Resistance studies and genetic characterization of the barley – *Pyrenophora teres* pathosystem

Genetisk karakterisering av *Pyrenophora teres* og studier av byggbrunflekkresistens i bygg

Philosophiae Doctor (PhD) Thesis

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List of papers

Paper I:

Wonneberger, R., Sønstebø, J. H., Vivian-Smith, A., Lillemo, M.: Genetic structure of the Norwegian *Pyrenophora teres* population (Manuscript)

Paper II:

Wonneberger, R., Ficke, A., Lillemo, M. (under review). Identification of quantitative trait loci associated with resistance to net form net blotch in a collection of Nordic barley germplasm. (Under review at Theoretical and Applied Genetics)

Paper III:

Wonneberger, R., Ficke, A., Lillemo, M. (2017). Mapping of quantitative trait loci associated with resistance to net form net blotch (*Pyrenophora teres* f. *teres)* in a doubled haploid Norwegian barley population. PLoS ONE 12(4): e0175773. https://doi.org/10.1371/journal.pone.0175773

Abstract

Net blotch is a major barley disease in Norway caused by the necrotrophic fungus *Pyrenophora teres*, which can cause yield losses of up to 40% under conducive conditions. Two forms of this pathogen, *P. teres* f. *teres* (*Ptt*) and *P. teres* f. *maculata* (*Ptm*), can be distinguished by the symptoms they cause upon infection. At present, net blotch resistance in Norwegian cultivars is insufficient and resistance sources in the Norwegian germplasm are poorly characterized. Therefore, the structure and biology of the Norwegian net blotch population was characterized in order to develop strategies to improve resistance breeding. In addition, a study was conducted to detect quantitative trait loci (QTL) associated with resistance in two mapping populations representing the germplasm most relevant to Norwegian barley breeding.

A collection of 339 single-conidia isolates from different regions in Norway were genotyped-by-sequencing using ddRADseq on the Ion Torrent platform and 4252 single nucleotide polymorphism (SNP) markers were used to characterize the genetic structure of the population. PCR-based assays showed that 95.9% of the isolates were *Ptt* while only 2.4% were *Ptm*, indicating that *Ptt* is the predominant form in Norway today. The mating type ratio was not significantly deviating from 1:1 and all isolates constituted distinct multilocus haplotypes, indicating a predominantly sexual reproduction. Index of association tests, however, suggested a predominantly clonal reproduction, which indicates that the population may have a mixed reproduction system. Analysis of genetic variation suggests that gene flow may occur between regions and within time periods of up to five years. Indications of adaptation to host cultivars underline the potential of rapid adaptation in the pathogen.

Resistance to three Norwegian *Ptt* isolates was assessed in a segregating biparental cross of the Norwegian cultivars Arve and Lavrans and an association mapping panel consisting of 209 mostly Nordic barley lines, including landraces, breeding lines and currently grown cultivars. Inoculation experiments were performed on seedlings in the greenhouse and on adult plants in the field. In the biparental population, a set of 589 SNP markers was used to map a major QTL on chromosome 5H that was stable in all environments and explained up to 48% and 55% of the phenotypic variation in seedlings and adult plants, respectively. Eight additional QTL explained up to 17% in seedlings and 15% in adult plants, and one of them was isolate-specific.

Most resistance alleles originated from the more resistant parent Lavrans. Association mapping in 209 Nordic barley lines genotyped with 5669 SNPs revealed 43 significant marker-trait associations corresponding to 15 QTL, each explaining less than 15% of the phenotypic variation. QTL on 3H and 6H were consistently found to be significant both in seedlings and in adult plants. These are promising candidates for breeding programs using marker-assisted selection strategies.

This work suggests that *Ptt* is a high-risk pathogen with a high evolutionary potential that can adapt to changing environmental conditions such as new host resistance quickly. The most successful breeding strategy is likely to be pyramiding different quantitative resistance genes in elite cultivars combined with a number of major resistance genes to achieve durable resistance against this genetically diverse fungus. A number of stable seedling and adult resistance loci have been identified and markers associated with these loci are available for marker-assisted selection. Since the correlation between seedling and adult resistance was between r = 0.35 and 0.49 in this study, seedling phenotyping is not a recommended method to predict adult field resistance.

Sammendrag

Byggbrunflekk forårsaket av *Pyrenophora teres* er en av de viktigste sykdommene på bygg i Norge, og kan gi avlingstap på opptil 40%. Det finnes to former av patogenet, *P. teres* f. *teres* (*Ptt*) og *P. teres* f. *maculata* (*Ptm*), som kan skilles basert på symptomene de forårsaker på plantene (nettflekk og ovalflekk). Resistensen i dagens norske byggsorter er utilstrekkelig og resistenskilder i norsk foredlingsmateriale er dårlig karakterisert. Strukturen og de biologiske egenskapene til den norske byggbrunflekkpopulasjonen ble derfor undersøkt for å utvikle strategier for bedre resistensforedling. I tillegg ble det utført genetiske studier for å identifisere QTL (quantitative trait loci) for resistens i to kartleggingspopulasjoner som representerer relevante genetiske materialer for norsk byggforedling.

En samling av 339 enkeltisolater fra forskjellige regioner i Norge ble genotypet ved sekvensering (ddRADseq) på Ion Torrent-plattformen og 4252 SNP (single nucleotide polymorphism)-markører ble brukt til å karakterisere populasjonens genetiske struktur. PCR-baserte analyser viste at 95.9% av isolatene var *Ptt* mens bare 2.4% var *Ptm*, noe som indikerer at *Ptt* er den dominerende formen i Norge i dag. Krysningstypene var ikke signifikant avvikende fra 1:1, og alle isolatene utgjorde forskjellige multilokus-haplotyper, hvilket indikerer en overveiende seksuell reproduksjon. Indeks for assosiasjonstester indikerte imidlertid en overvekt av klonformering, noe som antyder at populasjonen kan ha et blandet reproduksjonssystem. Analyse av genetisk variasjon viser at genflyt kan forekomme mellom regioner og innenfor tidsperioder på opptil fem år. Indikasjoner på tilpasning til ulike byggsorter understreker potensialet for rask tilpasning i patogenet.

Resistens mot tre norske *Ptt*-isolater ble undersøkt i en spaltende krysningspopulasjon fra de norske byggsortene Arve og Lavrans og i en assosiasjonskartleggingspopulasjon av 209 for det meste nordiske bygglinjer, inkludert landsorter, foredlingslinjer, historiske og dagens sorter. Inokuleringsforsøk ble utført på småplanter i veksthus og på voksne planter i felt. I Arve x Lavrans-populasjonen ble et sett med 589 SNP markører brukt til å kartlegge et hoved-QTL på kromosom 5H som var stabilt i alle miljøer og forklarte opptil 48% og 55% av den fenotypiske variasjonen i henholdsvis småplanter og voksne planter. Ytterligere åtte QTL forklarte opptil 17% av variasjonen i småplanter og 15% i voksne planter, og et av dem var isolatspesifikt. De

fleste resistensalleler stammer fra den mer resistente foreldresorten Lavrans. Assosiasjonskartlegging i 209 nordiske bygglinjer genotypet med 5669 SNP'er viste 35 signifikante markør-resistens-assosiasjoner, som tilsvarer 13 QTL. Hver av dem forklarer mindre enn 15% av den fenotypiske variasjonen. QTL på 3H og 6H var signifikante både i småplanter og i voksne planter. Disse er lovende kandidater for markørbasert seleksjon i foredlingsprogrammer.

Dette arbeidet viser at *Ptt* er et høyrisikopatogen med et høyt evolusjonært potensial som raskt kan tilpasse seg endrede miljøforhold som ny vertsresistens. Den beste foredlingsstrategien er sannsynligvis pyramidisering av forskjellige kvantitative resistensgener i samme sort kombinert med resistensgener med stor effekt for å oppnå varig resistens mot denne genetisk mangfoldige soppen. Flere QTL i småplanter og voksne planter ble identifisert, og markører assosiert med disse er tilgjengelige for markørbasert seleksjon. Siden korrelasjonen mellom småplanteresistens og resistens i voksne planter var mellom r = 0.35 og 0.49 i denne undersøkelsen, anbefales ikke resistenstesting av småplanter som en metode for å forutsi feltresistens i voksne planter.

Abbreviations

AFLP	Amplified fragment length polymorphism
AM	Association mapping
AMOVA	Analysis of molecular variance
AxL	Arve x Lavrans mapping population
bp	base pairs
cM	centiMorgan
ddRAD	double digest restriction associated DNA (sequencing)
DH	Days to heading
GWAS	Genome-wide association study
LD	Linkage disequilibrium
MAGIC	Multiparent advanced generation intercross
MAS	Marker-assisted selection
MTA	Marker-trait association
NAM	Nested association mapping
NB	Net blotch
NBP	Nordic breeding panel
NE	Necrotrophic effector
NFNB	Net form net blotch
PCA	Principal component analysis
PCR	Polymerase chain reaction
PH	Plant height
Ptt	*Pyrenophora teres* f. *teres*
Ptm	*Pyrenophora teres* f. *maculata*
RAPD	Random amplified polymorphic DNA
RIL	Recombinant inbred line
RFLP	Restriction fragment length polymorphism
QTL	Quantitative trait locus/loci
SFNB	Spot form net blotch
SNP	Single nucleotide polymorphism

1. Introduction

1.1. Barley

Barley (*Hordeum vulgare* ssp. *vulgare* L.) is a diploid (2n=2x=14) self-pollinated grass of the *Triticeae* tribe in the *Poaceae* family. It is considered one of the first domesticated plants (Verstegen et al. 2014). Recent research shows that it was likely domesticated at least twice from related wild populations of *H. vulgare* ssp. *spontaneum* C. Koch in the Fertile Crescent and beyond (Badr et al. 2000; Morrell and Clegg 2007; Poets et al. 2015).

Barley is, after corn (*Zea mays* L.), wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.), the fourth most important cereal crop in terms of global production (USDA 2016). In 2014, it was grown on an area of 49.4 million hectares and the global production amounted to 144.5 million metric tons (Food and Agriculture Organization of the United Nations 2016) (Table 1). The major barley producers are the Russian Federation (20.4 million metric tons in 2014), France (11.7 million metric tons) and Germany (11.5 million metric tons). Today, barley is mainly used for animal feed (65-75%) and malting while only 2% are dedicated to human consumption, although in some regions such as Asia and northern Africa it remains an important part of the human diet (Baik and Ullrich 2008; Blake et al. 2011). The adaptability of barley allows for cultivation in a wide range of different climates and latitudes worldwide (Poehlman 1987). In Norway, on average 0.54 million metric tons were produced on 49% of the total area used for cereals in the last five years (Statistics Norway 2016a, b), making it the country's most important cereal crop (Table 2).

Table 1 Barley production worldwide and per country 2014. (Food and Agriculture Organization of the United Nations 2016)

Country	Production (million metric tons)	Area (million hectares)	Yield (tons/hectare)
World	49.4	144.5	2.9
Russian Federation	20.4	9.0	2.3
France	11.7	1.8	6.6
Germany	11.6	1.6	7.3
Australia	9.2	3.8	2.4
Ukraine	9.0	3.0	3.0
Canada	7.1	2.1	3.3
Spain	7.0	2.8	2.5
United Kingdom	6.9	9.0	6.3
Turkey	6.3	2.7	2.3
USA	4.0	1.0	3.9

Table 2 Cereal production in Norway: Yield and area. Average values from 2012-2016 (Adapted from: Statistics Norway, Tables 04607 and 07479)

Cultivar	Area (hectares)	% of total cereal area	Yield in 1000 tonnes
Barley	140580	49.1	540
Oats	69850	24.4	271
Spring wheat	54578	19.0	332 (total wheat)
Winter wheat	16130	5.6	
Rye and triticale	5108	1.8	28
Total	284490		1171

Barley can be classified by a number of characteristics such as spring or winter type, presence or absence of hulls and awns as well as row type (Schulte et al. 2009; Ullrich 2011). In two-rowed barleys, only the central spikelets are fertile, whereas in six-rowed barley, all three spikelets of a triplet are fertile. Two-rowed barleys have a higher thousand kernel weight, higher starch content and lower protein content than six-rowed barleys (Verstegen et al. 2014). Globally, spring barley dominates, especially in colder climates where winter varieties might not survive the winter, such as Scandinavia (Verstegen et al. 2014). Spring barley yields about 2 tons/ha less than winter barley, but matures in 90-120 days and has a broad adaptability to different climates and little requirements to soil quality (Verstegen et al. 2014).

Today, almost all spring barley in Central Europe is two-rowed, whereas in Northern Europe and North America, six-rowed spring cultivars are grown as well (Verstegen et al. 2014).

Being a diploid and a major crop plant, barley is an important model in many research fields such as genetics, breeding and plant pathology (International Barley Genome Sequencing Consortium 2012; Schulte et al. 2009). The barley genome has a size of 5.1 Gbp (International Barley Genome Sequencing Consortium 2012). A high-throughput single nucleotide polymorphism (SNP) genotyping platform including two barley oligonucleotide arrays was developed (Close et al. 2009), and these markers are included in the Illumina iSelect 9k Barley SNP Chip developed by Comadran et al. (2012). A consensus map of these markers based on twelve maps is available (Comadran et al. 2012; Muñoz-Amatriaín et al. 2014; Muñoz-Amatriaín et al. 2011). Draft genomes of the cultivars Morex, Bowman, Barke, Haruna Nijo and a Tibetan line are available, and Morex is currently used as a reference genome (International Barley Genome Sequencing Consortium 2012; Sato et al. 2016; Zeng et al. 2015). A physical map of Morex covering 4.98 Gbp of the barley genome was created, into which sequence data from more than 6000 bacterial artificial chromosome (BAC) clones as well as transcriptome sequences were integrated (International Barley Genome Sequencing Consortium 2012). In total, the positions of contiguous genome sequences (contigs) of a total size of 3.9 Gb were anchored to a genetic map (International Barley Genome Sequencing Consortium 2012). Mascher et al. (2013) used an approach termed POPSEQ to increase the number of contigs anchored to an existing sequence assembly by exploiting genetic segregation in two biparental populations. The authors sequenced the Morex x Barke recombinant inbred line (RIL) and the Oregon Wolfe Barley populations at a shallow depth and mapped the reads to the Morex genome and created a new genetic map of the SNP markers obtained. This method allowed the ordering of 500000 to 600000 contigs with almost 1000 Mbp of sequence. Recently, the first high-quality barley reference sequence covering 4.79 Gbp of the Morex genome was published, which will greatly benefit barley research in the future (Mascher et al. 2017).

Grain yield is undoubtedly the most important breeding trait, and breeding efforts used to achieve an average yield increase of 0.5% per year, but considerable efforts are also made to improve nutrient stability and harvestability as well as resistance to biotic and abiotic stresses (Verstegen et al. 2014). The different end uses of barley (animal feed, malting and human

consumption) have varying demands for quality traits like content of protein, starch and fiber. Especially the malting barley industry has a number of quality requirements such as a large grain size and carbohydrate and enzyme content (see Fox et al. (2003) for an overview). Selection for traits has to be considered carefully, since many important traits are negatively correlated, such as yield and protein content.

Barley is a host for a number of fungal, bacterial and viral pathogens. The most important fungal barley diseases are powdery mildew (*Blumeria graminis*), scald (*Rhynchosporium commune*), net blotch (*Pyrenophora teres*), spot blotch (*Cochliobolus sativus*), head blight (*Fusarium* spp.), rusts (*Puccinia* spp.) and *Fusarium* spp. (Friedt et al. 2011; Schweizer 2014; Verstegen et al. 2014). Since these pathogens have differing optimal conditions for growth and reproduction, different diseases are predominant in different climatic regions. In Norway, net blotch is one of the most severe barley diseases.

1.2. Barley net blotch

Net blotch is caused by the fungal pathogen *Pyrenophora teres* Smedeg. (anamorph: *Drechslera teres* (Sacc.) Shoem.), which occurs in the two forms *P. teres* f. *teres* (*Ptt*) and *P. teres* f. *maculata* (*Ptm*) (see section 1.2.1). It is an ascomycete in the class of Dothideomycetes and belongs to the order Pleosporales, which also hosts a number of other agronomically important cereal pathogens such as *Cochliobolus sativus* and *Parastagonospora nodorum*, the causal agents of barley spot blotch and *Septoria nodorum* blotch on wheat, respectively. The first description of *P. teres* dates back to 1923 when the sexual stage was discovered by Drechsler (Drechsler 1923). The asexual stage was initially believed to belong to the *Helminthosporium* genus but was later reclassified as *Drechslera* (Shoemaker 1959). It was only in 1971 that Smedegård-Petersen suggested to distinguish between *Ptt* and *Ptm* as different forms of the same species (Smedegård-Petersen 1971), so that all research on *P. teres* before the early 1970s is considered to be based on *Ptt*. In Norway, the spot form was described to be caused by *P. teres* already in the 1960s, and disease surveys from the mid-1960s distinguished clearly between both forms (see section 1.2.2) (Hansen and Magnus 1969).

1.2.1. The two forms *P. teres* f. *teres* and f. *maculata*

The two *P. teres* forms *teres* and *maculata* are morphologically indistinguishable (McLean et al. 2009). Only minor differences have been observed in conidia size, but these are too insignificant to constitute a reliable distinction criterion (Crous et al. 1995). Differentiation is only possible by observation of symptoms on infected leaves (see section 1.2.3) or by polymerase chain reaction (PCR)-based methods. Williams et al. (2001) and Leisova et al. (2005) developed PCR primers based on amplified fragment length polymorphisms (AFLPs) that can differentiate *Ptt* and *Ptm*, and both primer sets reliably produced the same results in Czech and Slovak isolates (Leišová-Svobodová et al. 2014). According to the biological concept of species, *Ptt* and *Ptm* are different forms of the same species since it is possible to produce fertile progeny from a cross between both forms under laboratory conditions (Serenius et al. 2007; Smedegård-Petersen 1971). It is currently unclear to what extent hybridization occurs under natural conditions. *Ptt* and *Ptm* have been shown to occur together in fields in many barley-growing regions, and they have been isolated even from the same lesion (Akhavan et al. 2015; Leišová-Svobodová et al. 2014). Campbell et al. (2002) described a South African isolate that showed both net form and spot form-specific bands in a PCR assay with random amplified polymorphic DNA (RAPD) primers. Additionally, this isolate clustered close to hybrid isolates produced *in vitro* (Campbell et al. 1999) and was thus considered a naturally occurring progeny from a *Ptt* x *Ptm* cross. However, normal spore development in *Ptt* x *Ptm* crosses is often disrupted and meiosis seems incomplete (McDonald 1967; Serenius et al. 2005), which might explain why natural hybrids are extremely rare, if they occur at all. Rau et al. (2007) suggested that the two forms have been separated genetically for a long time and deemed natural hybridization very unlikely, and Ellwood et al. (2012) estimated the divergence time to be about 519000 years ago. In most studies, *Ptt* and *Ptm* fall into two distinct phylogenetic groups and should be treated as different pathogens (Akhavan et al. 2016b; Bogacki et al. 2010; Lehmensiek et al. 2010).

1.2.2. Agronomical significance

Barley is considered the main host for *P. teres* and the only one with agronomic importance. In addition, naturally *Ptm*-infected *H. vulgare* ssp. *spontaneum* as well as several species from the genera *Avena*, *Bromus* and *Triticum* have been reported, among them oats (*A. sativa*) and wheat (*T. aestivum*) (Shipton et al. 1973). *Ptt* seems to have an even broader host range and is able to infect species in the genera *Agropyron*, *Brachypodium*, *Elymus*, *Cynodon*, *Deschampsia*, *Hordelymus* and *Stipa,* the latter four also under field conditions (Brown et al. 1993) (reviewed in Liu et al., 2011).

The pathogen can cause yield losses of up to 44% in addition to quality losses such as reduced kernel weight, decreased grain density and grain shriveling (Jayasena et al. 2007; Liu et al. 2011; Mathre 1997), which can have a detrimental effect on malting and feed quality of the grain (Grewal et al. 2008; Paulitz and Steffenson 2010; Shipton 1966). For *Ptm*, Jayasena et al. (2007) reported that a 10% increase of disease severity of the three topmost leaves resulted in an average yield loss of 0.4 t/ha. By comparing *Ptt*-inoculated and fungicide-treated hillplots planted in the same location in Finland, Robinson (2000) determined yield losses of 20-35% due to net blotch in the Norwegian cultivar Arve. In Norway, yield losses due to *P. teres* are not well-documented and reliable data is lacking.

While the disease was not considered a serious threat for barley production until the 1960s, the increased application of reduced or no-tilling practices in combination with the use of susceptible cultivars has led to an increased significance in recent years (Mathre 1997; McLean et al. 2009; Paulitz and Steffenson 2010). Whereas *Ptt* still was considered to be a greater problem than *Ptm* for barley cultivation in Australia before the 2000s (Gupta and Loughman 2001), *Ptm* has been on the rise in recent years, especially in Australia and parts of North America where it has taken on epidemic proportions (McLean et al. 2009, 2010; Murray and Brennan 2009; Neupane et al. 2015). Local isolates possess high virulence on a number of current local barley cultivars, which indicates a recent change in virulence and adaptation to widely grown cultivars (Neupane et al. 2015). Conversely, some decades ago *Ptm* seemed to be more common than *Ptt* in some European countries including France, Norway, Finland and Denmark (Arabi et al. 1992; Hansen and Magnus 1969; Mäkelä 1972; Smedegård-Petersen 1971). Today, most Nordic studies focus on *Ptt*. In recent population genetics studies

from Finland, only *Ptt* isolates were found (Serenius et al. 2005). In 2009, *P. teres* was found in 86% of all sampled barley fields in Finland, and although it is not stated which of the two forms was sampled, it was presumably *Ptt* (Jalli et al. 2011).

The oldest findings of *Ptt*-infected barley samples in Norway date back to 1880 and originate from Hedmark in Eastern Norway (Jørstad 1945). A severe *Ptt* epidemic was reported in Southern Norway in 1927, and by the end of the 1960s, it was found in all barley-growing regions up to Nordland county (Hansen and Magnus 1969; Jørstad 1930). The presence of *Ptm* in Norway was only confirmed after 1965, but at the end of the 1960s it was found four times as often as *Ptt* (Hansen and Magnus 1969).

1.2.3. Symptoms, life cycle and infection biology of *P. teres*

The two forms can easily be distinguished by the symptoms they produce on susceptible cultivars. Within a day after infection, *Ptt* causes small necrotic spots on leaves, which elongate into longitudinal and transverse stripes and later form nets across the infected leaf, often surrounded by chlorotic areas (net form net blotch; NFNB)(reviewed in Liu et al., 2011). In highly susceptible genotypes, symptoms can cover the whole leaf (Fig. 1A, Fig. 2). To a lesser extent, symptoms can also occur on leaf sheaths and seeds (Liu et al. 2011). *Ptm* causes elliptical or circular lesions, often surrounded by a chlorotic halo (spot form net blotch; SFNB, reviewed in McLean et al., 2009) (Fig. 1B).

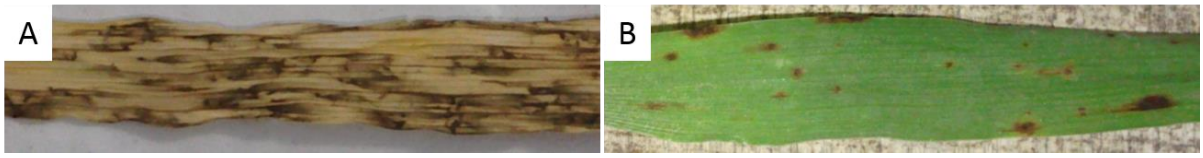

Fig. 1 Net blotch symptoms. A: net-form net blotch, fully infected leaf. B: spot-form net blotch (Photos: R. Wonneberger)

Fig. 2 Net-form net blotch symptoms in the field (Photos: R. Wonneberger)

The fungus overwinters as mycelium or pseudothecia on stubble and crop residues in the field or on alternative hosts. The primary inoculum is sexual ascospores, and, to a lesser extent, asexual conidia (McLean et al. 2009). The use of infected seed harvested from infected plants can be an inoculum source for *Ptt*, while it is commonly believed that only *Ptt* is seed-transmitted (McLean et al. 2009). However, *Ptm* was found on seed in at least one study (Louw 1996). Ascospores are discharged and transported by wind and rain splash to young seedlings (Deadman and Cooke 1989; Liu et al. 2011; McLean et al. 2009). After the fungus has spread within the plant, it produces a large number of conidia that serve as mainly wind-dispersed secondary inoculum (Deadman and Cooke 1991). There is controversy about the distance conidia can travel and to what extent infection of neighboring fields occurs (Deadman and Cooke 1989, 1991; Liu et al. 2011). Whereas Liu et al. (2011) claim that conidia are “dispersed by strong wind or rain to cause new infections on plants locally, or can be carried longer distances potentially to new barley fields”, other studies suggest that a dispersal range of a few meters is more likely, although dependent on wind conditions (Deadman and Cooke 1989; Piening 1968). The production and spread of conidia takes approximately two to three weeks, which allows for several infection cycles during the growth season (McLean et al. 2009). Conidia germinate at temperatures higher than 2°C, and the optimal conditions for infection are temperatures between 15°C and 25°C and a period of leaf wetness for 3 to 8.5 hours (Van den Berg and Rossnagel 1990, 1991). Since *P. teres* is a heterothallic fungus, two strains of opposite mating types need to be present for sexual

recombination and ascospore formation to occur. The two mating type alleles MAT1-1 and MAT1-2 are determined by a single mating type (MAT) locus (Kronstad and Staben 1997; Rau et al. 2005).

Lightfoot and Able (2010) showed that *Ptt* and *Ptm* possess different infection strategies and grow differently within the plant. *Ptm* hyphae germinating from conidia usually enter the plant by penetrating into epidermal cells followed by intracellular vesicle formation, which is a characteristic feeding strategy of biotrophic fungi. Subsequently, the fungus spreads sub-epidermally and then extracellularly within the mesophyll. Epidermal and mesophyll cell death is observed in cells directly adjacent to hyphae. In contrast, *Ptt* hyphae show a prolonged growth on the leaf surface before entering the tissue between the epidermal cells. *Ptt* also grows intercellularly throughout the mesophyll, but hyphal growth is extended and covers a larger area than *Ptm* hyphae. Necrosis and chlorosis can occur within a distance of 10 cells from the hyphae, which results in the characteristic necrotic net-like pattern in *Ptt*-infected plants. From these observations the authors conclude that the lifestyle of *Ptm* is initially biotrophic in the epidermis before switching to necrotrophic in the mesophyll, while *Ptt* skips the biotrophic stage altogether (Lightfoot and Able 2010). The symptoms are believed to be caused at least partly by necrotrophic effectors (NEs) (previously called host-selective toxins, see section 1.4.1) secreted by the pathogen that induce programmed cell death. Sarpeleh et al. (2007) hypothesize that proteinaceous metabolites are responsible for the necrotic symptoms, while low molecular weight compounds produce the chlorosis. Neupane et al. (2015) attributed the high variability in symptoms caused by different isolates on the same host or by the same isolate on different hosts to different NEs and their effect of different host genotypes.

1.2.4. Management strategies

The knowledge of the fungal biology allows the development of management strategies to control the pathogen in the field. The most sustainable way to reduce infection pressure is by cultural practices such as crop rotation, the eradication of alternative hosts and the choice of soil cultivation practices. The most important source of inoculum is stubble that remains on

the soil surface after harvest and allows the fungus to overwinter as mycelium and pseudothecia. Infected stubble can infect new seedlings for at least three years (Jordan and Allen 1984), so that in areas with heavy infection pressure, ploughing and breaks in barley cultivation of at least three years may be necessary. A narrow crop rotation, i.e. cultivation of barley in the same field in consecutive years, often seems to promote infection pressure more than reduced-tillage practices (Turkington et al. 2006; Turkington et al. 2012). The effect of alternative hosts on infection pressure has not yet been established (McLean et al. 2009). As seed transmission has been reported for at least *Ptt*, human activities such as seed trade are very likely to play a role in long distance distribution of pathotypes to new regions. A number of foliar fungicides have been shown to be effective against net blotch. Single applications of fungicides such as pyraclostrobin, epoxiconazole and propiconazole and a mixture of propiconazole and iprodione efficiently controlled *Ptm* in the presence of moderate disease pressure (Jayasena et al. 2002), but in cases of rapid disease progression on susceptible cultivars, several timed applications may be necessary (Van den Berg and Rossnagel 1990). Foliar propiconazole application was also associated with an initial reduction of *Ptt* infection, but resulted in a subsequent acceleration of infection rate (Sutton and Steele 1983). Seed treatments with carboxin and thiram or carbendazim and thiram had such a great effect against *Ptt* that the disease was no longer considered a problem in New Zealand in the late 1970s (Hampton 1980). Disease control should however not rely solely on fungicide application due to the immanent risk of mutations in the pathogen population that can lead to fungicide resistance. In early 2017, researchers from the Centre for Crop and Disease Management and from Curtin University in Australia reported that *Ptt* isolates were found in Western Australia that are very insensitive to tebuconazole and somewhat insensitive to epoxiconazole, prothioconazole and propiconazole (Kay et al. 2017). In addition, two Canadian *Ptt* isolates were recently found insensitive to propiconazole and one *Ptm* isolate was insensitive to pyraclostrobin (Akhavan et al. 2017).

1.2.5. The *P. teres* genome

In addition to controlling crop diseases by agronomical measures, the study of the genetic properties of pathogens and the molecular mechanisms of the interactions with their hosts will contribute to our knowledge of pathosystems and allow us to draw conclusions for disease management, risk assessment and resistance breeding. Today, almost 1100 fungal genomes are publicly available, approximately 10% of which are crop pathogens (Aylward et al. 2017), and the availability of fungal genomes has greatly benefitted the study of pathogen emergence, adaptation and host-pathogen interactions (Imam et al. 2016; Thynne et al. 2015).

Ellwood et al. (2010) sequenced the genome of the *Ptt* strain 0-1 from Ontario, Canada, with Solexa 75 sequencing of 75 bp paired-end reads. The assembly consists of 41.95 Mbp including 11.799 gene models of 50 amino acids or more, which is slightly bigger than that of the related pathogens *P. tritici-repentis* (37.8 Mbp) (Manning et al. 2013) and *P. nodorum* (37.1 Mbp) (Hane et al. 2007). The authors confirmed the presence of at least nine chromosomes and created a genetic map based on a cross of the isolates 0-1 and 15A with 243 AFLP, simple sequence repeat (SSR) and RAPD markers. This map consists of 25 linkage groups with a total genetic length of 2477.7 cM. Another mapping population derived from a 15A x 0-1 cross was later genotyped with double digest restriction associated DNA (ddRAD) sequencing on the Ion Torrent platform and a new genetic map was produced with 1393 SNP markers and anchored SSR and AFLP markers on 15 linkage groups (Leboldus et al. 2015). Leboldus et al. (2015) also genotyped a natural *Ptm* population of 38 isolates collected in North Dakota, USA, and obtained 16441 high quality SNPs at 5783 loci. Another *Ptt* linkage map was developed by Shjerve et al. (2014) from a cross between isolates 6A and 15A containing 468 AFLP and SNP markers spanning 1799.77 cM on 18 linkage groups. The *Ptm* isolate SG1-1 was also sequenced (Ellwood et al. 2012). Together, these genomes and maps provide useful tools for genomic studies, population studies and mapping of important loci such as virulence, avirulence or fungicide resistance loci.

1.3. Population genetics

1.3.1. Theoretical background of pathogen population genetics

The ability of fungal pathogens to compromise yield quantity and quality by overcoming host resistance or developing fungicide resistance is determined by the biological and genetic properties of the population as a whole (McDonald and McDermott 1993). According to McDonald and Linde (2002), the genetic structure of a population is defined as the "amount and distribution of genetic variation within and among populations". This in turn is a result of the evolutionary forces mode of reproduction, gene flow, genetic drift, mutation and selection acting on the population (McDonald and Linde 2002). The most dangerous pathogens are those that are able to adapt to changing environmental conditions quickly (McDonald and Linde 2002). These pathogens possess a high evolutionary potential that is determined by a mixed reproduction system, a short generation span, a high propagation rate, a high mutation rate, a large effective population size and long-distance dispersal of spores (high amount of gene flow).

A high mutation rate is the main driver for the development of new alleles and thus for genetic variation. Mutations usually occur rarely (mutation rates of 10^{-6} are common), but in populations consisting of millions of individuals as in fungal populations, they can have a substantial impact on creating new genotypes (McDonald and Linde 2002; McDonald and McDermott 1993). Genomic studies of pathogens have shown that effectors and virulence genes are often found in rapidly evolving genomic regions, e.g. regions with a high number of retrotransposons and repetitive regions which promote repeat induced point mutations and errors during crossing over (Oliver and Solomon 2010; Rep and Kistler 2010). Such a mutation will however only become a threat when there is strong directional selection on the pathogen population caused by the widespread use of single major resistance genes, which will lead to an increase in frequency of the virulence gene in the population. Additionally, the selected mutants need to be capable of long-distance travel and successful establishment in a new environment (McDonald and Linde 2002).

Most plant pathogenic fungi have a mixed reproduction system, i.e. both sexual and asexual propagation occur in the life cycle (Giraud et al. 2008). Sexual recombination can lead to the

combination of virulence loci by the generation of new haplotypes and therefore contributes to genetic variation. The asexual stage of fungi usually involves the production and dispersal of large numbers of clonal spores such as conidia. In fungi such as *P. teres*, many cycles of conidia production can occur during the season, leading to a dramatic increase in population size (see section 1.2.3). This mixed reproduction system confers to the pathogen the ability to overcome host resistance quickly, as sexual recombination can lead to the formation of genotypes with a selective advantage (virulence), which can then quickly rise to high frequency in the population via clonal propagation (McDonald and Linde 2002). The dispersal range of spores is one of the determinants of gene flow. Airborne spores of some species can travel over large distances and allow for the exchange of selective advantageous genotypes over large geographic areas (Brown and Hovmøller 2002). Another important factor that determines the exchange of pathogen genotypes is anthropogenic activity such as trade of seeds, plants and soil between regions, countries and continents, and on a small scale, the transmission of spores and mycelium between fields by contaminated machines.

The biology and the evolutionary potential of a pathogen determines which strategy to apply in resistance breeding (McDonald 2014; McDonald and Linde 2002). Pathogen populations with such properties as described above are usually genetically very diverse and consist of different strains with different pathotypes. Resistance breeding against such pathogens requires the accumulation of several quantitative resistance genes in elite cultivars since the resistance of these cultivars needs to hold up against different pathotypes. The risk of overcoming host resistance in these populations is high. Multigenic resistance is less likely to be overcome since it requires a series of mutations to occur in the pathogen population. If resistance relies on only one major resistance gene, a pathogen population with a high evolutionary potential will overcome the resistance quickly (“boom-and-bust cycle”). Clonal populations on the other hand are more stable and evolve at a much slower rate. Changes in these populations mostly occur via mutation, gene flow or a change in selection pressure. Pathogens with a small population size, a short range of dispersal and a clonal reproduction system are usually considered low-risk pathogens. McDonald and Linde (2002) established an evolutionary risk model to classify pathogens by the threat they pose based on their biological properties. In this model, where group 1 contains pathogens with a low risk and group 9 those with a high risk, *P. teres* should be placed in the risk groups 5-7 if moderate gene flow occurs

or even in the groups 7-9 where gene flow over larger distances occurs. These groups include pathogens with a mixed reproduction system, high effective population size and medium range dispersal such as *Parastagonospora nodorum* and *Rhynchosporium commune (McDonald and Linde 2002)*. The effect of mutation was not considered in this model since the mutation rate is considered low and similar between pathogen species.

1.3.2. Population genetics of *P. teres*

The genetic structure of *P. teres* populations has been analyzed mostly with AFLP markers (Lehmensiek et al. 2010; Rau et al. 2003; Serenius et al. 2007; Serenius et al. 2005; Statkeviciute et al. 2012; Stefansson et al. 2012), but also restriction fragment length polymorphisms (RFLP) (Wu et al. 2003), RAPD (Jonsson et al. 2000) and SSR markers (Akhavan et al. 2016b; Bogacki et al. 2010; Leišová-Svobodová et al. 2014) have been used. *P. teres* populations from different countries, e.g. Finland, Iceland, Hungary, South Africa, Canada, Australia and Czech and Slovak Republic have been studied (Akhavan et al. 2016b; Ficsor et al. 2014; Lehmensiek et al. 2010; Leišová-Svobodová et al. 2014; Serenius et al. 2005; Stefansson et al. 2012).

The majority of population genetics studies conducted in the last years report that *P. teres* populations possess a great amount of variability. Usually, the genetic variation within populations is larger than between them, and genetic differentiation is usually higher between distant than between adjacent populations (Akhavan et al. 2016b; Campbell et al. 2002; Jonsson et al. 2000; Peever and Milgroom 1994; Serenius et al. 2007; Serenius et al. 2005; Stefansson et al. 2012). This suggests that only a small amount of gene flow occurs between distant populations, although exceptions exist. For example, Leišová-Svobodová et al. (2014) found high genetic variation (G_{ST}=0.29-0.31) between adjacent populations (7 m and 5 km) and low variation between populations 250 km apart, so that the relation between these two factors has not been well established and may also depend on other unknown factors.

In many studies, the ratio of the two mating types is not significantly different from 1:1, indicating that sexual recombination occurs frequently under natural conditions (Bogacki et

al. 2010; Rau et al. 2003; Serenius et al. 2007; Stefansson et al. 2012). The percentage of individuals with unique allele combinations (multilocus genotypes) in sampled populations is usually very high (88-100%) (Akhavan et al. 2016b; Jonsson et al. 2000; Leišová-Svobodová et al. 2014; Serenius et al. 2007; Serenius et al. 2005; Statkeviciute et al. 2012; Stefansson et al. 2012) and only occasionally lower (36-68%) (Campbell et al. 2002; Rau et al. 2003). Only a few authors report that they sampled populations that only consist of one mating type (Leišová-Svobodová et al. 2014; Serenius et al. 2007). Despite a mating type ratio of 1:1, the hypothesis of random mating is often rejected based on tests of association indices, which often suggest clonal reproduction, possibly because of the presence of substructure within the population (Bogacki et al. 2010; Serenius et al. 2007; Statkeviciute et al. 2012).

1.4. Plant resistance against fungal pathogens

1.4.1. Theoretical background of plant resistance

The use of resistant cultivars is a very important means to control fungal pathogens and can have a direct impact on yield (Turkington et al. 2006; Østergård et al. 2008). Plant resistance is usually divided into two different forms. Race-specific resistance, also termed monogenic, qualitative or vertical resistance, is effective against one or a few races of the same pathogen species (Van der Plank 1968). Our classical understanding of disease resistance follows the gene-for-gene model, according to which pathogens produce virulence gene products that interact with corresponding receptors in the plant (Flor 1956; Flor 1971). If the receptor is able to recognize the pathogen molecule, a defense response often involving a hypersensitive reaction will be elicited to ward off the pathogen (incompatible reaction). If no recognition occurs because one of the gene products is missing, the pathogen will be able to evade recognition by the immune system and infect the plant (compatible reaction) (Jones and Dangl 2006). Examples of cereal diseases with a gene-for-gene relationship with their host are rusts and powdery mildew in cereals, and there are numerous examples of how this kind of resistance can be overcome very quickly, leading to susceptibility and high yield losses in a very short time (reviewed in McDonald and Linde, 2002). Whereas this type of defense is largely effective against biotrophic pathogens, some necrotrophic pathogens have evolved

NEs to deliberately induce a hypersentitive response, so that the pathogen can thrive on the dead plant tissue (Friesen et al. 2008; Oliver and Solomon 2010; Tan et al. 2010). NEs have been extensively studied in pathogens related to *P. teres* such as *Parastagonospora nodorum* and *Pyrenophora tritici-repentis*, the causal agents of *Septoria nodorum* blotch and tan spot in wheat, respectively (Ciuffetti et al. 2010; McDonald et al. 2013; Oliver et al. 2012).

The second type of resistance, quantitative or horizontal resistance, is usually effective against all races of a pathogen species and is usually governed by several genes, most of them with small effects (St. Clair 2010). These genes often encode pathogenesis-related (PR) proteins, phytoalexins, etc. (Ahuja et al. 2012; Golshani et al. 2015; van Loon et al. 2006) or developmental and morphological features (Melotto et al. 2006; Zhu et al. 1999). Genomic regions harboring loci that affect quantitative traits are termed quantitative trait loci (QTL). Since quantitative resistance is conferred by a number of genes, it is usually more stable since many mutations in the pathogen population are required to overcome this resistance (McDonald and Linde 2002). Quantitative resistance is often dependent on environmental factors (genotype x environment effects), and often only effective in certain growth stages or plant tissues (Miedaner et al. 2001; Steffenson et al. 1996).

1.4.2. Identification of resistance loci in plant genomes

In order to understand the genetic mechanisms of host-pathogen interactions and to exploit resistance genes to improve resistance in commercial varieties, knowledge of the genomic location of resistance or susceptibility genes is advantageous. A causative locus can be localized with the help of molecular or genetic markers (see below). Common types of genetic markers are SSR markers, insertions, deletions and SNPs (reviewed in Mammadov et al., 2012). Many different types of markers such as RAPD, AFLP and RFLPs have been used for mapping causative loci in the genome, but their detection was usually expensive and time-consuming and required a great amount of labor (reviewed in Mammadov et al., 2012). The advent of next-generation sequencing technologies and the decreasing genotyping price per sample has made it feasible to genotype large genomes of crop plants for large numbers of SNP markers with relatively little input of time, labor and money (Ansorge 2009; Mammadov

et al. 2012). SNPs are usually biallelic markers that show nucleotide variation at a specific position. A nucleotide variation is usually considered a SNP if its frequency in the studied population is at least 1% (Vignal et al. 2002). SNPs are the most abundant type of polymorphisms in genomes, but the amount of SNPs in a region can vary greatly (Brookes 1999). The ideal marker is so close to the causative locus that no recombination occurs between them, or preferably, the causal locus itself (Holland 2007).

Today, two main methods are used to map causative regions in the genome: Linkage mapping and association mapping (AM; linkage disequilibrium mapping). The terms used to describe these methods can be misleading, as both methods rely on the fragmentation of the genome by recombination, and the genetic linkage of markers with the trait of interest (Myles et al. 2009). Sexual recombination, more specifically meiosis, leads to the recombination of fragments of the genome and generates genetic variation. Regions in which no recombination occurs are called linkage blocks, and SNPs in these blocks are in high linkage disequilibrium (LD), which is defined as the non-random association of alleles at different loci in the genome (Lewontin and Kojima 1960; Slatkin 2008; Waugh et al. 2009). If a linkage block contains a causative region for a trait, the SNPs in this block will be linked to it and can be used to detect this region (Slatkin 2008).

Linkage mapping is still the most common method to detect causative genetic regions in plants (Holland 2007), although AM studies are gaining popularity quickly (Waugh et al. 2009; Xiao et al. 2017). Linkage mapping is performed in populations derived from a cross of two parental lines segregating for a trait of interest (Myles et al. 2009). In inbreeding species such as many cereals the progeny lines are usually selfed for a few generations to obtain homozygous lines (RILs), or the F1 generation can be used to produce doubled haploid lines. Since the population is derived from only two parents, the crosses are genetically narrow and only the two parental alleles can be investigated at each locus (in diploid organisms). The number of polymorphic markers depends on the genetic similarity of the parents. Only very few recombination events occur in the population, so linkage blocks are usually large and the LD decays slowly, thus the mapping resolution in these populations can be quite low.

Association mapping can be performed on any type of population, from natural populations to breeding line or cultivar collections and multi-parental crosses such as multiparent

advanced generation intercross (MAGIC) or nested association mapping (NAM) populations (Cockram et al. 2015; Nice et al. 2017; Vatter et al. 2016). For resistance breeding purposes, screening of natural populations can be useful to find resistance sources in wild relatives of crop plants. The use of collections of breeding lines and cultivars allows the direct implementation of the methodology and the results in breeding programs (Begum et al. 2015). AM circumvents some of the drawbacks of linkage mapping. There is no need for the time-consuming process of creating and maintaining mapping populations. Since AM panels are genetically diverse, many alleles can be present at one locus. As AM exploits the historical recombination events occurring in the population, AM genetic maps usually have a higher resolution because LD decays more rapidly, more polymorphisms are present in the population, and more causative loci can be detected (Myles et al. 2009).

In order to obtain accurate results with AM, statistical models that correct for population structure must be implemented to detect and exclude false-positive marker-trait associations (MTAs). Population structure in breeding populations is often increased due to human selection efforts that divide the germplasm into distinct groups, such as two-rowed and six-rowed barley or spring and winter types in barley and wheat (Wang et al. 2012). Another problematic feature of AM is that rare alleles, alleles that only occur in very few individuals of the population, are often not detectable because their effect on the total variation is low (Gupta et al. 2014). By excluding markers with a MAF < 0.05, these alleles are often filtered out at the beginning of the analysis, although they are often associated with the trait of interest, as is the case for a number of human diseases (Tennessen et al. 2012). As a result, the more common alleles detected in a GWA study often explain only a small percentage of the total genetic variation, since not all causative loci have been detected. To overcome these limitations, a number of solutions have been suggested, such as the use of biparental mapping, potentially combined with AM, a large AM panel population size or new analysis methods (Gupta et al. 2014). In plants, however, the importance of rare alleles in disease resistance has not yet been studied in detail.

1.4.3. Resistance against *P. teres* in barley

Research on net blotch resistance dates back to the 1920s when Geschele (1928) discovered that it followed Mendelian inheritance. By the end of the 1950, the presence of at least three genes conferring incomplete dominant resistance was known (Mode and Schaller 1958; Schaller 1955). The first resistance loci that could be localized in the genome were found by Bockelman et al. (1977) on chromosomes 1H, 2H and 3H in the cultivars Tifang, CI7584 and CI9819. Based on these early studies, net blotch resistance was mainly understood as a gene-for-gene relationship involving major-effect genes. In the late 1980s and early 1990s, a number of studies were conducted on adult plants, which found that resistance was quantitatively inherited under field conditions (Arabi et al. 1990; Douglas and Gordon 1985; Harrabi et al. 1993; Robinson and Jalli 1997; Steffenson et al. 1996; Steffenson and Webster 1992). With recent advances in molecular marker techniques, the location of resistance loci can be determined in a much more exact way, and we have learned that the mechanisms underlying this pathosystem are much more complex than initially thought. Today, resistance genes/QTL are known on all seven chromosomes, and many of them are specific to either *Ptt* or *Ptm* (reviewed in Liu et al., 2011 and McLean et al., 2009). Many of these QTL have been projected onto consensus maps, which facilitates the comparison of loci across different studies and populations (Richards et al. 2017; Wang et al. 2015). The majority of the resistance QTL found in these mapping studies confer dominant resistance, but a number of recessive resistance genes have also been identified. Ho et al. (1996) showed that resistance to two *Ptt* isolates in the Leger x CI9831 mapping population is conferred by one and three recessive resistance genes, respectively. Abu Qamar et al. (2008) detected two dominant susceptibility loci on chromosome 6H in the Rika x Kombar mapping population that are linked in repulsion and confer susceptibility to the *Ptt* isolates 15A (from Kombar) and 6A (from Rika), respectively. In a mapping population of the parental isolates 6A and 15A, Shjerve et al. (2014) identified four putative virulence genes, two of which confer virulence on Rika and two on Kombar, and hypothesized that the previously identified 6H region contains four closely linked susceptibility genes. The locus was subsequently fine-mapped to a 0.24 cM interval in the centromeric region of 6H (Richards et al. 2016).

Chromosome 6H is considered a hotspot for both major resistance genes and small-effect QTL, although the exact number of loci still remains to be determined (Abu Qamar et al. 2008; Friesen et al. 2006a; Gupta et al. 2011; Ma et al. 2004; Manninen et al. 2000; Steffenson et al. 1996). Some of the genes found on 6H are pathotype-specific (Abu Qamar et al. 2008; Friesen et al. 2006b). Chromosome 6H also harbors the first putative susceptibility gene to a *Ptt* NE (Liu et al. 2015). This QTL named SPN1, which was identified in the Hector x NDB112 mapping population after inoculation with the *Ptt* isolate 0-1, explained 31% of the phenotypic variation. The same QTL was also found after infection with five other globally collected *Ptt* isolates, indicating that isolates producing the corresponding NE may be found around the world. It remains to be elucidated whether other known dominant susceptibility genes also encode susceptibility to NEs. No NEs have been identified in *Ptm* yet, but it seems likely that this form also secretes them, most likely during later stages of infection. Both chromosomes 3H (Cakir et al. 2003; Grewal et al. 2012; Liu et al. 2015), and 7H are also considered hotspots for large-effect resistance QTL (König et al. 2014; Wang et al. 2015).

In the last years, it has become feasible to genotype large populations with thousands of SNP markers and GWAS has gained popularity in plant pathology (1.4.2). Currently, there are three GWA studies on *Ptm* resistance and one on *Ptt* resistance, reflecting the increasing importance of *Ptm* in many regions worldwide. The continuous distribution of disease severity in populations and the presence of between eight and 29 QTL per population underline the quantitative nature of resistance mechanisms in the pathosystem (Burlakoti et al. 2016; Richards et al. 2017; Tamang et al. 2015; Wang et al. 2015).

Most of these studies are performed on seedlings under controlled growth conditions, and more knowledge is required about how the resistance found in these studies holds up under field conditions (Williams et al. 2003), where genotype x environment effects may play a major role. Many studies found QTL that confer resistance consistently in both seedling and adult plants under field conditions (Cakir et al. 2003; Gupta et al. 2002), but some of the resistance was specific to a developmental stage. In a GWA study on four Australian breeding populations, 75% of the QTL conferred resistance both in seedlings and adult plants, while 17% were only effective in adult plants and 7% in seedlings only (Wang et al. 2015).

Sato and Takeda (1997) identified *P. teres* resistance in many *Hordeum* species, especially in *H. spontaneum*, which thus constitutes an interesting source for improved resistance, provided that closely linked markers are available. Progress is currently made in characterizing the genomes of wild relatives of barley (Wendler et al. 2014), and a NAM population generated from a cross between *H. spontaneum* and *H. agriocrithon* and the cultivar Barke is currently being used in a GWA study to map resistance to *P. teres* (Vatter et al. 2016).

Apart from the aforementioned putative effector PttNE1 and the putative virulence genes in the *Ptt* isolate 6A and 15A, little is known about genes conferring virulence or avirulence in the pathogen. Lai et al. (2007) identified the locus AvrHar conferring avirulence to the cultivars Tifang and Canadian Lake Shore in the isolate 15A and the loci AvrPra1 and AvrPra2 conferring virulence to the cultivar Prato in the isolate 0-1. AvrHar and AvrPra2 co-segregate, but it is currently not known if these loci are alleles of the same gene or two different genes.

