysis of field resistance, the confounding traits plant height, heading date and maturity were used as covariates to disease score in MapQTL6 as described by Lu and Lillemo (2014).

P. nodorum isolates: DNA extraction and screening for *SnTox* genes

Four isolates of *P. nodorum* were selected for the study (Table 1). Sn4 is a North American isolate known to produce SnToxA, SnTox1, SnTox2 and SnTox3, as described by Faris et al. (2011) and Crook et al. (2012). NOR4 was collected in Romerike, Akershus, Norway in 2011, from the spring wheat variety Zebra. Isolate 201593 was collected from the leaf blotch field trials at Vollebekk, Ås, Norway in 2014 from the Norwegian spring wheat cultivar Demonstrant (sensitive to SnTox3). Isolate 201618 was collected in Øsaker, Østfold in 2012 from the cultivar Quarna. The three Norwegian isolates were collected from leaves with visible leaf blotch symptoms, and grown on V8-PDA in 24h light (white + near ultraviolet (NUV)) to enhance sporulation before mycelial plugs were harvested with a cork borer and dried before storage at −80 °C. For

Table 1 List of isolates included in the study, with SnTox-profile (presence/absence based on PCR) and disease range and mean in the RIL population

Isolate	Presence (+) or absence (−) of SnToxA, SnTox1 and SnTox3, respectively	Disease range in the RILs	Population mean reaction
Sn4	+++	0.17–3.83	2.23
NOR4	+++	0.00–4.00	2.13
201593	−−+	0.00–5.00	3.37
201618	−−−	0.00–4.80	2.7

DNA extraction, the isolates NOR4, 201593 and 201618 were grown in the dark on PDA for 1–2 weeks and DNA extracted from the mycelium with the DNEasy plant kit (Qiagen). PCR screenings for *SnTox* genes and actin were performed as described in Gao et al. (2015).

Inoculum preparation and seedling inoculation

Dried plugs of the *P. nodorum* isolates were plated on V8-PDA agar and grown for approximately one week in incubation chambers with constant light (white fluorescent + NUV) and temperature around 21 °C until sporulation. The plates were flooded with distilled water and scraped with a sterilized inoculation loop to release pycnidiospores, and the final concentration of spores was adjusted to 1×10^6 spores/ml. One drop of Tween 20 (polyoxy-ethylene-20-sorbitan monolaureate) was added per 50 ml inoculum to reduce surface tension.

Seeds of the mapping population were planted in plastic cone-tainers (Stuewe and sons, Tangent, Orlando, USA), with potting mixture (peat soil with clay and sand, Gartnerjord, Tjerbo, Norway), and grown in the greenhouse under 18 °C day/15 °C night temperature and 16 h light cycle until the second leaf was fully expanded—approximately 14 days after planting. Three seeds were planted per cone. The susceptible cultivar Brakar was used as a border to reduce edge effect.

The 14-day-old plants were spray inoculated with a paint sprayer until runoff, placed in a mist chamber with 100% RH for 24 h in constant light before they were returned to the greenhouse. Seven days after inoculation, the second leaf of each plant in the accessions was evaluated for disease reactions on a scale from 0 to 5 (Liu et al. 2004), where 0 is highly resistant and 5 is highly susceptible.

Infiltrations

Two seeds per RIL were planted in individual cones in racks fitting 98 cones and grown in the greenhouse under similar conditions as for the inoculation experiments. The experiments were repeated three times.

Liquid cultures of the isolates were produced in Fries 3 medium as described in Friesen and Faris (2012). After three weeks in stationary phase, the cultures were filter sterilized and infiltrated into the fully expanded second leaf of 12–14-day-old seedlings, using a 1-mL needleless syringe. The infiltrated areas were marked with a non-toxic felt marker. After five days, the reactions were scored according to a 0–3 scale (Friesen and Faris 2012). These experiments were repeated three times with two infiltrated plants per genotype in each replicate.

Infiltration with purified SnToxA, SnTox1 and SnTox3

With partly purified SnToxA, SnTox1 and SnTox3, 12–14-day-old lines of the population were infiltrated. Approximately 25 µL of the partly purified NE was infiltrated into the fully expanded secondary leaf using a needleless syringe. The infiltrations were done in Fargo, North Dakota in 2013 with effectors produced by *Pichia pastoris* using the pGAPzA expression vector (Liu et al. 2009), and repeated in Ås, Norway with effectors provided by Dr. Richard Oliver. SnToxA from Dr. Oliver was expressed in *Escherichia coli* BL21E using the pET21a expression vector (Tan et al. 2012), while SnTox1 and SnTox3 were produced as above. All protein preparations containing the expressed effectors were desalted (Waters et al. 2011) prior to infiltration (Liu et al. 2009). The plants were evaluated after 3 to 5 days and scored on a 0–3 scale (Friesen and Faris 2012).

Gene annotations

The contextual sequences of the SNP markers with the closest linkage to *Snn3* were downloaded from https://triticeaetoolbox.org/ and BLASTED at http://plants.ensembl.org/Multi/Tools/Blast and https://urgi.versailles.inra.fr/Tools/BLAST. Annotated genes were identified, and the sequences were aligned against rice orthologues available through the rice genome annotation project http://rice.plantbiology.msu.edu/ in order to compare the results with previously reported genes in Shi et al. (2016a).

Results

Seedling inoculations and infiltrations

The frequency distribution histograms (Fig. 1) show that inoculation with isolate 201593 produced more severe necrosis (reaction type 5) than inoculation with the other isolates. Correlations between the SnTox3-positive isolates were highly significant after inoculation (Pearson's correlations 0.623–0.785, $P < 0.0001$, Table 2), while correlations between the SnTox3-negative isolate 201618 and the others were lower, but still significant. Also, the correlation between seedling inoculations and sensitivity data based on purified SnTox3 infiltration was high except for the SnTox3-negative isolate, as expected (Table 2).

Correlation between infiltration experiments with different isolates indicated that SnTox3 was the single effector produced in liquid culture by SnTox3-positive isolates causing sensitivity in the SHA3/CBRD × Naxos population (Table 3). Based on reactions on differential lines, we assume that Sn4 and NOR4 also produced SnTox1 and

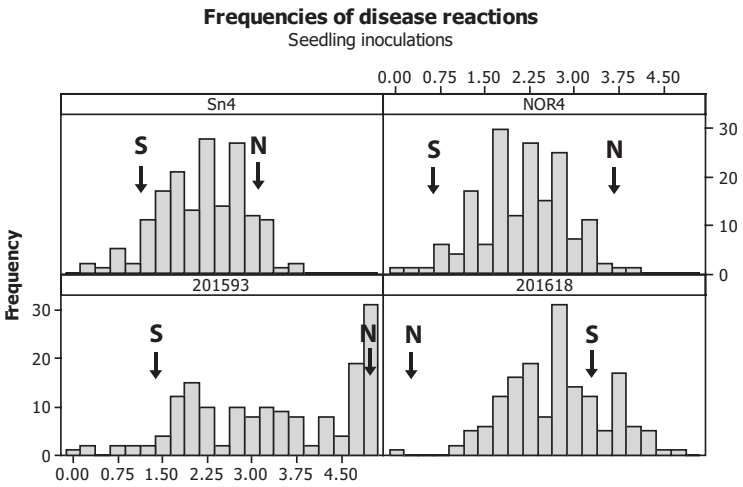


Fig. 1 Frequency distributions of disease reaction types for the SHA3/CBRD × Naxos RIL, after seedling inoculations. Parental phenotypes are indicated by *arrows*

Table 2 Pearson's correlation coefficients between single isolate inoculations at the seedling stage and correlation with reaction to purified SnTox3

	NOR4	201593	Sn4	SnTox3
201618	0.260**	0.300***	0.325***	0.062
Sn4	0.785***	0.623***		0.559***
201593	0.670***			0.741***
NOR4				0.626***

***<0.0001, **<0.001, *<0.01

Table 3 Pearson's correlation coefficients between sensitivity scores after single isolate culture filtrate (CF) infiltration and correlation between CF reactions and reactions to purified SnTox3 infiltration

	NOR4	201593	Sn4	SnTox3
201618	0.012	−0.097	−0.002	−0.07
Sn4	0.924***	0.863***		0.912***
201593	0.890 ***			0.952***
NOR4				0.935***

***<0.0001, **<0.001, *<0.01

SnTox2 and 201593 and 201618 produced SnTox2 and SnTox6 (data not shown) as well as unpublished effectors, but the population did not segregate for sensitivity to these.

Correlation between adult plant and seedling stage results

The correlation was highly significant ($P < 0.0001$) between disease reaction scores based on single isolate inoculations with SnTox3 positive isolates NOR4, Sn4 and 201593 and field disease severities in 2010 and 2011 and for the mean over years (Table 4). The correlation was lower between these isolates and field scores for 2012 and 2013. The correlation between field scores and the North American isolate Sn4 was as significant as the Norwegian isolates except for 2012. Correlation between isolate 201618 and field scores was only significant in 2010.

Table 4 Pearson's correlation coefficients between corrected leaf blotch severities in the field trials (years, 2010–2013 and mean) and disease reactions after seedling inoculations with single isolates, and infiltration with purified SnTox3

Year	Inoculation with single spore isolates				SnTox3
	NOR4	Sn4	201593	201618	
2010	0.486***	0.519***	0.615***	0.335***	0.486***
2011	0.344***	0.360***	0.291***	0.092	0.222**
2012	0.262**	0.182	0.243*	0.036	0.080
2013	0.235*	0.264**	0.334***	0.161	0.205**
mean	0.387***	0.366***	0.432***	0.154	0.262**

***<0.0001, **<0.001, *<0.01

Frequency distribution and mapping of *Snn3*

The RILs segregated for SnTox3 sensitivity as either completely sensitive (reaction type 3) or insensitive (reaction type 0), with 75 insensitive to 82 sensitive, which is not significantly different from 1:1 ($\chi^2 = 0.312$, $P = 0.576$). Eleven lines (of 168) were coded as missing, due to inconsistent reactions, to avoid misclassification of the alleles. The susceptibility was inherited from parent Naxos.

The phenotypic scores for SnTox3 sensitivity were used to infer allele variants (a and b for parent SHA3/CBRD and Naxos, respectively) and the position of the sensitivity locus mapped with linkage analysis (Fig. 2). The locus could not previously be mapped with SSR markers polymorphic in the population (Lu and Lillemo 2014). Only with the improved resolution and coverage provided by the SNP markers, the locus could be mapped as Fig. 2 shows. The population was insensitive to SnToxA and SnTox1.

QTL—seedling resistance

The major QTL at the *Snn3* locus on 5BS explained up to 51.8% of the phenotypic variation when the population was inoculated with SnTox3-positive, SnTox1-negative isolate 201593, and was also highly significant after inoculation with SnTox1-positive Sn4 and NOR4 (Table 2; Fig. 3) where suppressed expression of SnTox3 was expected according to the literature (Phan et al. 2016). The QTL on 5BS was the only significant genomic region after inoculation with isolates NOR4 and 201593 (Table 5; Fig. 3). After inoculation with Sn4, a QTL on 7B was also detected, but not after infiltration. After inoculation with 201618, QTL were detected on 1A, 1B and 2D. However, all three had only moderate or minor effects and did not correspond to the adult plant QTL on 1A and 1B (Table 7; Figure S1). Interestingly, the QTL showing significance on 7B after Sn4-inoculation corresponded to the only significant QTL after infiltration with 201618 (Tables 5, 6).

QTL—adult plant resistance

Seven significant and one putative QTL for adult plant resistance to SNB were previously reported in the population, based on the field evaluations from 2010 to 2013 (Lu and Lillemo 2014). The major QTL was found on 3BL flanked by

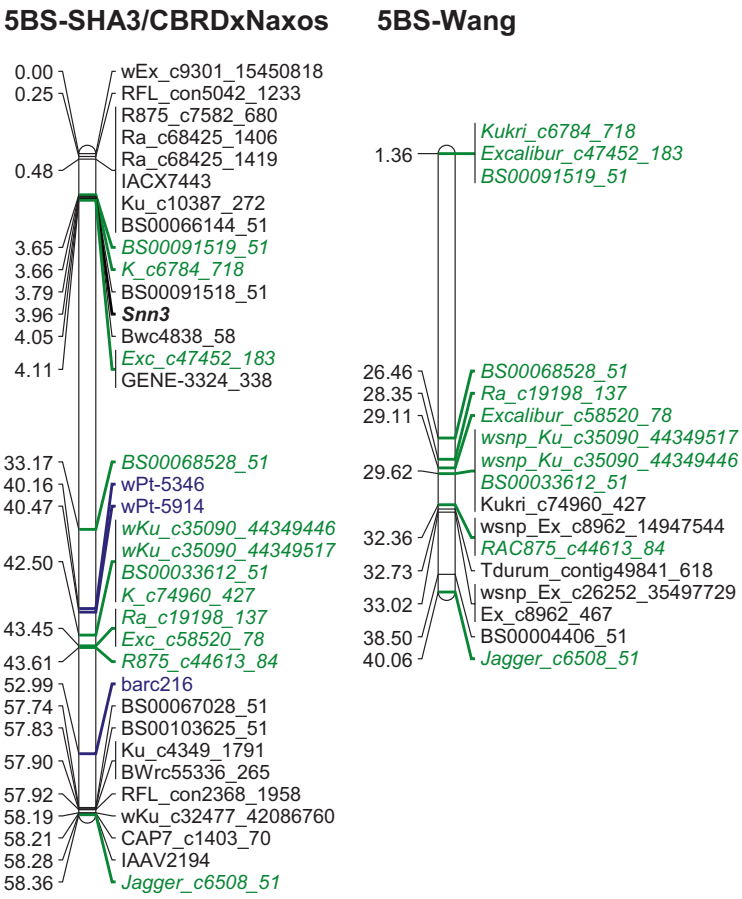


Fig. 2 *Left* Mapping of the *Snn3* locus on chromosome 5BS in SHA3/CBRD × Naxos based on segregation of SnTox3-sensitivity. *Right* region of 5BS in the Wang et al. (2014) consensus map covered by polymorphic SNPs in SHA3/CBRD × Naxos. Common markers are indicated in *italics*. The maps are drawn in Mapchart v. 2.2 (Voorrips 2002)

wpt-4933. However, improved map resolution and reanalysis of QTL captured a total of 11 significant QTL, with four being new (Table 7, Figure S1).

The QTL explaining most of the variation in any environment was located on the telomeric end of 5BS (Table 7; Fig. 4), not mapped with the initial set of SSR and DaRT markers in the study by Lu and Lillemo (2014). This QTL is located at the *Snn3* locus (Fig. 2) and explained as much as 24.0 and 9.0% of the phenotypic variation in 2010 and 2011, respectively. It was also significant across years (mean) and had an effect in 2013. However, in 2012 the *Snn3* region was not significant in QTL analysis. These results are also reflected by the correlations between infiltration with purified SnTox3 and field trials (Table 4), where the correlation is highly significant ($p < 0.0001$) between SnTox3-sensitivity for 2010 and across years, and significant at $p < 0.05$ in 2011, but not significant for 2012.

A novel QTL was detected on 1A in 2012 (Table 7). Higher map resolution and MQM mapping also revealed that 3A harbors at least two QTL (3AS.1 and 3AS.2), the most significant QTL in 2013. The 3AS.2 QTL was also significant in 2011 and across years (mean). The region covering 3AS.2 was not well covered in the SSR/DArT map.

The originally putative QTL on 3BS, important in 2013 (3BS.1) and 2013 (3BS.2), respectively, appear to be two distinct QTL although located approximately 8 cM apart. The QTL on 3BL was highly significant in 2011 and marker *wPt-4933* showed an effect in all years except 2012. In addition to the major QTL explained by *Snn3*, the QTL on 5B flanked by *wPt-5346* detected before, was also significant in 2013.

Gene annotations

Most of the SNPs co-segregating with *Snn3* could be matched to genes on scaffold TGACv1_scaffold_423631_5BS (Table 8). Although *Traes_6DL_388658304.1* was reported to be located on 6DL and *Traes_5AS_905D6F817.1:1* on 5AS, our mapping results as well as Wang et al. (2014) indicate that they are located on 5BS. Some of the genes share hallmarks of R-genes, i.e., coiled-coil (CC) (*Traes_5BS_C460CEDFB*), leucine-rich repeats (LRR) (*Traes_5BS_E0680D15E.2.path1*) and nucleotide binding sites (NBS) (*Traes_5BS_C460CEDFB, Traes_5AS_905D6F817.1:1*) domains (Table 8).

