QTL for Leaf Blotch Resistance in Spring Wheat, and a Method to Inoculate Wheat Seedlings with Stagonospora nodorum

QTL For bladflekkresistens i vårhvete, og en metode for inokolering av småplanter av Hvete med Stagonospora nodorum

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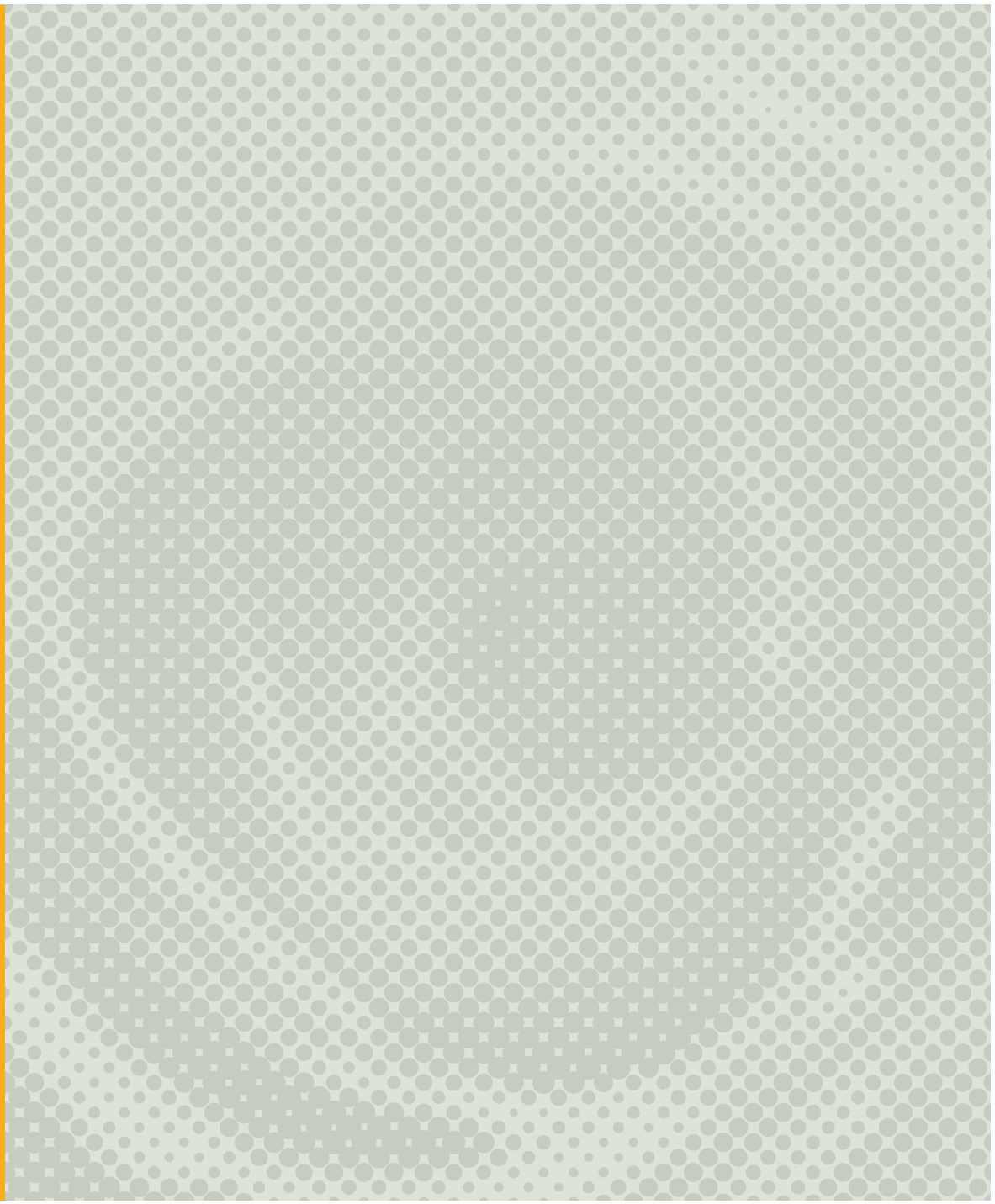

Preface and acknowledgments

This thesis was written at the Norwegian University of Life Sciences, Department of Plant and Environmental Sciences (IPM). The field trials were carried out at Østre Voll, Vollebekk research farm in Ås 2010-2012. In 2010 and 2011 the assessments were done by Dr. Morten Lillemo and Yalew Tarkegne, in 2012 by the author of this thesis if not specified in the text. Laboratory equipment and facilities were provided by Bioforsk Plantehelse, and growth chamber and greenhouse facilities were available at Senter for Klimaregulert Planteforskning (SKP). The thesis is part of the project "Norwegian bread wheat with high baking quality and sustainable disease resistance through marker-assisted selection" (NFR 185046/I10). Yara Norge AS provided me with 50 000 NOK in 2013, for which I am very grateful. The inoculation and infiltration experiments on the mapping population were carried out at the Department of Plant Pathology at North Dakota State University (NDSU) in Fargo in 2013. This work was led by adjunct professor Dr. Timothy Friesen. I am very thankful for the opportunity to use these results in my thesis.

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Summary

Leaf blotch diseases caused by *Stagonospora nodorum*, *Septoria tritici* and *Pyrenophora tritici-repentis* are important foliar diseases in wheat. The inheritance of resistance to these diseases appears to be quantitative, but recent research indicates that several host-specific interactions are involved. In this thesis, quantitative resistance was evaluated on a doubled haploid spring wheat population derived from a cross between Arina × NK93604. Adult plant resistance to leaf blotch diseases was evaluated in three years of field studies with natural infection. Seedling resistance experiments were conducted in Fargo (ND, USA) in a controlled environment by inoculation and culture filtrate infiltrations with two single isolates of *S. nodorum*. Seven QTL were significantly associated with adult plant resistance in one or more environments and located on chromosome 1D, 2B, 2D, 4D, 5A, 6D and 7A. The QTL on 4D and 6D were the most significant across environments, while the QTL on 2B and 7A may have been described in previous studies. The percentage of phenotypic variation explained by a single QTL ranged from 18.0 to 35.4. Several QTL were significantly associated with seedling resistance, the major being located on chromosome 1B, 2B, 3A, 3D, 4B, 5A, 5B, 6A and 6B. The range of phenotypic variation explained by a single of these QTL varied from 14.1 % to 42.9 %. Associations to characterized host-specific interactions were found to SnToxA, SnTox1, SnTox2, SnTox5 and possibly SnTox4. SnTox4 was detected simultaneously with SnToxA, contradicting a previous assumption. QTL that appeared to be important for both seedling and adult plant resistance were located on 1D and 5A. A method for inoculation of wheat seedlings with single isolates of *S. nodorum* was established and preliminary results from inoculation of on wheat differential lines with collected Norwegian isolates indicate production of hitherto unknown necrotrophic effectors.

Sammendrag

Bladflekksykdommer forårsaket av *Stagonospora nodorum*, *Septoria tritici* og *Pyrenophora tritici-repentis* er viktige sykdommer på hvete. Nedarvingen av resistens ser ut til å være kvantitativ, selv om nyere forskning indikerer at vertsspesifikke interaksjoner er involvert. I denne oppgaven ble kvantitativ resistens evaluert på en dobbel haploid populasjon av vårhvete, utviklet fra en krysning mellom Arina × NK93604. Resistens i voksne planter ble evaluert i tre års feltstudier med naturlig infeksjon. Småplanteresistens ble undersøkt i et kontrollert miljø i Fargo (ND, USA) ved at kartleggingspopulasjonen ble inokulert og infiltrert med kulturfiltrat av to enkeltisolat av *S. nodorum*. Sju QTL var signifikant assosiert med voksenplanteresistens og lokalisert på kromosom 1D. 2B, 2D, 4D, 5A, 6D og 7A. QTL på 4D og 6D var de mest signifikante over flere miljø, mens QTL på 2B og 7A kanskje er beskrevet i tidligere studier. Prosent fenotypisk variasjon forklart av hvert enkelt QTL varierte fra 18.0 til 35.4. Mange QTL var assosiert med småplanteresistens, de viktigste var lokalisert på kromosom 1B, 2B, 3A, 3D, 4B, 5A, 5B, 6A og 6B. Fenotypevariasjon forklart av et enkelt av disse QTL varierte fra 14.1 % til 42.9 %. Assosiasjoner til karakteriserte vertsspesifikke interaksjoner ble funnet relatert til SnToxA, SnTox1, SnTox2, SnTox5 og antagelig SnTox4. SnTox4 ble detektert samtidig som SnToxA, i motsetning til en tidligere antagelse. QTL som kan være av betydning både i voksen- og småplanteresistens ble lokalisert på 1D og 5A. En metode for inokulering av småplanter med enkeltisolater av *S. nodorum* ble etablert, og foreløpige resultater etter inokulering av differensiallinjer av hvete med innsamlede norske isolat antydet produksjon hittil ukjente nekrotrofe effektorer.

Table of contents

1 Introduction

The leaf blotch disease (LBD) complex *Stagonospora nodorum* blotch (SNB), *Septoria tritici* blotch and tan spot caused by *Pyrenophora tritici-repentis* causes leaf and glume blotch diseases in wheat (*Triticum* spp.). Leaf spot diseases can cause significant yield losses and reduce grain quality (Eyal et al. 1987). In Norway, *S. nodorum* is the dominant disease of the complex. Reduced tillage and rainy growth seasons increase the disease pressure, and recently discovered resistance to fungicides in Norwegian pathogen populations (Ficke et al. 2011b) call for sustainable solutions.

Breeding for resistance against *S. nodorum* have been difficult. Although monogenetic inheritance have been reported (Frecha 1973), the resistance is usually quantitatively inherited with several minor genes involved (Czembor et al. 2003; Friesen et al. 2009; Xu 2004). Robust quantitative trait loci (QTL) for SNB resistance have been reported on chromosome 1A, 1B, 2A, 2B, 2D, 3B, 4B, 5A, 5B, 6A and 7B (Francki 2013). Although differences in resistance between wheat cultivars, difference in pathogenicity between *S. nodorum* isolates and significant cultivar × isolate interactions have been interpreted as possible host-specific interactions by some authors (Rufty et al. 1981; Scharen et al. 1985), the pathosystem has been poorly understood, and thought to be mostly nonspecific (Johnson 1992; Oliver & Solomon 2010). However, more recent research suggests that very specific, inverse gene-for-gene actions are involved (Friesen & Faris 2012). So far, at least 15 host-selective toxins - necrotrophic effectors (NEs) - and corresponding sensitivity genes (*Snn*) in the host - have been identified in the *S. nodorum* pathosystem (Friesen, T. et al. 2008).

Only NEs from isolates from the upper Great Plains in the Mid-West, USA have been characterized so far. In a screening of isolates from the Southeastern region of USA, several novel NEs were found, indicating that yet uncharacterized NEs and corresponding sensitivity genes are more important in this region (Crook et al. 2012). Further research is needed to characterize the interactions of the *S. nodorum*-pathosystem in different geographic regions.

No study that genetically maps QTL for resistance against LBD or SNB has been published for the Scandinavian region, and we have no knowledge of NE production of the Norwegian *S. nodorum* population. The primary aim of my master thesis was to do QTL mapping of adult plant resistance to LBD, and seedling resistance to SNB, in the doubled haploid (DH) spring

wheat mapping population Arina × NK93604. The basis for investigating adult plant resistance was three years of phenotype data for leaf blotch severity were provided from field trials in Ås, Akershus, Norway. Seedlings of the mapping population were inoculated and infiltrated with culture filtrates of two single *S. nodorum* isolates (Sn4 and NOR4) and the results were used to investigate seedling resistance. The expectation was that new QTL would be discovered, because the trials were conducted in a geographical region where no previous mapping studies for leaf blotch disease have been performed. Also, the mapping population had not been used in earlier studies of leaf blotch resistance.

The second aim of the thesis was to establish a method to inoculate wheat seedlings with conidiospores of *S. nodorum*. When the method is established, it can be used to provide new knowledge of the genetic factors responsible for effects of specific QTLs. Additive and epistatic effects between interactions can also be examined. Differential lines sensitive to single effectors can be identified, and used to characterize pathogen isolates. Also genetic markers suitable for marker-assisted selection and genotyping can be identified (Friesen & Faris 2012).

A trait like leaf blotch resistance is considerably affected by morphological and developmental traits like earliness, maturity and plant height. Ideally, phenotyping for such traits should be done parallel with the disease severity scoring, and included in the modeling of true resistance level. However, only data for earliness was registered for all three years of field studies, and the emphasis was mostly put on this factor when calculating the resistance level. It would have been interesting to have included more factors in the model.

The QTL analysis was done using interval mapping for additive QTL in iciMapping (ICIM) (www.isbreeding.net). Other methods for QTL mapping are available, like composite interval mapping and inclusive composite interval mapping. However, the inclusive composite interval mapping used random cofactors in the calculations and seemed to "over compensate" and maybe detect "false" QTL. It would, however, have been interesting to compare the results from different mapping methods and software, because the algorithms vary.

2 Background

2.1 Wheat

Wheat (*Triticum* spp.) is a genus in the grass family *Gramineae* (*Poaceae*). The modern wheat species are either tetraploid (durum, AABB, 2n = 4x = 28) or hexaploid (common and club wheat, AABBDD, 2n = 6x = 42), with a basic haploid set of seven chromosomes (CFIA 2012). Hexaploid bread wheat (*T. aestivum*) is widely grown in temperate areas and provides 20 % of the human calorie consumption (FAO 2011).

The AA genome is probably donated by diploid wild einkorn *T. urartu* (Dvořák et al. 1993; Hong-Qing et al. 2013). The origin of the BB genome is unknown, but the closest living relative is *Aegilops speltoides* (Dvorák & Zhang 1990). *Ae. tauschii* is the progenitor of the DD genome (Jia et al. 2013; Salamini et al. 2002). The allopolyploid nature of the wheat genome contribute to the species' performance and ability to adapt to different environments (Salamini et al. 2002), but makes functional and genetic analyses highly complex (Hong-Qing et al. 2013).

2.2 Wheat production in Norway

2.2.1 Agro-ecological background

Norway is situated between latitudes 57°58' and 71°10' N, but the climate is warmer than in other areas of similar latitude due to the warm ocean currents of the Gulf Stream. Only 3 % of the total land area (324 000 km^2) is arable land (Lillemo & Dieseth 2011). Most of the cereal production is located in the south-eastern part of the country, with a more continental climate than the coastal areas. This implies less rainfall and that the difference between summer and winter temperatures is higher than near the coast.

The agricultural soil in Norway was formed by glacial deposits 10-20 000 years ago, and most wheat is grown on young clay soils of marine origin. The level of precipitation in the growth season is rarely limiting agricultural production, but can vary considerably from year to year (Lillemo & Dieseth 2011).

In the 1970ies, only 4-5 % of the wheat consumed by humans in Norway was produced domestically. In the 2000s, the percentage has been almost 80 % in good years. This is a result of political incentives, and the introduction of new stiff-strawed and pre-harvest sprouting resistant varieties better adapted to the extended drying period in the field after introduction of the

combined harvester (Lillemo & Dieseth 2011). In 2007, 381 000 tons of wheat were grown in Norway, covering an area of roughly 90 000 ha. 303 000 tons were of food quality (YARA 2010).

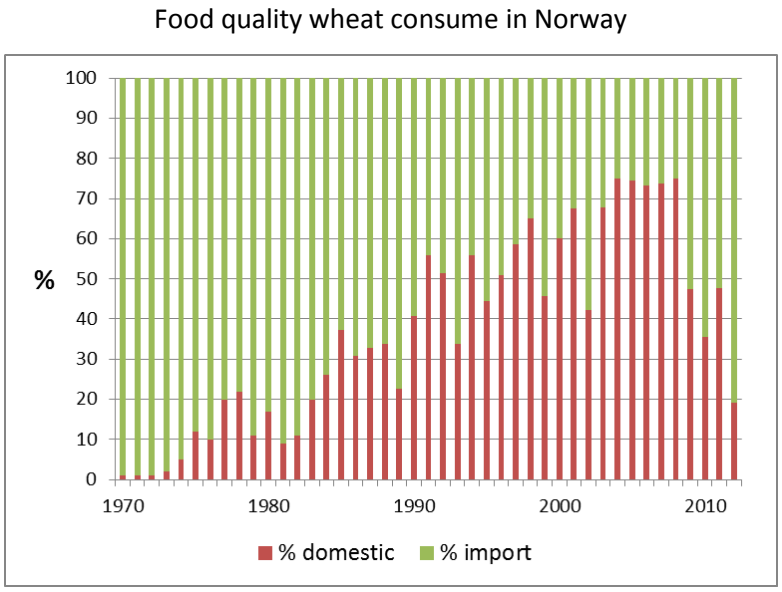


Figure 2.1 Consume of domestically grown (red bars) and imported (green bars) food quality wheat in Norway 1970-2012 (SLF 2012).

Figure 2.1 shows the percentage of domestically grown and imported wheat of food quality consumed from 1970-2012 (SLF 2012). The last four growing seasons have been difficult with significant losses due to leaf blotch diseases and pre-harvest sprouting.

2.2.2 Cultivation techniques

Spring wheat covers an area of 50-55 000 ha in Norway and is usually planted in the last week of April or early May. The crop is harvested in late August or early September (Lillemo & Dieseth 2011). The winter wheat area is more variable. The recommended planting of winter wheat in late August – first week of September may be delayed by a late pre-crop or rainy weather. Winter wheat is usually harvested in mid – late August. Due to warmer autumns in recent years, there is a tendency towards later planting of winter wheat, which benefits the crop. The potential for prolonged growing season for spring wheat is limited by increased precipitation in both spring and autumn (Lillemo & Dieseth 2011).

Oats, oilseed rape and potatoes are good pre-crops to wheat. But pre-crop requirements have been compromised by increased interest in wheat production as compared to the other crops.

Reduced tillage, including spring plowing and chisel plowing, has become more common in later years in order to reduce soil erosion, but problems with weeds and soil borne diseases increase with this practice (Lillemo & Dieseth 2011). More plant residues are usually left on the soil surface and may serve as an inoculum source the next growing season (McMullen & Adhikari 2009).

The typical requirement of fertilizer per season is 150-180 kg N, 30 kg P and 60 kg K per ha. P and K are usually added at planting for spring wheat, and first dressing for winter wheat. Nitrogen fertilization is usually split in order to optimize the utilization (protein content) and prevent leakage of nitrate from the soil (Lillemo & Dieseth 2011).

2.2.3 Pests and diseases

Weeds are controlled with herbicides. Severe attacks of aphids are controlled by insecticides, but this is not necessary every year. Fungal diseases are among the most important threats to wheat crops in Norway. Powdery mildew (*Blumeria graminis* f.sp. *tritici*), leaf blotch diseases ("Septoria Leaf Blotch") and *Fusarium* Head Blight (FHB) are the most common (Lillemo & Dieseth 2011).

Most farmers apply fungicides just after heading to protect the wheat crop against leaf blotch diseases (Lillemo & Dieseth 2011). The most common fungicide groups are strobilurins, triazoles, anilinopyrimidines and carboxamides. In later years, reduced sensitivity of leaf blotch pathogens to fungicides has been reported from Denmark and Skåne, Sweden, as well as other European countries. Studies have shown that mutations in the mitochondrial genome of the pathogens compromise the effects of the strobilurin and triazole fungicide groups (Blixt 2009). In Norway, resistance of *S. nodorum* isolates to strobilurins has been reported, as well as indications of reduced sensitivity to triazoles (Ficke et al. 2011b).

Integrated disease management is considered the best alternative to prevent fungicide resistant pathogens and losses due to leaf blotch diseases. This approach combines the utilization of crop rotations, seed quality, fungicide application and host plant resistance. Crop rotation with appropriate pre-crops (see above) reduce the amount of inoculum in the field (Blixt 2009). Seed are often treated with fungicides, as infected seed can be an important source of primary inoculum (Blixt 2009). Some wheat varieties have good resistance to leaf blotch pathogens. The genetic background for the resistance can be identified in mapping studies, genotyping with diagnostic markers and seedling tests with differential isolates.

2.3 Leaf blotch disease in wheat

2.3.1 The *"Septorias"*

The "*Septoria* leaf blotch disease complex" is responsible for up to 2 % of the annual global yield losses in wheat (ARC 2010). The average yield loss in Western Australia due to this disease is 9 % (Murray & Brennan 2009). *Stagonospora* (syn. *Septoria*) *nodorum* blotch (SNB) can be responsible for a yield loss of up to 31 % (Bhathal et al. 2003). The complex includes *Stagonospora* (syn. *Septoria*) *nodorum* (Berk.) glume and leaf blotch, *Septoria tritici* blotch and, of less importance, *S. avenae* blotch.