2. The thesis

2.1. Background and main objectives

Disease resistance is an important agronomical trait in all crop plants and the use of resistant cultivars is often the most economically and environmentally friendly means to control a disease. Knowledge of the local pathogen population is useful to inform resistance breeding strategies to exploit the available genetic resources in the most effective way. Depending on their mode of reproduction and their genetic structure and properties, pathogen populations have a high or low evolutionary potential. Populations with a high evolutionary potential can adapt to changing environmental conditions faster and are more prone to overcome host resistance quickly through sexual reproduction, mutations, gene flow etc. The beneficial allele is subsequently selected for and its frequency in the population increases and can potentially become fixed. Resistance breeding against pathogens with a high evolutionary potential is dependent on the accumulation of different resistance QTL in elite varieties, so that many genetic changes in the pathogen population are necessary to overcome resistance. If one of the resistance genes is no longer effective, others will still hold up.

In order to assess the evolutionary potential of the Norwegian *P. teres* population, we genotyped a collection of 339 Norwegian and 61 global isolates with 4252 SNP markers in order to analyze the genetic structure of the population. We investigated whether the reproductive system is predominantly sexual, asexual or mixed, and how diverse the isolates are: We looked for substructure in the population that might be determined by geographical sampling region or host cultivar, and we included a number of isolates from 1995 to see whether a recent change in the pathogen population could be observed. Since *P. teres* has been reported to have a mixed propagation system, large population sizes (high spore production), and, at least in the case of *Ptt,* seed transmission, we hypothesized to find an equal amount of isolates for each mating type, and a highly genetically diverse population. It has not been clearly established how far the spores can be dispersed by wind. If they are not capable of long-distance travel as suggested by experiments (see section 1.2.3), the amount of variation between different regions will mostly depend on the amount of seed exchange. Since leaf samples were chosen based on net form symptoms and since *Ptm* does usually not appear to be seed-transmitted, we expected to find only a very low number of *Ptm* isolates in the collection.

Net blotch resistance of the cultivars currently grown in Norway is insufficient and resistance sources have not yet been systemically exploited. The aim of this study was therefore to determine the resistance levels of barley cultivars currently grown in Norway as well as breeding lines used in breeding programs and to identify loci associated with resistance in these genotypes. To this end, we assessed resistance of a biparental mapping population and a collection of current cultivars, landraces and breeding lines at the seedling and adult plant stages against different net blotch isolates in order to identify genotypes that showed stable resistance. These genotypes are potential sources of resistance and could serve as parents to create mapping populations. The genetic characterization allowed us to map resistance QTL in both populations and to assess their contribution to resistance at both developmental stages. The markers associated with these QTL will, after validation in other populations, be applicable in marker-assisted selection (MAS) for net blotch resistance.

2.2. Main results and discussion

Detailed results and discussion are included in each paper. This section provides a combined overview and discussion of all results and focuses on the main findings and their implication for resistance breeding in Norwegian barley.

2.2.1. The Norwegian *P. teres* population has a high evolutionary potential (Paper I)

Among the 339 Norwegian *P. teres* isolates used, 95.9% were *Ptt* and only 2.4% were *Ptm*, all from Akershus county. Since leaf samples were mainly selected based on net form symptoms and since it has not been established yet to what extent *Ptm* is seed-transmittable, the proportion of *Ptt* isolates found in this study may overestimate the true proportion of *Ptt* found in Norwegian barley fields. However, conidia were collected from leaves regardless of the presence of symptoms, and both forms have been shown to occur within the same lesions (Leišová-Svobodová et al. 2014). Furthermore, *Ptm* has been successfully isolated from barley seed (Louw 1996), so that it can still be assumed that *Ptt* is truly the dominant form in Norway today. This suggests that there has been a change in the predominant *P. teres* form since the 1960s, when *Ptm* was four times as abundant as *Ptt* in Norway (Hansen and Magnus 1969). A similar development has been reported in Finland, where both forms were equally abundant in the late 1960s (Mäkelä 1972), whereas a more recent study only found *Ptt* in the sampled fields (Serenius et al. 2005). Conversely, an increased occurrence of *Ptm* has been observed in other regions of the world such as Australia or North America (Lartey et al. 2013; Liu and Friesen 2010; Marshall et al. 2015; McLean et al. 2009; Murray and Brennan 2009). The reasons for these changes are still unknown, but it is speculated that changes in environmental conditions or in pathogen virulence as well as changes in cultivar use may have an influence on the pathogen population. Louw (1996) suggested that a change in the South African *P. teres* population from predominantly *Ptt* to *Ptm* might have been caused or supported by a change in cultivars grown in the region, from the *Ptt*-susceptible cultivars Elsa and Swaneck to the *Ptm*-susceptible Clipper, which was grown on 93% of commercial fields in the 1990s. The prevalence of *Ptm* in Norway in the late 1960s was at least partly attributed to the widespread cultivation of the cultivar Herta, which was said to have a “certain amount

of resistance" to *Ptt* (Hansen and Magnus 1969). No statements were made about the susceptibility of Herta to *Ptm*, but recently it was shown to be susceptible to 24 out of 27 Canadian *Ptm* isolates (Akhavan et al. 2016a), which gives some support to the hypothesis that Herta is indeed susceptible to *Ptm,* and that its widespread cultivation may have had an influence on the dominance of *Ptm* in Norway in the 1960s.

Consistent with most other *P. teres* population studies, *Ptt* and *Ptm* were found to be distinct groups as shown by distinct clusters in PCA and Structure analyses and a high amount of form-specific alleles (11.7% for *Ptm* and 22.0% for *Ptt*, respectively). Nei's G_{ST} was 0.42, indicating that 42% of the genetic variation among the Norwegian isolates can be explained by differences due to the form. In addition, subclades within the forms may exist as indicated by isolates clustering between the *Ptt* and *Ptm* isolates in the PCA analysis. A substructure within the *Ptt* population was discernible in PCA and Structure analysis and a distance matrix-based dendrogram and could be explained by the cultivar from which the isolates were obtained. The cultivars that were best represented in the collection were Tiril (113 isolates) and Helium (121 isolates), and isolates from these two cultivars formed two distinct groups, although not all isolates followed this pattern. The isolates derived from Helium and Tiril may have undergone sorting or selection on their host. Some genome scaffolds had high F_{ST} values, indicating that the two genetic groups have been previously isolated and have come into secondary contact recently. Low nucleotide diversity and negative Tajima's D suggest that the groups have undergone a genetic bottleneck followed by an increase in population size. The more intermediate frequency of the two groups in Tiril, along with the presence of apparently admixed isolates shows that the two genetic groups probably interbreed.

Table 3 Market shares (%) for barley cultivars in Norway 2005-2016 (Åssveen et al. 2016, 2017)

Year	Brage	Helium	Heder	Fairytale	Tiril	Tyra	Salome	Edel	Marigold	Iver
2005	0	0	0	0	0	11.4	0	29.0	0	12.7
2006	0	0.2	0	0	9.5	10.9	0	32.2	0	9.9
2007	0	1.1	0	0	11.9	13.2	0	29.9	0	9.8
2008	0	11.1	0	0	15.4	12.8	0	26.1	0	10.3
2009	0	17.2	4.8	0	12.6	14.4	0	21.4	0	10.0
2010	0	13.9	9.3	0	13.5	13.3	0	25.7	1.8	7.8
2011	0	20.4	11.6	0	13.0	13.7	0	9.0	4.9	8.9
2012	6.6	21.3	12.6	0	15.6	10.0	0	4.1	4.1	5.4
2013	16.3	22.5	11.5	1.3	11.7	8.6	0	6.3	4.6	4.7
2014	25.2	19.2	12.7	2.4	10.9	8.5	4.5	4.9	4.4	4.1
2015	30.4	13.9	12.0	9.8	7.4	7.4	6.1	4.6	3.6	2.6
2016	37.8	10.9	10.3	14.0	4.2	5.6	7.2	4.2	3.8	1.2

Table 3 shows the market shares of Norwegian barley cultivars from 2005 to 2016. Helium and Tiril were both released as cultivars in 2004 and gained significant market share in the following years. Helium was the cultivar with the biggest market share in 2011-2013, and Tiril had the second highest market share in 2011 and 2012. Our results indicate that the pathogen population is able to adapt to different host cultivars within less than 10 years. In a virulence study of a Swedish isolate collection, Jonsson et al. (1997) found that some pathotypes predominantly occurred on certain cultivars. One of them, the cultivar Golf, had been cultivated for a similar period (released in 1984) as Helium and Tiril. Specialization to cultivars has also been shown for other pathogens like *Zymoseptoria tritici*. Resistance in the cultivar Gene was overcome three years after its introduction and cultivation in Oregon, as shown in pathogenicity tests (Cowger et al. 2000). In addition, the authors showed that isolates from the same collection were virulent on other cultivars, but avirulent on Gene, indicating cultivar-specificity. To test the hypothesis of specialization to host cultivars in the Norwegian *P. teres* population, isolates from other cultivars should be included in the genetic analysis, ideally from cultivars that have been released recently and have gained a high market share such as Brage. In addition, pathogenicity tests need to be performed to conclude whether the different subpopulations on Helium and Tiril represent different pathotypes and whether they possess different virulence profiles on these cultivars. With Helium being a two-rowed cultivar and Tiril being six-rowed, the question needs to be addressed whether the observed host specificity is due to genetic differences in these two types of barley rather than due to differences between cultivars. Since the breeding programs for two- and six-rowed barley are

usually separated, it is possible that certain resistance genes are only found in one of these germplasm groups.

No geographical substructure was discernible within the Norwegian *P. teres* population as shown in the PCA and supported by the finding that only three subpopulations contained private alleles that were not found in any other subpopulation. Instead, analysis of molecular variance (AMOVA) showed that moderate but significant genetic differentiation occurred between isolates collected in 1995 and between 2011 and 2014, which indicates that the pathogen population has changed within the last 15 to 20 years. However, an effect of cultivar specialization cannot be ruled out, since the cultivar of the 1995 samples is unknown. No significant genetic differentiation was found within the isolates collected between 2011 and 2014, indicating that a substantial amount of gene flow can occur within 5-year periods. This has important implications for disease management in the field, as it underlines the importance of using wide crop rotations in order to control disease pressure. Turkington et al. (2006) demonstrated the importance of crop rotation by showing that the likelihood of higher net blotch infection can be 4.5 times as high if the previous crop was barley instead of a nonhost crop, although this effect was not found in all years.

Mating type analyses showed that 158 of the Norwegian isolates had MAT1-1 and 181 had MAT1-2. This ratio was not significantly different from 1:1 (Exact binomial test for goodness of fit, $p<0.05$). Mating type ratios different from 1:1 were only found in the population collected in 1995 and in two populations collected in Nord-Trøndelag in 2013 from the cultivars Skagen and Corniche, respectively. The occurrence of both mating types at equal amounts in most subpopulations indicates that frequent genetic exchange may occur between them via sexual recombination. This is supported by the finding that each isolate represented a distinct haplotype. Based on standard tests of association, however, the hypothesis of random mating had to be rejected in all but six subpopulations, which is somewhat contradictory but not uncommon (Bogacki et al. 2010; Serenius et al. 2007; Stefansson et al. 2012). A possible explanation may be the presence of undetected isolated subpopulations within the sample, which may suggest the presence of linkage disequilibrium (Maynard Smith et al. 1993). This may be the case for the subpopulations derived from seed samples since these seed samples represented a mixture of seed from the same cultivar from different farms within a postal code region. It is therefore still likely that sexual reproduction

plays a major role in the Norwegian *P. teres* population, but the proportion of sexual and asexual propagation still remains to be determined. Since *P. teres* is potentially able to generate large population sizes, the effect of mutations on the genetic diversity may also be considerable.

Taken together, the analysis of the Norwegian *P. teres* population indicated that the predominant form is *Ptt* and that sexual recombination is frequent enough to result in a genetically diverse population, which in addition shows a substantial amount of gene flow between regions. These findings confirm the characterization *Ptt* as a high-risk pathogen with a high evolutionary potential (McDonald and Linde 2002).

2.2.2. Net blotch resistance of Norwegian cultivars (Paper II and III)

The resistance tests of the AM panel revealed that none of the current cultivars tested is completely resistant to the three isolates at the adult stage (Fig. 3). The average range of disease scores in the panel over four years was between 13% and 45% diseased leaf area. On average, Fairytale had the highest resistance level of the current cultivars with 21.1% diseased leaf area whereas Tyra, Iver and Tiril were highly susceptible (34.5%, 36.2% and 43.0%, respectively). This corresponds fairly well with the official variety trials conducted in Norway in different regions, where Fairytale was usually one of the most resistant cultivars, and Iver, Tyra and Tiril were often the most susceptible cultivars, although not in all years (Table 4). In three out of four years, none of the current cultivars was among the 70 most resistant lines. The older cultivars Arve and Lavrans, the parents of the QTL mapping population, were more resistant than all current cultivars. Especially Lavrans (18.3% of diseased leaf area) was consistently resistant in all years and also had a comparatively high amount of seedling resistance (Fig. 4). Although the level of resistance differed between years, Lavrans was consistently more resistant than Arve in the official variety trials and in most of the experiments in paper II and III.

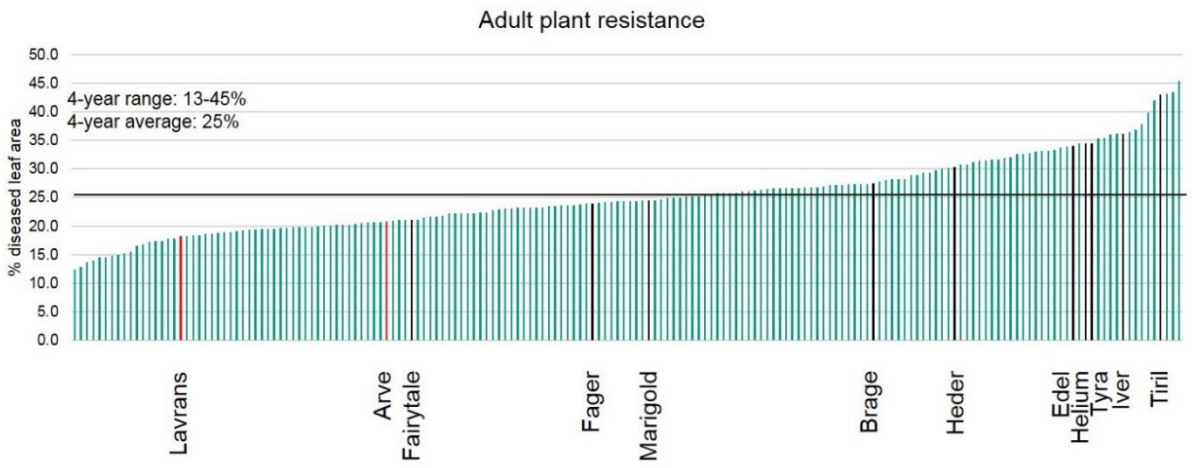

Fig. 3 Adult plant resistance of the AM panel. Percentage of diseased leaf area was measured over four years of field trials. Highlighted in black are cultivars currently grown in Norway and the historically important breeding parent Fager. Highlighted in red are the parents of the biparental mapping population Arve and Lavrans. A table with the scores of all lines of the AM panel can be found as a supplement to Paper III.

Since data from the official trials is not available for all cultivars for all years, comparisons should be made with caution. To better compare the disease severities of the current cultivars, average severities for 2013 to 2015 were calculated (Table 4). The rankings of the current cultivars from the field trials (Paper II; Fig. 3) correspond quite well with the rankings based on the official trials (Table 4), especially with the data from Eastern Norway. This indicates that the three isolates used for field inoculations are quite representative of the natural *P. teres* population at least in Eastern Norway, and suggests that the results from paper II will be of practical relevance for Norwegian barley breeders (Peever et al. 2002).

Table 4 Net blotch severities (%) from official variety trials in Norway

Eastern Norway											
Year	Arve	Lav-rans	Edel	Tiril	Heder	Brage	Fairy-tale	Mari-gold	Helium	Iver	Tyra
1999	38	19									19
2002	4	1	0/3 (diff. trials)							6	7
2003	8	3	7							12	12
2004	10	3	8	9					2	5	8
2005	5	5	6	5					2	13	14
2007	2	1	5/5	5	4			1	2	6	5
2008			2	1	2						
2009			4	2	1						
2010			4					2	3	5	5
2012			17	17	17	10					
2013			23	35	8	5	5	5	8	15	20
2014			33	51	14	21	3	3	13	40	40
2015			1	2	14	1	0	1	3	2	0
Average 2013-2015			19	29	12	9	3	3	6	19	20
Central Norway											
Year	Arve	Lav-rans	Edel	Tiril	Heder	Brage	Fairy-tale	Mari-gold	Helium	Iver	Tyra
1999	20	10									1
2003	19	10	2							4	5
2005	5	4	4	6						6	6
2006	5	3	1	7							
2007	18	2	3/4	8	3			1	1	2	3
2009			6/4	25	8			2	1	10	10
2010			2	5	4	3					
2011			7	19	5	8					
2012			3	5	3	3					
2013			2	9	1	2	1	1	2	2	1
2014			0	8	0	1	0	0	0	0	0
2015			3	38	9	8	1	1	1	21	18
Average 2013-2015			2	18	3	4	1	1	1	8	6
Southwest Norway											
Year	Arve	Lav-rans	Edel	Tiril	Heder	Brage	Fairy-tale	Mari-gold	Helium	Iver	Tyra
2009			4		3			3	3	3	
2010			9		4	5	2	3	4	5	
2011			45		9	50	8	6	43		
2012			8		12	7	12	13	21		
2013			21		35	24	40	25	72		
2014			2		3	2	1	1	5		
2015			12		16	12	8	9	17		
Average 2013-2015			12		18	13	16	12	31		

Similar to the field trials, Fairytale was also the most resistant current cultivar in the greenhouse experiments (Fig. 4). Several current cultivars, especially Helium, had much better resistance at the seedling stage than at the adult stage. This might be an indication that the disease mechanisms in these cultivars are different at different developmental stages, or that environmental factors affect resistance negatively in the field. Environmental conditions are known to have a great influence on net blotch severity (Shipton et al. 1973; Steffenson and Webster 1992) and may influence the significance of QTL in different years.

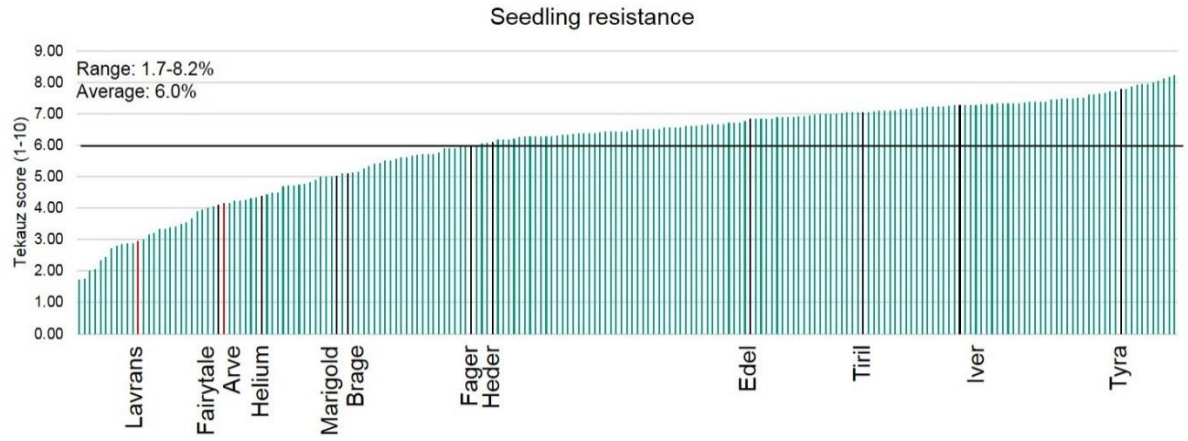

Fig. 4 Average seedling plant resistance of the AM panel determined by separate inoculation with three different *Ptt* isolates. Disease was scored on the Tekauz scale ranging from 1 (resistant) to 10 (susceptible) (Tekauz 1985). Highlighted in black are cultivars currently grown in Norway and the historically important breeding parent Fager. Highlighted in red are the parents of the biparental mapping population Arve and Lavrans. A table with the scores of all lines of the AM panel can be found as a supplement to Paper II.

No breeding line with good resistance at both developmental stages was identified. Lines 145 and 108 were among the 20 most resistant lines in all years in the field trials but did not have sufficient seedling resistance. Breeding line 71 was among the 20 most resistant lines in all seedling tests, but adult plant resistance differed greatly between years. These three lines may be interesting candidates for further investigations since they may be suitable crossing parents for the development of new varieties.

The cultivar Arve showed good resistance at both the adult plant and especially the seedling stage. In the 1990s and 2000s, however, it was one of the most susceptible cultivars (Bakkegard and Abrahamsen 2004; Robinson 2000)(M. Lillemo, pers. comm.). Conversely,

Herta showed good resistance to *Ptm* in the 1960s (Hansen and Magnus 1969), but was average resistant in our experiments. Bjørnstad and Aastveit (1990) reported that resistance of the cultivar Agneta was not effective anymore in Northern Europe, whereas Clermont showed very good resistance. In our studies, however, the resistance of Agneta was slightly below the AM mapping panel average, and Clermont was highly susceptible in all years. These results support the hypothesis in Paper I that changes in the pathogen population may occur within a period of 15-20 years, and resistance in currently grown cultivars can be overcome by the pathogen and thus quickly rendered ineffective. The scorings from the official field trials, however, indicate that differences in susceptibility between years have occurred to a similar extent in most cultivars tested. Therefore, these differences in susceptibility may rather be caused by different conditions for pathogen viability between years rather than by overcoming host resistance or by selection pressure.

2.2.3. Usefulness of seedling tests to predict adult stage resistance (Paper II and III)

We tested whether seedling screenings can be a reliable method to predict adult plant resistance in the field in order to facilitate selection. In the AxL population, the correlation between seedling resistance to single isolates and field resistance of adult plants ranged from r=0.29 to r=0.59 and was significant in all cases ($p<0.01$). In the AM panel, it was substantially lower (r=-0.05-0.47) and not always significant (cv. Papers II and III). The correlation between the average of all years of field experiments and the average of all seedling inoculations was r=0.49 in the AM panel and r=0.35 in AxL (Fig. 5). In most pairwise correlations between adult and seedling tests, the AM panel lines that were most susceptible at the adult plant stage also had low seedling resistance, but in the lines with average or high field resistance, there was usually no correlation to seedling resistance. In the AxL population, the lines with the highest seedling resistance did not always have good adult plant resistance. Based on these findings, it is not recommended to predict adult plant resistance solely by scoring resistance in seedlings the way it was done in these studies. However, the availability of genetic markers associated with resistance loci enables breeders to screen the breeding lines at the seedling stage for the presence of resistance alleles. Here, the selection efforts should focus on

markers associated with adult plant stage or developmental stage-independent QTL, some of which have been identified in Paper II and III (see section 2.2.4). This allows the reduction of breeding lines and shorter breeding cycles.

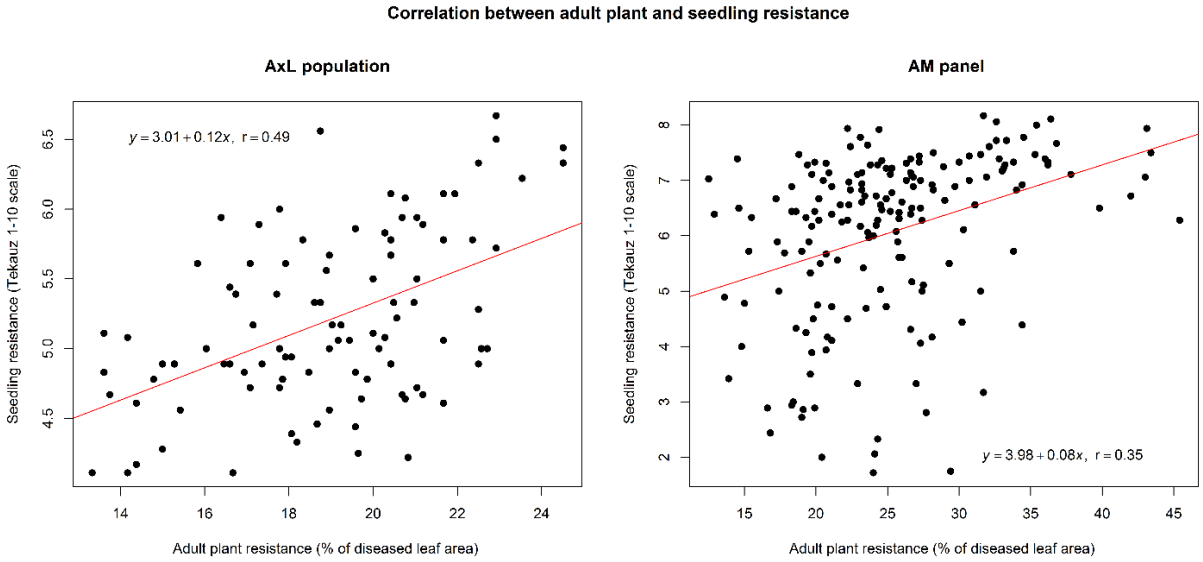

Fig. 5 Correlation between adult plant and seedling resistance in the AxL mapping population (left) and the AM panel (right).

2.2.4. Resistance loci in Norwegian barley (Paper II and III)

In order to map genomic loci associated with resistance and to identify markers linked to these causative loci, both barley populations were genotyped for SNP markers with the Illumina iSelect 9k Barley SNP Chip. Due to the close relatedness of the parents of the biparental population, no polymorphic markers were found on chromosome 1H in this population, therefore this chromosome could not be screened for resistance loci. In AxL, nine QTL associated with resistance were found on all chromosomes except 1H and 2H (Table 5). A major QTL on chromosome 5H explained between 16 and 55% of the genetic variation and was significant in all experiments at both developmental stages. The eight other QTL explained between 10% and 17%. Four QTL were adult plant-specific and three were only found in seedlings. One of these QTL was race-specific, as no association of markers in that region with resistance to the isolate 6949B was observed. All other seedling QTL were race non-specific, which supports the expectation based on Lavrans being consistently more

resistant than Arve, despite different disease severities between years (Table 4). All QTL except for AL_QRPtt4-1 are from the more resistant parent Lavrans, which makes this cultivar a promising crossing parent.

In the AM panel, we found 35 marker-trait associations corresponding to 13 QTL on all chromosomes, each explaining 5-14% of the genetic variation (Table 5**Fig. 4**). Of the 13 loci, seven (54%) of the QTL were found only in adult plants and four (27%) only in seedlings. Two QTL on 3H and 6H were significant during seedling inoculations and in two out of four years of adult stage field trials. These QTL are in regions that were associated with net blotch resistance in a number of previous studies (Abu Qamar et al. 2008; Burlakoti et al. 2016; Cakir et al. 2003; Grewal et al. 2008; Gupta et al. 2010; Koladia et al. 2016; König et al. 2013; Liu et al. 2015; Richards et al. 2016; Richards et al. 2017; Tamang et al. 2015; Wang et al. 2015)(See paper III for discussion). The exact number of genes at these loci is still unknown, so it remains to be elucidated whether these regions are hotspots for resistance genes or whether only a few genes are responsible for conferring broad-range resistance to a large number of pathotypes in different genetic backgrounds in all these studies. In addition to dominant resistance, this region on chromosome 6H also harbors the two loci *SPN1* and *Spt1* conferring dominant susceptibility to net blotch (Liu et al. 2015; Richards et al. 2016). These loci are currently hypothesized to be susceptibility genes involved in an inverse gene-for-gene interaction with a necrotrophic effector of *P. teres*.

Three genomic regions were associated with resistance in both populations (Table 5). NBP_QRPtt4-2 was associated with seedling resistance to LR9 and plant height (PH) in 2016 in the AM panel and AL_QRPtt4-1 with seedling resistance to LR9 and 5050B as well as days to heading (DH) in 2016 and PH in 2015 and 2016 in AxL. The major AxL QTL AL_QRPtt5-2 co-localized with NBP_QRPtt5-2 associated with adult plant resistance in 2014 in the AM panel. The NBP_QRptt7-1 QTL significant in the uninoculated field trial co-localized with the AL-QRptt7-2 QTL found in 2016 and in inoculations with LR9.

Several studies have reported that some resistance loci are only effective at one developmental stage, while others are stable during the whole lifespan of the plant (Grewal et al. 2012; Steffenson et al. 1996; Wang et al. 2015). Often, plants that are resistant at the seedling stage also possess this resistance at the adult plant stage, but also seedling stage-

specific QTL have been reported (Friesen et al. 2006a; Grewal et al. 2008; Wang et al. 2015). In the AM panel and AxL, four QTL were found at both the seedling and the adult plant stage (NBP_QRPtt3-2, NBP_QRPtt6-1, AL_QRPtt5-2 and AL_QRPtt7-2/NBP_QRptt7-1), whereas the others were stage-specific. Developmental stage-specific resistance QTL have also been reported for other cereal diseases such as leaf rust (*Puccinia hordei*) in barley (Singh et al. 2015), spot blotch (*Cochliobolus sativus*) in barley (Steffenson et al. 1996), leaf rust (*Puccinia triticina*) in wheat (Gao et al. 2016) and powdery mildew (*Blumeria graminis* f. sp. *tritici)* in wheat (Wang et al. 2005).

Table 5 Overview of all genetic regions associated with net blotch resistance, PH or DH in both populations.

QTL AxL	QTL NBP	Chr	Position (cM) [a]	Position (cM) [b]	AxL										NBP								AxL				NBP				AxL			NBP		
					NB14	NB15_1	NB15_2	NB15	NB16_1	NB16_2	NB16	LR9	5050B	6949B	NB13	NB13_nat	NB14	NB15	NB16	LR9	5050B	6949B	DH14	DH15	DH16	DH13	DH14	DH15	DH16	PH14	PH15	PH16	PH14	PH15	PH16
	QRptt1-1	1H		43-53											x			x	x							x		x								
		1H		115																							x	x	x							
	QRptt1-2	1H		137-143												x																				x
	QRptt2-1	2H		14																		x														
		2H	70-72																												x					
		2H		177																						x			x							
	QRptt3-1	3H		6											x																					
	QRptt3-2	3H		58-61											x			x		x	x	x												x		x
		3H		87																								x	x							
QRPtt3-1		3H	129-136	134		x																												x		
		3H	129-151																																x		
		3H	160-164																					x	x											
	QRptt4-1	4H	1-59	3																x												x	x			
Qtt4-1	QRptt4-2	4H	53-76	47-54									x	x						x					x							x	x			x
		5H		26-39																								x	x							
	QRptt5-1	5H		52-54											x	x																				
QRptt5-1		5H	128-132									x	x	x																							
QRptt5-2	QRptt5-2	5H	153-170	166	x	x	x	x	x	x	x	x	x	x			x																				
		6H		8																							x									
	QRptt6-1	6H	52-58	58-59													x		x		x	x														x	
QRptt6-1		6H	79-80									x	x																								
QRptt6-2		6H	80-102			x	x	x																	x											
Qrptt6-3		6H	113-115	111			x	x																			x									

		6H		129																							x									
QRptt7-1		7H	3		x																														
		7H	5-73																								x	x	x						
QRptt7-2	QRptt7-1	7H	30-38	35						x	x	x				x																			
	QRptt7-2	7H		117												x																			
	QRptt7-3	7H		149																x															

NB: Wherever QTL for different traits overlap, the positions of the bigger QTL are given. The exact position of the QTL can be found in the respective paper. x: Locus is significantly associated with corresponding trait. [a] Position of AxL QTL on the consensus map, [b] Position of NBP QTL on the consensus map

2.2.5. How do DH and PH influence net blotch resistance?

In some barley diseases such as Fusarium crown rot and Fusarium Head Blight (FHB) the effect of other agronomical traits such as PH or DH has been examined. A region on chromosome 2H harbors QTL for both FHB resistance and DH (Nduulu et al. 2007). QTL for both Fusarium diseases were found to co-localize with PH QTL (Chono et al. 2003; Saville et al. 2012) and FHB is negatively correlated with PH (Lu et al. 2013; Zhu et al. 1999). No studies have as of yet examined the effect of PH on net blotch resistance. The only indication of a possible interaction between DH and net blotch resistance is a multi-disease resistance locus against stem rust, scald and net blotch on chromosome 4H, which is in close vicinity to an DH QTL (Spaner et al. 1998).

No clear conclusions can be drawn about the influence of DH or PH on net blotch severity in the two populations we studied. In AxL, the Pearson correlation coefficient between DH and disease severity was very low in 2014 (r=0.06) and slightly negative in 2015 (r=-0.10). However, in 2016, the correlation was positive (r=0.19) and significant at $p < 0.05$. In the AM panel, it was significant and negative in three out of four years (r=-0.17--0.31; $p<0.05$) and close to zero in the other year. Similarly, the correlation between PH and disease resistance differed between the populations as well. In AxL it was positive and significant in two years (r=0.25-0.31; $p<0.05$) and slightly negative in the other year, while in the AM panel it was negative in all years (r=-0.08--0.26), one of them being significant. When both adult plant resistance as well as DH and PH are averaged over all four years of field trials, the correlation coefficient was between -0.27 and 0.25 in both populations (Fig. 6). The relation between both cofactors and disease severity is slightly positive in AxL and slightly negative in the AM panel.

Some of the net blotch resistance QTL found in the two populations co-localized with QTL for either DH (NBP_QRPtt1-1, AL_QRPtt6-2), PH (NBP_QRPtt3-2, NBP_QRptt4-2), or both (AL_QRPtt4-1), indicating a possible genetic interaction of these traits that may explain the high correlations in single years. Out of these QTL, AL_QRPtt4-1, NBP_QRPtt3-2 and NBP_QRPtt4-2 were also significantly associated with seedling resistance against one or several isolates. In single years, DH, PH or both traits were significantly associated with seedling resistance. These findings together support the

hypothesis that the interaction between disease severity and the cofactors may be partly genetic. However, further work is necessary to unravel the mechanisms behind these findings. It is possible that these loci harbor one gene that determines both traits in a pleiotropic manner, or two or more genes that are closely linked. Fine-mapping these regions will shed light on the mechanisms and the number of genes involved.

In the AxL population, the allele conferring resistance at the AL_QRPtt4-1 conferred a shorter PH. Since short plants are preferred due to a higher lodging resistance and better harvestability, it may be possible to positively affect two important traits by introducing this QTL in new cultivars. This QTL may be favored over AL_QRPtt3-1, where the resistance allele is linked to increased PH.

The observed correlation between adult disease severity and DH or PH does not necessarily need to be entirely genetically determined but could also be caused by the scoring method and timepoint. Early lines develop faster, so that the pathogen will have more time to colonize leaf tissue and to establish itself and spread within the plant, which will manifest itself in higher disease scores. In the specific case of necrotrophic pathogens, early maturity may also benefit the development of the pathogen. Thus, to exclude an effect of DH, all plants should be scored at the same growth stage, but this is not feasible in a field trial. Similarly, in order to detect “true” genetic association between PH and disease resistance, disease should be scored in an experimental setup where all plants have the same height or where an inoculation method is used that is largely independent on the effect of height, such as spray inoculations in the greenhouse. As *P. teres* is mainly wind-dispersed, it can be expected that the effect of PH is lower than in diseases for which rain splash is an important dispersal factor within the canopy, such as *Fusarium* or *Phytophthora* species (Paul et al. 2004; Ristaino and Gumpertz 2000).

In summary, these results suggest that DH and PH do not have a strong influence on disease severity in general, and that it may not be necessary to correct for these cofactors when assessing the level of resistance. In single years, though, these correlations can be highly significant, and may differ from year to year and in different populations. This suggests that in some years other environmental factors may be more

important in determining disease severity and that they may mask any possible causal genetic relation between disease resistance and the cofactors.

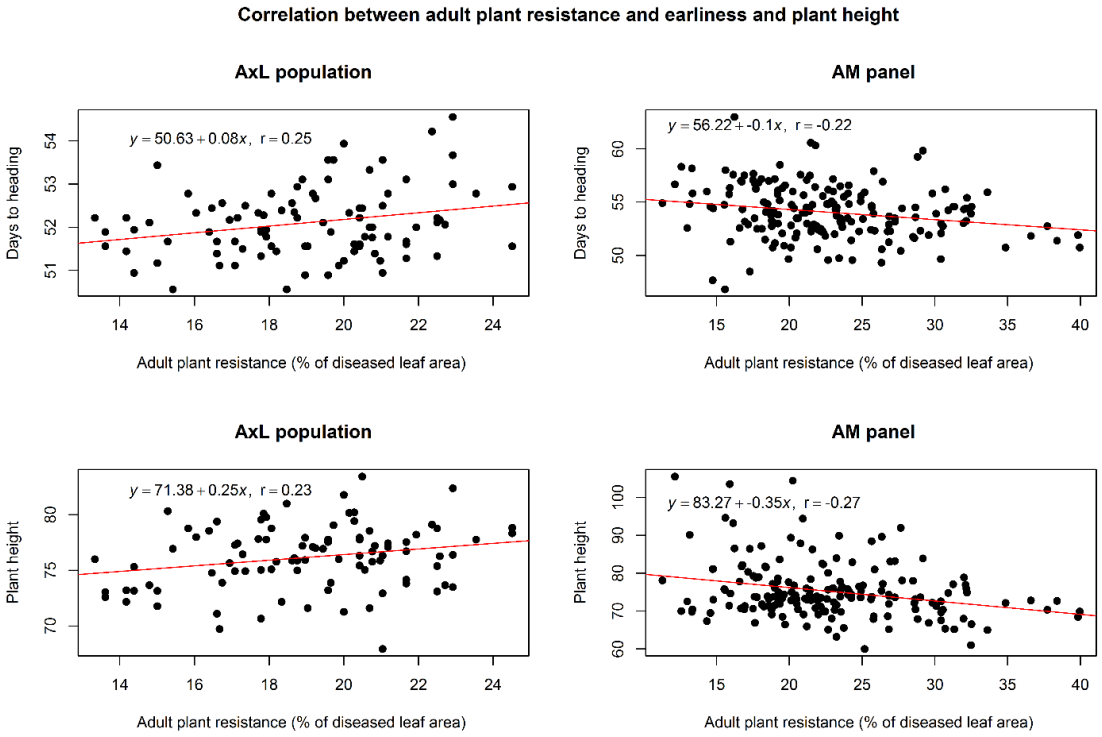

Fig. 6 Correlation between adult plant resistance and DH and PH in both populations averaged over all years of field trials.

3. Conclusions and future perspectives

In order to breed cultivars with durable resistance to diseases, knowledge of the biological properties of the pathogen is required. Depending on the mode of dispersal and genetic diversity, different breeding strategies may be preferred. Our studies have shown that the Norwegian *P. teres* population shows signatures of high genetic diversity and a high evolutionary potential. Its biological properties such as a mixed mating system, abundant spore production and potentially high gene flow (possibly by anthropogenic activities rather than natural spore dispersal) classify *P. teres* as a high risk pathogen (McDonald and Linde 2002). Since pathogens with such properties are able to accumulate virulence genes in different genetic backgrounds, single major resistance genes will not confer long-term resistance. High gene flow will allow new virulent

genotypes to travel over long distances to new populations where they may quickly increase in frequency and become a severe threat to previously resistant cultivars. To successfully breed cultivars resistant to this kind of pathogens, McDonald and Linde (2002) proposed a mixed breeding strategy focusing on the accumulation of quantitative resistance, but also on using major resistance genes that should be used in cultivar mixtures and multilines. In addition, the occurrence of gene flow between years and the potential of the pathogen to adapt quickly to cultivars requires the use of crop and also cultivar rotations to decrease inoculum carry-over and to keep selection pressure low. This means that farmers should not rely on a single cultivar in a growing season, and that they should grow different cultivars in different years. In practice, this is a more feasible method than the use of cultivar mixtures.

In the Nordic barley material used in this study, we identified 167 markers corresponding to 22 QTL associated with net blotch resistance in at least one experiment in at least one of the populations. Before these markers can be used in MAS, they need to be validated in other populations for association with resistance to verify that they are not false positives. If the QTL are found to be true, these loci can be fine-mapped with a larger number of markers in order to find markers that are even more closely associated with the causal locus, which will increase prediction accuracy. It will also help in elucidating the number of genes per locus, especially at loci that are associated with both resistance and cofactors, which will shed light on the genetic mechanisms at these loci.

Efforts should focus on:

1) QTL stable in different environments and developmental stages such as NBP_QRptt1-1, NBP_QRPtt3-2, NBP_QRPtt6-1, AL_QRPtt5-2/NBP_QRPtt5-2, AL_QRPtt7-2/NBP_QRPtt7-1 and AL_QRPtt7-2,

2) QTL that positively affect several traits such as AL_RPtt4-1, and

3) major-effect QTL such as AL_QRPtt5-2.

Seedling screenings are not a recommended method to predict the resistance of adult plants under differing field conditions. Screening seedlings for the presence of resistance alleles will however increase selection efficiency and shorten breeding cycles. However,

while with MAS it is only possible to accumulate known QTL with available markers, phenotypic selection has the advantage of allowing for the implementation of small-effect, previously unknown QTL into cultivars or breeding lines. Therefore, a combined strategy is likely to be most successful in resistance breeding to barley net blotch.

In addition, breeding lines with good resistance should be tested for their suitability as crossing parents for new cultivars. Further work should also include the search for more resistance sources that will be suitable for the introduction into adapted elite germplasm, Furthermore, the Norwegian *P. teres* population needs to be continuously surveyed in order to detect changes in virulence such as the emergence of strains with new virulences. This work will include extensive virulence screens as well as mapping of virulence genes in the *P. teres* genome and the functional and molecular analysis of pathogen-host interactions.

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Paper I

Paper I

Genetic structure of the Norwegian *Pyrenophora teres* population

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Abstract

Net blotch caused by the fungal pathogen *Pyrenophora teres* constitutes a major barley disease in Norway. The pathogen occurs in two forms, *Pyrenophora* f. *teres* (*Ptt*) and f. *maculata* (*Ptm*), which cause different types of necrotic symptoms upon the infection of barley leaves. The pathogen reproduces via asexual and sexual spores, but sexual recombination can only occur between strains of opposite mating types. We analyzed the genetic diversity and structure of the Norwegian net blotch population using 339 isolates collected from different regions in Norway in addition to the comparative analysis of 61 globally collected isolates. We found 325 *Ptt* (95.9%) and 8 *Ptm* (2.4%) isolates in the Norwegian collection, the latter ones originating exclusively from Southeastern Norway, indicating that *Ptt* is more abundant in Norway today than *Ptm*. Using a Bayesian approach and a principal component analysis, we found three groups within the Norwegian population, dividing it into one spot form and two net form subgroups. The net form groups clustered further into two main groups which were mostly defined by the cultivars which the isolates were collected from. There was no geographic differentiation, indicating that long-distance gene flow occurs. No significant genetic differentiation was found over a period of five years, indicating that gene flow occurs over years. Conversely, isolates collected 20 years apart were significantly different. Both mating types were evenly distributed within the sampling regions and the mating type ratio was not significantly different from 1:1 (158:181), but further analysis indicated that clonal reproduction may play a role in many subpopulations. The results from this study indicate that a mixed reproduction system is found in *Ptt*, and there is distinct

evidence for sorting or selection of pathogen lineages on specific cultivars. Evidence of sorting may indicate a coupling with vertical inheritance through seed-borne transmission. Alternatively the observed *Ptt* subpopulation association with host cultivar suggests that the pathogen is able to adapt to different cultivars of the host relatively quickly, potentially underscoring a high evolutionary potential of the pathogen that needs to be taken into account in resistance breeding.

Introduction

Net blotch (NB), caused by the haploid necrotrophic fungal pathogen *Pyrenophora teres*, is a major barley disease in Norway. Under conducive conditions, it can cause yield losses up to 40% (Jayasena et al. 2007; Liu et al. 2011; Mathre 1997) as well as grain quality losses. There is insufficient resistance in the barley cultivars currently grown in Norway (Wonneberger et al. under review). Two forms of the pathogen are apparent, and *P. teres* f. *teres* (*Ptt*) and *P. teres* f. *maculata* (*Ptm*) can be differentiated based on the unique symptoms they produce when the host is challenged. Once infected, *Ptt*-infected plants develop necrotic net-like symptoms (net form net blotch, NFNB), while *Ptm*-infected plants have spot-shaped necrosis with a chlorotic halo (spot form net blotch, SFNB) (Liu et al. 2011; Smedegård-Petersen 1976). Globally, NB has been on the rise in the past years due to the increasing use of reduced or no-till practices (McLean et al. 2009; Paulitz and Steffenson 2010). Earlier, *Ptt* was considered to be the predominant form in most countries, but recently it was reported that the occurrence of *Ptm* has increased in several regions worldwide, especially in Northern America and Australia (Lartey et al. 2013; Liu and Friesen 2010; Marshall et al. 2015; McLean et al. 2009). Conversely, it was reported that 40 to 50 years ago, *Ptm* was the more common form in Norway (Hansen and Magnus 1969), Finland (Mäkelä 1972) and Denmark (Smedegård-Petersen 1971). In Norway, *Ptt* was first documented in 1880 and a severe epidemic was reported in 1927 (Hansen and Magnus 1969; Jørstad 1930). Historically the first findings of *Ptm* date back to 1965, but by the end of the 1960s, *Ptm* was four times more abundant than *Ptt* (Hansen and Magnus 1969). No recent systematic surveys have been performed on NB in Norway, thus, it is not known which form of *P. teres* is now

predominant. Specific knowledge on the pathogen population structure is required to define the *Pyrenophora* disease complexes and types, as well as for resistance breeding.

Despite their morphological similarity (Liu et al. 2011), *Ptt* and *Ptm* are genetically distinct and have been proposed to be classified as different species (Rau et al. 2007). It was shown that laboratory crosses between the two forms can produce fertile progeny (Smedegård-Petersen 1971, 1976), but it remains unknown to what extent hybridization occurs under natural conditions. Symptoms may overlap or have an intermediate form (Lu et al. 2013) and may be easily confused with symptoms caused by *Cochliobolus sativus*, the causal agent of spot blotch, making a visual identification of the isolate form difficult (McLean et al. 2009). Even though many resistance loci seem to be associated with resistance to both forms (Richards et al. 2017; Wonneberger et al. under review), many resistance genes seem to be effective against only one of the forms (reviewed in Liu et al., 2011), suggesting discrete subpopulations and/or the employment of divergent disease modes, such as through the expression of different virulence factors. Therefore, the identification of the pathogen form, and any association with population substructure, is a crucial step in the development of successful disease management strategies including the development and choice of resistant cultivars and reliable prediction modelling.

Both forms of *P. teres* are able to reproduce sexually and asexually. Primary infection during the season usually occurs via the dispersal of sexually produced ascospores, after which a large number of asexual conidia are produced which serve as secondary inoculum. Several cycles of conidia production can occur before the fungus produces pseudothecia on plant debris for overwintering. As in all heterothallic ascomycetes, sexual reproduction requires the interaction of strains of opposite mating types (Kronstad and Staben 1997; McDonald 1967). The mating type trait is conferred by the mating type (MAT) locus and is manifested as mating type 1-1 (MAT1-1) or mating type 1-2 (MAT1-2) (Kronstad and Staben 1997; Rau et al. 2005). Knowledge of mating type frequencies is necessary for monitoring the disease in the field as the mating type ratio will give an indication of whether asexual or sexual recombination is the predominant mode of reproduction. Sexually reproducing species generally evolve faster and have a higher ability to adapt to their environment, such as to overcome resistance in their host or to develop fungicide resistance (McDonald and Linde 2002). Knowledge of the genetic structure, the rate of evolution

and the mode of propagation of the pathogen is important when choosing breeding strategies to develop varieties with durable resistance. The structure of *P. teres* populations has been studied in many barley-growing regions such as Australia (Bogacki et al. 2010; Lehmensiek et al. 2010; Serenius et al. 2007), South Africa (Campbell et al. 2002), Canada (Akhavan et al. 2016), Italy (Rau et al. 2003), Lithuania (Statkeviciute et al. 2012), Czech Republic and Slovakia (Leišová-Svobodová et al. 2014). In Northern Europe, *P. teres* populations have been analyzed in Sweden (Jonsson et al. 2000), Finland (Serenius et al. 2007; Serenius et al. 2005) and Iceland (Stefansson et al. 2012).

Generally, *P. teres* populations are characterized as genetically variable, often also within small sampling regions. Usually, the genetic differentiation increases with geographic distance, indicating that gene flow between adjacent populations is higher than between distant populations (Akhavan et al. 2016; Peever and Milgroom 1994; Serenius et al. 2007; Stefansson et al. 2012). However, exceptions exist (Leišová-Svobodová et al. 2014), so that the relation between these two factors has not been well established. Even though the mating type ratio is very often not different from 1:1, multilocus analyses often lead to the rejection of the hypothesis of random mating (Bogacki et al. 2010; Statkeviciute et al. 2012; Stefansson et al. 2012). *Ptt* and *Ptm* usually form distinct phylogenetic groups (Akhavan et al. 2016; Bogacki et al. 2010; Lehmensiek et al. 2010). Currently, knowledge about the genetic structure of the Norwegian *P. teres* population is lacking.

The aim of this study was to assess the genetic diversity and population structure of *P. teres* samples collected from different regions in Norway in addition to a number of global isolates. More specifically, we aimed to 1) establish which form is the dominant one in Norway today, 2) analyze the mode of reproduction and 3) examine whether distinct subpopulations exist, e.g. due to geography or other factors. To our knowledge, this is the first comprehensive study of the genetic diversity and composition of the Norwegian *P. teres* population, and the first population study of *P. teres* using SNP markers.

Material and methods

Fungal material

Three-hundred and thirty-nine isolates were collected from naturally infected barley leaves and seed from various regions in Norway, including the main barley growing regions Akershus, Østfold and Trøndelag. Most isolates were sampled from seed from the two-rowed cultivar Helium and the six-rowed cultivar Tiril. Seed samples were kindly provided by Kimen seed laboratory, Ås, Norway (www.kimen.no). Isolates derived from straw samples from 1995 were included in the study to infer changes in the population over time. An additional set of 61 isolates from Denmark, Finland, Iceland, USA, Australia and Canada were included in the study. Suppl. 1 lists all isolates used in this study and their geographical origin, year of collection and the barley variety from which they were isolated. All foreign isolates except for the Icelandic ones were provided as mycelium-covered agar plugs by our collaborators. The Norwegian and Icelandic isolates were isolated directly from infected barley material as described by Wonneberger et al. (2017).