Discussion

General

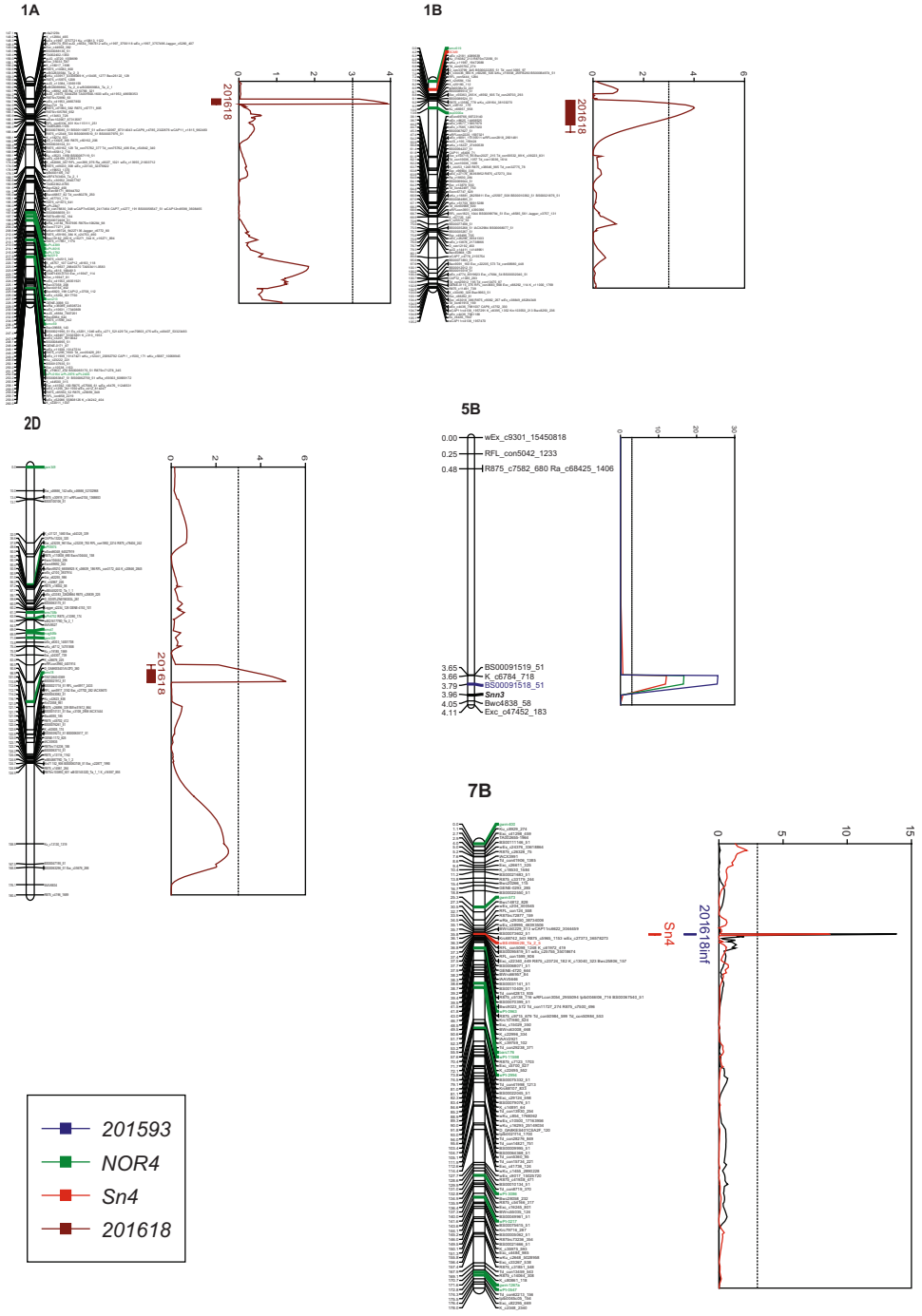
In this study, we mapped the *Snn3* locus (Fig. 2) in the SHA3/CBRD × Naxos population and identified it as a major determinant of susceptibility to SNB both under natural field infection at the adult stage and single spore isolate inoculations of seedlings (Tables 5, 6, 7; Figs. 3, 4). In the previous study by Lu and Lillemo (2014), the effect of this interaction was not identified, due to lack of segregating SSR and DART markers in the chromosome area. Although the locus has been mapped in other populations, this is, to our knowledge, the first time the effect of the SnTox3–*Snn3* interaction has been detected under natural infection in the field (Table 7; Fig. 4). We also identified SNP markers tightly linked to *Snn3*, some of which are located within putative NBS-LRR genes (Table 8).

Seedling QTL

The most significant interaction after seedling inoculation was SnTox3–*Snn3*, explaining as much as 51.8% of the phenotypic variation (Table 5) and producing strong necrosis on the leaves of susceptible lines after inoculation with SnTox3-positive isolates. Prior to screening the entire population, a selection of differential lines from SHA3/CBRD × Naxos, segregating for single field resistance QTL, were screened with several locally collected isolates to test for differential segregation (data not shown). However, very few isolates produced higher reaction scores than 2.5 on the lines unless they were also SnTox3-positive. One exception was isolate 201618 which was selected to possibly capture different QTL than the one explained by *Snn3*. QTL on 1A, 1B and 2D were detected after inoculation with 201618 (Table 5; Fig. 3). The QTL on 1A overlap partly with the QTL on 1A detected in 2012 (Table 7), but the resistance source was opposite. The QTL on 1B also seems to be specific to this particular isolate. After infiltration, a QTL on 7B corresponding to the QTL detected after inoculation with Sn4 was discovered, indicating a putative new NE/*Snn* interaction that will be investigated in further studies.

Of the three major interactions SnToxA/*Tsn1*, SnTox1/*Snn1* and SnTox3–*Snn3*, SHA3/CBRD × Naxos only segregated for *Snn3*. The limited number of genes segregating in a two-parent cross is a limitation to the range of the results, and several important interactions may not be detected due to monomorphism in the population. On the other hand, it also allows better investigation of interactions that may be statistically undetectable in the presence of other genes and epistatic interactions.

It has been suggested that presence of SnTox1 suppresses SnTox3 production (Phan et al. 2016). We found that the SnTox3–*Snn3* interaction was highly significant in all relevant inoculation experiments, and that infiltration with CF with SnTox3 positive isolates produced the same necrotic symptoms regardless of SnTox1 presence. However, the frequency of RIL with reaction type 5 was


1A
1B
2D
5B
7B
201618
201618
201618
Sn4
201618inf
0.00 wEx_c9301_15450818
0.25 RFL_con5042_1233
0.48 R875_c7582_680 Ra_c68425_1406
3.65 BS00091519_51
3.66 K_c6784_718
3.79 BS00091518_51
3.95 Snn3
4.05 Bwc4838_58
4.11 Exc_c47452_183
201593
NOR4
Sn4
201618

◂**Fig. 3** From top: QTL detected on 1A, 1B and 2D after inoculation with 201618. QTL on 5B after inoculation with NOR4, Sn4 and 201593. QTL on 7B detected after infiltration with 201618 and inoculation with Sn4. 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 maps are drawn in Mapchart v.2.2 (Voorrips 2002). (Color figure online)

much higher after inoculation with the SnTox1-negative isolate 201593 (Fig. 1).

Effect of *Snn3* in the field

Saturation of the genetic map with the 90 K SNP chip showed that *Snn3* can explain up to 24% of the phenotypic variation in the field (Table 7; Fig. 4: 2010). The results favor the hypothesis that host-specific interactions also play a role in adult plant susceptibility to *P. nodorum* leaf blotch. It also serves as a confirmation that the multiple regression approach where confounding traits (plant height, heading date and maturity) are included as covariates, works well. However, the SnTox3–*Snn3* interaction was only significant in two out of four years of field trials—illustrating the complexity of the disease. One definition of a robust QTL is that it is significant in two or more environments (Francki 2013). Under this definition, selection against lines carrying *Snn3* would be recommended based on our findings.

Since the field experiments depended on natural infection, the results capture a more realistic picture of the situation in farmers' fields rather than after artificial inoculation with single isolates. Nevertheless, very few QTL studies rely on natural inoculum, where one takes a higher risk of large variability between environments.

Mapping of other QTL for field resistance

The fine mapping improved the coverage of the chromosomes and led to the discovery of a significant novel QTL for field resistance on 3A (3A.2, Table 7; Figure S1). Lu and Lillemo (2014) reported that MQM or CIM mapping did not improve the results for the field resistance QTL. However, with the new maps, we found that the significance and precision increased with MQM mapping for several field QTL [1B, 3A, 3BL, 5B (Table 7; Fig. 4; Figure S1)], although different cofactors were used for different years. In 2012, the use of cofactors did not improve the results. Improved coverage of the chromosomes also revealed that some QTL are probably linked and that different underlying genes may be involved in different years, for instance the two on 3BS (Table 7; Figure S1). The novel QTL detected on 1A (Table 7) was below significance threshold when mapped on the original SSR and DArT map.

Although the effect of SnTox3–*Snn3* was highly significant in 2010 and in 2011, the variation between years shown both in correlation coefficients and relative importance of individual QTL, also emphasizes the need to screen the plants in multiple environments and/or locations as discussed by Francki (2013), before selecting genotypes or markers for marker-assisted selection (MAS). The variation illustrates the complexity of the trait and diversity of the natural pathogen population. For some QTL, the % explained variation was lower with the new maps.

Correlation field—seedling trials

A main objective of this study was to investigate the correlation between seedling and adult plant resistance to SNB. Based on the Pearson correlation coefficients between field

Table 5 Significant QTL (LOD > 3.0) for seedling resistance to SNB in inoculation experiments with single isolates, after MQM mapping

Chromosome	Markers (cofactors)	Isolate				R-source
		Sn4	NOR4	201593	201618	
1A	*RAC875_c10083_800*				**11.7**	Naxos
1B	*psp3000*				**10.4**	SHA3/CBRD
2D	*wsnp_RFL_Contig3960_4401914*				**11.1**	Naxos
5B (*Snn3*)	*BS00091518_51*	**27.5**	**35.4**	**51.8**		SHA3/CBRD
7B	*wsnp_BE498662B_Ta_2_5*	**15.5**				Naxos

% phenotypic variance (PEV) explained for significant QTL is listed

Table 6 Marker correlations after infiltration with culture filtrate from single isolates

Chromosome	Markers	Isolate				R-source
		Sn4	NOR4	201593	201618	
5BS (*Snn3*)	*BS00091518_51*	**82.7**	**87.2**	**73.4**		SHA3/CBRD
7B	*wsnp_BE498662B_Ta_2_5*				**32.6**	Naxos

The % phenotypic variance (R^2 values) is listed for the significant interactions

Table 7 List of significant QTL with close markers based on 4 years and the mean of field scorings at Vollebekk, Norway

Chr.	Markers	2010	2011	2012	2013	Mean	R-source
1A	*wsnp_Ex_ c25734_34995416*		2.4	10.3		3.0	SHA3/CBRD
1B.1RS	*SCM9*		5.2		**8.1**	**7.7**	Naxos
3AS.1	*gwm2 IAAV6676*	6.5			**11.5**	3.7	Naxos
3AS.2	*Ku_c41007_116 Excalibur_ c52446_519*		6.6		**9.4**	2.2	SHA3/CBRD
3BS.1	*BS00030534_51*				**5.7**		SHA3/CBRD
3BS.2	*wBE445348B_ Ta_2_1*	**6.9**					
3BL	*wPt-4933*	4.6	**11.2**		3.5	3.9	Naxos
5BS	*BS00091518_51*	**24.0**	**9.0**		4.7	**9.9**	SHA3/CBRD
5B.2	*wPt-5914*	4.8	3.4		**5.6**	2.4	SHA3/CBRD
7 A	*RAC875_ c14195_1155*	2.9	4.1	3.4	**6.5**	**6.2**	Naxos
7B	*BobWhite_rep_ c50229_413*			**8.4**		2.7	Naxos

The % explained phenotypic variation (R^2) is listed if above the LOD threshold of 3 in at least one environment. QTL detected above the LOD threshold in the corresponding environment are indicated in bold. The phenotypic data are identical to the dataset used for the analysis published by Lu and Lillemo (2014)

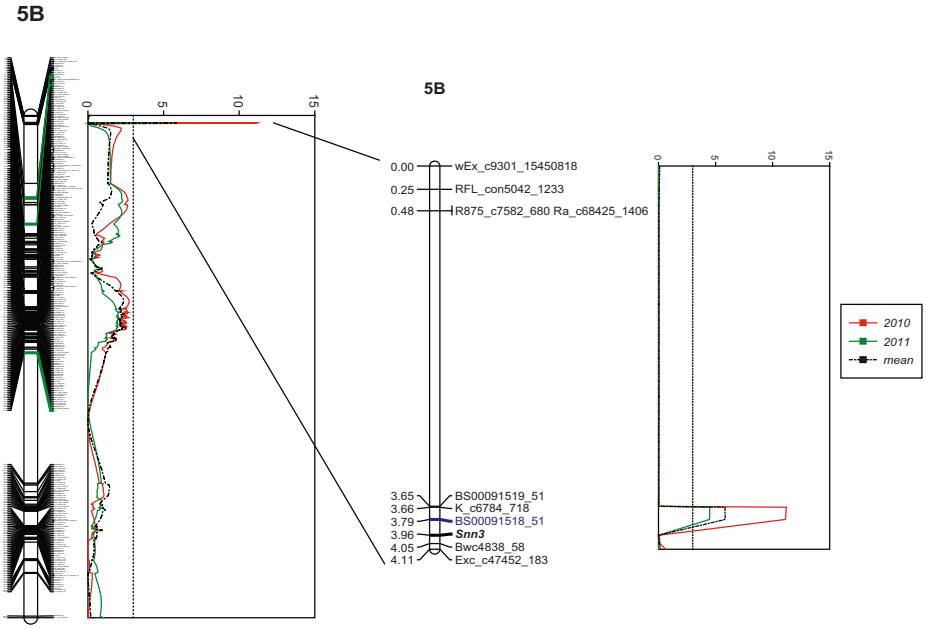


Fig. 4 Linkage group 5B with LOD curves for the major QTL for field susceptibility to SNB at the *Snn3* locus detected in the field trials at Vollebekk, Ås, Norway in 2010, 2011 and across years (mean). Genetic distances are shown in centimorgans to the left of the chromosomes. A threshold of 3.0 is indicated by a dashed vertical line in the LOD graphs. The maps are drawn in Mapchart v.2.2 (Voorrips 2002). (Color figure online)

Table 8 List of SNPs tightly linked to *Snn3* in the SHA3/CBRD × Naxos population and gene annotations based on the draft genome sequence (Mayer et al. 2014) unless otherwise noted (in hexaploid wheat within scaffold TGACv1_scaffold_423631_5BS)

SNP marker	NCBI *Triticum aestivum* gene	Rice orthologue	Function	Reference
BS00091519_51	*Traes_5BS_C460CEDFB*	*Os06g30380.1*	P-loop containing nucleoside triphosphate hydrolases superfamily protein GTP-binding domain GTPase	http://plants.ensembl.org/ (Kersey et al. 2016) http://www.uniprot.org/uniprot/Q656A4
Excalibur_c47452_183	*Traes_5BS_E0680D15E.2.path1* *TRIAE_CS42_5BS_TGACv14236631_AA1380950.1*	*Os12g44000*	Ubiquitin-conjugating enzyme 15-like Panther: Leucine-rich repeat-containing protein (PTHR23155) (Traes_5BS_E0680D15E.2.path1)	http://plants.ensembl.org/ (Kersey et al. 2016) https://urgi.versailles.inra.fr http://www.uniprot.org/ http://www.pantherdb.org/ (Mi et al. 2016)
Kukri_c6784_718	*Traes_6DL_388658304.1*	*Os05g05354*	Trypsin-like cysteine/serine peptidase domain superfamily	http://plants.ensembl.org/ (Kersey et al. 2016)
BS00091518_51	*Traes_5BS_C460CEDFB*	*Os06g30380.1*	P-loop containing nucleoside triphosphate hydrolases superfamily protein	http://plants.ensembl.org/ (Kersey et al. 2016)
BobWhite_c4838_58	100% BLAST match to *Traes_5BS_C460CEDFB*	*Os12g44000 (MSU)* *Os06g30380.1 (IRGSP)*	Coiled-coil superfamily (based on *Arabidopsis thaliana* match)	http://plants.ensembl.org/ (Kersey et al. 2016) http://rice.plantbiology.msu.edu/ http://rgp.dna.affrc.go.jp/IRGSP/
GENE-3324_338	*Traes_5AS_905D6F817.1:1*	*Os06g30380.1*	Nontranslating coding sequence (CDS) GTP-binding domain P-loop NTPase	https://urgi.versailles.inra.fr/ http://www.uniprot.org/

years and single isolates (Table 4), the correlation seems to be highest between SnTox3-producing isolates and years where *Snn3* was significant (2010, 2011 and mean). However, correlation was also significant between the SnTox3-negative isolate 201618 and the field scores in 2010, indicating that other infection mechanisms or effectors may also play a role. Interestingly, the correlation between this isolate and field resistance was negligible for all other years. Although the correlation between 201593 and 2013 was significant ($p < 0.0001$), no significant QTL were shared between the field and seedling resistance. In other words, correlation alone is a fairly rough mean to compare experiments compared to genetic analysis. Interestingly, the correlation between the North American isolate and the field trials conducted in Norway was as high as for Norwegian isolates, illustrating the global relevance of the disease and host resistance mechanisms.

Genetic mapping of *Snn3*

The markers linked to *Snn3* mapped to the telomeric end of 5BS, about 30 cM from the nearest markers in SHA3/CBRD × Naxos (Figs. 2, 4). In the consensus map (Wang et al. 2014), several markers that clustered in this distal group were not assigned to any chromosome, or mapped to different chromosomes (like *Kukri_c6784_718*, assigned to 6DL) in the different populations used to build the consensus map. The high recombination frequency in this region challenges the mapping algorithms, and we want to underline the importance of including unassigned and unmapped markers in the analysis (i.e., association mapping or linkage maps) before filtering.

We did not observe recombination between *Snn3* and the markers *BS00091518_51, BS00091519_51, BobWhite_c4838_58, Excalibur_c47452_183* or *GENE-3324_338* in the RIL lines. However, a small number of missing data points contributed to the minor distances between the markers in the map (Figs. 2, 3, 4).

Gene annotations

The SNP markers *BS00091518_51* and *BS00091519_51* are located 20 bp apart from each other in an exon of a P-loop containing nucleoside triphosphate hydrolases superfamily protein (Table 8, *Traes_5BS_C460CEDFB*, https://triticeaetoolbox.org/jbrowse). The P-loop is a common motif

in NTP-binding proteins including NBS-LRRs (Marone et al. 2013). *Excalibur_ c47452_183* is located within a gene (*Traes_5BS_E0680D15E.2.path1*) expressing a protein with leucine-rich repeats (LRR, Table 8), also a feature of the classical R-genes. The genes in which *Excalibur_ c47452_183* and *BobWhite_c4838_58* are located, corresponded to rice orthologue *Os12g44000* (http://rice.plantbiology.msu.edu/) (Table 8). This rice gene was also reported by Shi et al. (2016a). Indeed, the sequence for marker XTC266536 (Table 1) in Shi et al. (2016a) corresponded to the same gene, *TRIAE_CS42_5BS_TGACv14236631_AA1380950.1*, as *Excalibur_c47452_183* and *BobWhite_c4838_58*. Interestingly, this gene has been annotated both as an NBS-LRR (PTHR23155) and ubiquitin-conjugating enzyme.

In the case of *BobWhite_c4838_58*, the rice orthologue is identified as *Os06g30380.1* by the International Rice Sequencing Project (IRGSP) (http://rgp.dna.affrc.go.jp/IRGSP/), which corresponds to the gene in which SNPs *BS00091518_51, BS00091519_51* and possibly *GENE-3324_338* are located (Table 8). We speculate whether the orthologues in reality correspond to different motifs in the same gene, allelic or splice variants or if more than one gene belonging to the same gene family are clustered within the scaffold.