In Norway, *S. nodorum* is the major causal agent of leaf blotch. *S. tritici* has become more common in recent years, probably due to warmer climate, and is more common in winter than spring wheat (Ficke et al. 2011a). Tan spot (*Pyrenophora tritici-repentis*) is also significant in some areas (Ficke et al. 2011a). Identification of the correct disease in field is difficult, and microscopic examination of the conidiospores (pycnidiospores) is often necessary.

In the following, I will focus on *S. nodorum* and *S. nodorum* blotch (SNB). The reasons are both because this fungus dominates the leaf blotch complex in spring wheat in Norway, and because the most extensive studies of specific interactions between host and a necrotrophic pathogen is done by using *S. nodorum* isolates and toxins. However, I'll also use the term leaf blotch disease (LBD) especially when referring to field studies where other leaf blotch causing pathogens can contribute to the disease level.

2.4 *Stagonospora nodorum* leaf and glume blotch

2.4.1 Taxonomy

Stagonospora (syn. *Septoria*) *nodorum* (Berk.) Castell, and Germano [teleomorph: *Phaeosphaeria* (syn. *Leptosphaeria*) *nodorum* (Müll), Hedjar.] belongs to the kingdom Fungi, phylum Ascomycota, subphylum Euascomycota, class Dothideomycetes, order Pleosporales, family Phaeosphaeriaceae, genus *Phaerosphaeria* and species *nodorum* (Solomon et al. 2006).

Although previously placed in the same genus, *S. nodorum* and *S. tritici* are not closely related. Analysis of the Internal Transcribed Spacer (ITS) Region of the ribosomal DNA, group *S. tritici* with Mycosphaerellae and Cladosporia. *S. nodorum* and *P. tritici-repentis* are placed in different sub-bins of the Pleosporales (Goodwin et al. 2001; Goodwin 2004).

2.4.2 Host range and distribution

The main hosts of *S. nodorum* are bread wheat (*T. aestivum*), durum wheat (*T. durum*) and triticale, but also other cereals and a range of wild grasses can host the pathogen. The pathogen is common in major geographical regions where wheat is grown, including USA, Australia and Europe (Francki 2013; Solomon et al. 2006).

2.4.3 Reproduction

The life cycle of *S. nodorum* includes both a sexual and an asexual cycle of reproduction. The sexual cycle is known from Europe, including Norway (Ficke et al. 2011a) and Sweden (Blixt et al. 2008), Australia (Bathgate & Loughman 2001) and North America (Cowger & Silva-Rojas 2006). The fungus is heterothallic with two mating types, and both have to be present for sexual recombination to occur (Halama & Lacoste 1991). When different mating types meet, one of them forms an antheridium, the other an ascogonium. The structures fuse and a pseudothecium (sexual fruiting body) is formed. The pseudothecium contains numerous asci, each containing eight ascospores. The ascospores are wind borne over long distances (Bathgate & Loughman 2001).

Asexual fruiting bodies, pycnidia, begin to form in infected tissue within a week, approximately. Pycnidiospores are released from the pycnidia and are splash dispersed within the canopy (Solomon et al. 2006). Both the sexual and asexual spores produce multiple germ tubes and penetrates the plant tissue directly through the cuticle and opportunistically through stomata (Solomon et al. 2006).

The mixed reproduction system of *S. nodorum* allows both great diversity due to sexual recombination, and fast replication of successful genotypes during the asexual cycles. Studies have shown that no single *S. nodorum* genotype dominates in any environment (Blixt et al. 2008; Francki 2013; Stukenbrock et al. 2006). The genetic diversity is large and it is likely that selection in different environments have given rise to high levels of variation in aggressiveness and pathogenicity (Ali & Adhikari 2008; Engle et al. 2006; Francki 2013).

2.4.4 Epidemiology

The primary inoculum sources of SNB are ascospores or pycnidiospores from infected seeds and wheat stubble. The wind-borne ascospores are released from stubble of previous year's crop, and pseudothecia are produced during the entire growing season (Blixt et al. 2008). Low temperature (≥10° C), rainfall and high relative humidity initiates the release of ascospores (Bathgate & Loughman 2001). The secondary inoculum is pycnidiospores that are spread by rain

splash or windblown rain (Eyal et al. 1987). Splash dispersal occurs when at least 5 mm rainfall and temperature higher than 10 °C is followed by 10 mm rainfall during the next 48 hours (Eyal et al. 1987), but dew and mist can be sufficient to cause spore release (Bathgate & Loughman 2001). To produce significant disease and infect the glumes, 2-4 cycles of infection by pycnidiospores are needed (Solomon et al. 2006).

Production of pseudothecia and pycnidia both depend on near ultra violet light (NUV) and release of both sexual and asexual spores depend on moisture (Blixt et al. 2008; Eyal et al. 1987).

2.4.5 Signs and symptoms

An initial symptom of Stagonospora blotch is chlorosis at the infection site, expanding into oval lesions, often with necrotic regions. Under controlled humidity, pycnidia begin to form in the lesions within a week (Solomon et al. 2006).

Figure 2.2 Pycnidia of *S. nodorum* oozing cirrhi with pycnidiospores. Photo: With courtesy of KC Tan (2008)

Pycnidiospores are released in a mass of usually pink cirrhus (a mucus-bound, ribbon like mass of spores (Dictionary.com 2013)) (Figure 2.2). Previous to the release of spores, a swelling of a single point of the pycnidium causes the plant cuticle to rupture. The necrotrophic pathogen then quickly invades the collapsing and chlorotic leaf (Solomon et al. 2006). Symptoms of SNB usually first appear on the lower leaves, then progress to the upper leaves and in the case of favorable wet weather after flowering, the glumes (McMullen & Adhikari 2009).

2.4.6 Significance of *Stagonospora* leaf blotch

The effect on the plant and yield depends on the disease progress on the three last leafs (Ficke et al. 2011a). Although disease severity on the upper 1-3 leaves is not always a good predictor of yield (Spadafora et al. 1987), grain filling and the rate of grain growth per ear are highly

correlated with the photosynthetic rate of the flag leaf (Sofield et al. 1977). If the flag leaf or other upper leaves are attacked by SNB, reduced photosynthesis and early maturity affects the grain filling and thus the yield and quality of the crop (Ficke et al. 2011a; Spadafora et al. 1987).

2.5 The plant-pathogen relationship

2.5.1 Host specific interactions

Necrotrophic pathogens like *S. nodorum* were earlier considered to rely on "simple" or unspecific mechanisms like lytic or degradative enzymes to destroy the host's cell wall. Studies during the last decade have, on the contrary, revealed that host specific interactions play an important role in these pathosystems (Oliver & Solomon 2010). The core of this research has been to "Mendelize" the host-pathogen system by deconstructing the components of a single interaction between the host and the pathogen (Friesen & Faris 2012).

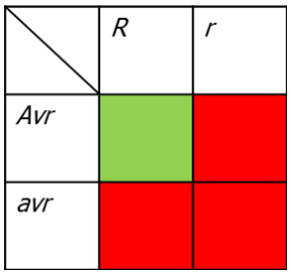


Figure 2.3.a. The classical gene for gene relationship in race specific resistance to biotrophs. A. Ruud, 2013

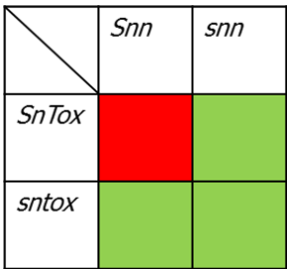


Figure 2.3.b. The inverse gene for gene interaction in the *S. nodorum*-wheat pathosystem. A. Ruud, 2013

The interactions resemble the gene-for-gene interactions described in the classical model of biotroph-plant pathosystems (Flor 1971) (Figure 2.3.a). In the classical model, presence of a dominant avirulence gene (*Avr*) in the pathogen and a corresponding dominant resistance gene (R) in the host, usually leads to a hypersensitive (HS) response, including an oxidative burst which triggers programmed cell death. As the biotrophs depends on living host tissue, infection is stopped (Figure 2.3.a). When a gene product from a necrotroph is recognized, the same HS response and cell death is advantageous to the pathogen which thrives on dead tissue. The interaction is therefore described as a mirror image of the classical model, or as an inverse gene-for-gene interaction (Friesen & Faris 2012) (Figure 2.3.b). The gene product of the pathogen is called a *host-selective toxin* (HST) or *necrotrophic effector* (NE, preferred), and the dominant gene in the host is called a sensitivity gene because its presence will give increased sensitivity to the pathogen. In the classical system, the presence of only one *Avr/R*-interaction will confer

complete resistance towards the pathogen race. In the inverse system, the effect of each *SnTox/Snn*-interaction is incomplete and usually additive in nature (Friesen & Faris 2010).

Table 2.1 Verified and characterized *SnTox-Snn*-interactions. From (Friesen & Faris 2010; Friesen et al. 2012)

Toxin	Host gene	Chromosome	Markers	Max R^2	Reference
SnToxA	*Tsn1*	5BL	*Xfcp1, Xfcp2, Xfcp394,Xfcp620*	95 %	(Friesen et al. 2006) (Zhang et al. 2009)
SnTox1	*Snn1*	1BS	*Xfcp618, Xpsp3000*	58 %	(Liu et al. 2004a) (Reddy et al. 2008)
SnTox2	*Snn2*	2DS	*XTC253803, Xcfd51*	47 %	(Friesen et al. 2007)
SnTox3	*Snn3*	5BS	*Xcfd20*	18 %	(Friesen et al. 2007)
SnTox4	*Snn4*	1AS	*XBG262267, XBG26975, Xcfd58*	41 %	(Abeysekara et al. 2009)
SnTox5	*Snn5*	4BL	*wmc349-cfd22/barc163*	63 %	(Friesen et al. 2012)

So far, at least 15 NEs - and corresponding sensitivity (*Snn*) -genes in the host - have been identified in the *S. nodorum* pathosystem (Friesen, T. et al. 2008). Six of the interactions have currently been characterized in detail (Table 2.1).

All the NEs are of a proteinaceous nature (Friesen & Faris 2010). The ToxA-*Tsn1* is the interaction investigated in most detail. Both *Pyrenophora tritici-repentis* and *S. nodorum* possess almost identical ToxA-genes, as a result of lateral gene transfer between the species (Friesen et al. 2006). The *Tsn1*-gene that confer sensitivity to ToxA-protein from both pathogens (Liu et al. 2006), has been cloned. The gene has resistance gene characteristics - including nucleotide-binding (NB) and leucine-rich repeat (LRR) domains, and a serine/threonine kinase domain (Faris et al. 2010). On the necrotrophic effector side, SnTox1 contains 16 cysteine residues, a feature shared by some avirulence effectors (Liu et al. 2012).

2.6 Quantitative trait loci (QTL) involved in *Stagonospora* blotch

Although some have reported monogenic inheritance of resistance to SNB in a common wheat line (Frecha 1973), most studies of resistance to SNB have shown quantitative and usually additive inheritance (Czembor et al. 2003; Friesen et al. 2009; Xu 2004). Several quantitative trait loci (QTL) have been described in a number of studies. Robust QTL detected in at least two different environments are summed up below.

2.6.1 Seedling resistance

Table2.2 List of QTL for seedling resistance to *S. nodorum* significant in at least two environments (Francki 2013).

Population[1)]	Inoculation[2)]	QTL-chr.	Markers	R^2	Allele[3)]	Reference
T. aestivum LiwillaxBegra, DH (*n* = 111)	Mixed isolate (15), CE	*QSnl.ihar-2B*	*gwm501–gwm410*	16 %	Liwilla	(Czembor et al. 2003)
		QSnl.ihar-5B	*barc32–gwm499*	30 %	Liwilla	
		QSnl.ihar-5D	*gwm205–gwm212*	37 %	Liwilla	
T. aestivum W7984xOpata85	Single isolate (Sn2000), Greenhouse	1B	*mwg938-snn1*	27-58 %	Opata85	(Liu et al. 2004b)
		4B	*cdo1312*	6-9 %	W7984	
T. aestivum AlbaxBegra, DH (*n* = 131)	Mixed isolate (15), CE	*QSnl.ihar-6A*	*gwm570–mwg934*	36 %	Alba	(Arseniuk 2004)
Triticum turgidium L. LDN x LDN (Dic-5B), RIL (*n* = 133)	Single isolate (Sn2000), Greenhouse	5B	*bcd9–fbb237*	37%	LDN (Dic-5B)	(Gonzalez-Hernandez et al. 2009)
T. aestivum BR34 x Grandin, RIL (*n* = 118)	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	

[1)] DH = doubled haploid population. RIL = recombinant inbred line, [2)]single isolate or number of isolates in mixed inoculation in parenthesis, CE = controlled environment, [3)] Resistance source (parent/allele)

Eight loci for seedling resistance have been identified in at least two environments on chromosome 1B, 2B, 2D, 4B, 5A, 5B, 5D and 6A (Table 2.2) using bi-parental mapping populations. In addition, two loci have been identified on 6A (marker: *wPt-7330*) and 7A (marker: *wPt-4515*) using association mapping, but are not shown in the table (Adhikari et al. 2011). In the studies by Czembor et al. (2003) and Arseniuk et al. (2004), different components of partial resistance – length of latent period (time from inoculation until development of pycnidia), incubation period (time from inoculation until the first visible symptoms) and disease severity (percent leaf area with lesions) – were analyzed. *QSnl.ihar-2B* was significantly associated with disease severity and latency period. *QSnl.ihar-5D* was significant for severity and incubation time while *QSnl.ihar-5B* was highly significant for all the components (Czembor et al. 2003). *QSnl.ihar-6A* was highly significant for disease severity, less for incubation period and not at all for the latent period (Arseniuk 2004).

The QTL on 1B associated with *snn1* (Table 2.2) is most likely an expression for the HST interaction between SnTox1 and *Snn1* and probably co-segregates with the sensitivity gene (Table 2.1) (Liu et al. 2004b). The QTL on 5B described by Gonzalez-Hernandez et al. (2009) is more likely linked to than identical with *Tsn1* (Gonzalez-Hernandez et al. 2009), although another study disagree (Faris & Friesen 2009) (see later under flag leaf resistance). The QTL on 2DS is probably identical with the *Snn2* locus, and the one on 5BL described in the same study co-segregates with *Tsn1* (Friesen et al. 2009). The latter is also significant in adult plant (flag leaf) resistance (Table 2.3) (Friesen et al. 2009).

2.6.2 Adult plant resistance

Table 2.3 Flag leaf resistance adapted from (Francki 2013)

Population[1)]	Inoculation[2)]	QTL-chr.	Markers	R^2	Allele[3)]	Reference
T. aestivum Forno x Oberkulmer, RIL (*n* = 226)	Mixed isolate (40), Field	*QSnl.eth-2D*	*psr932–psr331a*	20 %	Oberkulmer	(Aguilar et al. 2005)
		QSnl.eth-4B	*glk348–psr921*	17 %	Oberkulmer	
		QSnl.eth-7B	*mwg710a–glk576*	12 %	Forno	
T. aestivum WAWHT2074x 6HRWSN125	Mixed isolate (6–10), Field	*QSnl.daw-2D*	*cfd11–gwm30*	8–17 %	6HRWSN125	(Shankar et al. 2008)
T. aestivum BR34 x Grandin, RIL (*n* = 118)	Single isolate (BBSSn5), Field	*QSnb.fcu-1BS*	*fcp267–barc240*	10 %	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 x P91193D1, RIL (*n* = 254)	Mixed isolate (10), Field	*QSnl.daw-2A*	*gwm614a–wPt7056*	11–21 %	P92201D5	(Francki et al. 2011)
T. aestivum EGA Blanco x Millewa, DH (*n* = 235)	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	

[1)] DH = doubled haploid population. RIL = recombinant inbred line, [2)]single isolate or number of isolates in mixed inoculation in parenthesis, CE = controlled environment, [3)] Resistance source (parent/allele)

QTL for adult plant (flag leaf) resistance have been consistently detected in at least two environments on chromosome 1B, 2A, 2D, 4A, 5A, 5B and 7B (Table 2.3) (Francki 2013). The QTL at 2D, 4B and 7B described in the study by Aguilar et al.(2005) are QTL for leaf blotch

resistance that not overlap with QTL for morphological traits investigated in the same experiments (Aguilar et al. 2005).

The QTL *QSnl.daw-2D* identified by Shankar et al. (2008) is located in the same region as *Snn2* (Francki 2013) and the QTL *QSnb.fcu-2DS* for seedling and flag leaf resistance (Friesen et al. 2009), but the distance between the flanking markers *cfd11* and *gwm30* is too large (73.5 cM) (Shankar et al. 2008) to conclude if the QTL could be identical or linked. The QTL *QSnl.eth-2D* is located on the long arm of 2D and is not linked to seedling resistance (Aguilar et al. 2005).

The QTL *QSnb.fcu-1BS* and *QSnb.fcu-5AL* are shown in several studies to be associated with seedling resistance as well as adult plant resistance (Table 2.2 and 2.3) (Friesen et al. 2009; Friesen, T. L. et al. 2008; Xu 2004). The QTL at *QSnb.fcu-5BL* and *QSnb.fcu-2DS* were detected as significant for both flag leaf and seedling resistance in the study by Friesen et al. (2009). These QTL are associated with the host specific interactions between SnToxA-*Tsn1* and SnTox2-*Snn2*, respectively. The *QSnl.daw-2A* is the only reported locus for flag leaf resistance on 2A (Francki et al. 2011). There are also reports of QTL for seedling resistance on the same region of 2AS (Abeysekara et al. 2009), but the use of different markers for the two mapping populations make direct comparison difficult (Francki et al. 2011).

QSnl.daw-1B is located near the centromere of 1B and not linked to the other QTL described for this chromosome (Francki et al. 2011; Francki 2013). *QSnl.daw-5B* is located in the same region as several other QTL for seedling and adult plant resistance, including the sensitivity locus *Tsn1*, and markers closely linked to *Tsn1* (*fcp001 (= fcp1), fcp620*) were associated to the QTL in at least one of the environments investigated. This indicates that the ToxA-*Tsn1* interaction contributed to disease in these environments and that the QTL possibly was identical with the sensitivity gene (*Tsn1*) (Francki et al. 2011). There is also evidence of a number of linked genes conferring SNB resistance in this region (Gonzalez-Hernandez et al. 2009) **(**Table 2.2), although this has been re-evaluated by Faris & Friesen (2009), using the same mapping population and pathogen isolates, but different experimental environments (Francki 2013). In this study the ToxA-*Tsn1* interaction was the only factor governing SNB susceptibility.

The damaging effect of SNB is largest during moist periods when the plant reaches physiological maturity. Evaluation and genetic analysis of adult plants under field conditions are therefore of great importance. Considerable genotype × environment interaction is expected, and many QTL (not shown here) have been detected only in one environment. To be of interest for breeders the QTL should be consistent in several environments (Francki 2013) .

2.6.3 Glume blotch resistance

Table2.4 QTL for *Stagonospora* glume blotch resistance adapted from (Aguilar et al. 2005; Francki 2013)

Population[1)]	Inoculation[2)]	QTL, chromosome	Markers	R^2	Allele[3)]	Reference
T. aestivum Arina x Forno	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 x P91193D1, RIL (*n* = 254)	Natural infection and mixed isolate (10), Field and Greenhouse	*QSng.pur-2DL.1*	*gwm526a–cfd50b*	12–38 %	P91193D 1	(Uphaus et al. 2007)
		QSng.pur-2DL.2	*cfd50c–wPt9848*	5–6 %	P92201D 5	
T. aestivum WAWHT2074 x 6HRWSN125, DH (*n* = 280)	Mixed isolate (6–10), Field	*QSng.daw-4B*	*Rht1–gwm495*	8–19%	6HRWSN 125	(Shankar et al. 2008)
T. aestivum Forno x Oberkulmer, RIL (*n* = 226)	Mixed isolate (40), Field	*QSng.eth-5AL*	*psr1194-psr918*	36 %	Oberkulm er	(Aguilar et al. 2005)

[1)] DH = doubled haploid population. RIL = recombinant inbred line, [2)]single isolate or number of isolates in mixed inoculation in parenthesis, CE = controlled environment, [3)] Resistance source (parent/allele)

Four QTL for glume blotch resistance are located on 2DL, 3B, 4B and 5AL (Table 2.4). The QTL for glume blotch resistance do typically not align to the same chromosomal regions as QTL for seedling or flag leaf resistance. Exceptions are *QSng.pur-2DL.1* (Uphaus et al. 2007), which is located in the same region as flag leaf resistance QTL *QSnl.eth-2D* (Aguilar et al. 2005) (Table 2.2), and *QSng.sfr-4B* (Schnurbusch et al. 2003) and *QSng.daw-4B* (Shankar et al. 2008) which are associated with the toxin seedling insensitivity QTL at 4BL described by Liu et al. (2004b) (Table 2.1) (Francki 2013). It is possible that the underlying gene of this QTL is *Snn5*, but further comparative mapping has to be done to confirm whether the SnTox5-*Snn5* interaction is involved (Friesen et al. 2012).