Fungal DNA extraction

Each isolate was grown separately from agar plugs on a V8 agar plate for 7 days at 20°C in the dark, for 24 hours at 21°C in the light and for 24 hours at 15°C in the dark to promote mycelium and conidia formation. The fungal biomass was scraped off the surface with a sterile inoculation loop. Genomic DNA was extracted using the DNeasy Plant DNA Extraction Kit (Qiagen) and eluted into 100 µl elution buffer. DNA samples were quantitated with the Qubit Broad Range quantification kit (Thermo Fisher #Q32850).

Determination of mating type and pathogen form

A polymerase chain reaction (PCR)-based approach was used to determine the mating type of each isolate and to assign an isolate as *Ptt* or *Ptm*. The oligonucleotide sequences are given in

Table 1. Form-specific primers developed by Williams et al. (2001) were used to determine the form of each isolate, yielding a 378 bp fragment for *Ptt* and a 411 bp fragment for *Ptm*, respectively. To identify the mating type, primers amplifying a 401 bp fragment from the alpha box and a 252 bp fragment from the HMG box within the mating type region were used for MAT1-1 and MAT1-2, respectively (D. Holmes, USDA-ARS, Fargo, North Dakota, USA, personal communication). A 586 bp fragment of the glyceraldehyde-3-phosphate dehydrogenase (GPD1) gene was amplified as a positive control to ensure the presence of genomic DNA in each PCR assay (Lu et al. 2010). The reactions were amplified in 20 µl which contained 1x PCR buffer I (Applied Biosystems), 0.25 mM of each dNTP, 10 pmol of each primer, 1U AmpliTaq™ DNA polymerase (Applied Biosystems) and 20 ng of template DNA. The samples were denatured at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing for 30 s and elongation at 72°C for 30 s. For the amplification with form-specific primers, a touch-down PCR protocol was employed, with the initial annealing step being performed at 60°C for 30 s with a reduction of by 1°C per cycle until the final temperature of 50°C. For all other amplifications, the annealing temperature was 61°C for 30 s. Each reaction was terminated by a final elongation at 72°C for 5 min. The amplified fragments were resolved in 1% agarose gels.

Library construction, adapters and sequencing

The Ion Torrent library method and adapters used in this study have been previously described by Leboldus et al. (2015), but with the following differences and modifications. Oligonucleotides were synthesized (Biolegio B.V., Nijmegen, The Netherlands; www.biolegio.com) and diluted to a stock concentration of 200 µM in nuclease-free water. Respective oligonucleotides were combined equally in annealing buffer (100mM Tris HCl, pH 8.0, 10 mM EDTA, 500 mM NaCl), denatured at 98°C for 2 min and annealed at a rate of 1°C min^{-1} to form double-stranded adaptors using a Veriti PCR thermocycler (Thermo Fisher). The annealed universal and barcode adaptors were each diluted 10x and stored at -20°C to provide working solutions as needed.

The DNA samples were normalized for libraries 1 to 7 to a concentration of 20 ng/µl, and for the remaining libraries 8, 9 and 10 the DNA concentration were adjusted to either 15, 10 or 5 ng/µl, due to the lower initial DNA concentrations from extractions. Two hundred nanograms of DNA were then transferred and suspended in 30 µl of water and used as the normalized DNA sample input for digestion with 20 units of *Hha*I enzyme for 3 hours at 37°C and subsequently with four units of *Ape*KI enzyme for 3 hours at 70°C as described by Leboldus et al. (2015). The reactions were cleaned with 1.1 volumes of Agencourt AMPure XP (Beckman Coulter) magnetic beads and washed three times with 200 µl freshly prepared 70% ethanol. The beads were air-dried for 5 minutes, the DNA was resuspended in 25 µl nuclease-free water and transferred to a new reaction tube away from the beads. The ligation of adapters and barcodes to each sample was performed in a 26 µl volume and contained 0.25 µl T4 DNA ligase (400 units/µl; NEB, Ipswich, MA), 2.6 µl 10x LigaseT4 buffer (NEB), 0.5 µl ATP (10 mM), 0.25 µM P1-*Hha*I adapter, 20 µl DNA and 0.25 µM of the *Ape*KI individual barcoded adaptor. The reaction proceeded at 21° for 3 hours and was terminated at 65° for 20 min. After the ligation, 13 µl of each barcoded sample in a library was pooled together into a single DNA Low Bind 1.5 ml Eppendorf tube, and the pooled library was cleaned with 1.1x volumes of AMPure XP, as described above but with 500 µl washes of freshly prepared 70% ethanol. Each pooled library was then size-selected for 275 bp fragments using the broad range settings on a Pippin Prep size selection system (Sage Science, Beverly, MA, USA) with a 2% agarose cassette using external markers as described by Leboldus et al. (2015). Size-selected libraries were further cleaned with AMPure XP, as above, and amplified in a 260 µl reaction volume consisting of 200 µl Platinum PCR Super Mix High Fidelity (Thermo Fisher), 10µl each of the P and A1 primer (20 µM; primers as described in IonXpress library amplification; Thermo Fisher) and 25 µl of the size-selected and pooled library using the following settings on a Veriti thermocycler: 95°C for 2 min, 10 cycles of 95°C for 30 s, 62°C for 30 s and 72°C for 30 s, followed by 72°C for 5 min. Finally, the amplification was cleaned with Ampure XP as described above and the size-selected library was quantitated on an Agilent 2100 Bioanalyzer. A concentration of 35 pM of DNA was used as the input for the sequencing reaction using an Ion Chef system (Thermo Fisher). The sequencing was performed at the Norwegian Institute for Bioeconomy Research (NIBIO) in Ås, Norway, on an Ion Torrent PGM sequencer (Thermo Fisher),

using Hi-Q sequencing chemistry (#A25948) and Ion 318™ v2 Chips (Ion 316™ v2 Chips for libraries 4 and 5) following the Ion Torrent PGM and Chef sequencing protocol (MAN0010919; Thermo Fisher). The internal sequencing calibration standards, customarily used for *de novo* sequencing, were omitted since the *P. teres* NCBI reference sequence (GenBank assembly accession, GCA 000166005.1) was used. Library 2 was sequenced twice to assess sequencing accuracy.

Quality control, alignment to the reference genome and SNP calling

The reads were aligned to the *P. teres* reference genome (GenBank assembly accession, GCA 000166005.1) (Ellwood et al. 2010) in Novoalign v3.02.13, with the homopolymer filter set to 20 (homopolymers with quality of less than 20 were filtered out), gap opening penalty of 15 and gap extension penalty of 6. Aligned reads were sorted and converted to BAM format files with Samtools v1.3.1. Freebayes v.1.0.2 was used to call variants with the ploidy set to 1, and vcffilter was used to filter out low quality and low coverage variants (QUAL<30 and depth <10). The indels, sites with more than 80% missing data and sites with minimum allele frequency of < 0.05 were filtered with Vcftools v.0.1.14. The quality checks were passed by 425 isolates (including duplicate isolates from repeated sequencing) and 4252 SNP markers.

Data analysis

Population structure of the isolate collection was analyzed using the Bayesian cluster analysis implemented in Structure v. 2.3.4 (Falush et al. 2003) based on a subset of the polymorphic markers collected with the 'thin' function in vcftools. In Structure, individuals are placed into K clusters that are in Hardy-Weinberg and linkage equilibrium and have distinctive allele frequencies without imposing *a priori* population information. The number of genetic groups K was varied between 1 and 6. The following parameters were used: we assumed correlated allele frequencies and an admixed origin of populations. For each K value the analysis was iterated 20 times, using an initial burn-in of 50000 with 50000 additional cycles. The best K was inferred from

the estimated likelihood values [LnP(D)] based on the ΔK approach by Evanno et al. (2005) in Structure Harvester (Earl and vonHoldt 2012). The similarity between runs within single K was determined with Clumpp (Jakobsson and Rosenberg 2007) implemented in CLUMPAK (Kopelman et al. 2015). Graphical presentation of the results was generated using R (R Core Team 2016). Alternatively, principal component analysis (PCA) was performed with the 'ad e4' package in the R to visualize population structures in different subsets of isolates (Dray and Dufour 2007).

Nei's distance matrix was created between all individuals and visualized in a dendrogram with the unweighted pair group method with arithmetic mean method and 2000 bootstrap replicates using the R package 'poppr' (Kamvar et al. 2014; Nei 1972, 1978; R Core Team 2016).

To calculate diversity statistics per population, we divided the Norwegian isolates into subpopulations based on location, cultivar and form. For isolates derived from seed samples, location was defined by the ZIP code obtained together with the seed as provided by Kimen Seed testing laboratory (Ås, Norway). This resulted in 76 populations, 27 of which had more than five individuals. For each of these populations, as well as across populations, we calculated the number of multilocus genotypes, the percentage of polymorphic markers, the number of private alleles and also Nei's gene diversity (Nei 1978). Additionally, the index of association (I_A), the standard index of association (r_d) using 1000 permutations and the corresponding p-values for the latter two indices were calculated to test the null hypothesis of random mating (Brown et al. 1980; Maynard Smith et al. 1993). These analyses as well as the analysis of molecular variance (AMOVA) were performed with the R package 'poppr'. An exact binomial test for goodness of fit was used to test whether the mating type ratio was different from the expected 1:1 ratio at a significance level of $p < 0.05$ under the assumption of random mating. Normally, this is tested with a Chi-squared test, but this test is not suitable if the expected sample size is very small (McDonald 2014). Nei's G_{ST} was calculated between subpopulations as well as between populations defined by form, cultivar, county or year (Nei 1978).

Results

Genotyping of the population

Sequencing of the eleven libraries yielded between 3,696,738 and 7,187,747 reads per library. The reads mapped to a total of 1262 scaffolds in the genome. The number of variants per isolate ranged from 54 to 14173 with an average of 2308. The number of SNPs per contig ranged from 1 to 105. In total, 88873 SNPs were identified after aligning the reads to the reference genome. After filtering, 4252 SNPs were retained and used for further analysis. Fourteen isolates were removed from further analysis due to more than 50 % of missing marker data. One library was sequenced twice in order to assess the reliability of the obtained results. The duplicated isolates clustered closely in a PCA, indicating that our results are reproducible and reliable (Suppl. 2A).

Geographic and spatial distribution of the isolate collection

Out of the 400 single-conidia isolates included in this study, 339 isolates were obtained from naturally infected barley leaves and seed from various regions in Norway in different years, representing most of the major barley-growing regions in Norway (Suppl. 1)(Statistics Norway 2016). One hundred twenty-five isolates (36.9%) were isolated from leaf samples and 213 (62.8%) from seed. The majority of samples (117 isolates; 34.5%) originates from Akershus county in Southeast Norway (Table 2). Other well-represented regions are Nord-Trøndelag county (59; 17.4%), Vestfold county (50; 14.7%) and Østfold and Sør-Trøndelag counties (33 and 34, respectively). Two hundred fifty-nine isolates (76.4%) are from 2013 and 36 (10.6%) from 2014, while less than 10 isolates are from 2011 and 2012. The oldest isolates were obtained from straw samples from Vollebekk Research Farm in Ås, Akershus, collected in 1995. The current commercial cultivars Tiril and Helium are very well represented in this study with 102 and 11 isolates obtained from Tiril and 110 and 11 from Helium in 2013 and 2014, respectively. Sixty-one isolates from other countries were also included in the study. Most of these isolates originate from other Nordic countries (Iceland: 23, Finland: 18, Denmark: 11). The Icelandic isolates were collected from three regions and three cultivars in 2013 and the Finnish isolates come from ten regions, four cultivars and four years. The exact geographical origin of the Danish isolates is

unknown. Seven isolates are from different regions in the USA (California: 3, Montana and North Dakota: 2 each). Canada and Australia are represented with one isolate each.

Population structure in the Norwegian and the global collection

Principal component analyses were performed on the whole isolate collection as well as on different subsets (Figs. 1 and 2). Using the whole dataset, most of the international and Norwegian *Ptm* isolates formed a very distinct group along the first principal component (PC) which explained 51.4% of the variance (Fig. 1A and B). All *Ptm* isolates except for two (6744C and 48I) formed a group separate from the *Ptt* isolates (See Suppl. 2B for a PCA including isolate names). Two *Ptt* isolates (94I and the Icelandic isolate 53IX) were found within the *Ptm* group. Within the *Ptt* isolates, the Danish isolates formed a distinct group together with two Californian isolates, while Icelandic and Finnish *Ptt* isolates clustered together with the Norwegian ones (Fig. 1C). Also in the Norwegian isolates, the most striking clustering was due to the pathogen form (Fig. 2A). Within the Norwegian *Ptt* isolates, two distinct subgroups were discernible, and the isolates from 1995 clustered together within one of the subgroups (Fig. 2B). The clustering of isolates originating from the two well-represented cultivars Helium and Tiril corresponded well to these two subgroups (Fig. 2C). No clusters were found based on geographic region within Norway (Fig. 2D) or mating type (data not shown).

The structure of the Norwegian population was additionally inferred by a Bayesian algorithm in Structure. With the ΔK method, K=2 was found to be the most likely structure, while ΔK was also higher for K=3 than K=4 (Suppl. 3). K=2 largely divided the population according to form (Fig. 3), but as in the PCA, the two *Ptt* isolates (94I and the Icelandic isolate 53IX) grouped with the Ptm and the two *Ptm* isolates (6744C and 48I) grouped with the *Ptt* isolates. One *Ptt* (CAWB05) and one *Ptm* (117LII) isolate were admixed with around 50% membership to each group. For K=3, the group with mostly *Ptt* isolates was divided into two groups with no clear structure detected based on geographical origin (data not shown). However, among cultivars and particularly among the widely sampled cultivars Helium and Tiril, there was a clear structuring in the membership of the two groups. Fig. 4 shows the membership coefficient of the three genetic groups in isolates from

Helium and Tiril. The green color represents a genetic group that dominates in isolates from Helium, while the red color represents a genetic group that dominates on Tiril. The average membership coefficient to the green group was 89.4% for isolates from Helium and 35.9% for isolates from Tiril, while the average membership to the red population was 9.7% for Helium-derived isolates and 63.2% for Tiril-derived isolates. The isolates colored in blue were 94I (Helium) and 53IX (Tiril), which were previously shown to group with the *Ptm* isolates.

A dendrogram of the Norwegian *Ptt* isolates based on Nei's genetic distance confirmed the findings from the PCA and Structure analyses (Fig. 5). The population was divided into two main groups, largely based on the cultivar that the isolates come from. The samples from 1995 (isolates starting with "117") clustered together in the Tiril-derived group. No geographic pattern was detectable. In a dendrogram including *Ptt* and *Ptm*, all *Ptm* isolates except for 48I and 6744C formed a distinct group, and the *Ptt* isolate 94I clustered with the *Ptm* isolates (Suppl. 4), which was in concordance with the PCA results.

Distribution of *Ptt* and *Ptm* and mating types in Norway

Out of the Norwegian isolates, 325 isolates (95.9%) were identified as *Ptt* and only eight (2.4%) were *Ptm* (Table 2). For six of the isolates (0.18%) we were not able to obtain any form-specific amplicons in the PCR assay. All eight *Ptm* isolates came from Akershus county in Southeast Norway, and six of them were collected from Vollebekk research farm in Ås. One of these was isolated from straw from an unknown cultivar from 1995, and the remaining five were isolates from different cultivars from a seed increase in 2012. All the *Ptm* isolates collected from Vollebekk research farm occurred in the same field together with *Ptt* isolates. No hybrids between *Ptt* and *Ptm* were identified in the collection by PCR.

The distribution of mating types in *P. teres* was analyzed by region, by year and by cultivar (Table 2). The ratio across all Norwegian populations was not significantly different from 1:1 (158:181) (Exact binomial test for goodness of fit, $p<0.05$). However, the mating type ratios of isolates collected in 1995 and 2014 were both 11:25, which differed from the expected 1:1 ratio. Similarly,

the mating type ratios of the isolates collected from cultivars Skagen and Corniche were 1:10 and 6:0, respectively, which was significantly different from 1:1.

Population diversity statistics

For further analysis, isolates were considered a subpopulation if they were collected from the same cultivar within the same region (as defined by postal code or location name) in the same year and belonged to the same form, which resulted in 76 subpopulations, 70 of which were *Ptt* subpopulations. Twenty-seven subpopulations contained more than five isolates and were further considered in the analysis of diversity statistics (Table 3). The number of *Ptm* isolates in this study was too low to calculate reliable population diversity statistics.

Among the Norwegian isolates, 3764 SNPs were polymorphic. The number of polymorphic markers was 3324 (88.3% of the total number of markers) and 2942 (78.2%) in *Ptt* and *Ptm*, respectively. Out of the 3764 markers, 441 alleles were only found in *Ptm* isolates and 827 alleles were specific to *Ptt*. In the *Ptt* populations with $n \geq 5$, the percentage of polymorphic loci was highest for subpopulation 11 consisting of seven isolates (75.7%) and lowest for subpopulation 38 (14.9%) (Table 3). Subpopulation 11 contained the *Ptt* isolate 94I which grouped with *Ptm* isolates in the PCA, which indicates that this isolate is genetically distinct from the rest of the subgroup and may explain the high number of polymorphic markers. Among the remaining populations, the highest percentage of polymorphic markers was 24.8% in subgroup 51 consisting of twelve isolates.

Nei's gene diversity ranged between 0.04 for subpopulation 7 and 0.22 for subpopulation 11. The second highest value was 0.09. Nei's gene diversity across all *Ptt* isolates was 0.08. The index of association (I_A) and the standard index of association (r_d) ranged from 3.11 to 1528 and from 0.004 to 0.6, respectively. In all but one of the subpopulations we rejected the null hypothesis of alleles being in linkage disequilibrium due to random mating ($p < 0.05$). The multilocus analysis suggests that each of the 339 Norwegian isolates represents a unique haplotype.

Nei's G_{ST} was calculated between between the two different forms as well as between all subgroups (Suppl. 5). The G_{ST} between *Ptt* and *Ptm* was 0.42, thus 42% of the genetic variation among the Norwegian isolates was due to the form. The G_{ST} values between subgroups with $n \geq 5$ ranged from 0.02 to 0.39, with an average of 0.10. The highest G_{ST} values were usually found between the isolates from 1995 and the other subpopulations.

The nucleotide diversity was slightly higher in isolates from Tiril compared with those from Helium (Fig. 6A). Average Tajima´s D values of isolates derived from Helium and Tiril were -0.133 (sd=1.08) and -0.02 (sd=1.07) respectively (Fig. 6B). For both cultivars, Tajima´s D showed large variation between the different scaffolds, but the mean was significantly different from zero. The genome wide genetic differentiation (average F_{ST} of all scaffolds) between isolates derived from Helium and Tiril was found to be 0.11, but ranged between -0.11 and 0.77 at the different scaffolds (Fig. 6C). The mean genetic diversity per SNP in each scaffold was 0.052 (sd=0.069) for Helium and 0.063 (0.075) for Tiril.

Analysis of molecular variance (AMOVA) showed that no significant genetic differentiation was found between sampling regions (Table 4a; p=0.947), and low but significant differentiation existed between cultivars within a region (4.35% of the total variation; p=0.026). The largest portion of genetic differentiation was however found within the cultivars (96.82%, p=0.003). Very similar results were found when only Helium and Tiril were included in the analysis (Table 4b)

Very low but significant differentiation was found between different sampling years (Table 4c). When we grouped the population into isolates collected in 1995 and isolates collected between 2011 and 2014 (Table 4d), genetic differentiation was still relatively low (PhiPT=0.10) but significant ($p<0.001$) with 11% of the variation due to the sampling year and 89% within the years. When the isolates from 1995 were excluded from the analysis (Table 4e), no significant different was observed between the years.

Genetic differentiation due to mating type was extremely low (PhiPT=0.00009) and not significant (p=0.483) (Table 4f). Only 0.009% of the genetic variation was found between the mating types. Similarly, no significant differential between mating types was observed within a sampling region (Table 4g, p=0.607).

Discussion

Prevalence of *Ptt* in Norway

In a survey performed in Norway in the 1960s, *Ptm* was four times as abundant as *Ptt* (Hansen and Magnus 1969). In our study, only 2.4% of all isolates were *Ptm*, indicating that there has been a shift in the *P. teres* population in the last 40 to 50 years. Similar findings are reported from Finland, where both forms were equally common in the early 1970s (Mäkelä 1972), but in a study from the mid 2000s, only *Ptt* isolates were found in two localities in Western and Southwestern Finland (Serenius et al. 2005).

The majority of the leaf samples in this study was collected because they displayed *Ptt* symptoms, so there is potentially a bias towards the sampling of *Ptt* in this study. However, it has been shown earlier that both forms can occur on the same leaf and even within the same lesion (Leišová-Svobodová et al. 2014), and conidia were picked from our leaf samples regardless of whether they formed within lesions or symptomless parts of the material. It can therefore be assumed that we would have been able to pick up *Ptm* conidia by chance if they had been present on the leaf. With 117 leaf samples being used in this study, our results can therefore be used as an indication that *Ptt* is the more abundant form in Norway today. Systematic, unbiased sampling of leaves regardless of visible symptoms will however be needed to obtain more reliable data.

While it has been shown that *Ptt* can be transmitted by infected seed, there is currently only one report that also *Ptm* has been successfully isolated from seed samples (Louw 1996). In our collection of Norwegian isolates, 213 (62.8%) isolates were obtained from infected seed, and two of the eight Norwegian *Ptm* isolates are derived from these seed samples. We are unable to trace the seed back to a farm or a field since we obtained them as a mixture from different places within a postal code, but our findings still indicate that seed transmission of *Ptm* can occur and should be investigated further.

Ptt and *Ptm* are genetically distinct, but different subclades may exist in Norway

Population structure analysis showed the presence of three clusters in the global net blotch collection. The most striking substructure was due to form (Fig. 1A), which is in concordance with earlier studies which obtained similar results and suggested that both forms are genetically very distinct (Akhavan et al. 2016; Leišová-Svobodová et al. 2014; Leisova et al. 2005). Nei's G_{ST} between *Ptt* and *Ptm* was 0.42, which means that 42% of the genetic variation among the Norwegian isolates can be explained by differences due to the form, which is identical to the amount of genetic variation between *Ptt* and *Ptm* found by Bogacki et al. (2010) in Australia. Compared with a number of other studies that found a level of differentiation between 27% and 79%, this indicates that the Norwegian population has a rather average level of differentiation due to form (Akhavan et al. 2016; Campbell et al. 2002; Lehmensiek et al. 2010; Leišová-Svobodová et al. 2014; Serenius et al. 2007). According to Ellwood et al. (2010), the two forms diverged approximately 519000 years ago, although there are indications that hybridization between the forms may rarely occur under natural conditions (Campbell et al. 2002; McLean et al. 2014). 11.7% and 22.0% of the SNP markers used in this study had alleles specific to *Ptm* and *Ptt* isolates, respectively, underlining the high level of genetic differentiation of the two forms. Some of these markers may be suitable for reliable routine molecular determination of isolate forms. We were not able to obtain any form-specific band from six isolates, which indicates that the nucleotide sequences at the targeted loci are very distinct to those from the other *P. teres* isolates.

Only very few isolates clustered within the opposite form group. Isolates 94I and 53IX were unambiguously identified as *Ptt* in the PCR test but grouped with *Ptm*, and 48I clearly produced a *Ptm*-specific amplicon and no *Ptt*-specific amplicon but grouped with *Ptt*. For isolate 6744C, which grouped with the *Ptt* cluster, we did not obtain any *Ptt*-specific PCR amplicon and a very weak *Ptm*-specific amplicon. There is thus little possibility that these isolates have been misclassified. Isolate 94I isolate belongs to the *Ptt* subpopulation 11 which consists of seven isolates. This group had both a strikingly high percentage of polymorphic markers (75.7%) and a high index of association (1527.8). This *Ptt* isolate is potentially more similar to *Ptm* than to other *Ptt* isolates. Inoculation of differential lines with these isolates and subsequent observation of

symptoms may aid in determining the form of these isolates. Previous studies also found isolates which did not cluster as expected based on form. Campbell et al. (2002) found two *Ptm* isolates which did not cluster with other *Ptm* isolates but formed a distinct separate clade, indicating that different substructures may exist within forms. Here we found that the *Ptt* isolate CAWB05 Pt-4 was located between the *Ptt* and *Ptm* group in the PCA (Fig. 1A, Suppl. 2B). This isolate was collected from diseased wild barley in California (Lu et al. 2013). In the study by Lu et al. (2013), it was morphologically indistinguishable from *Ptt* or *Ptm*, and it produced intermediate symptoms which manifested themselves as necrotic spots smaller than the typical *Ptm* spots and lacking the *Ptm*-characteristic chlorotic halos. This isolate showed a *Ptt*-specific PCR band for MAT1-2, which is in accordance with the present study. The MAT1-2 idiomorph of this isolate, however, possessed two previously unidentified SNPs, and it mapped to a distinct phylogenetic clade within the *Ptt* isolates analyzed. Specific primers which distinguish between these different groups of *P. teres* are available and may also be used to further characterize the isolates which showed unexpected clustering in our study, such as 53IX, 94I, 6744C and 48I, but also V1794 and 117LII which were located between the two forms in the PCA (Fig. 1A, Suppl. 2B).

Genetic diversity of the Norwegian *Ptt* population

Nei's gene diversity within the 27 subpopulations ranged between 0.04 and 0.09, which is very low compared to most other studies. Bogacki et al. (2010) reported values of 0.38 and 0.40 for *Ptt* and *Ptm*, respectively, and Liu et al. (2012) found a value of 0.62 for *Ptt*. A number of studies found gene diversity between 0.1 and 0.2 (Akhavan et al. 2016; Jonsson et al. 2000; Leišová-Svobodová et al. 2014; Stefansson et al. 2012), and only rarely similar values to those of our study are reported (Campbell et al. 2002; Peever and Milgroom 1994; Rau et al. 2003). These different results indicate that a wide range of different levels of population differentiation may exist worldwide, but these differences may also be due to differences in the experimental setup and sampling strategy, such as the geographic distance between populations, and is also partly attributable to the number and type of markers used in the study (Bogacki et al. 2010). Nei's gene diversity was 0.08 within the *Ptt* isolates and on average 0.07 within subpopulations, which

indicates that the majority of the genetic variation is found within *Ptt* subpopulations, which agrees well with findings by Akhavan et al. (2016) and Serenius et al. (2005). This is also in good agreement with the AMOVA results, where between 89% and 100% of the genetic variation was found within samples, regardless of how the isolates were clustered (Table 4).

No substructure due to geographical origin, but subdivision due to sampling year

No geographical clustering was observed within the Norwegian net blotch population (Fig. 2D). The genetic differentiation between regions was low but significant (PhiPT=0.018; p=0.007) and only 1.8% of the variation was attributable to sampling location (data not shown), but when cultivars were included in the analysis, the effect of region disappeared and the effect of cultivar became significant instead (Tables 4a and b). This is possibly due to a bias in the dataset, since not all cultivars were sampled from all regions (Suppl. 1). Many studies report that the majority of genetic differentiation is found within populations (Akhavan et al. 2016; Leišová-Svobodová et al. 2014; Serenius et al. 2007; Serenius et al. 2005), but the effect of geographical distance on population differentiation and gene flow has not yet been conclusively established. G_{ST} was 0.46 between populations from Europe and North America and decreased to 0.05 when only two populations form Alberta, Canada, were compared (Peever and Milgroom 1994). Jonsson et al. (2000) found a similar level of differentiation of *Ptt* populations from fields 20 km apart (Nei's G_{ST}=0.053). Rau et al. (2003) found a significant correlation between genetic differentiation and geographic distance of populations predominantly consisting of *Ptt* isolates, but not for populations of *Ptm*. Serenius et al. (2007) did not find genetic differentiation among different populations within states in Australia or within sampling sites in Finland. In a study by Leišová-Svobodová et al. (2014), Nei's G_{ST} was 0.31 for two populations 7 m apart, but genetic differentiation was insignificant between populations 250 km apart. This indicates that there must be factors present which can impair gene flow between *Ptt* under certain circumstances.

Moderate but significant genetic differentiation was observed between 1995 and 2011-2014 (Fig. 2B, Table 4d; PhiPT=0.10, p=0.001). When comparing these two periods, 11% of the genetic differentiation were explained by the difference between years (Table 4d), and this value

increased to 19% when only isolates from the same location (Ås, Akershus, postal code 1430) were considered (PhiPT=0.18; P=0.001; data not shown). Additionally, the isolates from 1995 had the highest G_{ST} values to the other subpopulations, indicating that this was the most distinct of all subpopulations (Suppl. 5). No clustering due to year of collection and no genetic differentiation was found within the isolates collected between 2011 and 2014 (Fig. 2B, Table 4e). Thus, changes in the population over time can be observed within periods of 15-20 years, but a substantial amount of gene flow seems to occur within 1-5 years. This has implications for resistance breeding and management strategies. Resistance breeding needs to keep up with the development of the pathogen by constantly incorporating new resistance sources into commercial cultivars, and wide crop rotations are needed to lower infection pressure in the field (Turkington et al. 2006). To further validate this hypothesis, gene flow between isolates collected from the same field in consecutive years should be analyzed.

Population substructure due to host cultivar suggests sorting or selection among isolates

The *Ptt* isolates clustered into two groups based on the cultivar from which the isolates were derived. The cultivars which were best represented in our collection were Tiril (113 isolates) and Helium (121 isolates). Both cultivars were released in 2004, and Tiril was developed in Norway while Helium is of Danish origin. Isolates from these two cultivars formed two distinct groups in both the Structure analysis, the PCA and the dendrogram based on Nei's distance matrix, although not all isolates followed this pattern. In the Structure analysis, Helium-derived isolates were more distinct than the Tiril-derived isolates that appeared more intermixed.

The genome-wide F_{ST} analysis between isolates from Helium and Tiril showed an overall relatively high genetic differentiation, with many scaffolds having very high F_{ST} values. This suggests that the two genetic groups have been previously isolated and have come into secondary contact recently. The more intermediate frequency of the two groups in Tiril, along with presence of apparent admixed isolates shows that the two genetic groups probably interbreed. In contrast to Tiril, isolates from Helium were assigned mostly to one genetic group, with low amount of admixture. As Helium show higher resistance to *Ptt* it may mean that only one of the genetic

groups can efficiently infect the Helium cultivar. Tajima's D is used to detect departures from the standard neutral model. A positive Tajima's D in single genetic regions is evidence for heterozygotes having a selective advantage, while negative values suggest directional selection for a specific allele. If, however, the majority of the genes have either a negative or a positive Tajima's D value, the most probable explanation is that the population underwent a recent expansion or a bottleneck, respectively. In both the Helium- and Tiril-derived isolates, the average Tajima's D was significantly below zero, which may, together with low nucleotide diversity, indicate that the genetic groups have gone through a bottleneck followed by an increase in population size. The effect of such a demographic event on Tajima´s D may vary within the genome depending on the level of diversity and this is probably the reason for the large variation in Tajima´s D values seen here.

Analysis of Helium- and Tiril-derived isolates shows indications for either sorting or selection on these cultivars. If the observed substructure is attributable to selection, this indicates that the pathogen population is able to adapt to different host cultivars within less than 10 years. In a virulence study of a Swedish isolate collection, Jonsson et al. (1997) found that some pathotypes predominantly occurred on certain cultivars. One of them, the cultivar Golf, had been cultivated for a similar period (released in 1984) as Helium and Tiril. Specialization to cultivars has also been shown for other pathogens like *Zymoseptoria tritici*. Resistance in the cultivar Gene was overcome within three years after its introduction and cultivation in Oregon, as shown in pathogenicity tests (Cowger et al. 2000). In addition, the authors showed that some isolates collected from other cultivars were virulent on these other cultivars, but avirulent on Gene, indicating cultivar-specificity. The observed substructure on Helium and Tiril can however also be explained by sorting due to vertical inheritance through seed-borne transmission.

Inoculation tests on Helium and Tiril will allow us to detect potential differences in virulence of the two subpopulations on the different hosts, which will give an indication of potential specialization and adaptation to the different host genotypes. Inoculation of a *Ptt* differential set will allow the identification of distinct pathotypes which will help to further characterize these isolates.

The Norwegian *Ptt* population likely reproduces both sexually and asexually

The mating type ratio was not significantly different from 1:1 in all but three Norwegian subpopulations, and in all geographic regions, the ratio did not differ from 1:1. Additionally, no genetic differentiation was observed between mating types within regions (PhiPT=-0.003, p=0.607, Table 4g), which is consistent with the situation in Finland (Serenius et al. 2005). This indicates that both mating types occur together and frequent genetic exchange may occur between them via sexual recombination. Genotype diversity was high, since each isolate represented a distinct multilocus genotype, which is consistent with studies by McLean et al. (2010), Jonsson et al. (2000), Stefansson et al. (2012), Statkeviciute et al. (2012) and Leišová-Svobodová et al. (2014) in which all individuals of Australian, Swedish, Icelandic, Lithuanian and Czech and Slovak populations were found to be distinct haplotypes. Akhavan et al. (2016) reported that 90% of the isolates analyzed in their study represented unique genotypes, indicating that 10% of the isolates were clonally derived. In a Finnish study, only three out of 72 *Ptt* isolates were clonal, but had different mating types (Serenius et al. 2005). In another study, Serenius et al. (2007) found 146 haplotypes among 167 Finnish isolates, and 87 haplotypes in a collection of 116 Australian isolates. Rau et al. (2003) found 117 unique haplotypes among 150 isolates in Sardinia. Our findings suggest that propagation in the Norwegian net blotch population occurs mostly via sexual recombination. The I_A and r_D tests, however, led us to the rejection of the null hypothesis of linkage equilibrium under random mating at $p<0.05$ in all but one subpopulations. Similarly, in several studies the hypothesis of random mating was rejected, even though the mating type ratio was not different from 1:1 (Bogacki et al. 2010; Serenius et al. 2007; Stefansson et al. 2012). High I_A values may be caused by linkage disequilibrium due to an unaccounted substructure within subpopulations (Maynard Smith et al. 1993). The substructure may be due to family structure in the samples or caused by selection, e.g. of different avirulence genes on different hosts (Serenius et al. 2007). Selection based on host genotype can be ruled out in our study since we grouped isolates into subpopulations based on the cultivar they were isolated from, as well as a common geographic origin and a common year of collection. However, after a selective sweep the level of LD will be high in the regions of the genome around the

beneficial mutation independent of population substructure. An unaccounted substructure may be present in subpopulations derived from seed samples in this study since the seed are likely to be a collection from different farms. Similarly, seed-transmitted inoculum may lead to the introduction of new genotypes into a field, which may constitute different subpopulations. These results give further evidence to the possibility that different subpopulations exist even in small sampling units such as farms or fields (Leišová-Svobodová et al. 2014). It is therefore still likely that sexual reproduction plays a major role in the Norwegian *P. teres* population, but the exact proportion of sexual and asexual propagation still remains to be determined.

Relationship of the Norwegian *Ptt* population to other Nordic populations

The Norwegian *Ptt* isolates clustered together with Icelandic and Finnish *Ptt* isolates in the PCA (Fig. 1C), which was supported by low G_{ST} values (0.02 and 0.03)(data not shown). The Danish isolates were not part of this group but formed a distinct cluster (G_{ST}=0.23). Earlier, Stefansson et al. (2012) found that genetic differentiation between Finnish and Icelandic *Ptt* isolates was high (F_{ST}=0.64) and concluded that the Icelandic *Ptt* population likely does not originate from mainland Scandinavia. Our results, however, indicate that there is a possibility that *Ptt* may have been introduced to Iceland via infected Scandinavian seed. A possible explanation might be that our Icelandic isolates are genetically different from those used by Stefansson et al. (2012). This appears likely since most of our isolates are from different localities in Iceland than those used by Stefansson et al. (2012), and the authors found that 51% of the genetic variation was found between the Icelandic populations, which indicates that they are distinct populations. In addition, 13 of our Icelandic isolates (out of 21) were isolated from the Norwegian cultivar Tiril and the Finnish cultivar Wolmari, which allows for the speculation that the Icelandic isolates were either introduced with infected seed from Norway and Finland, or that host-specific selection has occurred in the pathogen population.

Conclusions

Our study of a population of 339 Norwegian *P. teres* isolates showed that there has been a shift in the Norwegian population in the last 50 years, since *Ptt* is likely to be the predominant form in Norway today, and *Ptm* was only found in Southeastern Norway. Both forms are genetically distinct, which underlines the necessity of treating them as different diseases with regard to management strategies. A high amount of gene flow within periods of five years indicates that the pathogen survives climatic conditions of Norwegian winters in the field and stresses the importance of wide crop rotations to reduce infection pressure and ensure high yields. Genetic differentiation is mainly found within subpopulations, but no geographical clustering was discernible, indicating that long-distance gene flow occurs, possibly by anthropogenic activities. The mixed reproduction system and the outcrossing between the two mating types allows the fungus to evolve quickly by the mass propagation of new recombinant genotypes, which justifies the classification of *P. teres* as a high risk pathogen in Norway. This knowledge is crucial for the development of effective breeding programs, which should focus on the combination of diverse resistance sources in elite cultivars to ensure longlasting resistance to *P. teres*.

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Table 1 Primer sequences and resulting amplicon sizes

Name	Forward primer sequence	Reverse primer sequence	Amplicon size	Reference
Ptt	5'-CTCTGGCGAACCGTTC-3'	5'-ATGATGGAAAAGTAATTTGTA-3'	378 bp	Williams et al., 2001
Ptm	5'-TGCTGAAGCGTAAGTTTC-3'	5'-ATGATGGAAAAGTAATTTGTG-3'	411 bp	Williams et al., 2001
MAT1-1 (matALPHABOX)	5'-CGCTTCATCGACCTTCCTTG-3'	5'-TGTCCGAAGTGGACTGGTGA-3'	401 bp	D. Holmes (pers. comm.)
MAT1-2 (matHMG)	5'-CAGCCTTCCGCTTCTTTTCG-3'	5'-TCGCGGAAGATGATCCAACA-3'	252 bp	D. Holmes (pers. comm.)
GPD1	5'-TATCGTCTTCCGCAAC-3'	5'-GAGAGCACCTCAATGT-3'	586 bp	Lu et al., 2010

Table 2 Mating types and pathogen forms by country, county, year and cultivar

Country	*Ptt* MAT1-1/ MAT1-2	*Ptm* MAT1-1/ MAT1-2	Unknown form MAT1-1/MAT1-2	Total MAT1-1/MAT1-2	Total no. of isolates
Norway	152/173	5/3	1/5	158/181	339
Iceland	8/15	0/0	0/0	8/15	23
Finland	9/6	2/1	0/0	11/7	18
Denmark	3/8	0/0	0/0	3/8	11
USA	1/3	2/1	0/0	3/4	7
Australia	0/0	0/1	0/0	0/1	1
Canada	0/1	0/0	0/0	0/1	1
Total	173/206	9/6	1/5	183/217	400
County (Norway)					
Akershus	43/63	5/3	1/2	49/68	117
Nord-Trøndelag	30/29	0/0	0/0	30/29	59
Vestfold	20/29	0/0	0/1	20/30	50
Sør-Trøndelag	14/19	0/0	0/1	14/20	34
Østfold	16/17	0/0	0/0	16/17	33
Hedmark	14/10	0/0	0/1	14/11	25
Buskerud	9/3	0/0	0/0	9/3	12
Telemark	3/1	0/0	0/0	3/1	4
Nordland	0/1	0/0	0/0	0/1	1
NA	3/1	0/0	0/0	3/1	4
Total	152/173	5/3	1/5	158/181	339
Year (Norwegian)					
1995	11/24	0/1	0/0	11/25 *	36
2011	1/0	0/0	0/0	1/0	1
2012	2/2	1/1	0/0	3/3	6
2013	127/121	4/1	1/5	132/127	259
2014	11/25	0/0	0/0	11/25 *	36
NA	0/1	0/0	0/0	0/1	1
Total	152/173	5/3	1/5	158/181	339
Cultivar/Line (Norwegian)					
Helium	58/60	0/0	1/2	59/62	121
Tiril	51/60	0/0	0/2	51/62	113
Skagen	1/10	0/0	0/0	1/10 *	11
Tyra	5/3	2/0	0/0	7/3	10
Cork	6/2	0/0	0/0	6/2	8
Iron	1/6	0/0	0/0	1/6	7
Corniche	6/0	0/0	0/0	6/0 *	6
Brage	0/3	0/0	0/0	0/3	3
Edel	2/0	0/0	0/0	2/0	2
Fløya	1/0	1/0	0/0	2/0	2
Agneta	0/0	0/0	0/1	0/1	1
Amber	1/0	0/0	0/0	1/0	1
Etu	0/1	0/0	0/0	0/1	1
Gammel dansk	0/0	1/0	0/0	1/0	1
Heder	1/0	0/0	0/0	1/0	1
Herta	1/0	0/0	0/0	1/0	1
Marigold	1/1	0/0	0/0	1/1	1

NA	17/27	1/3	0/0	18/30	48
Total	152/173	5/3	1/5	158/181	339

* Mating type ratio is significantly different from the expected 1:1 under the hypothesis of random mating (Exact binomial test for goodness of fit, $p<0.05$)

Table 3 Population diversity statistics for all Ptt subpopulations with n ≥ 5

Sub-pop.	Cultivar	Place	ZIP code	County	Year	n total[a]	n geno-typed[b]	MAT 1-1[c]	MAT 1-2	% poly-morphic markers	I_A[d]	r_D[e]	p rd[f]	Nei's gene diversity[g]
2	Helium	NA	974	Akershus	2013	8	8	3	5	21.3	26.57	0.038	0.001	0.08
5	Helium	Vollebekk (Ås)	1430	Akershus	2014	8	8	2	6	15.4	32.54	0.064	0.001	0.05
7	NA	Vollebekk (Ås)	1430	Akershus	1995	35	35	**11**	**24**	20.5	14.13	0.023	0.001	0.04
8	Tiril	Vollebekk (Ås)	1430	Akershus	2014	12	11	4	8	24.6	20.21	0.025	0.001	0.08
10	Tiril	NA	1957	Akershus	2013	10	10	6	4	23.2	8.27	0.011	0.001	0.08
11	Helium	NA	2296	Akershus	2013	7	7	3	4	75.7	1527.79	0.610	0.001	0.22
12	Helium	NA	3097	Akershus	2013	10	10	5	5	22.7	30.01	0.040	0.001	0.07
13	Helium	NA	5798	Akershus	2013	10	10	6	4	21.4	22.35	0.032	0.001	0.07
14	Helium	NA	2661	Buskerud	2013	7	6	5	2	17	19.46	0.035	0.001	0.07
15	Tiril	NA	4320	Buskerud	2013	5	5	4	1	16.4	15.90	0.030	0.003	0.07
18	Tiril	NA	2800	Hedmark	2013	8	8	4	4	19.8	8.74	0.013	0.001	0.07
35	Skagen	Holthe (Verdal)	NA	Nord-Trøndelag	2013	11	11	**1**	**10**	20.2	92.60	0.140	0.001	0.07
36	Corniche	Holthe (Verdal)	NA	Nord-Trøndelag	2013	6	6	**6**	**0**	16.8	54.19	0.097	0.001	0.07
38	Cork	Værnes	NA	Nord-Trøndelag	2013	8	8	6	2	14.9	56.63	0.116	0.001	0.06
44	Tiril	Værnes	NA	Nord-Trøndelag	2013	6	6	2	4	19.7	4.88	0.007	0.01	0.08
46	Tyra	Eidum (Stjørdal)	NA	Nord-Trøndelag	2014	7	7	4	3	19.4	3.67	0.006	0.825	0.07
49	Tiril	NA	2794	Sør-Trøndelag	2013	9	9	2	7	22.7	3.11	0.004	0.025	0.08
50	Tiril	NA	4069	Sør-Trøndelag	2013	7	6	5	2	21.4	22.75	0.032	0.001	0.09
51	Tiril	NA	7176	Sør-Trøndelag	2013	12	12	7	5	24.8	19.12	0.024	0.001	0.08
56	Tiril	NA	1287	Vestfold	2013	10	10	5	5	23.8	21.18	0.027	0.001	0.08
57	Helium	NA	2956	Vestfold	2013	10	10	6	4	15.9	8.59	0.017	0.004	0.05
58	Helium	NA	2960	Vestfold	2013	11	11	4	7	22.9	42.22	0.057	0.001	0.07
60	Tiril	NA	4102	Vestfold	2013	8	8	3	5	20.9	22.07	0.032	0.001	0.08
62	Iron	Viken	NA	Vestfold	2014	7	7	1	6	15	49.57	0.100	0.001	0.05
63	Helium	NA	899	Østfold	2013	8	7	7	1	15	11.46	0.023	0.001	0.09
65	Helium	NA	1602	Østfold	2013	5	5	2	3	15.5	39.65	0.079	0.001	0.07
66	Helium	NA	1639	Østfold	2013	9	8	4	5	18.6	37.83	0.062	0.001	0.07
All										88.3*	158.20			0.08

[a] Number of isolates in the subpopulation, [b] Number of isolates in the subpopulation that passed the quality tests for genotyping, [c] Numbers in bold indicate a significant deviation from a mating type ratio of 1:1, [d] Index of association (Brown et al. 1980; Maynard Smith et al. 1993), [e] Standard index of association. [f] P values for the index of association, [g] Nei's gene diversity (Nei 1978), * Percentage of polymorphic markers for all 325 *Ptt* isolates

Table 4 Analyses of molecular variance (AMOVA).

Source	Df [a]	SS [b]	MS [c]	Estimated variance	Percentage %	PhiPT [d]	p value
a) by county and cultivar							
Between counties	7	3020	431	-3.9	-1.17	-0.012	0.947
Between cultivars within county	19	8085	426	14.4	4.35	0.043	0.026
Within cultivar	248	79520	321	320.6	96.82	0.032	0.003
Total	274	90625	331	331.2	100.00		
b) by county and cultivar, only Helium and Tiril							
Between counties	7	2775	396	-10.6	-3.10	-0.031	0.972
Between cultivars within county	5	3048	610	19.1	5.60	0.054	0.001
Within cultivar	210	69744	332	332.1	97.51	0.024	0.001
Total	222	75566	3340	340.6	100.00		
c) by year							
Between years	4	3690	923	19.4	5.72		
Within years	313	99959	319	319.4	94.28		
Total	317	103649	327	338.7	100.00	0.057	0.001
d) by year (1995 vs. 2011-2014)							
Between years	1	2771	2771	39.4	11.00		
Within years	316	100879	319	319.2	89.02		
Total	317	103650	327	358.6	100.00	0.10	0.001
e) by year (2011-2014)							
Between years	3	918	306	-1.0	-0.31		
Within years	279	91948	330	329.6	100.31		
Total	282	928866	329	328.6	100.00	-0.003	0.483
f) by mating type							
Between mating types	1	332	332	0.03	0.009		
Within mating types	317	103707	327	327.2	99.99		
Total	318	104039	327	327.2	100.00	0.00009	0.348
g) by mating type and county							
Between counties	8	4059	507	6.3	1.92	0.019	0.001
Between mating types within county	8	2440	305	-1.0	-0.31	-0.003	0.607
Within mating type	298	96571	324	324.1	98.39	0.016	0.014
Total	314	103069	328	329.4	100.00		

[a] Degrees of freedom, [b] Sum of squares, [c] Mean square, [d] Phi statistics

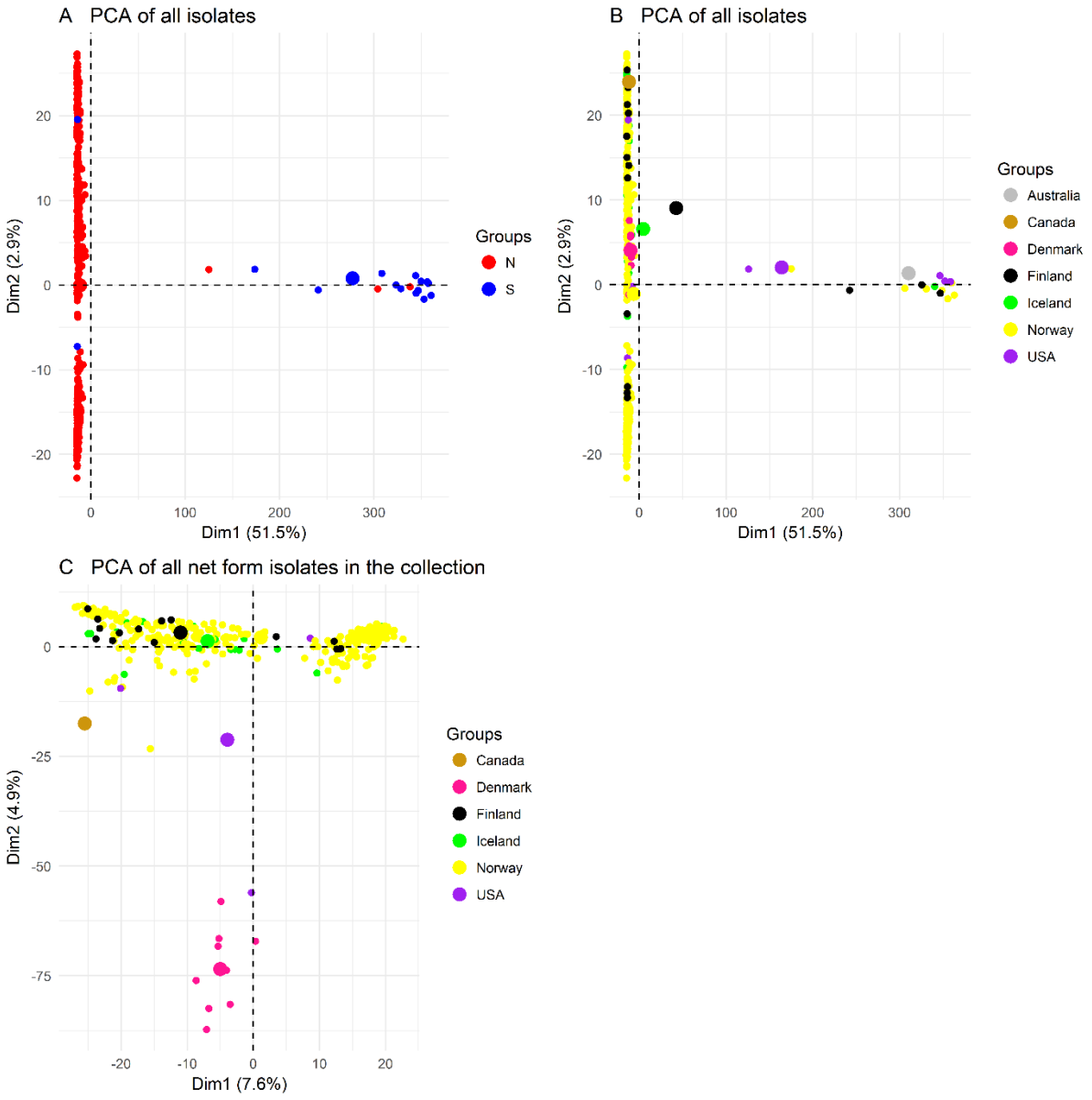


Fig. 1 PCAs of the global isolate collection. A: PCA of all isolates in the global collection, color-coded by form. B: PCA of all isolates in the global collection, color-coded by country. C: PCA of all *Ptt* isolates in the global collection, color-coded by country.