The markers *Excalibur_c47452_183, Kukri_c6784_718, BobWhite_c4838_58* and *GENE-3324_338* also co-segregate with the loose smut resistance gene *UtBW278*, conferring resistance to *Ustilago tritici* race T9 (Kassa et al. 2015). Since the *Snn* genes confer dominant susceptibility and the NE-*Snn*-interactions are described as hijacking traditional R-genes to biotrophs, it has been speculated that they may counteract with these. However, SnTox3-resistant cultivars like BR34 are also resistant to T9 (Kassa et al. 2015), while T9-susceptible lines like Sumai3 and Grandin also carry *Snn3*. Clustering of NBS-LRR genes after duplications and the following evolution through local rearrangements and gene conversions is common, as is the irregular distribution of the gene family across chromosomes (Marone et al. 2013). Screening of SnTox3-sensitivity in a wide association mapping panel of spring wheat (MASbasis) revealed that the markers are not diagnostic or that there may be more than one sensitivity locus present (data not shown). Hence, it is likely that several NBS-LRR-like genes, including *UtBW278, Traes_5BS_C460CEDFB* and *Traes_5BS_E0680D15E.2.path1* are clustered within scaffold TGACv1_scaffold_423631_5BS, and further work is needed to identify *Snn3*, potential splice variants, allelic variants and other genes within its proximity.

Author contribution statement AKR conducted seedling inoculation, culture filtrate infiltrations and validation of infiltration with purified effectors, analyzed the data from seedling experiments, refined linkage mapping of chromosome 5B in JoinMap, performed QTL mapping, reanalyzed the field data and wrote the manuscript. SW analyzed and scored the SNP genotyping results in Genome Studio and performed linkage mapping in JoinMap. TB performed linkage mapping in MSTmap and assigned linkage groups to chromosomes based on BLASTn hits. TF was responsible for seedling inoculations and infiltrations with isolate Sn4 and NOR4 and screening with purified SnToxA, SnTox1 and SnTox3. ML obtained the funding, supervised the work and edited the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Paper III

Identification of consistent loci for *Fusarium* head blight resistance in Northern European spring wheat through genome-wide association mapping

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Core ideas

GWAS of a wheat collection consisting of 299 lines with a broad genetic background could reveal eight QTL, mapped to seven chromosomes, significant for both Fusarium head blight (FHB) and deoxynivalenol resistance (DON) in three or more environments. Of these eight QTL, six coincided with QTL detected for anther extrusion (AE).

Abstract

Fusarium head blight resistance is quantitative, highly complex and divided into several different resistance types. QTL that are effective against several of the resistance types would be a valuable contribution for resistance breeding against this devastating wheat disease. A panel of 299 spring wheat lines with different geographical origin was tested in spawn-inoculated field trials and subjected to visual FHB assessment. In addition, DON level was analysed in the harvested seed. Anther extrusion (AE) was also assessed, in separate field trials. The panel was genotyped with the Affymetrix 35K SNP chip. Eight QTL, significant in three or more testing environments, were detected associated with both FHB and DON. These QTL

were detected on chromosomes 1AS, 1AL, 2BL, 3B, 4AL, 5AL, 7AS and 7BS. AE was negatively correlated with FHB and DON, and association mapping could reveal seven AE QTL that coincided with the QTL detected for FHB and DON. The lines tested in the wheat panel harboured from zero to all the detected QTL, and the results show that resistance can be significantly increased by combining several of these resistance alleles. This information enhances the possibility to select crossing parents to obtain varieties more resistant to FHB and DON.

Keywords

Fusarium head blight (FHB), Deoxynivalenol (DON), Affymetrix 35K SNP Chip, Association mapping, spring wheat

Author contributions

Susanne S. Windju performed field work, analysed the structure of the wheat collection, performed and analysed the results from the association mapping, wrote the manuscript.

Tatiana Belova filtered the markers after genotyping and assigned markers to positions. Developed the merged map of the Illumina 90K and Affymetrix 35K.

Jon Arne Dieseth executed the field design and experiment, reviewed the manuscript.

Muath Alsheikh obtained funding, supervised the work, edited the manuscript.

Morten Lillemo obtained funding, designed and led the project, designed and performed fieldwork, supervised the work, edited the manuscript.

Abbreviations

AE	Anther extrusion
DH	Days to heading
DON	Deoxynivalenol
FHB	Fusarium head blight
LD	Linkage disequilibrium
MAS	Marker assisted selection
PH	Plant height
QTL	Quantitative trait loci
SNP	Single nucleotide polymorphism
MTA	Marker-trait association
GWAS	Genome-wide association study

Introduction

Fusarium head blight (FHB) is a devastating fungal disease affecting wheat worldwide. Disease attacks can cause severe yield loss due to failed kernel development or because infected kernels are shrivelled, discoloured and light in test weight (McMullen et al., 2012). *Fusarium graminearum* which produces the mycotoxin deoxynivalenol (DON) is found to be the most causal agent of FHB in wheat (Hofgaard et al., 2016; McMullen et al., 1997). DON may cause feed refusal and poor feed weight gain in animals, and may also cause immunological problems in humans (McMullen et al., 2012). Threshold levels of DON concentration set by the European Union ranges between 200 µg/kg for processed cereal based foods and baby foods for infants and young children to 750 µg/kg for cereals intended for direct human consumption and cereal flour. For unprocessed cereals the threshold has been set to 1250 µg/kg (EC, 2006).

Resistance to FHB has been divided into active and passive resistance mechanisms. The active mechanisms are further divided into five different types; Type I: resistance against initial infection, Type II: resistance to pathogen spread in infected tissue (Schroeder et al., 1963), Type III: resistance to kernel infection, Type IV: tolerance, Type V: resistance to toxins in ears by degradation (Mesterházy, 1995; Miller et al., 1985). The passive resistance mechanisms are divided into 4 different types: Type I: plant height, Type II: presence of awns increase disease severity while absence decreases disease severity, Type III: spikelet density within the head Type IV: escape, flowering in boot stage and the ability for spikes to extrude awns (Mesterházy, 1995). The different resistance mechanisms are under quantitative control and highly influenced by the environment, making breeding for resistance in traditional ways difficult. In addition, due to incomplete understanding of factors that influence the disease development and difficulty in efficient application, use of fungicides for controlling FHB is limited (Goswami et al., 2004; McMullen et al., 1997). Breeding for disease resistance is the most cost-effective method to control this disease (Buerstmayr et al., 2002).

New marker technologies, enabling quantitative trait loci (QTL) detection, association mapping and subsequently marker detection, have the potential to enhance the resistance breeding for FHB and in addition dissect and enhance the understanding of the genetic basis of the complex resistance mechanisms.

Many QTL mapping studies for FHB resistance in wheat have been performed over the past two decades, and with the single nucleotide polymorphism (SNP) marker technology, more markers are available and it is possible to more precisely map resistance QTL in linkage maps.

These studies have been performed on different bi-parental populations and have revealed chromosomal regions harbouring FHB resistance loci. Some years ago, Buerstmayr et al. (2009) and Liu et al. (2009) compared and assembled the information from several of these studies in maps displaying interesting chromosomal regions harbouring FHB resistance QTL, which can be further tested and potentially utilised in resistance breeding. The possibility to more precisely map the resistance QTL also led scientists closer to understanding genes and mechanisms underlying the resistance trait. The *Fhb1* QTL on 3BS for FHB resistance derived from the Chinese wheat cultivar Sumai 3 has been detected in QTL studies (Waldron et al., 1999), and recently Rawat et al. (2016) reported to have cloned the gene responsible for the *Fhb1* resistance (Rawat et al., 2016).

In recent years, genome-wide- association (GWAS) mapping studies for detection of resistance QTL have been reported. The benefit of these studies is the ability to capture historic recombination events and utilize collections with a wide genetic background. This increases the possibility for breeders to detect interesting QTL for resistance in collections and enable incorporation of resistance QTL into breeding programs. In addition, new marker technologies enable the detection of thousands of SNPs in the genome. Linkage maps with markers covering more parts of the genome can be developed, and the possibility to detect a marker tightly associated with a trait is higher. This makes it possible to use markers for resistance breeding.

Both plant height (PH) and anther extrusion (AE) have been demonstrated to be negatively correlated to FHB. Skinnes et al. (2010) detected a consistent and negative correlation between AE and FHB and AE and DON in the Arina x NK93604 mapping population. A study by Lu et al. (2013) performed on the mapping population Shanghai3/Catbird x Naxos confirmed the negative correlation between AE and FHB and the QTL analysis further demonstrated the relationship; eight out of ten AE QTL detected in the study coincided with FHB severity. Kubo et al. (2013) demonstrated that partially extruded anthers were a good source for FHB infection, while rapid extrusion and ejection of the anthers contributed to the avoidance of infection by FHB. A meta- analysis performed by Mao et al. (2010) confirmed a negative association between PH and FHB, where coincident QTL for PH and FHB were detected on chromosomes 2D, 3A, 4B, 4D and 7A.

QTL for both DON and FHB can serve as a valuable source for disease resistance in wheat. SNP markers closely linked to the resistance QTL could be further tested and used in resistance-breeding for FHB and DON resistance.

The aim of this study was to identify QTL for both FHB and DON resistance in a diverse panel of 299 spring wheat lines by GWAS, to study the consistency of these QTL across environments and their association with anther extrusion.

Material and Methods

Plant material

The germplasm in the study was a collection of 299 hexaploid spring wheat accessions relevant for Norwegian spring wheat breeding. In addition to 186 Norwegian lines, the collection included 40 lines from Sweden, 37 from CIMMYT and some additional accessions from Australia, Brazil, Canada, China, Czech Republic, Denmark, Finland, France, Germany, Netherlands, Poland. Russia, Slovakia, South Africa, Switzerland, United Kingdom and USA.

Fusarium field design, inoculation and scoring

The wheat lines were planted in alpha lattice block design at two locations in Norway; Vollebekk research farm at the Norwegian University of Life Sciences, Ås (59°N, 90 m above sea level) in 2013, 2014, 2015 and Staur research farm close to Hamar (60°N, 153 m above sea level) in 2015.

Seedlings of the collection were grown in the greenhouse in Ås, Norway and genomic DNA was extracted from fresh young leaves using the DNeasy plant DNA extraction kit (Qiagen).

Spawn inoculation

To ensure good Fusarium disease pressure, spawn inoculation of the fields was performed. Grain spawn, oat kernels infected with *F. graminearum*, was prepared and distributed in the field based on a modified protocol from Dr. Bernd Rodemann, Julius Kuhn Institue, Braunchweig and as described by Lu et al. (2013). A mist irrigation (10-15 min/h) system was applied in the evenings from 19:00-23:00 every day from spawn inoculation at the booting stage to 3-4 weeks after flowering to ensure high disease pressure.

Phenotypic scoring

FHB disease assessments

FHB disease assessments were performed at the beginning of maturity, when the stems of the plants in the individual plots were turning yellow, but the head still green. At Staur research farm five random heads at three different positions in each plot were evaluated. At Vollebekk research station ten random heads at two different positions in each plot were evaluated. The evaluation was performed visually by counting the number of *Fusarium*-infected spikelets and

divide this by the total number of spikelets giving a percentage of infected spikelets in each plot.

DON measurements

The field plots were combine harvested, and the level of DON in each sample was evaluated by GC-MS at the University of Minnesota DON testing lab (Mirocha et al., 1998). Results from the DON level assessment were transformed by log(DON level +1) transformation (DON) to more normally distribute the data.

Days to heading, plant height and anther extrusion

Days to heading (DH) was scored in the same field as the Fusarium disease evaluation. DH was scored at the time where 50% of the heads in the plot had emerged. DH was evaluated in every testing environment

Plant height (PH) was evaluated in the same field as the fusarium nurseries at Vollebekk in 2013 and 2014 and Staur 2015.

Anther extrusion (AE) was evaluated at Vollebekk in 2013 in both greenhouse and small field plots and in 2014 in hill plots, and at Staur in 2014 and 2015 in hill plots in different nurseries adjacent to the fusarium disease assessment field, avoiding the confounding effects of mist irrigation on AE. AE was assessed visually by a scale from 0-9, where 0 represented no anther extrusion and 9 full anther extrusion as described by Skinnes et al. (2010).

Statistical Analysis

The FHB disease severity data were analysed using proc MIXED procedure in SAS v. 9.4 (SAS Institute Inc., Cary, NC, USA), where a correction for variation in the fields also were incorporated. Least square means of FHB disease severity, DON level, DH, PH and AE were calculated in addition to correlation between the traits using MINITAB 17 (Minitab, 2010). Analysis of variance (ANOVA) and heritability (H^2) of FHB and DON was calculated in Agrobase Generation II from Agronomix Software Inc (AgronomixSoftware, 2016).

To correct for confounding effects, regression for FHB and DON was performed in MINITAB 17 (Minitab, 2010) with DH and PH as factors for correction. The resulting residuals were used for detecting marker-trait associations.

Maps

The wheat collection was genotyped with the 35K SNP Chip from Affymetrix (Allen et al., 2017), KASP markers for key agronomic and disease resistance traits (Rasheed et al 2016), and

Illumina 90K SNP markers (Wang et al 2014) previously found to be associated with FHB resistance in wheat (Sørensen, 2016).

After genotyping with the Affymetrix 35K SNP Chip, markers were filtered based on the presence in more than 90% of wheat lines and minor allele frequency >=5%. Positional information was assigned using the consensus 35K SNP map (Allen et al., 2017).

Structure analysis

To detect the population structure of the wheat collection, STRUCURE 2.3.4 (Pritchard et al., 2000) was utilised. A simplified map of the SNP markers was made. A SNP was chosen every 5 cM on each chromosome, and a subset of 938 SNP markers were used in the analysis. The structure analysis was run on the 299 spring wheat lines in the collection. The parameters set for STRUCTURE were k1-k10, 5 000 Burnin Period and 50 000 Markov-Chain Monte-Carlo and 3 iterations per run. The model used was an admixture model.

Linkage Disequilibrium

To calculate intra-chromosomal linkage disequilibrium (LD) the software Haploview (Barrett et al., 2005) was used. The parameters used for the LD analysis in Haploview were Hardy-Weinberg p-value cut off: 1, minimum individuals genotyped: 10%, minor allele-frequency (MAF): 0.01.

Calculation of the LD over the entire genome was performed using the software TASSEL 5 (Bradbury et al., 2007). The LD was calculated with every mapped marker over the 21 chromosomes, in a sliding window approach, with 800 as the window size.

LD was calculated as the squared frequency correlation r^2 between marker pairs. The average genome-wide LD decay was visualized by plotting all intra-chromosomal r^2 values of all chromosomes against genetic distance. A critical r^2 value was set to 0.1.

Association Analysis

Association analysis was performed for the traits AE, FHB corrected for PH and DH (FHB) and DON corrected for PH and DH (DON) using the software TASSEL 5 (Bradbury et al., 2007). The analysis was done on the least square means of the data for all traits and in addition on single years for FHB and DON.

More than 48% of the reported QTL for FHB resistance are derived from Asian sources (Liu et al., 2009).

The effect of the Asian and CIMMYT line QTL might possibly mask resistance QTL not yet fully elucidated in the European and Nordic material, and there might be Asian and CIMMYT line QTL not yet fully utilised in the Nordic and European material. Therefore, the wheat population was divided into two subsets based on the results from STRUCTURE. One set of the total wheat collection and one subset consisting of the Nordic and European lines in the collection (subpop.1 and subpop., 2 with the one CIMMYT line excluded, Table 2).

Several statistical methods were tested for association mapping in TASSEL, the method chosen for the association analysis was mixed linear model (MLM) (Henderson, 1975) with kinship matrix from TASSEL and Q-matrix obtained from STRUCTURE (MLM + K +Q).

The significance threshold for marker-trait association (MTA) was set to the bottom 0.1 percentile of p- values for each trait. This approach gave p-values between $3.55*10^{-3}$ and $1.42*10^{-2}$ from association analysis in the total collection, and p-values between $5.13*10^{-3}$ and $1.96*10^{-2}$ from association analysis in the European subset of the collection.

Markers not mapped were placed on a fictive chromosome 22 in the initial association analysis. The markers associated with DH, PH, AE, FHB or DON were added to the rest of the marker set and analysed in Haploview. High LD (high r^2) between these unmapped markers and markers in the developed linkage map suggested their position. The unmapped markers were placed on the map based on these LD results, and the association analysis was run again with these markers on the linkage map. Not all unmapped markers were possible to map based on LD, these markers remained on the fictive chromosome 22 in the final association analysis.

After association analyses, markers displaying MTA in several environments for both FHB and DON, and SNP markers from the Illumina 90K SNP Chip (Wang et al., 2014) were BLASTed against the wheat pseudomolecules (the latest release from IWGCS) (www.wheatgenome.org)" to "were BLAST againt the wheat chromosome sequences (IWGSC, http://www.wheatgenome.org).

And a suggestive “merged” map was developed to possibly place the significant Affymetrix SNP markers on the map together with the Illumina 90K map (Wang et al., 2014).

Results

Phenotypic analysis

Histogram of least square means for the distribution of FHB severity, DON content, AE, PH, and DH all show continuous distribution (Fig 1). The DON values were transformed (log(DON+1)) to normalize the data.

Correlation and heritability

The FHB severity was positively correlated with DON and DH, and negatively correlated with PH and AE. DON was positively correlated with DH, and negatively correlated with PH and AE (Table 1). Heritability (H^2) calculated from the ANOVA table revealed a H^2 of 0.315 for FHB and 0.594 for DON.

35K SNP Chip analyses

After filtering and removing redundant markers, in total 14095 markers were remained for the association mapping. The map covered all chromosomes (Supplementary Table 1 in supplementary material: Map of the spring wheat association panel with 14095 mapped markers and 41 unmapped markers assigned to fictive chromosome 22).

Population structure

Results of the populations structure analysis showed K=2 or K=3 as the most likely subdivision (Fig. 2, Table 2). For K = 2, one subpopulation consisted of lines mainly from CIMMYT. The second subpopulation consisted mainly of lines from Norway and Sweden. For K = 3, the group of mostly Norwegian and Swedish lines split in two, with one groups consisting almost exclusively of Norwegian lines and the other consisting mostly of lines from Norway and Sweden, while the group of mostly CIMMYT lines remained the same (Table 2). The Q-matrix for K =3 was used in the downstream studies because any more subdivision only divided the Norwegian lines into more subpopulations.

Linkage disequilibrium

A rapid LD decay was observed in all chromosomes of the spring wheat panel (Supplementary fig. S1) LD decay in the A genome spanned form 1-5 cM, in the B genome from 1-10 cM and in the D- genome from 2-15 cM (Supplementary fig S1). The genome-wide half-decay of LD was calculated to 1 cM. (Fig. 3).

Association mapping

The association mapping for AE was performed on the lsmeans of the data from all environments.

AE

Total spring wheat panel

For mean AE data over all years, the MTA p-value threshold was set to $7.82*10^{-3}$. Chromosomes 1A, 1B, 1D, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 5A, 5B, 5D, 6A, 7A, 7B, 7D all harboured QTL associated with AE, and in addition the unmapped marker IAAV5302 was also associated to AE. (Fig. 4, Supplementary Table 2). Chromosomes 2A, 2B, 3B and 6A had QTL above the -log10(p) = 3 threshold (Fig. 4, Supplementary Table 2).