QSng.daw-4B for glume blotch resistance is probably linked to the dwarfing gene *Rht-B1b (Rht1),* but the chromosomal position of this gene indicates that the resistance is not a pleiotropic effect of plant height (Shankar et al. 2008). *QSng.sfr-3B* is located in the telomeric region of the short arm of chromosome 3B, and was inherited from the Arina parent (Schnurbusch et al. 2003). *QSng.pur-2DL.2* is also an unique QTL for glume resistance, and was detected in several environments (Uphaus et al. 2007).

2.7 Molecular markers and linkage mapping

2.7.1 Molecular markers

Mapping of and breeding for complex quantitative traits like leaf blotch resistance have been considered difficult. Each locus usually have moderate to low contribution to the phenotype, more than one gene contributes to the same phenotypic trait and can be masked by dominant single genes. The utilization of molecular markers has changed this. Molecular markers can be determined at all levels of the plant (cellular to whole plant), there are relatively large number of alleles at molecular marker loci, and usually no deleterious effects of the markers. The markers are often co-dominant and it is possible to distinguish all genotypes, with fewer epistatic or pleiotropic effects than for phenotypic markers (Tanksley 1983).

Molecular markers can be categorized as either hybridization based – i.e. the DNA fragments are hybridized with labeled probes, or polymerase chain reaction (PCR) based. In the latter reaction, small and well defined pieces of DNA are enzymatically amplified. The technique has been used since 1983 (Semagn et al. 2006a).

Restriction fragment length polymorphism (RFLP) markers were first used in 1975 and were the most widely used hybridization technique. Bacterial restriction enzymes digest DNA and reveal a pattern difference between DNA fragment sizes and numbers. The technique can identify variation on individual, population and species levels (Semagn et al. 2006a), but is of very limited use today because it is expensive, labor-intensive and low throughput.

AFLP combines the strength of RFLP with the flexibility of PCR by ligating primer-recognition sequences to restricted DNA. PCR amplification will only occur where the primers are able to anneal to fragments which have the adaptor sequence plus the complementary base pairs to the additional nucleotides, called selective nucleotides. Because of the high selectivity, primers differing by only a single base in the AFLP extension amplify a different subset of fragments (Semagn et al. 2006a).

DArT is an open source technology, and a microarray hybridization-based technique that enables typing of several hundred polymorphic loci spread over the genome, simultaneously (Jaccoud et al. 2001; Wenzl et al. 2004). First, genomic representations are prepared by restriction enzyme digestion of genomic DNA, before the restriction fragments are ligated to adapters (Semagn et al. 2006a). Then the complexity is reduced by PCR, fragments from representations are cloned and cloned inserts are amplified, purified and arrayed in a “discovery array” (solid support microarray) (Semagn et al. 2006a).

SSRs, microsatellites, short tandem repeats (STRs) or simple sequence repeats are the smallest class of simple repetitive DNA sequences. 2-6 base pair (bp) repeats is the original definition by Litt & Luty (1989). The predominant mechanism of mutation in microsatellite tracts is "slipped strand mispairing" (Levinson & Gutman 1987). Forward and reverse primers that anneal to the 5' and 3' end of the DNA, respectively, are used in the PCR. The efficiency of the markers depends on the abundance of (polymorphic) repeats in the target species and how these repeats can be developed into informative markers. Tri- or tetra-nucleotide repeats make fewer stutter bands than dinucleotide repeats, but are less abundant (Semagn et al. 2006a).

A single nucleotide polymorphism occur for every 100-300 bp in any genome, making SNPs the most abundant of the molecular markers (Gupta et al. 2001). The development of SNP markers has been facilitated by the availability of genome-wide sequences and expressed sequence tags (ESTs) (Gupta et al. 2001). KASP Markers are a method of SNP genotyping developed by KBioscience. KASP stands for Kompetitive Allele Specific PCR. Advantages of KASP over other systems may be less expense, greater flexibility, and higher conversion rate (USDA 2012).

The ideal marker is a functional marker that is developed from functional domains within the target gene and thus is completely linked to the gene (Andersen & Lubberstedt 2003).

2.7.2 Linkage mapping

Loci on the same chromosome are defined as linked, and do not undergo independent assortment, because they are inherited together in the meiosis. However, during the first meiotic prophase, cross over between homologs occur, and the result is recombination. The frequency of recombination is used to calculate the relative distances between loci (genes, markers) and create maps of linkage groups or entire chromosomes. The map unit is centiMorgan (cM) (Klug et al. 2007). The longer the distances between two loci, the more inaccurate do the mapping estimates become. The reason for this is double crossover, and the result is that the relative distances between genes usually are underestimated (Klug et al. 2007).

3 Materials and Methods

3.1 Analysis of Quantitative Trait Loci (QTL) for leaf blotch resistance

3.1.1 The mapping population

The Arina × NK93604 doubled haploid (DH) mapping population was developed by Semagn et al. (2006b) using the maize by wheat crossing system (Laurie & Bennett 1988). 'Arina' is a Swiss winter wheat variety released in 1981, with excellent resistance to *S. nodorum* leaf and glume blotch (Paillard et al. 2003). NK93604 is a Norwegian spring wheat breeding line with high productivity (Semagn et al. 2006b).The DH population in the field studies includes 109 different lines. Lines with strong winter wheat characeristics have been excluded.

DNA was extracted by Semagn et al (2006b) from young leaves of the parents and 93 of the DH lines using the DNeasy Plant DNA extraction kit (Qiagen, Mississauga, Ont.), used to genotype a total of 624 molecular markers (AFLP, DArT and SSR) and construct the first published map for the population (Semagn et al. 2006b).

Table 3.1: New markers genotyped in 2012-2013, and their chromosomal assignment

Chromosome	Markers
1A	*cfd058, barc10b*
1B	*psp3000, fcp618*
1D	*gdm033*
2A	*barc10a*
2B	*mag681*
2D	*TC253803*
3A	*wmv489a*
3B	*barc068b, wmc653b*
4A	*barc020*
4B	*wmc679, cfa2149, wmc652, gwm006a*
4D	*Rht-D1, wmc473a, wmc331*
5A	*wmc489b*
5B	*fcp1, fcp620, gwm234*
5D	*cfd018*
6B	*GPC*
7B	*wmc182b*
7D	*Lr34, gwm437, gwm473, wmc182a, cfd014a, SWM10, cssfr5, wmc463*
None	*barc068a, wmc182c, gwm165a/b (4B or 4D)*

The mapping population has succesfully been used to identify QTL for resistance to Fusarium head blight (Semagn et al. 2006b) and for anther extrusion (Skinnes et al. 2010). 38 new SSR, KASP and functional markers were genotyped on the population in 2012-13 (Table 3.1). This work was done by Anne Guri Marøy, to improve the resolution in areas of known loci for *S. nodorum* toxin sensitivity, and to increase the marker density in interesting areas after initial QTL analysis of the field data from 2010-12.

3.1.2 Field trials 2010-12

Figure 3.1 The leaf blotch hill plot site at Østre Voll 10.07.2012, with mist irrigation system. Photo: A. Ruud 2012

109 (108 in 2011) DH lines from the Arina × NK93604 cross, 9 checks and the spring wheat parent NK93604 were planted in hill-plots in a randomized complete block design at Østre Voll, Vollebekk research farm, Ås, Akershus, Norway (Figure 3.1). The α-lattice experimental design had 12 plots in each of 10 blocks, three replicates and three repeats (2010, 2011 and 2012). Each replicate consisted of 120 plots, a total of 360 each year. The distances between each row was 40 cm, between each plot 50 cm. For 2012 the first replicate (120 plots) was not included in the final data. In this replicate the plants were of poor quality, yellow and small, which made correct disease scoring very difficult.

Vollebekk research farm is located at 50°N, 90 m above sea level. The soil type at the experimental site is a Mollic Gleysol (ISSS 1998; Lillemo et al. 2006). The plots were mist irrigated (Figure 3.1) 5 minutes every half hour to create optimal conditions for leaf blotch disease and limit powdery mildew (*B. graminis* f.sp *tritici*) infections. For disease development the experiments relied on natural infection by the present pathogen population.

Table3.2 Dates for sowing, first and last registered heading date, and dates for leaf blotch severity scorings in 2010-2012

Year	Sowing date	First heading	Last heading	First disease scoring	Second disease scoring
2010	12.5.	4.7.	20.7.	31.7.	6.8.
2011	3.5.	1.7.	20.7.	28.7.	2.8.
2012	2.5.	3.7.	22.7.	3.8.	6.8.

Sowing dates, dates for the first and last heading and for disease assessment are shown in Table 3.2.

3.1.3 Weather data 2010-2012

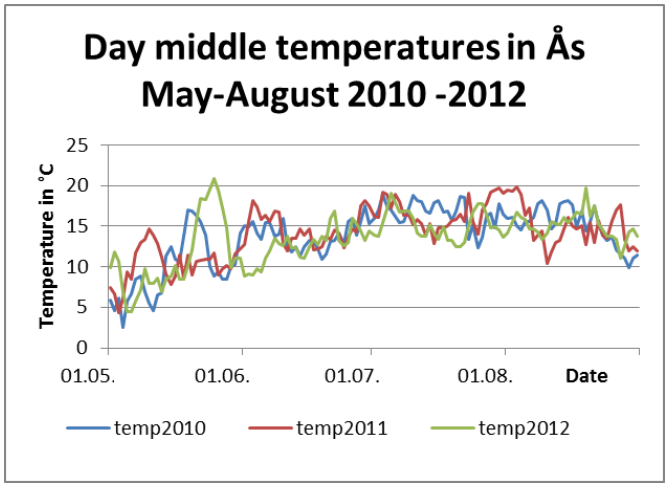


Figure3.2 Day middle temperature in 2 m height from 1.5.-30.8 2010-12. There was an early temperature peak in the beginning of May in 2012, not in 2010 and 11. The temperatures are mostly lower (from the beginning of June) in 2012 than 2010 and 2011, and 2010 (blue) is warmest from mid-July to early August.

Figure 3.2 shows day middle temperature at 2 m height in Ås, from the 1st of May – 31st of August 2010-2012 (VIPS 2013).

3.1.4 Soil and plant treatments

The experimental sites were autumn plowed before each season, and the seedbed harrowed before sowing. The pre-crop for 2010 was oats (*Avena sativa*) and barley (*Hordeum vulgare*) for 2011 and 2012.

In 2010, the site was fertilized with 75 kg/daa 22-3-10 YaraMila™ (YARA) April 15th, and 53 kg/da 22-3-10 YaraMila™ May 11th. In 2011 the site was fertilized with 8.5 kg/da 22-3-10 YaraMila™ at April 28th. In 2012 the site was fertilized with 28.5 kg/da 22-3-10 YaraMila™ at the 1st of May, and 20 kg/da Calcium Nitrate (Kalksalpeter™) 3rd of July.

In 2010, the site was sprayed against insects with 80 ml/da Perfekthion ® (BASF) June 15th and 100 ml/da Prokoz ® Zenith™ June 26th. The field was sprayed with 250 ml/da herbicide Ariane S ® June 1st. In 2011, the site was sprayed with the herbicide Granstar ® (concentration not registered) 8th of June. In 2012, the site was sprayed with 250 ml/da Ariane S ® and 80 ml/da Perfekthion ® May 24th, and 20 g/da Karate ® against aphids July 3rd.

3.1.5 Heading date

The heading date for each DH line was registered in 2010, 2011 and 2012. The day of heading was defined as the day when the heads of most plants in a plot were fully emerged (Zadok stage 58) (Zadoks et al. 1974). In 2010 the assessment was done by Dr. Morten Lillemo, in 2011 by Yalew Tarkegne. In 2012 the assessment was done by master student Anja Karine Ruud. Registrations were done every third day.

The Least Square Means (LSM) for days from sowing to heading (days until heading, DH) for each DH line were calculated in SAS ® 9.2 (SAS Institute, Cary, NC). These data were regressed against the leaf blotch severity data. The formula for the regression line was used to correct the disease severity data against the confounding effect of earliness on leaf blotch disease severity.

3.1.6 Physiological maturity

Physiological maturity (Zadoks stage 87) (Zadoks et al. 1974) is a better measure of maturity than days from sowing to heading, especially in a population like Arina × NK93604.

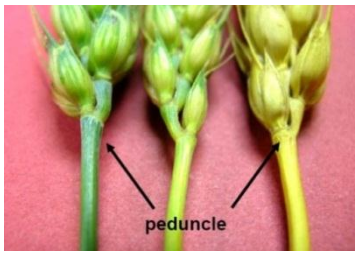


Figure3.3 Wheat spikes before (left) and at (right) physiological maturity (http://www.nwroc.umn.edu 2013)

Physiological maturity for each plot was only registered in 2012 and defined as the day when most of the plants in the plot had reached Zadok stage 87. The whole plant is yellow at this stage, except the internodes which still may be green (Figure 3.3). Registrations were done every third day. The Least Square Means (LSM) for days from sowing until physiological maturity (days until maturity, YM) for each line were calculated and used as for days to heading (above).

3.1.7 Disease severity

The severity of leaf blotch was assessed by visual evaluation of the whole canopy in each plot. The percentage of diseased tissue compared to healthy tissue was registered at two different days (Table 3.2) after the visible level of disease had reached at least 40 % on the earliest lines. The least square means (LSM) of the means between the first and second registration were calculated as for days to heading (above), and regressed against heading date and against physiological maturity (in 2012) to get a measure of adult plant resistance to *S. nodorum* leaf blotch.

3.1.8 Plant height

Plant height was measured in 2011 and 2012 with a measuring stick before harvest (dates: 02.08.2011 and 05.09.2012) for each plot. The height was measured from the soil to the base of the spikes in each plot. The measurement was conducted by Yalew Tarkegne in 2011 and by Dr. Qiongxian Lu and master student Anja Karine Ruud in 2012. The LSM were calculated as described above for days to heading.

3.2 Seedling plant resistance (Fargo, North Dakota, USA)

3.2.1 Inoculation and infiltration with culture filtrates

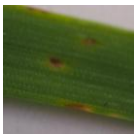
The Arina × NK93604 DH population (109 lines), plus the parents, three important Norwegian spring wheat varieties (Bjarne, Demonstrant, Zebra), a resistant line (RE714) and Naxos (parent in another mapping population used in leaf blotch studies at Østre Voll, Sha3/CBR x Naxos), were screened for seedling reactions to two *S. nodorum* isolates – Sn4 and NOR4. This work was conducted by Dr. Timothy Friesen's group in Fargo, North Dakota (North Dakota State University, NDSU).

3.2.2 The *S. nodorum* isolates

Sn4 is a North American isolate known to produce NEs SnToxA and SnTox2 (Faris et al. 2011). NOR4 was collected in Romerike (NAPE14061111-002), Akershus, Norway, 29.06.2011, from spring wheat variety Zebra. The field where the isolate was collected was not treated by fungicides, and the collection was a part of Nordic Field Trials, testing of fungicides (Andrea Ficke, personal comment). NOR4's production of NEs was unknown.

3.2.3 Inoculation

Table3.3 Disease reaction types (Liu et al. 2004b). Photos: A. Ruud 2013

Photo	Score	Symptoms	Reaction type
	0	Absence of visible lesions	Highly resistant (HR)
	1	Few penetration points, lesions small dark spots	Resistant (R)
	2	Lesions dark spots with little surrounding necrosis/chlorosis	Moderately resistant (MR)
	3	Dark lesions completely surrounded by necrosis/chlorosis, 2-3 mm	Moderately susceptible (MS)
	4	Larger necrotic/chlorotic lesions ≥4 mm, little coalescence	Susceptible (S)
	5	Large coalescing lesions with very little green tissue remaining	Highly susceptible (HS)

The seedlings were planted in cones and inoculated with $1 \cdot 10^6$ spores/mL conidiospores of a single *S. nodorum* isolate at the 2-3 leaf stage until runoff. The experiments were done according to the methods described in Liu et al. (2004b) and Friesen and Faris (2012). Disease symptoms were scored seven days after inoculation, using the 0-5 scale (Table 3.3).The experiment was repeated three times – for NOR4 18.01.-25.01.2013, 25.01.-02.02.2013 and 08.02-15.02.2013. For Sn4 the inoculations were done 04.02.-11.02.2013, 15.02.-22.02.2013 and 22.02.-01.03.2013.

3.2.4 Infiltration

The seedlings were planted in plastic cones and infiltrated with ≈25 µl of partially purified toxin using a 1-ml syringe with the needle removed, when the second leaf was fully expanded. The culture filtrate production and partial purification of toxins were done according to Liu et al. (2004a) Five days after infiltration the leaf reactions were scored using the 0-3 scale described in Friesen & Faris (2012), Table 3.4. The results from this part of the experiment are based on one repetition and must be seen as preliminary.

Table 3.4 0-3 disease scale for leaf reaction to culture filtrate of *S. nodorum* conidiospores (Friesen & Faris 2012)

Score	Reaction
0	No reaction
1	Mottled chlorosis
2	Chlorosis/necrosis without tissue collapse
3	Necrosis and complete tissue collapse

3.3 Data analysis

3.3.1 Statistical analysis

Least square means (LSM) were calculated in SAS ® 9.2 (SAS Institute, Cary, NC) using the LSmean statement in proc mixed (mixed model).

Analysis of variance (ANOVA) was done using the GLM procedure in SAS ® 9.2.

The linear regressions for the relationships between earlin ess, plant height and leaf blotch severity were calculated in Minitab 16 (*Minitab 16 Statistical Software* 2010).

3.3.2 Calculation of heritability

The narrow sense heritability h^2 is a measure of the fraction of phenotype variability contributed by additive genetic effects. Estimates of the narrow sense heritability were calculated using the formula from (Singh 1995).

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

$\sigma_g^2 =$ additive genetic variance, estimated from ANOVA table as $\frac{\sigma_L^2 - \sigma_E^2}{r}$,

$$\sigma_L^2 = variance\ of\ the\ DH\ lines = var(error) + r \cdot var(line)$$

$$\sigma_E^2 = Mean\ Squared\ Error\ (MSE) = var(error)$$

$$r = the\ observed\ number\ of\ replications$$

$$\sigma_p^2 = phenotypic\ variance = \sigma_g^2 + \frac{\sigma_E^2}{r}$$

For 2010-12 the heritability was calculated for LBD corrected for heading date (DHcLBD). In 2012, the heritability for LBD corrected against physiological maturity date (DYMcLBD) was also calculated.

3.3.3 Estimation of gene numbers

The number of quantitative genes involved was estimated using Wright's method

(Lillemo et al. 2006; Wright 1968):

$n = D^2/[8\sigma^2_g/(2 - 1/2^{(g-2)})]$

D = the genotypic range of the lines in generation F_g.

Doubled haploids equal a F_∞ generation of inbreeding (Snape et al. 1984), thus the adjusted formula was:

$n = D^2/[8\sigma^2_g/(2\text{-}0)] = D^2/(4\ \sigma^2_g)$

D was estimated as the genetic range (difference between the extreme genotypes) of DH line means multiplied by the heritability. This tends to eliminate the environmental influence and give more stable gene number estimates.

The assumptions of the calculation are 1) no linkage, 2) no epistasis, 3) no dominance, 4) equal effects of all loci and 5) no transgressive segregation. Failure to meet any of the criteria leads to an underestimation of genes (Snape et al. 1984).

3.3.4 Linkage mapping

The construction of linkage maps was done in JoinMap ® 4 (van Oijen 2006). Map distances were calculated using Kosambi's mapping function. This mapping method adjusts the map distance based on interference which changes the proportion of double crossovers (DCO). The relationship between genetic distance in cM (m) and recombination frequency (r) is given by

$m = 25\ln(\frac{1+2r}{1-2r})$, or $r = \frac{1}{2} \cdot \frac{e^{4m}-2}{e^{4m}+2}$ (Helms 2000).