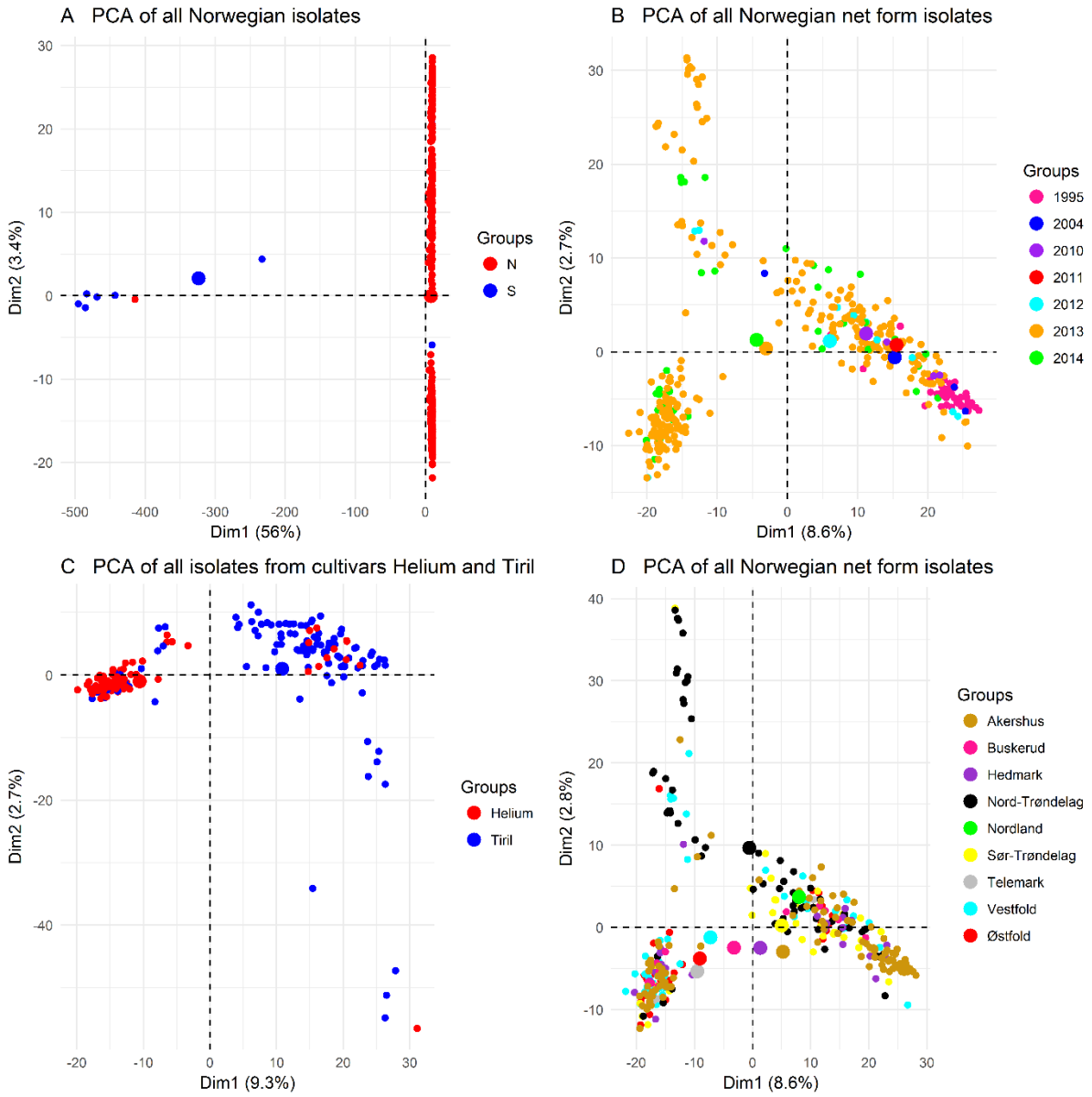


Fig. 2 PCAs on different subpopulations of the Norwegian isolate collection. A: PCA of all Norwegian isolates, color-coded by form. B: PCA of all Norwegian *Ptt* isolates, color-coded by year of collection. C: PCA of all of all *Ptt* isolates from the cultivars Tiril and Helium. D: PCA of all Norwegian *Ptt* isolates, color-coded by region. For better visualization, outlier isolates 53IX and 94I were excluded

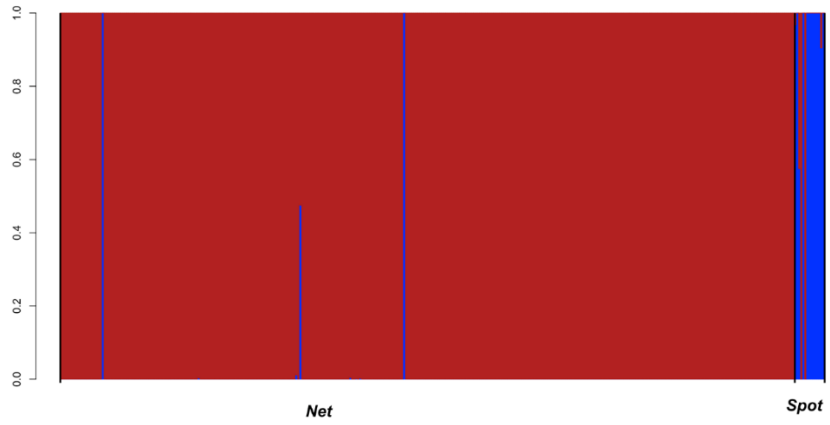


Fig. 3 Estimated genetic structure for K=2 obtained with the STRUCTURE program. Each vertical line represents one fungal isolate. The two genetic groups are largely divided into *Ptt* and *Ptm*.

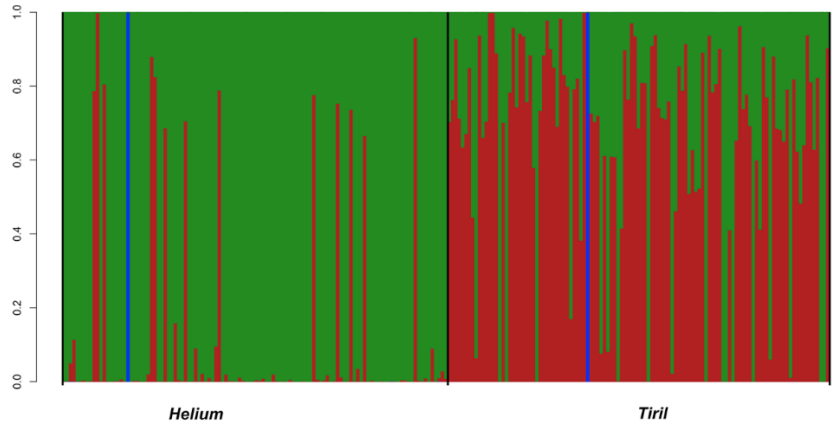


Fig. 4 Estimated genetic structure for K=3 with only cultivars Helium and Tiril shown. Each vertical line represent one fungal isolate where the blue group represent the isolate grouping with the spot form, the green group is largely isolated from the Helium cultivar and the red group is mostly found on the Tiril cultivar.

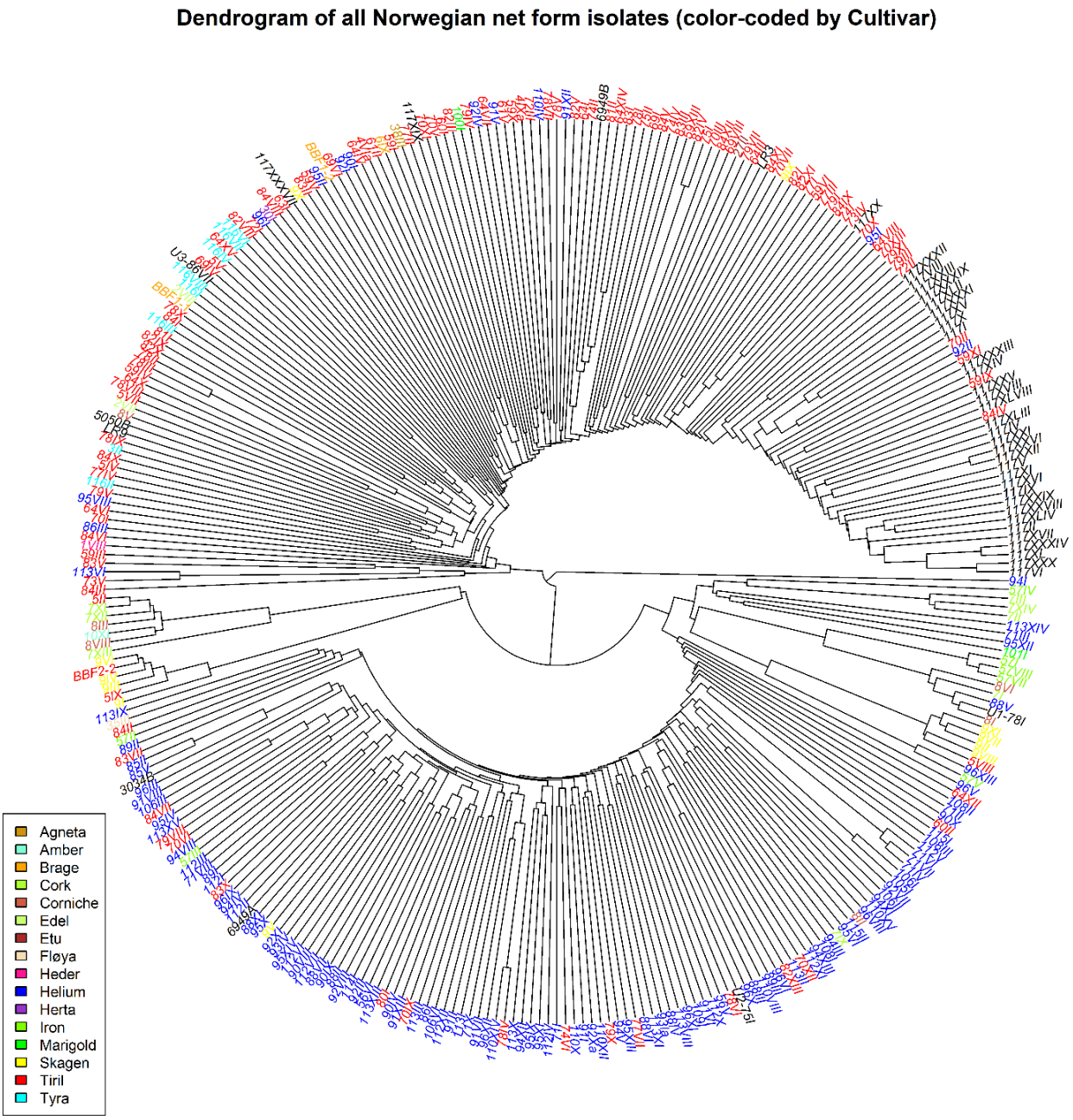


Fig. 5 UPGMA- based dendrogram created from Nei's distance matrix of all *Ptt* isolates, color-coded by the cultivar they were sampled from.

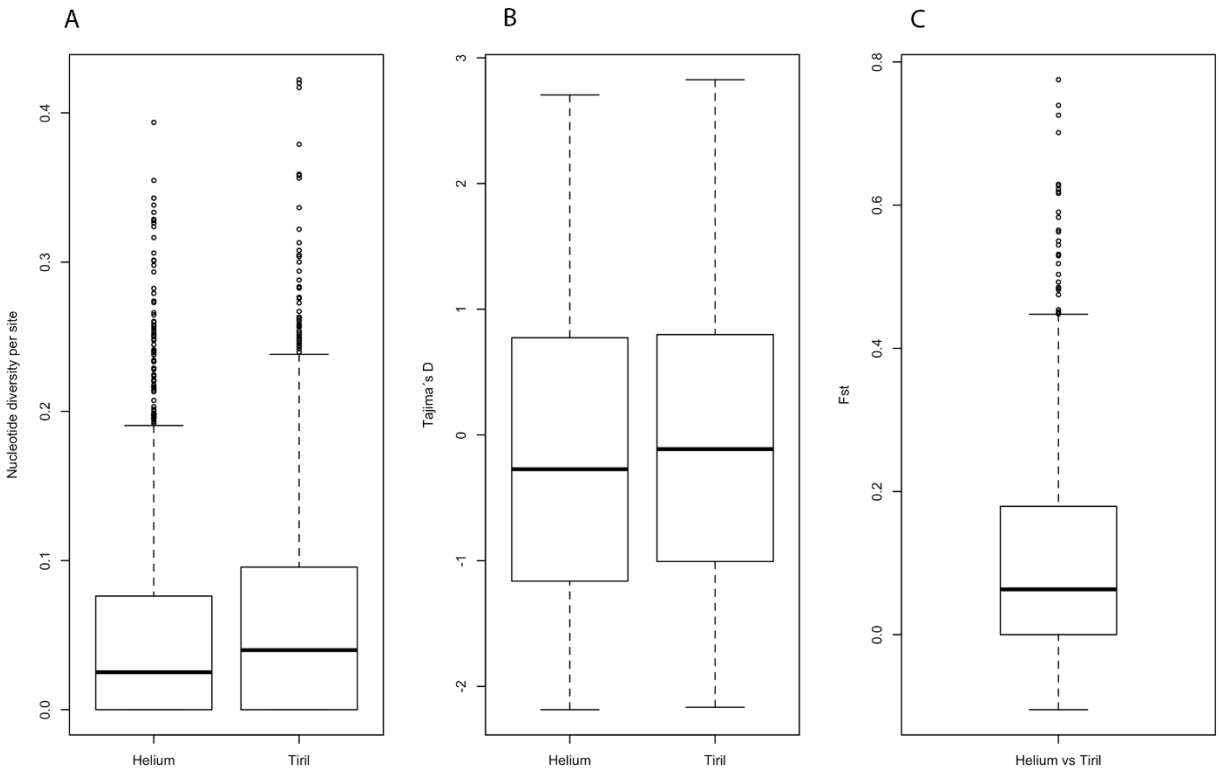


Fig. 6 Boxplot showing the Nucleotide diversity per site (A), Tajima´s D (B) in isolates cultivated from Helium and Tiril and the pairwise F_{ST} (C) value between isolates from Helium and Tiril.

S1 List of *P. teres* isolates, including the barley cultivar and material they were isolated from, geographic origin, year of collection, form and mating type

Isolate	Cultivar	Place	County/State	Country	Mate-rial	Year	Net/ spot form	MAT	Ref-
1VIII	Heder	Lånke (Stjørdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
2VII	Edel	Eidum (Stjørdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
2VIII	Edel	Eidum (Stjørdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
3II	Tyra	Mona (Stjørdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
4VI	Tiril	Eidum (Stjørdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
4IX	Tiril	Eidum (Stjørdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
5II	Tiril	Værnes	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
5IV	Tiril	Værnes	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
5V	Tiril	Værnes	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
5VII	Tiril	Værnes	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
5VIII	Tiril	Værnes	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
5IX	Tiril	Værnes	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
6IX	Brage	Eidum (Stjørdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
7I	Cork	Værnes	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
7II	Cork	Værnes	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
7III	Cork	Værnes	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
7IX	Cork	Værnes	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
7XI	Cork	Værnes	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
7XII	Cork	Værnes	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
7XIII	Cork	Værnes	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
7XIV	Cork	Værnes	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
8I	Corniche	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
8II	Corniche	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
8III	Corniche	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
8V	Corniche	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
8VI	Corniche	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
8VIII	Corniche	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
9I	Skagen	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
9II	Skagen	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
9III	Skagen	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
9IV	Skagen	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
9V	Skagen	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
9VI	Skagen	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
9VII	Skagen	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
9VIII	Skagen	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
9IX	Skagen	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
9X	Skagen	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-2	
9XI	Skagen	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
10XI	Amber	Holthe (Verdal)	Nord-Trøndelag	Norway	Leaves	2013	N	1-1	
24III	Gammel dansk	Vollebekk (Ås)	Akershus	Norway	Leaves	2013	S	1-1	
27II	Etu	Vollebekk (Ås)	Akershus	Norway	Leaves	2013	N	1-2	
30I	Herta	Vollebekk (Ås)	Akershus	Norway	Leaves	2013	N	1-1	
35I	Fløya	Vollebekk (Ås)	Akershus	Norway	Leaves	2013	S	1-1	
35III	Fløya	Vollebekk (Ås)	Akershus	Norway	Leaves	2013	N	1-1	
38III	Agneta	Vollebekk (Ås)	Akershus	Norway	Leaves	2013	NA	1-2	
41II	Tyra	Vollebekk (Ås)	Akershus	Norway	Leaves	2013	S	1-1	
41III	Tyra	Vollebekk (Ås)	Akershus	Norway	Leaves	2013	S	1-1	

48I	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	2013	S	1-2	
56V	Helium	Råde	Østfold	Norway	Leaves	2014	N	1-2	
56VI	Helium	Råde	Østfold	Norway	Leaves	2014	N	1-2	
57I	Iron	Viken	Vestfold	Norway	Leaves	2014	N	1-2	
57II	Iron	Viken	Vestfold	Norway	Leaves	2014	N	1-1	
57III	Iron	Viken	Vestfold	Norway	Leaves	2014	N	1-2	
57IV	Iron	Viken	Vestfold	Norway	Leaves	2014	N	1-2	
57V	Iron	Viken	Vestfold	Norway	Leaves	2014	N	1-2	
57VII	Iron	Viken	Vestfold	Norway	Leaves	2014	N	1-2	
57VIII	Iron	Viken	Vestfold	Norway	Leaves	2014	N	1-2	
59I	Tiril	NA	Hedmark	Norway	Seeds	2013	N	1-1	
59III	Tiril	NA	Hedmark	Norway	Seeds	2013	N	1-1	
59IV	Tiril	NA	Hedmark	Norway	Seeds	2013	NA	1-2	
59V	Tiril	NA	Hedmark	Norway	Seeds	2013	N	1-1	
59VI	Tiril	NA	Hedmark	Norway	Seeds	2013	N	1-2	
59IX	Tiril	NA	Hedmark	Norway	Seeds	2013	N	1-2	
59X	Tiril	NA	Hedmark	Norway	Seeds	2013	N	1-2	
59XI	Tiril	NA	Hedmark	Norway	Seeds	2013	N	1-2	
59XIII	Tiril	NA	Hedmark	Norway	Seeds	2013	N	1-1	
60I	Tiril	NA	Hedmark	Norway	Seeds	2013	N	1-2	
60II	Tiril	NA	Hedmark	Norway	Seeds	2013	N	1-1	
61II	Tiril	NA	Hedmark	Norway	Seeds	2013	N	1-1	
61V	Tiril	NA	Hedmark	Norway	Seeds	2013	N	1-2	
63I	Tiril	NA	Akershus	Norway	Seeds	2013	N	1-1	
64I	Tiril	NA	Akershus	Norway	Seeds	2013	N	1-1	
64III	Tiril	NA	Akershus	Norway	Seeds	2013	N	1-2	
64IV	Tiril	NA	Akershus	Norway	Seeds	2013	N	1-1	
64VI	Tiril	NA	Akershus	Norway	Seeds	2013	N	1-1	
64VII	Tiril	NA	Akershus	Norway	Seeds	2013	N	1-1	
64VIII	Tiril	NA	Akershus	Norway	Seeds	2013	N	1-1	
64X	Tiril	NA	Akershus	Norway	Seeds	2013	N	1-2	
64XII	Tiril	NA	Akershus	Norway	Seeds	2013	N	1-2	
64XIV	Tiril	NA	Akershus	Norway	Seeds	2013	N	1-1	
64XV	Tiril	NA	Akershus	Norway	Seeds	2013	N	1-2	
69I	Tiril	NA	Østfold	Norway	Seeds	2013	N	1-1	
69IV	Tiril	NA	Østfold	Norway	Seeds	2013	N	1-2	
69VI	Tiril	NA	Østfold	Norway	Seeds	2013	N	1-2	
69VIII	Tiril	NA	Østfold	Norway	Seeds	2013	N	1-2	
70I	Tiril	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-2	
70II	Tiril	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-2	
70III	Tiril	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-2	
70V	Tiril	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-1	
70VI	Tiril	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-2	
70VII	Tiril	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-1	
70VIII	Tiril	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-2	
70IX	Tiril	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-1	
70X	Tiril	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-2	
70XI	Tiril	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-2	
70XII	Helium	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-2	
70XIII	Tiril	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-1	
71I	Helium	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-2	
71II	Helium	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-2	
71III	Helium	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-1	
71IV	Helium	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-2	

71VI	Helium	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-2	
71VII	Helium	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-2	
71VIII	Helium	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-1	
71X	Helium	Vollebekk (Ås)	Akershus	Norway	Leaves	2014	N	1-2	
72I	Tiril	NA	Nord-Trøndelag	Norway	Seeds	2013	N	1-2	
72II	Tiril	NA	Nord-Trøndelag	Norway	Seeds	2013	N	1-2	
72III	Tiril	NA	Nord-Trøndelag	Norway	Seeds	2013	N	1-2	
73IV	Tiril	NA	Nord-Trøndelag	Norway	Seeds	2013	N	1-2	
73V	Tiril	NA	Nord-Trøndelag	Norway	Seeds	2013	N	1-2	
74I	Tiril	NA	Nord-Trøndelag	Norway	Seeds	2013	N	1-2	
74II	Tiril	NA	Nord-Trøndelag	Norway	Seeds	2013	N	1-2	
74IV	Tiril	NA	Nord-Trøndelag	Norway	Seeds	2013	N	1-1	
74VI	Tiril	NA	Nord-Trøndelag	Norway	Seeds	2013	N	1-1	
75I	Tiril	NA	Nord-Trøndelag	Norway	Seeds	2013	N	1-1	
75II	Tiril	NA	Nord-Trøndelag	Norway	Seeds	2013	N	1-1	
77I	Tiril	NA	Buskerud	Norway	Seeds	2013	N	1-1	
77II	Tiril	NA	Buskerud	Norway	Seeds	2013	N	1-1	
77III	Tiril	NA	Buskerud	Norway	Seeds	2013	N	1-1	
77IV	Tiril	NA	Buskerud	Norway	Seeds	2013	N	1-1	
77VII	Tiril	NA	Buskerud	Norway	Seeds	2013	N	1-2	
78I	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-2	
78II	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-1	
78III	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-1	
78IV	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-2	
78V	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-2	
78VI	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-1	
78VII	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-2	
78VIII	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-2	
78IX	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-1	
78X	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-1	
79I	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-2	
79III	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-1	
79IV	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-1	
79V	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-1	
79VII	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-2	
79VIII	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-2	
79X	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-2	
79XI	Tiril	NA	Vestfold	Norway	Seeds	2013	N	1-2	
80I	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
81I	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
81II	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	NA	1-2	
82I	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
82III	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
82IV	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
82V	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-1	
82VIII	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
82IX	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
82X	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-1	
82XI	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
82XIII	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
83I	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-1	
83II	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-1	
83III	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-1	
83V	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	

83VI	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-1	
83VII	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
83X	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-1	
84I	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-1	
84II	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
84III	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
84IV	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-1	
84V	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-1	
84VI	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
84VII	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-1	
84VIII	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-1	
84IX	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
84X	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-1	
84XI	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-2	
84XII	Tiril	NA	Sør-Trøndelag	Norway	Seeds	2013	N	1-1	
85II	Helium	NA	Telemark	Norway	Seeds	2013	N	1-2	
85V	Helium	NA	Telemark	Norway	Seeds	2013	N	1-1	
86I	Helium	NA	Telemark	Norway	Seeds	2013	N	1-1	
86III	Helium	NA	Telemark	Norway	Seeds	2013	N	1-1	
88I	Helium	NA	Østfold	Norway	Seeds	2013	N	1-1	
88II	Helium	NA	Østfold	Norway	Seeds	2013	N	1-1	
88III	Helium	NA	Østfold	Norway	Seeds	2013	N	1-1	
88IV	Helium	NA	Østfold	Norway	Seeds	2013	N	1-1	
88V	Helium	NA	Østfold	Norway	Seeds	2013	N	1-1	
88VI	Helium	NA	Østfold	Norway	Seeds	2013	N	1-1	
88VII	Helium	NA	Østfold	Norway	Seeds	2013	N	1-2	
88XI	Helium	NA	Østfold	Norway	Seeds	2013	N	1-1	
89I	Helium	NA	Østfold	Norway	Seeds	2013	N	1-2	
89II	Helium	NA	Østfold	Norway	Seeds	2013	N	1-2	
89III	Helium	NA	Østfold	Norway	Seeds	2013	N	1-2	
90I	Helium	NA	Østfold	Norway	Seeds	2013	N	1-2	
90IV	Helium	NA	Østfold	Norway	Seeds	2013	N	1-2	
90VIII	Helium	NA	Østfold	Norway	Seeds	2013	N	1-1	
90IX	Helium	NA	Østfold	Norway	Seeds	2013	N	1-2	
90X	Helium	NA	Østfold	Norway	Seeds	2013	N	1-1	
91I	Helium	NA	Østfold	Norway	Seeds	2013	N	1-2	
91II	Helium	NA	Østfold	Norway	Seeds	2013	N	1-1	
91III	Helium	NA	Østfold	Norway	Seeds	2013	N	1-2	
91V	Helium	NA	Østfold	Norway	Seeds	2013	N	1-2	
91VI	Helium	NA	Østfold	Norway	Seeds	2013	N	1-2	
91VII	Helium	NA	Østfold	Norway	Seeds	2013	N	1-1	
91VIII	Helium	NA	Østfold	Norway	Seeds	2013	N	1-2	
91XII	Helium	NA	Østfold	Norway	Seeds	2013	N	1-1	
91XIII	Helium	NA	Østfold	Norway	Seeds	2013	N	1-1	
92I	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
92II	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
92III	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
92IV	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
92VII	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
92VIII	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
92X	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
92XI	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
93I	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
93IV	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	

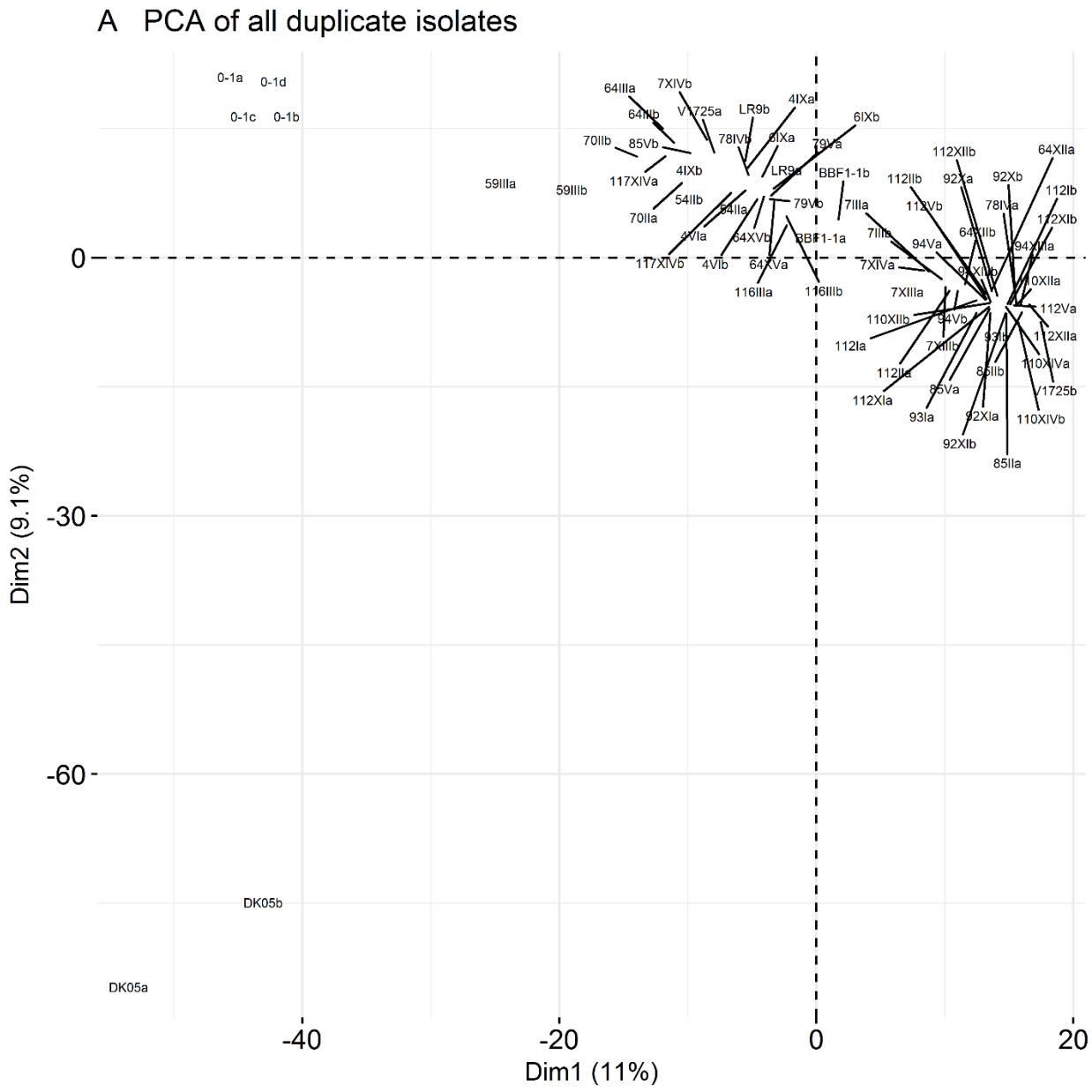
94I	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
94III	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
94IV	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
94V	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
94VIII	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
94XI	Helium	NA	Akershus	Norway	Seeds	2013	NA	1-2	
94XII	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
94XIII	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
95I	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
95II	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
95IV	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
95VI	Helium	NA	Akershus	Norway	Seeds	2013	NA	1-1	
95VIII	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
95IX	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
95X	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
95XI	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
95XII	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
95XIII	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
95XIV	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
96I	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
96III	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
96V	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
96VI	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
96VIII	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
96IX	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
96X	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
96XI	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
96XII	Helium	NA	Akershus	Norway	Seeds	2013	N	1-1	
96XIII	Helium	NA	Akershus	Norway	Seeds	2013	N	1-2	
100I	Marigold	NA	Hedmark	Norway	Seeds	2013	N	1-1	
101I	Marigold	NA	Hedmark	Norway	Seeds	2013	N	1-2	
105I	Helium	NA	Hedmark	Norway	Seeds	2013	N	1-1	
105II	Helium	NA	Hedmark	Norway	Seeds	2013	N	1-2	
106I	Helium	NA	Hedmark	Norway	Seeds	2013	N	1-1	
106II	Helium	NA	Hedmark	Norway	Seeds	2013	N	1-2	
106III	Helium	NA	Hedmark	Norway	Seeds	2013	N	1-1	
106VI	Helium	NA	Hedmark	Norway	Seeds	2013	N	1-1	
108I	Helium	NA	Hedmark	Norway	Seeds	2013	N	1-2	
108II	Helium	NA	Hedmark	Norway	Seeds	2013	N	1-1	
108III	Helium	NA	Hedmark	Norway	Seeds	2013	N	1-1	
109I	Helium	NA	Hedmark	Norway	Seeds	2013	N	1-1	
110IV	Helium	NA	Buskerud	Norway	Seeds	2013	N	1-1	
110VIII	Helium	NA	Buskerud	Norway	Seeds	2013	N	1-1	
110X	Helium	NA	Buskerud	Norway	Seeds	2013	N	1-1	
110XII	Helium	NA	Buskerud	Norway	Seeds	2013	N	1-1	
110XIII	Helium	NA	Buskerud	Norway	Seeds	2013	N	1-1	
110XIV	Helium	NA	Buskerud	Norway	Seeds	2013	N	1-2	
110XV	Helium	NA	Buskerud	Norway	Seeds	2013	N	1-2	
112I	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-1	
112II	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-1	
112III	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-1	
112IV	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-2	
112V	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-1	
112VI	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-2	

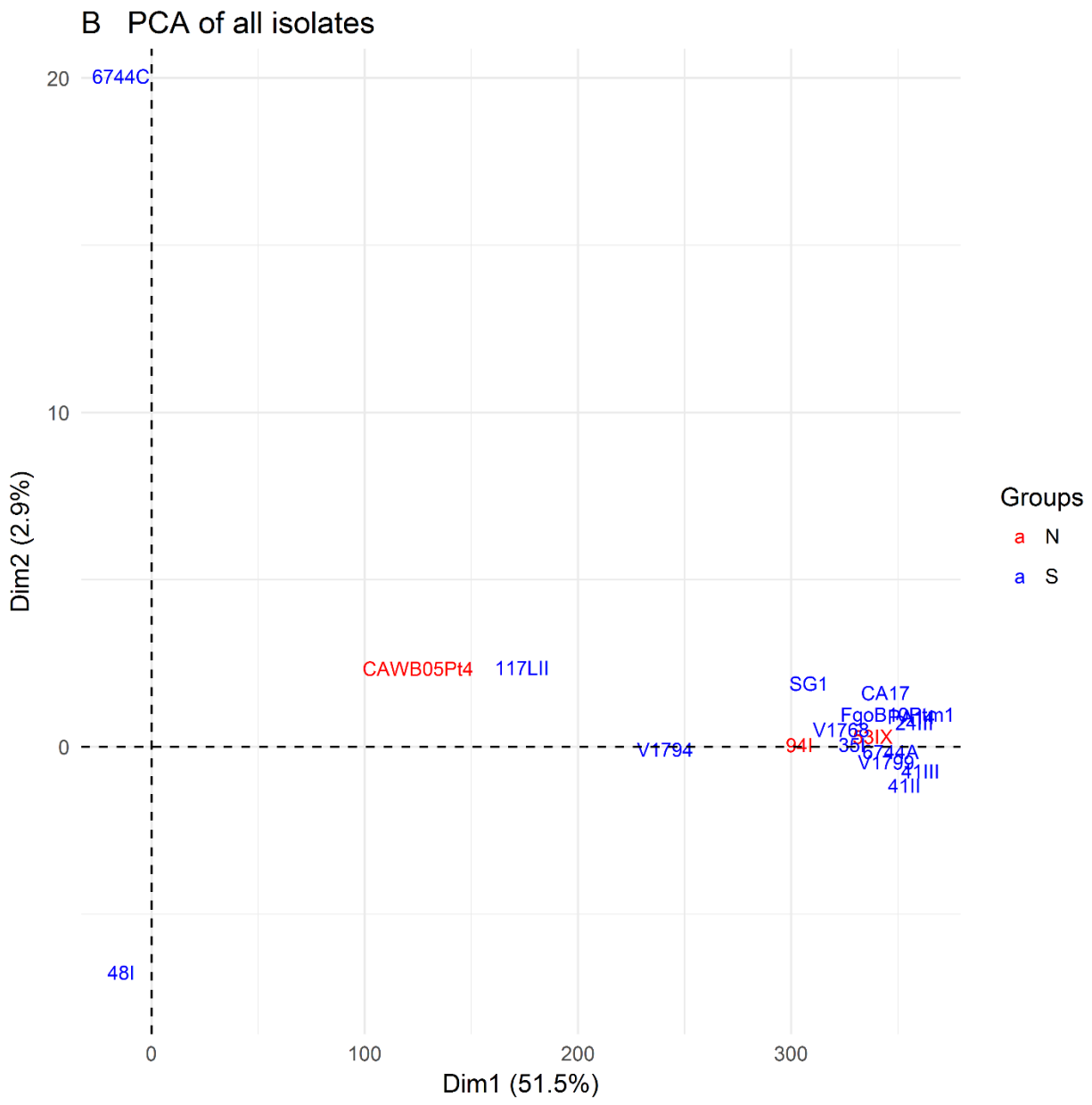
112VII	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-1	
112X	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-2	
112XI	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-2	
112XII	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-1	
113I	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-2	
113II	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-2	
113III	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-1	
113VI	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-1	
113VIII	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-2	
113IX	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-2	
113X	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-1	
113XI	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-1	
113XIII	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-2	
113XIV	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-2	
113XV	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-2	
114I	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-2	
114II	Helium	NA	Vestfold	Norway	Seeds	2013	NA	1-2	
115I	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-2	
115II	Helium	NA	Vestfold	Norway	Seeds	2013	N	1-1	
116I	Tyra	Eidum (Stjørdal)	Nord-Trøndelag	Norway	Leaves	2014	N	1-2	
116II	Tyra	Eidum (Stjørdal)	Nord-Trøndelag	Norway	Leaves	2014	N	1-1	
116III	Tyra	Eidum (Stjørdal)	Nord-Trøndelag	Norway	Leaves	2014	N	1-1	
116IV	Tyra	Eidum (Stjørdal)	Nord-Trøndelag	Norway	Leaves	2014	N	1-1	
116VI	Tyra	Eidum (Stjørdal)	Nord-Trøndelag	Norway	Leaves	2014	N	1-2	
116VII	Tyra	Eidum (Stjørdal)	Nord-Trøndelag	Norway	Leaves	2014	N	1-1	
116VIII	Tyra	Eidum (Stjørdal)	Nord-Trøndelag	Norway	Leaves	2014	N	1-2	
117I	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117II	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117IV	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-1	
117V	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-1	
117VI	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117VIII	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117IX	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-1	
117X	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-1	
117XI	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-1	
117XIV	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XV	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-1	
117XVI	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-1	
117XVII	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XVIII	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XIX	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XX	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XXI	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XXII	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XXIII	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XXV	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-1	
117XXVI	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XXVIII	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XXIX	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XXX	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XXXI	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-1	
117XXXII	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XXXIII	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XXXIV	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	

117XXXVII	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XXXIX	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-1	
117XL	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XLII	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XLIII	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XLIV	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-2	
117XLVIII	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	N	1-1	
117LII	NA	Vollebekk (Ås)	Akershus	Norway	Leaves	1995	S	1-2	
3034B	NA	Ørje	Østfold	Norway	Seeds	2012	N	1-1	
5050B	NA	Hemnes	Nordland	Norway	Seeds	2012	N	1-2	
6744A	NA	Fenstad	Akershus	Norway	Seeds	2012	S	1-2	
6744C	NA	Fenstad	Akershus	Norway	Seeds	2012	S	1-1	
6949A	NA	NA	NA	Norway	NA	NA	N	1-2	
6949B	NA	Rakkestad	Østfold	Norway	Seeds	2012	N	1-1	
BBF1-1	Brage	Meldal	Sør-Trøndelag	Norway	Leaves	2013	N	1-2	
BBF1-2	Brage	Meldal	Sør-Trøndelag	Norway	Leaves	2013	N	1-2	
BBF2-2	Tiril	Meldal	Sør-Trøndelag	Norway	Leaves	2013	N	1-2	
LR3	NA	Værnes	Nord-Trøndelag	Norway	Leaves	2011	N	1-1	
LR9	NA	Værnes	Nord-Trøndelag	Norway	Leaves	2012	N	1-2	
U1-78I	NA	NA	NA	Norway	Seeds	2013	N	1-1	
U2-75I	NA	NA	NA	Norway	Seeds	2013	N	1-1	
U3-86VII	NA	NA	NA	Norway	Seeds	2013	N	1-1	
14-DT-DK-01-01	NA	NA	NA	Denmark	NA	NA	N	1-2	L. Nistrup Jørgensen
14-DT-DK-01-02	NA	NA	NA	Denmark	NA	NA	N	1-2	L. Nistrup Jørgensen
14-DT-DK-01-03	NA	NA	NA	Denmark	NA	NA	N	1-2	L. Nistrup Jørgensen
14-DT-DK-01-04	NA	NA	NA	Denmark	NA	NA	N	1-1	L. Nistrup Jørgensen
14-DT-DK-01-05	NA	NA	NA	Denmark	NA	NA	N	1-2	L. Nistrup Jørgensen
14-DT-DK-01-06	NA	NA	NA	Denmark	NA	NA	N	1-1	L. Nistrup Jørgensen
14-DT-DK-01-07	NA	NA	NA	Denmark	NA	NA	N	1-2	L. Nistrup Jørgensen
14-DT-DK-01-08	NA	NA	NA	Denmark	NA	NA	N	1-2	L. Nistrup Jørgensen
14-DT-DK-01-09	NA	NA	NA	Denmark	NA	NA	N	1-2	Lise Nistrup Jørgensen
14-DT-DK-01-10	NA	NA	NA	Denmark	NA	NA	N	1-2	L. Nistrup Jørgensen
BB25	NA	NA	NA	Denmark	NA	NA	N	1-1	L. Nistrup Jørgensen
V204	Rolfi	Mietoinen	NA	Finland	NA	2004	N	1-1	M. Jalli
V228	Rolfi	Forssa	NA	Finland	NA	2004	N	1-2	M. Jalli
V238	Rolfi	Tammela	NA	Finland	NA	2004	N	1-1	M. Jalli
V278	Arve	Ruukki	NA	Finland	NA	2000	N	1-2	M. Jalli
V1707	Annbell	Jokionen	NA	Finland	NA	2010	N	1-1	M. Jalli
V1725	Pilvi	Ylistaro	NA	Finland	NA	2010	N	1-1	M. Jalli
V1732	Pilvi	Ylistaro	NA	Finland	NA	2010	N	1-2	M. Jalli
V1737	Pilvi	Ylistaro	NA	Finland	NA	2010	N	1-1	M. Jalli
V1768	Annbell	Jokionen	NA	Finland	NA	2011	S	1-2	M. Jalli
V1794	Annbell	Jokionen	NA	Finland	NA	2011	S	1-1	M. Jalli

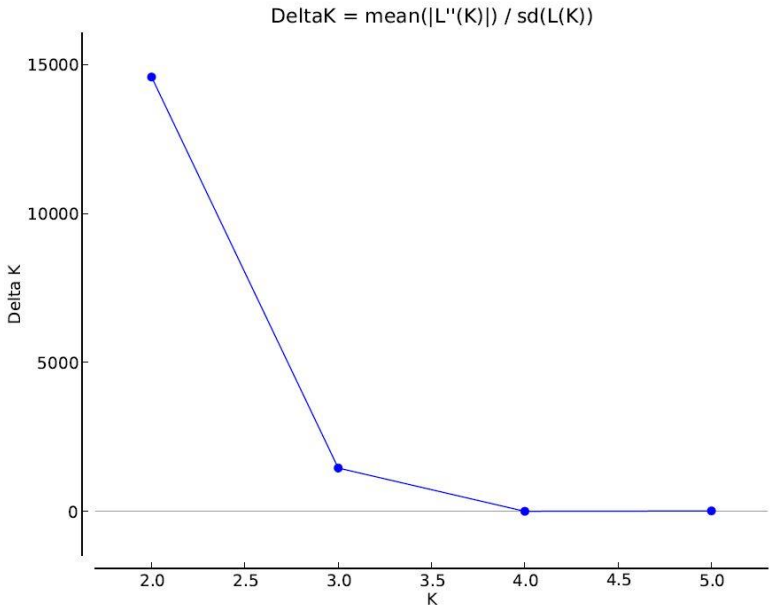
V1799	Annbell	Jokionen	NA	Finland	NA	2011	S	1-1	M. Jalli
V1849	NA	Muhos	NA	Finland	NA	2012	N	1-2	M. Jalli
V1851	NA	Laihia	NA	Finland	NA	2012	N	1-2	M. Jalli
V1857	NA	Sotkamo	NA	Finland	NA	2012	N	1-1	M. Jalli
V1866	NA	Jokionen	NA	Finland	NA	2012	N	1-1	M. Jalli
V1867	NA	Jokionen	NA	Finland	NA	2012	N	1-1	M. Jalli
V1868	NA	Jokionen	NA	Finland	NA	2012	N	1-2	M. Jalli
V1907	NA	Inkoo	NA	Finland	NA	2014	N	1-1	M. Jalli
51II	Tjaldur	Korpa	NA	Iceland	Leaves	2013	N	1-1	
51III	Tjaldur	Korpa	NA	Iceland	Leaves	2013	N	1-1	
52I	Tjaldur	Möðruvellir	NA	Iceland	Leaves	2013	N	1-1	
52VIII	Tjaldur	Möðruvellir	NA	Iceland	Leaves	2013	N	1-1	
52X	Tjaldur	Möðruvellir	NA	Iceland	Leaves	2013	N	1-2	
52XI	Tjaldur	Möðruvellir	NA	Iceland	Leaves	2013	N	1-2	
52XII	Tjaldur	Möðruvellir	NA	Iceland	Leaves	2013	N	1-2	
53II	Tiril	Möðruvellir	NA	Iceland	Leaves	2013	N	1-2	
53IV	Tiril	Möðruvellir	NA	Iceland	Leaves	2013	N	1-1	
53V	Tiril	Möðruvellir	NA	Iceland	Leaves	2013	N	1-2	
53VI	Tiril	Möðruvellir	NA	Iceland	Leaves	2013	N	1-2	
53IX	Tiril	Möðruvellir	NA	Iceland	Leaves	2013	N	1-1	
54I	Wolmari	Vindheimar	NA	Iceland	Leaves	2013	N	1-2	
54II	Wolmari	Vindheimar	NA	Iceland	Leaves	2013	N	1-2	
54III	Wolmari	Vindheimar	NA	Iceland	Leaves	2013	N	1-2	
54IV	Wolmari	Vindheimar	NA	Iceland	Leaves	2013	N	1-1	
54V	Wolmari	Vindheimar	NA	Iceland	Leaves	2013	N	1-2	
54VII	Wolmari	Vindheimar	NA	Iceland	Leaves	2013	N	1-2	
54VIII	Wolmari	Vindheimar	NA	Iceland	Leaves	2013	N	1-1	
54IX	Wolmari	Vindheimar	NA	Iceland	Leaves	2013	N	1-2	
55IX	Tjaldur	Vindheimar	NA	Iceland	Leaves	2013	N	1-2	
55XI	Tjaldur	Vindheimar	NA	Iceland	Leaves	2013	N	1-2	
55XII	Tjaldur	Vindheimar	NA	Iceland	Leaves	2013	N	1-2	
0-1	NA	NA	Ontario	Canada	NA	NA	N	1-2	T. Friesen
15A	NA	California	California	USA	NA	NA	N	1-1	B. Stef-fenson
6A	NA	California	California	USA	NA	NA	N	1-2	B. Stef-fenson
CA17	NA	Montana	Montana	USA	NA	NA	S	1-2	T. Friesen
CAWB05 Pt-4	NA	California	California	USA	NA	NA	N	1-2	T. Friesen
FgoB10 Ptm-1	NA	Fargo	North Dakota	USA	NA	NA	S	1-1	T. Friesen
FgoH04 Pt-21	NA	Fargo	North Dakota	USA	NA	NA	N	1-2	T. Friesen
PA14	NA	Montana	Montana	USA	NA	NA	S	1-1	T. Friesen
SG1	NA	NA	NA	Australia	NA	NA	S	1-2	R. Oliver

S2 A: PCA of all isolates that were sequenced twice. B: PCA of all isolates including the isolate names, color-coded by form. For better visualization, all *Ptt* isolates were omitted, except for those that clustered with *Ptm*. The remaining *Ptt* isolates would localize along the y axis (cv. Fig. 1A)

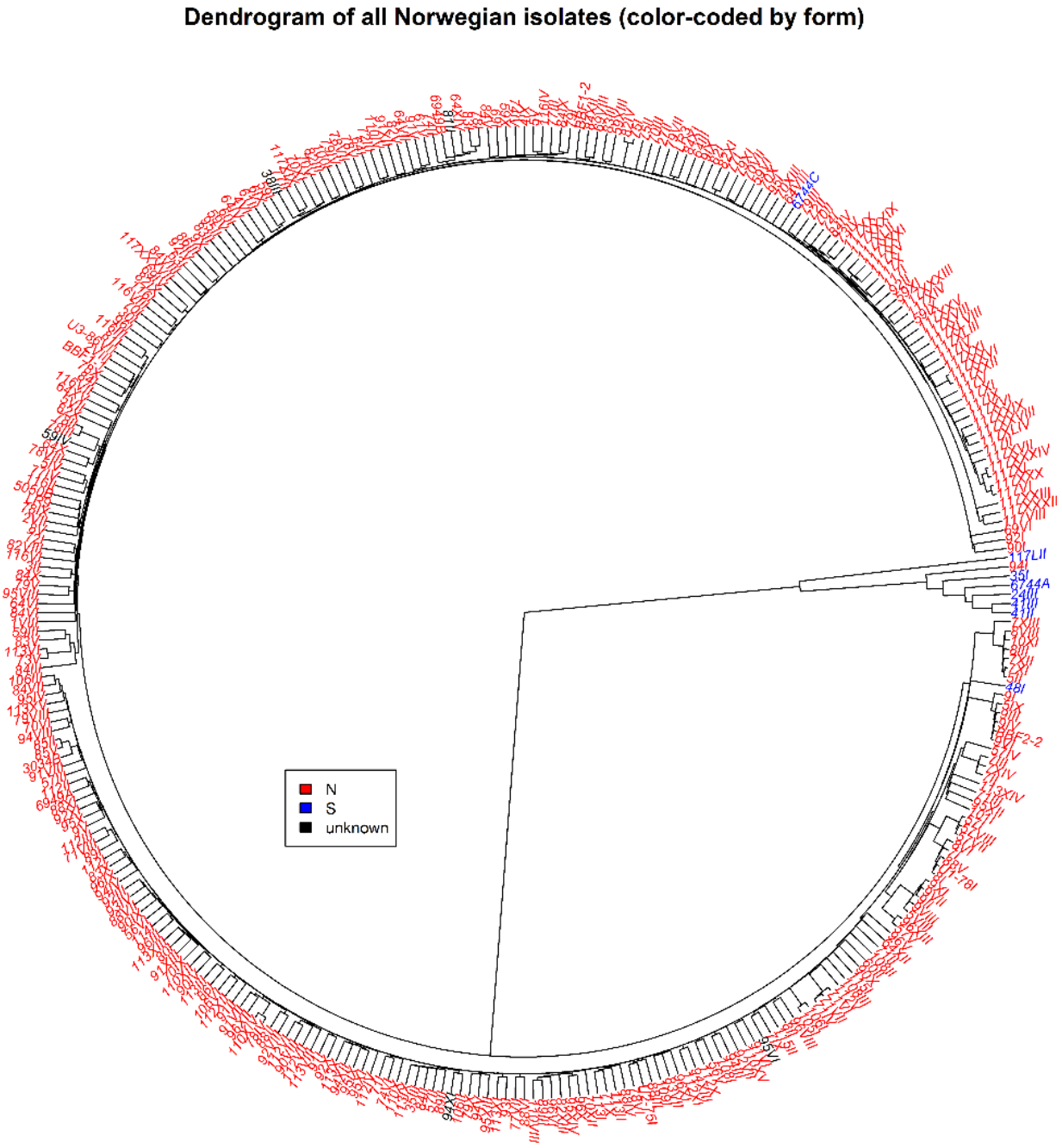



B PCA of all isolates
20
10
0
Dim2 (2.9%)
6744C
CAWB05Pt4
117LII
SG1
CA17
V1794
94I
41II
48I
0
100
200
300
Dim1 (51.5%)
Groups
a N
a S

S3 Estimated population structure of the AM panel



S4 Dendrogram of all Norwegian isolates



S5 Hedrick's G_{ST} for different subpopulations

Sub-pop.	2	5	7	8	10	11	12	13	14	15	18	35	36	38	44	46	49	50	51	56	57	58	60	62	63	64	65
2																											
5	0.08																										
7	0.17	0.35																									
8	0.03	0.14	0.10																								
10	0.05	0.16	0.11	0.03																							
11	0.05	0.05	0.18	0.08	0.09																						
12	0.02	0.06	0.21	0.05	0.07	0.04																					
13	0.04	0.05	0.28	0.09	0.11	0.04	0.03																				
14	0.04	0.07	0.28	0.08	0.12	0.04	0.03	0.03																			
15	0.07	0.17	0.14	0.04	0.05	0.08	0.07	0.12	0.12																		
18	0.10	0.24	0.06	0.04	0.05	0.13	0.12	0.18	0.17	0.07																	
35	0.08	0.13	0.27	0.11	0.12	0.08	0.08	0.09	0.10	0.13	0.17																
36	0.08	0.12	0.28	0.11	0.11	0.07	0.08	0.09	0.10	0.13	0.18	0.10															
38	0.12	0.14	0.34	0.14	0.15	0.09	0.11	0.11	0.12	0.18	0.22	0.12	0.05														
44	0.05	0.12	0.20	0.06	0.05	0.07	0.05	0.07	0.08	0.07	0.11	0.04	0.05	0.07													
46	0.07	0.17	0.18	0.05	0.04	0.09	0.08	0.11	0.11	0.06	0.09	0.12	0.11	0.15	0.05												
49	0.05	0.15	0.10	0.02	0.03	0.09	0.06	0.10	0.10	0.04	0.04	0.11	0.11	0.15	0.05	0.05											
50	0.04	0.13	0.13	0.03	0.04	0.07	0.05	0.08	0.08	0.05	0.06	0.10	0.10	0.14	0.06	0.06	0.04										
51	0.03	0.12	0.12	0.02	0.03	0.07	0.04	0.08	0.07	0.03	0.05	0.09	0.09	0.14	0.04	0.04	0.02	0.03									
56	0.04	0.14	0.14	0.03	0.03	0.08	0.05	0.09	0.08	0.05	0.07	0.10	0.10	0.14	0.05	0.05	0.03	0.03	0.02								
57	0.06	0.06	0.35	0.13	0.16	0.04	0.05	0.03	0.05	0.17	0.24	0.13	0.11	0.14	0.12	0.17	0.15	0.12	0.12	0.13							
58	0.04	0.06	0.28	0.09	0.12	0.04	0.03	0.02	0.03	0.13	0.18	0.10	0.09	0.12	0.08	0.12	0.11	0.09	0.08	0.09	0.03						
60	0.04	0.15	0.11	0.02	0.03	0.08	0.05	0.09	0.09	0.05	0.05	0.10	0.11	0.15	0.05	0.05	0.03	0.04	0.02	0.03	0.14	0.10					
62	0.10	0.12	0.34	0.14	0.16	0.07	0.09	0.08	0.10	0.18	0.23	0.11	0.08	0.10	0.09	0.16	0.15	0.14	0.13	0.13	0.11	0.09	0.13				
63	0.06	0.07	0.34	0.13	0.16	0.04	0.04	0.03	0.05	0.17	0.23	0.11	0.09	0.13	0.11	0.16	0.14	0.12	0.12	0.12	0.03	0.03	0.13	0.09			
64	0.09	0.08	0.39	0.15	0.19	0.03	0.07	0.06	0.07	0.21	0.26	0.16	0.15	0.18	0.14	0.19	0.17	0.14	0.14	0.15	0.06	0.05	0.16	0.16	0.08		
65	0.04	0.09	0.26	0.08	0.10	0.04	0.03	0.04	0.05	0.11	0.17	0.11	0.10	0.14	0.08	0.11	0.09	0.07	0.07	0.08	0.06	0.05	0.08	0.12	0.06	0.11	
66	0.03	0.06	0.25	0.07	0.10	0.04	0.02	0.03	0.03	0.11	0.15	0.10	0.09	0.13	0.07	0.11	0.09	0.06	0.06	0.08	0.05	0.03	0.08	0.10	0.05	0.08	0.04

Paper II

Paper II

Identification of quantitative trait loci associated with resistance to net form net blotch in a collection of Nordic barley germplasm

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Abstract

Net blotch, caused by the necrotrophic fungus *Pyrenophora teres*, is one of the major diseases in barley in Norway causing quantitative and qualitative yield losses. Resistance in Norwegian cultivars and germplasm is generally insufficient and resistance sources have not been extensively explored yet. In this study we mapped quantitative trait loci (QTL) associated with resistance to net blotch in Nordic germplasm. We evaluated a collection of 209 mostly Nordic spring barley lines for reactions to net form net blotch (NFNB; *Pyrenophora teres* f. *teres)* in inoculations with three single conidia isolates at the seedling stage and in inoculated field trials at the adult stage in four years. Using 5669 SNP markers genotyped with the Illumina iSelect 9k Barley SNP Chip and a mixed linear model accounting for population structure and kinship, we found a total of 35 significant marker-trait associations for net blotch resistance, corresponding to 13 QTL, on all chromosomes. Out of these QTL, seven conferred resistance only in adult plants and four were only detectable in seedlings. Two QTL on chromosomes 3H and 6H were significant during both seedling inoculations and adult stage field trials. These are promising candidates for breeding programs using marker-assisted selection strategies. The results elucidate the genetic background of NFNB resistance in Nordic germplasm and suggest that NB resistance is conferred by a number of genes each with small to moderate effects, making it necessary to pyramid these genes in order to achieve sufficient levels of resistance.