European and Nordic subpopulation

The MTA threshold was set to $8.03*10^{-3}$. Chromosomes 1A, 1B, 1D, 2A, 2B, 2D, 3A, 3B, 5A, 5B, 5D, 6A, 6B, 7B and 7D displayed significant QTL for AE. And in addition, the unmapped marker BS00022459_51 was also associated with AE. The chromosomes with -log10(p)-values above 3 were 1D, 2A, 2D and 6A for the mean AE data (Fig. 4, Supplementary Table 2).

FHB and DON

Association mapping was performed on FHB data corrected for PH and DH and transformed DON data corrected for DH and PH year by year to study the consistency of QTL across field trials. The association mapping was also performed on the mean data over all trials for these traits. For simplicity, FHB corrected for PH and DH, and transformed DON corrected for PH and DH, will hereafter be referred to as FHB and DON.

Association mapping results detected QTL on many chromosomes and several environments. In the total wheat collection, twelve QTL on nine chromosomes, significant in more than three environments were detected for FHB (Fig 5). For DON, fifteen QTL on ten chromosomes were detected that were significant in three or more environments (Fig. 5) (Supplementary Table 4 for description of the QTL and Supplementary Table 3 for markers associated with the different QTL). In the European subpopulation, 11 QTL were detected on 10 chromosomes that were significant in three environments both in the results for FHB and DON (Fig. 6) (Supplementary Table 6 for description of the QTL and Supplementary Table 5 for markers associated with the different QTL).

Description of the association mapping results will focus on QTL detected in three or more environments for both traits. A summary of the QTL and the environments are listed in Table 3. Details for each QTL are described in Supplementary Table 4 and 6.

1A

On chromosome 1A, two QTL were detected. One QTL was mapped to the 49-59 cM interval. In the total population, this QTL was significant for FHB in 2013, 2014, 2015Vb and 2015St. For DON, this QTL was detected as significant in the mean results and in the results from 2013, 2014, 2015Vb and 2015St. This QTL was significant only in 2015Vb for FHB and 2015Vb and 2015St for DON in the European subpopulation. In the European subpopulation, an AE QTL was detected in this area of the 1A map. The second QTL detected on chromosome 1A, was mapped to the 74-77 cM region. In the total wheat population, this QTL was significant for FHB in the mean data, 2013, 2014 and 2015St. For DON in the total population this QTL was detected as significant in the mean data, 2013, 2014 and 2015Vb. A QTL was also detected for AE in this area of the map in the total population. In the European subpopulation, this 1A QTL mapped to 74-77 cM was significant in the mean dataset, 2013, 2014 and 2015Vb for FHB. For DON, this QTL was significant in the mean dataset and in 2014Vb. Also in the European subpopulation, a QTL for AE was detected as significant in this area of the map.

2B

On chromosome 2B, a QTL was detected in the 102-105 area of the map. This QTL was consistently significant for both FHB and DON in both population sets. The only environment this QTL was not detected as significant was for DON in 2014. In both population sets; a QTL for AE was also detected in this area of the map.

3B

On chromosome 3B a QTL was detected in the 85-89 cM area of the map. In the total wheat population, this QTL was significant for FHB in the mean data, 2013, 2015Vb and 2015St. For DON, this 3B QTL was detected as significant in all testing environments and in the mean dataset. In the European subpopulation, this QTL was significant for FHB in the mean dataset, in 2013, 2015Vb and 2015St. For DON, this QTL was significant all testing environments in the European subpopulation. For AE, a QTL was detected in the 85-90 cM area of 3B in the association mapping in the total population and in the European subpopulation.

4A

On 4A, a consistent QTL was detected in the 73-78 cM area of the map. In the total population, this QTL was significant for FHB in the mean data, in 2013, 201 and 2015St. For DON, this QTL was significant in the mean data, in 2013, 2014 and 2015St in the results from the association mapping of the total population. In the European subpopulation, this QTL was significant for FHB in 2013, 2014 and 2015St. For DON, this QTL was detected as significant for DON in the mean dataset.

5A

On 5A, a QTL in the 69-82 cm of the map was detected as significant for FHB and DON in all testing environments and in the mean data. A QTL for AE was also detected in this area of the map in the results from the total population. In the European subpopulation, this QTL was significant in 2013, 2014 and 2015Vb for FHB and in the mean data, 2013 and 2014 for DON.

7A

On chromosome 7A, a QTL in the 25-38 cm area was significant in all testing environment and in the mean data for both FHB and DON in the total population and in the European subpopulation. Also, a QTL for AE was detected in this area of the map in the total population.

7B

On 7B, in the 27-33 cM area of the map, a QTL was significant for FHB in 2014 and 2015St and for DON in 2013, 2014, 2015Vb and 2015St in the total population. In the European subpopulation, a QTL in this area was detected in 2014, 2015Vb and 2015 St for FHB and for DON in the mean data and all testing environments. QTL for AE were detected in this area of 7B in both the total population and in the European subpopulation.

The GWAS results revealed eight QTL that were significant more than three environments for both DON and FHB (Table 3). For FHB, the number of resistance alleles in the lines had an additive effect on resistance. A higher resistance was observed in the lines containing six of the resistance alleles compared to the lines containing one resistance allele (Fig. 7a). The same effect was observed for DON. An enhanced resistance was observed in the lines displaying six QTL compared to the lines containing zero or one of the resistance alleles (Fig. 7b). More resistance alleles present in the lines gave in general better resistance. A plateau seemed to be reached at the accumulation of six resistance alleles for both FHB and DON (Fig. 7 a and b) in the material tested. The Nordic breeding lines displayed the highest level of resistance both for

FHB and DON. Different lines and varieties in the mapping panel, together with resistance alleles and frequencies are listed in Supplementary Table 7.

The frequencies of each of the eight resistance alleles varied in the population. The allele frequency of the 1AS QTL was the highest with 90% of the lines carrying the allele associated with resistance. The 7BS allele frequency was the lowest in the population, with only 11% of the lines carrying the resistance allele (Supplementary Table 8).

Discussion

Correlation

Days to heading has been reported to be negatively correlated with FHB (Emrich et al., 2008). In the present study correlation between DH and DON was slightly positive and significant, while the correlation between DH and FHB was very low and positive, and not significant. A negative correlation between DH and DON and FHB may hamper association results, because in wheat breeding one is after the lines heading sooner, not later. Even though we observed a slightly positive correlation, we decided to correct both DON and FHB for DH in the association analyses to avoid any confounding effects of this trait.

Also, PH has been reported to be associated with FHB (Kubo et al., 2013; Lu et al., 2013; Mao et al., 2010). The correlation analyses in the present study further confirm these reports, both FHB and DON were negatively correlated to PH. In wheat production, the taller plants may lodge when fertilized and taller plants may also make use of modern machines difficult, the preferred plants are therefore the lower ones. To avoid the effect of PH, this trait was also corrected for with in the association analysis.

Correlation between AE and FHB and AE and DON were both negative in the wheat collection. High anther extrusion has been proposed to be a valuable escape trait to avoid FHB infection. Many studies have reported the correlation between AE and FHB and DON (Buerstmayr et al., 2015; He, Lillemo, et al., 2016; He, Singh, et al., 2016; Kubo et al., 2013; Lu et al., 2013; Skinnes et al., 2010). Skinnes et al. (2010) detected a lower FHB and DON infection rate in lines with high AE, and suggested high AE to contribute to Type I resistance. They suggested that lines with low FHB could be achieved by selecting the genotypes that displayed high anther extrusion. But they also pointed to that lines that shed anthers well, also got infected by FHB. These findings were further confirmed by Lu et al. (2013). They reported AE to be positively correlated with Type I resistance to FHB in a biparental mapping population. Also, they detected several AE QTL to coincide with FHB QTL. In the association mapping in the present

study, of the eight QTL detected as significant for both FHB and DON, seven coincided with AE QTL. These findings further validate the correlation between AE and FHB and DON. Therefore, searching for genotypes with high AE, could possibly be a valuable contribution to the resistance breeding for FHB.

Several studies have reported correlation between FHB and DON in wheat both in segregating material and in collections of varieties with different resistance level (Bai et al., 2001; Hofgaard et al., 2016; Ji et al., 2015; Miedaner et al., 2003; Snijders, 2004). The correlation between FHB and DON in the present study was significant and positive with a correlation coefficient of 0.747. Reports of the correlation between FHB and DON suggests a complicated relationship, Bai et al. (2001) performed a study with 116 cultivars and breeding lines of wheat. The results from their study show that cultivars moderately resistant and moderately susceptible to FHB usually had higher DON levels than resistant cultivars, but that there also were exceptions, especially for cultivars with a moderate Type II resistance. In the present study, we assume to have assessed a combination of both Type I and Type II FHB resistance in the field, because the field scoring was performed at a late stage in the development of the plants when both the initial infection (Type I) and spread (Type II) had occurred.

Heritability

Heritabilities for FHB and DON were low, especially for FHB, with a calculated H^2 of 0.315. The heritability of DON was calculated to be 0.594. The QTL mapping for FHB revealed many QTL over all chromosomes each year, but only few of these were consistent in three or more environments. For DON, the disease assessment is performed by GC-MS, and probably not subjected to the same rate of error as the visual scoring of FHB in the field. The QTL mapping for DON also revealed many QTL in each environment, but only few that were consistent over three or more environments. These findings suggest that also in the spring wheat panel tested in our study, G x E interactions play a significant role. The QTL that were detected and significant in three or more environments for FHB and DON are therefore very interesting for resistance breeding.

Population structure

The population structure analysis displayed both 2, 3 and 7 subpopulations as possible solutions. The division into 7 subpopulations would divide the Norwegian lines into many different populations, this was evaluated as a too stringent solution. The results from K=2 subpopulations were evaluated to be a too mild subdivision because, it left many of the European lines separated between the two subpopulations. For the association analysis, the

division into 3 subpopulations was utilised. It grouped the geographically distinct CIMMYT and Asian lines into one subpopulation, separated from the European lines.

Association mapping

After analysing the Affymetrix SNP linkage map of the spring wheat panel and the merged map of the significant Affymetrix and 90K Illumina SNPs, the chromosome arm positioning of the QTL was suggested where that was possible.

Many of the FHB resistance QTL are derived from Asian sources (Buerstmayr et al., 2009; Steiner et al., 2017). Association mapping was therefore performed on two different sets of the wheat collection in the present study; one was performed on the whole collection and the other on a subset consisting of the Nordic and European lines. This was done to possibly detect resistance sources within the adapted material that might not appear when performing association analysis on the total collection of lines. And with this approach it was also possible to detect QTL in the exotic material not yet integrated into the European or Nordic material. These QTL will be interesting for breeders to evaluate and possible utilise in resistance breeding.

Being highly influenced by the environment, the resistance QTL need to be consistent across several environments to be interesting for breeding. QTL that are significant across multiple locations or years for both FHB and DON will be valuable for developing new more resistant varieties. Studying the association results for each environment many QTL turned up as significant. When comparing every environment and their significant QTL, many of these QTL were only significant for one or two environments.

Comparisons with previous reports

Previous studies have reported QTL for FHB resistance on all 21 chromosomes of hexaploid wheat (Buerstmayr et al., 2009; Liu et al., 2009).

Chromosome 1A has been reported to harbour QTL from several sources. We detected two QTL on 1A in the present study. One on the short arm of 1A and one on the long arm of the chromosome.

The Chinese source CJ9306, a derivative of Sumai 3, has been shown to harbour resistance QTL for Type II (disease spread within spike) and III (toxin accumulation) resistance (Buerstmayr et al., 2009; Jiang, Dong, et al., 2007; Jiang, Shi, et al., 2007; Liu et al., 2009).

QTL for Type II resistance on 1AS have been detected in the lines Pirat (Holzapfel et al., 2008) and Wheaton (Liu et al., 2009; Yu et al., 2007).

On chromosome 1AL, there has been a report of a QTL originating from the Norwegian breeding line NK93604, associated with Type III resistance (Toxin accumulation) (Semagn et al., 2007).

Mapping studies have revealed several QTL for FHB on the 2B chromosome. On 2BL, the lines Dream (Schmolke et al., 2005) and Ning 7840 (Zhou et al., 2002) have been reported to harbour QTL for Type II resistance and Goldfield have been reported to harbour QTL for Type I resistance (Gilsinger et al., 2005). Chromosome 3B harbours the *Fhb1* gene. This gene has recently been cloned and shown to encode a pore-forming toxin-like (PFT) domain (Rawat et al., 2016). This gene is mapped to the short arm of 3B (Buerstmayr et al., 2009; Liu et al., 2009; Rawat et al., 2016). In the present study, the 3B chromosome linkage map developed of the wheat collection after genotyping with the Affymetrix 35K SNP Chip (Allen et al., 2017), spanned from 0 to 246 cM. The QTL we detected mapped in the 72-88 cM interval, which might correspond to the centromeric region of 3B where Type II resistance has been mapped in the lines Wangshuibai (Yu et al., 2007; Zhou et al., 2004) , Massey (Liu et al., 2009), Arina (Paillard et al., 2004) , Ernie (Abate et al., 2008; Li et al., 2008) and Apache (Holzapfel et al., 2008).

On chromosome 4AL, the lines Pirat (Holzapfel et al., 2008) and Arina (Paillard et al., 2004) have been reported to harbour QTL for Type II resistance.

On 5AL, there have been reports of several lines and varieties harbouring QTL. Renan (Gervais et al., 2003), Arina (Paillard et al., 2004), Apache and Pirat (Holzapfel et al., 2008) all have been reported to have QTL for type II resistance.

On 7AS the line Frontana (Mardi et al., 2006) have been reported to have a QTL associated with Type II resistance.

There are several reports of QTL on 7BS. The lines CJ9306 (Jiang, Dong, et al., 2007), Ning8026 (Häberle et al., 2009), Cansas (Klahr et al., 2007), Dream (Häberle et al., 2007; Schmolke et al., 2005) and Rubens (Holzapfel et al., 2008) have been reported to harbour QTL for Type II resistance on this chromosome arm.

Resistance breeding

In this study, many lines and varieties have been identified that harbour one or several of the eight consistently significant QTL for FHB and DON. The identification of which of the lines harbours which resistance allele gives a possibility to choose crossing parents. This will make accumulation of the correct resistance alleles possible and potentially segregate offspring with higher resistance. Some of the Nordic breeding lines displayed a high level of resistance, even though these lines not always displayed most resistance alleles. This is possibly because these lines are more adapted to the Nordic climate. The known resistance sources Sumai 3 and CJ 9306 both contained 6 and 7 resistance alleles for FHB and 5 and 6 for DON respectively, but still we could find breeding lines that displayed levels of resistance comparable to these. FHB is highly influenced by the environment, and a better adaptation to the environment gives better resistance. Accumulation of resistance alleles alone will not be enough to develop the best resistance material, one must also always consider the environmental factor.

The resistance allele detected on 1AS had a frequency of 0.9. This allele has been utilised greatly in breeding for resistance. The resistance allele at 7AS, had a frequency of 0.5 for DON and 0.22 for FHB, with different markers associated with the traits. This 7AS QTL has a potential to be better utilised in resistance breeding. Several of the Nordic breeding lines with the highest resistance did not harbour the 2BL QTL for FHB or DON. It would be very interesting to incorporate this QTL into more of the Nordic material.

Combination of several resistance alleles for FHB and DON was shown to give a positive effect for the resistance against both FHB and DON. Boxplot in Fig. 7 displays the effect of stacking from zero to eight of the resistance alleles detected in the tested material. The resistance seems to reach a plateau at six QTL, where adding more of the detected QTL in the present material does not result in any measurable improvements in resistance.

With the information of which of the lines in the spring wheat panel are containing which resistance allele, it would be possible to select crossing parents more accurate and, after testing the SNP markers associated with the resistance alleles, to be able to screen which offspring contain which resistance allele.

QTL in the present study can be utilised using MAS in the breeding program after testing and validation of associated SNP markers. Together with an additional phenotypic evaluation in the field, accumulation of the minor QTL not detected in the GWAS could further contribute to the

resistance. A selection of lines with high AE would indirectly add to the resistance against FHB and DON, these traits are correlated and several of the QTL for FHB, DON and AE coincide.

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Figure captions:

Figure1 Frequency distribution of FHB, transformed DON, AE, PH and DH. The histograms are based on the mean frequency data over all testing environments.

Figure2 Population structure in the 299 spring wheat panel. The clustering divided the 299 spring wheat panel into 3 subpopulations.

Figure 3 Genome-wide LD decay

Figure 4 Association mapping of AE mean data in the a) total wheat collection and in the b) European and Nordic subpopulation. Dots above the horizontal line represents MTA with p-value below the 0.01 percentile, and are considered significant.

Figure 5 Association mapping of FHB and DON in the total wheat panel. Dots above the horizontal line represents MTA with p-value below the 0.01 percentile, and are considered significant.

a) FHB_mean data f) DON_mean data

b) FHB_2013 Vollebekk g) DON_2013 Vollebekk

c) FHB_ 2014 Vollebekk h) DON_2014 Vollebekk

d) FHB_2015 Vollebekk i) DON_2015 Vollebekk

e) FHB_2015 Staur j) DON_2015 Staur

Figure 6 Association mapping of FHB and DON in the European subpopulation. Dots above the horizontal line represents MTA with p-value below the 0.01 percentile, and are considered significant.

a) FHB_mean data f) DON_mean data

b) FHB_2013 Vollebekk g) DON_2013 Vollebekk

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d) FHB_2015 Vollebekk i) DON_2015 Vollebekk

e) FHB_2015 Staur j) DON_2015 Staur

Figure 7 Effect of number of QTL for a) FHB corrected for DH and PH and b) tDON corrected for DH and PH

Tables

Table 1 Pearson correlation coefficients calculated on the mean data among FHB, tDON, AE, DH and PH.