69 linkage groups covering the 21 chromosomes were created, based on the calculations in JoinMap 4 and comparison to the Wheat Consensus Map (Somers et al. 2004), the published Arina × NK93604 map (Semagn et al. 2006b) and control of marker positions in the calculated map versus registered position in the Grain Genes Database (GrainGenes2.0 2013). 547 markers were included in the map. The rest of the genotyped markers (107) did not assign to a linkage group.

3.3.5 QTL analysis

The QTL analysis was conducted in ICIM v3.2 (http://www.isbreeding.net), using the Interval Mapping for additive QTL (IM-ADD) option for bi-parental populations. For a QTL to be statistically significant the LOD value had to be larger than 3.0 at p = 0.05. The linkage maps with QTL and LOD curves were constructed in MapChart 2.2 (Voorrips 2002).

3.4 A method for inoculation of wheat seedlings with *S. nodorum* isolates

3.4.1 Collection of isolates

10 lines of the Arina × NK93604 population were chosen based on the leaf blotch resistance level in 2010: From the two most susceptible via moderately susceptible and average, to moderately susceptible and most resistant. Leaves with visible symptoms were collected at two times. The first sample was taken from the third leaf, the second from the flag leaf. The leaves were collected in paper bags and pressed flat between newspapers, until dry (at least 48 hours), then stored at room temperature.

3.4.2 Surface sterilization

The dried leaves were surface sterilized using the following protocol: Five dry leaves from one sample were rinsed in 100 ml distilled water in a sterile hood. Then they were put in 70 % Ethanol (C_2H_5OH) for 10 seconds, rinsed in 100 ml distilled water before put in 0.5 % Sodium Hypochlorite (NaOCl) for 90 seconds. Finally, the leaves were rinsed in 100 ml distilled water (Figure 3.4).

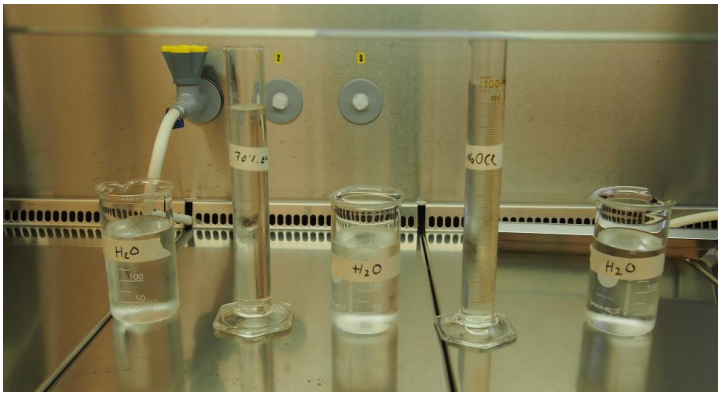


Figure 3.4 Set up for surface sterilization of wheat leaves. From left: 100 ml distilled water, 50 ml 70 % Ethanol, 100 ml distilled water, 50 ml 0.5 % NaOCl, 100 ml distilled water. Photo: A. Ruud 2012

3.4.3 Incubation

The surface sterilized leaves were put in a plastic box on a layer of five filter papers moisturized with 30 ml distilled water and covered by a plastic lid (Figure 3.5). The plastic box was placed in an incubation locker with 25 ° C, fluorescent white light and near ultra violet (NUV) light (24 h). The exact wavelengths and intensity of the light varied through the experimental period, and were not registered. After 48 h, the leaves were examined under the stereo microscope for pycnidia oozing pycnidiospores (Figure 2.2).

Figure 3.5 Plastic box with surface sterilized wheat leaves on moist filter paper, for incubation. Photo: A. Ruud 2012

3.4.4 Making single spore isolates

A sterilized needle was used to remove oozing spore masses from the leaf to a drop of lactic acid on an objective glass slide. The slide was examined in the microscope to confirm that the pathogen was *S. nodorum*. The pycnidiospores of *S. nodorum* measure 15-32 x 2-4 µm with 0-3 septa, while the significantly longer spores of *S. tritici* are 35-98 x 1-3 µm and with 3-5 septa (Eyal et al. 1987).

After correct identification of *S. nodorum*, the sterilized needle was dipped into the spore masses oozing from one single pycnidium, and the spores were put in 50 µL distilled water in a 1.5 µL Eppendorf (www.eppendorf.com) tube. The rest of the procedure took place in the sterile hood: The spore suspension in the Eppendorf tube was shaken by hand and a sterilized loop was used to spread a drop of the suspension on a 9 cm petri dish with potato dextrose agar (PDA) medium (Appendix 1).

After 48 h in an incubation locker with 25 °C and 24 h fluorescent white and NUV light, the petri plate was examined in the stereo microscope. The location of germinating, single spores was marked with a marker pen on the bottom of the petri dish. In the sterile hood, a sterilized cork borer was used to cut out plugs of PDA containing single spores. The plugs were placed on fresh PDA agar medium in 9 cm petri dishes, and the dishes sealed with Parafilm (Bernis ® Flexible Packaging, Neenah, WI).

3.4.5 Sporulation on PDA after isolation from leaves

The PDA plates were put in an incubation locker with 20 ° C and 24 h white light and NUV light. The light conditions were not constant during the experimental period, and are not registered. The plates were checked regularly (every 2-3 days) for development of pycnidia and sporulation. When sporulation occurred, the spores were examined in the light microscope

(following the procedure described in Single spore isolation) to confirm that the isolate was *S. nodorum*. The time from isolation from leaf until sporulation on PDA was registered (see results).

Isolates that were not sporulating after approximately six weeks were thrown.

3.4.6 Storing

(1) After confirming that the single spore isolates were *S. nodorum*, the initial method I used for storing was as following: In the sterile hood, plugs of mycelium from each single spore isolate were cut out with a sterile cork borer and put on fresh PDA plates, the plates sealed with Parafilm and grown in darkness at 9°C. Then plugs of mycelium were cut out the same way, put five each in 1.5 µL Eppendorf tubes and frozen at -80° C.

(2) As the first method proved to be unreliable this method should be preferred (personal comment from Timothy Friesen): **Sporulating** tissue (on V8 [Appendix 1] or PDA) was cut out with a sterilized cork borer (as above). The plugs were placed on the lid of a petri dish, covered with the bottom of the dish, leaving an opening for circulation of air, and left in a running laminar flow hood eight hours to dry. Then the plugs could be stored as described above.

3.4.7 Growth from stored isolates

Plugs of culture were taken from the freezer, thawed and smeared on 9 cm PDA or V8 plates. The plates were placed in the incubation locker at 20°C and 24 h white and NUV light. After 5-7 days the cultures were expected to sporulate, but this varied considerably.

3.4.8 Making spore suspensions

From a sporulating culture, spore suspensions were made by collecting oozing spore masses (as described above) with a sterilized needle and put in 0.25 ml sterilized water in a 1.5 ml eppendorf tube. The suspension was shaken by hand, and smeared on 9 cm PDA or V8 plates. The plate was left to dry for 5-15 minutes in the laminar flow hood, with an opening between the lid and bottom, before sealed with Parafilm (Bernis ® Flexible Packaging, Neenah, WI) and placed at 20°C and 24 h white and NUV light.

3.4.9 Re-inoculation and re-isolation of cultures

To (theoretically) maintain the sporulation frequency and aggressiveness of the isolates, they can be "run through" wheat plants after 2-3 cycles of sporulation. I collected healthy leaves from the susceptible variety Brakar grown in the greenhouse at SKP for three weeks (18° C day/15° C night, 16 h daylight from 06.00-22.00). The leaves were put in a wet paper bag and an autoclave bag, and autoclaved for 90 minutes at 121°C, two times.

The leaves were stored in the closed autoclave bag at room temperature. Then they were put in 100 ml distilled water for 30 seconds, before placed on water agar (WA) (Appendix 1) plates (9 cm petri dishes). After that, either 1) A plug of thawed culture from the freezer was smeared on the agar or leaves or 2) 0.25 ml spore suspension was spread on the leaves and agar. Then the plates were placed in incubation lockers at 20°C and 24 h white and NUV light, and evaluated for development of pycnidia during the next days and weeks.

3.5 Inoculation

3.5.1 Making inoculum

Petri dishes with sporulating pycnidia were washed with distilled water and the suspension filtrated through three layers of cheesecloth. The spore concentration was counted using a haemocytometer (www.hycorbiomedical.com). Only spores inside the grid (not on the lines of the grid) were counted. The concentration was adjusted to $1 \cdot 10^6$ spores/mL and one drop of Tween ® 20 (Polyethylene glycol sorbitan monolaurate) was added to the final suspension See calculation/result section for example of calculation.

3.5.2 Inoculation and post-inoculation facilities

Initially, wheat seedlings of six different varieties and lines (Arina, NK93604, SHA3/CBRD, Naxos, RE714 and Bjarne) and a border row of Brakar were randomized and grown in VEFI (VP53/54, VEFI Norway 1998) plastic trays, filled with soil (Gartnerjord, Tjerbo, Norway) (Figure 3.6). In the final design, the wheat seedlings were randomized and grown in recycled plastic cones with volume 164 ml, diameter 3.8 cm and depth 21 cm (Stuewe & Sons, Tangent, Orlando, USA). The cones were placed in trays, 98 in each tray, filled with soil (Gartnerjord, Tjerbo, Norway) and one seedling was sown in each cone (Figure 3.7).

The seedlings were grown in the greenhouse at 18° C day/15° C night and 16 h day length until they had reached the 2-3 leaf stage.

Figure 3.6 VEFI tray with wheat seedlings on the carousel, ready for inoculation. Photo: A. Ruud 2012

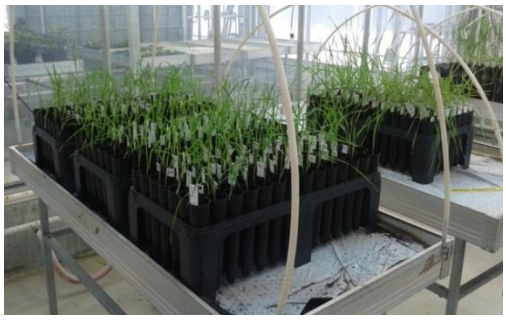


Figure 3.7: Plastic cones with wheat seedlings on tables in the greenhouse after inoculation with *S. nodorum* isolates. Photo: A. Ruud 2013

When the conditions were stable enough for consistent development of disease, ten wheat lines with known sensitivities to certain necrotrophic effectors (NEs) (Table 3.4) were tested briefly.

Table 3.4 List of differential lines of wheat, with known sensitivities to NEs produced by *S. nodorum*

Line	Source	Provider	Sensitivity
BR34	2008 increase	T. Friesen	Universal Insensitive
BG 261	2008 increase	T. Friesen	Tox A
M6	2008 increase	T. Friesen	Tox 1
BG 223	2008 increase	T. Friesen	Tox 2
BG 220	J02P 2667	J. Faris	Tox 3
AF 89	GH increase	J. Faris	Tox 4
CS(DIC1B)	J02S 559	J. Faris	Tox 5D (unpub)
ITMI 44	2008 increase	T. Friesen	Tox 4Ba (unpub)
ITMI 37	2008 increase	T. Friesen	Tox 6A (unpub)
LP 29		S. Xu	Tox 4Bb (unpub)

A paint sprayer with air pressure 1 Bar was used to inoculate the seedlings at the 2-3 leaf stage. The tray with seedlings was placed on a carousel (Figure 3.6) and sprayed with $1 \cdot 10^6$ pycnidiospores/mL until runoff. 50 ml inoculum was used per tray, and the sprayer was rinsed with 70 % ethanol, then with distilled water, after each inoculation

After inoculation, the trays were placed (carefully so the drops of inoculum would stay on the leaves) in a growth chamber with 21°C, 24 hours artificial light and 99 % relative humidity, for 24 hours, to make optimal conditions for *S. nodorum* spore germination and infection. After 24 hours the trays were placed in the greenhouse at 21 °C and 16 h day length.

3.5.3 Disease assessment

After seven days the second leaf of each plant was evaluated for disease reaction type by the. a 0–5 scale (Liu et al. 2004b) (Table 3.3). Disease reaction types are described as the best method to measure necrotic lesion size as a result of the toxin–host gene interaction. The percent diseased leaf area is expected to give similar QTL results (Friesen et al. 2009).

4 Results

4.1 Leaf blotch field trials at Østre Voll

4.1.1 Leaf blotch severity 2010-12

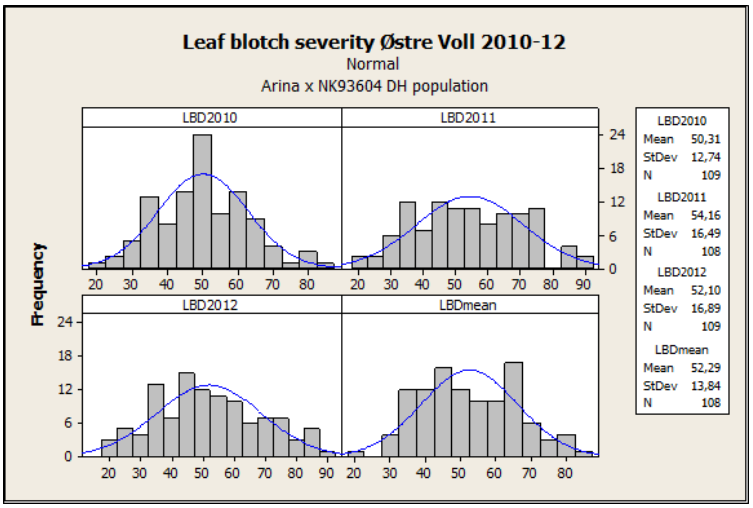


Figure 4.1 Frequency distributions of the least squared means (LSM) for leaf blotch severity % in the Arina x NK DH population, Østre Voll 2010-12.

Figure 4.1 shows the frequency distributions for uncorrected leaf blotch severity in the DH population from the field trials at Østre Voll 2010-12. The number of lines in each class varied from year to year. The shape of the histograms indicates the disease level at the time of evaluation. In 2010, the frequency seemed to follow the normal distribution, although with many lines in the mean class, and fewer with lower and higher values. In 2011, there was a relatively even distribution of lines in each class from approximately 25 to 75 % severity. 2012 have more lines in the lowest and highest classes.

Table 4.1 Pearson correlation coefficient for uncorrected leaf blotch severity between years

	2010	2011
2011	0.80	
2012	0.59	0.756

Table 4.1 shows the Pearson correlation coefficient between the different years. The correlation was high between 2010 and 2011 and indicates more similarities in the results for these two

years, than between 2010 and 2012 which had the lowest correlation coefficient (0.59), although the correlation was still high between 2010 and 2012 per definition.

4.1.2 Leaf blotch severity mean as a function of earliness

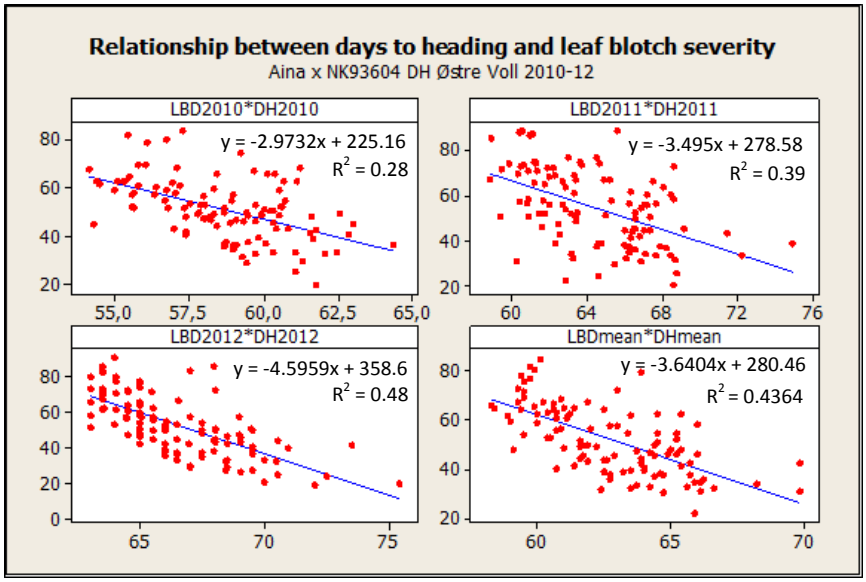


Figure4.2a) to d) Scatterplots showing leaf blotch severity (LBD, in %) as a function of days to heading (DH) for the least square means (LSM) in the field trial at Østre Voll, 2010, 2011, 2012 and for the mean of the three years (LSM). The LSM for the earliest heading line in 2010 was 54.1 days, and for the latest included line 64.4 days. 2011: The LSM for the earliest heading line was 58.8, and for the latest included line 72.2 days. 2012: The LSM for the earliest heading line was 63.0, and for the latest included line 75.5 days. Mean: The LSM for the earliest heading line was 58.2, and for the latest included line 69.8 days.

Figure 4.2.a) to d) shows % leaf blotch severity (mean of two registrations each season) as a function of heading for the Arina × NK93604 DH leaf blotch trials at Østre Voll, Vollebekk research farm, in 2010, 2011, 2012 and the mean of 2010-2012. Each red dot represents the Least Square Mean (LSM) of leaf blotch disease and days from sowing to heading for a DH line, computed in SAS 9.2. Extremely late heading lines were excluded from further analysis. The expressions for the regression lines were used to correct the registered mean level of leaf blotch and get a better expression for the resistance level. Lines that are more resistant than the average are placed below the trend line, susceptible lines above, and the distance to the line indicates how resistant or susceptible the line is. In 2012, physiological maturity was used as an additional correction factor (Figure 4.3).

4.1.3 Leaf blotch mean as a function of maturity

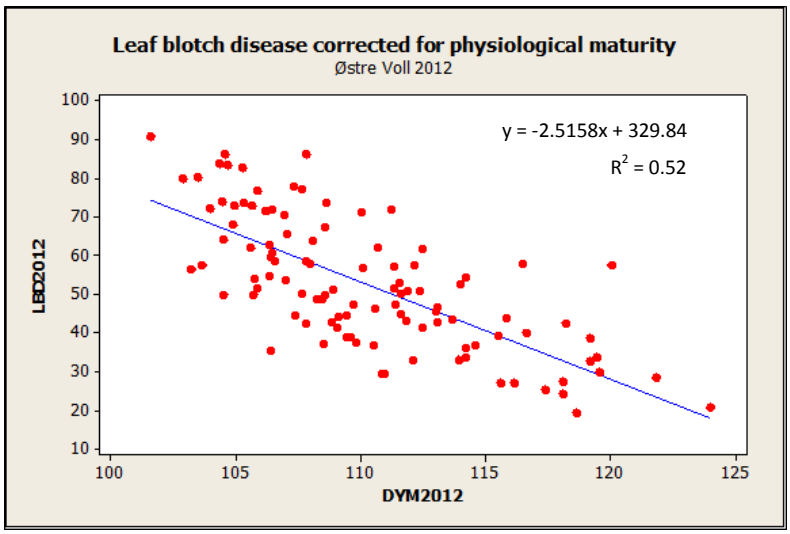


Figure 4.3 Scatterplot showing leaf blotch severity (LBD, in %) as a function of physiological maturity (DYM) for the least square means (LSM) in the field trial at Østre Voll, 2012. The LSM for the earliest line was 101.6 days, and for the latest included line 124.0 days.

Figure 4.3 shows % leaf blotch severity (mean of two registrations) as a function of physiological maturity for the Arina × NK93604 DH leaf blotch trials at Østre Voll, Vollebekk research farm, in 2012. Each dot represents the Least Square Mean (LSM) of leaf blotch disease and days from sowing to heading for a DH line, computed in SAS 9.2. Extremely late lines were excluded from further calculations. The regression line expressions were used to correct the registered mean level of leaf blotch and get a better expression for the resistance level.

Table 4.2 Pearson correlation between years for leaf blotch severity corrected for earliness

	2010	2011
2011	0.72	
2012	0.40	0.60

Table 4.2 displays the Pearson correlation coefficient between the different years. The correlation was highest between 2010 and 2011 and indicated more similarities in the results these years, than between 2010 and 2012 which had the lowest correlation coefficient (0.4).