Introduction

Barley (*Hordeum vulgare* L.), together with wheat (*Triticum aestivum* L.) and oat (*Avena sativa* L.), is one of the most important cereals grown in Norway. Grown on 44% of the total area used for cereal cultivation in 2015, it is the major cereal in Norway in terms of cultivated area (Statistics Norway 2016). However, most of the currently grown barley cultivars in Norway possess insufficient resistance to barley net blotch (NB), a major foliar disease caused by the fungal pathogen *Pyrenophora teres* Drechsler (anamorph *Drechslera teres* (Sacc.) Shoemaker), which causes severe grain shriveling and yield losses in barley growing regions worldwide, especially in regions with a temperate and humid climate (Mathre 1997). The fungus is further classified into two forms based on the symptoms they cause: The form *P. teres* f. *teres* produces net-like necrotic lesions (net form net blotch, NFNB), whereas lesions caused by the form *P. teres* f. *maculata* have a round or oval, spot-like shape (spot form net blotch, SFNB) (Smedegård-Petersen 1971). Both forms of the disease have been isolated from barley leaves collected in different regions in Norway, but there are indications that NFNB is the dominant form (unpublished data). Both forms are stubble-borne and have therefore been on the rise with the increase of reduced-tillage practices during the last years (McLean et al. 2009). Control strategies include tillage, crop rotation and fungicide treatment, and, most economically and environmentally sound, the use of resistant varieties. The success of resistance breeding, however, relies on thorough knowledge of resistance mechanisms and the availability of resistance genes in locally adapted germplasm.

Many studies have been conducted to detect genomic regions associated with resistance against both NFNB and SFNB, and quantitative trait loci (QTL) for resistance have been found on all chromosomes (Liu et al. 2011). The majority of these studies used linkage mapping in biparental mapping populations. This method is currently the most common tool to map causative genomic regions in plants (Holland 2007). It is based on linkage and thus co-segregation between markers and traits in a cross between two parents segregating for the trait of interest. These crosses are usually genetically narrow because they rely on only two parental genotypes. Only the parental alleles can be examined, neglecting all other alleles occurring in the population from which the parents were sampled, so the QTL found in these studies may be very specific to the material

used. The linkage disequilibrium (LD) in these populations is very high due to the limited amount of recombination events, and as a result, the resolution of genetic maps generated with this method is relatively low. Association mapping (AM), as it is employed in genome-wide association studies (GWAS), on the other hand, is based on LD in large natural populations (Zhu et al. 2008). In these populations, LD usually decays faster due to a high number of ancestral recombination events that break down linkage between alleles at different loci. All these alleles can be accounted for by AM simultaneously. As GWAS can be performed in any collection of individuals of a species such as advanced elite lines, it can be directly implemented in breeding programs by exploring the existing germplasm (Begum et al. 2015).

The application of GWAS can be hampered by several factors such as population structure and extended blocks of LD. In AM panels with a high degree of population structure, false-positive marker-trait associations (MTA) may be found which are not based on physical linkage between the marker and the trait of interest but on population stratification (Wang et al. 2012). Due to its inbreeding nature, barley has a high degree of population structure. Spring and winter barley as well as two-rowed and six-rowed barley are usually distinct subpopulations due to separate breeding programs. Since AM relies on the breakdown of linkage between loci through recombination events, slow linkage decay may negatively influence the mapping resolution. In inbreeding species like barley, LD decay is usually slow (Nordborg et al. 2002), but differs greatly between elite cultivars and landraces (Caldwell et al. 2006).

Despite these challenges, GWAS is a well-established and useful method to map various important morphological and agronomic traits in barley (Matthies et al. 2014; Stracke et al. 2009; Wang et al. 2012), among them resistance to spot blotch (*Cochliobolus sativus*; Roy et al., 2010) and Fusarium head blight (Massman et al. 2011). However, so far there exist only three GWA studies on SFNB and one on NFNB resistance in barley (Burlakoti et al. 2016; Tamang et al. 2015; Wang et al. 2015) Tamang et al. (2015) mapped seedling stage resistance against four SFNB isolates from different countries in 2062 lines from the world barley core collection (USDA-ARS National Small Grains Collection). In a GWA study with an Australian elite barley breeding germplasm panel, Wang et al. (2015) analyzed seedling reactions to two SNFB isolates and adult plant reactions in a field trial inoculated with one of the isolates. Burlakoti et al. (2016) evaluated

resistance against one SFNB isolate in 376 advanced breeding lines from the US Upper Midwest breeding programs at the seedling stage. Richards et al. (2017) mapped resistance to three NFNB isolates from the USA against 1050 barley genotypes from the National Small Grains Collection at the seedling stage.

Although a few Norwegian barley cultivars have been included in different studies on resistance to NB (Robinson 2000; Robinson and Jalli 1999), this is the first comprehensive study of NB resistance sources in the Norwegian barley germplasm and the first NFNB GWA study including both seedling and adult plant reaction and focusing on resistance under field conditions. We performed GWAS on 209 mostly Nordic barley varieties, breeding lines and historic cultivars that were assessed for resistance to NFNB at the seedling stage under greenhouse conditions and at the adult stage in mist-irrigated and inoculated hillplots over four years. In addition, we mapped genetic variation associated with plant height (PH) and heading date (DH) and investigated whether these agronomic traits have an influence on NB resistance. We aimed at (1) mapping QTL in Nordic germplasm associated with resistance to NB, (2) establishing whether these QTL are stable in different years and developmental stages, (3) identifying SNP markers associated with NB resistance, and (4) evaluating whether small-scale seedling assays under controlled conditions can be a useful tool for breeders to predict field resistance.

Material and methods

Plant material

The study was based on a Nordic AM panel (Nordic Barley Panel; NBP) consisting of 209 spring barley cultivars, landraces, landrace selections and breeding lines predominantly originating from Norway, Sweden, Denmark and Finland with a few accessions derived from other countries (Online resources 1 and 2). The collection consisted of 72 two-rowed and 137 six-rowed barley lines. The cultivars most commonly grown in Norway (Brage, Edel, Fairytale, Heder, Helium, Iver, Marigold, Tiril and Tyra) were included in the panel.

Field experiments

Five *P. teres* single conidia isolates were originally used in this study. The isolates 6949B, 6744A and 6744C were isolated from barley seed collected in southeastern Norway in 2012 provided by Kimen seed laboratory in Ås, Norway. Isolate 5050B comes from barley seed collected in Northern Norway in 2012. Isolate LR9 was obtained from barley leaves collected in central Norway in 2011. The isolates 6744A and 6744C are SFNB isolates while LR9, 5050B and 6949B are NFNB, which was confirmed by a polymerase chain reaction (PCR)-based test developed by Williams et al. (2001). The isolation of single conidia isolates from infected barley material as well as the preparation of inoculum for the field trials was performed as described by Wonneberger et al. (2017).

The barley lines were sown in hillplots in an alpha lattice design with three replications at Vollebekk research farm, Ås, Norway, over four years (2013-2016). The moderately susceptible cultivar Heder was planted at the borders of the field trials to minimize border effects in the trial plots. After approximately one month, the infected straw was spread in the field trials when the plants had reached approximately Zadoks growth stage 24. In 2013, all five isolates were used to inoculate the field, while in the other years, only the isolates LR9, 5050B and 6949B were used since 6744A and 6744C were found to be spot form isolates. In addition, in 2013 a second field trial was set up which was not inoculated with infected straw. The trials were mist-irrigated daily for 10 minutes per hour from 7 to 10 pm in order to promote NB development. In 2015 and 2016 the trial was sprayed regularly with Talius (proquinazid, 40 g/ha) against powdery mildew (*Blumeria graminis* f. sp. *hordei*). Talius is not known to have any effect on NB (DuPont 2016).

Disease severity was scored as percentage of infected leaf area based on the whole hillplot at two (2013, 2014 and 2016) or three (2015) different time points. The first scoring was done when the most resistant lines had reached approximately 25% disease severity and the second and third scorings approximately one week to ten days later when they had reached up to 60% and 80%, respectively. Scoring at early time points of disease development was necessary because later in the season accurate scoring would become more difficult due to lodging, maturation and

infection with competing diseases such as powdery mildew or leaf rust (*Puccinia hordei*). DH were recorded in all four years and PH in 2014-2016.

Greenhouse experiments

Greenhouse experiments were performed as described by Wonneberger et al. (2017). The phenotypic data collected in this study is available in Online resource 3.

Statistical analysis of phenotypic data

The statistical analysis (analysis of variance, linear regression and calculation of Pearson coefficients) was performed as described by Wonneberger et al. (2017).

DNA extraction and SNP genotyping

Genomic DNA was extracted from young leaves of all individuals using the DNeasy Plant DNA Extraction Kit (Qiagen). The population was genotyped for 7864 markers on the Illumina iSelect 9k Barley SNP Chip (Illumina) at Trait Genetics GmbH (Gatersleben, Germany). Marker positions were taken from the barley consensus map published by Muñoz-Amatriaín et al. (2014), and SNP markers that were not mapped on the consensus map were assigned to an artificial eighth chromosome with an arbitrary distance of 0.1 centimorgans (cM) between adjacent markers. Monomorphic markers and markers with more than 10% missing data were removed. The sites filter function in the software Tassel v. 5.2.24 (Bradbury et al. 2007) was used to filter out markers with a minor allele frequency (MAF) < 0.05. The remaining 5669 markers (4149 mapped and 1520 unmapped) were used for association studies. The map positions of the mapped markers are available as supplementary information (Online resource 4). For markers that were not mapped on the consensus map, the POPseq position was used, if available (Mascher et al. 2013).

Population structure and Q-matrix

Population structure of the barley mapping panel was analyzed by a Bayesian cluster analysis in STRUCTURE v. 2.3.4 (Pritchard et al. 2000) based on the genotypic data of a subset of 319 markers with a spacing of approximately 3 cM to reduce computing time. The number of subpopulations (k) in the panel was inferred using an admixture model with correlated allele frequencies and a burn-in length of 25000 and 50000 Markov Chain Monte Carlo (MCMC) repetitions. Five independent analyses were run for each k between 1 and 15. The estimated likelihood values [LnP(D)] were imported into STRUCTURE HARVESTER (Earl and vonHoldt 2012) in order to infer the best k based on the Δk approach by Evanno et al. (2005). For this optimal k value, the population structure matrix (Q-matrix) containing membership coefficients for each individual was exported from STRUCTURE to be used for AM in Tassel.

As an alternative approach, a principal component analysis (PCA) was conducted in Tassel to account for population structure. The first 3 principal components (PCs) explained 40% of the cumulative variance of the markers and were included in the PCA.

LD analysis and LD decay

Analysis of intra-chromosomal LD was performed by pairwise comparison of all 4149 mapped markers in the software Haploview 4.2 (Barrett et al. 2005) using the following parameters: Hardy-Weinberg p-value cutoff: 0; Minimum individuals genotyped: 10%; MAF: 0.05. LD was calculated as the squared allele frequency correlation r^2 between marker pairs and the LD decay was fitted using a formula by Hill and Weir (1988). LD was considered to be significant at a p-value < 0.001, other r^2 values were not considered further. These r^2 values were plotted against the corresponding genetic distance between markers using R (R Core Team 2016). The average genome-wide LD decay was visualized by plotting all significant intra-chromosomal r^2 values of all chromosomes against genetic distance. A critical r^2 value beyond which LD is assumed to be due to genetic linkage was set to 0.1 in accordance with Richards et al. (2017) and Tamang et al. (2015).

Association analysis

AM was performed in Tassel using the following formula: Y=Xa+Qb+Kv+e where Y is a vector for the phenotypic values, X is a matrix of the genotypic values of the marker, a is the vector of fixed effects of the marker, Q is the population structure and can either be the Q-matrix or the PCs from the PCA, b is a vector of fixed effects of the population structure, K is the number of subpopulations, v is a vector of random effects due to the relatedness of the individuals, and e is a vector of residual effects. Six different statistical models were tested on NB scores from three years to detect significant MTAs including both GLM (General Linear Model) and MLM (Mixed Linear Model) approaches: 1) Naïve GLM: GLM without correction for population structure; 2) GLM + Q: GLM with Q-matrix to account for population structure; 3) GLM + PCA: GLM with PCA to account for population structure; 4) MLM + K + Q: MLM with both K- and Q-matrix, 5) MLM + K + PCA: MLM with both K-matrix and PCA; 6) MLM + K: MLM with K-matrix only.

Several approaches were considered to determine the significance threshold for the MTA p-values. A common method to correct for multiple testing is the Bonferroni correction, where the significance threshold is divided by the total number of tests, i.e. in this case the total number of markers. At a significance level of 0.05 for a single test and 5669 markers, this threshold would be at $8.8*10^{-6}$, which most markers would fail to achieve. The Bonferroni correction is considered a very stringent method and its applicability in GWAS is disputed (Gupta et al. 2014). Similarly, the false discovery rate was calculated for each trait but was also found to be quite stringent. As we chose a rather stringent model to account for population structure, we believe that most of the false positives are already accounted for and therefore chose a more liberal approach to find the p-value threshold. Chan et al. (2010) suggested the bottom 0.1 percentile of the p-values of each trait to be significant, which gave p-values between 0.008 and $2.1*10^{-8}$ in our traits (Online resource 5). Significant markers within a distance of 13 cM were defined to be a single QTL.

For adult plant stage trials, QTL for average disease scores per year are shown and discussed. QTL associated with single scorings are reported in Online resources 6 and 7. Additionally, we mapped DH and PH in every year. In seedlings, resistance to the three isolates was mapped separately.

We used the trait spike row number (two-rowed or six-rowed) as a validation for the accuracy of our mapping approach.

QTL nomenclature

We followed the QTL nomenclature established by Grewal et al. (2008), but we did not differentiate between seedling stage and adult stage QTL. A suffix was added to distinguish different QTL on the same chromosome, and the prefix "NBP_" was added to designate that the QTL was found in the Nordic Barley Panel.

Results

Disease assessment in the greenhouse

In seedlings, Tekauz scores ranged from 1.2 to 9.5, and all three isolates caused a similar range of disease severities (Fig. 1, Online resource 8). Plants inoculated with LR9 had the highest average scores (7.1) followed by plants inoculated with 5050B (5.9). Isolates LR9 and 5050B started to cause small point lesions as soon as 1-2 days past inoculation (dpi) and produced fully developed typical net-like symptoms at approximately 4-5 dpi. Point lesions from 6949B developed between 2-4 dpi and at 5 dpi, net-like lesions were usually much smaller and less pronounced than on plants inoculated with the other two isolates. To account for this, 6949B-inoculated trials were scored one day later than the other experiments. Among the current cultivars on the Norwegian market, Fairytale and Helium were the most resistant ones in inoculations with each of the isolates (Online resource 3). When inoculated with LR9, Iver was among the 10 most susceptible lines. Tiril and Edel were the most susceptible cultivars when inoculated with 5050B while Tyra was the most susceptible to 6949B of all current cultivars.

Phenotypic assessment in the field

Especially in 2013 and 2014, field disease scorings showed a right-skewed distribution due to the scoring taking place at early time points (Fig. 2). Early scoring was necessary to avoid inaccuracies due to confusion of symptoms with competing diseases and natural senescence at later time points. Mean scores ranged from 17% in 2013 to 32% diseased leaf area in 2014 (Online resource 8). The disease severity scores of the most resistant lines in each year ranged from 6% to 15%, and in the most susceptible lines between 45% and 69% of the leaf area showed symptoms. The disease scores of the current cultivars on the Norwegian market are shown in Table 1. In 2013, 2014 and 2016, none of the current cultivars was among the most resistant 70 lines. A number of lines from the panel showed good resistance to NB in several years, and some of them were also resistant against one or several isolates at the seedling stage. Among the 25 most resistant lines in field trials we found three breeding lines and Seger in all four years and Audrey, Chevron, Elmeri, Iron, KWS Olof, Malz, Oppdal and Seijo 17 in three years. Out of these lines, Seijo 17 and Chevron ranged among the 25 most resistant lines in seedling inoculation with three and two isolates, respectively, while Seger and KWS Olof showed good resistance against one isolate (Online Resource 3).

In addition to NB resistance, the population also segregated for DH and PH (Online resource 9). The average time to heading was 51 days in 2013, 46 days in 2014, 69 days in 2015 and 49 days in 2016 (Online resource 8). PH differed greatly between the years as well. In 2014, the average height was 72 cm and in 2015 the plants were 20 cm taller on average (92 cm), while the average PH in 2016 was 63 cm.

Correlation between seedling and field evaluations

We found significant correlations between disease evaluations in seedlings and adult resistance in three out of four years ($p<0.01$, Table 2), and also the un-inoculated trial. The correlations between resistance in seedlings inoculated with LR9 and 5050B and adult plants in 2014-2016 ranged from 0.23 to 0.44 and was highly significant ($p<0.001$). The correlation between 6949B-inoculated seedlings and adult plants was lower (0.04-0.19) and only significant in 2014 ($p<0.01$).

There was a low but significant correlation between the un-inoculated trial scores and seedling inoculations with 5050B and 6949B. The symptoms caused by LR9 and 5050B correlate more with each other (0.7) than with 6949B (0.48 and 0.45, respectively), which is in agreement with the observation in seedling tests that 6949B produced different symptoms than the other isolates.

The average disease severity scores in adult plants in 2014 to 2016 were quite well correlated (0.63-0.76, $p<0.001$) while the scorings in 2013, albeit still significantly correlated ($p<0.01$), appeared to be less similar to the other years. The disease scorings in the un-inoculated field in 2013 show a slightly higher correlation with 2014 and 2015 scorings and a slightly lower correlation with 2016 than the inoculated 2013 trials. We did not find a consistent correlation between disease severity and the potential covariates DH and PH. For DH, there was a significant negative association to disease severity in 2013, 2015 and 2016 (-0.18, -0.31 and -0.17, respectively) but no correlation in 2014. The correlation between disease severity and PH was highly significant and negative in 2014 (-0.26) but insignificant in 2015 (positive) and 2016 (negative).

Heritability for NB resistance across years was 70% (Table 3). The heritability for DH and PH was 88% and 85%, respectively. The heritability within years for NB disease response ranged from 0.80 in 2013 to 0.96 in 2016 in adult plants and from 0.93 to 0.96 in seedling inoculations (Table 2).

Genotyping

Out of the 7864 SNP markers the panel was genotyped for, 5200 markers were mapped on the consensus map by Muñoz-Amatriaín et al. (2014). Among these, 239 markers had more than 10% missing data, 305 did not segregate in the panel and another 507 had a MAF<0.05, resulting in 4149 mapped markers passing the quality checks (Online resource 10). These markers spanned 1108.7 cM over all seven chromosomes (Online resource 11). The number of markers per chromosome varied from 402 markers on 1H to 847 markers on 5H. The average distance between markers was 0.27 cM. Chromosome 5H had the highest marker density (0.22 cM between markers) while chromosome 1H had a marker every 0.36 cM. Of the 2664 unmapped

markers, 790 had more than 10% missing data, 139 were monomorphic and another 215 had a MAF>0.05. The remaining 1520 markers were assigned to an artificial eighth chromosome and spaced 0.1 cM apart.

LD and population structure

Chromosomes 4H and 6H showed a rapid LD decay (7.0 and 9.3 cM, respectively), while 2H exhibited the slowest decay (55.9 cM) (data not shown). Genome-wide, the LD decay threshold was reached at 13.0 cM (Online resource 12).

The most likely number of subpopulations was k=2 (Online resource 13) as determined in STRUCTURE, with 137 lines clustered into group 1 and 72 lines assigned to group 2, which divides the panel into six-rowed and two-rowed barleys (Fig. 3, Online resource 14). Two six-rowed lines were assigned to group 2, and one two-rowed line was assigned to group 1. The ΔK method alternatively suggested three and ten subpopulations within the AM panel. At k=3, the 6-rowed barleys were split into 2 groups (n=38 and 90, respectively). The first subgroup of the six-rowed lines mainly consisted of old Norwegian cultivars, landraces and varieties developed in a breeding program in central Norway (Trøndelag) before 1990, in addition to a few old cultivars from Finland. The other subgroup was mainly composed of Norwegian breeding lines in addition to some old and new cultivars from Norway. Seven six-rowed barleys were considered mixed as their population estimation coefficient did not exceed 0.5 for any of the subgroups.
To test the fit of alternative models for AM, three PCs explaining 40% of the variation were included as cofactors in both GLM and MLM models to account for substructure in the panel. The first PCA explained 31% and the second one 6% of the variation.

Model validation

The results of the six AM models were compared in a QQ-plot to find the model with the best fit to our data (Online resource 15). Ideally, the dots representing the association p-values should follow the red identity line, indicating a good concordance between expected and observed p-

values. Although the naïve GLM model fitted the 2013 data best, all GLM models showed a substantial underestimation of p-values (an indication of false positive associations) in 2014 and 2015 and were thus not considered suitable to describe the data. The MLM models showed a good fit in 2014 and a slight overestimation of p-values in 2013 and 2015, thus decreasing the amount of spurious background associations. All MLM models performed in a similar manner regardless of whether the Q matrix or the PCs were included as cofactors or whether cofactors were omitted altogether. Similarly, the assumption of three or ten subpopulations did not alter the model fit considerably (data not shown). This suggests that population stratification does not play an important role in the AM panel, however, we decided to use the MLM + K + Q model for further AM to minimize the chance of detecting false positive associations.

In order to validate the accuracy and usefulness of our GWAS setup, we mapped the spike row number trait and identified three markers on chromosome 1H and two markers on 2H as well as one unmapped marker that were significantly associated with this trait, representing five different QTL (Fig. 4, Online resource 16). The unmapped marker had the same POPseq position as one of the markers on chromosome 1H, thus these markers represent the same QTL.

Association mapping

In total, we found 13 QTL associated with NB resistance, four of which (27%) were only found in seedlings and seven (54%) only in adult plants. Two QTL on chromosomes 3H and 6H were significant at both growth stages (Figs. 5 and 6, Tables 4 and 5). At the seedling stage, we found between two and four QTL per isolate used. Two markers were not mapped on the consensus map, but their POPseq position did not indicate that they represent additional QTL that were not detected by mapped markers.

Table 4 lists the markers that were significantly associated with NB resistance at the seedling stage. A QTL (*NBP_QRptt3-2*) at 60-62 cM on 3H was the only QTL that was highly significant during inoculations with all three isolates, explaining up to 15% of the phenotypic variation. For inoculations with 5050B and 6949B, an additional QTL at 58-59 cM on 6H (*NBP_QRptt6-1*) was significantly associated with resistance and explained up to 10% of the variation. Resistance QTL

against LR9 were also found at two loci on 4H (*NBP_QRptt4-1* and *NBP_QRptt4-2*) and one on 7H (*NBP_QRptt7-3*). Resistance against 6949B was also mapped to *NBP_QRptt2-1*.

At the adult plant stage, four QTL were found in more than one scoring (Table 5). The *NBP_QRptt3-2* QTL which was significant at the seedling stage was also found in 2013 (6%), and the POPseq position of the unmapped marker SCRI_RS_221644 significant in 2015 suggests that this marker maps to this QTL region. The *NBP_QRptt6-1* significant in different seedling inoculations was also significant at the adult stage in 2014 and 2016. At this stage, it explained 9% of the phenotypic variation. A significant MTA at 43 cM on chromosome 1H was found in 2015 and 2016 (*NBP_QRptt1-1*). In 2013, a MTA was found at 53 cM on 1H, and since the QTL interval cutoff was set to 13 cM, this MTA was also within the *NBP_QRptt1-1* range. This QTL explained up to 14% of the phenotypic variation. A QTL at 51-54 cM on chromosome 5H (*NBP_QRptt5-1*) was found in both the inoculated and uninoculated field trials in 2013 and explained up to 11%. This was the only QTL common to both inoculated and inoculated trials. Another adult stage QTL was found in chromosome 5H in 2014 (*NBPQRptt5-2*; 10%) Three QTL were unique to the uninoculated trial: *NBP_QRPtt1-2* (6%), *NBP_QRptt7-1* (10%) and *NBP_QRptt7-2* (6%).

In the inoculated field trials, six QTL for net blotch resistance were found, but none of the lines had all the alleles conferring resistance. Fig. 7 shows how the number of resistance alleles per line affects the average adult stage disease response of four years. One line, the Finnish cultivar Rolfi, had only one allele for resistance and was also the most susceptible line at the adult stage (46% diseased leaf area). The lines with two resistance alleles had on average 36% lower disease severity compared to that line, and in lines with three or four beneficial alleles, disease severity was reduced by 41% and 47%, respectively. The presence of five resistance alleles decreased disease severity by 56%. The presence of an additional resistance allele did not increase resistance significantly, but the differences in disease severity between groups differing by two or more resistance alleles were significant (Tukey Honest Significant Differences, $p<0.05$).

In general, the more resistance alleles are present in a line, the more resistant the line was at the adult stage (Fig. 7), underlining the quantitative nature of net blotch resistance. Among lines with good resistance, Vada had the resistance alleles at the *NBP_QRptt3-1*, *NBP_QRptt5-1* and

NBP_QRptt6-1 loci, while CI11577 and Birgitta additionally had the *NBP_QRptt1-1* resistance allele. Annabell also had the *NBP_QRptt5-2* allele in addition to the other four resistance alleles. Chevron, Oppdal, Olli and Lavrans had all resistance alleles at all adult plant stage QTL except for *NBP_QRptt5-2*. These lines are all available from gene banks (accession numbers are listed in Online resource 1).

In total, we found five QTL associated with PH and ten associated with DH (Tables 6 and 7, Figs. 8 and 9). The most significant QTL for PH in 2014 was found at 58.3 cM on 3H, in the same position as *NBP_QRptt3-2*, and explained 15% of the phenotypic variation. In 2015, two significant markers explaining 12% each were found on 3H and 5H in addition to a number of unmapped markers, two of which were mapped to respectively 1.6 cM and 97.4 cM on 5H on the POPseq map. Albeit not significant, a putative QTL on 1H was found in the same region as the NB resistance QTL in 2013 and 2015. In 2016, PH was influenced by the regions harboring the resistance loci *NBP_QRptt1-2*, *NBP_QRptt3-2* and *NBP_QRptt4-2*. Furthermore, there are indications for additional putative PH QTL on 2H, 4H, and 7H which were insignificant in this study. QTL associated with DH were found on chromosomes 1H, 2H, 3H, 5H and 6H. Two QTL on 1H at the *NBP_QRptt1-1* locus and at 115 cM were significant in 2013 and 2015, and in 2014-2016, respectively. A QTL at 178 cM on 2H was detectable in all years and significant in 2013 and 2016. Significant MTAs were found at 87 cM on 3H in 2015 and 2016. A QTL on 5H was significant in 2015 and 2016, and three QTL were found on 6H in 2014. For an unmapped marker which was significant in 2015 and 2016, neither a consensus map nor a POPseq map position were found.

Discussion

Phenotypic evaluation

At both seedling and adult plant stage, the 209 lines evaluated in this study showed a substantial variation in resistance to barley NB. This suggests a polygenic nature of this trait which is consistent with a number of previous studies (Liu et al. 2011; Tamang et al. 2015; Wang et al. 2015).

Field evaluations of adult plant disease reactions and greenhouse trials for seedling stage disease reactions showed that the currently grown cultivars have largely insufficient resistance to barley NB. The disease scores of the cultivars Tiril, Tyra and Iver exceeded the average disease scores during all seedling and adult stage evaluations, and Tiril was among the ten most susceptible lines in 2014-2016. Fairytale was the most resistant of all current cultivars except for 2015 and was the only cultivar that was more resistant than the average in all scorings. Brage was above average resistant in all seedling and adult stage evaluations except for 2014 and 2016.

The highly significant correlations between different years of field trials indicate a good reproducibility of the data. Especially the 2015 and 2016 scorings are well correlated (r=0.76). The correlation between adult plant and seedling resistance was not very high but still very significant in all years except for 2013, indicating that some resistance mechanisms are present at all developmental stages, while others may be stage-specific. However, we used different scales to evaluate resistance at different stages, and environmental factors such as climatic conditions and natural infection pressure also play a role in the field trials. The lack of correlation between seedling and adult plant disease reactions in 2013 might be due to the inclusion of two spot form isolates in the field trial inoculations that year. Since both seedling resistance and adult resistance were usually stronger correlated with the un-inoculated trial 2013 than with the inoculated trial in 2013, it is possible that the spot form isolates are not representative of the natural pathogen population found in the area.

Validation of association mapping panel

The spike row number trait was used to evaluate the quality of our GWAS results. A number of genes determining this trait have been mapped and are well described, among others *vrs3* on 1H and *vrs1* on 2H (Franckowiak and Lundqvist 1997; Lundqvist and Franckowiack 1997). We found markers on chromosomes 1H and 2H that were significantly associated with spike row number (Fig. 4, Online resource 16). Markers 11_21000 and SCRI_RS_170542 are located 1-2 cM apart from markers that were found to be linked to the *vrs3* locus by Pasam et al. (2012) and Muñoz-Amatriaín et al. (2014). The significant markers on 2H are not associated with the *vrs1* locus but

the marker SCRI_RS_175300 is in close vicinity to a region reported to be associated with row number by Pasam et al. (2012). Also the other two 1H markers are in close vicinity to row number QTL found by Pasam et al. (2012). Additionally, we identified several other markers on 4H and 5H that were associated with row number, although not significantly, which have been previously described by Pasam et al. (2012) and Muñoz-Amatriaín et al. (2014). This indicates that our mapping resolution was sufficient to detect common variants in the AM panel.

Association mapping

The collection of 209 mostly Nordic barley cultivars, breeding lines and landraces used in this study allowed us to exploit the historic recombination events in the Nordic barley germplasm to find genomic regions associated with phenotypic variation in resistance to barley NB in seedlings and adult plants, DH and PH. We considered markers which were located less than 13 cM apart as belonging to the same QTL and found between two and five QTL per trait. In total, we discovered 23 QTL, 13 of which were associated with NB resistance. In addition, one unmapped marker was associated with DH and one with PH. A number of NB resistance QTL were very consistent throughout different years and developmental stages. The *NBP_QRptt3-2* locus was common to all seedling inoculations as well as adult plant evaluations in 2013 and 2015. It was also detectable in 2014 and 2016, although the MTAs were not significant. In addition, we found MTAs with PH in this region, which were significant in 2014 and 2016. Similarly, the *NBP_QRptt6-1* QTL was found in 2014 and 2016 and seedling inoculations with 5050B and 6949B, while it was prominent but not significant in 2013, 2015 and in LR9 inoculations. The *NBP_QRptt1-1* locus was significant in several scorings (2013, 2015 and 2016), although the MTA that was significant in 2013 is located approximately 10 cM from the 2015 and 2016 QTL, so it remains to be elucidated how many resistance genes are located in this region. This QTL was also significant for DH in 2013 and 2015 and co-located with the *vrs3* locus determining spike row number in barley. By using the POPseq map position, the three unmapped markers SCRI_RS_221644, SCRI_RS_103515 and SCRI_RS_150517 were localized to the NBPQRptt3-2, *NBP_QRptt2-1 and NBP_QRptt7-1* QTL, respectively. They do thus not represent additional QTL.

Of the 13 disease resistance loci, at least eight have been described previously, while the others are putatively novel. Tamang et al. (2015) found a significant MTA within the *NBP_QRptt3-2* interval at 53.42 cM on 3H common to the SFNB isolates DEN 2.6 and NZKF2 from Denmark and New Zealand, respectively, and another significant MTA at 65.16 cM for DEN 2.6. This region was also significantly associated with resistance to the NFNB isolates BB06 from Denmark, NB50 from Australia and BrPteres from Brazil (Liu et al. 2015). Burlakoti et al. (2016) found that this region harbors seedling resistance to a SFNB isolate from Montana, USA, in a collection of Upper Midwestern breeding lines. The marker SCRI_RS_221644 which is likely to be located in this region was found to be associated with dominant seedling resistance against two Japanese NFNB isolates in the barley line CI5791 and against the isolates 15A, 6A, BrPteres and BB06 in the cultivar Tifang (Koladia et al. 2016). In the first GWA study on NFNB resistance, Richards et al. (2017) recently mapped resistance to the isolates 15A and LDN from California and North Dakota to this region. This suggests the presence of one or several genes at this locus conferring resistance to both NFNB and SFNB isolates from diverse origins. Additionally, several other studies have reported resistance loci on 3H, indicating that this chromosome might harbor one or more hotspots for NB resistance genes (Cakir et al. 2003; Gupta et al. 2010; König et al. 2013).

In multiple QTL and AM studies, the *NBP_QRptt6-1* region was associated with resistance to several different isolates. Tamang et al. (2015) mapped resistance to the SFNB isolates FGO, NZKF2 and DEN2.6 at 59.01-59.21 cM on 6H and also mapped the seedling and adult stage QTL *QRpt6* previously described by Grewal et al. (2008) in this region. One of the markers in this interval was also highly associated with 2014 resistance in our study. Koladia et al. (2016) mapped dominant resistance in the barley line CI5791 against nine NFNB isolates from different countries to this region. This region was additionally associated with resistance to three NFNB isolates from the USA (Richards et al. 2017). The QTL for the first sensitivity gene to NB necrotrophic effectors, *SPN1*, is in close vicinity to the *NBP_QRptt6-1* markers (Liu et al. 2015). Richards et al. (2016) recently fine-mapped the dominant susceptibility locus *Spt1* to the centromeric region of 6H, and the marker SCRI_RS_165041 which was closely linked to it is located less than 0.3 cM apart from markers that were significantly associated with resistance in 2014 and to isolate 6949B. Also this locus is hypothesized to be an effector sensitivity gene. This may indicate that isolate 6949B

possesses the same necrotrophic effector(s) found in the isolates 15A and 6A used by Richards et al. (2016) or at least necrotrophic effectors that target the *Spt1* region, and that *Spt1* is present in our AM panel. Currently, chromosome 6H is considered to be the chromosome with the highest number of genes influencing NFNB and SFNB resistance (Abu Qamar et al. 2008; Cakir et al. 2003; Friesen et al. 2006; Grewal et al. 2008; Grewal et al. 2012; Gupta et al. 2010; Gupta et al. 2011; Somers et al. 2006) and it has been shown that both dominant resistance and dominant susceptibility genes can be found in this region. Since different marker types were used in these studies, the number, interaction and exact position of these genes on 6H still remains to be elucidated.

In a QTL mapping study in a biparental mapping population of the two Norwegian cultivars Arve and Lavrans tested under the same conditions as the AM panel in this study, the *NBP_QRptt4-2* region showed association with resistance under seedling inoculation with 5050B and 6949B and was also strongly linked to PH in 2015 and 2016 (Wonneberger et al. 2017) . This region was also associated with resistance to two NFNB isolates from the USA (Richards et al. 2017). Tamang et al. (2015) reported resistance QTL common to the SFNB isolates DEN 2.6 from Denmark and NZKF2 from New Zealand in this region and located the seedling resistance QTL *QRpts4* previously described by Grewal et al. (2008) in this region.

Under 2014 field conditions we found a significant QTL at 166 cM on 5H (*NBP_QRptt5-2*). This region harbored the major resistance locus *AL_QRptt5-2* in the above mentioned mapping study in the Arve x Lavrans population. This locus showed association with adult plant disease reaction in three years and seedling resistance to all three isolates (Wonneberger et al. 2017). *NBP_QRptt3-1* was less than 1 cM apart from a QTL found by Liu et al. (2015) which conferred resistance against the NFNB isolate LDN07Pt5 from North Dakota, USA. Richards et al. (2017) also mapped resistance to another North Dakota isolate to this region. Other QTL found in this study that co-located with previously described NB resistance QTL were *NBP_QRptt1-1* (co-locating with a resistance QTL against SG1) and *NBP_QRptt7-2* (co-locating with a resistance QTL against NZFK2) (Tamang et al. 2015). NBPQRptt7-2 was also associated with resistance to all three NFNB isolates used by Richards et al. (2017). In addition, *NBP_QRptt2-1* also co-located with the *SFNB-2H-8-10* QTL that was identified by Burlakoti et al. (2016).

Many of the QTL found in this study have previously been reported to be associated with disease reactions to SFNB isolates. Thus, even though the two forms of *P. teres* diverged about 519000 years ago (Ellwood et al. 2012), it appears as if they still share a number of virulence or avirulence genes, while others appear to have evolved after the pathogen has developed into the two forms. This sheds new light on the discussion whether the two forms should be considered as two different pathogens. Similarly, a number of the QTL found in this study were in close vicinity to QTL associated with resistance to other diseases in barley. Within the range of the resistance QTL *NBP_QRptt1-1* discovered in this study, Gutiérrez et al. (2015) mapped the spot blotch (*Cochliobolus sativus*) resistance QTL *Rcs-qtl-1H-6-7* which was originally described by Steffenson et al. (1996). In the same study, Gutiérrez et al. (2015) found a novel stripe rust (*Puccinia striiformis* f. sp. *hordei*) resistance QTL in several environments that co-locates with the previously described NB QTL *QRpts4* which we also found in our study. The *NBP_QRptt7-3* locus for seedling resistance to LR9 was located less than 2 cM apart from another novel multi-environment stripe rust QTL reported by the same authors. It remains to be elucidated whether the genes underlying these QTL confer broad-range non-host resistance to different diseases or if these QTL harbor two or more genes, each conferring resistance to a different disease. These common resistance loci may be of special interest for barley breeders.

Each of the QTL found under field conditions explained less than 14% of the phenotypic variation and most of them had p-values slightly below or above the significance threshold. At least two resistance alleles are necessary to significantly increase adult stage disease resistance (Fig. 7). This indicates that each QTL contributes only a small effect to resistance, which substantiates earlier findings by Wang et al. (2015) that barley NB resistance is conferred by a range of genes, each with a small effect. The small effect of the QTL can also partly be due to the early scoring time points. The QTL can be expected to become more significant at later scoring time points when the differences in resistance between the genotypes become more pronounced. As at least part of NB resistance appears to be dependent on the developmental stage, additional loci can be expected to become significant at the late stages, while some of the seedling resistance may break down. To capture this development, more scorings should be done regularly, covering the whole period from early stages up to maturity.

In three out of four years, there was a significant negative correlation between DH and NB severity, meaning that early lines show less resistance than late lines. An explanation for this might be that the leaves of early lines start to senesce earlier, which might facilitate fungal penetration and growth. We found ten loci associated with DH in this study, among them one that co-locates with the *HvCO3* locus influencing flowering time. The *NBP_QRptt1-1* NB resistance locus was associated with DH in two years, but further studies are required to examine whether this association is due to close linkage of DH and resistance genes or due to pleiotropic effects of one gene influencing both traits.

We found five QTL associated with PH, one of which co-located with *NBP_QRptt3-2* that conferred resistance to seedlings and adult plants. This region also harbors the semi-dwarfing locus *uzu*, which leads to shorter straw and was also found to be associated with increased resistance to a number of necrotrophic diseases (Chono et al. 2003; Goddard et al. 2014). However, we found a negative correlation between PH and adult plant disease severity which was highly significant in 2014, indicating that taller plants seem to be able to outgrow the disease. This suggests that the effect of *uzu* on resistance was masked by other genes or environmental factors which lead to the observed negative correlation between PH and resistance. Interestingly, in the Arve x Lavrans population grown under the same conditions we found a significant positive correlation between PH and severity in two years and a non-significant negative correlation in one year (Wonneberger et al. 2017).

Implications for resistance breeding

A number of both breeding lines and cultivars were identified that showed good adult stage resistance in three or four years. These lines are potential crossing parents and promising candidates for implementation in breeding programs. Our findings support the notion that only a few resistance genes seem to have a major influence on resistance (Friesen et al. 2006; Somers et al. 2006; Wang et al. 2015). As a consequence, breeders need to accumulate and combine numerous small-effect QTL in an effective way in elite breeding material to obtain sufficient and durable disease resistance. Our results show that as little as two additional resistance alleles can

significantly improve adult stage disease response, and each additional resistance allele further increases resistance (Fig. 7). The cultivars Annabell, Chevron, Oppdal, Olli and Lavrans had different combinations of resistance alleles at five different adult stage QTL and are potential resistance sources. Hotspots for NB resistance loci have been identified on 3H, 6H and 7H (Wang et al. 2015), and it will be necessary to determine whether these loci are constituted by different resistance genes or different alleles of the same gene, since this will determine which breeding strategy to apply.

One of our objectives in this study was to test if seedling screenings can be used by breeders as a time- and space-efficient tool to select genotypes with good field resistance to NB. The correlation between seedling and adult plant disease reaction was between 0.12 and 0.44, indicating that only a small portion of the adult plant disease reaction can be predicted by seedling tests, and that this portion is highly dependent on the environmental conditions and the pathogen population in the field. However, *NBP_QRptt3-2* and *NBP_QRptt6-1*, which were the most significant QTL at the seedling stage, were also significant in the inoculated field trials in two out of four years. In this study, *NBP_QRptt5-2* was only significant in adult plants, but in the Arve x Lavrans mapping population tested under the same conditions (Wonneberger et al. 2017), it influenced resistance in both adults and seedlings. Seedling screenings, thus, can still be useful for pre-screening for NB resistance and to assess consistency of the QTL and their robustness in different environments. This is especially relevant when using pathogen isolates in the seedling tests which represent the natural NB population. Seedling tests will also give an indication of whether the underlying resistance gene is involved in a general resistance mechanism or whether it is specific to a certain developmental stage.