Correlation		
	tDON	**FHB**
FHB	0.747**	
DH	0.210**	0.021
PH	-0.323**	-0.374**
AE	-0.441**	-0.519**

** 0.0001 level

Table 2 Structure results. Composition and origin of lines in the 3 subpopulations

K=3					
Origin	**Nr.**	**Origin**	**Nr.**	**Origin**	**Nr.**
Subpop. 1 (K1)		**Subpop. 2 (K2)**		**Subpop. 3 (K3)**	
Finland	2	CIMMYT	1	Australia	5
Norway	100	Finland	2	Brazil	1
Sweden	2	Germany	5	Canada	1
		Norway	82	China	7
		Poland	2	CIMMYT	36
		Slovakia	1	Czech. Republic	1
		Sweden	38	Finland	2
		Switzerland	1	Norway	4
		UK	1	South Africa	1
				UK	1
				USA	3

Table 3 QTL significant in three or more environments for both FHB corrected for PH and DH and tDON corrected for PH and DH

Chrom	cM	Significant traits total population			Significant traits European sub-population		
		FHB	DON	AE	FHB	DON	AE
1A	49-59	2013, 2014, 2015Vb, 2015St	Mean, 2013, 2014, 2015Vb, 2015St	-	2015Vb,	2015Vb, 2015St	Mean
1A	74-77	Mean, 2013, 2014, 2015St	Mean, 2013, 2014, 2015Vb	Mean	Mean, 2013, 2014, 2015Vb, 2015St	Mean, 2014Vb	Mean
2B	102-105	Mean, 2013, 2014, 2015Vb, 2015St	Mean, 2013, 2015Vb, 2015St	Mean	Mean, 2013, 2014, 2015Vb, 2015St	Mean, 2013, 2014, 2015Vb, 2015St	Mean
3B	85-90	Mean, 2013, 2015Vb, 2015St	Mean, 2013, 2014, 2015Vb, 2015St	Mean	Mean, 2013, 2015Vb, 2015St	Mean, 2013, 2014, 2015Vb, 2015St	Mean
4A	73-78	Mean,2013, 2014, 2015St	Mean, 2013, 2014, 2015St	-	2013, 2014,2015St	Mean	-
5A	69-82	Mean, 2013,2014,2015Vb, 2015St	Mean, 2013,2014,2015Vb, 2015St	Mean	2013, 2014, 2015Vb	Mean, 2013, 2014	-
7A	25-38	Mean, 2013,2014,2015Vb, 2015St	Mean, 2013,2014,2015Vb, 2015St	Mean	Mean, 2013, 2014, 2015Vb, 2015St	Mean, 2013, 2014, 2015Vb, 2015St	-
7B	27-33	2014, 2015St	2013,2014,2015Vb, 2015St	Mean	2014, 2015Vb, 2015St	Mean, 2013, 2014, 2015Vb, 2015St	Mean

Figures

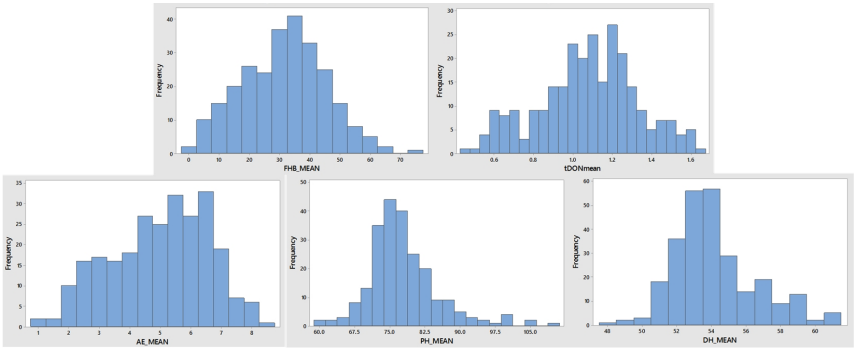


Figure 1. Frequency distribution of FHB, transformed DON, AE, PH and DH. The histograms are based on the mean frequency data over all testing environments.

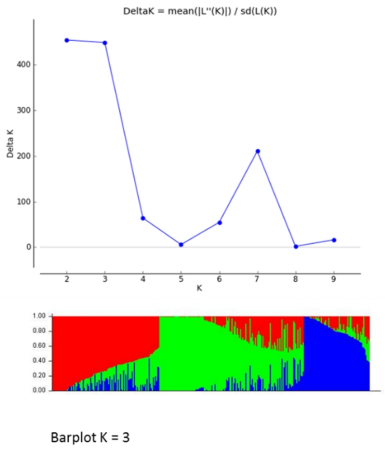


Figure 2. Population structure in the 299 spring wheat panel. The clustering divided the 299 spring wheat panel into 3 subpopulations.

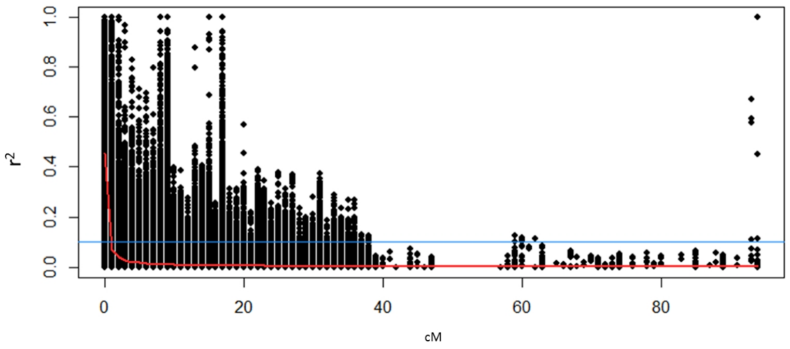


Figure 3. Genome-wide LD decay.

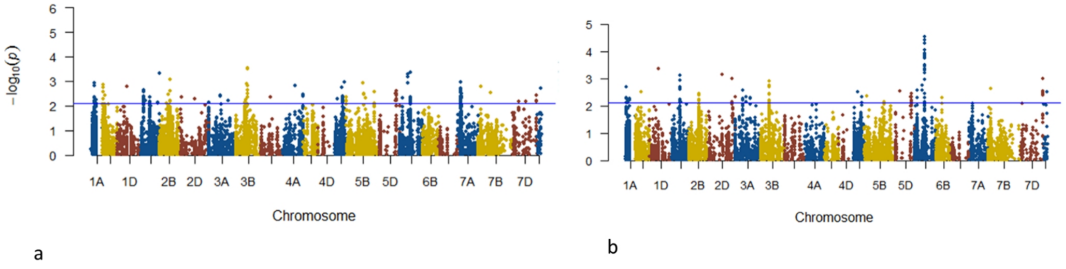


Figure 4. Association mapping of AE mean data in the a) total wheat collection and in the b) European and Nordic subpopulation. Dots above the horizontal line represents MTA with p-value below the 0.01 percentile, and are considered significant.

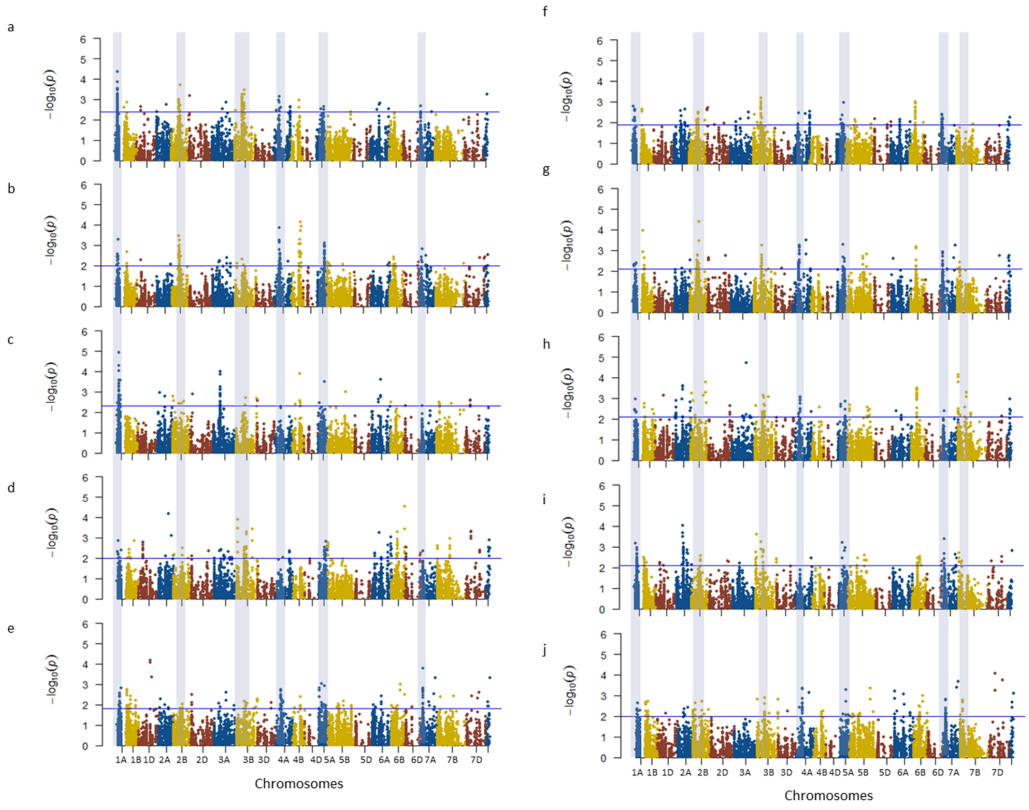


Figure 5. Association mapping of FHB and DON in the total wheat panel. Dots above the horizontal line represents MTA with p-value below the 0.01 percentile, and are considered significant.

a) FHB_mean data

b) FHB_2013 Vollebekk

c) FHB_ 2014 Vollebekk

d) FHB_2015 Vollebekk

e) FHB_2015 Staur

f) DON_mean data

g) DON_2013 Vollebekk

h) DON_2014 Vollebekk

i) DON_2015 Vollebekk

j) DON_2015 Staur

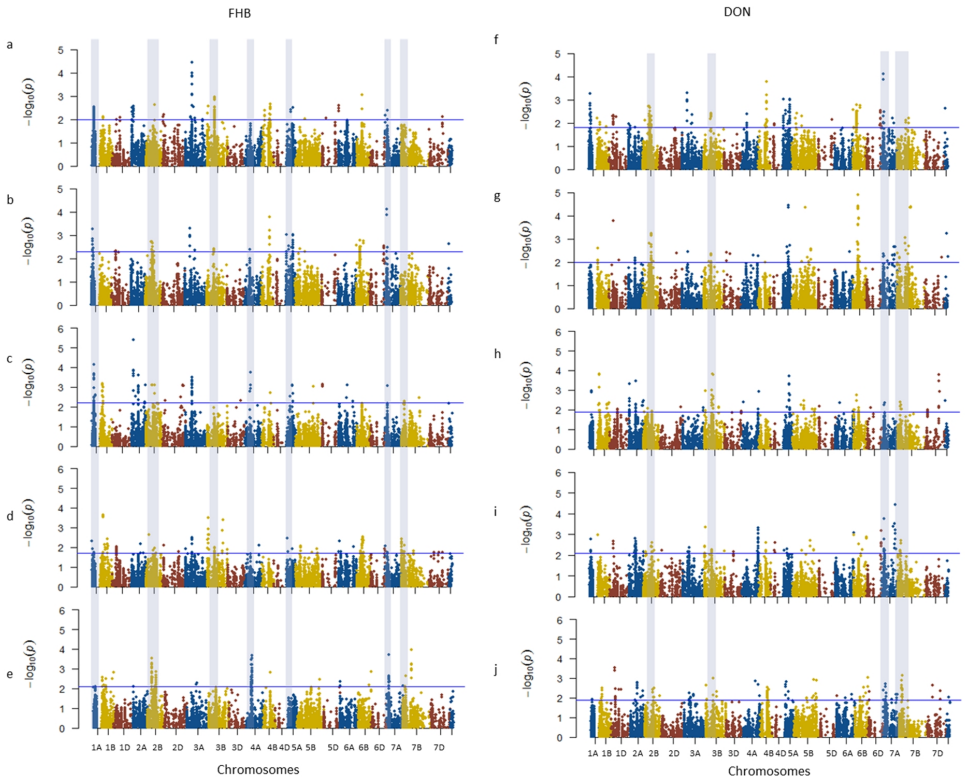


Figure 6 Association mapping of FHB and DON in the European subpopulation. Dots above the horizontal line represents MTA with p-value below the 0.01 percentile, and are considered significant.

a) FHB_mean data

b) FHB_2013 Vollebekk

c) FHB_ 2014 Vollebekk

d) FHB_2015 Vollebekk

e) FHB_2015 Staur

f) DON_mean data

g) DON_2013 Vollebekk

h) DON_2014 Vollebekk

i) DON_2015 Vollebekk

j) DON_2015 Staur

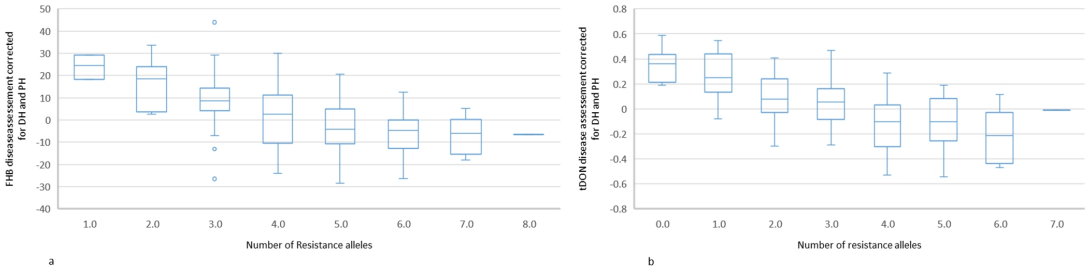
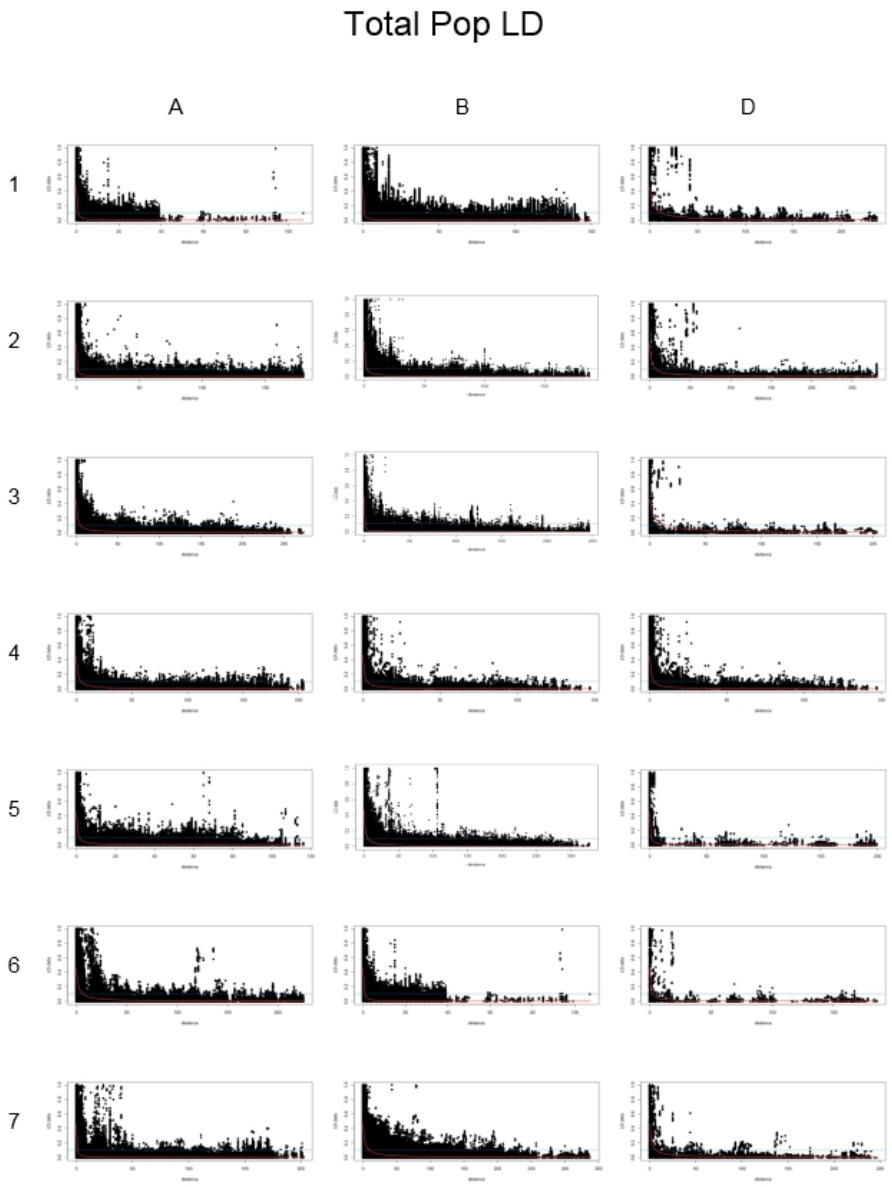


Figure 7 Effect of number of QTL for a) FHB corrected for DH and PH and b) tDON corrected for DH and PH

Supplementary material



Supplementary Figure 1. LD decay in the different chromosomes in the spring wheat collection.