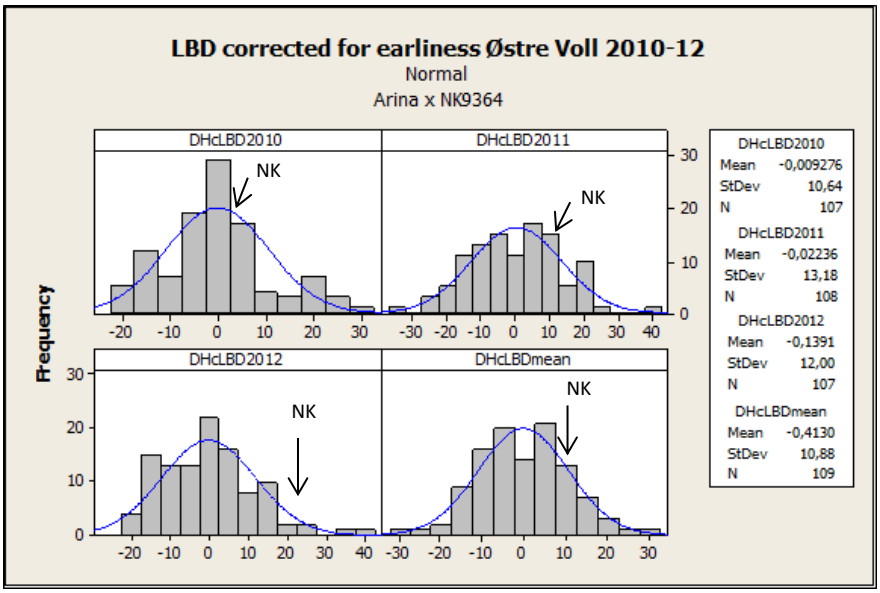


Figure 4.4 Frequency distribution and the genotypic ranges for leaf blotch disease corrected for days to heading 2010: -21.9 (most resistant, ID 20077) - 28.9 (most susceptible, ID 20049), 2011: -36.8 (ID 20035) – 38.1 (ID 20050), 2012: -21.3 (ID 20028) – 39.9 (ID 20050). The resistance level of parent NK93604 (NK) is indicated in each year (1.2 in 2010, 16.2 in 2011, 25.5 in 2012 and 10.3 for 2010-12). Winter wheat Arina was not included in the field trials.

Figure 4.4 shows the frequency distributions for resistance to leaf blotch disease (LBD or SNB) corrected for maturity (days to heading, DH)) for the field trials at Østre Voll 2010-2012. The frequencies follow the normal distribution. The one parent (NK93604) evaluated in the field trials was average (2010) to moderately susceptible (2011-12, mean) to LBD compared to the DH offspring. The figure also illustrates the genotypic ranges of the population, from the most susceptible to the most resistant lines (values for each year given in the figure).

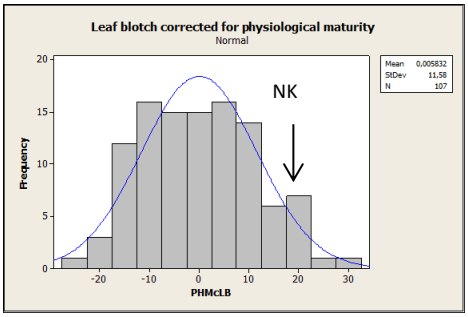


Figure 4.5 Frequency distribution and genotypic ranges for leaf blotch disease corrected for physiological maturity in 2012. The genotypic range was -26.7 (ID 99342) - 29.5 (ID 20042). The resistance level of parent NK93604 (NK) is indicated (17.3).

Figure 4.5 shows the frequency distributions for resistance to leaf blotch disease (LBD or SNB) corrected for physiological maturity for the field trials at Østre Voll 2010-2012. The frequencies seem to follow the normal distribution. The one parent (NK93604) evaluated in the field trials was moderately susceptible (17.3) to LBD compared to the DH offspring. The figure also illustrates the genotypic ranges of the population, from the most susceptible to the most resistant lines (values for each year given in the figure).

Table 4.3 Narrow sense heritability for DHcLBD in the Arina × NK93604 DH population, based on data from the field trials at Vollebekk 2010-12. Genetic range = value of most susceptible line - value of most resistant line – see figure 4.4) and estimated number of genes (n) involved in the trait are also listed.

year	h^2	Genotypic range (*d*)	*D* (h^2**d*)	$n = D^2/4 \cdot \sigma_g$
2010	0.84	50.5	42.4	4.12
2011	0.65	74.9	48.7	3.36
2012	0.40	61.2	24.5	2.14
2010-12	0.79			

Table 4.3 shows the narrow sense heritabilities for leaf blotch disease corrected for heading date in 2010, 2011, 2012 and the mean of 2010-12, for the Arina × NK93604 DH trials at Østre Voll. The narrow sense heritability (h^2) was lowest in 2012 with a value of 0.40, and the highest h^2 was observed in 2010, with a value of 0.84. The heritability for the mean of 2010-12 was 0.79 and reveals the genotype × environment interaction between years. The table also shows the genotypic ranges (range of the DH means from the most susceptible to the most resistant line) for the trait in respective years, *D* which is the genotypic range multiplied by the narrow sense heritability, and the estimated minimum number of genes (*n*) involved in the quantitative trait. The estimated gene numbers were 4.12 in 2010, 3.36 in 2011 and 2.14 in 2012.

Table 4.4 Narrow sense heritability for leaf blotch disease corrected for days from sowing to physiological maturity in the Arina × NK93604 DH population, based on data from the field trials at Vollebekk 2012. Genetic range = value of most susceptible line - value of most resistant line) and estimated number of genes (n) involved in the trait are also listed.

year	h^2	Genotypic range (*d*)	*D* (h^2**d*)	$n = D^2/4 \cdot \sigma_g$
2012	0.43	56.2	24.2	2.22

Table 4.4 shows the narrow sense heritabilities for leaf blotch disease corrected for physiological maturity for the Arina × NK93604 DH trials at Østre Voll in 2012. The narrow sense heritability (h^2) was 0.43 and the range between the most susceptible and resistant line was 56.2 in 2012. The estimated number of genes (*n*) involved in the quantitative trait was 2.22 (which correspond quite well with the estimate for disease level corrected for heading in 2012).

4.1.4 QTL for adult plant resistance to leaf blotch disease

Table 4.5 Summary of major QTL for resistance level corrected for days to heading 2010-12. The significance level was set to LOD ≥ 3.0 at p = 0.05. A QTL is listed as one if the positions in different environments are < 20 cM apart. R^2 value is given for each QTL, in bold letters when LOD ≥ 3.0 and in grey when the LOD value was 1.5-2.0. Additive interval mapping was performed in ICIM v3.2 (www.isbreeding.net).

Chr.	2010	2011	2012	Mean	Position	Left marker	Right marker	Allele
1DL	-	-	**28.8**	-	19	*gwm191a*	*gwm337a*	Arina
2B	15.2	20.0	**26.9**	21.3	14-25	*wPt-0408*	*wmc770*	Arina
2DL	29.0	**35.4**	10.8	29.0	22-25	*P4M49-280*	*P2M49-255*	NK96304
4DS	12.7	**18.0**	-	**19.3**	3	*wmc473a*	*barc334*	Arina
5AS	19.6	**21.7**	13.9*	16.5	56-61	*cfd17a*	*gwm156*	Arina
6DL	22.5	22.6	-	**26.0**	77-80	*gwm55b*	*barc273*	NK93604
7A	-	-	**28.2**	18.5	10	*wPt-7299*	*gwm260a*	Arina

* = R^2 when physiological maturity was used as a correction factor

[1)] Donor parent of allele for resistance

Chr. = chromosome

Table 4.5 is a summary of significant QTL from the field trials at Østre Voll 2010-2012. The major QTL in 2010 were located on chromosome 2DL, 4DS, 5AS and 6DL (Table 4.5, Figure 4.6), however the LOD values were < 3.0 for all the loci (Figure 4.6 blue lines). In 2011, the same QTL were detected: the QTL on 2DL, 4DS and 5AS were significant (LOD ≥ 3.0) while 6DL had LOD = 2.6 (Figure 4.6 red lines).

In 2012, three different significant QTL were detected on 1DL, 2B and 7A (Table 4.5, Figure 4.6 green lines). The QTL from 2010-2011 on 2DL was detected, but not significant, with LOD = 2.0, and when using physiological maturity as the correction factor for resistance level instead of days to heading, the QTL on 5AS from 2010-2011 also appeared to have minor effects in 2012, with LOD = 2.1. The significant QTL on 2B in 2012 was also detected with low LOD thresholds in 2010 and 2011 (1.7 and 1.6, respectively), although there may be two different QTL within the marker interval (Table 4.5, Figure 4.6 upper right).

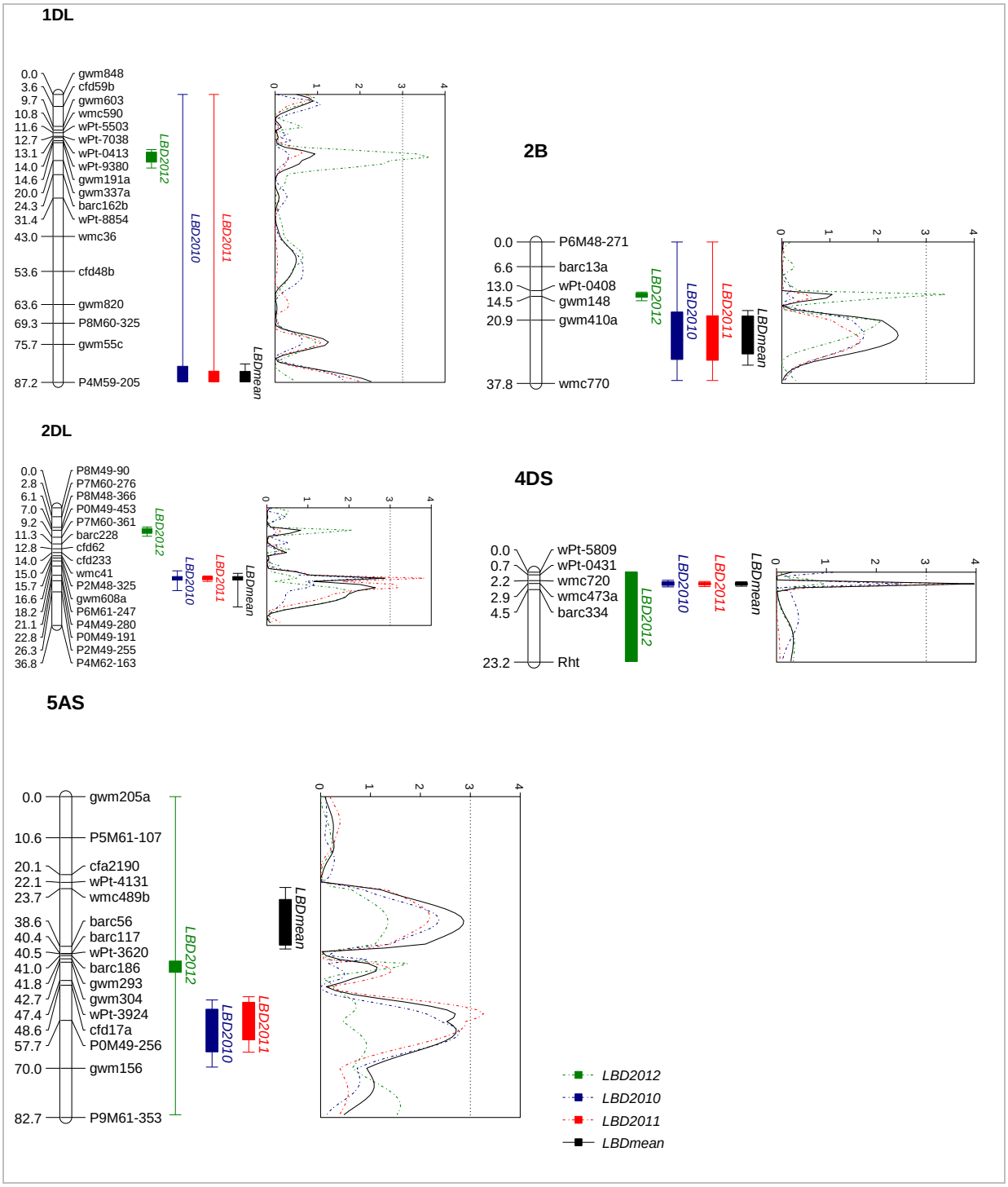


Figure 4.6 Linkage groups with significant QTL for adult plant leaf blotch resistance detected in one or more environments (years) at Østre Voll 2010-12. The map unit is cM. The QTL bar between the linkage group and LOD-graph shows the 1-LOD (thick) and 2-LOD (thin) interval for the QTL. The LOD threshold (3.0) is illustrated by the dotted line. From upper left: Linkage group on 1DL with a significant QTL for adult plant resistance to LBD in 2012 (green). The LOD value in 2012 was 3.6. Linkage group on 2B, with a significant QTL in 2012 (LOD value 3.4). We can also see a suggestive QTL 2010-11 (see table 4.5). The interval from *wPt0408-wmc770* was counted as one QTL. QTL position 22-25 on 2DL highly significant in 2011 and major in 2010, also with minor effects in 2012. QTL on 4DS significant in 2011 (LOD 3.7) and for the mean over three years (LOD 4.0). Also important in 2010 and a minor effect is visible for 2012. Dwarfing gene (*Rht*) in position 23.2. QTL on 5AS between *cfd17a* and *gwm156*. Also, a QTL with LOD < 3.0 is observed flanked by *wmc489b* and *barc56*. A significant QTL for plant height was also detected within this interval (Table 4.7).

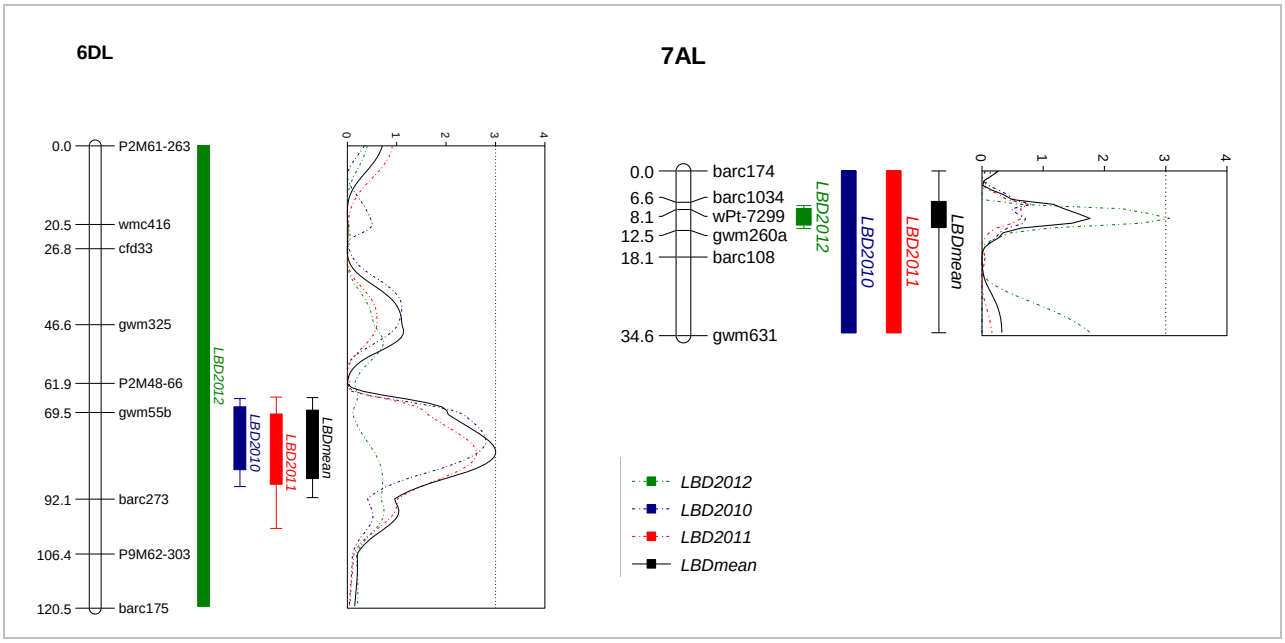


Figure 4.6 (continued) Linkage groups with significant QTL for adult plant leaf blotch resistance detected in one or more environments (years) at Østre Voll 2010-12. The map unit is cM. The QTL bar between the linkage group and LOD-graph shows the 1-LOD (thick) and 2-LOD (thin) interval for the QTL. The LOD threshold (3.0) is illustrated by the dotted line.From left: QTL on 6DL important in 2010 and 2012 although the LOD values were below < 3.0 except for the mean (3.0). In 2012 the QTL had almost no effect. QTL on 7AL significant in 2012.

4.1.5 QTL for resistance level corrected for physiological maturity 2012

Table4.6 QTL for resistance level corrected for physiological maturity. LOD above 3.0 indicated with bold R^2, LOD<2.5 in grey. Additive interval mapping perfomed in ICIM v3.2.

Chr	R^2	Position	Left marker	RM	Allele[1)]
1AS	14.8	44	*wPt-9402*	*P4M49-258*	NK93604
2BL	**24.9**	14	*wPt-0408*	*gwm148*	Arina
4BL	24.3	45	*wmc679*	*wmc349*	Arina
5AS	18.2	34	*wmc489b*	*barc56*	NK93604
5BS	**24.0**	15	*DuPw115*	*P2M59-242*	NK93604
6A	13.5	39	*P7M62-214*	*wmc621b*	NK93604
6A	15.2	43	*wmc661*	*wmc764*	Arina

[1)] Donor parent of allele for resistance

Table 4.6 shows the most significant QL detected in 2012 when physiological maturity was used as the correction factor. The QTL on 2BL was detected when this correction was used as well as earliness (Table 4.5), while the QTL on 1DL and 7A were below the LOD threshold. A QTL on 5BS was also significant. This QTL was not detected in 2010-12 when earliness was used as the correction factor.

4.1.6 QTL for plant height, heading and physiological maturity

Table 4.7 QTL with LOD above 2.5 for plant height in 2011 and 2012. R^2 value of highly significant QTL (LOD > 3.0) in bold letters. Additive interval mapping in ICIM v3.2.

Chr	2011	2012	Position	Left marker	Right marker	Allele[1)]
1A	**26.6**	18.6	3-5	*P7M60-267*	*P2M59-129*	Arina
2BL	16.7	22.4	25	*wPt-9350*	*wPt-0950*	NK93604
3BS	**28.2**	18.4	51	*wPt-5640*	*wmc291*	Arina
3DS	**18.3**	**29.3**	9-10	*gwm161*	*P1M59-199*	Arina
4BL	**16.5**	11.9	21-39	*wPt-5564*	*wmc413*	NK93604
5AS	**32.9**	**27.2**	31	*wmc489b*	*barc56*	Arina
6BL	31.3	28.5	29	*wPt-8183*	*P9M59-325*	Arina
6DL	30.8	19.8	80-100	*gwm55b*	*P9M62-303*	Arina

[1)]Donor parent of allele for short straw

Table 4.7 shows the major QTL for plant height in 2011 and 2012. QTL on 3DS and 5AS are highly significant in both years. The QTL on 6DL was located within the same marker interval as a major QTL for leaf blotch resistance corrected for earliness in 2010 and 2011 (Table 4.5). The QTL on 5AS corresponded to a minor QTL for leaf blotch resistance detected in all environments (Figure 4.6, chromosome 5AS).

Table 4.8 QTL for heading (earliness) in the Arina × NK population 2010-2012. R^2 values for each QTL are listed under respective years. The analysis was performed in ICIM, using additive interval mapping. QTL with LOD above 2.0 are listed.

Chr	2010	2011	2012	Pos.	Left Marker	Right Marker	Allele[1)]
5AS			19.5	48	*wPt-3924*	*cfd17a*	NK93604
5BL	12.2	13.7		3	*P6M61-240*	*fcp620*	NK93604
5D			11.1	0	*cfd78*	*wmc608*	NK93604
7BL		21.5		51	*wPt-2356*	*gwm577*	NK93604

[1)] Donor parent of allele for early heading

Table 4.8 shows QTL detected for earliness in 2010-2012. Generally, the LOD values were low (< 2.5). The QTL on 5AS was located within the same region as a QTL for adult plant resistance detected in all environments (Figure 4.6 chromosome 5AS). None of the other QTL corresponded to QTL for corrected resistance in 2010-2012.

Table 4.9 QTL for physiological maturity in the Arina × NK population 2010-2012. The analysis was performed in ICIM, using additive interval mapping. QTL with LOD above 2.5 are listed. R^2 values for each QTL are listed under respective years.

Chr	2012	Pos.	LM	RM	Allele[1)]
2DS	17.1	14	*cfd34*	*gwm191c*	NK93604
2DL	26.4	10	*gwm349*	*wmc167a*	NK93604
3BS	27.5	24	*P1M59-265*	*P1M59-265*	Arina
7AL	19.5	21	*P7M61-290*	*P6M48-114*	Arina

[1)] Donor parent of allele for early maturity

Table 4.9 shows the major QTL for physiological maturity detected in 2012. QTL were found on 2DS, 2DL, 3BS and 7AL. None of the QTL were located on the same linkage groups as QTL for corrected leaf blotch resistance (Table 4.6, Figure 4.6). However, the QTL on 2DL and 7AL are located on the same chromosomal region as resistance QTL and the possibility of significant linkage should not be excluded.