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Table 1 Mean net blotch disease scores for the main current barley cultivars on the Norwegian market and a few historically important cultivars. Please refer to Online resource 3 for the complete data set

Line number	Name	Mean scores from field trials (%)						Mean scores from seedling inoculations (1-10)[a]			
		2013	2013 Nat[b]	2014	2015	2016	Avg.	LR9	5050B	6949B	Avg.
6	Asplund	NA	8.3	41.6	NA	27.6	NA	8.5	7.3	3.3	6.4
110	Maskin	29.3	11.3	38.9	37.7	22.3	NA	3.8	4.0	1.7	3.2
63	Herse	19.3	10.4	32.9	29.9	22.2	26.1	7.2	6.8	5.3	6.4
191	Varde	NA	5.2	41.6	NA	28.3	NA	8.5	6.5	2.8	5.9
22	Herta	16.4	12.1	37.1	NA	19.5	NA	7.3	4.8	7.0	6.4
208	Domen	31.9	12.0	20.2	24.1	15.1	22.8	7.0	4.5	7.5	6.3
140	Jarle	14.9	5.7	26.7	36.0	21.2	24.7	8.3	6.2	5.2	6.6
101	Mari	19.3	22.9	37.1	41.4	33.5	32.8	9.0	6.8	8.3	8.0
160	Lise	8.6	5.3	48.2	40.2	26.0	30.8	9.0	8.2	5.2	7.5
113	Gunilla	10.5	7.5	31.5	37.7	25.3	26.3	8.3	6.2	6.5	7.0
178	Pernilla	14.4	8.6	40.3	34.4	19.5	27.2	8.7	4.5	5.7	6.3
144	Bamse	19.7	20.0	45.9	24.2	18.6	27.1	6.3	3.8	2.0	4.0
49	Tyra	20.9	15.0	41.0	42.7	32.8	34.4	8.5	6.8	8.0	7.8
83	Arve	14.7	13.8	23.9	27.6	19.0	21.3	7.0	3.2	2.3	4.2
99	Lavrans	5.8	7.8	26.4	21.7	13.7	16.9	3.5	3.3	2.0	2.9
81	Fager	21.3	15.3	32.3	23.3	19.9	24.2	7.3	4.8	5.8	6.0
162	Gaute	18.0	11.4	56.4	44.8	27.4	36.7	8.5	8.2	5.3	7.3
195	Iver	21.5	14.4	46.0	40.4	35.2	35.8	9.0	7.3	5.5	7.3
5	Annabell	27.5	13.4	23.4	18.6	14.4	21.0	4.7	2.8	4.3	3.9
166	Edel	31.9	25.7	42.1	32.8	30.5	34.3	7.0	7.5	6.5	7.0
188	Helium	18.3	15.2	55.5	34.7	26.7	33.8	4.7	3.3	5.2	4.4
20	Tiril	29.0	20.7	55.6	49.1	37.8	42.9	8.0	7.5	5.7	7.1
172	Heder	46.3	22.9	33.8	20.8	22.8	30.9	7.8	4.5	6.0	6.1
123	Fairytale	16.2	15.0	28.4	27.1	18.0	22.4	4.2	3.3	4.8	4.1
79	Marigold	20.1	11.5	32.3	24.3	21.4	24.5	5.3	3.5	6.8	5.2
43	Brage	16.2	10.2	36.7	30.4	26.5	27.5	6.2	4.2	5.0	5.1
203	Iron	18.1	9.0	19.3	18.8	11.9	17.0	6.3	5.3	6.0	5.9

[a] Tekauz et al., 1985, [b] natural infection

Table 2 Pearson correlation coefficients for net blotch severities in the field and greenhouse and DH and PH in the AM panel and broad sense heritabilities for each trait

	Percentage of diseased leaf area in adult plants					Infection type (1-10)[a] in seedlings			DH[b]				PH[c]		
	2013	2013 Nat[d]	2014	2015	2016	LR9	5050 B	6949 B	2013	2014	2015	2016	2014	2015	2016
2013nat	0.52 ***														
2014	0.36 ***	0.41 ***													
2015	0.23 **	0.27 ***	0.63 ***												
2016	0.37 ***	0.34 ***	0.64 ***	0.76 ***											
LR9	0.14	0.08	0.26 ***	0.32 ***	0.44 ***										
5050B	0.12	0.17 *	0.23 **	0.38 ***	0.42 ***	0.70 ***									
6949B	0.13	0.21 **	0.19 **	0.04	0.14	0.48 ***	0.45 ***								
DH2013	-0.18 *	-0.07	0.00	-0.29 ***	-25 ***	-0.18 **	-0.13	0.34 ***							
DH2014	-0.16 *	-0.05	0.02	-0.19 **	-0.13	-0.11	-0.05	0.31 ***	0.83 ***						
DH2015	-0.2 **	-0.09	-0.05	-0.31 ***	-0.24 ***	-0.13	-0.15 *	0.32 ***	0.84 ***	0.84 ***					
DH2016	-0.22 **	-0.22 **	-0.08	-0.22 **	-0.17 *	-0.09	-0.05	0.25 ***	0.78 ***	0.78 ***	0.81 ***				
PH2014	-0.3 ***	-0.49 ***	-0.26 ***	-0.04	-0.18 **	-0.13	-0.11	-0.24 ***	0.04	0.04	0.11	0.31 ***			
PH2015	-0.23 **	-0.32 ***	-0.19 **	0.08	-0.01	0.03	0.04	-0.41 ***	-0.43 ***	-0.34 ***	-0.36 ***	-0.15 *	0.65 ***		
PH2016	-0.30 ***	-0.47 ***	-0.22 **	-0.05	-0.17	-0.15 *	-0.17 *	-0.34 ***	-0.05	0.01	0.50	0.27 ***	0.86 ***	0.73 ***	
h^2	0.80	0.88	0.93	0.94	0.96	0.96	0.93	0.96	1.00	0.96	1.00	0.98	0.97	0.97	0.98

* <0.05. ** <0.01. *** <0.001. [a] Tekauz et al., 1985 [b] DH: days to heading, [c] PH: plant height, [d] nat: natural infection

Table 3 Analysis of variance table for net blotch severity. DH and PH and heritabilities in the AM panel

Trait	Source	df	Mean square	F value	p-value	Heritability
NB[a]	Genotype	208	334.13	4.61	<0.0001	0.70
	Year	3	18344.62	253.13	<0.0001	
	Genotype x year	606	72.47	2.29	<0.0001	
	Rep(Year)	7	668.62	21.16	<0.0001	
	Block(Rep x year)	187	62.42	1.98	<0.0001	
	Error	1186	31.59			
DH[b]	Genotype	208	83.79	13.98	<0.0001	0.88
	Year	3	71184.75	11881.15	<0.0001	
	Genotype x year	619	5.99	3.46	<0.0001	
	Rep(Year)	6	11.83	6.83	<0.0001	
	Block(Rep x year)	51	3.88	2.24	<0.0001	
	Error	1697	1.73			
PH[c]	Genotype	208	475.19	8.64	<0.0001	0.85
	Year	2	137430.90	2499.14	<0.0001	
	Genotype x year	415	54.99	3.32	<0.0001	
	Rep(Year)	6	969.24	57.01	<0.0001	
	Block(Rep x year)	149	42.92	2.52	<0.0001	
	Error	1108	17.00			

[a] NB: net blotch severity, [b] DH: days to heading, [c] PH: plant height

Table 4 SNP markers significantly associated with seedling resistance against the isolates LR9, 5050B and 6949B

Marker	QTL name	Chr.	Pos. [a]	POPseq pos. [b]	R^2 [c]	MAF	p-value	-log10 (p-value)
LR9								
11_10728	*NBP_QRptt3-2*	3H	60.84	52.76	0.10	0.345	7.86E-05	4.1
SCRI_RS_152172	*NBP_QRptt3-2*	3H	61.29		0.09	0.336	1.04E-04	4.0
SCRI_RS_186102	*NBP_QRptt3-2*	3H	61.29	53.26	0.08	0.373	2.85E-04	3.5
SCRI_RS_154517	*NBP_QRptt4-1*	4H	3.31	1.13	0.08	0.166	3.27E-04	3.5
SCRI_RS_135637	*NBP_QRptt4-2*	4H	53.87	50.99	0.07	0.361	7.46E-04	3.1
SCRI_RS_16316	*NBP_QRptt7-3*	7H	148.65	128.68	0.08	0.123	4.29E-04	3.4
5050B								
11_10728	*NBP_QRptt3-2*	3H	60.84	52.76	0.12	0.345	1.11E-05	5.0
SCRI_RS_152172	*NBP_QRptt3-2*	3H	61.29		0.14	0.336	1.33E-06	5.9
SCRI_RS_186102	*NBP_QRptt3-2*	3H	61.29	53.26	0.10	0.373	5.80E-05	4.2
12_30441	*NBP_QRptt6-1*	6H	58.24	53.33	0.10	0.345	5.95E-05	4.2
12_31005	*NBP_QRptt6-1*	6H	58.24	53.33	0.09	0.356	1.00E-04	4.0
SCRI_RS_182195	*NBP_QRptt6-1*	6H	58.24		0.09	0.475	1.39E-04	3.9
6949B								
SCRI_RS_167465	*NBP_QRptt2-1*	2H	13.79	7.44	0.09	0.302	1.16E-04	3.9
11_10728	*NBP_QRptt3-2*	3H	60.84	52.76	0.13	0.345	2.62E-06	5.6
SCRI_RS_152172	*NBP_QRptt3-2*	3H	61.29		0.15	0.336	8.68E-07	6.1
SCRI_RS_186102	*NBP_QRptt3-2*	3H	61.29	53.26	0.11	0.373	1.67E-05	4.8
11_10513	*NBP_QRptt6-1*	6H	59.33	55.67	0.09	0.164	1.90E-04	3.7
SCRI_RS_103515	*NBP_QRptt2-1*	2H		7.44	0.10	0.282	8.74E-05	4.1

[a] Marker position based on consensus map by Munõz-Amatriaín et al. (2014), [b] Marker position based on POPseq map by Mascher et al. (2012), [c] Amount of phenotypic variance explained by this QTL

Table 5 SNP markers significantly associated with adult NB resistance under field conditions in 2013-2016

Marker	QTL name	Chr.	Pos. [a]	POPseq pos. [b]	R^2 [c]	MAF	p-value	-log10 (p-value)
2013 - Average								
11_21333	*NBP_QRptt1-1*	1H	53.3	52.55	0.06	0.471	0.00511	2.3
12_31448	*NBP_QRptt3-1*	3H	5.60	2.41	0.05	0.302	0.00649	2.2
11_21109	*NBP_QRptt3-2*	3H	58.31	49.65	0.06	0.169	0.00503	2.3
SCRI_RS_221999	*NBP_QRptt5-1*	5H	51.73	55.62	0.11	0.29	7.36E-05	4.1
SCRI_RS_205235	*NBP_QRptt5-1*	5H	51.83		0.10	0.292	8.63E-05	4.1
12_20350	*NBP_QRptt5-1*	5H	53.77		0.07	0.121	0.00245	2.6
2013 - Uninoculated								
SCRI_RS_4928	*NBP_QRptt1-2*	1H	142.54		0.06	0.232	0.00228	2.6
SCRI_RS_221999	*NBP_QRptt5-1*	5H	51.73	55.62	0.06	0.29	0.00243	2.6
SCRI_RS_205235	*NBP_QRptt5-1*	5H	51.83		0.06	0.292	0.00267	2.6
11_20993	*NBP_QRptt7-1*	7H	34.74		0.10	0.174	7.48E-05	4.1
SCRI_RS_161285	*NBP_QRptt7-2*	7H	117.15	106.44	0.06	0.286	0.00169	2.8
SCRI_RS_150517	*NBP_QRptt7-1*	7H		29.96	0.09	0.175	3.34E-04	3.5
2014 - Average								
12_20867	*NBP_QRptt5-2*	5H	165.57	155.56	0.10	0.104	5.35E-05	4.3
SCRI_RS_179841	*NBP_QRptt5-2*	5H	165.57	155.56	0.07	0.088	2.59E-04	3.6
SCRI_RS_165290	*NBP_QRptt5-2*	5H	165.57	155.62	0.07	0.103	2.78E-04	3.6
SCRI_RS_219810	*NBP_QRptt6-1*	6H	58.34	53.90	0.09	0.165	1.44E-04	3.8
12_30120	*NBP_QRptt6-1*	6H	58.34	55.03	0.09	0.162	1.90E-04	3.7
SCRI_RS_186193	*NBP_QRptt6-1*	6H	59.21	55.38	0.09	0.115	1.06E-04	4.0
2015 - Average								
SCRI_RS_170878	*NBP_QRptt1-1*	1H	43.41	42.21	0.09	0.434	4.12E-04	3.4
SCRI_RS_170869	*NBP_QRptt1-1*	1H	43.41	42.21	0.09	0.449	4.20E-04	3.4
SCRI_RS_153785	*NBP_QRptt1-1*	1H	43.41	41.64	0.08	0.452	0.00107	3.0
SCRI_RS_189483	*NBP_QRptt1-1*	1H	43.62	42.35	0.09	0.44	4.21E-04	3.4
11_10764	*NBP_QRptt1-1*	1H	43.62	42.35	0.09	0.437	4.47E-04	3.4
SCRI_RS_221644	*NBP_QRptt3-2*	3H		52.03	0.08	0.169	5.15E-04	3.3
2016 - Average								
SCRI_RS_170878	*NBP_QRptt1-1*	1H	43.41	42.21	0.14	0.434	1.76E-06	5.8
SCRI_RS_153785	*NBP_QRptt1-1*	1H	43.41	41.64	0.13	0.452	3.51E-06	5.5
SCRI_RS_170869	*NBP_QRptt1-1*	1H	43.41	42.21	0.10	0.449	5.20E-05	4.3
11_10764	*NBP_QRptt1-1*	1H	43.62	42.35	0.14	0.437	1.58E-06	5.8
SCRI_RS_189483	*NBP_QRptt1-1*	1H	43.62	42.35	0.10	0.44	4.80E-05	4.3
SCRI_RS_210025	*NBP_QRptt6-1*	6H	54.10	49.08	0.09	0.421	1.28E-04	3.9

[a] Marker position based on consensus map by Munõz-Amatriaín et al. (2014), [b] Marker position based on POPseq map by Mascher et al. (2012), [c] Amount of phenotypic variance explained by this QTL

Table 6 SNP markers significantly associated with PH

Marker	QTL name	Chr.	Pos. [a]	POPseq pos. [b]	R^2 [c]	MAF	p-value	-log10 (p-value)
2014								
11_21129	*NBP_QRptt3-2*	3H	58.31	51.63	0.15	0.138	6.07E-07	6.2
SCRI_RS_128706	*NBP_QRptt3-2*	3H	58.31	51.06	0.11	0.138	1.92E-05	4.7
SCRI_RS_125581		5H		97.35	0.11	0.063	1.58E-05	4.8
2015								
12_21386		3H	133.66	132.93	0.12	0.063	7.12E-06	5.1
SCRI_RS_237352		5H	89.82	95.49	0.12	0.055	7.51E-06	5.1
12_30978		5H		1.64	0.13	0.186	3.31E-06	5.5
SCRI_RS_125581		5H		97.35	0.12	0.063	7.79E-06	5.1
SCRI_RS_147618		NA			0.12	0.053	4.79E-06	5.3
2016								
SCRI_RS_196025	*NBP_QRptt1-2*	1H	136.75	126.13	0.15	0.07	4.97E-07	6.3
11_21129	*NBP_QRptt3-2*	3H	58.31	51.63	0.13	0.138	4.43E-06	5.4
11_10365	*NBP_QRptt3-2*	3H	58.31		0.12	0.162	7.63E-06	5.1
SCRI_RS_190764	*NBP_QRptt4-2*	4H	46.57	43.48	0.12	0.055	8.18E-06	5.1
12_10371	*NBP_QRptt4-2*	4H	46.87		0.12	0.055	8.18E-06	5.1
11_20180	*NBP_QRptt4-2*	4H	46.87	43.48	0.12	0.055	8.18E-06	5.1

[a] Marker position based on consensus map by Munõz-Amatriaín et al. (2014), [b] Marker position based on POPseq map by Mascher et al. (2012), [c] Amount of phenotypic variance explained by this QTL

Table 7 SNP markers significantly associated with DH

Marker	QTL name	Chr.	Pos. [a]	POPseq pos. [b]	R^2 [c]	MAF	p-value	-log10 (p-value)
2013								
SCRI_RS_149726	*NBP_QRptt1-1*	1H	50.00	48.09	0.09	0.424	2.19E-04	3.7
SCRI_RS_204579		2H	177.38	147.45	0.14	0.145	2.41E-06	5.6
SCRI_RS_170753		2H	177.38	147.31	0.04	0.155	4.21E-04	3.4
BK_04		5H		136.06	0.01	0.367	1.98E-04	3.7
12_30306		1H		47.83	0.03	0.43	2.44E-04	3.6
SCRI_RS_207423		5H		67.40	0.01	0.072	1.33E-04	3.9
2014								
12_30014		1H	114.98	104.25	0.08	0.065	2.58E-04	3.6
SCRI_RS_138977		6H	8.09	9.84	0.07	0.475	6.96E-04	3.2
11_20725		6H	110.79	100.99	0.10	0.267	4.70E-05	4.3
12_31498		6H	129.22	118.56	0.09	0.277	1.34E-04	3.9
11_11111		6H	129.22	119.06	0.08	0.456	5.27E-04	3.3
11_21112		6H	129.32	118.56	0.08	0.154	4.85E-04	3.3
2015								
SCRI_RS_124377	*NBP_QRptt1-1*	1H	50.00	48.23	0.12	0.102	7.74E-06	5.1
12_30014		1H	114.98	104.25	0.16	0.065	3.01E-07	6.5
SCRI_RS_206202		3H	86.87	83.92	0.13	0.082	4.29E-06	5.4
SCRI_RS_172730		3H	86.87	83.92	0.12	0.068	5.22E-06	5.3
12_11318		5H	38.84	43.76	0.12	0.131	6.34E-06	5.2
SCRI_RS_233901		NA			0.13	0.075	4.38E-06	5.4
2016								
12_30014		1H	114.98	104.25	0.23	0.065	6.51E-10	9.2
SCRI_RS_204579		2H	177.38	147.45	0.23	0.145	5.98E-10	9.2
SCRI_RS_206202		3H	86.87	83.92	0.18	0.082	3.58E-08	7.4
SCRI_RS_85089		5H	26.36	23.61	0.19	0.109	1.07E-08	8.0
SCRI_RS_189323		5H	26.36		0.19	0.109	1.07E-08	8.0
SCRI_RS_233901		NA			0.21	0.075	3.36E-09	8.5

[a] Marker position based on consensus map by Munõz-Amatriaín et al. (2014)

[b] Marker position based on POPseq map (Cantalapiedra et al. 2015; Mascher et al. 2013)

[c] Amount of phenotypic variance explained by this QTL

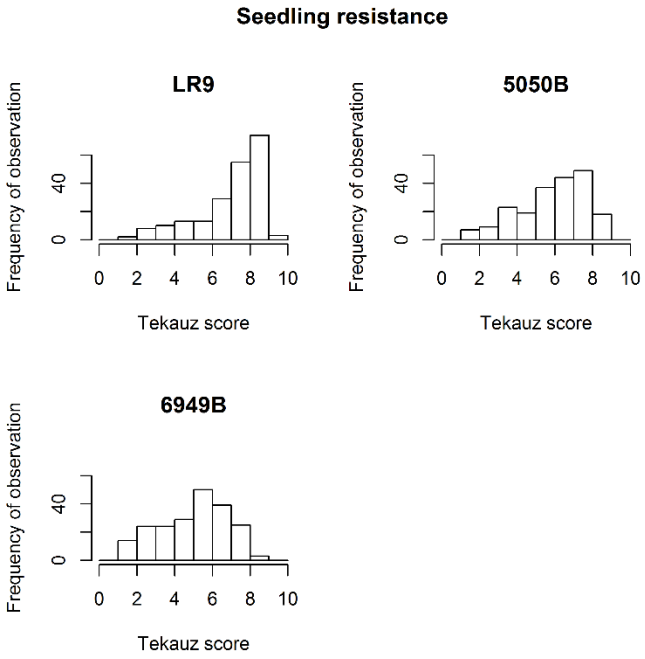


Fig. 1 Frequency distributions for disease responses in seedling inoculations with three different isolates LR9. 5050B and 6949B

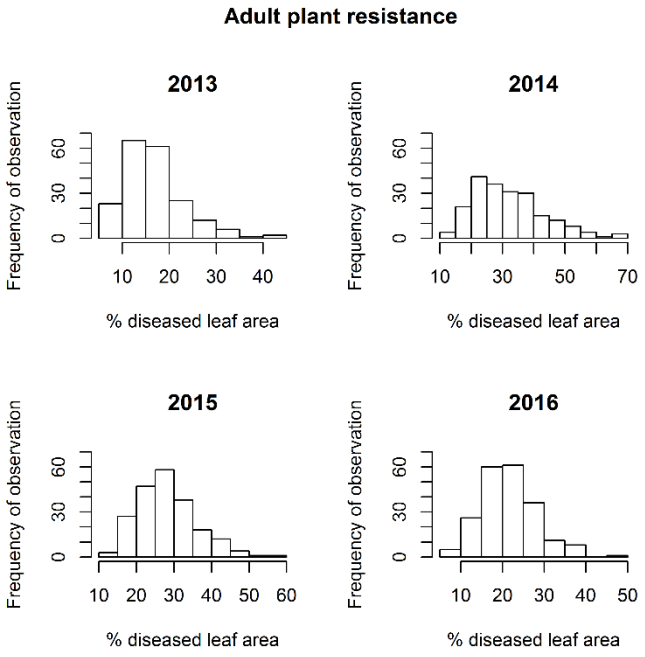
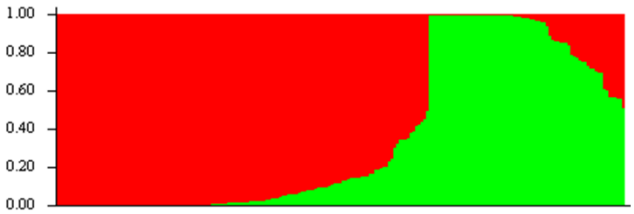


Fig. 2 Frequency distributions for adult plant disease responses under inoculated field conditions in three years



	Group 1	Group 2
Two-rowed	1	70
Six-rowed	136	2

Fig. 3 Population structure in the AM panel. Bayesian clustering divided the panel mainly into two subgroups corresponding to two-rowed (green) and six-rowed (red) barleys

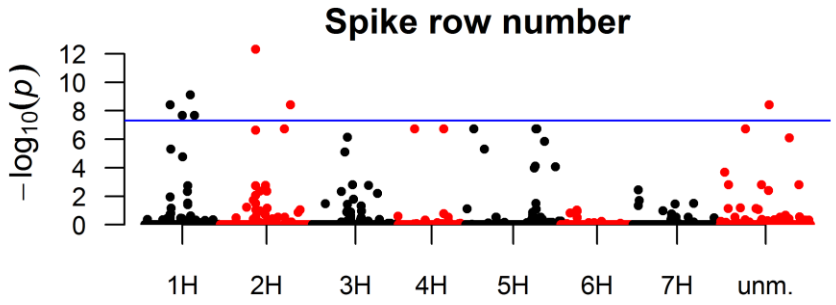


Fig. 4 Association mapping of spike row number using the MLM + K + Q model. Vertical axes show the –log(10) value of MTA p-values. Dots above the horizontal lines represent MTAs with a p-value within the 0.1 percentile quantile and are considered significant

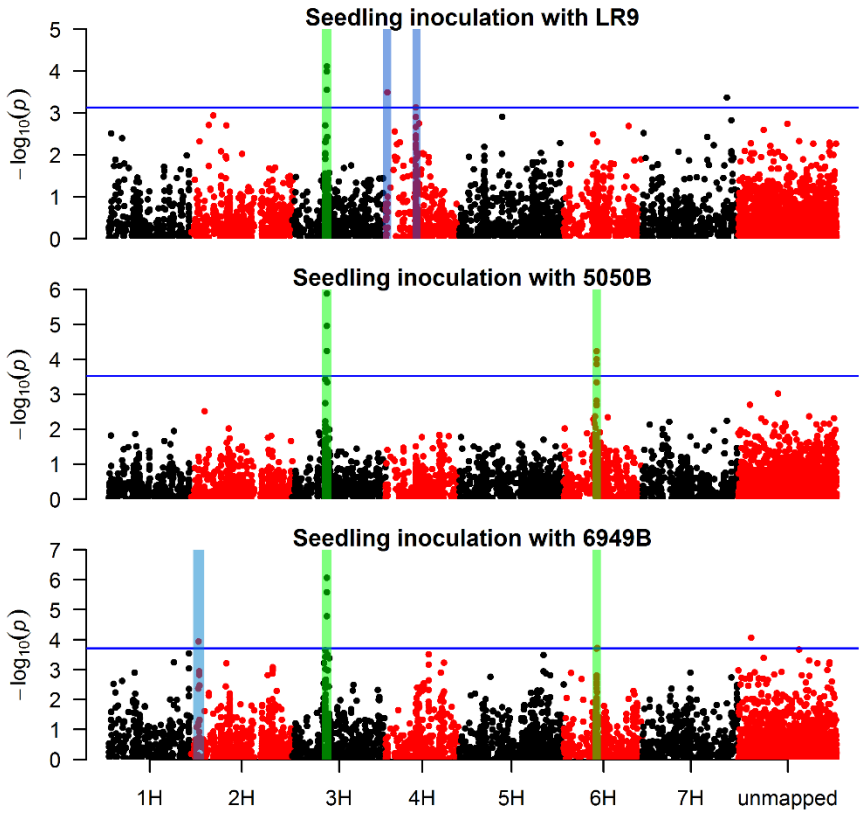


Fig. 5 Association mapping of net blotch scorings in seedlings using the MLM + K + Q model. Vertical axes show the –log(10) value of MTA p-values. Dots above the horizontal lines represent MTAs with a p-value within the 0.1 percentile quantile and are considered significant. Green vertical bars indicate QTL significant in more than one seedling test, blue vertical bars indicate QTL only found in inoculations with one isolate

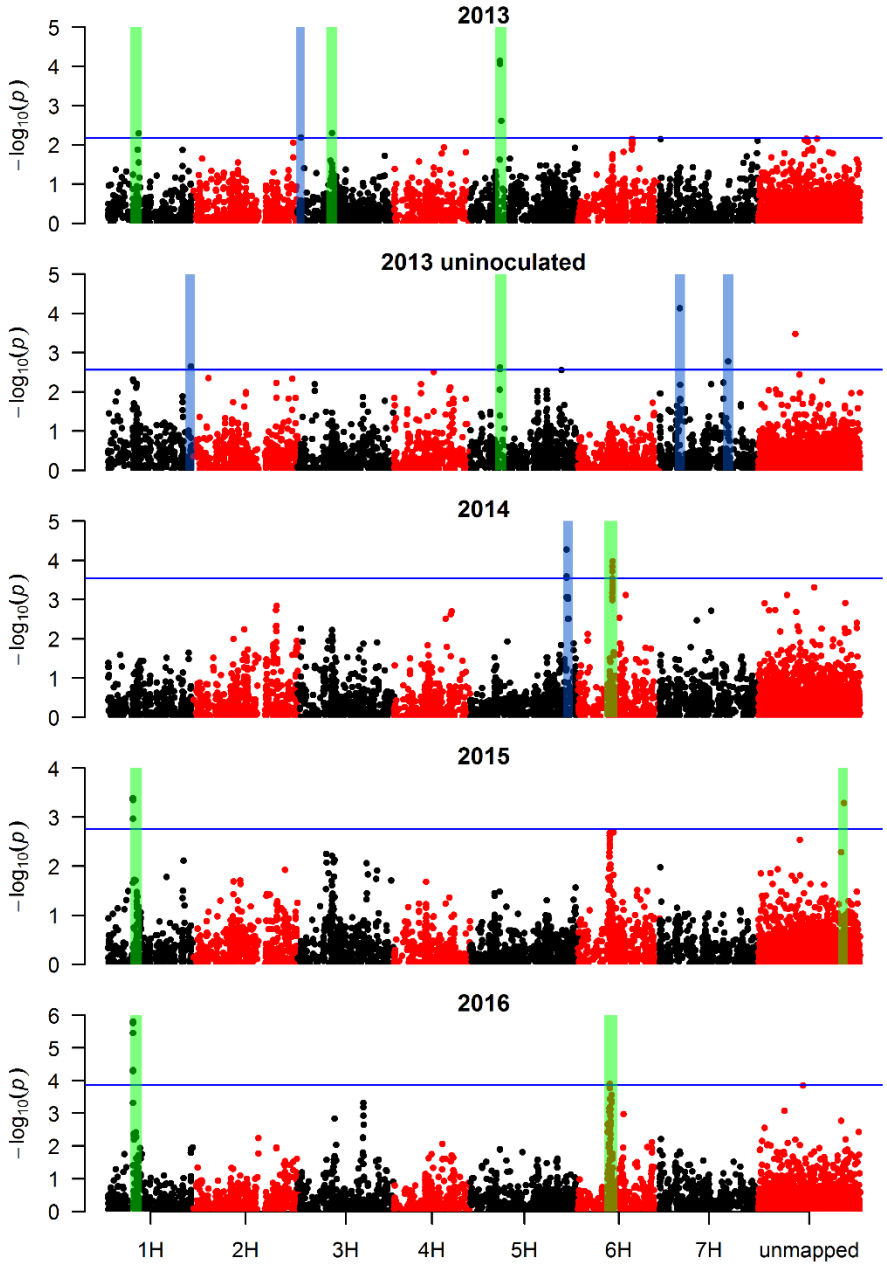


Fig. 6 Association mapping of net blotch scorings in adult plants using the MLM + K + Q model. Vertical axes show the –log(10) value of MTA p-values. Dots above the horizontal lines represent MTAs with a p-value within the 0.1 percentile quantile and are considered significant. Green vertical bars indicate QTL significant in more than one trial, blue vertical bars indicate QTL only found in one trial. The significant unmapped marker in 2015 maps to the *NBP_QRptt3-2* region which was also significant in 2013 and is therefore marked in green

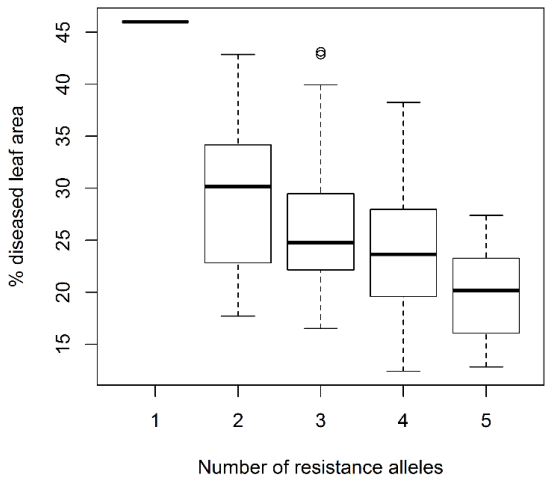


Fig. 7 Effect of QTL number on adult plant stage disease response

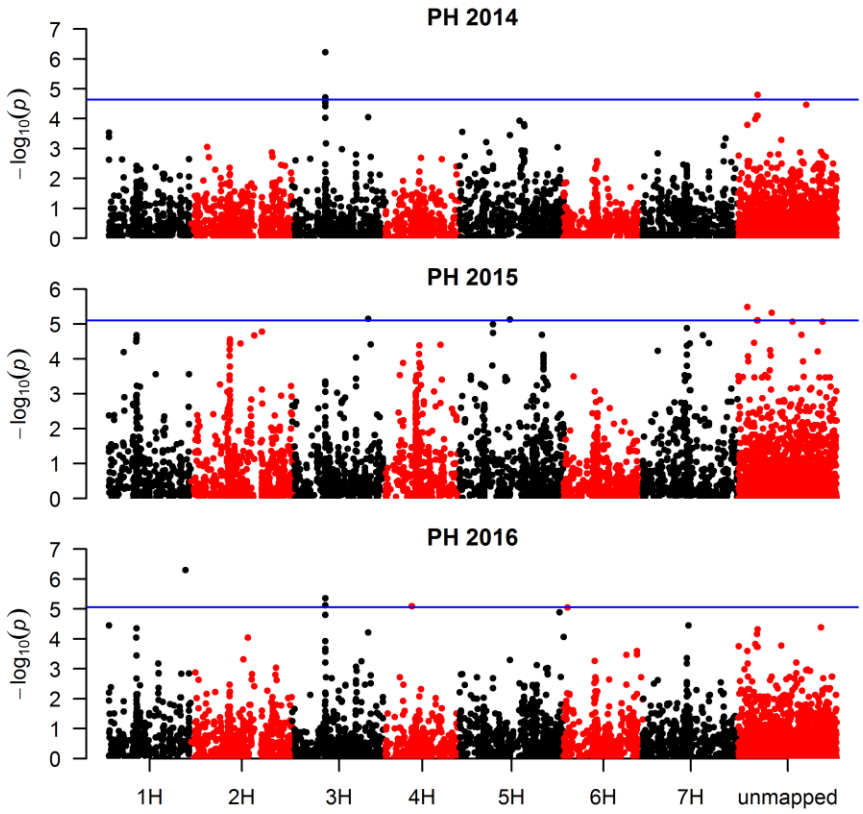


Fig. 8 Association mapping of PH using the MLM + K + Q model. Vertical axes show the –log(10) value of MTA p-values. Dots above the horizontal lines represent MTAs with a p-value within the 0.1 percentile quantile and are considered significant

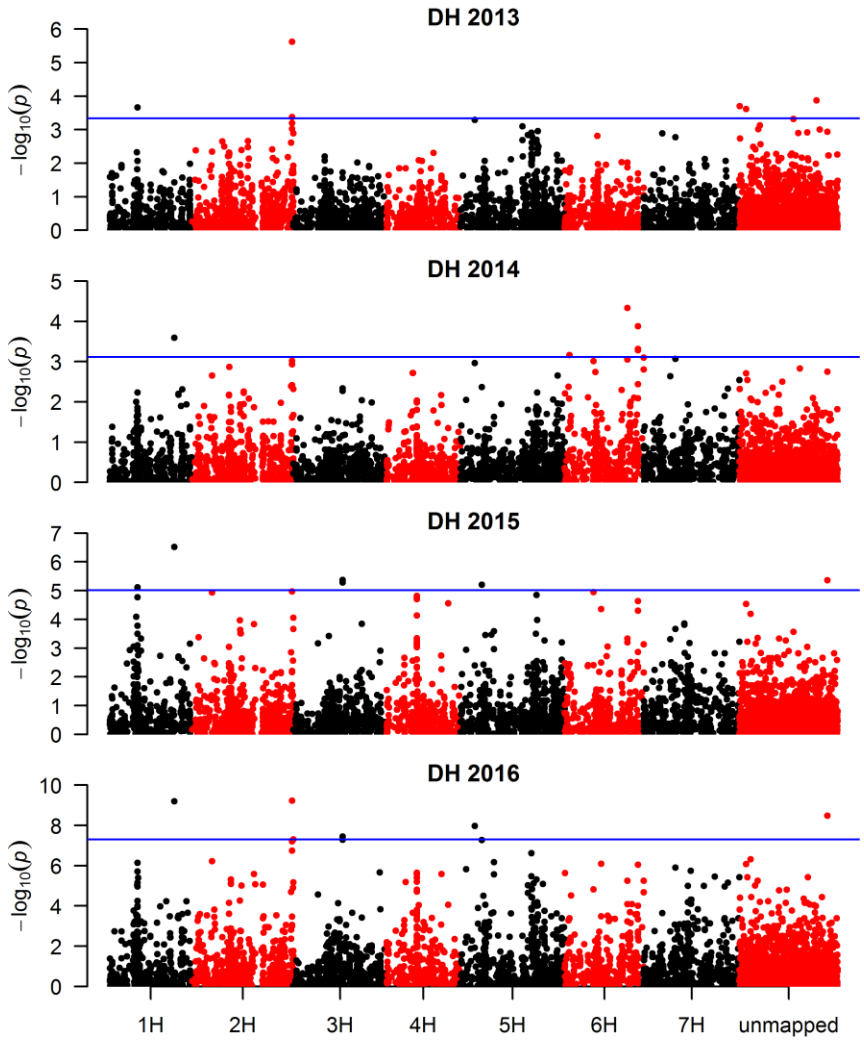


Fig. 9 Association mapping of DH using the MLM + K + Q model. Vertical axes show the –log(10) value of MTA p-values. Dots above the horizontal lines represent MTAs with a p-value within the 0.1 percentile quantile and are considered significant

S1 Details about the barley lines used in this study

Line number	Name	Accession number	Origin	Released	Spike row number	Status
1	Breeding line		Sweden		6	Breeding line
2	Ida	SNP049	Sweden	1979	2	Cultivar
3	Fløya	NGB2064	Norway	1939	6	Landrace selection
4	Akka	NGB2665	Sweden	1970	2	Cultivar
5	Annabell	NGB13916	Germany	2002	2	Cultivar
6	Asplund		Sweden	1910	6	Landrace selection
7	Elmeri		Finland	2009	6	Cultivar
8	Tampar		Faroe Islands		6	Landrace
9	Oppdal	NGB13670	Norway		6	Landrace
10	Breeding line		Norway		6	Breeding line
11	Breeding line		Denmark		2	Breeding line
12	Finne	NGB6924	Norway		6	Landrace
13	Breeding line		Norway		6	Breeding line
14	Breeding line		Norway		6	Breeding line
15	Tore		Norway	1986	6	Cultivar
16	Breeding line		Norway		6	Breeding line
17	KWS Olof		Germany		2	Cultivar
18	Breeding line		Norway		6	Breeding line
19	Breeding line		Norway		6	Breeding line
20	Tiril		Norway	2004	6	Cultivar
21	Breeding line		Norway		6	Breeding line
22	Herta		Sweden	1949	2	Cultivar
23	Breeding line		Norway		6	Breeding line
24	Juli	NGB4585	Denmark	1909	6	Landrace selection
25	Breeding line		Sweden		2	Breeding line
26	Tunga	SNP030	Norway	1975	6	Cultivar
27	Chevron	NGB8866	Switzerland		6	Cultivar
28	Birgitta	NGB14667	Sweden	1966	2	Cultivar
29	Bonus	NGB14657	Sweden	1950	2	Cultivar
30	Nordlys	NGB2076	Norway	1962	6	Cultivar
31	Breeding line		Norway		6	Breeding line
32	Breeding line		Norway		6	Breeding line
33	Breeding line		Norway		6	Breeding line
34	Thule		Norway	1993	6	Cultivar
35	Breeding line		Norway		6	Breeding line
36	Breeding line		Norway		6	Breeding line
37	Breeding line	NGB2108	Norway		2	Breeding line
38	Breeding line		Norway		6	Breeding line
39	Breeding line		Norway		6	Breeding line
40	Bode	SNP010	Norway	1978	6	Cultivar
41	Breeding line		Norway		6	Breeding line
42	Breeding line		Norway		6	Breeding line
43	Brage		Norway	2010	6	Cultivar
44	Breeding line		Norway		6	Breeding line
45	Gull	NGB1480	Sweden	1913	2	Landrace selection
46	Seger	NGB9467	Sweden	1926	2	Cultivar
47	Breeding line		Norway		6	Breeding line
48	Breeding line		Norway		6	Breeding line
49	Tyra		Norway	1988	2	Cultivar
50	Aktiv		Czech Republic		2	Cultivar
51	Breeding line		Norway		6	Breeding line
52	Breeding line		Norway		6	Breeding line
53	Tocada		Germany		2	Cultivar

54	Breeding line		Norway		6	Breeding line
55	Axelina		Sweden	2008	2	Cultivar
56	Henni	NGB12281	Germany		2	Cultivar
57	Breeding line		Norway		6	Breeding line
58	Forus	NGB2065	Norway	1959	6	Cultivar
59	H354-333-7-5		Norway		6	Breeding line
60	Atlas 46	JIC7795	USA		6	Cultivar
61	Breeding line		Norway		6	Breeding line
62	Golf	SNP093	UK	1984	2	Cultivar
63	Herse	NGB2067	Norway	1939	6	Cultivar
64	Yrjar	NGB2084	Norway	1975	6	Cultivar
65	Breeding line		Norway		6	Breeding line
66	Rambler		unknown		2	Cultivar
67	Breeding line		Norway		6	Breeding line
68	Breeding line		Norway		6	Breeding line
69	Breeding line		Norway		6	Breeding line
70	Breeding line		Norway		6	Breeding line
71	Breeding line		Norway		6	Breeding line
72	H82009-1-2		Norway		6	Breeding line
73	Breeding line		Norway		6	Breeding line
74	CI11577	Clho11577	Greece		6	Landrace
75	Jotun	NGB466	Norway	1930	6	Landrace selection
76	Meltan	NGB9948	Sweden	1991	2	Cultivar
77	Asahi 5		Japan		2	Cultivar
78	Seijo 17		Japan		2	Cultivar
79	Marigold		France	2009	2	Cultivar
80	Vada	JIC3615	Netherlands		2	Cultivar
81	Fager		Norway	2000	6	Cultivar
82	Tammi	NGB6925	Finland	1937	6	Cultivar
83	Arve		Norway	1990	6	Cultivar
84	Opal	NGB4619	Denmark	1922	2	Cultivar
85	Breeding line		Norway		6	Breeding line
86	Einar		Finland	2008	6	Cultivar
87	Uforædlet Jämtland	NGB6927	Sweden		6	Landrace
88	Kinnan		Sweden	1991	2	Cultivar
89	Vilde		Norway	2004	6	Cultivar
90	Polarbygg	NGB8892	Norway	1933	6	Landrace selection
91	Delphi		Denmark		2	Cultivar
92	Vera	NGB11312	Norway	1988	6	Cultivar
93	Ven		Norway	1999	6	Cultivar
94	Otra	NGB291	Finland	1959	6	Cultivar
95	Breeding line		Norway		6	Breeding line
96	Breeding line		Norway		6	Breeding line
97	Vega ABED	SNP035	Denmark	1976	2	Cultivar
98	Stine		Germany		2	Cultivar
99	Lavrans	NGB16727	Norway	1999	6	Cultivar
100	Varde		Norway	1941	6	Cultivar
101	Mari	NGB1491	Sweden	1963	2	Cultivar
102	Breeding line		Norway		6	Breeding line
103	Audrey		unknown		2	Cultivar
104	Dønnes	NGB9448	Norway		6	Landrace
105	Breeding line		Norway		6	Breeding line
106	Breeding line		Norway		6	Breeding line
107	Breeding line		Norway		6	Breeding line
108	Breeding line		Norway		6	Breeding line
109	Jyvä		Finland	2000	6	Cultivar

110	Maskin	NGB9588	Norway	1918	6	Landrace selection
111	Saana		Finland	1996	2	Cultivar
112	Habil		Norway	2007	6	Cultivar
113	Gunilla		Sweden	1973	2	Cultivar
114	Breeding line		Norway		6	Breeding line
115	Breeding line		Norway		6	Breeding line
116	Etu	NGB0332	Finland	1970	6	Cultivar
117	Breeding line		Norway		6	Breeding line
118	Breeding line		Sweden		6	Breeding line
119	Olsok		Norway	1994	6	Cultivar
120	Breeding line		Norway		6	Breeding line
121	Pallas	SNP077	Sweden	1958	2	Cultivar
122	Breeding line		Norway		6	Breeding line
123	Fairytale		Denmark	2009	2	Cultivar
124	Trysil	NGB9346	Norway		6	Landrace
125	Breeding line		Norway		6	Breeding line
126	Maja	NGB8815	Denmark	1927	2	Cultivar
127	Breeding line		Norway		6	Breeding line
128	H572-8		Norway		2	Breeding line
129	Vigdis	NGB2083	Norway	1964	6	Cultivar
130	Møyar		Norway	1969	2	Cultivar
131	Breeding line		Norway		6	Breeding line
132	Bjørneby	NGB469	Norway		6	Landrace
133	Breeding line		Norway		6	Breeding line
134	Gustav		Sweden	2009	2	Cultivar
135	Frisco		Denmark	2005	2	Cultivar
136	Vena	NGB2082	Norway	1975	6	Cultivar
137	Stolt		Sweden	1999	6	Cultivar
138	Breeding line		Norway		6	Breeding line
139	Olve		Norway	1994	2	Cultivar
140	Jarle	NGB2070	Norway	1960	6	Cultivar
141	Toria		Norway	2011	6	Cultivar
142	Breeding line		Norway		6	Breeding line
143	Balder	SNP172	Sweden	1945	2	Cultivar
144	Bamse		Sweden	1983	6	Cultivar
145	Breeding line		Norway		6	Breeding line
146	Harbinger		Finland	2009	2	Cultivar
147	IS-046	SNP006	Iceland		2	Cultivar
148	Breeding line		Norway		6	Breeding line
149	Rolfi		Finland	1997	6	Cultivar
150	Breeding line		Norway		6	Breeding line
151	Breeding line		Denmark		2	Breeding line
152	Shirley		unknown		2	Cultivar
153	Edvin		Finland	2008	6	Cultivar
154	H82011-2-2		Norway		6	Breeding line
155	Breeding line		Norway		6	Breeding line
156	Breeding line		Germany		2	Breeding line
157	Fræg	SNP011	Norway	1948	6	Cultivar
158	Jazz		Netherlands		2	Cultivar
159	Trine	SNP166	Norway	1986	6	Cultivar
160	Lise		Norway	1964	6	Cultivar
161	Luhkas		France		2	Cultivar
162	Gaute		Norway	2000	6	Cultivar
163	Verner	NGB9943	Sweden	1991	6	Cultivar
164	Breeding line		Norway		6	Breeding line
165	Breeding line		Norway		6	Breeding line
166	Edel		Norway	2002	6	Cultivar

167	Breeding line		Sweden		2	Breeding line
168	Ingrid		Sweden	1956	2	Cultivar
169	Agneta	SNP031	Sweden	1978	6	Cultivar
170	Rigel	NGB8818	Denmark	1941	2	Cultivar
171	Fredrickson		Japan		2	Landrace
172	Heder		Norway	2007	6	Cultivar
173	Breeding line		Norway		6	Breeding line
174	Barke	JIC20611	Germany		2	Cultivar
175	Baronesse		Germany	1997	2	Cultivar
176	H3003	SNP139	Norway		2	Breeding line
177	Breeding line		Norway		6	Breeding line
178	Pernilla		Sweden	1982	2	Cultivar
179	CIho4196		China		2	Landrace
180	Breeding line		Denmark		2	Breeding line
181	Delibes	JIC20770	UK		2	Cultivar
182	Svanhals	NGB1482	Sweden	1903	2	Landrace selection
183	Olli	NGB13660	Finland	1927	6	Landrace selection
184	Breeding line		Norway		6	Breeding line
185	Kunnari		Finland	2001	6	Cultivar
186	Binder	NGB9440	Denmark	1913	2	Landrace selection
187	Atlas	JIC7794	USA		6	Cultivar
188	Helium		Denmark	2004	2	Cultivar
189	Clermont		France		6	Cultivar
190	Breeding line		Norway		6	Breeding line
191	Vega	NGB15238	Sweden	1920	6	Landrace selection
192	Breeding line		Norway		6	Breeding line
193	Breeding line		Norway		6	Breeding line
194	Dore	NGB6272	Sweden	1932	6	Landrace selection
195	Iver		Norway	2001	2	Cultivar
196	Malz		Czech Rebublic		2	Cultivar
197	Breeding line		Norway		6	Breeding line
198	Breeding line		Norway		6	Breeding line
199	Triumph	SNP067	Germany		2	Landrace selection
200	Sunnita		Sweden	1992	2	Cultivar
201	Quench		UK		2	Cultivar
202	Breeding line		Norway		6	Breeding line
203	Iron		Denmark	2011	2	Cultivar
204	Gammel dansk	NGB4613	Denmark		2	Landrace
205	Breeding line		Norway		6	Breeding line
206	Breeding line		Norway		6	Breeding line
207	Breeding line		Norway		6	Breeding line
208	Domen	NGB1493	Norway	1952	2	Cultivar
209	Breeding line		Norway		6	Breeding line

S2 Properties of the Nordic association mapping panel used in this study

Information	Number of lines
Spike row number	
2	72
6	137
Improvement status	
Cultivars	101
Landraces	11
Landrace selections	13
Breeding lines	84
Origin	
Norway	119
Sweden	31
Denmark	15
Finland	12
Germany	9
Other/unknown	23

S3 Least square means based on phenotypic scores collected in this study. Percentage of diseased leaf area, DH and PH scored in adult plants in field trials 2013-2016; Tekauz scale scores in seedlings inoculated with three NFNB isolates; spike row number

Line no.	Name	2013 first scoring	2013 second scoring	2013 average	2013 uninoculated	2014 first scoring	2014 second scoring	2014 average	2015 first scoring	2015 second scoring	2015 third scoring	2015 average	2016 first scoring	2016 second scoring	2016 average	Adult stage average	Days to heading 2013	Days to heading 2014	Days to heading 2015	Days to heading 2016	Plant height 2014	Plant height 2015	Plant height 2016	Seedling inoculation, isolate LR9	Seedling inoculation, isolate 5050B	Seedling inoculation, isolate 6949B	Seedling stage average	Spike row number
1	Breeding line	9	14	11	11	19	45	32	12	37	50	33	9	34	22	24	52	46	70	49	74	90	63	9	7	4	7	6
2	Ida	7	15	12	13	17	27	22	13	31	37	27	8	33	21	20	52	46	70	48	72	87	62	7	5	4	6	2
3	Fløya	14	14	14	13	17	28	23	10	32	48	30	4	34	19	22	48	42	66	46	78	115	75	5	6	3	5	6
4	Akka	21	21	21	14	17	42	30	19	36	57	35	7	43	25	28	47	41	66	45	79	100	69	8	7	6	7	2
5	Annabell	23	33	27	13	13	33	23	9	17	30	19	5	24	14	21	55	47	73	50	67	82	56	5	3	4	4	2
6	Barke	11	NA	NA	8	27	56	42	19	39	NA	NA	9	46	28	NA	51	47	70	52	78	100	72	9	7	3	6	6
7	Elmeri	8	18	14	15	9	19	14	4	15	28	15	2	18	11	13	49	44	69	48	71	88	59	7	9	6	7	6
8	Tampar	NA	NA	NA	NA	47	59	53	14	38	65	40	8	34	21	NA	NA	35	62	42	67	102	61	10	8	4	7	6
9	Oppdal	9	NA	NA	7	12	31	22	6	22	32	20	3	18	11	NA	46	41	69	48	83	106	69	7	6	3	5	6
10	Breeding line	17	25	21	16	18	31	24	12	34	40	30	8	33	20	24	49	44	68	47	67	91	54	9	9	5	7	6
11	Breeding line	15	22	19	11	14	30	22	11	29	37	26	5	24	15	20	56	49	72	50	71	89	57	6	5	6	6	2
12	Finne	6	15	11	8	14	25	20	13	41	NA	NA	7	32	19	NA	53	50	72	57	95	113	95	6	8	4	6	6
13	Breeding line	18	21	20	15	12	30	21	7	24	40	22	4	28	16	20	50	46	69	48	65	99	58	8	6	6	6	6
14	Breeding line	14	18	16	10	35	65	50	10	23	33	21	9	37	23	27	53	47	70	50	71	96	58	8	7	8	7	6
15	Tore	9	14	12	15	26	63	44	16	38	57	37	11	43	27	30	55	47	72	53	75	92	65	7	5	4	5	6
16	Breeding line	15	17	15	18	16	54	35	11	29	36	26	9	38	24	25	52	47	70	48	67	90	59	7	6	7	7	6
17	KWS Olof	10	15	12	9	10	16	13	5	16	23	15	5	17	11	13	59	47	73	54	69	80	61	4	4	8	5	2
18	Breeding line	14	20	19	11	18	51	35	10	32	41	27	12	32	22	26	49	45	68	47	63	86	59	9	8	6	7	6
19	Breeding line	19	18	19	14	12	29	20	9	25	34	22	4	24	14	19	50	45	70	48	64	91	59	8	NA	3	NA	6
20	Tiril	28	30	29	21	38	73	56	24	53	71	49	14	61	38	43	48	44	67	47	67	91	58	8	8	6	7	6
21	Breeding line	10	19	15	15	17	40	29	7	26	39	24	4	20	12	20	49	46	68	47	67	87	57	7	5	6	6	6
22	Herta	13	20	16	12	19	54	37	17	35	NA	NA	7	32	19	NA	55	47	71	53	84	94	72	7	5	7	6	2
23	Breeding line	13	27	20	22	21	45	33	15	30	51	32	7	32	20	26	49	45	68	48	70	90	60	9	7	7	7	6
24	Juli	7	18	13	6	18	31	25	7	36	NA	NA	4	31	18	NA	52	49	73	57	80	108	96	6	4	3	4	6