Supplementary Table 2. Results association mapping of the mean AE data a) in the total spring wheat collection and b) in the European subpopulation

Total population AE_mean			
Chrom	cM	Phenotypic variation (%)	-log10(p-value)
1A	74	2.9-4.4	2.1-2.9
1A	89	3.8-3	2.1-2.4
1B	8.0-12.0	3.1-4.1	2.3-2.8
1B	25	3.4	2.4
1D	96	4.3	2.8
2A	20-24	2.9-3.8	2.2-2.7
2A	41	3.1	2.2
2A	84	2.9-3.2	2.1-2.3
2A	159	2.8-3	2.1
2A	176	5	3.3
2B	83	3.5	2.5
2B	104-105	2.9-5.4	2.2-3.1
2D	26	3.4	2.4
2D	161	3.1	2.3
3A	2	2.9	2.1
3A	116-117	3.3	2.4
3A	198-199	3	2.2
3B	85	2.9	2.1
3B	98-110	3.1-5.3	2.3-3.5
3D	92	3.4	2.4
4A	133	4	2.8
4A	215	3.3-3.5	2.4
5A	22	3.1	2.3
5A	68-75	2.8-3.9	2.1-2.8
5A	94	4.3	3
5B	0	3-3.3	2.2-2.4
5B	164-170	3.4-4.4	2.5-2.9
5B	185	3.2	2.3
5B	274	2.9-3.6	2.1-2.6
5D	160-164	3-3.7	2.2-2.6
6A	27	3.1-3.2	2.3
6A	78	4.2-4.8	3-3.3
6A	101	2.8-5.1	2.1-3.6
7A	26-38	2.9-3.9	2.1-3
7B	30	3.9	2.8
7B	120	4.1	2.5
7D	63	3	2.2
7D	133	2.9	2.2
7D	237	2.9-3.4	2.2-2.4
IAAV5302	-	3.9	2.7

Nordic and European subpopulation AE_mean			
Chrom	cM	Phenotypic variation (%)	-log10(p-value)
1A	54-55	3.9-4.8	2.1-2.7
1A	74	3.6-3.7	2.1-2.2
1A	89	3.9	2.2-2.3
1B	59	4.4	2.5
1D	96	7.3	3.4
2A	80-91	3.5-5.5	2.1-3.1
2B	104-105	3.7-4.7	2.2-2.5
3A	161	5.9	3.1
3A	266	3.6-5.5	2.1-3
3A	295	4	2.3
3B	84	3.7-4.6	2.2-2.8
	116-117	3.7-4	2.1-2.3
3B	161	3.9	2.3
3D	85	3.5-5.6	2.1-2.9
5A	43	4.4	2.5
5A	87-88	3.6-4	2.1-2.3
5B	24	4.2	2.3
5B	208	3.6	2.1
5D	44	4.4	2.5
5D	160-164	3.6-4.3	2.1-2.5
6A	27	4-4.5	2.3-2.6
6A	78	4.5-4.8	2.6-2.7
6A	104	3.6-9.4	2.1-4.5
6B	63	4	2.3
7B	30	4.6	2.6
7D	18	3.5	2.1
7D	237	4.3-5.6	2.4-3
BS00022459_51	-	4.3	2.5

Supplementary Table 4. MLM results association mapping of a) FHB and b) DON in the total wheat collection.

a	FHBmean			2013Vb_FHB			2014Vb_FHB			2015Vb_FHB			2015St_FHB		
Chrom.	cM	Phenotypic variation (%)	-log10 (p-value)	cM	Phenotypic variation (%)	-log10 (p-value)	cM	Phenotypic variation (%)	-log10(p-value)	cM	Phenotypic variation (%)	-log10(p-value)	cM	Phenotypic variation (%)	-log10(p-value)
1A	-	-	-	52-59	3.8	2	54	4.2	2.5	49-55	4.1-5.6	2.2-2.8	59	4.1	2
1A	74	4.0-8.5	2.4-4.3	74	3.9-7.0	2.1-3.3	74-75	3.6-9.5	2.3-4.9	-	-	-	74-76	3.7-5.7	1.9-2.6
1A	-	-	-	-	-	-	85	4.0-6.3	2.4-3.6	91	4.8	2.4	80-90	4.7-6.2	2.0-2.8
2B	102-105	4.7-6.5	2.7-3.7	105	5.1	2.4	105	2.4	2.4	105	4.1-4.8	2.1-2.5	102-105	4.0-4.5	1.9-2.0
3A	-	-	-	73-78	4.2-4.4	2.2	78-84	3.7-7.7	2.3-4.0	84	4.6-4.7	2.4	73	4	1.9
3B	85	4.0-5.6	2.5-3.2	78	5.4	2.3	-	-	-	85-88	4.0-5.1	2.2-2.5	86	4.5	2.2
3B	98-110	4.1-6.0	2.5-3.5	98	3.8	2	104-109	3.9-4.6	2.4-2.7	98-110	4.0-6.9	2.2-3.3	102	3.9-4.5	1.9-2.1
4A	75-78	4.9-5.3	2.5-3.1	73-78	4.0-8.3	2.1-3.3	78	3.6	2.3	-	-	-	75-78	3.9-6.1	1.9-2.7
4B	97	4.2-4.9	2.6-2.9	92-97	4.0-6.5	2.1-3.1	87-97	3.7-6.9	2.3-3.9	-	-	-	87-88	3.8-5.1	1.9-2.4
5A	82	4.0-4.5	2.5-2.6	82	4	2.1	79	4	3.5	71-72	3.9-5.0	2.1-2.5	72-82	3.6-6.5	1.8-2.9
6B	-	-	-	2	3.8	2	2	4.2	2.5	-	-	-	2	4.1	2
7A	30	4.5	2.7	27-35	4.5-5.7	2.3-2.8	30	3.9	2.3	30	4.7	2.4	25-30	3.7-9.1	1.9-3.8

b	tDONmean			2013Vb_tDON			2014Vb_tDON			2015Vb_tDON			2015St_tDON		
Chrom.	cM	Phenotypic variation (%)	-log10(p-value)	cM	Phenotypic variation (%)	-log10(p-value)	cM	Phenotypic variation (%)	-log10(p-value)	cM	Phenotypic variation (%)	-log10(p-value)	cM	Phenotypic variation (%)	-log10(p-value)
1A	52-59	3-4- 4.6	2.1-2.8	52	4.7	2.3	52-54	4.1	2.4-2.5	52	6.6	3.2	52-59	4.3-5.6	2.1-2.6
1A	74	3.1-4.2	2.1-2.7	74-77	5.7-5.0	2.3-2.4	72-74	3.7-3.9	2.3	72-74	4.0-6.2	2.1-3.0	-	-	-
1B	8	4.0-4.2	2.5-2.6	8	6.2-8.7	2.9-4.0	16	4.1-5.1	2.6-2.8	8	4.0-4.9	2.1-2.4	8	4.1-5.8	2.0-2.7
1B	-	-	-	36	4	2.1	-	-	-	32	4.2	2.2	35-39	4.1-5.8	2.0-2.7
2A	75-83	2.7-4.1	1.9-2.6	83	4.4	2.1	73-83	5.1-6.9	3.0-3.6	72-84	4.2-7.6	2.1-4.0	73-83	4.3-4.8	2.1-2.3
2B	102-105	2.8-4.0	1.9-2.5	102-105	4.6-10.1	2.1-4.4	-	-	-	102-105	4.6-4.9	2.4-2.5	105	4.6-5.8	2.3-2.7
3B	8	3.9	2.4	-	-	-	8	4.4	2.6	1	3.9-7.5	2.1-3.6	1	6.1	2.8
3B	85	2.7-5.4	1.9-3.2	85	4.0-6.9	2.1-3.2	85	3.4-3.9	2.2-2.4	85	4.2-5.7	2.3-2.9	85	4.1-6.5	2.0-2.9
4A	73-78	2.7-3.9	1.9-2.4	75-78	4.2-7.0	2.2-3.3	75-78	3.7-5.2	2.3-3.1	-	-	-	78	4.3-7.5	2.1-3.5
4A	210-214	2.8-4.0	1.9-2.5	214	4.2	2.1	215	3.8	2.4	206	4.6	2.4	-	-	-
5A	72-78	2.8-5.0	1.9-3.0	72	6.9	3.3	82	3.3-4.8	2.1-2.9	72-79	5.5-6.9	2.8-3.0	72	5.7-7.3	2.7-3.3
6B	63	2.9-5.0	1.9-3.0	60-63	4.3-6.9	2.2-3.2	60-63	3.3-5.9	2.1-3.5	63	4.0-4.6	2.2-2.4	63-75	4.0-5.5	2.1-2.5
7A	30-37	2.8-3.3	1.9-2.4	30-31	4.4-6.0	2.3-2.9	38	3.8	2.4	30	4.0-6.9	2.2-3.4	30-38	4.1-6.2	2.1-2.8
7A	174	2.7	1.9	174	7.2	3.2	-	-	-	174	4.7	2.5	174	8.4	3.7
7B	-	-	-	30	4.8-4.9	2.2-2.4	27	3.4	2.2	30-33	4.3-4.4	2.2-2.3	30	4.9-5.9	2.3-2.8

Supplementary Table 6. Results association mapping a) FHB and b) DON in the European and Nordic subpopulation of the spring wheat collection

a		FHBmean			2013Vb_FHBcPHDH			2014Vb_FHB			2015Vb_FHB			2015St_FHB	
Chrom.	cM	Phenotypic variation (%)	-log10(p-value)	cM	Phenotypic variation (%)	-log10(p-value)	cM	Phenotypic variation (%)	-log10(p-value)	cM	Phenotypic variation (%)	-log10(p-value)	cM	Phenotypic variation (%)	-log10(p-value)
1A	74-75	4.4-5.8	2.0-2.5	74	7	2.4	74-75	4.8-10.2	2.3-3.7	74	4.7	1.7	-	-	-
1B	39	4.3-4.5	2.0-2.1	-	-	-	26-39	4.9-10.6	2.3-3.2	39	4.5-11.3	1.7-3.6	26	6.2-6.6	2.2
1D	52	4.3	2	51	6.6	2.3	-	-	-	51-52	4.5-5.8	1.7-2.0	-	-	-
2B	-	-	-	80-84	6.5-8.1	2.3-2.7	76	7.1	3.1	83	5.8	1.8	83	6.2	2.1
2B	102	5.9	2.6	105	7	2.3	105	6.0-7.1	2.7-3.1	102	5.3	2	102	6.0-8.8	2.1-2.9
3B	85	4.2-7.4	2.0-3.0	82-85	6.5-8.3	2.3-2.4	-	-	-	85-90	4.5-5.5	1.8-2.0	-	-	-
4A	-	-	-	78	6.8	2.4	78	6.6-9.0	2.8-3.7	-	-	-	73-78	6.0-12.2	2.1-3.7
4B	106-108	5.3-6.0	2.5-2.7	104-111	7.7-11.9	2.9-3.8	111	6.3	2.7	-	-	-	-	-	-
5A	-	-	-	69	7.1	2.5	69-79	4.9-7.1	2.3-3.1	69	5.2	1.9	-	-	-
7A	30	4.1-5.2	2.0-2.4	30-35	6.9-13.3	2.3-4.1	30	7.3	3.1	30	4.4	1.7	30-38	6.2-12.5	2.1-3.7
7B	-	-	-	-	-	-	31	4.8-5.8	2.2-2.3	30-31	4.5-6.2	1.8-2.1	33	7.8	2.6

b		tDONmean		2013Vb_tDON			2014Vb_tDON			2015Vb_tDON			2015St_tDON		
Chrom.	cM	Phenotypic variation (%)		cM	Phenotypic variation (%)		cM	Phenotypic variation (%)		cM	Phenotypic variation (%)		cM	Phenotypic variation (%)	
1B	8	3.9	1.9	8	5.7-7.7	2.0-2.6	12	5	2.2	1	9	3	-	-	-
1D	-	-	-	55	12.2	3.8	-	-	-	45	6.8-7.7	2.3-2.7	45-49	6.2-12.9	2.0-3.5
2A	-	-	-	83	5.6-6.8	2.0-2.2	83-84	4.0-8.2	1.9-3.4	73-84	5.8-8.2	2.1-2.8	83	7.5-9.7	2.2-2.8
2B	102	3.9-5.2	1.9-2.8	102-105	5.5-10.2	2.0-3.2	102	4	1.9	102-108	6.0-7.6	2.1-2.6	105-108	6.5-8.2	2.0-2.5
3B	08-14.0	3.8-5.5	1.9-2.4	-	-	-	8.0-10.0	4.2-7.1	2.0-2.9	2.0-8.0	6.9-10.2	2.4-3.3	1.0-8.0	5.9-8.7	1.9-2.6
3B	85	3.8-7.8	1.8-3.3	85	5.5-6.7	2.0-2.4	85	4.8-7.0	2.2-3.0	85	5.9-6.3	2.1-2.3	85	7.1-10.6	2.1-3.0
4A	214	4.2-7.3	2.0-3.0	215	6.4	2.3	214-215	4.4-6.9	2.0-2.9	210-214	5.8-10.5	2.1-3.3	214	6.6	2.1
4B	77	4.2	2	87	5.6	2.0-2.4	-	-	-	87	5.9-7.5	2.1-2.6	87	6.6-8.2	2.0-2.5
6B	60-63	3.9-7.0	1.9-3.0	60-63	5.3-14.6	2.0-4.9	60-63	4.0-4.5	1.9-2.1	63	7.2	2.5	63	5.8-6.3	1.9-2.0
7A	30-38	3.9-5.9	1.8-2.3	30-35	6.4-6.8	2.3-2.4	30-38	4.9-5.2	2.2-2.3	30-38	5.9-11.6	2.2-3.7	30-38	6.4-9.1	1.9-2.7
7B	30	5.8	2.4	30	6.2-6.5	2.2	27-34	4.8-5.3	2.0-2.4	30-33	6.0-7.9	2.2-2.7	30-33	6.5-10.7	2.0-3.1

Supplementary Table 7a. Resistance alleles and frequencies in the spring wheat panel detected for DON

		Freq:	0.9	0.12	0.41	0.51	0.5	0.28	0.5	0.11	
		Chrom:	1AS	1AL	2BL	3B	4AL	5AL	7AS	7BS	
		SNP:	AX-94768180	AX-94757176	AX-94402860	AX-95652038	AX-94760880	AX-94568413	AX-95252445	AX-94859831	
line #	line	tDONmeancPHDH									
1560	Breeding line_0060	-0.545354682	1	0	0	1	1	1	1	0	5
1561	Breeding line_0067	-0.530435275	1	0	0	1	0	1	1	0	4
1551	Breeding line_0081	-0.512730938	1	0	1	0	0	1	1	0	4
1559	Breeding line_0058	-0.503779763	1	0	0	1	1	1	1	0	5
1546	Breeding line_0092	-0.500632989	1	0	1	1	0	0	1	0	4
1541	Breeding line_0056	-0.488651603	1	0	0	0	1	0	1	1	4
1569	Breeding line_0085	-0.467677059	1	0	0	1	1	1	1	1	6
1558	Breeding line_0076	-0.455970005	1	0	1	0	0	0	1	1	4
1549	Breeding line_0063	-0.454237549	1	0	1	1	1	1	1	0	6
1547	Breeding line_0093	-0.440759892	1	1	1	1	0	1	1	0	6
1566	Breeding line_0074	-0.432477455	1	0	0	0	1	1	1	0	4
1627	N894037	-0.429283315	1	1	1	1	1	0	1	0	6
1543	Breeding line_0078	-0.427485219	1	0	0	1	1	1	0	0	4
1562	Breeding line_0061	-0.42248433	1	0	0	1	1	1	1	0	5
1567	Breeding line_0065	-0.411078969	1	0	0	1	1	0	1	1	5
1539	Breeding line_0051	-0.392230378	1	0	0	1	1	0	1	0	4
1555	Breeding line_0098	-0.373174961	1	1	1	1	1	1	0	0	6
1542	Breeding line_0077	-0.371596443	1	1	1	0	0	0	1	0	4
1427	Anniina	-0.363786401	1	0	0	1	1	0	1	0	4
1540	Breeding line_0055	-0.359397592	1	0	0	1	1	0	1	0	4
1564	Breeding line_0071	-0.359313291	1	0	0	1	0	0	1	1	4
1552	Breeding line_0082	-0.329740759	1	0	1	0	0	1	1	1	5
1568	Breeding line_0084	-0.326108682	1	0	0	1	1	1	1	1	6
1557	Breeding line_0050	-0.322837396	1	1	1	1	0	1	0	0	5
1577	Breeding line_0054	-0.312205589	1	0	0	0	1	1	1	0	4
1565	Breeding line_0072	-0.304407071	1	0	0	1	1	0	1	0	4
1536	Breeding line_0047	-0.297472759	1	0	0	0	1	0	0	0	2
1544	Breeding line_0079	-0.288819264	1	0	0	1	1	1	0	0	4
1563	Breeding line_0070	-0.288252233	1	0	0	1	1	0	0	0	3
1571	Breeding line_0052	-0.281839913	1	0	1	1	1	1	0	0	5
1556	Breeding line_0048	-0.279433418	1	0	0	1	0	1	0	0	3
1611	Breeding line_0075	-0.265410203	1	0	0	1	1	0	1	0	4
1548	Breeding line_0096	-0.264372735	1	0	1	1	0	0	1	0	4
1091	Sumai 3 (18.)	-0.247623288	1	0	1	1	1	0	1	0	5
1537	Breeding line_0045	-0.223491453	1	0	0	1	1	0	0	0	3
1085	512-87	-0.219581046	1	0	1	1	0	0	1	0	4
1605	Breeding line_0053	-0.217483575	1	0	0	0	1	1	0	0	3
1538	Breeding line_0066	-0.215603758	1	0	1	0	1	1	1	1	6
1545	DC623/07-14/08	-0.208804164	1	0	1	0	0	1	1	1	5
1305	Breeding line_0016	-0.204403355	1	0	1	1	1	1	0	0	5
1082	512-50	-0.204132138	1	0	1	1	1	0	1	0	5
1578	Breeding line_0073	-0.202596668	1	0	0	1	0	0	1	0	3
1142	BCN*2//CROC_1/AE	-0.196060068	0	1	1	0	1	1	0	0	4
1570	Breeding line_0049	-0.190597373	1	0	1	1	0	1	1	0	5
1554	Breeding line_0097	-0.18850758	1	0	1	1	0	0	0	0	3
1587	Breeding line_0091	-0.177597598	1	0	1	0	0	1	1	0	4
1574	Breeding line_0059	-0.175789096	1	0	0	1	1	1	1	0	5
1550	Breeding line_0062	-0.172018781	1	0	0	1	0	0	1	0	3
1191	QUARNA	-0.169004975	1	0	1	1	0	0	1	0	4
1573	Breeding line_0046	-0.151303551	1	0	0	0	0	0	1	0	2
1328	Breeding line_0108	-0.140485208	1	0	1	0	0	0	1	0	3
1111	Nanjing 7840 - PI.4	-0.139493598	1	0	1	1	1	0	0	1	5
1172	NK01513	-0.136730469	1	0	1	1	1	1	1	0	6
1409	Breeding line_0032	-0.133252816	1	0	0	1	0	1	1	0	4
1325	Breeding line_0105	-0.128308466	1	0	1	0	0	1	1	0	4