4.2 Seedling resistance to single *S. nodorum* isolates (Fargo)

Table 4.10 QTL detected after inoculation (inoc.) and infiltration (infiltr.) with the Norwegian *S. nodorum* isolate NOR4 LOD threshold 3.0. Additive interval mapping (IM-ADD) in ICIM 3.2 (www.isbreeding.net). R^2 values in bold letters were highly significant, in grey when under the LOD threshold. When the same chromosome is listed more than once the QTL are on different linkage groups on the chromosome.

Chr	Inoc.	Infiltr.	Pos.	Left marker	Right marker	Allele[1)]
1AS	16.2		39	*wPt-3983*	*P4M60-159*	NK93604
1B	23.5		35	*gwm11*	*wPt-1374*	NK93604
1BS	**20.0**	16.3	4-17	*fcp618*	*barc128a*	Arina
2BL	**15.8**		31	*wPt-7200*	*wPt-4968*	Arina
3A		**26.9**	1	*gwm155*	*wPt-3697*	Arina
3DS	18.5		12	*wPt-6358*	*wPt-1336*	NK96304
4BL		**35.4**	2	*P7M62-86*	*P2M59-225*	NK93604
4BL	14.9		19	*wPt-6209*	*wPt-8107*	Arina
5BL	14.1	14.6	0	*P6M61-240*	*fcp620*	Arina
6AS	18.0	**36.7**	1-3	*barc171a*	*barc3*	Arina
6BS		30.1	26	*P1M59-148*	*wPt-4706*	NK93604
6BL		32.1	20	*barc198*	*P1M50-302*	NK93604
7DS	20.5		30	*gwm111a*	*cfd46*	Arina

[1)] Donor parent of allele for resistance

Table 4.10 and figure 4.7 shows the R^2 values, LOD curves, position and marker intervals for the most significant QTL detected after inoculation with conidiospores and infiltration of culture filtrate of the Norwegian *S. nodorum*-isolate NOR4. Major QTL were located on 1BS and 2BL after inoculation, 3A, 4BL and 6AS after infiltration. The QTL on 1BS and 6AS had effect in both the inoculation and infiltration trials, as did a QTL on 5BL. The other QTL detected were only significant for either the infiltration or the inoculation experiment.

Table 4.11 QTL detected after inoculation (inoc.) and infiltration (infiltr.) with the North American *S. nodorum* isolate Sn4. LOD threshold 4.0. Additive interval mapping (IM-ADD) in ICIM 3.2 (www.isbreeding.net). R^2 values in bold letters were highly significant. R2 values in gray are less significant in one of the experiments. When the same chromosome is listed more than once the QTL are on different linkage groups on the chromosome.

Chr	Inoc	Infiltr.	Pos.	Left marker	Right marker	Allele[1)]
1B	32.7	**38.4**	39-42	*P2M59-192*	*P2M61-456*	Arina
1BS	**32.6**	26.6	4-17	*fcp618*	*barc128a*	Arina
1DL	27.9	**37.5**	25	*barc162b*	*wPt-8854*	Arina
2BL	**42.9**	22.4	29	*wPt-7200*	*wPt-4968*	Arina
3A	19.1	21.8	0	*gwm155*	*wPt-3697*	Arina
3DS	**36.4**	31.9	5	*cfd34*	*gwm191c*	Arina
4BL	26.4		19	*wPt-6209*	*wPt-8107*	Arina
5AS	**33.8**		22	*cfa2190*	*wPt-4131*	Arina
5BL	**37.1**	18.9	1	*P6M61-240*	*fcp620*	Arina
6AS	22.4	32.0	1	*barc171a*	*barc3*	Arina
6B	**32.0**	23.9	22	*wPt-3605*	*wPt-5333*	Arina
7A	29.0	22.1	15	*gwm276*	*DuPw226*	Arina
7DS	25.3		33	*gwm111a*	*cfd46*	Arina

[1)] Donor parent of allele for resistance

Table 4.11 and figure 4.7 shows the R^2 values, LOD curves, position and marker intervals for the most significant QTL detected after inoculation with conidiospores and infiltration of culture filtrate with the North American *S. nodorum*-isolate Sn4. Highly significant QTL were detected on 1B, 1BS, 1DL, 2BL, 3DS, 5AS, 5BL and 6B (Table, 4.11 Figure 4.7). Except for 5AS, all these QTL had LOD values above 3.0 in both the infiltration and inoculation experiments (Figure 4.7). Many of the same QTL were detected after both inoculation and infiltration with Sn4.

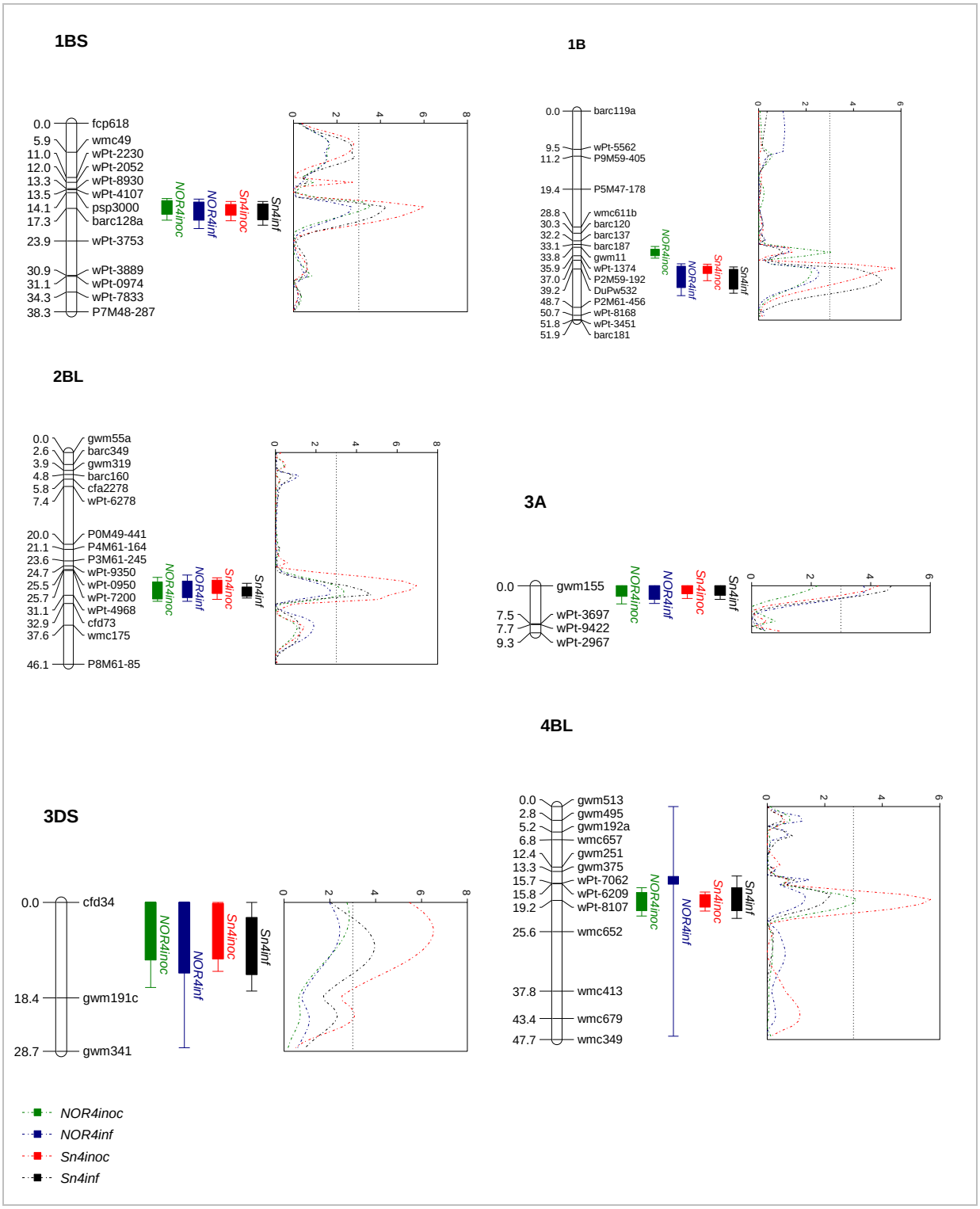


Figure 4.7 Linkage groups with highly significant QTL for seedling resistance to *Stagonospora* blotch, detected after single isolate inoculation ($1{\cdot}10^6$ spores/mL) and infiltration experiments with *S. nodorum* isolates NOR4 and Sn4 in Fargo. The map unit is cM. The QTL bar between the linkage group and LOD-graph shows the 1-LOD (thick) and 2-LOD (thin) interval for the QTL. The LOD threshold (3.0) is illustrated by the dotted line. From upper left: Significant QTL on 1BS in both Sn4 and NOR4 interactions (*Snn1*-locus). Significant QTL 1B for Sn4-interactions, suggestive QTL for NOR4 interactions. QTL on 2BL significant in all interactions. QTL on 3A significant in Sn4 inoculation and infiltration, and NOR4 infiltration. QTL on 3DS significant in Sn4 interactions and suggestive for NOR4 interaction. However, the map resolution on the linkage groups on 3A and 3DS is low. QTL on 4BL particularly significant in the inoculations, suggestive for infiltrations (may be an unpublished Tox-sensitivity).

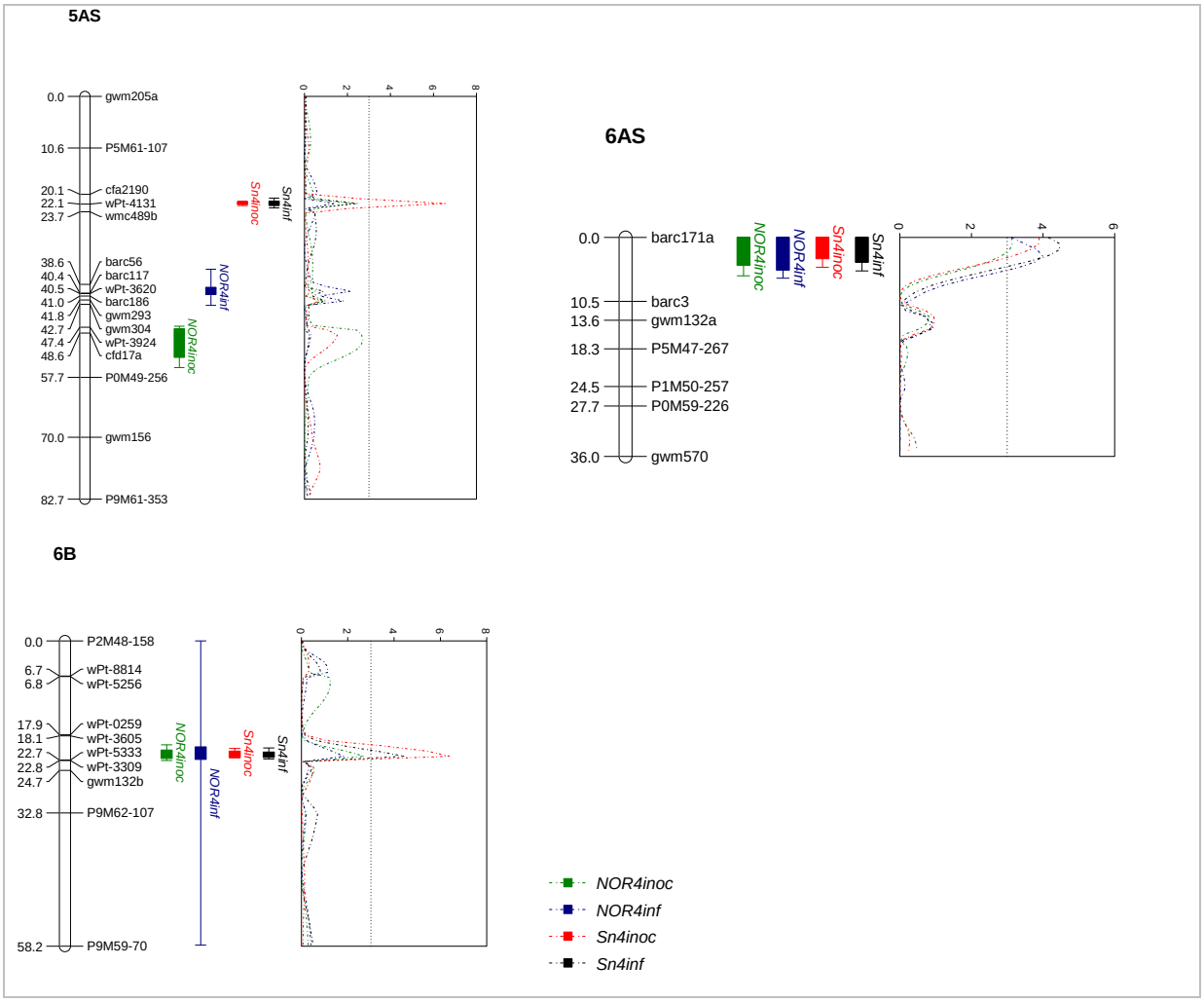


Figure 4.7 (continued) Linkage groups with highly significant QTL for seedling resistance to *Stagonospora* blotch, detected after single isolate inoculation ($1 \cdot 10^6$ spores/mL) and infiltration experiments with *S. nodorum* isolates NOR4 and Sn4. The map unit is cM. The QTL bar between the linkage group and LOD-graph shows the 1-LOD (thick) and 2-LOD (thin) interval for the QTL. The LOD threshold (3.0) is illustrated by the dotted line. From upper left: QTL on 5AS highly significant when the population was inoculated with Sn4, suggestive in the other interactions. QTL on 5BL significant in all interactions (*Tsn1*-locus). QTL on 6AS also significant for all interactions. QTL on 6B significant for Sn4 inoculation and infiltration, suggestive for NOR4 interactions. The thin bars show the confidence interval for the QTL.

Figure 4.7 shows the linkage groups with major QTL detected in the seedling experiments in Fargo. The LOD-curves show many similar patterns for both NOR4 and Sn4, but the LOD-scores for Sn4 were usually higher than for NOR4. This indicates that many of the same interactions were involved for both isolates.

4.3 QTL significant for both adult plant and seedling resistance

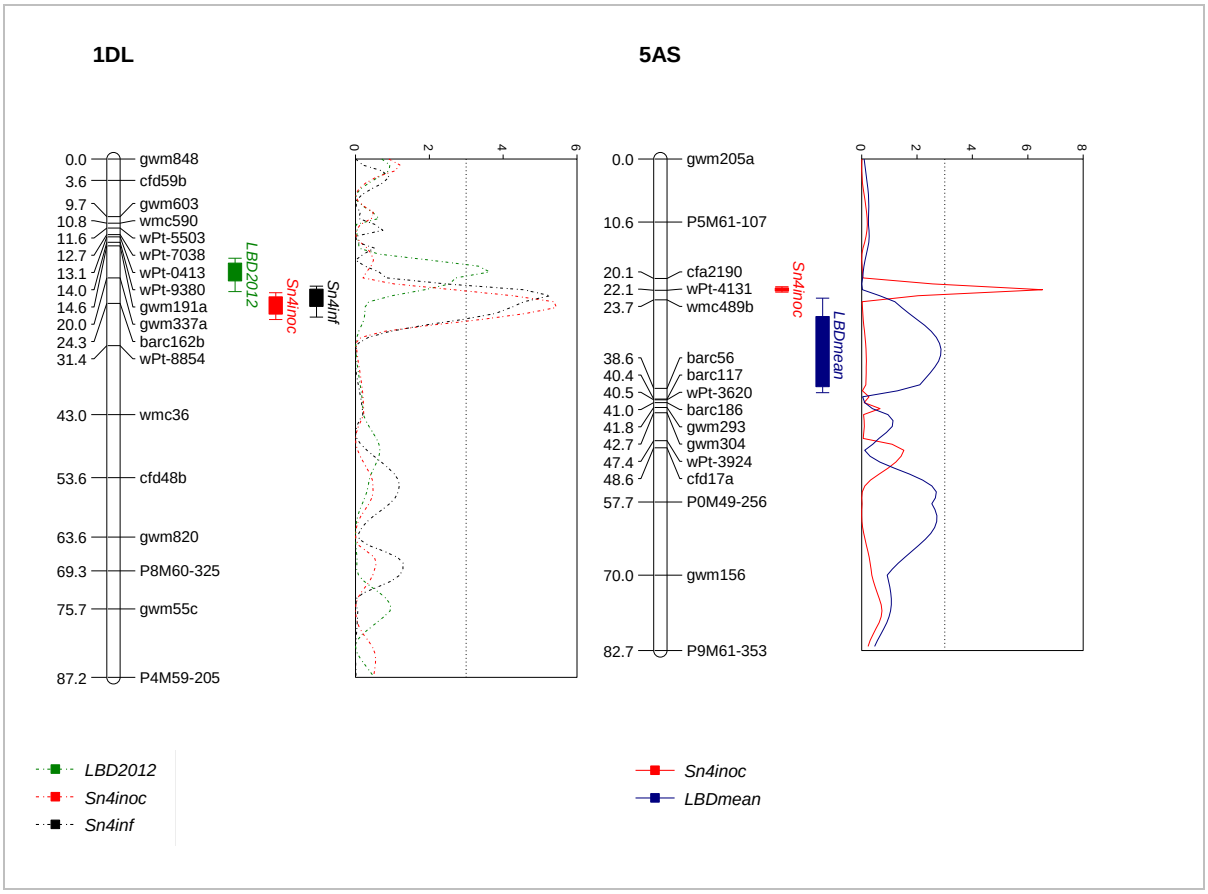


Figure 4.8 Linkage groups on 1DL (left) and 5AS (right) with QTL that were significant for both seedling and adult plant resistance. The map unit is cM. The QTL bar between the linkage group and LOD-graph shows the 1-LOD (thick) and 2-LOD (thin) interval for the QTL. The LOD threshold (3.0) is illustrated by the dotted line.

Figure 4.8 show linkage groups with significant interactions for both seedling and adult plant resistance. The position and shape of the QTL on 1DL indicate that the same locus was involved both in seedling and adult plant resistance. The QTL for adult plant resistance (blue) on 5AS overlapped the QTL for seedling resistance (red), although the shape and significance is different. This is discussed later.

4.4 Association to characterized toxin sensitivities

Table 4.12 R^2 values of QTL with association to characterized sensitivity genes (see table 3.1 in Background). In bold letters if significant, grey if below the significance threshold (LOD = 3.0).

Host gene	Toxin	Chr	Field			Nor4		Sn4		Markers
			2010	2011	2012	Inoc.	Infiltr.	Inoc.	Infiltr.	
Tsn1	SnToxA	5BL				**14.1**	14.6	**37.1**	18.9	*fcp620*
Snn1	SnTox1	1BS				20.0	16.3	**32.6**	26.6	*fcp618,psp3000*
Snn2	SnTox2	2DS	18.3	19.7		14.9		14.1		*cfd51-TC253803*
Snn3	SnTox3	5BS								
Snn4	SnTox4	1AS				16.2				*wPt-3983, P4M60159*
Snn5	SnTox5	4BL			24.3*					*wmc349*

* physiological maturity as a correction factor

Table 4.12 shows QTL from the field (Østre Voll) and single isolate (Fargo) experiments with marker association to characterized SnTox-*Snn*-interactions. Significant interactions were observed associated with SnToxA-*Tsn1* and SnTox1-*Snn1* (Table 4.12). Also, minor QTL were associated to SnTox2-*Snn2* in both field and single isolate inoculation trials, and possibly a QTL on 4BL when physiological maturity was used as a correction factor for resistance level in 2012 (Table 4.12). Also, a QTL was detected on 1AS in the same region as *Snn4*, although the markers were not identical with previously used markers.

4.5 Isolate/inoculation results

4.5.1 Isolates

Table 4.13 List of *S. nodorum* isolates collected from Arina × NK93604 doubled haploid lines at Østre Voll 2012. The first number in the isolate name indicates from which DH line the isolate was collected. The second number indicates different single spore isolates from different samples of the same line.