25	Breeding line	18	21	20	9	15	28	22	8	25	33	20	6	25	15	19	60	49	68	53	70	84	59	4	4	6	5	2
26	Tunga	14	12	13	6	26	58	42	22	45	63	46	14	45	30	33	52	45	70	50	70	97	68	9	8	5	7	6
27	Chevron	5	4	4	5	13	28	20	3	23	25	15	2	30	16	14	54	47	72	53	103	117	96	5	3	2	3	6
28	Birgitta	4	12	8	6	18	34	26	10	30	42	27	5	30	18	20	52	45	71	50	81	102	64	7	3	3	4	2
29	Bonus	10	11	11	9	16	51	34	12	53	NA	NA	6	43	25	NA	57	48	71	54	77	88	67	9	7	8	8	2
30	Nordlys	14	25	20	7	25	62	43	20	49	65	39	8	46	28	32	45	42	64	46	68	99	59	9	8	6	7	6
31	Breeding line	13	22	17	12	18	41	30	17	32	46	32	16	52	34	28	50	44	69	47	68	97	64	8	8	5	7	6
32	Breeding line	11	17	14	19	34	71	52	19	42	67	44	16	46	31	36	52	47	70	49	76	95	64	9	8	7	8	6
33	Breeding line	10	21	16	17	23	51	37	12	40	55	36	9	31	20	27	50	47	68	48	68	98	64	8	6	8	7	6
34	Thule	12	13	12	11	19	46	33	13	35	47	31	10	38	24	25	53	47	70	50	68	94	64	7	6	4	6	6
35	Breeding line	6	10	8	13	14	36	24	10	32	43	28	10	43	27	22	51	46	69	48	69	88	56	9	8	6	8	6
36	Breeding line	14	19	17	10	21	49	34	11	32	48	31	8	42	25	27	53	46	70	50	69	87	61	6	6	4	5	6
37	Breeding line	22	28	24	15	20	53	37	15	43	58	40	13	49	31	33	49	44	69	47	67	83	51	9	8	5	7	2
38	Breeding line	23	27	25	9	27	65	47	16	37	51	36	8	43	25	33	52	47	70	49	70	93	60	8	9	6	8	6
39	Breeding line	10	24	18	18	17	56	37	11	28	43	28	8	34	21	26	51	44	69	48	69	94	59	6	7	5	6	6
40	Bode	18	11	14	7	20	32	26	9	33	33	23	6	32	19	20	50	45	68	49	77	99	63	9	8	4	7	6
41	Breeding line	10	16	14	9	16	33	26	12	29	40	27	7	28	18	21	51	47	70	48	72	104	67	8	8	6	7	6
42	Breeding line	13	30	22	19	23	60	42	9	35	44	29	9	39	24	29	49	47	67	48	60	83	52	8	6	6	7	6
43	Brage	16	16	16	10	22	50	37	12	33	48	30	14	39	26	27	51	46	69	49	71	91	64	6	4	5	5	6
44	Breeding line	21	26	23	15	15	40	27	9	27	34	24	9	36	22	24	48	40	67	47	71	89	59	8	5	4	6	6
45	Gull	13	14	13	11	21	46	33	9	26	55	39	9	36	23	27	49	45	71	50	84	94	72	8	3	3	4	2
46	Seger	10	15	14	7	10	29	19	6	22	35	19	5	23	14	17	52	46	70	51	93	101	78	5	5	2	4	2
47	Breeding line	18	21	19	21	17	38	27	14	34	49	32	8	37	23	25	49	44	66	47	72	92	58	8	8	6	7	6
48	Breeding line	10	16	13	15	19	36	28	9	24	36	22	5	23	14	19	49	47	70	49	69	89	56	7	7	7	7	6
49	Tyra	18	24	21	15	26	56	41	21	42	62	43	13	53	33	34	50	45	70	47	69	85	54	9	7	8	8	2
50	Aktiv	22	26	24	23	30	73	50	9	23	34	22	NA	NA	NA	NA	56	47	71	49	75	87	61	4	3	4	3	2
51	Breeding line	14	9	12	6	13	30	22	7	27	35	23	4	23	12	17	54	46	70	49	68	97	62	9	4	3	5	6
52	Breeding line	15	16	15	6	23	46	34	11	29	51	30	11	42	26	27	53	46	70	50	69	92	57	9	7	4	6	6
53	Tocada	10	15	13	10	16	25	21	10	29	34	24	8	27	17	19	58	48	72	51	69	84	59	6	7	8	7	2
54	Breeding line	12	21	21	21	18	42	30	15	38	58	37	9	37	23	28	51	46	69	49	68	94	60	8	7	7	7	6
55	Axelina	30	31	30	13	32	64	48	15	43	62	40	13	41	27	37	51	47	71	48	76	89	64	9	9	7	8	2
56	Henni	15	20	17	21	37	57	46	15	29	50	31	6	23	14	27	58	49	72	52	66	85	54	6	6	7	6	2
57	Breeding line	13	13	13	13	26	48	37	12	31	44	29	12	40	26	26	52	47	70	49	65	88	62	9	5	3	6	6
58	Forus	20	19	23	13	16	45	31	11	31	43	27	5	39	22	26	54	49	71	53	71	90	64	8	8	6	7	6
59	H354-333-7-5	21	31	31	25	46	80	63	12	40	NA	NA	14	47	31	NA	47	42	67	46	63	85	57	9	9	8	8	6
60	Atlas 46	11	17	14	12	11	27	19	11	39	72	52	6	24	15	25	51	45	67	46	84	95	69	5	7	2	5	6
61	Breeding line	12	31	22	15	21	36	28	17	35	42	31	9	40	24	26	54	48	71	50	71	90	59	8	7	7	7	6
62	Golf	12	20	16	3	24	51	37	6	21	38	22	5	33	19	24	58	47	72	57	73	84	58	5	4	7	5	2
63	Herse	20	18	19	10	21	46	33	8	39	54	30	10	35	22	26	48	44	68	49	76	104	70	7	7	5	6	6
64	Yrjar	10	14	12	11	30	58	44	18	41	58	40	14	41	28	31	49	45	66	48	73	110	65	8	8	5	7	6

65	Breeding line	17	24	21	16	32	73	53	19	46	68	45	22	73	48	42	48	45	67	47	67	91	60	8	7	6	7	6
66	Rambler	17	25	21	23	15	28	21	14	35	46	31	6	31	19	23	53	46	71	50	65	82	56	7	8	6	7	2
67	Breeding line	27	60	44	14	35	57	46	16	34	54	35	16	54	35	40	47	44	67	47	63	96	60	8	6	6	7	6
68	Breeding line	9	18	13	17	19	48	33	16	38	47	34	10	34	22	25	50	46	69	47	66	93	53	8	8	6	7	6
69	Breeding line	24	27	26	22	20	55	38	13	32	49	30	10	35	22	29	51	44	69	48	66	92	62	8	8	5	7	6
70	Breeding line	10	19	15	14	13	19	16	9	29	37	25	7	30	18	18	49	45	69	48	68	91	56	8	7	5	7	6
71	Breeding line	18	21	20	15	17	49	35	7	19	30	20	6	25	16	23	50	44	68	48	64	90	61	2	2	1	2	6
72	H82009-1-2	NA	NA	NA	NA	22	48	35	19	43	68	43	13	41	27	NA	NA	49	73	59	62	65	49	7	8	7	7	6
73	Breeding line	6	15	11	7	17	29	23	11	27	36	25	5	30	17	19	49	45	68	48	72	99	62	3	4	2	3	6
74	CI11577	5	10	7	5	13	42	28	10	25	NA	NA	6	19	13	NA	51	47	69	49	79	90	65	6	8	5	6	6
75	Jotun	13	NA	NA	10	21	50	36	5	37	73	38	6	33	19	NA	45	42	66	48	85	111	75	4	5	2	4	6
76	Meltan	9	22	16	12	20	53	37	15	30	40	29	10	36	23	26	57	47	70	48	67	77	54	8	7	6	7	2
77	Asahi 5	18	16	17	8	10	19	15	8	29	38	25	3	21	12	17	44	39	65	42	73	105	64	2	4	1	2	2
78	Seijo 17	14	19	17	11	11	24	18	7	20	41	21	2	16	9	16	46	38	64	41	69	98	63	3	3	2	3	2
79	Marigold	16	23	20	11	20	45	32	9	26	39	24	9	35	21	25	54	47	71	49	70	85	56	5	4	7	5	2
80	Vada	19	20	19	11	15	35	24	7	29	37	24	0	22	11	20	54	48	72	56	79	89	70	2	3	3	3	2
81	Fager	18	22	21	15	28	37	32	12	34	33	23	6	34	20	24	49	45	68	47	68	91	63	7	5	6	6	6
82	Tammi	6	NA	NA	6	20	40	30	11	32	65	36	9	40	25	NA	43	40	62	43	77	104	67	8	7	4	7	6
83	Arve	10	21	15	14	21	29	24	10	28	46	28	6	32	19	21	48	43	66	46	67	96	60	7	3	2	4	6
84	Opal	11	12	12	5	15	32	24	11	26	NA	NA	7	31	19	NA	52	45	71	50	86	93	71	8	2	6	5	2
85	Breeding line	11	13	12	13	15	37	25	11	33	42	29	5	27	16	21	52	47	68	49	68	94	61	8	7	7	7	6
86	Einar	11	29	19	17	18	26	22	12	35	42	29	10	41	25	24	50	45	69	48	68	96	59	8	6	6	6	6
87	Uforædlet Jämtland	9	20	15	9	12	21	17	7	32	NA	NA	12	45	29	NA	47	41	67	48	86	106	71	8	8	5	7	6
88	Kinnan	13	17	16	9	16	29	23	8	34	43	28	7	33	20	22	52	46	71	48	64	94	57	8	6	7	7	2
89	Vilde	10	20	15	14	29	68	50	15	37	53	35	14	41	27	32	51	46	69	48	61	90	60	9	8	7	8	6
90	Polarbygg	19	NA	NA	12	18	51	35	8	50	82	47	13	51	32	NA	46	41	65	46	81	108	71	9	NA	4	NA	6
91	Delphi	19	23	20	10	18	45	31	5	15	28	16	3	18	10	19	55	48	73	52	67	78	55	7	4	6	6	2
92	Vera	14	21	17	9	24	39	31	8	26	45	29	4	28	16	23	45	43	66	45	71	97	65	6	2	2	3	6
93	Ven	19	21	21	17	31	66	48	20	39	57	40	10	42	26	34	54	47	71	51	69	91	57	8	8	6	7	6
94	Otra	15	14	15	8	15	32	24	11	58	75	47	11	41	26	28	46	42	66	45	87	111	74	8	5	2	5	6
95	Breeding line	14	16	16	10	20	37	29	15	34	43	30	8	36	22	24	51	47	71	49	76	92	62	7	5	3	5	6
96	Breeding line	19	21	20	12	25	46	38	13	30	NA	NA	9	38	24	NA	52	45	70	48	72	93	60	7	7	7	7	6
97	Vega ABED	13	15	14	5	20	49	34	12	32	44	29	9	40	24	26	60	49	74	59	78	89	67	9	7	6	7	2
98	Stine	14	19	17	11	15	38	27	4	26	30	20	7	27	17	20	52	46	72	49	67	84	62	3	3	3	3	2
99	Lavrans	8	7	6	8	17	35	26	6	23	35	22	6	22	14	17	48	44	67	46	67	92	66	4	3	2	3	6
100	Varde	17	NA	NA	5	33	50	42	13	31	NA	NA	11	46	28	NA	47	45	67	48	81	102	67	9	7	3	6	6
101	Mari	16	22	19	23	21	53	37	17	48	60	41	11	56	33	33	50	43	69	47	63	84	54	9	7	8	8	2
102	Breeding line	11	17	14	21	32	64	48	13	37	61	37	16	50	33	33	52	47	70	49	62	83	53	9	7	6	7	6
103	Audrey	6	9	8	7	16	31	23	2	19	29	16	4	15	9	14	56	46	71	51	69	83	60	8	5	10	8	2
104	Dønnes	15	25	21	7	15	52	32	13	37	NA	NA	5	30	17	NA	43	41	62	45	73	98	69	8	5	2	5	6

105	Breeding line	11	12	11	15	28	55	41	16	37	55	36	9	41	25	28	52	46	70	48	64	86	55	9	7	6	7	6
106	Breeding line	12	18	15	13	14	42	28	12	29	38	27	13	36	24	23	53	46	70	50	68	96	56	8	6	4	6	6
107	Breeding line	9	9	9	12	14	28	20	6	23	33	20	3	20	12	15	52	46	70	50	63	90	56	7	5	5	6	6
108	Breeding line	5	7	6	6	12	20	16	7	21	29	19	3	14	9	12	52	47	71	50	71	98	64	7	6	6	6	6
109	Jyvä	28	38	34	17	21	59	40	10	37	58	35	7	48	28	34	46	41	66	46	68	86	62	8	6	4	6	6
110	Maskin	21	33	29	11	18	57	39	9	40	63	38	7	37	22	32	46	42	67	47	93	111	75	4	4	2	3	6
111	Saana	14	36	30	25	33	49	40	16	41	52	36	16	45	30	34	52	47	70	48	66	84	51	9	8	5	7	2
112	Habil	13	17	16	12	14	37	26	8	25	40	25	4	31	17	21	53	47	69	50	69	93	60	7	4	2	5	6
113	Gunilla	12	9	10	7	17	46	31	14	48	49	38	9	41	25	26	52	45	69	48	72	92	61	8	6	7	7	2
114	Breeding line	10	18	13	17	20	37	28	14	32	41	29	7	31	19	22	49	46	68	48	70	92	60	8	7	5	7	6
115	Breeding line	9	7	8	7	11	28	20	9	28	39	25	5	25	15	17	51	47	69	50	70	88	60	9	7	4	7	6
116	Etu	21	23	22	14	26	36	31	15	45	52	36	12	49	30	30	49	45	68	48	64	85	57	8	8	5	7	6
117	Breeding line	16	24	19	14	18	33	26	14	34	56	35	7	34	21	25	48	44	68	48	63	87	60	7	4	7	6	6
118	Breeding line	40	NA	NA	24	51	77	64	23	52	74	50	17	61	39	NA	47	43	67	47	62	89	57	8	7	5	7	6
119	Olsok	26	NA	NA	13	18	40	28	11	28	41	27	6	28	17	NA	48	44	68	47	70	93	60	7	4	2	4	6
120	Breeding line	11	11	10	15	23	51	38	12	29	41	26	9	27	18	23	48	45	69	47	69	90	59	9	7	8	8	6
121	Pallas	10	22	16	11	20	55	38	10	33	45	32	11	35	23	27	57	47	70	50	75	87	66	9	6	5	7	2
122	Breeding line	11	12	11	12	20	41	31	16	40	50	36	11	37	24	25	55	48	71	52	73	94	66	8	7	5	7	6
123	Fairytale	14	19	16	15	17	40	28	10	31	41	27	7	29	18	22	54	47	71	51	68	83	57	4	3	5	4	2
124	Trysil	8	14	12	5	14	30	21	4	31	33	21	7	37	22	19	52	48	73	57	95	94	90	4	2	2	3	6
125	Breeding line	7	16	12	13	19	37	28	16	35	43	31	9	37	23	24	49	45	69	48	69	93	59	8	6	7	7	6
126	Maja	10	21	16	10	15	32	24	4	27	33	20	6	33	19	20	55	46	71	55	76	92	67	9	6	4	6	2
127	Breeding line	18	34	26	21	22	52	37	14	34	61	37	15	42	28	32	51	47	70	48	66	94	54	9	8	7	8	6
128	H572-8	16	15	16	10	23	48	36	13	31	47	31	7	27	17	25	62	49	74	57	78	75	59	5	7	8	6	2
129	Vigdis	17	22	27	12	18	46	32	12	33	NA	NA	12	38	25	NA	51	45	68	49	76	103	68	9	6	6	7	6
130	Møyar	8	12	10	6	18	50	34	11	32	39	27	7	30	19	22	55	46	71	53	82	86	66	8	6	7	7	2
131	Breeding line	9	19	14	12	14	30	22	10	29	41	27	6	32	19	20	54	48	71	51	72	99	61	6	6	3	5	6
132	Bjørneby	6	9	6	11	13	33	23	9	46	79	45	6	34	20	24	47	44	70	48	81	103	74	3	2	2	2	6
133	Breeding line	34	31	34	17	29	65	47	14	36	54	36	10	46	28	36	49	46	69	49	71	95	63	9	8	6	8	6
134	Gustav	11	19	15	16	23	64	44	10	33	45	30	6	42	24	28	57	47	71	49	57	73	50	3	2	4	3	2
135	Frisco	23	16	19	18	60	76	68	28	51	56	45	10	31	21	38	54	47	73	50	65	75	56	8	5	8	7	2
136	Vena	15	14	17	11	21	55	38	18	46	59	41	13	44	29	31	49	46	70	50	77	102	70	8	6	5	7	6
137	Stolt	12	21	17	11	19	40	30	14	31	42	29	8	27	17	23	49	44	69	47	69	88	63	8	7	7	8	6
138	Breeding line	12	18	15	16	52	81	67	27	58	73	52	21	55	38	43	50	46	66	48	62	88	60	9	9	6	8	6
139	Olve	9	21	15	9	17	41	29	9	36	49	31	8	32	20	24	47	44	68	47	74	89	59	8	6	7	7	2
140	Jarle	13	16	15	6	20	34	27	14	38	55	36	5	38	21	25	49	46	70	51	91	115	75	8	6	5	7	6
141	Toria	13	22	20	13	18	33	25	10	25	33	23	6	27	17	21	50	46	68	48	62	87	59	7	6	6	6	6
142	Breeding line	39	27	23	21	22	56	39	16	34	50	34	13	43	28	31	50	45	68	48	68	88	54	9	7	7	7	6
143	Balder	8	11	9	8	17	25	21	10	27	37	25	4	29	17	18	54	48	71	55	79	96	70	7	8	7	7	2
144	Bamse	16	26	20	20	27	56	46	6	27	41	24	4	34	19	27	49	44	68	47	74	105	64	6	4	2	4	6
145	Breeding line	9	6	7	12	13	26	21	9	20	32	20	6	21	14	15	51	46	71	49	73	103	66	8	7	4	6	6

146	Harbinger	17	29	23	13	19	27	23	12	31	35	26	5	29	17	22	57	47	70	52	66	76	56	NA	7	6	NA	2
147	IS-046	29	30	32	26	33	70	52	23	62	70	50	15	59	37	43	49	44	67	47	67	86	54	9	8	7	8	2
148	Breeding line	14	14	15	16	25	53	40	20	51	69	45	13	42	27	32	49	45	67	47	73	96	65	9	7	6	7	6
149	Rolfi	46	44	38	18	37	80	59	27	62	81	57	13	49	31	46	47	43	66	46	63	91	54	8	7	3	6	6
150	Breeding line	21	20	19	21	24	49	36	16	36	59	35	9	34	21	28	48	44	67	47	65	96	63	6	5	2	4	6
151	Breeding line	18	34	26	10	23	31	27	15	34	44	31	6	28	16	25	56	48	73	51	67	79	54	9	8	8	8	2
152	Shirley	13	17	15	7	14	31	22	6	19	28	17	2	16	9	16	59	48	73	52	69	82	58	5	2	8	5	2
153	Edvin	13	20	16	16	15	32	24	12	26	41	24	9	32	20	21	52	47	70	50	67	94	64	7	5	5	6	6
154	H82011-2-2	28	24	26	15	26	57	41	26	39	56	41	20	52	36	36	51	47	69	48	61	73	49	7	9	8	8	6
155	Breeding line	17	15	16	18	15	42	29	7	20	32	20	4	26	15	20	49	45	69	49	71	89	61	8	7	8	8	6
156	Breeding line	21	18	20	17	33	71	52	14	34	43	31	3	28	16	29	53	46	71	41	69	81	67	2	2	1	2	2
157	Fræg	27	NA	NA	16	15	39	26	12	38	61	37	9	42	26	NA	47	43	66	46	82	116	72	5	4	2	4	6
158	Jazz	11	20	16	13	15	24	19	8	26	34	23	5	29	17	19	58	48	72	51	71	83	58	6	5	7	6	2
159	Trine	6	12	9	10	24	49	37	14	39	56	36	12	44	28	27	56	48	71	54	71	95	63	8	7	7	7	6
160	Lise	8	11	9	5	29	64	48	16	42	64	40	11	41	26	31	51	47	70	51	85	104	75	9	8	5	7	6
161	Luhkas	12	21	17	19	24	51	37	15	32	37	25	6	27	16	24	57	47	71	50	68	77	52	8	6	9	7	2
162	Gaute	21	19	18	11	33	77	56	21	50	65	45	12	43	27	37	51	48	70	50	73	94	65	9	8	5	7	6
163	Verner	15	24	20	17	18	23	21	7	23	32	21	5	27	16	20	47	42	68	46	75	94	64	8	8	6	7	6
164	Breeding line	19	16	17	13	15	26	21	14	33	42	28	10	35	22	22	50	47	69	48	68	91	56	8	8	4	7	6
165	Breeding line	13	17	15	19	18	41	30	12	31	40	28	8	40	24	24	50	43	69	49	68	100	64	8	5	7	6	6
166	Edel	17	50	32	26	24	61	42	14	38	47	33	14	47	31	34	54	47	71	50	68	96	60	7	8	7	7	6
167	Breeding line	14	21	16	19	20	32	26	7	27	44	26	7	32	20	22	58	47	72	53	73	92	60	9	8	8	8	2
168	Ingrid	20	16	18	7	22	35	29	5	29	39	25	3	19	11	21	55	46	71	53	82	88	68	9	6	7	7	2
169	Agneta	12	17	13	11	10	NA	NA	9	25	45	29	5	29	17	NA	49	42	68	47	71	105	69	8	4	2	5	6
170	Rigel	27	13	20	8	15	32	24	5	27	33	21	6	33	20	21	52	47	71	51	80	94	76	9	5	6	7	2
171	Fredrickson	8	10	9	6	13	36	24	9	29	39	25	5	27	16	19	52	48	71	54	103	117	90	8	6	5	6	2
172	Heder	45	50	46	23	22	46	34	10	25	32	21	8	37	23	31	48	44	67	47	64	90	60	8	5	6	6	6
173	Breeding line	13	16	15	14	18	30	24	8	32	48	29	7	33	20	22	50	45	69	48	65	92	61	7	6	6	6	6
174	Barke	37	30	34	15	23	48	35	15	37	NA	NA	8	36	22	NA	55	48	73	53	70	83	64	9	6	8	7	2
175	Baronesse	13	28	20	13	17	37	27	12	32	41	28	4	32	18	23	56	47	71	51	70	86	63	3	2	3	2	2
176	H3003	12	18	15	15	19	45	32	9	29	41	26	7	45	26	25	52	46	71	48	61	76	50	9	5	7	7	2
177	Breeding line	10	24	17	18	10	29	19	9	26	37	24	7	32	19	20	49	43	68	48	68	94	57	7	7	5	6	6
178	Pernilla	11	17	14	9	26	55	40	18	34	51	34	7	32	20	27	50	44	70	49	77	89	63	9	5	6	6	2
179	CIho4196	9	21	15	10	19	35	27	11	29	57	33	5	35	20	24	54	48	70	52	107	115	90	9	7	6	7	2
180	Breeding line	13	20	16	16	13	29	20	5	29	34	22	5	27	16	19	56	48	73	52	73	83	58	3	3	5	4	2
181	Delibes	15	24	19	23	30	65	47	17	39	54	36	3	33	18	30	56	50	73	57	66	79	61	4	4	6	5	2
182	Svanhals	7	13	10	6	27	53	40	17	35	NA	NA	6	38	22	NA	52	46	70	51	94	118	86	8	9	6	7	2
183	Olli	8	NA	NA	7	12	13	12	11	48	NA	NA	9	42	25	NA	42	40	61	43	75	98	68	8	7	4	6	6
184	Breeding line	14	20	17	16	12	NA	NA	7	24	35	22	3	24	11	NA	51	45	69	49	69	82	62	4	4	2	3	6
185	Kunnari	13	18	16	12	15	19	16	8	27	41	26	8	35	21	20	49	45	69	47	71	91	62	6	4	2	4	6
186	Binder	12	16	14	12	21	42	31	9	25	30	21	5	33	19	21	56	46	72	54	92	91	76	5	5	5	5	2

187	Atlas	10	18	15	14	13	23	18	8	25	66	32	8	34	21	21	49	45	65	45	74	93	62	2	3	1	2	6
188	Helium	13	24	18	15	38	73	55	16	40	50	35	12	42	27	34	57	47	71	50	66	76	55	5	3	5	4	2
189	Clermont	16	18	17	19	30	62	46	13	42	52	37	9	42	25	31	61	49	72	58	79	101	71	6	6	4	5	6
190	Breeding line	23	25	24	17	12	42	27	8	28	36	24	6	29	18	23	51	46	70	49	70	92	57	8	7	4	6	6
191	Vega	NA	NA	NA	NA	14	28	21	4	22	NA	NA	4	38	21	NA	NA	42	67	49	97	117	74	7	6	4	5	6
192	Breeding line	17	20	19	12	20	39	29	12	28	40	26	9	29	19	23	52	45	70	50	72	96	60	7	8	6	7	6
193	Breeding line	16	34	27	18	25	53	39	15	36	46	33	15	39	27	32	49	45	69	47	68	93	59	9	8	7	8	6
194	Dore	15	14	15	9	13	19	16	5	29	49	27	5	29	17	19	45	39	65	44	79	109	69	7	7	4	6	6
195	Iver	20	21	22	14	27	66	46	22	40	60	40	13	57	35	36	50	44	70	47	68	81	55	9	7	6	7	2
196	Malz	12	15	14	13	14	24	19	5	20	26	17	3	20	12	15	55	47	71	50	61	82	58	7	5	7	7	2
197	Breeding line	10	19	15	16	34	57	45	10	31	42	28	8	36	22	27	54	47	70	50	70	93	57	4	4	2	3	6
198	Breeding line	14	20	18	16	16	34	25	12	32	34	21	9	35	22	21	49	45	67	47	64	93	59	8	7	4	6	6
199	Triumph	16	15	15	18	18	50	35	8	38	49	32	9	34	21	26	55	46	71	50	77	85	65	8	6	8	7	2
200	Sunnita	10	12	12	8	18	39	28	12	31	45	29	6	36	21	23	50	45	70	50	72	88	60	8	6	6	7	2
201	Quench	NA	NA	NA	NA	NA	NA	NA	15	36	43	32	8	28	18	NA	NA	NA	71	51	NA	75	58	NA	NA	NA	NA	2
202	Breeding line	8	13	10	9	14	42	28	8	40	70	43	9	37	23	26	48	40	66	45	73	100	67	8	7	4	6	6
203	Iron	11	25	18	9	16	23	19	9	21	27	19	3	21	12	17	56	46	72	48	67	85	61	6	5	6	6	2
204	Gammel dansk	7	12	9	10	18	26	22	4	34	39	25	4	24	14	17	61	52	78	61	88	96	76	6	6	5	6	2
205	Breeding line	11	14	11	12	13	41	27	11	36	50	32	9	38	24	24	51	44	69	48	72	91	58	7	7	4	6	6
206	Breeding line	12	12	12	9	47	69	58	12	32	40	26	11	32	22	30	52	46	70	49	67	94	60	8	7	7	7	6
207	Breeding line	24	33	29	11	21	42	31	13	35	NA	NA	12	48	30	NA	50	46	68	48	64	86	54	8	6	4	6	6
208	Domen	28	35	32	12	16	25	20	9	28	35	24	5	25	15	23	56	49	72	54	86	100	76	7	5	8	6	2
209	Breeding line	21	20	21	15	22	62	42	19	45	65	44	11	62	37	36	50	48	69	49	71	94	59	8	7	7	7	6

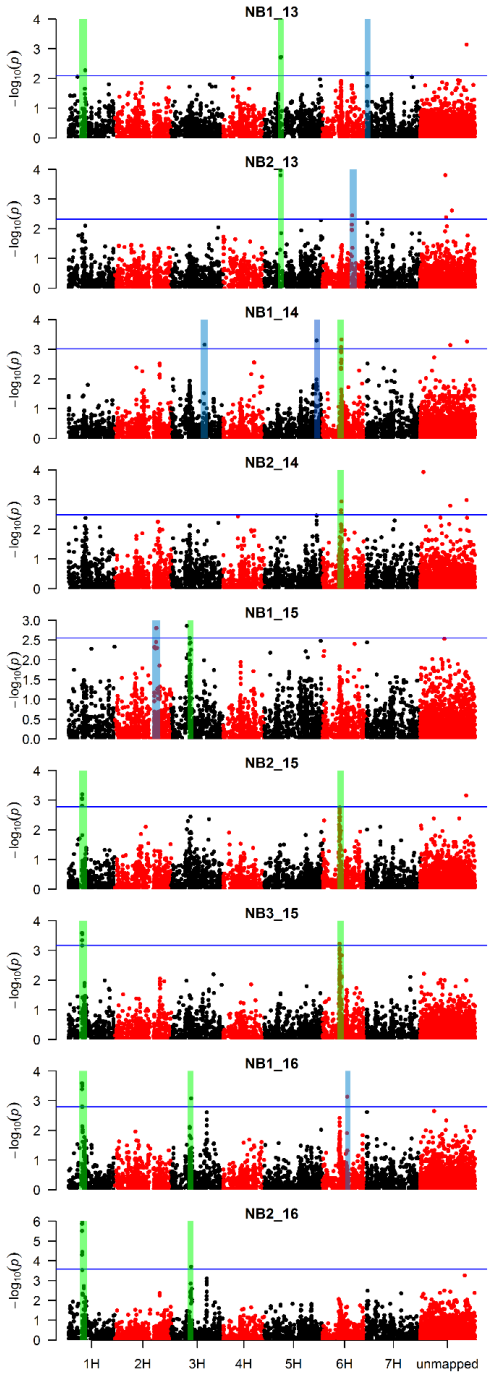
S4 Overview of markers used for association mapping of net form net blotch resistance. Marker positions refer to the barley consensus map by Muñoz-Amatriaín et al. (2014)

NB: This file is too large to be printed. Please refer to supplementary table S3 by Muñoz-Amatriaín et al. (2014) instead

S5 MTA significance thresholds for all traits according to Chan et al. (2010). The 0.1 percentile quantile of marker p-values were considered significant and are given together with the –log(10)-transformed p-value

Trait	0.1 percentile quantile	-log(10) p-value
Seedling inoculation with isolate:		
LR9	0.000749	3.1
5050B	0.000299	3.5
6949B	0.000196	3.7
Field trial, first scoring 2013	0.008096	2.1
Field trial, second scoring 2013	0.004785	2.3
Field trial, average 2013	0.006824	2.2
Field trial, 2013, uninoculated	0.002764	2.6
Field trial, first scoring 2014	0.000959	3.0
Field trial, second scoring 2014	0.003248	2.5
Field trial, average 2014	0.000287	3.5
Field trial, first scoring 2015	0.00283	2.5
Field trial, second scoring 2015	0.001687	2.8
Field trial, third scoring 2015	0.000675	3.2
Field trial, average 2015	0.001731	2.8
Field trial, first score 2016	0.001627	2.3
Field trial, second score 2016	0.000262	3.6
Field trial, average 2016	0.000137	3.9
Days to heading 2013	0.000458	3.3
Days to heading 2014	0.000767	3.1
Days to heading 2015	9.78E-06	5.0
Days to heading 2016	4.56E-08	7.3
Plant height 2014	2.29E-05	4.6
Plant height 2015	8.03E-06	5.1
Plant height 2016	8.7E-06	5.1
Spike row number	2.1E-08	7.7

S6 Association mapping of net blotch scorings using the MLM + K + Q model. The panels show Manhattan plots for all scorings of adult plant reactions in field trials inoculated with LR9, 5050B and 6949B in all four years. Vertical axes show the –log(10) value of MTA p-values. Dots above the horizontal lines represent MTAs with a p-value within the 0.1 percentile quantile and are considered significant. Green vertical bars indicate QTL significant in more than one trial, blue vertical bars indicate QTL only found in one trial



S7 SNP markers significantly associated with adult NB resistance under field conditions in all scorings different years

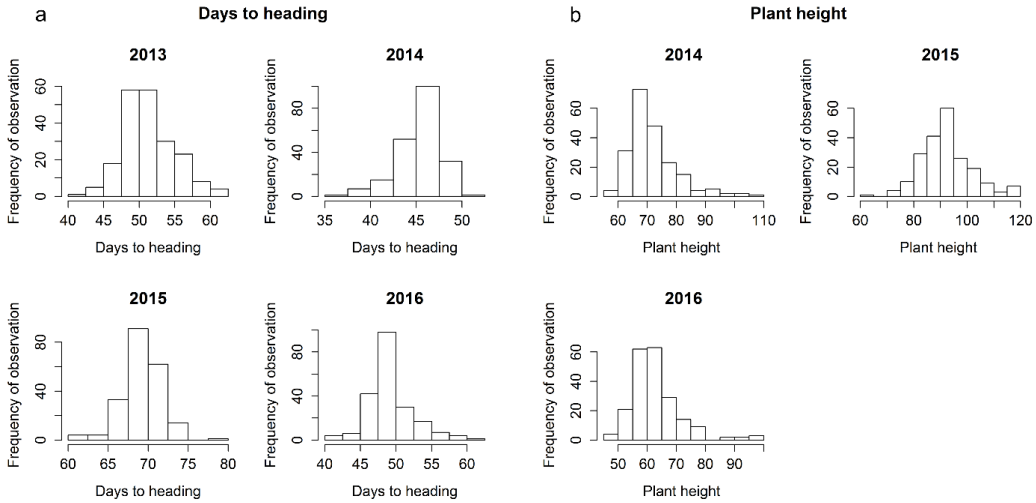
Marker	QTL name	Chr.	Pos. [a]	POPseq pos. [b]	R^2 [c]	MAF	p-value	-log10 (p-value)
2013 - First scoring								
11_21333	*NBP_QRptt1-1*	1	53.3	52.55	0.05	0.471	0.00526	2.3
SCRI_RS_221999	*NBP_QRptt5-1*	5	51.73	55.62	0.06	0.29	0.0019	2.7
SCRI_RS_205235	*NBP_QRptt5-1*	5	51.83		0.06	0.292	0.00198	2.7
12_20350	*NBP_QRptt5-1*	5	53.77		0.06	0.121	0.00187	2.7
11_20710		7	2.47	1.91	0.05	0.13	0.00674	2.2
SCRI_RS_222377		5		107.08	0.07	0.123	7.22E-04	3.1
2013 - Second scoring								
SCRI_RS_221999	*NBP_QRptt5-1*	5	51.73	55.62	0.10	0.29	1.06E-04	4.0
SCRI_RS_205235	*NBP_QRptt5-1*	5	51.83		0.09	0.292	1.58E-04	3.8
SCRI_RS_169374		6	93.22	87.31	0.06	0.211	0.00351	2.5
SCRI_RS_160179					0.09	0.104	1.57E-04	3.8
SCRI_RS_161652		6		85.84	0.06	0.237	0.00409	2.4
SCRI_RS_175709		6		87.76	0.06	0.208	0.00243	2.6
2014 - First scoring								
11_10584		3	105.98	101.86	0.07	0.456	7.00E-04	3.2
12_20867	*NBP_QRptt5-2*	5	165.57	155.56	0.08	0.104	5.06E-04	3.3
12_30120	*NBP_QRptt6-1*	6	58.34	55.03	0.07	0.162	8.37E-04	3.1
SCRI_RS_186193	*NBP_QRptt6-1*	6	59.21	55.38	0.08	0.115	4.68E-04	3.3
SCRI_RS_171997	*NBP_QRptt6-1*	6	59.21	55.38	0.07	0.417	7.20E-04	3.1
SCRI_RS_224245		2		6.3	0.08	0.063	5.48E-04	3.3
2014 - Second scoring								
SCRI_RS_219810	*NBP_QRptt6-1*	6	58.34	53.90	0.06	0.165	0.00229	2.6
12_30120	*NBP_QRptt6-1*	6	58.34	55.03	0.06	0.162	0.00278	2.6
SCRI_RS_186193	*NBP_QRptt6-1*	6	59.21	55.38	0.07	0.115	0.00115	2.9
12_30115		8			0.09	0.446	1.18E-04	3.9
SCRI_RS_171997	*NBP_QRptt6-1*	6	59.21	55.38	0.07	0.417	0.00161	2.8
SCRI_RS_222377		5		107.08	0.07	0.123	0.00104	3.0
2015 - First scoring								
SCRI_RS_151349		2	131.66	109.28	0.06	0.191	0.00158	2.8
SCRI_RS_610		2	131.86	108.22	0.06	0.191	0.00158	2.8
11_10404		2	132.76	110.20	0.06	0.191	0.00158	2.8
SCRI_RS_199987	*NBP_QRptt3-2*	3	49.55		0.07	0.444	0.0014	2.9
2015 - Second scoring								
SCRI_RS_170878	*NBP_QRptt1-1*	1	43.41	42.21	0.07	0.434	8.65E-04	3.1
SCRI_RS_170869	*NBP_QRptt1-1*	1	43.41	42.21	0.07	0.449	9.17E-04	3.0
SCRI_RS_153785	*NBP_QRptt1-1*	1	43.41	41.64	0.06	0.452	0.00156	2.8
11_10764	*NBP_QRptt1-1*	1	43.62	42.35	0.07	0.437	6.36E-04	3.2

SCRI_RS_189483	*NBP_QRptt1-1*	1	43.62	42.35	0.07	0.44	8.79E-04	3.1
SCRI_RS_221644		3		52.03	0.07	0.169	6.97E-04	3.2
2015 - Third scoring								
SCRI_RS_170878	*NBP_QRptt1-1*	1	43.41	42.21	0.09	0.434	2.59E-04	3.6
SCRI_RS_170869	*NBP_QRptt1-1*	1	43.41	42.21	0.08	0.449	4.59E-04	3.3
11_10764	*NBP_QRptt1-1*	1	43.62	42.35	0.09	0.437	2.85E-04	3.5
SCRI_RS_189483	*NBP_QRptt1-1*	1	43.62	42.35	0.08	0.44	4.55E-04	3.3
SCRI_RS_210025	*NBP_QRptt6-1*	6	54.1	49.08	0.08	0.421	5.95E-04	3.2
SCRI_RS_143259	*NBP_QRptt6-1*	6	54.1	48.94	0.08	0.422	6.57E-04	3.2
2016- First scoring								
SCRI_RS_170878	*NBP_QRptt1-1*	1	43.41	42.21	0.09	0.434	2.60E-04	3.6
SCRI_RS_153785	*NBP_QRptt1-1*	1	43.41	41.64	0.08	0.452	4.11E-04	3.4
SCRI_RS_170869	*NBP_QRptt1-1*	1	43.41	42.21	0.06	0.449	0.00158	2.8
11_10764	*NBP_QRptt1-1*	1	43.62	42.35	0.08	0.437	3.17E-04	3.5
11_10281	*NBP_QRptt3-2*	3	62.97	54.53	0.07	0.256	8.37E-04	3.1
SCRI_RS_231790		6	77.70		0.07	0.451	7.44E-04	3.1
2016 - Second scoring								
SCRI_RS_170878	*NBP_QRptt1-1*	1	43.41	42.21	0.14	0.434	1.36E-06	5.9
SCRI_RS_153785	*NBP_QRptt1-1*	1	43.41	41.64	0.13	0.452	3.09E-06	5.5
SCRI_RS_170869	*NBP_QRptt1-1*	1	43.41	42.21	0.10	0.449	4.93E-05	4.3
11_10764	*NBP_QRptt1-1*	1	43.62	42.35	0.14	0.437	1.14E-06	5.9
SCRI_RS_189483	*NBP_QRptt1-1*	1	43.62	42.35	0.11	0.44	3.59E-05	4.4
11_10281	*NBP_QRptt3-2*	3	62.97	54.53	0.09	0.256	2.01E-04	3.7

S8 Estimation of mean, minimum and maximum values for disease resistance in adult plants (shown as % diseased leaf area) and seedlings (scores on the Tekauz scale), DH (days) and PH (cm)

Trait	Mean	Min	Max
Seedling inoculation with isolate:			
LR9	7.1	1.8	9.5
5050B	5.9	1.8	8.8
6949B	5.1	1.2	9.0
Field trial, first scoring 2013	15	3	46
Field trial, second scoring 2013	20	5	60
Field trial, average 2013	17	6	45
Field trial, 2013, uninoculated	13	4	27
Field trial, first scoring 2014	21	8	60
Field trial, second scoring 2014	44	15	83
Field trial, average 2014	32	11	69
Field trial, first scoring 2015	12	2	28
Field trial, second scoring 2015	33	15	63
Field trial, third scoring 2015	46	23	83
Field trial, average 2015	30	15	57
Field trial, first score 2016	8	1	23
Field trial, second score 2016	35	14	73
Field trial, average 2016	22	9	48
DH 2013	51	42	62
DH 2014	46	35	52
DH 2015	69	61	78
DH 2016	49	41	61
PH 2014	72	58	108
PH 2015	92	64	118
PH 2016	63	49	96

S9 Frequency distributions for DH (a) and PH (b) in the AM panel



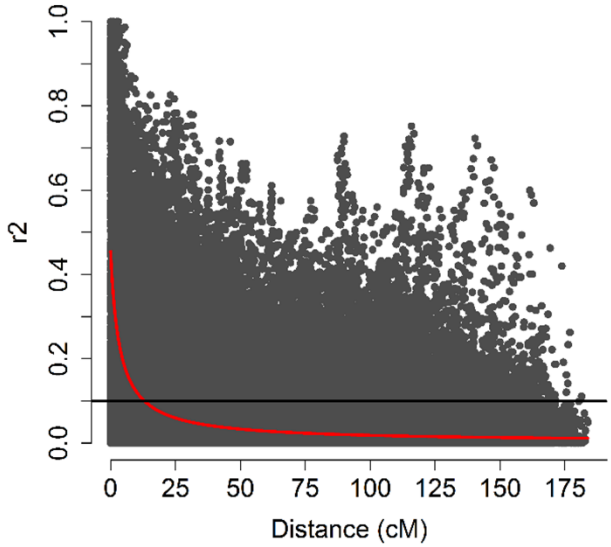
S10 Overview of numbers of mapped and unmapped markers passing different quality checks

	Mapped markers	Unmapped markers	Total number of markers
Total	5200	2664	7864
Less than 10% missing data	4961	1874	6835
Polymorphic	4656	1735	6391
MAF > 0.05	4149	1520	5669

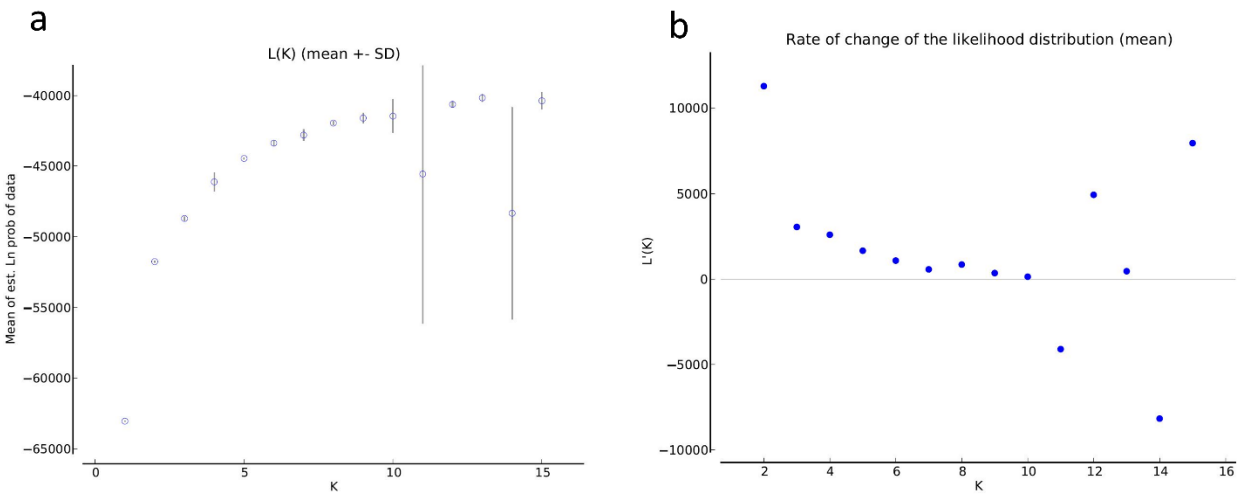
S11 SNP coverage and distribution across all chromosomes after filtering

Chromosome	cM	Markers	Marker coverage (cM/marker)
1H	144.52	402	0.36
2H	178.33	654	0.27
3H	164.42	634	0.26
4H	129.27	445	0.29
5H	183.83	847	0.22
6H	139.39	591	0.24
7H	168.94	575	0.29
Total	1108.70	4148	0.27

S12 Intra-chromosomal LD decay (r^2) of marker pairs averaged over all chromosomes. A critical r^2 value beyond which LD is assumed to be due to genetic linkage was arbitrarily set to 0.1 (black line). The red curve shows the LD decay as a function of genetic distance (cM)



S13 Estimated population structure of the AM panel. a: Mean log likelihood of the data [L(K)]. b: Δk method suggesting k=2 as the best k.