		Freq:	0.9	0.12	0.41	0.51	0.5	0.28	0.5	0.11	
		Chrom:	1AS	1AL	2BL	3B	4AL	5AL	7AS	7BS	
		SNP:	AX-94768180	AX-94757176	AX-94402860	AX-95652038	AX-94760880	AX-94568413	AX-95252445	AX-94859831	
line #	line	tDONmeancPHDH									
1588	Breeding line_0043	-0.12802396	1	0	1	1	0	1	1	0	5
1535	Breeding line_0008	-0.119799134	1	0	1	1	0	0	0	0	3
1081	512-21	-0.119740297	1	0	1	1	0	0	1	0	4
1170	BAJASS-5	-0.118261719	1	0	0	1	1	1	0	0	4
1402	Rabagast	-0.114575829	1	0	1	0	0	0	1	0	3
1403	Seniorita	-0.114562793	1	0	0	0	0	0	1	0	2
1413	Berlock	-0.114024086	1	0	1	0	0	0	1	0	3
1174	Krabat	-0.113814517	1	0	0	1	0	0	1	0	3
1114	Ning 8343 - Pl.4	-0.113004205	1	0	1	0	1	0	0	0	3
1327	Breeding line_0107	-0.111256208	1	0	1	0	0	0	1	0	3
1183	Breeding line_0012	-0.106483147	1	0	0	0	0	0	1	0	2
1608	Breeding line_0057	-0.105384406	1	0	1	0	1	0	1	0	4
1083	512-54	-0.103795984	1	0	1	1	0	0	1	0	4
1084	512-70	-0.102814229	1	0	0	0	0	0	1	0	2
1193	Breeding line_0102	-0.102168674	1	0	1	1	0	0	1	0	4
1044	Paros/NK93602	-0.097946477	1	0	0	1	0	0	0	0	2
1607	Breeding line_0068	-0.096497476	1	0	0	1	0	1	1	0	4
1575	Breeding line_0069	-0.088890877	1	0	1	1	1	0	0	0	4
1592	Breeding line_0083	-0.085807153	1	0	1	1	0	0	0	1	4
1418	Breeding line_0041	-0.078797601	1	0	0	0	0	0	0	0	1
1039	DH 49-18 Bastian/Ad	-0.074918254	1	1	1	1	1	0	0	0	5
1031	T9040 (1995)	-0.074685159	1	1	1	0	1	0	0	0	4
1079	CJ9306	-0.069721475	1	1	1	1	1	0	1	0	6
1303	Breeding line_0010	-0.065071449	1	0	1	0	0	0	0	0	2
1148	AC Somerset	-0.063157571	1	0	0	0	0	0	0	1	2
1194	Breeding line_0005	-0.058486057	1	0	0	1	0	0	1	0	3
1080	CJ9403	-0.057182424	1	0	1	1	1	0	0	1	5
1016	Berserk	-0.057102918	1	0	0	1	1	1	1	0	5
1406	Breeding line_0017	-0.054623931	1	0	0	0	1	0	0	0	2
1036	NK93602(1995)	-0.052788775	1	0	1	1	1	0	0	0	4
1301	NK01565	-0.052786695	1	0	0	1	0	0	0	0	2
1032	T10014	-0.048560362	1	0	0	1	1	0	0	0	3
1407	Breeding line_0023	-0.041494765	1	0	0	1	1	0	1	0	4
1152	Sport	-0.038422265	1	0	1	1	1	1	1	0	6
1137	NG8675/CBRD	-0.037966268	1	0	1	0	0	0	0	1	3
1576	Breeding line_0080	-0.034821637	1	0	0	1	1	1	0	0	4
1401	Mirakel	-0.032789569	1	0	0	1	0	0	1	0	3
1189	Amulett	-0.030935492	1	0	1	1	0	0	1	0	4
1405	Breeding line_0019	-0.029154166	1	0	0	1	0	0	0	0	2
1330	Tom	-0.02866766	1	0	0	0	1	0	0	0	2
1416	Breeding line_0020	-0.027980266	1	0	0	0	0	1	0	0	2
1171	NK00521	-0.024903732	1	0	0	1	1	0	0	0	3
1630	80456/YANGMAI 5//	-0.021339797	1	1	1	1	1	0	0	0	5
1009	Avle	-0.017960173	1	0	1	0	0	0	1	0	3
1308	Breeding line_0024	-0.01779787	1	0	0	1	1	0	0	0	3
1572	Breeding line_0044	-0.017778985	1	0	1	1	1	1	1	0	6
1323	Breeding line_0099	-0.013837946	1	0	1	0	0	0	1	0	3
1087	SABUF/5/BCN/4/RAI	-0.013837216	1	0	1	1	1	1	0	0	5
1043	Paros	-0.011383862	1	1	1	0	1	1	0	0	5
1628	SHA5/WEAVER//804	-0.010836043	1	1	1	1	1	0	1	1	7
1275	J03	-0.009229726	1	0	0	0	0	0	0	0	1
1307	Breeding line_0022	-0.007559078	1	0	0	1	1	1	0	0	4
1591	PS-1	-0.006610883	1	0	0	1	0	0	0	0	2
1329	Granary	-0.002110596	1	0	0	0	0	1	1	0	3
1606	Breeding line_0064	0.006472852	1	0	0	0	0	0	1	0	2
1120	DH20097	0.010511037	1	0	0	0	1	0	0	0	2
1304	Breeding line_0013	0.012286202	1	0	0	1	1	0	0	0	3
1433	Scirocco	0.01251556	1	0	0	1	0	0	0	0	2
1086	SHA3/CBRD	0.014166102	1	0	1	1	1	0	0	0	4
1102	Nobeokabouzu (Mh	0.017145597	0	0	1	1	1	0	1	0	4

		Freq:	0.9	0.12	0.41	0.51	0.5	0.28	0.5	0.11	
		Chrom:	1AS	1AL	2BL	3B	4AL	5AL	7AS	7BS	
		SNP:	AX-94768180	AX-94757176	AX-94402860	AX-95652038	AX-94760880	AX-94568413	AX-95252445	AX-94859831	
line #	line	tDONmeancPHDH									
1589	Breeding line_0094	0.024583298	1	0	1	1	0	0	0	0	3
1414	Arabella	0.025075936	1	0	1	0	1	0	1	0	4
1175	Breeding line_0003	0.030531142	1	0	1	0	0	0	1	0	3
1179	Breeding line_0101	0.030839301	1	0	1	0	0	1	1	0	4
1420	Avans	0.03157001	1	0	0	1	0	0	0	0	2
1176	Breeding line_0007	0.032110092	1	0	0	1	1	0	1	0	4
1005	Bjarne	0.034811038	1	0	0	0	1	0	0	0	2
1334	Møystad	0.03969642	1	0	0	1	1	0	0	0	3
1322	Sabin	0.043216226	1	1	0	0	1	1	0	0	4
1430	Marble	0.046798125	1	0	1	0	0	0	1	0	3
1429	Kruunu	0.053787015	1	0	0	1	1	0	0	0	3
1316	Breeding line_0033	0.054840471	1	0	0	1	0	0	0	0	2
1636	T.DICOCCON PI9462	0.056287163	1	0	0	0	1	0	1	0	3
1324	Breeding line_0100	0.056421419	1	0	1	0	0	0	1	0	3
1590	Breeding line_0095	0.060229275	1	0	0	1	1	0	1	1	5
1181	Breeding line_0004	0.061292977	1	0	0	1	0	0	1	0	3
1119	DH20070	0.070427946	1	0	0	0	1	0	0	0	2
1173	Demonstrant	0.072371939	1	0	0	1	0	0	1	0	3
1434	Dragon	0.074011469	1	0	1	0	0	0	1	0	3
1124	MILAN/SHA7	0.074996644	0	0	0	0	1	0	1	0	2
1060	Gondo -1	0.07890999	1	1	0	0	1	0	0	0	3
1045	Paros/T9040	0.079006658	1	1	1	0	1	0	1	0	5
1038	MS 273-150	0.079862621	1	0	1	0	1	0	0	0	3
1421	BJY/COC//CLMS/GEI	0.080518946	1	0	0	0	0	0	0	0	1
1187	Breeding line_0015	0.08142858	1	0	0	1	1	0	1	0	4
1411	Willy	0.082880084	1	0	0	1	0	0	0	0	2
1326	Breeding line_0106	0.085786724	1	0	1	0	0	1	1	0	4
1410	Breeding line_0040	0.086289943	1	0	0	1	1	1	0	0	4
1178	Breeding line_0011	0.088576579	1	0	0	0	0	0	0	0	1
1063	Catbird -2	0.093194118	1	1	1	1	1	1	0	0	6
1312	Breeding line_0028	0.094862283	1	0	1	1	0	1	1	0	5
1180	Breeding line_0103	0.095889738	1	0	1	0	0	0	1	0	3
1426	Bjarne/LW91W86	0.097550242	0	0	0	0	1	0	1	0	2
1417	Breeding line_0039	0.098188186	1	0	0	0	0	0	1	0	2
1041	Naxos	0.101129357	1	0	1	1	1	1	0	0	5
1424	T7347	0.103031464	1	0	0	1	0	0	0	0	2
1309	Breeding line_0025	0.104749136	1	0	1	0	0	1	0	0	3
1064	Croc_1/Ae.squarros	0.107251985	1	1	1	1	1	0	0	0	5
1631	NG8675/CBRD//SHA	0.112937528	1	1	1	1	1	1	0	0	6
1315	Breeding line_0031	0.114106862	1	0	1	1	1	0	0	0	4
1408	Breeding line_0038	0.120965366	1	0	0	1	1	0	1	0	4
1633	IVAN/6/SABUF/5/BC	0.121608678	1	1	0	1	1	1	0	0	5
1415	Breeding line_0109	0.123850415	1	0	1	0	0	0	1	0	3
1629	VERDE/3/BCN//DOY	0.127329661	1	1	0	1	1	0	0	0	4
1317	Breeding line_0034	0.129824453	1	0	1	0	0	0	1	0	3
1177	Breeding line_0009	0.133261861	1	0	1	1	0	0	0	0	3
1404	Breeding line_0018	0.141898644	1	0	0	0	1	0	0	0	2
1310	Breeding line_0026	0.149038922	1	0	1	1	0	0	0	0	3
1337	Fram II	0.149347993	1	0	0	0	0	0	0	0	1
1029	T9040	0.152557002	1	0	0	1	0	0	1	0	3
1068	Altar84/Ae.sq(219)//	0.158702898	1	0	0	1	1	0	1	1	5
1188	Breeding line_0104	0.167563295	1	0	1	0	0	0	1	1	4
1626	EMB16/CBRD//CBRD	0.169727234	1	1	0	1	1	1	0	0	5
1075	NK93604	0.169891164	1	0	0	0	1	0	0	0	2
1313	Breeding line_0029	0.172556171	1	0	0	0	1	0	1	0	3
1003	Bastian	0.177472453	1	0	0	0	0	0	0	0	1
1006	Tjalve	0.178654199	1	0	1	0	0	0	0	0	2
1635	WHEAR/2*KRONSTA	0.181160137	1	0	0	0	1	0	0	0	2
1186	Breeding line_0014	0.183223767	1	0	0	1	0	0	1	0	3
1318	Breeding line_0035	0.18844563	1	0	1	0	0	0	1	0	3

		Freq:	0.9	0.12	0.41	0.51	0.5	0.28	0.5	0.11	
		Chrom:	1AS	1AL	2BL	3B	4AL	5AL	7AS	7BS	
		SNP:	AX-94768180	AX-94757176	AX-94402860	AX-95652038	AX-94760880	AX-94568413	AX-95252445	AX-94859831	
line #	line	tDONmeancPHDH									
1311	Breeding line_0027	0.188687809	0	0	0	0	0	0	0	0	0
1182	Breeding line_0006	0.191014262	1	0	0	1	1	1	1	0	5
1121	GONDO	0.198263464	1	1	0	0	1	1	0	0	4
1134	GUAM92//PSN/BOW	0.202823606	0	0	0	0	1	0	0	0	1
1190	Bombona	0.20648906	1	0	1	0	0	0	0	0	2
1314	Breeding line_0030	0.207254465	1	0	1	0	0	0	1	0	3
1011	Zebra	0.208413761	1	0	1	0	0	0	0	0	2
1320	Breeding line_0037	0.210708685	1	0	1	0	0	1	0	0	3
1192	Breeding line_0002	0.211410331	1	0	0	1	0	0	1	0	3
1018	Brakar - Pl.1	0.21245302	1	0	0	0	0	0	1	0	2
1306	Breeding line_0021	0.215020235	1	0	0	0	1	1	1	0	4
1050	Filin	0.220663507	0	0	0	0	0	0	0	0	0
1145	MAYOOR//TK SN108	0.220915332	1	1	0	0	1	0	0	0	3
1106	Frontana (95)	0.220991112	1	1	0	0	1	0	1	0	4
1412	Breeding line_0042	0.221571733	1	0	0	0	1	0	1	0	3
1428	Aino	0.223078428	1	0	0	1	1	0	0	0	3
1127	CBRD/KAUZ	0.231774856	1	1	0	0	1	1	0	0	4
1071	Kariega	0.241014532	0	0	0	0	1	0	0	0	1
1331	RB07	0.242641679	0	0	0	0	1	0	0	0	1
1321	TJALVE/Purpur seed	0.252522812	0	0	0	0	1	1	0	0	2
1431	Wanamo	0.253803357	1	0	0	0	0	0	0	0	1
1319	Breeding line_0036	0.257717604	1	0	0	0	1	0	0	0	2
1161	Chara	0.264259568	1	0	0	0	0	0	1	0	2
1116	Vinjett	0.26807061	1	0	1	0	0	0	1	0	3
1419	Polkka	0.278501813	1	0	0	1	0	0	1	0	3
1166	Naxos/2*Saar	0.283410048	0	0	1	0	1	0	0	0	2
1336	Norrøna	0.285943069	1	0	0	0	0	0	0	0	1
1058	Dulus	0.287293237	1	0	0	0	1	1	0	1	4
1020	Runar	0.288040807	1	0	0	1	0	0	1	0	3
1057	Bau/Milan -2	0.293043888	1	1	0	0	0	0	0	0	2
1335	Rollo	0.305959379	1	0	0	1	0	0	1	0	3
1302	Breeding line_0001	0.311365486	1	0	1	0	0	0	0	0	2
1046	T9040/Paros	0.314096595	1	1	0	0	0	0	0	0	2
1333	C80.1/3*QT4522//2*	0.326697273	0	0	0	0	1	0	0	0	1
1052	Milan	0.334638494	0	0	0	0	1	1	0	0	2
1158	CD87	0.336686921	0	0	0	0	0	1	0	1	2
1048	Saar	0.338647003	0	0	0	0	0	0	0	0	0
1027	T2038	0.369035381	1	0	0	1	0	0	0	0	2
1054	Pfau/Milan	0.37892578	0	0	0	0	0	0	0	0	0
1422	HAHN/PRL//AUS140	0.384194345	0	0	0	0	0	0	0	0	0
1168	ONPMSYDER-05	0.390828641	0	0	0	1	0	0	0	0	1
1073	Avocet YrA	0.406651017	0	0	1	0	0	0	1	0	2
1425	Reno	0.439681784	1	0	0	0	0	0	0	0	1
1164	Kukri	0.448512064	1	0	0	0	0	0	0	0	1
1141	ALTAR 84/AE.SQUAR	0.465982871	1	0	0	1	1	0	0	0	3
1066	Altar84/Ae.squarros	0.477676602	0	0	0	0	1	0	0	0	1
1332	C80.1/3*QT4522//2*	0.529397495	0	0	0	0	0	0	0	1	1
1423	TUI/RL4137	0.548126693	0	0	0	0	0	0	0	1	1
1634	GAMENYA	0.587503198	0	0	0	0	0	0	0	0	0

Supplementary Table 7b. Resistance alleles and frequencies in the spring wheat panel detected for FHB.

		Freq:	0.9	0.91	0.51	0.59	0.9	0.44	0.22	0.11	
		Chrom:	1AS	1AL	2BL	3B	4AL	5AL	7AS	7BS	
		SNP:	AX-94768180	AX-95632825	AX-94634298	AX-95080400	AX-94478215	AX-94693418	AX-94806305	AX-94859831	
line	line	FHBmeancPHDH									
1541	Breeding line_0056	-28.54	1	1	1	0	1	0	0	1	5
1562	Breeding line_0061	-26.48	1	0	0	1	0	1	0	0	3
1578	Breeding line_0073	-26.35	1	1	1	1	1	1	0	0	6
1563	Breeding line_0070	-24.12	1	1	1	0	1	0	0	0	4
1546	Breeding line_0092	-23.58	1	1	0	1	1	0	0	0	4
1569	Breeding line_0085	-22.68	1	1	0	0	1	1	0	1	5
1558	Breeding line_0076	-22.53	1	1	0	0	1	0	0	1	4
1561	Breeding line_0067	-22.34	1	0	1	1	1	1	0	0	5
1539	Breeding line_0051	-22.22	1	1	1	1	1	0	0	0	5
1537	Breeding line_0045	-22.17	1	1	1	1	1	0	0	0	5
1427	Anniina	-21.97	1	1	1	1	1	0	0	0	5
1565	Breeding line_0072	-21.89	1	1	0	1	1	1	0	0	5
1567	Breeding line_0065	-20.39	1	1	1	0	1	0	0	1	5
1536	Breeding line_0047	-20.24	1	1	0	1	1	1	0	0	5
1559	Breeding line_0058	-19.81	1	1	0	0	1	1	0	0	4
1555	Breeding line_0098	-19.34	1	0	0	1	1	1	0	0	4
1560	Breeding line_0060	-19.03	1	1	0	0	1	1	0	0	4
1566	Breeding line_0074	-18.68	1	1	0	0	1	1	0	0	4
1550	Breeding line_0062	-18.29	1	1	0	1	1	0	0	0	4
1605	Breeding line_0053	-18.06	1	1	1	1	1	1	1	0	7
1591	PS-1	-17.67	1	1	0	1	1	0	0	0	4
1611	Breeding line_0075	-17.55	1	1	1	1	1	0	0	0	5
1607	Breeding line_0068	-17.42	1	1	1	1	1	1	0	0	6
1551	Breeding line_0081	-17.36	1	1	1	0	1	1	0	0	5
1544	Breeding line_0079	-17.34	1	1	1	1	1	1	0	0	6
1086	SHA3/CBRD	-17.30	1	1	1	0	1	1	1	0	6
1552	Breeding line_0082	-17.24	1	1	1	0	1	1	0	1	6
1587	Breeding line_0091	-17.19	1	1	0	0	1	1	0	0	4
1568	Breeding line_0084	-17.03	1	0	1	1	1	1	0	1	6
1137	NG8675/CBRD	-17.00	1	1	1	1	1	0	1	1	7
1570	Breeding line_0049	-16.34	1	1	0	1	1	1	0	0	5
1085	512-87	-16.25	1	1	1	0	1	0	0	0	4
1079	CJ9306	-16.22	1	1	1	1	1	1	1	0	7
1574	Breeding line_0059	-16.06	1	1	0	0	1	1	0	0	4
1124	MILAN/SHA7	-15.74	0	1	1	1	1	1	1	0	6
1577	Breeding line_0054	-14.44	1	1	1	0	0	1	0	0	4
1627	N894037	-14.35	1	1	1	1	1	1	1	0	7
1191	QUARNA	-13.96	1	1	1	0	1	1	0	0	5
1557	Breeding line_0050	-13.57	1	1	1	1	1	1	0	0	6
1091	Sumai 3 (18.)	-13.20	1	1	1	1	1	0	1	0	6
1542	Breeding line_0077	-13.08	1	0	1	0	1	0	0	0	3
1535	Breeding line_0008	-13.04	1	1	1	1	1	0	0	0	5
1571	Breeding line_0052	-12.55	1	1	1	1	1	1	0	0	6
1548	Breeding line_0096	-12.43	1	1	0	1	1	0	0	0	4
1082	512-50	-12.21	1	1	1	0	1	0	0	0	4
1543	Breeding line_0078	-12.02	1	1	1	1	1	1	0	0	6
1573	Breeding line_0046	-11.74	1	1	1	0	1	0	0	0	4
1564	Breeding line_0071	-11.43	1	1	1	1	1	0	0	1	6
1171	NK00521	-11.32	1	1	1	1	1	0	1	0	6
1628	A5/WEAVER//80456/YANGMA	-10.83	1	1	1	0	1	1	1	1	7
1080	CJ9403	-10.64	1	1	1	0	1	0	0	1	5
1038	MS 273-150	-10.48	1	1	1	1	1	0	0	0	5
1538	Breeding line_0066	-10.36	1	1	0	0	1	1	0	1	5
1608	Breeding line_0057	-10.13	1	1	0	0	1	1	0	0	4
1540	Breeding line_0055	-10.04	1	1	1	0	1	0	0	0	4
1556	Breeding line_0048	-9.83	1	1	0	1	1	1	0	0	5