Isolate	Collection date	Resistance in DH line (based on 2010 data)	Sporulation time*	Color	Sporulation
Voll10.4	27.07.2012	Moderately resistant	15 days	white	few pycnidia/low sporulation
Voll10.8	06.08.2012	Moderately resistant	30 days	white	few pycnidia/low sporulation
Voll10.9	06.08.2012	Moderately resistant	20 days	white	few pycnidia/low sporulation
Voll11.4	27.07.2012	Moderately suceptible	14 days	white/grey	Only on V8,pycnidia buried in the agar, not surface
Voll15.2	01.08.2012	Moderately resistant	25 days	white	Slow/unreliable, V8, not on PDA
Voll28.2	27.7.2012	Moderately resistant	10 days	white	Rich sporulation
Voll28.3	27.7.2012	Moderately resistant	9 days	white	Rich and reliable sporulation,moderately aggressive
Voll28.8	6.8.2012	Moderately resistant	10 days	white	Rich sporulation
Voll28.10	6.8.2012	Moderately resistant	16 days	white/grey	Good sporulation
Voll49.5	27.7.2012	Susceptible	15 days	white	not very aggressive
Voll49.6	6.8.2012	Susceptible	30 days	grey/pinkish	Slow
Voll48.3	1.8.2012	Susceptible	> 30 days	white	Slow, few pycnidia
Voll48.4	1.8.2012	Susceptible	12 days	white	Not reliable
Voll48.10	6.8.2012	Susceptible	15 days	white	Not reliable, low sporulation
Voll73.1	1.8.2012	Resistant	15 days	pinkish	Good sporulation
Voll73.3	01.08.2012	Resistant	14 days	white/pinkish	Rich sporulation, strong indication of unknown effectors
Voll73.4	1.8.2012	Resistant	14 days	white/pinkish	Rich sporulation, strong indication of unknown effectors
Voll86.1	1.8.2012	Resistant	13 days	white	Rich sporulation
Voll86.2	2.8.2012	Resistant	9 days	orange	Rich sporulation, reduced mycelial growth
Voll86.3	3.8.2012	Resistant	13 days	orange/grey	Rich sporulation, reduced mycelial growth
Voll86.4	3.8.2012	Resistant	13 days	orange/grey	Rich sporulation, reduced mycelial growth
Voll86.10	15.8.2012	Resistant	10 days	orange	Rich sporulation, reduced mycelial growth
Voll86.15	15.8.2012	Resistant	9 days	orange	Rich sporulation, reduced mycelial growth
Voll101.1	1.8.2012	Average	14 days	white	Ok sporulation on V8, not PDA
Voll101.3	1.8.2012	Average	12 days	white	Rich sporulation

***) Sporulation time:** Time from isolation from leaf until sporulation.

Table 4.13 is a list of *S. nodorum* isolates collected from diseased leaves of the DH lines at Østre Voll in July-August 2012. Samples were made from 10 lines, but I was unable to retrieve any *S. nodorum* isolates from one line (57). The number of successful isolates from each of the selected

DH line varied from 1 (line 11, 15) to 6 (line 86), as the table shows. If the isolate did not sporulate on agar after the initial single spore isolation from a pycnidium, it was not registered as successful. In some cases (like line 57) no sporulating pycnidia developed on the leaves. The level of resistance of each DH line was also registered, based on data from 2010, and the color of the isolate (as a crude morphological character). The amount of sporulation on agar could imply if the isolate would be suitable for inoculation experiments, and possibly say something about the isolate's aggressiveness and preference for mycelial versus sporulating tissue.

4.5.2 Inoculations

Table 4.14 Results of inoculation of wheat seedlings on the 2-3 leaf stage with $1 \cdot 10^6$spores/mL *S. nodorum* isolate NOR4. The results 7 d.a.i. (days after inoculation) and 15 d.a.i. are from my experiments at SKP, the "Fargo mean disease score (m.d.s)" are results from inoculations with NOR4 done in Fargo, North Dakota.

Line #	Line/variety	M.d.s		Fargo m.d.s (7 d.a.i)
		7 d.a.i.	15 d.a.i.	
1	SHA3/CBR	0.5	0.5	-
2	Naxos	1	1.3	3.2
3	NK93604	1.17	3.7	2.5 (3.8)*
4	Arina	0	1	1.2
5	RE714	0	0	1.5
6	Bjarne	1.5	2.7	3.8

*) different means for two different sources of NK93604

Table 4.15 Inoculations with *S. nodorum* isolate Voll28.3 at two separate experiments (1. and 2.) to confirm stable conditions in the growth chamber/experimental set up. Each line had three replicates in each experiment, and the mean disease score is based on this.

Line #	Line/Variety	Mean disease score	
		24.01-04.02	05.02.-12.02
1	SHA3/CBR	1	1.6
2	Naxos	2.3	3
3	NK93604	2.5	2
4	Arina	1	1.2
5	RE714	0	0.6
6	Bjarne	3	3

NOR4 was used as a control when testing the experimental set up at SKP, because this isolate had been successfully tested in inoculation studies in Fargo, North Dakota (column to the right, Table 4.14) on five of the wheat varieties/lines used in my screenings. The trends are almost the

same as in Friesen's results, but the disease scores are lower. Table 4.15 shows the results of two separate inoculation experiments (24.01-04.02.12 and 05.02-12.02.12) with *S. nodorum*-isolate Voll28.3. The results display a similar trend for both experiments, and the conditions were evaluated to be stable.

Table 4.16 Results from inoculation with 10^6 spores/mL of *S. nodorum* isolate Voll73.3. Seedling inoculation of 10 wheat lines differing in toxin sensitivity, and of 10 other lines.

Line	Name	Sensitivity	m.d.s*
1	AF89	Tox4	0.0
2	ITMI44	Tox4Ba (unpub)	0.7
3	BR34	Univ.insens.	2.7
4	ITMI37	Tox6A (unpub)	0.8
5	CS(DIC1B)	Tox5D(unpub)	1.3
6	M6	Tox1	0.3
7	BG220	Tox3	1.0
8	LP29	Tox4Bb(unpub)	0.0
9	BG261	ToxA	2.3
10	BG223	Tox3	1.0
11	NK93604		1.0
12	Arina		0.0
13	SHA3/CBR		0.3
14	Naxos		1.7
15	Demonstrant		1.7
16	Bjarne		1.0
17	Zebra		1.0
18	Laban		1.3
19	Krabat		1.3
20	RE714		0.0

*) m.d.s = mean disease score

Table 4.16 shows the results of an inoculation of wheat lines with different, known sensitivities to NEs produced by *S. nodorum*. The differential lines (1-10 in the table) and 10 other lines (11-20) were inoculated with $1 \cdot 10^6$ spores/mL of *S. nodorum* isolate 73.3, and the disease level was evaluated after 7 days, using the 0-5 scale by Liu et al. (2004b) (Table 3.3). The mean disease level varied from 0.0 in the resistant lines RE714 and AF89, to 2.7 in BR34 (Table 4.16).

5 Discussion

5.1 Adult plant resistance - field trials 2010-2012

5.1.1 Leaf blotch severity

The mean leaf blotch severity (Figure 4.1) for 2010 and 2011 were highly positively correlated and the correlation was also high between 2011 and 2012 (Table 4.1). The correlation between 2010 and 2012 was lower, although still highly correlated per definition (> 0.5). The disease level at the time of evaluation can affect the quality of the results. Leaf blotch disease develops exponentially during a short period (1-2 weeks). If the assessment is done too early or too late in the disease development, it is difficult to differentiate between lines that really vary in resistance level.

Morphological and developmental traits like plant height, earliness and maturity (Table 4.7-4.9) can have profound effects on accurate evaluation of leaf blotch resistance (Scott et al. 1982). In a winter × spring cross like Arina × NK93604, large variation in earliness is expected and it is important to find suitable correction factors in order to get a good estimation of the true resistance level. Earliness was measured as days from sowing to heading and used to correct the severity data (Figure 4.2a-d). In addition, physiological maturity was used in 2012 (Figure 4.3). Plant height was phenotyped in 2011 and 2012 and a QTL analysis was performed to find QTL for height (Table 4.7), but not used as a correction factor in this thesis.

5.1.2 Corrected level of resistance

When the severity level was corrected (Figure 4.4), we observed that the frequency distributions changed compared to the uncorrected frequencies (Figure 4.1). The uncorrected LBD severity is a measure of the disease level at the time of measurement, not the true level of resistance in the line. The correlation between 2010 and 2011 was lowered, but still highly positive, when the severity data were corrected (Table 4.2). The same was true for the correlation between 2011 and 2012, while the correlation between 2010 and 2012 was moderately positive for the corrected data (Table 4.2).

The DH population followed the normal distribution for leaf blotch resistance regregation (Figure 4.4). Spring wheat parent NK93604 was average to moderately susceptible. The offspring probably displayed transgressive segregation for the resistance trait, with many lines being more susceptible than the parent. The exact relationship could not be investigated because the other parent Arina was excluded from the field trials because of its winter wheat habit. However, Arina is known to possess a high level of leaf blotch resistance (Paillard et al. 2003).

5.1.3 Heritability and gene number estimates

The narrow sense heritability of leaf blotch resistance varied from moderate (0.40) in 2012 to high (0.84) in 2010 (Table 4.3). The heritability for 2010-12 was 0.79. Corrected for physiological maturity in 2012, the heritability was 0.43 (Table 4.4). Heritability for *Stagonospora* blotch is known to vary, and has been reported to be low, moderate or high in different studies (Aguilar et al. 2005; Francki et al. 2011; Schnurbusch et al. 2003; Shankar et al. 2008; Uphaus et al. 2007; Wicki et al. 1999). This illustrates a complex genetic inheritance of the trait (Francki 2013). However, an explanation for the moderate heritability in 2012 can be that only two replicates were included in the ANOVA, adding to the uncertainty and letting less of the phenotypic variance be explained. Also, the variation between the replicates due to environmental conditions was possibly larger than normal in 2012. This was supported by the fact that one replicate had to be excluded because the plants looked chlorotic and with strong leaf tip necrosis from early in the season. Overall, the heritability seemed to be high, a requirement for efficient breeding to improve the resistance level.

Estimation of gene numbers in quantitative traits is associated with uncertainty and the estimates given in table 4.3 and 4.4 are minimum values. Failure to meet the assumptions of the model will give lower estimates. The number of QTL detected each year (Table 4.5) was higher than the estimates. Obviously, some of the model assumptions were not met: The effect of each allele is not equal (different R^2 values), epistasis and dominance may be present (the *Snn* genes are dominant/recessive), some of the QTL are linked and there is probably transgressive segregation (Figure 4.4). In a complex trait like leaf blotch resistance, the gene number estimations were not very informative.

5.1.4 Short evaluation of correction factors

As discussed above, earliness was used as the correction factor for all three years, and physiological maturity was used in 2012. QTL analysis for plant height, earliness and maturity were also performed (Table 4.7 – 4.9), to control that the QTL listed for corrected ("true") resistance were not actually expressions for pleiotropic effects of height or development stage.

For the correction of the different confounding factors, I could have used multiple linear regression. The regression would have combined the different factors (height, heading and physiological maturity) in one mathematical expression. In 2011 the regression would have included both plant height and days to heading, and in 2012 plant height, days to heading and physiological maturity. However, because earliness was the only correction trait that had been

phenotyped all three years and could be used to compare data for 2010-2012, the priority was on this single factor.

I used QTL iciMapping (ICIM) to do the QTL analysis. In MapQTL ® 6 (van Oijen 2009), an alternative to ICIM for the QTL analysis, the correction for multiple factors could be performed in the software. In retrospect, it might have been better to learn how to use MapQTL® in addition to ICIM, and corrected for multiple traits in each QTL analysis where it was possible.

5.1.5 QTL for adult plant resistance

The same QTL on 2DL, 4DS, 5AS and 6DL were dominating both 2010 and 2011 (Table 4.5, Figure 4.6), while the significant QTL in 2012 were located on 1DL, 2B and 7A (Table 4.5, Figure 4.6). The QTL on 2B also had minor effects in the previous years. The QTL on 2B was significant in 2012 also when physiological maturity was used as the correction factor (Table 4.6). When days to heading were used to correct the level of resistance, only two of six QTL with LOD $\geq$ 1.5 in 2012 corresponded to QTL in 2010-2011 (Table 4.5). A minor QTL was detected on 2DL in 2012 in the same position as in 2010-2011 (Table 4.5, Figure 4.6).

When physiological maturity was used as the correction factor for resistance level in 2012, the QTL on 5AS was detected in all three years (Table 4.5). Another QTL was observed on the same linkage group (Figure 4.6). A highly significant QTL for plant height shared the same marker interval (Table 4.7). The source of resistance for this second QTL was NK93604, while the source of short straw was Arina (Table 4.7). Plant height has a pleiotropic effect on leaf blotch disease level due to the rain-splashed spread from lower to upper parts of the plants (Francki 2013; Shankar et al. 2008) and may explain this QTL, instead of a true resistance mechanism. This QTL will also be discussed under seedling resistance.

All in all, 2012 looked quite different from the two previous years, in particular different from 2010. This is also confirmed by the correlation coefficients (Table 4.2). 2010 and 2011 were highly correlated while the correlation between 2010 and 2012 was lower (Table 4.1 and 4.2). 2011 and 2012 had higher correlation than 2010 and 2012 (Table 4.1 and 4.2). It is known from previous studies that QTL for leaf blotch are largely affected by genotype × environment interactions (Francki 2013). Weather conditions like air temperature and leaf wetness affect the disease progress, and have profound effects on relative rankings of resistance (Kim & Bockus 2003). The mist irrigation system minimized the effect of natural variation in rainfalls across the years. In 2012, a short period of temperatures above 10 °C very early in the growing season may have triggered early ascospore release before the host plants were available to the pathogen

(early May, Figure 3.2). If this happened, it could have been a bottleneck for the pathogen population and to an extent explained the different genotype expression reflected in the QTL analysis for 2012 than 2010-11. Other events, like the conditions for overwintering, may also have influenced the survival and changed the population from one year to another.

Another factor that could explain some of the variation between years is that different raters phenotyped the population in 2010-11 (M. Lillemo) versus 2012 (A. Ruud). Unfortunately, no control measures were taken to compare the reliability and accuracy of the two raters. However, other mapping populations at Østre Voll were evaluated for leaf blotch by one person all three years, and showed the same trend with similar results in 2010-11 and different in 2012 (Q. Lu, personal comment).

5.1.6 Association of major QTL to previously reported loci for LBD/SNB resistance

No QTL for *Stagonospora* blotch resistance have previously been reported on 1DL. As this QTL was only detected in 2012 and had LOD value ≤ 1.5 in 2010 and 2011, more field studies of the population should be executed to investigate the presence of consistent QTL in the region.

On 2B, a QTL for glume blotch resistance has been detected in one environment in a previous study (Shankar et al. 2008), with one marker (*gwm148*) shared with the major QTL in 2012 (Table 4.5, Figure 4.6). A robust QTL for seedling resistance has also been reported between *gwm501-gwm410* (Czembor et al. 2003) (Table 2.2). Marker *gwm410* is included in the marker interval (*gwm148-wmc770*) for the 2012 QTL (Figure 4.6). The map resolution in the linkage group is low and could be increased by adding suitable markers. The population could be evaluated again to confirm whether there are consistent QTL for seedling, leaf and glume blotch in the region. The QTL had moderate effects in 2010 and 2011 as well as 2012 (Table 4.5, Figure 4.6). A QTL that is detected in many environments is more interesting to breeders (Francki 2013), and the underlying host-pathogen interaction should be investigated.

Two QTL on 2DL for adult plant resistance, *QSnl.daw-2D* (Shankar et al. 2008) and *QSnl.eth-2D* (Aguilar et al. 2005), have been reported earlier (Table 2.3). These QTL are probably located closer to the centromere than the QTL found in 2010-11 (GrainGenes2.0 2013; Somers et al. 2004; Sourdille et al. 2004). However, in the Arina × NK93604 map, many markers in the region deviate significantly ($P<0.001$) from the expected 1:1 ratio (Semagn et al. 2006b), and the linkage group had few markers in common with the consensus maps (GrainGenes2.0 2013; Somers et al. 2004). This makes the position of the QTL uncertain. The problem with segregation distortion is a well-known issue in wide crosses like a winter × spring wheat cross,

and when developing a population using the maize x wheat system (Semagn et al. 2006b). Lines with strong winter wheat characteristics were excluded from the population. This contributes to the degree of distortion, especially for chromosomal regions with vernalization genes. In the ArinaxNK9306 map from 2006, the total segregation distortion was 20.4 %, and 2D was one of the chromosomes with a high degree of distortion (Semagn et al. 2006b).

A QTL for *Stagonospora* glume blotch has also been reported on the distal part of 2DL (Table 2.4), but is assumed to be unique for glume resistance (Uphaus et al. 2007). Also, it was probably located more to the telomeric end of 2DL than the QTL I found (GrainGenes2.0 2013; Semagn et al. 2006b; Sourdille et al. 2004; Uphaus et al. 2007).

No QTL for seedling, adult plant or glume blotch resistance have previously been consistently assigned to 4DS. More studies should be conducted to further evaluate the QTL found here in 2010 and 2011. A dwarfing gene (*Rht-D1*) is assigned to the same linkage group (Figure 4.6, marker name *Rht*), but not closely linked to the resistance QTL.

A major QTL was detected on 5AS in 2010 and 2011 (Table 4.5, Figure 4.6). Flanking marker *gwm156* showed significant segregation distortion in the map from 2006 (Semagn et al. 2006b) and is placed on 3B according to the physical map (GrainGenes2.0 2013). This makes the results more uncertain, and more markers should be assigned to the region to clarify the correct marker order and linkage groups. No robust QTL for *Stagonospora* blotch resistance are reported on 5AS in previous studies.

No robust QTL have been reported on 6DL either, but a QTL for seedling resistance was listed on 6D flanked by markers *gdm46* and edm*148a* in a greenhouse environment (Shankar et al. 2008). However, the marker *gdm46* has also been assigned to 7D (GrainGenes2.0 2013).

A QTL has been reported on 7AS using association mapping (Adhikari et al. 2011). Although the markers are different at direct comparison difficult, it is possible that this is the same QTL detected in 2012. According to the published Arina × NK93604 map, the QTL is located at ≈ 50 cM on 7AS (Semagn et al. 2006b). The marker associated to the previously described QTL is located at 55.1 cM (Adhikari et al. 2011). However, the map distances are relative.

In summary; few of the detected QTL have been described in previous studies. This study is conducted in a different geographic region than for the published QTL (USA, continental Europe, Australia), and one should expect a different pathogen population. The DH population had not been used for mapping of leaf blotch resistance previously either, although Arina was

one of the parents in the winter spring cross Arina × Forno studied by Schnurbusch et al. (2003). The QTL on 1DL, 4DS, 5AS and 6DL are not described earlier to my knowledge. The QTL on 2B, 2D and 7A are maybe identical with previously described loci.

5.2 QTL for seedling resistance to SNB (Fargo)

5.2.1 QTL detected

A major QTL was detected on 1BS after inoculation and infiltration with both NOR4 and Sn4, although the LOD value was < 3.0 in the NOR4 infiltration (Table 4.10 and 4.11, Figure 4.7). This locus is discussed in detail under host specific interactions (2.5.1). Other QTL detected after inoculation and infiltration with both isolates were located on 5BL (also discussed under host specific interactions), and 6AS (Table 4.10-11, Figure 4.7). No robust QTL for *Stagonospora* blotch resistance has previously been assigned to 6AS. The major QTL on 2BL explaining 42.9 % of the phenotypic variation for the Sn4 inoculation was also detected in all the experiments, although the LOD score after NOR4 infiltration was below 3.0 (2.7) (Figure 4.7). No robust QTL or host specific interaction have previously been reported on 2BL.The QTL is located on another linkage group than the QTL on 2B from the field studies (see above). A major QTL on 3AS was detected after infiltration with NOR4 and Sn4, and inoculation with Sn4 (Table 4.10-11, Figure 4.7). The LOD value for NOR4 inoculation was 2.2, indicating that the QTL played a role in this interaction as well.

Two QTL were detected after inoculations with pycnidiospores of NOR4 and Sn4, but not after infiltration: on 4BL and 7DS (Table 4.10-11). A sensitivity gene (*Snn5*) has recently been characterized on 4BL (Friesen et al. 2012). However, the QTL on 4BL between *wPt-6209* and *wPt-8107* is probably not associated with this sensitivity gene (see discussion under host specific interactions), but two other, unpublished host-specific interactions are known on 4B. These toxins are called Tox4Ba and b, and the differential wheat lines ITMI44 and LP29 (Table 3.4) possess sensitivity to Tox4a and b, respectively.

No QTL for *Stagonospora* blotch resistance have been described previously on 7D (Table 4.10-11). The reason why these QTL were only detected after inoculation may be that the interaction is dependent on a mechanism in the living pathogen.

Generally, the LOD values for QTL detected after inoculation and infiltration with Sn4 had notably higher LOD values than NOR4. However, the LOD curves in Figure 4.7 strongly indicate that NOR4 is involved in the same interactions as Sn4. If the Sn4 isolate gave a clear differentiation in symptoms that were easy to assess using the range of 0-5 and 0-3 categories,

and the NOR4 isolate gave symptoms that not were as easy to score, this can be an explanation. Also, different isolates can produce different amounts of the same NE, which may affect the level of disease (Faris et al. 2011).

Interestingly, the inoculation versus infiltration experiments with NOR4 gave few common QTL of significance (Table 4.10), only the three mentioned above. However, this is mostly explained by lower LOD values in the NOR4 infiltration analysis, and Figure 4.7 indicates that the reaction pattern for NOR4 filtrate resembles that of the inoculation. The infiltration experiments have only been performed once for each of the isolates on the Arina × NK93604 population, which increase the possibility of random errors and uncertainty of the results. Also, to prepare the culture filtrate, the pathogen was grown in liquid media for several weeks (Friesen & Faris 2012; Liu et al. 2004b). This may affect and change the production of pathogenic compounds compared to a natural environment.

5.2.2 Indications of novel NE/*Snn*-interactions

New QTL that appear to be important in all or most of the experiments, for instance the QTL on 2BL, 3AS, 4BL, 6AS and 6B (Figure 4.7), may be indications of novel NE/*Snn*-interactions, although the relationships must be investigated. Further studies should include isolation of the pathogenic compound by chromatography, and exact mapping of the host gene. Unpublished host-specific interactions are known on 4B, and may or may not correspond to the one detected in my study.

5.3 QTL detected in both field and seedling experiments

5.3.1 General

Few QTL were significant in both field and inoculation/infiltration trials. However, the QTL on 1DL significant for adult plant resistance in 2012 (Table 4.5) and for seedling resistance to Sn4 inoculum and culture filtrate (Table 4.11) are probably identical. The position of the QTL for adult plant resistance was 19, for seedling resistance 25, on the same linkage group (Figure 4.8).

On 5AS, a highly significant QTL was detected when the population was inoculated with Sn4 (Table 4.10, Figure 4.7-8). The interaction is also observable, but under the LOD threshold, for the filtrate and NOR4 trials (Figure 4.7). Interestingly, the position of this QTL corresponds to the position of a minor resistance QTL observed in the field trials (Figure 4.8). In field, accurate scoring and interpretation of the resistance may have been confused by the presence of a significant QTL for plant height within the same marker interval (Table 4.7). Inoculation and

disease assessment of the adult population in a greenhouse to eliminate the effect of plant height might be useful to confirm whether the QTL has effect on adult plant resistance.

It is not surprising that the field and seedling experiments yielded different results. From previous studies, it is known that some genes for seedling resistance have minor effects on adult plant resistance, while other genes are independent (Francki 2013). Also, the natural pathogen population at Østre Voll was probably quite different and much more complex than the two single isolates used for the seedling resistance screening. This addresses the problem with using single isolates to evaluate resistance: The diversity of a field environment is not reflected. This is particularly important in a multigenic trait like SNB resistance.

5.3.2 Association of detected QTL to characterized HST interactions

Tsn1: For all the seedling experiments conducted in Fargo, a QTL was detected on 5BL flanked by marker *fcp620* (Table 4.12). This marker is closely associated (≈0.05 cM) with the *Tsn1* sensitivity gene causing susceptibility to SnToxA (Friesen & Faris 2010; Zhang et al. 2009). The Sn4 isolate is known to produce SnToxA (Faris et al. 2011), and the LOD values of the QTL detected after Sn4 inoculation and culture filtrate infiltration indicate a major and highly significant interaction (Table 4.11-12, Figure 4.7). The QTL is located at the end of the linkage group (Figure 4.7). To improve the map we could genotype other closely associated markers, like *fcp394* (Zhang et al. 2009). In conclusion, this QTL is most likely *Tsn1*.

In previous studies, robust and major QTL have been detected in the same region conferring both seedling (Czembor et al. 2003; Friesen et al. 2009; Gonzalez-Hernandez et al. 2009) and adult plant (Friesen et al. 2009) resistance (Table 2.2-3). Although a number of linked genes involved in SNB resistance in this region have been reported in one study (Gonzalez-Hernandez et al. 2009), the SnToxA-*tsn1/Tsn1*-interaction is possibly the only, or most important, factor involved (Faris & Friesen 2009; Francki 2013). In the field trial, a QTL for physiological maturity was linked to *fcp620* (Table 4.12).

Snn1: A QTL was detected on 1BS in the single isolate inoculation and infiltration experiments within a marker interval including both *fcp618* and *psp3000* (Table 4.10-12, Figure 4.7). The distance between the markers is significantly larger in my map than in the high density map of the region (Reddy et al. 2008). The exact order and position of the markers vary between populations and some disagreement between maps should be expected (Somers et al. 2004). The larger distance between *psp3000* and *fcp618* in my map could be due to errors in the genotyping or coding of the marker data. However, the marker data was evaluated again 25.04.2013 and did

not contain obvious errors. Under all circumstances, the unsuspected positions of the markers make it difficult to conclude on the exact position of *Snn1*.

No QTL with direct marker association to *Tsn1* or *Snn1* were detected in the Østre Voll field trials of adult plant resistance. This may either indicate that these loci are less important in adult plant than seedling resistance, or that SnToxA and SnTox1 not were produced by the local pathogen population at Østre Voll in 2010-2012.

Snn2: The Sn4 isolate is known to produce SnTox2 (Faris et al. 2011) which interacts with the *snn2* sensitivity gene on 2DS. However, only a very minor QTL (LOD 1.7, R^2=14.1) was detected flanked by *Snn2*-associated markers *cfd51-TC253803* when the Arina × NK93604 population was inoculated with Sn4 at the seedling stage (Table 4.12). The *snn1*-gene has been reported to affect adult plant resistance under field conditions (Friesen et al. 2009). A suggestive QTL was also detected in the same position when the population was inoculated with NOR 4 and in the field studies at Østre Voll in 2010 and 11 (Table 4.12). The detection in different environments and conditions indicate a robust QTL in spite of the moderate LOD values. Reasons for the modest LOD scores may be that the marker resolution of the linkage group was poor or that the SnTox2-expression of Sn4 (Fargo) and the natural pathogen population (Østre Voll) was low. It may also be that the DH population not segregates for *snn2/Snn2* and that the QTL represents another, linked gene. The distance between *cfd51* and *TC253803* in my map was 5.1 cM, which corresponds well to the 4.0 cM interval between the same markers, mapped by Zhang et al. (2009).

Snn5: When physiological maturity was used in 2012 as a correction factor for level of leaf blotch resistance, a minor QTL was detected flanked by *wmc413* and *wmc349* (Table 4.12),The marker *wmc349* is associated with the newly characterized sensitivity locus *Snn5*, which has been mapped 2.8 cM proximal to *wmc349* (Friesen et al. 2012). The position of the QTL in 2012 was 2.7 cM distance proximal to *wmc349*. The position of several other markers on the linkage group also correspond well to the linkage group assigned to *Snn5* (Friesen et al. 2012). However, in the original chromosome map for the Arina × NK93604 population (Semagn et al. 2006b) and in the consensus map (Somers et al. 2004), the linkage group is placed on the short arm of 4B. This contrasts the location on 4BL reported by Friesen et al. (2012), which is based on the physical map (Sourdille et al. 2004). The interaction does not appear to be very important in adult plant resistance, but it would be interesting to do seedling screenings of the Arina ×

NK93604 population with inoculation and culture filtrate infiltration of *S. nodorum* isolates that produce SnTox5.

Snn3 and ***Snn4***: No QTL with direct marker association to *Snn3* or *Snn4* were detected in any of the trials. The SnTox3-*Snn3*-interaction is reported to be epistatic with the SnTox2-*Snn2*-interaction (Friesen & Faris 2010), and this interaction appears to be present, if not very significant, in 2010, 2011 and in the single isolate inoculations (Table 4.11). When corrected for physiological maturity in 2012, a significant QTL for leaf blotch resistance was detected on the end of 5BS (Table 4.6), possibly in the same region as *snn3*. This QTL was also detected with LOD < 2.0 in 2012 (corrected for heading) and for the mean of the three years of field trials (data not shown). But it cannot be concluded what the association to *snn3* is.

The SnTox4-*Snn4* interaction is assumed to be significant only if the SnToxA-*Tsn1* interaction is absent (Friesen & Faris 2010). However only one population (Arina × Forno) has been tested (Abeysekara et al. 2009). If both NOR4 and Sn4 produced SnToxA, this may explain the absence of a reaction for Tox4. However, the SnToxA-*Tsn1*-interaction was not present in the field trials. A resistance QTL was also detected at the end of 1AS when the DH seedlings were inoculated with NOR4 (Table 4.10). Because the susceptibility is inherited from Arina, this QTL is probably *Snn4*. The result also indicates that the Tox4-*Snn4*-interaction can be significant even in the presence of ToxA.

5.3.3 Evaluation of methods for disease assessment

Disease was also measured in different ways in the adult plants versus seedlings: In field (adult plants) we used a quantitative continuous % scale to score the disease *severity*, and corrected the data for a confounding factor (earliness). The seedlings were assessed qualitatively for disease *reaction type* by using discrete classes (0-5 for inoculation, 0-3 for culture filtrate). These measurements are not directly comparable – they measure different aspects of the disease (% diseased plant versus reaction type), the range in values is different and different statistic methods should be used to evaluate discrete and continuous variables (Madden et al. 2007).

The assessment of adult plant disease can also be performed in different ways. A common method for measuring LBD is to score % diseased flag leaf at a certain development stage. We evaluated the whole canopy, which may or may not be comparable to registrations of flag leaf only. Registration of development stage was not done at the time of disease scoring, but extrapolated from days to heading or to physiological maturity. More accurate scorings could have been performed, but would have been very time consuming.

5.4 Isolation and inoculation part

5.4.1 The work process

Several months (August 2012-February 2013) were spent in the lab at Bioforsk Plantehelse and greenhouse at SKP trying to establish a method for inoculating of wheat seedlings with *S. nodorum* pycnidiospores. The isolation of *S. nodorum* from diseased leaves was straight forward and resulted in the isolates listed in Table 4.13. But for the remaining work two major challenges were encountered:

1) Unpredictable sporulation of many *S. nodorum* isolates
2) Creating a suitable environment 0-24 h post inoculation to get successful infection

To control if the conditions in the greenhouse and growth chamber were suitable for infection and would reproduce results corresponding to those achieved in Fargo, I tried to use the *S. nodorum* isolate NOR4 as a control. This isolate had been used to inoculate several Norwegian wheat lines in Fargo 2012 (Table 4.14). However, the isolate appeared to be very slow to sporulate under the conditions we could provide here (challenge number 1). NOR4 had been treated as described in 3.4.1-3.4.6 (1) prior to the testing. The isolate refused to sporulate on PDA, and sporulated sparsely after a month on V8 (Appendix 2).This delayed the initial testing significantly. Most of the isolates, including NOR4, were less prone to sporulate after 2-3 cycles of spore suspension on agar. I re-isolated the isolates from autoclaved wheat leaves to try to maintain the pathogenicity and willingness to sporulate, however the results varied (Appendix 3).

5.4.2 Conditions for inoculation

When, finally, I was able to produce inoculum and inoculate the wheat seedlings, challenge 2) was encountered. The conditions in the growth chamber were not stable enough to meet the requirements of the pathogen. It was important to find a relative humidity (RH) level where water droplets (i.e. inoculum) would stay on the leaves, not evaporate because of too low humidity, or drip off the leaves if the humidity or mist input was too high. However, the main problem with the growth chamber was that it had an air flow involved in the temperature regulation. The air was retrieved from the outdoor environment and thus affected by changing RH depending on weather and temperature outside. This gave unstable and unpredictable conditions and it was impossible to set parameters for mist intervals that would provide the environment needed for infections. Also, the airflow gave an unwanted effect of drying/wetting of the leaves because the RH level varied through the 24 h post inoculation, which probably

killed the pycnidiospores in several inoculation attempts. When the technicians and I finally figured out that it was possible to minimize the airflow to almost zero, it was easier to adjust the added humidity (mist) to a suitable level, and to get a stable RH of 99 %.

Then, in December 2012/January 2013, it was finally possible to do successful inoculations. The six wheat lines were inoculated with NOR4. I evaluated the results from Table 4.14 and concluded that the trends were the same in both mine and the Fargo experiments, although the disease scores were slightly higher in Fargo than mine (Table 4.14). Also, it took more days to develop good symptoms on my plants than in Fargo. Maybe the isolate I used had less production of pathogenic factors, possibly due to the prolonged period it grew on agar until it sporulated. Also the viability of the pycnidiospores may have been compromised for some reason.

Now it was time to test selected isolates from the 2012 Vollebekk collection (Table 4.13). However, challenge 1), the problem with unpredictable sporulation, was still present, although a few isolates, like Voll28.3 and Voll73.3 were reliable. In January/February 2013, wheat seedlings were inoculated with Voll28.3 (Table 4.15) and 73.3 (table 4.16).

5.4.3 Discussion of results

The inoculation with Voll28.3 on the six "test lines" was repeated to confirm stable conditions because the air flow had been changed since the NOR4 inoculations. Bjarne was the most susceptible line (Table 4.15) with a moderately susceptible (MS) reaction (Table 3.3). Naxos was MS while the other parent in the Sha3/CBR × Naxos mapping population was resistant (R.). NK93604 was moderately resistant (MR) while Arina was R. For the offspring to segregate for a trait, different resistance level in each parent is wanted. However, the parents can have different alleles for resistance and the offspring can segregate for the trait even when the parents are equally resistant or susceptible. The level of resistance towards this isolate

When inoculating the 10 differential lines, the information we can get is whether the isolate *not* produces the toxin the differential line is known to be sensitive to. If we get a susceptible reaction, the reasons can be either 1) production of the known toxin, 2) production of another, maybe hitherto unknown toxin or 3) other mechanisms of pathogenicity. The results from the inoculation with Voll73.3 (Table 4.16) indicate that the isolate not produced Tox1, Tox4, Tox 4Ba and b and Tox6A (unpublished) due to highly resistant reactions in the lines with these sensitivities (Table 3.4/4.16). The isolate probably neither produced Tox2, Tox3 or Tox5D (unpublished). However, it produced a MS reaction on BR34 which is known as universally

resistant to known North American *S. nodorum*-isolates (Table 3.4./Table 4.16). This strongly indicates that the Norwegian isolate Voll73.3 is different from the North American isolates of *S. nodorum* and produces NEs unknown in USA. Therefore, Voll73.3 appeared to be an interesting isolate for continued work on identifying novel NEs in the Norwegian pathogen population. The other wheat lines (11-20) tested in the experiment varied from 0-1.7, i.e. HR-MR reactions. In other words, none of the Norwegian wheat lines were very susceptible to the isolate. Arina and the universally resistant line RE1714 were most resistant with disease scores of 0. The experiment was only conducted once due to time limitations and should be repeated to further investigate the relationships.

5.4.4 Summary of isolation/inoculation part

Clearly, more work has to be done on the pathogen side. It was very difficult to get many of the isolates to sporulate. Maybe some isolates of *S. nodorum* are unsuitable for inoculation experiments because they don't sporulate readily on agar. Or maybe the conditions needed for optimal sporulation were not met. I was advised to grow the isolates on 9 °C after the first sporulation on agar before storing them (-80 °C), but this might have enhanced the preference of the fungus to grow mycelium instead of sporulating tissue. It is probably better to dry and freeze the sporulating tissue, not mycelium. The isolates should, if necessary, be re-isolated from wheat tissue to maintain pathogenicity, not grown in repeated cycles on agar.

A consideration for further work will be whether it is practical to grow and screen many isolates at the same time, or if it is better to work with few isolates. If the number of isolates proves to be a constraint because they are unpredictable, the method may not be very efficient for screening and characterizing many collected isolates within a reasonable time span. Molecular characterization, i.e. fingerprinting, can be an alternative method. However, I believe the methods for growing and storing the isolates can be improved considerably and that the methods I used not were optimal. Other constraints for testing many different isolates on differential lines may be a limited availability of seed and the time and greenhouse space consumed. However, the seedling inoculation method can also be used in pre-selection screening of wheat genotypes in breeding programs, or to map QTL and specific host-pathogen interactions in mapping populations using single or mixed isolates.

5.5 Future prospects: Improving resistance to leaf blotch in wheat

The impacts of leaf blotch diseases will probably increase with more rainy growth season due to predicted climatic changes, resistance and loss of sensitivity to important fungicide groups and further implementation of reduced tillage. This calls for sustainable and efficient methods to prevent yield and quality losses.

The multigenic property of the resistance trait, variable heritability and the significant influence of genotype × environment interactions are challenging to breeders. The effect of each gene is relatively small, and varies from environment to environment, as illustrated in this thesis as well as in previous research. The leaf blotch diseases are most devastating when the plants reaches physiological maturity and an approach to improve the genetic gain for adult plant resistance is field based screenings in multiple environments. Pre-selection of wheat genotypes through simplified screening systems is an alternative approach. However, good correlation to field performance is necessary. The identification of host-specific interactions is an important contribution to the understanding of the *S. nodorum*-wheat pathosystem; but their role in identifying resistance and the correlation to adult plant resistance is not well understood at time being.

The use of molecular markers, including functional physiological markers that can discriminate against pleiotropy and true resistance, will be of help in the breeding. However, identification of QTL that are expressed across different environments is necessary, and good phenotyping of the resistance level is required to identify these QTL and suitable markers.

6 Conclusion

The many new QTL discovered in both adult plant and seedling evaluations in this study illustrate the complex inheritance of resistance to LBD and SNB, as well as the diversity of the pathogen population. Genotype × environment interactions contribute to the complexity of adult plant resistance. The correlation between seedling and adult plant resistance need further investigation. Even though the results of the seedling inoculations with Norwegian isolates have to be reproduced, indications are strong that we will find novel necrotrophic effectors that differs from the North American effectors hitherto reported. For practical breeding where adult plant resistance is the objective, continuous selection of resistant genotypes in multiple field environments will probably be more efficient than selecting against host-specific interactions in seedling screenings.

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Appendixes

1. Agar recipies

1. Difco Potato Dextrose Agar (PDA)

Per liter:

Potato starch from infusion: 4.0 g

Dextrose: 20.0 g

Agar: 15.0 g

Final pH: 5.6 ± 0.2

2. V8-PDA Agar (V8)

Per liter:

V8 juice 150 mL

Difco PDA 10 g

$CaCO_3$ 3g

Agar 10g

Distilled H_2O 850 mL

3. Water Agar (WA)

Per liter: 15 g agar.

2. Spore suspension sporulation time

Time from spore suspension of selected isolates was spread on agar (PDA or V8) to sporulation.

Isolate #	PDA	V8	Comments
Voll28.8	23.11-29.11 (5 days)	23.11-29.11 (5 days)	Richer sporulation on V8, more mycelium, fewer pycnidia on PDA
Voll28.2	Contaminated, thrown 29.11	23.11-29.11 (5 days)	The contaminated sample was an inoculum test
Voll49.5	No	16.11-29.11	Sporulates, but sparsely
Voll73.3	14.11-19.11	14.11-19.11	Very rich sporulation on both media
NOR4	No (from 24.9.12)	14.11-22.11	Rich sporulation from suspension

3. Re-isolation from autoclaved leaves

Re-isolation via autoclaved wheat leaves placed on water agar (WA) from 4.1.13: If no end date -> no sporulation

Isolate number	Origin	Days until pycnidia formation on leaf	Comments	Days until sporulation (V8)	Comments
Voll28.3		18.12.12-4.1.13	Reliable isolate, many pycnidia	4.1.-12.1	Rich sporulation
Voll10.4	Mycel plug from freezer	18.12.12-	-	-	-
NOR4		4.1.13-12.02.13	Did not look like *S.nodorum*, possibly *Aschocyta* – contamination?	-	-
Voll73.3	From spore suspension -> leaf	6.2.13-12.2.13	Some sporulation	12.2.-20.2	Rich sporulation
Voll48.4	Mycel plugs from freezer rubbed against wet autoclaved leaves	6.2.13-	-	-	-
Voll49.5	-"-	6.2.13-26.2.13	Some sporulation	26.2 -	-
Voll86.1	-"-	6.2.13-	-	-	-
Voll86.15	-"-	6.2.13-12.02.13	Rich sporulation	12.2-	-