		Freq:	0.9	0.91	0.51	0.59	0.9	0.44	0.22	0.11	
		Chrom:	1AS	1AL	2BL	3B	4AL	5AL	7AS	7BS	
		SNP:	AX-94768180	AX-95632825	AX-94634298	AX-95080400	AX-94478215	AX-94693418	AX-94806305	AX-94859831	
line	line	FHBmeancPHDH									
1325	Breeding line_0105	-9.77	1	1	0	0	1	0	1	0	4
1084	512-70	-9.56	1	1	1	1	1	0	0	0	5
1027	T2038	-9.22	1	1	1	1	1	1	1	0	7
1588	Breeding line_0043	-9.04	1	1	1	1	1	1	0	0	6
1176	Breeding line_0007	-8.87	1	1	1	1	1	0	0	0	5
1547	Breeding line_0093	-8.07	1	0	0	1	1	1	0	0	4
1114	Ning 8343 - Pl.4	-7.91	1	1	1	1	1	0	1	0	6
1031	T9040 (1995)	-7.76	1	1	0	1	1	1	0	0	5
1545	DC623/07-14/08	-7.74	1	1	1	0	1	1	0	1	6
1606	Breeding line_0064	-7.69	1	1	1	0	1	0	0	0	4
1575	Breeding line_0069	-7.42	1	1	0	1	1	0	1	0	5
1631	\G8675/CBRD//SHA5/WEAVEI	-7.40	1	1	1	0	0	1	1	0	5
1102	Nobeokabouzu (Mhazy)	-6.94	0	1	1	0	1	0	0	0	3
1152	Sport	-6.89	1	1	0	1	1	1	0	0	5
1120	DH20097	-6.64	1	1	0	1	1	1	1	0	6
1046	T9040/Paros	-6.44	1	0	1	1	1	1	0	0	5
1111	Nanjing 7840 - Pl.4	-6.42	1	1	1	1	1	1	1	1	8
1549	Breeding line_0063	-6.27	1	1	0	0	1	1	0	0	4
1554	Breeding line_0097	-6.19	1	1	0	1	1	0	1	0	5
1630	ANGMAI 5//SHA5/WEAVER/3,	-5.94	1	1	1	1	1	1	1	0	7
1334	Møystad	-5.79	1	1	1	1	1	0	0	0	5
1410	Breeding line_0040	-5.32	1	1	1	1	1	1	0	0	6
1589	Breeding line_0094	-4.90	1	1	0	1	1	0	1	0	5
1337	Fram II	-4.78	1	1	0	1	1	1	1	0	6
1305	Breeding line_0016	-4.77	1	1	0	1	1	1	0	0	5
1071	Kariega	-4.27	0	1	1	1	1	1	0	0	5
1063	Catbird -2	-4.20	1	1	1	0	0	1	0	0	4
1121	GONDO	-3.99	1	1	1	1	1	1	1	0	7
1064	roc_1/Ae.squarrosa (205)//Ka	-3.66	1	1	1	1	1	1	1	0	7
1173	Demonstrant	-3.64	1	1	1	1	1	1	0	0	6
1330	Tom	-3.64	1	1	0	1	1	0	0	0	4
1592	Breeding line_0083	-3.63	1	1	1	1	1	0	0	1	6
1075	NK93604	-3.24	1	1	0	1	1	0	0	0	4
1590	Breeding line_0095	-3.15	1	1	1	1	1	0	0	1	6
1060	Gondo -1	-3.04	1	1	1	1	1	0	1	0	6
1119	DH20070	-2.52	1	1	0	1	1	0	0	0	4
1172	NK01513	-2.28	1	1	1	1	1	1	0	0	6
1166	Naxos/2*Saar	-2.19	0	1	0	1	1	0	1	0	4
1409	Breeding line_0032	-2.14	1	1	1	0	1	1	0	0	5
1307	Breeding line_0022	-1.88	1	1	0	1	1	1	0	0	5
1087	\/4/RABI//GS/CRA/3/AE.SQUA	-1.80	1	0	0	1	1	1	1	0	5
1036	NK93602(1995)	-1.69	1	1	1	1	1	0	0	0	5
1145	)R//TK SN1081/ AE.SQUARROS	-1.32	1	1	0	0	1	0	0	0	3
1429	Kruunu	-1.20	1	1	1	1	1	0	1	0	6
1405	Breeding line_0019	-1.17	1	1	1	1	1	0	0	0	5
1081	512-21	-1.02	1	1	1	0	1	0	1	0	5
1174	Krabat	-0.92	1	1	1	1	1	0	0	0	5
1322	Sabin	-0.67	1	1	1	1	1	1	1	0	7
1572	Breeding line_0044	-0.60	1	1	0	1	1	1	0	0	5
1179	Breeding line_0101	-0.56	1	1	0	0	1	1	0	0	4
1335	Rollo	-0.50	1	1	1	1	1	0	0	0	5
1194	Breeding line_0005	-0.46	1	1	1	1	1	1	0	0	6
1635	WHEAR/2*KRONSTAD F2004	-0.45	1	1	1	1	1	0	1	0	6
1142	*2//CROC_1/AE.SQUARROSA (	-0.34	0	1	1	1	1	1	1	0	6
1414	Arabella	-0.33	1	1	0	0	1	0	0	0	3
1016	Berserk	0.06	1	1	0	0	1	1	0	0	4
1148	AC Somerset	0.20	1	1	1	1	1	0	0	1	6
1275	J03	0.77	1	1	0	1	1	1	0	0	5
1083	512-54	0.81	1	1	1	0	1	0	0	0	4
1020	Runar	0.90	1	1	1	0	1	0	0	0	4

		Freq:	0.9	0.91	0.51	0.59	0.9	0.44	0.22	0.11	
		Chrom:	1AS	1AL	2BL	3B	4AL	5AL	7AS	7BS	
		SNP:	AX-94768180	AX-95632825	AX-94634298	AX-95080400	AX-94478215	AX-94693418	AX-94806305	AX-94859831	
line	line	FHBmeancPHDH									
1057	Bau/Milan -2	1.20	1	1	1	1	1	1	1	0	7
1029	T9040	1.26	1	0	1	1	1	0	0	0	4
1044	Paros/NK93602	1.52	1	1	1	1	1	0	0	0	5
1626	EMB16/CBRD//CBRD	1.56	1	1	0	0	1	1	0	0	4
1418	Breeding line_0041	1.86	1	0	0	0	1	1	0	0	3
1009	Avle	1.97	1	1	0	0	1	0	0	0	3
1636	T.DICOCCON PI94625/AE.SQU	2.07	1	1	1	1	1	1	1	0	7
1127	CBRD/KAUZ	2.47	1	1	1	1	0	1	1	0	6
1407	Breeding line_0023	2.60	1	1	0	0	0	0	0	0	2
1413	Berlock	2.92	1	1	1	0	1	0	1	0	5
1403	Seniorita	2.94	1	1	0	0	1	0	0	0	3
1048	Saar	3.08	0	1	0	0	0	0	1	0	2
1032	T10014	3.34	1	1	1	1	1	0	0	0	5
1328	Breeding line_0108	3.53	1	1	1	0	1	0	0	0	4
1306	Breeding line_0021	3.54	1	0	0	0	0	1	0	0	2
1629	/3/BCN//DOY1/AE.SQUARROS	3.72	1	1	1	0	1	0	0	0	4
1433	Scirocco	3.94	1	1	0	0	1	0	1	0	4
1424	T7347	4.17	1	1	1	0	1	0	0	0	4
1331	RB07	4.43	0	1	0	1	1	1	1	0	5
1317	Breeding line_0034	4.53	1	1	0	0	1	0	0	0	3
1018	Brakar - Pl.1	4.56	1	1	1	0	1	0	0	0	4
1177	Breeding line_0009	4.96	1	1	0	1	1	0	0	0	4
1043	Paros	5.03	1	1	1	0	1	1	0	0	5
1417	Breeding line_0039	5.03	1	1	0	0	1	0	0	0	3
1327	Breeding line_0107	5.14	1	1	0	0	1	0	0	0	3
1426	Bjarne/LW91W86	5.19	0	1	1	1	1	0	0	0	4
1134	GUAM92//PSN/BOW	5.20	0	1	1	1	1	1	0	0	5
1039	DH 49-18 Bastian/Adder	5.32	1	1	1	1	1	1	1	0	7
1329	Granary	5.36	1	1	0	0	1	1	0	0	4
1401	Mirakel	5.43	1	1	0	1	1	0	0	0	4
1576	Breeding line_0080	5.48	1	1	1	1	1	1	0	0	6
1326	Breeding line_0106	5.58	1	1	0	1	1	1	1	0	6
1041	Naxos	5.70	1	1	0	1	1	1	1	0	6
1419	Polkka	5.75	1	1	1	1	1	0	0	0	5
1183	Breeding line_0012	5.79	1	0	0	0	1	0	0	0	2
1402	Rabagast	5.88	1	1	1	0	1	0	0	0	4
1319	Breeding line_0036	5.95	1	1	0	0	1	0	0	0	3
1633	/BCN/4/RABI//GS/CRA/3/AE.S	6.38	1	1	1	0	0	1	0	0	4
1189	Amulett	6.64	1	1	0	1	1	0	1	0	5
1190	Bombona	6.68	1	1	0	0	1	0	0	0	3
1186	Breeding line_0014	6.69	1	1	1	1	1	0	0	0	5
1005	Bjarne	6.72	1	1	1	1	1	0	0	0	5
1187	Breeding line_0015	6.89	1	0	0	1	1	0	0	0	3
1406	Breeding line_0017	8.07	1	1	1	0	1	0	0	0	4
1054	Pfau/Milan	8.16	0	1	1	1	0	1	1	0	5
1415	Breeding line_0109	8.31	1	0	1	0	1	0	0	0	3
1303	Breeding line_0010	8.43	1	1	1	0	1	0	0	0	4
1421	BJY/COC//CLMS/GEN	8.43	1	1	1	0	0	0	0	0	3
1175	Breeding line_0003	8.49	1	1	0	0	1	0	0	0	3
1411	Willy	8.50	1	1	1	1	1	1	0	0	6
1323	Breeding line_0099	8.96	1	1	0	0	1	0	0	0	3
1416	Breeding line_0020	9.08	1	0	0	0	1	1	0	0	3
1158	CD87	9.19	0	1	0	1	0	1	0	1	4
1193	Breeding line_0102	9.22	1	1	0	1	1	0	0	0	4
1058	Dulus	9.58	1	1	0	1	1	1	0	1	6
1324	Breeding line_0100	10.13	1	1	0	0	1	0	0	0	3
1301	NK01565	10.38	1	0	1	1	1	1	0	0	5
1318	Breeding line_0035	10.81	1	1	0	0	1	0	0	0	3
1106	Frontana (95)	11.15	1	1	1	0	1	0	0	0	4
1310	Breeding line_0026	11.22	1	1	0	1	1	0	0	0	4

		Freq:	0.9	0.91	0.51	0.59	0.9	0.44	0.22	0.11	
		Chrom:	1AS	1AL	2BL	3B	4AL	5AL	7AS	7BS	
		SNP:	AX-94768180	AX-95632825	AX-94634298	AX-95080400	AX-94478215	AX-94693418	AX-94806305	AX-94859831	
line	line	FHBmeancPHDH									
1181	Breeding line_0004	11.24	1	1	1	1	1	1	0	0	6
1321	TJALVE/Purpur seed	11.31	0	1	0	1	1	1	0	0	4
1006	Tjalve	11.34	1	1	0	0	1	0	0	0	3
1045	Paros/T9040	11.69	1	1	0	1	1	1	0	0	5
1316	Breeding line_0033	11.84	1	1	0	1	1	0	0	0	4
1188	Breeding line_0104	12.22	1	1	0	0	1	0	0	1	4
1170	BAJASS-5	12.23	1	1	0	1	1	1	0	0	5
1309	Breeding line_0025	12.35	1	1	0	1	1	1	0	0	5
1192	Breeding line_0002	12.38	1	0	1	1	1	0	0	0	4
1052	Milan	12.43	0	1	1	1	1	1	1	0	6
1302	Breeding line_0001	12.61	1	1	0	0	1	1	0	0	4
1304	Breeding line_0013	13.10	1	1	0	1	1	0	0	0	4
1404	Breeding line_0018	13.13	1	0	1	1	1	0	0	0	4
1180	Breeding line_0103	13.87	1	1	0	0	1	0	0	0	3
1313	Breeding line_0029	13.89	1	1	0	0	1	0	0	0	3
1050	Filin	13.93	0	1	1	1	0	0	0	0	3
1336	Norrøna	14.01	1	1	0	0	1	0	0	0	3
1011	Zebra	14.03	1	1	0	0	1	1	0	0	4
1428	Aino	15.18	1	1	1	1	1	0	0	0	5
1311	Breeding line_0027	15.62	0	0	0	1	1	0	0	0	2
1178	Breeding line_0011	15.65	1	1	0	0	1	0	0	0	3
1182	Breeding line_0006	15.90	1	0	0	0	1	1	0	0	3
1315	Breeding line_0031	15.94	1	1	0	1	1	1	0	0	5
1003	Bastian	15.95	1	1	0	1	1	0	0	0	4
1408	Breeding line_0038	16.14	1	1	0	1	1	0	0	0	4
1430	Marble	17.24	1	1	0	0	1	1	0	0	4
1161	Chara	17.42	1	1	0	1	0	0	1	0	4
1308	Breeding line_0024	17.57	1	1	1	1	1	0	0	0	5
1420	Avans	17.91	1	1	0	1	0	0	0	0	3
1168	ONPMSYDER-05	18.37	0	0	0	1	0	0	0	0	1
1073	Avocet YrA	18.49	0	1	0	1	0	0	0	0	2
1116	Vinjett	18.69	1	1	0	0	1	0	0	0	3
1066	:ar84/Ae.squarrosa(219)// 2*S	20.00	0	0	0	1	1	0	0	0	2
1312	Breeding line_0028	20.56	1	1	0	1	1	1	0	0	5
1320	Breeding line_0037	20.72	1	1	0	0	1	1	0	0	4
1068	ar84/Ae.sq(219)//2*Seri/3/ A	22.09	1	0	1	0	1	0	0	1	4
1332	C80.1/3*QT4522//2*ATTILA	22.42	0	1	1	1	0	0	0	1	4
1434	Dragon	22.62	1	1	0	0	1	0	0	0	3
1423	TUI/RL4137	22.72	0	0	0	0	0	0	1	1	2
1164	Kukri	24.07	1	0	0	0	0	0	1	0	2
1422	HAHN/PRL//AUS1408	24.51	0	0	0	0	0	0	1	0	1
1333	C80.1/3*QT4522//2*PASTOR	26.91	0	1	0	0	1	0	0	0	2
1412	Breeding line_0042	27.65	1	0	1	0	1	0	0	0	3
1634	GAMENYA	29.15	0	0	0	0	0	1	0	0	1
1314	Breeding line_0030	29.27	1	1	0	0	1	0	0	0	3
1141	\R 84/AE.SQUARROSA (224)//E	29.91	1	1	1	1	0	0	0	0	4
1425	Reno	33.72	1	0	0	0	1	0	0	0	2
1431	Wanamo	43.97	1	1	0	0	1	0	0	0	3

Errata

Page nr	Paragraph	Changed from	Changed to
5	L 17	This screening displayed on average that 23 % of the SNPs on “The Wheat Breeder array” were predicted to be polymorphic SNPs between these collections (Allen et al., 2017).	A screening displayed on average that 23 % of the SNPs on “The Wheat Breeder array” were predicted to be polymorphic SNPs between two random accessions (Allen et al., 2017).
21	L 2	Type I: resistance against initial infection; Type II: resistance to pathogen spreading in infected tissue (Schroeder et al., 1963); Type III: resistance to kernel infection; Type IV: tolerance; and Type V: resistance to toxins in ears by decomposing them (Mesterházy, 1995; Miller et al., 1985).	Type I: Resistance to initial infection; Type II: Resistance to fungal spread (Schroeder et al., 1963); Type III: Resistance to toxin accumulation; Type IV: Resistance to kernel infection; and Type V: Tolerance (Mesterházy, 1995; Miller et al., 1985).
22	L 8	Sumai-3	Sumai 3
33	L 17	Septoria can cause several diseases; Septoria nodorum leaf and glume blotch caused by *Parastagonospora nodorum* (*P. nodorum*), tan spot caused by *Pyrenophora tritici-repentis, ZymoSeptoria tritici* leaf blotch and *Parastagonospora avenae* blotch.	Septoria nodorum leaf and glume blotch are caused by *Parastagonospora nodorum* (*P. nodorum*).
43	L 11	*Affymetrix 35K Breeders SNP chip*	*Affymetrix 35K SNP chip*
43	L 12	Affymetrix 35K Breeders SNP chip	Affymetrix 35K SNP chip
43	L 25	*Affymetrix 35K Breeders SNP chip*	*Affymetrix 35K SNP chip*

43	L 26	Affymetrix 35K Breeders SNP chip	Affymetrix 35K SNP chip
58	L 3	Sumai#3	Sumai 3
PaperIII 7	L 30	35K Breeders SNP Chip	35K SNP Chip
PaperIII 8	L 3	Affymetrix 35K Chip	Affymetrix 35K SNP Chip
Paper III 18	L8	Sumai#3	Sumai 3

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