S14 Membership coefficients for subpopulation 1 (Q1) and 2 (Q2) based on which the individual lines were assigned to a subpopulation

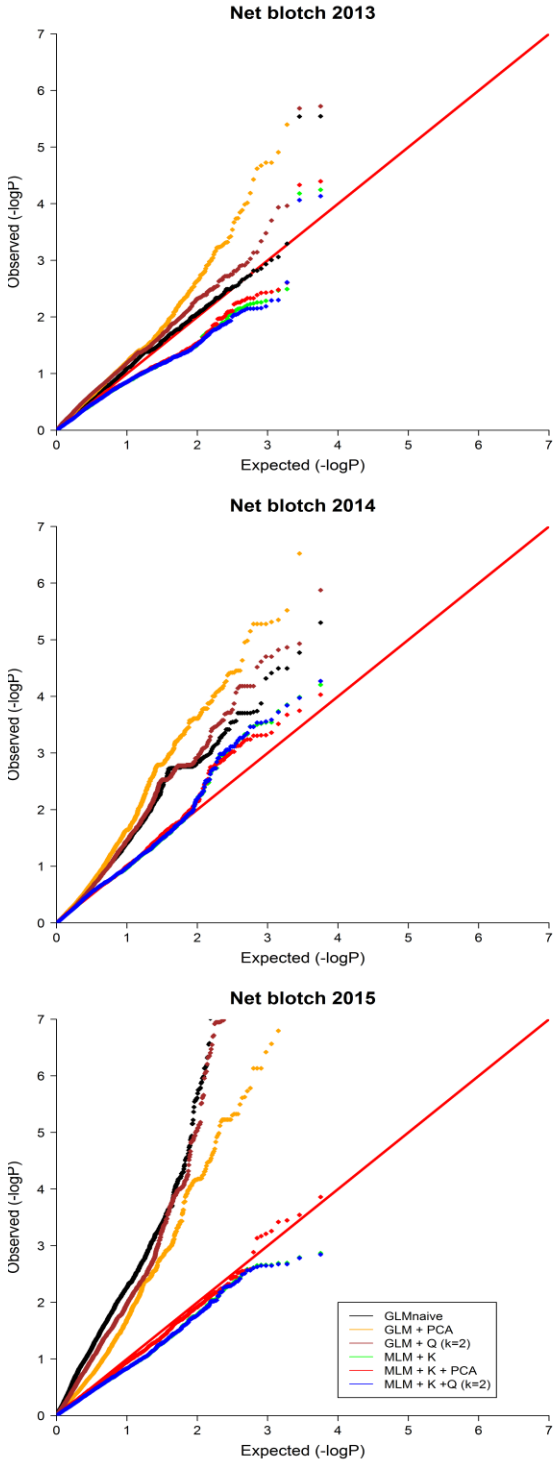
Line no.	Name	Q1	Q2	Subpopulation
1	Breeding line	0.27	0.73	2
2	Ida	0.249	0.751	2
3	Fløya	0.999	0.001	1
4	Akka	0.657	0.343	1
5	Annabell	0.001	0.999	2
6	Asplund	0.998	0.002	1
7	Elmeri	0.855	0.145	1
8	Tampar	0.999	0.001	1
9	Oppdal	0.999	0.001	1
10	Breeding line	0.997	0.003	1
11	Breeding line	0.002	0.998	2
12	Finne	0.977	0.023	1
13	Breeding line	0.995	0.005	1
14	Breeding line	0.857	0.143	1
15	Tore	0.951	0.049	1
16	Breeding line	0.993	0.007	1
17	KWS Olof	0.108	0.892	2
18	Breeding line	0.848	0.152	1
19	Breeding line	0.984	0.016	1
20	Tiril	0.999	0.001	1
21	Breeding line	0.94	0.06	1
22	Herta	0.001	0.999	2
23	Breeding line	0.99	0.01	1
24	Juli	0.997	0.003	1
25	Breeding line	0.04	0.96	2
26	Tunga	0.8	0.2	1
27	Chevron	0.944	0.056	1
28	Birgitta	0.294	0.706	2
29	Bonus	0.001	0.999	2
30	Nordlys	0.998	0.002	1
31	Breeding line	0.794	0.206	1
32	Breeding line	0.969	0.031	1
33	Breeding line	0.882	0.118	1
34	Thule	0.996	0.004	1
35	Breeding line	0.937	0.063	1
36	Breeding line	0.999	0.001	1
37	Breeding line	0.001	0.999	2
38	Breeding line	0.979	0.021	1
39	Breeding line	0.926	0.074	1
40	Bode	0.999	0.001	1
41	Breeding line	0.754	0.246	1
42	Breeding line	0.892	0.108	1
43	Brage	0.999	0.001	1
44	Breeding line	0.865	0.135	1
45	Gull	0.008	0.992	2
46	Seger	0.038	0.962	2
47	Breeding line	0.996	0.004	1
48	Breeding line	0.853	0.147	1
49	Tyra	0.145	0.855	2
50	Aktiv	0.01	0.99	2
51	Breeding line	0.972	0.028	1

52	Breeding line	0.999	0.001	1
53	Tocada	0.002	0.998	2
54	Breeding line	0.998	0.002	1
55	Axelina	0.217	0.783	2
56	Henni	0.004	0.996	2
57	Breeding line	0.999	0.001	1
58	Forus	0.579	0.421	1
59	H354-333-7-5	0.39	0.61	2
60	Atlas 46	0.614	0.386	1
61	Breeding line	0.996	0.004	1
62	Golf	0.149	0.851	2
63	Herse	0.999	0.001	1
64	Yrjar	0.975	0.025	1
65	Breeding line	0.999	0.001	1
66	Rambler	0.001	0.999	2
67	Breeding line	0.999	0.001	1
68	Breeding line	0.971	0.029	1
69	Breeding line	0.997	0.003	1
70	Breeding line	0.929	0.071	1
71	Breeding line	0.998	0.002	1
72	H82009-1-2	0.646	0.354	1
73	Breeding line	0.915	0.085	1
74	CI11577	0.543	0.457	1
75	Jotun	0.999	0.001	1
76	Meltan	0.004	0.996	2
77	Asahi 5	0.429	0.571	2
78	Seijo 17	0.43	0.57	2
79	Marigold	0.06	0.94	2
80	Vada	0.138	0.862	2
81	Fager	0.984	0.016	1
82	Tammi	0.999	0.001	1
83	Arve	0.999	0.001	1
84	Opal	0.003	0.997	2
85	Breeding line	0.926	0.074	1
86	Einar	0.865	0.135	1
	Uforædlet			
87	Jämtland	0.958	0.042	1
88	Kinnan	0.284	0.716	2
89	Vilde	0.907	0.093	1
90	Polarbygg	0.999	0.001	1
91	Delphi	0.002	0.998	2
92	Vera	0.999	0.001	1
93	Ven	0.911	0.089	1
94	Otra	0.999	0.001	1
95	Breeding line	0.847	0.153	1
96	Breeding line	0.989	0.011	1
97	Vega ABED	0.15	0.85	2
98	Stine	0.001	0.999	2
99	Lavrans	0.988	0.012	1
100	Varde	0.999	0.001	1
101	Mari	0.001	0.999	2
102	Breeding line	0.905	0.095	1
103	Audrey	0.001	0.999	2
104	Dønnes	0.999	0.001	1
105	Breeding line	0.83	0.17	1
106	Breeding line	0.997	0.003	1
107	Breeding line	0.976	0.024	1

108	Breeding line	0.809	0.191	1
109	Jyvä	0.896	0.104	1
110	Maskin	0.998	0.002	1
111	Saana	0.302	0.698	2
112	Habil	0.999	0.001	1
113	Gunilla	0.214	0.786	2
114	Breeding line	0.998	0.002	1
115	Breeding line	0.883	0.117	1
116	Etu	0.795	0.205	1
117	Breeding line	0.654	0.346	1
118	Breeding line	0.999	0.001	1
119	Olsok	0.995	0.005	1
120	Breeding line	0.916	0.084	1
121	Pallas	0.018	0.982	2
122	Breeding line	0.883	0.117	1
123	Fairytale	0.008	0.992	2
124	Trysil	0.991	0.009	1
125	Breeding line	0.808	0.192	1
126	Maja	0.001	0.999	2
127	Breeding line	0.996	0.004	1
128	H572-8	0.226	0.774	2
129	Vigdis	0.561	0.439	1
130	Møyar	0.004	0.996	2
131	Breeding line	0.85	0.15	1
132	Bjørneby	0.983	0.017	1
133	Breeding line	0.998	0.002	1
134	Gustav	0.029	0.971	2
135	Frisco	0.027	0.973	2
136	Vena	0.999	0.001	1
137	Stolt	0.768	0.232	1
138	Breeding line	0.978	0.022	1
139	Olve	0.302	0.698	2
140	Jarle	0.963	0.037	1
141	Toria	0.92	0.08	1
142	Breeding line	0.938	0.062	1
143	Balder	0.022	0.978	2
144	Bamse	0.999	0.001	1
145	Breeding line	0.501	0.499	1
146	Harbinger	0.002	0.998	2
147	IS-046	0.4	0.6	2
148	Breeding line	0.999	0.001	1
149	Rolfi	0.999	0.001	1
150	Breeding line	0.999	0.001	1
151	Breeding line	0.002	0.998	2
152	Shirley	0.001	0.999	2
153	Edvin	0.996	0.004	1
154	H82011-2-2	0.578	0.422	1
155	Breeding line	0.862	0.138	1
156	Breeding line	0.016	0.984	2
157	Fræg	0.999	0.001	1
158	Jazz	0.003	0.997	2
159	Trine	0.9	0.1	1
160	Lise	0.999	0.001	1
161	Luhkas	0.003	0.997	2
162	Gaute	0.94	0.06	1
163	Verner	0.699	0.301	1
164	Breeding line	0.999	0.001	1

165	Breeding line	0.993	0.007	1
166	Edel	0.98	0.02	1
167	Breeding line	0.003	0.997	2
168	Ingrid	0.002	0.998	2
169	Agneta	0.999	0.001	1
170	Rigel	0.001	0.999	2
171	Fredrickson	0.441	0.559	2
172	Heder	0.997	0.003	1
173	Breeding line	0.675	0.325	1
174	Barke	0.002	0.998	2
175	Baronesse	0.487	0.513	2
176	H3003	0.035	0.965	2
177	Breeding line	0.991	0.009	1
178	Pernilla	0.164	0.836	2
179	CIho4196	0.433	0.567	2
180	Breeding line	0.005	0.995	2
181	Delibes	0.006	0.994	2
182	Svanhals	0.439	0.561	2
183	Olli	0.999	0.001	1
184	Breeding line	0.977	0.023	1
185	Kunnari	0.852	0.148	1
186	Binder	0.239	0.761	2
187	Atlas	0.616	0.384	1
188	Helium	0.004	0.996	2
189	Clermont	0.652	0.348	1
190	Breeding line	0.985	0.015	1
191	Vega	0.991	0.009	1
192	Breeding line	0.95	0.05	1
193	Breeding line	0.999	0.001	1
194	Dore	0.998	0.002	1
195	Iver	0.138	0.862	2
196	Malz	0.001	0.999	2
197	Breeding line	0.998	0.002	1
198	Breeding line	0.998	0.002	1
199	Triumph	0.002	0.998	2
200	Sunnita	0.129	0.871	2
201	Quench	0.016	0.984	2
202	Breeding line	0.901	0.099	1
203	Iron	0.002	0.998	2
204	Gammel dansk	0.25	0.75	2
205	Breeding line	0.835	0.165	1
206	Breeding line	0.958	0.042	1
207	Breeding line	0.979	0.021	1
208	Domen	0.283	0.717	2
209	Breeding line	0.984	0.016	1

S15 Quantile-quantile (Q-Q) plots of the difference between the distribution of observed and expected p-values. Six different GWAS models are compared for the traits net blotch disease severity 2013, 2014 and 2015



S16 SNP markers significantly associated with spike row number

Marker	Chr.	Pos. [a]	POPseq pos. [b]	R^2 [c]	MAF	p-value	-log10 (p-value)
Spike row number							
11_21000	1H	48.09	50.00	0.21	0.321	3.86E-09	8.4
11_20121	1H		73.59	0.22	0.393	2.09E-08	7.7
SCRI_RS_170389	1H	86.54	89.04	0.27	0.362	7.79E-10	9.1
SCRI_RS_175300	2H	58.64	69.00	0.34	0.345	4.82E-13	12.3
11_21220	2H	113.31	136.66	0.21	0.104	3.85E-09	8.4
SCRI_RS_170542 [c]	1H	48.09		0.21	0.321	3.86E-09	8.4

Paper III

Paper III

Mapping of quantitative trait loci associated with resistance to net form net blotch (*Pyrenophora teres* f. *teres)* in a doubled haploid Norwegian barley population

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PLOS ONE

RESEARCH ARTICLE

Mapping of quantitative trait loci associated with resistance to net form net blotch (*Pyrenophora teres* f. *teres)* in a doubled haploid Norwegian barley population

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Abstract

Barley net blotch caused by the necrotrophic fungus *Pyrenophora teres* is a major barley disease in Norway. It can cause grain shriveling and yield losses, and resistance in currently grown cultivars is insufficient. In this study, a set of 589 polymorphic SNP markers was used to map resistance loci in a population of 109 doubled haploid lines from a cross between the closely related Norwegian cultivars Arve (moderately susceptible) and Lavrans (moderately resistant). Resistance to three net form net blotch (*P. teres* f. *teres*) single spore isolates was evaluated at the seedling stage in the greenhouse and at the adult plant stage under field conditions during three years. Days to heading and plant height were scored to assess their influence on disease severity. At the seedling stage, three to four quantitative trait loci (QTL) associated with resistance were found per isolate used. A major, putatively novel QTL was identified on chromosome 5H, accounting for 23–48% of the genetic variation. Additional QTL explaining between 12 and 16.5% were found on chromosomes 4H, 5H, 6H and 7H, with the one on 6H being race-specific. The major QTL on 5H was also found in adult plants under field conditions in three years (explaining up to 55%) and the 7H QTL was found in field trials in one year. Additional adult plant resistance QTL on 3H, 6H and 7H were significant in single years. The resistance on chromosomes 3H, 5H, 6H and 7H originates from the more resistant parent Lavrans, while the resistance on 4H is conferred by Arve. The genetic markers associated with the QTL found in this study will benefit marker-assisted selection for resistance against net blotch.

Introduction

The necrotrophic fungus *Pyrenophora teres* Drechsler (anamorph *Drechslera teres* (Sacc.) Shoemaker) is the causal agent of net blotch (NB), a foliar disease on barley, which occurs predominantly in cool and humid barley growing regions around the world [1]. Yield losses up to 44% have been reported under conducive conditions [2]. There are two forms of the pathogen, *P. teres* f. *teres and P. teres* f. *maculata*, which cause net form net blotch (NFNB) and spot form

net blotch (SFNB), respectively. The stubble-born disease has been on the rise globally with the increased usage of reduced tillage practices in recent years [3]. In Norway, NB is an important barley disease with varying severity in different years and regions [4], but yield losses due to *P. teres* are not well-documented and reliable data is lacking. Both forms of the pathogen are found in Norway but it is not known which one is the dominant form.

Controlling the disease by the use of resistant varieties is desirable, but at present resistance of Norwegian cultivars is insufficient. All currently grown Norwegian cultivars are susceptible, moderately susceptible or moderately resistant to NB. Under these circumstances, crop rotation, tilling and pesticide treatment are the only effective means to control the disease. Resistance breeding is a more sustainable measure to reduce yield losses due to NB and even small increments in resistance will complement and enhance the effects of other control measures. More in-depth knowledge of this host-pathogen interaction will be of great benefit for resistance breeding.

Resistance to NB is usually governed by multiple genes, and several different resistance mechanisms are present in the pathosystem. Resistance can be dominant [5–7], recessive [8, 9] or incompletely dominant [10], and both major genes and minor effect quantitative trait loci (QTL) can be involved (reviewed in [11]). QTL harboring resistance to NB have been found on all chromosomes [11]. Since resistance to NFNB and SFNB is inherited independently [11, 12] and the two forms are genetically distinct, it is important to investigate both diseases separately. As is the case for many diseases, resistance to NB depends on the developmental stage of the plant. Some resistance QTL are only found in seedlings or adult plants, while others are reported to be associated with resistance at all stages [12–14]. Resistance under field conditions is often more complex than in seedlings tested under greenhouse conditions [15], in addition to being dependent on environmental conditions during the growth season and inoculum concentration [16]. A promising approach to breeding for long-lasting polygenic resistance is thus to pyramid different genes effective in seedlings and adult plants and against a wide range of isolates of both forms of the pathogen.

To our knowledge, this is the first QTL mapping study of resistance against NB in Norwegian cultivars. A biparental mapping population of 109 doubled-haploid lines segregating for NB resistance was created from a cross of the moderately susceptible cultivar 'Arve' and the moderately resistant cultivar 'Lavrans'. Arve and Lavrans were widely grown during the 1990s and 2000s and are parents to some of the cultivars grown currently in Norway. Arve was previously characterized as highly susceptible to net blotch whereas Lavrans possessed moderate resistance [17](M. Lillemo, pers. comm.). Even though the susceptibility of both cultivars has changed since their release, Lavrans has always been consistently more resistant than Arve, which indicates that resistance in Lavrans is likely race non-specific. The population was tested for adult plant resistance under field conditions in inoculated and mist-irrigated hillplots over three years and for seedling resistance under greenhouse conditions. The objectives of this study were (1) to identify and map QTL associated with resistance to NB in Norwegian barley cultivars, (2) to test whether these QTL are stable throughout different environments, years and developmental stages and (3) to assess whether resistance screenings at the seedling stage can be used to predict adult resistance under field conditions.

Material and methods

Plant material

The study was based on 109 doubled haploid lines from a cross between the closely related Norwegian six-rowed barley cultivars Arve (released in 1990, moderately susceptible to NB) and Lavrans (released in 1999, moderately resistant) obtained by microspore culture from F_1

seeds. The pedigrees of Arve and Lavrans are 'Otra/Vigdis//Agneta' and 'Vera/4/Arve/3/Sold/Alva//Mø75-288', respectively, with Vera being a sister line of Arve.

Fungal isolates

Three *P. teres* single conidia isolates were used in all experiments in this study. The isolates 5050B and 6949B were isolated from barley seeds collected in Southeastern (5050B) and Northern (6949B) Norway in 2012 and provided by Kimen seed laboratory in Ås, Norway. Isolate LR9 was obtained from barley leaves collected in the Trøndelag area in Norway in 2011. All isolates were confirmed to be NFNB by a polymerase chain reaction (PCR)-based test developed by Williams et al. [18]. The infected plant material was surface sterilized in 70% ethanol for 10 seconds and 0.5% NaOCl for 90 seconds and placed on moist filter paper at 21˚C and 12h UV light for approximately 3–5 days until conidia started to develop. Single conidia were transferred to V8 agar plates (150 ml V8 Juice, 10.0 g Difco PDA, 3.0 g $CaCO_3$, 10.0 g agar, 850 ml distilled H_2O) and after sufficient mycelium development agar plugs with a diameter of 0.6 cm were excised, air-dried and stored at -80˚C until further use.

Field experiments

To produce inoculum for field experiments, each single spore isolate was grown separately from agar plugs on V8 agar plates for 7 days at 20˚C in the dark, for 24 hours at 21˚C in the light and for 24 hours at 15˚C in the dark to promote conidia formation. The plates were flooded with water and the conidia were scraped off the surface with a sterile inoculation loop. For each isolate, the inoculum was diluted to a volume of ca. 3 liters with 1 drop Tween 20 added for every 50 ml of inoculum. The highly susceptible cultivar 'Tiril' was grown in trays in the greenhouse at 20–25˚C. Each tray was spray-inoculated with one of the three isolates. The inoculation was repeated twice during the course of five weeks to ensure sufficient disease development. After maturation all above ground biomass was harvested, dissected into 5 cm long pieces and the straw inoculated with the different isolates was mixed at equal shares.

The Arve x Lavrans population was sown in hillplots in an alpha lattice design at Vollebekk research farm, Ås, Norway, over three years with two (2014) or three (2015 and 2016) replications. The moderately susceptible cultivar 'Heder' was planted at the borders of the field trial to minimize border effects. After approximately one month the plants were inoculated with the infected straw. The field trial was mist-irrigated daily for 10 minutes per hour from 7 to 10 pm in order to promote disease development. In 2015 and 2016 the trial was sprayed with Talius (proquinazid, 40 g/ha) at three-week intervals to control powdery mildew (*Blumeria graminis* f. sp. *hordei*). Disease severity was scored as percentage of infected leaf area based on the whole hillplot at two different timepoints. The first scoring was done when some lines had reached approximately 25% disease severity and the second scoring approximately one week later when they had reached up to 40%. Scoring at early timepoints of disease development was necessary because later in the season accurate scoring would be hampered due to lodging and infection with competing diseases such as powdery mildew or leaf rust (*Puccinia hordei*). In 2014, the population was scored only once due to heavy powdery mildew infection. In addition, days to heading (DH) and plant height (PH) were recorded in all years.

Greenhouse experiments

For disease phenotyping on seedlings in the greenhouse, the isolates LR9, 5050B and 6949B were grown on V8 agar as described above. The inoculum was diluted to 2000 spores/ml and 1 drop of Tween 20 was added per every 50 ml.

Two seeds per barley line were sown in SC10 plastic cones (Stuewe and Sons, Inc., Corvallis, Oregon, USA) placed into racks of 98 and the plants were grown in the greenhouse at 22˚/16˚C (day/night), 16 hours light and 65% relative humidity (RH) for two weeks. The susceptible cultivar Tiril was used as a border to minimize border effects and to serve as a control to ensure even inoculation. When the second leaf had fully expanded, the plants were spray-inoculated with the spore suspensions until the leaves were at the point of inoculum runoff. The infected plants were kept in mist chambers at 100% RH, 21˚C and continuous light for 24 hours. After 24 hours the plants were moved back to greenhouse chamber conditions. Four to five days after inoculation, the second leaves of both plants from each line were scored together for disease development according to the Tekauz disease reaction type scale where a score of 1 denotes small lesions (resistance) and 10 complete necrosis (susceptibility) [19]. The experiments were performed three times with each isolate.

Statistical analysis

The PROC GLM procedure in the SAS software package 9.4 (SAS Institute Inc.) was used for Analysis of Variance (ANOVA) analysis. Broad sense heritability within and across years was estimated from the ANOVA table using the formulas $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_E^2/r)$ and $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{gxy}^2/y + \sigma_E^2/ry)$, respectively, with σ_g^2 = genetic variance, σ_{gxy}^2 = genotype-by-environment interaction variance, σ_E^2 = error variance, r = number of replicates and y = number of years. The LSMEANS function in PROC MIXED was used to calculate the mean NB severity, mean DH and mean PH of each line. To determine whether DH and PH influence the disease development under field conditions, the mean NB severity of every line in every year was regressed to the mean DH and mean PH in the corresponding year using the PROC REG procedure. PH was found to have a significant impact in 2014 and both scorings in 2015 and was used as a covariate in QTL mapping. The Pearson correlation coefficients were calculated with the PROC CORR function.

Map construction and QTL mapping

Genomic DNA was extracted from young leaves of the parents and all doubled haploid lines using the DNeasy Plant DNA Extraction Kit (Qiagen). The population was genotyped for 7864 markers on the Illumina iSelect 9k Barley SNP Chip (Illumina) at Trait Genetics GmbH (Gatersleben, Germany). SNP markers for which the genotyping failed in more than 10% of the individuals were excluded from further analysis. Out of the remaining 6888 markers, 589 markers were polymorphic and segregated in the population and were used to construct linkage maps. Heterozygous SNPs were treated as missing values. Two lines with more than 10% missing marker data were omitted from further analysis. A genetic linkage map was constructed using the Kosambi function in the software JoinMap 4.0 [20]. Initially, linkage groups were created at an independence LOD (logarithm of odds) score of 3.0. In a second step, the LOD was lowered to 1.9 to obtain separate linkage groups for each chromosome. A recently published consensus map [21] was used to determine which chromosomes the obtained linkage groups represent. Maximum Likelihood mapping was used with default parameters to produce linkage maps.

QTL mapping was performed with the software MapQTL 6 [22]. First, interval mapping (IM) was performed to detect major QTL for NB resistance and then the most closely linked markers to these QTL were used as cofactors for multiple-QTL models (MQM) mapping. In this study, we report on the IM results since MQM did not produce more significant results than IM. The LOD threshold of 2.5 for significance of a QTL was determined by permutation test based on 1000 permutations with $\alpha = 0.05$ for type 1 error rate. Linkage maps and LOD

curves were created with MapChart 2.3 [23]. To allow the comparison of QTL found in this paper with previously described QTL, the marker positions on the consensus map by Muñoz-Amatriaín et al. [24] and on the POPseq map [25] are given wherever appropriate.

QTL nomenclature

We followed the QTL nomenclature established by Grewal et al. [13], but we did not differentiate between seedling stage and adult stage QTL. A suffix was added to distinguish different QTL on the same chromosome, and the prefix "AL_" was added to designate the name of the population the QTL was found in (Arve x Lavrans).

Results

Disease severity

Despite the genetic similarity of the parents Arve and Lavrans, the mapping population segregated for NB resistance in seedlings and adult plants, as well as for DH and PH (Fig 1, S1 and S2 Figs). The disease severity followed a normal distribution with transgressive segregation. The adult plant disease scores ranged from 5–30% diseased leaf area in 2014, from 14–33% in 2015 and from 13–30% in 2016, with average disease scores of 14%, 23% and 20%, respectively. On average, the plants were 68 cm in 2014, 94.5 cm in 2015, and 65 cm in 2016, and the average time to heading was 45 days, 66 days, and 45 days, respectively. Seedling inoculations with the LR9 and 5050B isolates yielded Tekauz scale disease scores between 4.2 and 8.2 (average: 6.1), and 4.0 and 7.0 (average: 5.3), respectively, while the 6949B isolate caused symptoms between 3.0 and 6.0 points (average: 4.2) on the scale and thus seems to be slightly less aggressive than the other two isolates. Whereas the isolates LR9 and 5050B produced typical NFNB

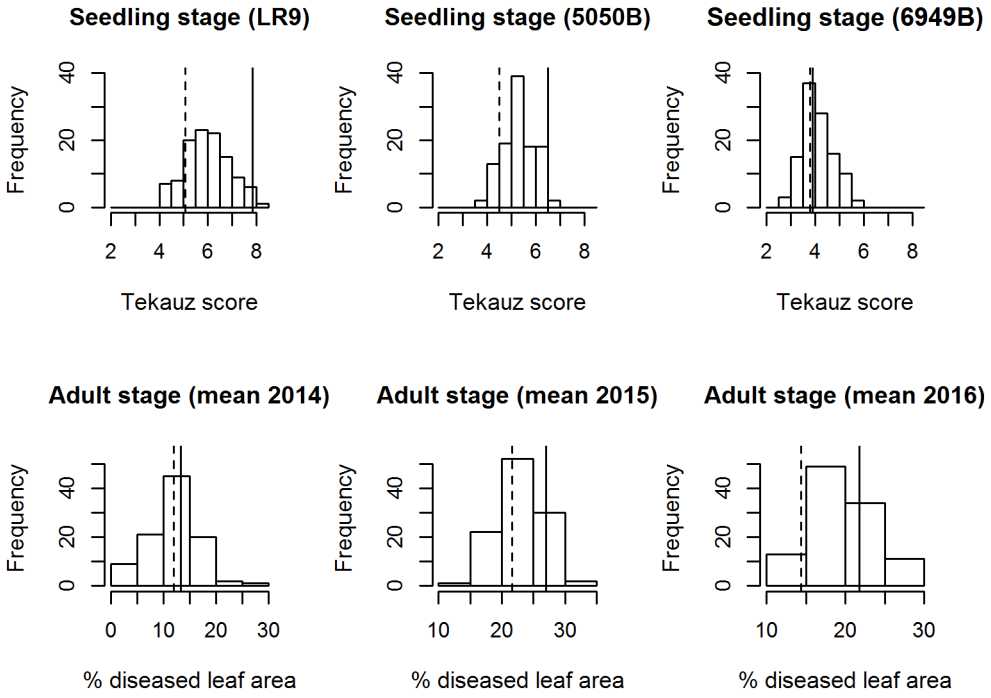

Fig 1. Frequency distributions for disease severities in the Arve x Lavrans mapping population. Disease responses are shown as Tekauz scores in seedling inoculations with three different isolates LR9. 5050B and 6949B and as percentage of diseased leaf area for adult plants under inoculated field conditions in three years. Vertical solid line represents the disease scores of Arve, vertical dashed lines represents disease scores of Lavrans.

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net-shaped symptoms ca. 4–5 days after inoculation, we observed that the symptoms caused by 6949B remained smaller and spot-shaped before expanding into the typical net symptoms at ca. 6–7 days after inoculation. Consistent with previous characterization of Arve as being more susceptible to NB than Lavrans, Arve reached higher disease scores in all environments. These differences were significant during infection with LR9 and 5050B at the seedling stage and in adult plants in 2016 (Fig 1). No significant differences in DH and PH were observed between the parental lines.

Significant correlations of adult plant disease severities were observed between all years (Table 1). The correlations between 2014 and 2016 and 2015 and 2016 were 0.41 and 0.48, respectively ($p<0.001$), while the correlation between 2014 and 2015 was slightly lower ($r = 0.23$, $p<0.05$). In the 2014 field trial, the NB severity was significantly correlated with PH ($r = 0.31$) but not with DH ($r = 0.06$). However, a significant correlation to the heading dates scored in 2015 and 2016 was found (0.33 and 0.29, respectively). The NB scores in 2015 were significantly correlated with PH ($r = 0.25$, $p<0.05$), but not with DH ($r = -0.10$). Conversely, NB scores in 2016 were significantly correlated with DH (0.19, $p<0.05$) but not with PH (-0.10). The correlation between resistance and PH was negative in that year. The correlation between seedling experiments and adult plant field trials ranged between $r = 0.31$ and $r = 0.59$ and was highly significant in all cases. Notably, there was also a significant correlation between seedling inoculations and DH in 2015 and 2016 as well as PH in 2015. Heritability of disease severity across years was 0.69 and that of DH and PH 0.79 and 0.73, respectively (Table 2). The heritability of resistance within year ranged from 0.77 to 0.89 under field conditions and from 0.90 to 0.95 in seedling inoculations (Table 1). Analysis of variance showed significant differences ($p<0.0001$) among genotypes and years for resistance.

Map construction

Two doubled haploid lines with more than 10% missing marker data were omitted from further analysis. A total of 589 SNP markers was polymorphic in the population and was used to construct a linkage map which spanned 644.9 cM in total (Table 3). Due to the close relatedness of the parental lines, no segregating markers were found on chromosome 1H, and chromosomes 2H, 4H, 5H and 6H contained major gaps of 58.5 cM, 33.3 cM, 43.3 cM and 50.5 cM, respectively. Two linkage groups were obtained for chromosome 3H and 5H. Marker density ranged from 0.4 cM (3H.1) to 2.9 cM between markers (linkage group 5H.2 on chromosome 5H). The marker positions in the Arve x Lavrans map were found to be in good agreement with the recently published consensus map [21] (see S1 Table for a comparison of the marker positions on both maps).

QTL mapping

In total, nine QTL significantly associated with resistance to NB were found on chromosomes 3H, 4H, 5H, 6H and 7H in different years and at different developmental stages using interval mapping (Fig 2 and Table 4). Chromosome 6H harbored three QTL, while chromosomes 5H and 7H contained two QTL and 3H and 4H one QTL each. In adult plants assessed under field conditions, 1–3 QTL were detected in the different scorings. Four QTL were found in seedling inoculations with LR9 and 5050B, while three QTL were detected in inoculations with 6949B. A major QTL on chromosome 5H (AL_QRptt5-2) peaking around 98.1 cM between the markers SCRI_RS_140499 and SCRI_RS_8410 was consistently found under inoculations with all three isolates at the seedling stage and in all field trials, explaining between 15.5% (2014) and 54.7% (first scoring 2016) of the genetic variation. Apart from AL_QRptt5-2, only one QTL was significantly associated with resistance in both seedlings and adult plants. AL_QRptt7-2 at

Table 1. Pearson correlation coefficients for net blotch severities, DH and PH and heritability (h^2) within years.

	NFNB severities in adult plants							NFNB severities in seedling tests			Days to heading (DH)			Plant height (PH)		
	2014	2015_1[a]	2015_2	2015	2016_1	2016_2	2016	LR9	5050B	6949B	2014	2015	2016	2014	2015	2016
NB2015_1	0.27**															
NB2015_2	0.17	0.58***														
NB2015	0.23*	0.79***	0.93***													
NB2016_1	0.43***	0.51***	0.44***	0.48***												
NB2016_2	0.39***	0.50***	0.40***	0.45***	0.78***											
NB2016	0.41***	0.53***	0.43***	0.48***	0.87***	0.99***										
LR9	0.39***	0.48***	0.32***	0.37***	0.49***	0.44***	0.47***									
5050B	0.46***	0.48***	0.37***	0.44***	0.43***	0.46***	0.47***	0.68***								
6949B	0.39***	0.35***	0.29**	0.31**	0.56***	0.56***	0.59***	0.52***	0.55***							
DH2014	0.06	0.00	-0.09	-0.08	0.07	0.15	0.14	0.21*	0.11	0.26						
DH2015	0.33**	-0.05	-0.09	-0.10	0.20*	0.17	0.19	0.28**	0.31**	0.28**	0.58***					
DH2016	0.29**	0.05	0.05	0.02	0.21*	0.18	0.19*	0.34***	0.35***	0.41***	0.64***	0.77***				
PH2014	0.31**	-0.06	0.02	-0.05	0.04	0.04	0.05	0.14	0.11	0.00	0.11	0.21*	0.12			
PH2015	0.28**	0.27**	0.22*	0.25*	0–09	0.12	0.12	0.24*	0.23*	0.14	0.13	0.33***	0.28**	0.42***		
PH2016	0.18	0.03	-0.06	-0.08	-0.06	-0.11	-0.10	0.10	0.05	0.08	0.12	0.34***	0.27	0.48***	0.68***	
h^2	0.77	0.80	0.80	0.80	0.87	0.89	0.89	0.91	0.90	0.95	0.74	0.95	0.91	0.73	0.90	0.92

* <0.05.

** <0.01.

*** <0.001

[a] Net blotch scores: Numbers before and after the underscore in the trait name represent the year and number of scoring, respectively

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Table 2. Analysis of variance table for net blotch severity (NB), days to heading (DH) and plant height (PH) and heritabilities in the AxL mapping population.

Trait	Source	df	Mean square	F value	P value	Heritability
NB[a]	Genotype	107	50.00	2.73	<0.0001	0.69
	Year	1	444.38	24.23	<0.0001	
	Genotype x year	102	18.34	1.39	0.0230	
	Rep(Year)	4	36.13	2.73	0.0299	
	Block(Rep x year)	54	19.60	1.48	0.0256	
	Error	228	13.23			
DH[b]	Genotype	108	5.58	5.57	<0.0001	0.79
	Year	3	27029.75	26974.99	<0.0001	
	Genotype x year	205	1.00	1.88	<0.0001	
	Rep(Year)	5	2.56	4.30	0.0008	
	Block(Rep x year)	67	0.77	1.29	0.0707	
	Error	420	0.60			
PH[c]	Genotype	108	70.81	4.09	<0.0001	0.73
	Year	3	1063755.86	61396.51	<0.0001	
	Genotype x year	205	17.33	1.42	0.0019	
	Rep(Year)	5	90.00	7.37	<0.0001	
	Block(Rep x year)	67	16.20	1.33	0.0555	
	Error	370	12.21			

[a] NB: net blotch severity

[b] DH: days to heading

[c] PH: plant height

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41–46 cM on 7H was detected in seedling inoculations with LR9 and in adult plants in 2016, explaining around 12% of the genetic variation. Four QTL were only significant in adult plants. AL_QRptt3-1 (0 cM) was significant only in the first scoring in 2015 and explained up to 10.8% of the genetic variation while AL_QRptt7-1 (3.8 cM) explained up to 11.9% in 2014. However, we cannot exclude the possibility that AL_QRptt7-1 was caused by faulty scoring in 2014 due to heavy infection with powdery mildew. The two adult stage QTL on 6H, AL_QRptt6-2 (110 cM) and AL_QRptt6-3 (~140 cM), were only significant in 2015 and explained up to 14.8% and 11.0%, respectively. Three QTL were only detectable at the seedling stage. AL_QRptt5-1 peaked at 33 cM on 5H and was significant during inoculations with all three isolates. It explained between 11.5% and 14.8% of the genetic variation in these experiments. AL_QRptt4-1 close to

Table 3. SNP coverage and distribution across all chromosomes after filtering.

Linkage group	cM	Markers	Marker coverage (cM/marker)
1H	-	-	-
2H	108.1	71	1.5
3H.1	23.4	57	0.4
3H.2	2.9	5	0.6
4H	156.2	139	1.1
5H.1	98.1	65	1.5
5H.2	31.7	11	2.9
6H	142.4	87	1.6
7H	82.1	154	0.5

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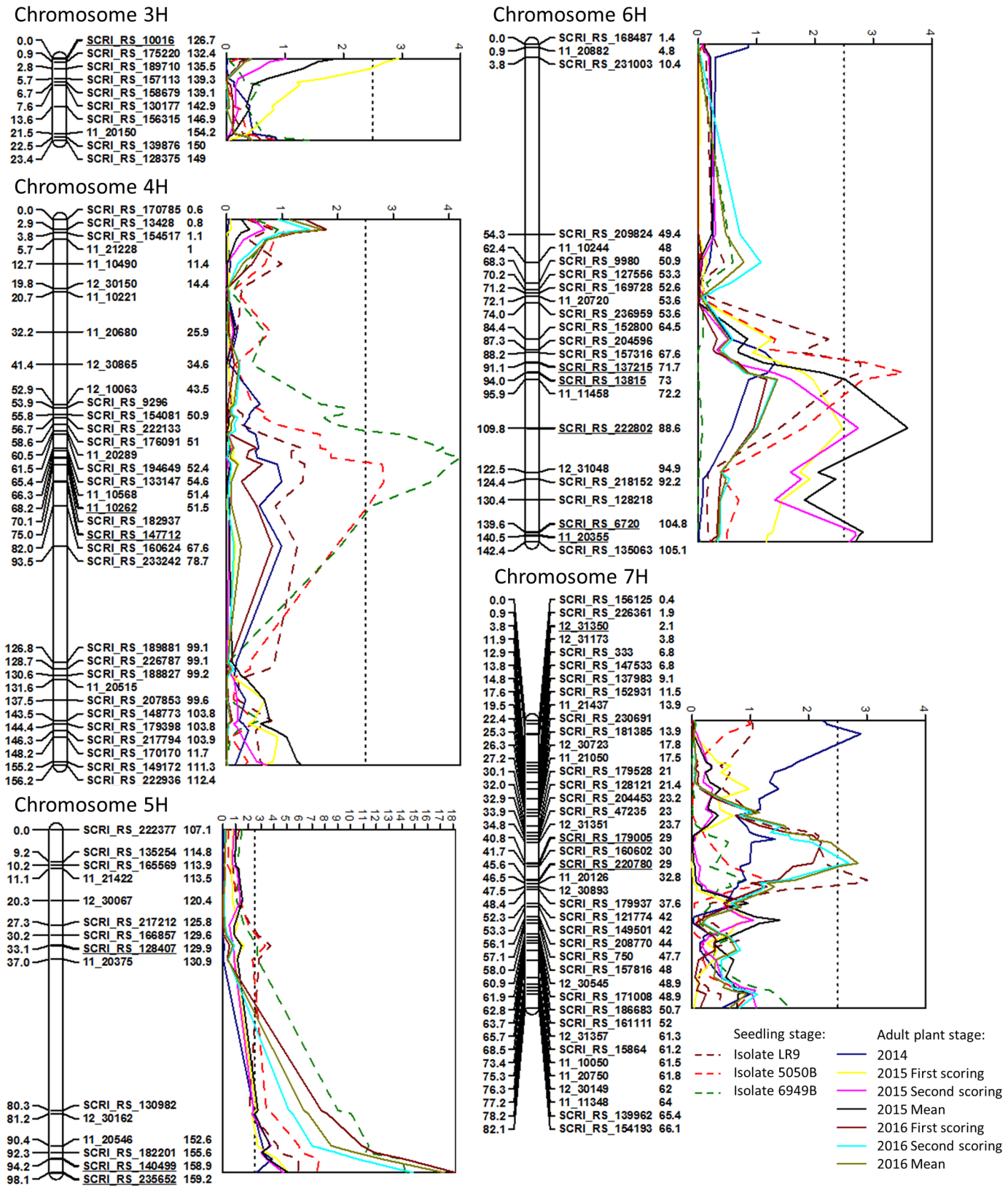

Fig 2. Chromosomes with significant QTL for net blotch resistance with LOD curves obtained with interval mapping. Genetic distances on the AxL map are given in cM on the left side of the linkage map bars. The numbers on the right side of the marker names refer to the POPseq position

of the marker [25]. Only one marker per position was kept. Markers most closely linked to QTL are underlined. The dashed lines indicate the LOD threshold of 2.5 determined by permutation test.

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the markers SCRI_RS_147712 and 11_10262 at 68.2–75.0 cM on 4H was significant under inoculations with 5050B and 6949B and explained up to 16.5% of the genetic variation. A QTL on 6H (AL_QRptt6-1) peaked at 94.0 cM in the vicinity of the marker SCRI_RS_13815. It explained up to 14.0% of the genetic variation and was detected in seedling resistance assessment with LR9 and 5050B. This QTL was not found during inoculation with 6949B, indicating a race-specific resistance mechanism at this locus. Except for AL_QRptt4-1, the resistance is conferred by the more resistant parent Lavrans at all loci.

In total, five QTL for DH were found on chromosomes 3H, 4H and 6H (Fig 3, S2 Table). On chromosome 7H, significant marker-trait associations (MTA) were found within an interval from 12 to 82 cM. The most significant markers were located at 46.5–48.4 cM and 62.8 cM on chromosome 7H and were significant in all three years and explained up to 57.8% and 40.2% of the genetic variation, respectively. A QTL at 22.5 cM on 3H was significant in 2015 and 2016 (13.5% and 17.2%). Chromosome 4H harbored three regions at 12.8 cM, 61.4 cM and 82.0 cM which were significantly associated with DH in 2016, explaining between 10.4% and 11.8%. Additionally, 11.3% of the variation were explained by a QTL at 122.5 cM on 6H.

Four PH QTL were found on 2H, 3H, 4H and 6H (Fig 4, S3 Table). The region significantly associated with PH on chromosome 4H spanned a region of 75 cM and it is not clear how many QTL are present. The LOD curve showed a peak at 58.6 cM in 2015 and 2016, explaining up to 22.4% of the genetic variation. In addition, two more peaks were at 12.7 cM and 41.4 cM on the same chromosome in 2015 and 2016, respectively, but it remains to be elucidated if these peaks represent separate QTL. In 2016, three additional QTL were located at 19.2 cM on 2H, 0.0 cM on 3H and 70.2 cM on 6H, explaining 14.5%, 18.3% and 19.7%, respectively. Most of these QTL were also observed in 2014, but did not reach the significance threshold.

The PH QTL on 3H and 4H co-located with the NB resistance QTL AL_QRptt3-1 and AL_QRptt4-1 and the 7H QTL for DH partly overlapped with AL_QRptt7-2. The AL_QRptt4-1 region coincided with markers significantly associated with DH in 2016 and PH in 2015 and 2016.

Discussion

Disease severity

In both seedlings and adult plants, quantitative variation in disease severity was observed in the Arve x Lavrans population, suggesting the involvement of multiple genes in NB resistance, which is confirmed by the results of the QTL analysis.

The field trials were inoculated with the same isolates used for seedling inoculations in the greenhouse. In spite of the likely presence of natural inoculum in the field, the different developmental stages of the plants tested and the different scales used to score the disease, a significant correlation of disease severity was observed between the two sets of experiments ($r = 0.31–0.59$). Even though the natural *P. teres* population in the field is likely to differ between years, this correlation was relatively stable across years, indicating that most of the observed disease symptoms were caused by the three isolates or genetically similar naturally occurring isolates. Seven of the 20 lines that were most resistant at the seedling stage were also among the 20 most resistant lines under field conditions. Similarly, 10 of the 20 most susceptible lines under greenhouse conditions were also among the 20 most susceptible lines in the field. However, among these 20 most resistant or susceptible lines under each condition were

Table 4. QTL for net blotch severity in the Arve x Lavrans mapping population.

	AL_QRPtt3-1			AL_QRptt4-1			AL_QRptt5-1			AL_QRptt5-2			AL_QRptt6-1			AL_QRptt6-2			AL_Qrptt6-3			AL_QRptt7-1			AL_QRptt7-2		
Closest marker	SCRI_RS_10016			SCRI_RS_147712 (5050B), 11_10262 (6949B)			SCRI_RS_128407			SCRI_RS_140499 (NB14, LR9, 5050B), SCRI_RS_235652			SCRI_RS_137215 (LR9), SCRI_RS_13815 (5050B)			SCRI_RS_222802			11_20355 (NB15_2), SCRI_RS_6720 (NB15)			12_31350			SCRI_RS_179005 NB16_2, NB16), SCRI_RS_220780 (LR9)		
AxL map position (cM)	0.0			75.0, 68.2			33.1			94.2, 98.1			91.1, 94.0			109.8			140.5, 139.6			3.8			40.7, 45.6		
Consensus map range of the most significant markers [24]	128.5–129.6			58.7–59.2; 55.0			128.8–130.4			NA; 170.0–170.1			77.7–78.1; 79.8–80.3			94.5–96.2			113.1; 112.8			3.21			NA; NA		
POPseq map range of the most significant markers [25]	126.0–128.6			57.5–59.7; 51.4			129.9–130.7			158.9; 159.2–159.8			71.7, 73.0			87.6–88.6			NA, 104.8			2.0			26.7–31.4; 29,0		
Trait	**LOD**	**Add [a]**	**R^2 (%) [b]**	**LOD**	**Add**	**R^2 (%)**	**LOD**	**Add**	**R^2 (%)**	**LOD**	**Add**	**R^2 (%)**	**LOD**	**Add**	**R^2 (%)**	**LOD**	**Add**	**R^2 (%)**	**LOD**	**Add**	**R^2 (%)**	**LOD**	**Add**	**R^2 (%)**	**LOD**	**Add**	**R^2 (%)**
NB14										3.8	2.0	15.5										2.9	1.7	11.9			
NB15_1[d]	2.9	1.2	10.8							5.1	1.4	18.0				2.6	1.2	9.7									
NB15_2										4.8	2.0	18.1				2.8	1.6	10.9	2.7	1.5	10.7						
NB15										5.1	1.6	19.0				3.7	1.5	14.8	2.8	1.2	11.0						
NB16_1										18.2	1.9	54.7															
NB16_2										14.9	5.4	47.6													2.7	2.7	11.1
NB16										17.3	3.7	53.1													2.9	1.8	11.8
LR9							3.7	0.3	14.8	6.2	0.5	23.4	2.9	0.3	11.6										3.0	0.4	12.1
5050B				3.0	-0.2	12.0	3.3	0.2	13.4	7.8	0.4	28.6	3.5	0.3	14.0												
6949B				4.2	-0.3	16.5	2.8	0.2	11.5	15.0	0.5	47.6															
Res. source	L[c]			A			L			L			L			L			L			L			L		

[a] Additive effect.

[b] Percent of phenotypic variance explained by QTL.

[c] A: Arve. L: Lavrans.

[d] Net blotch scores: Numbers before and after the underscore in the trait name represent the year and number of scoring, respectively.

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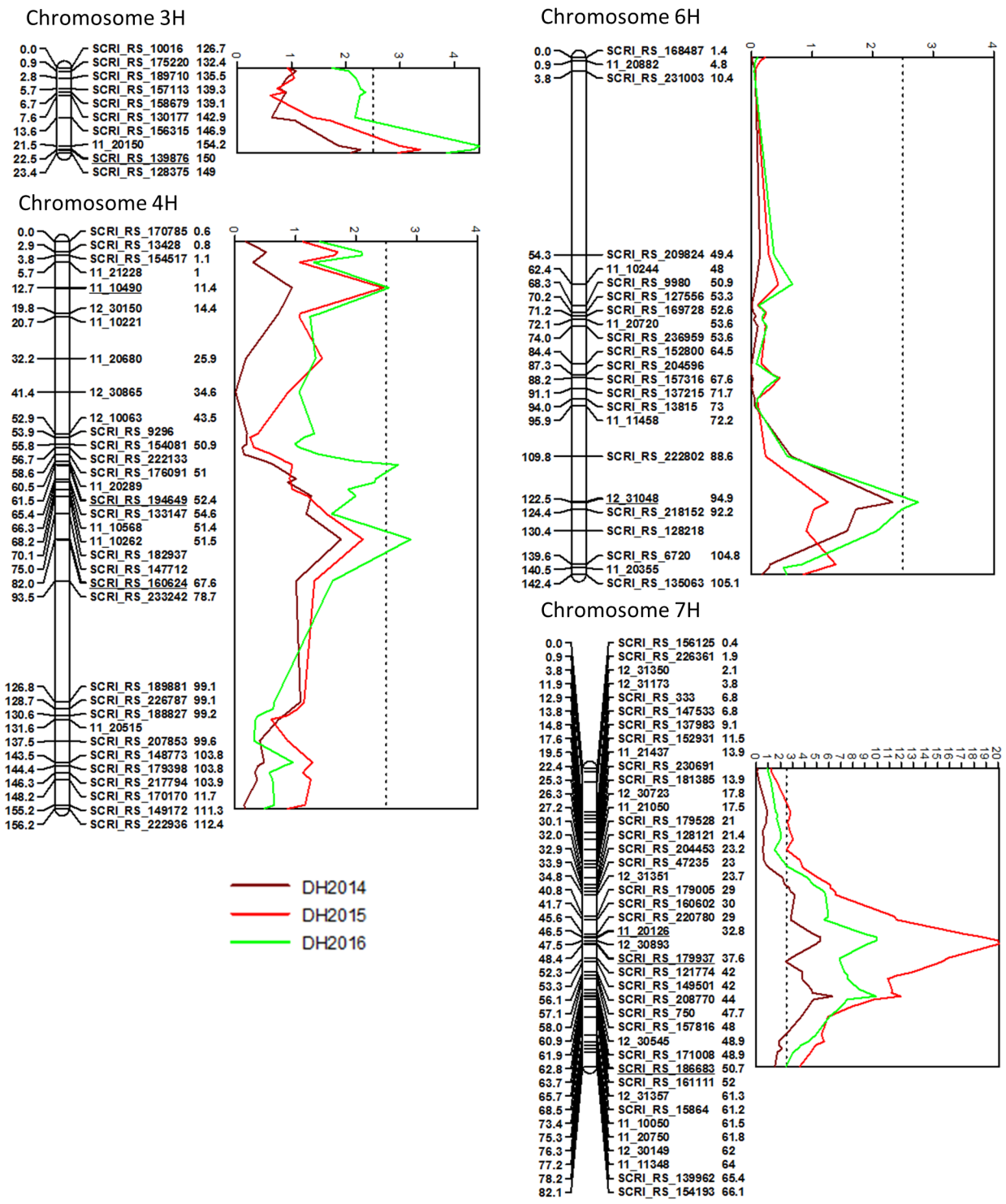

Fig 3. Chromosomes with significant QTL for DH in the Arve x Lavrans population with LOD curves obtained with interval mapping. Genetic distances on the AxL map are given in cM on the left side of the linkage map bars. The numbers on the right side of the marker names refer to the POPseq position of the marker [25]. Only one marker per position was kept. The dashed lines indicate the LOD threshold of 2.5 determined by permutation test.

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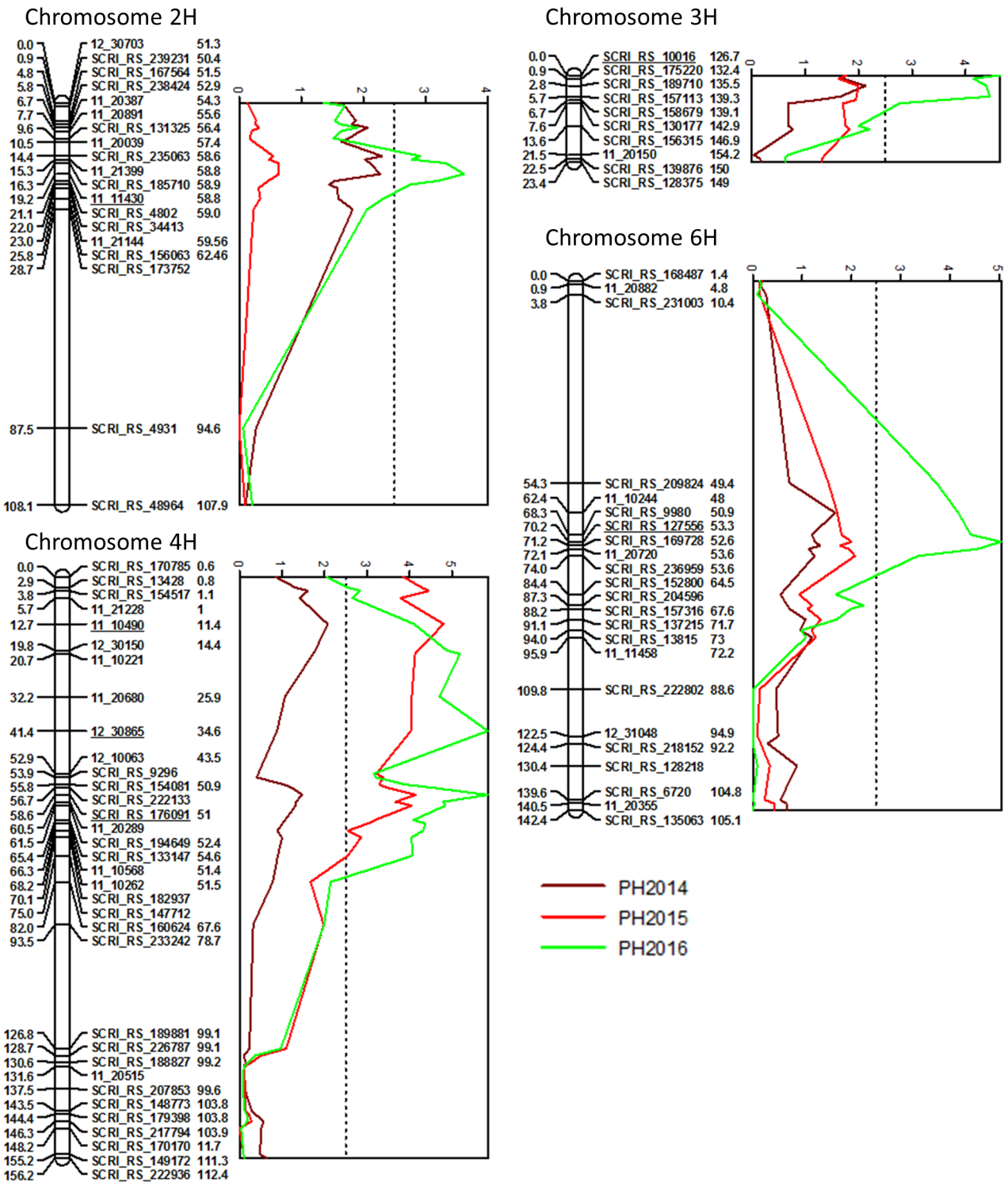

Fig 4. Chromosomes with significant QTL for PH in the Arve x Lavrans population with LOD curves obtained with interval mapping. Genetic distances on the AxL map are given in cM on the left side of the linkage map bars. The numbers on the right side of the marker names refer to the POPseq position of the marker [25]. Only one marker per position was kept. The dashed lines indicate the LOD threshold of 2.5 determined by permutation test.

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usually one or two lines that ranged in the opposite group under the respective other condition. Thus, a line that is resistant at the seedling stage may still show substantial susceptibility under field conditions. These observations indicate that it is possible to use seedling screenings with isolates representative of the natural pathogen population to pre-screen breeding material before field testing, under the condition that the selection criteria are not too strict. In a similar study, Grewal et al. reported a higher correlation (r = 0.65–0.71) between seedling inoculations with different isolates and adult plant-stage reactions in non-inoculated field experiments than in our study [13]. The reason for this difference could be that the isolates used in Grewal's study are more representative for the natural NB population present at the field site. Another reason could be that the disease scale the authors used for scoring the field trial correlated better with the scale used in seedling experiments than in our study.

QTL mapping

Despite the close relatedness of the parents, we were able to identify nine NB resistance QTL in this study, demonstrating that QTL mapping can be a powerful tool even in populations derived from narrow crosses. One QTL was highly significant in all scorings, five QTL occurred in more than one experiment and three QTL were present under one condition each. The putatively novel QTL AL_QRptt5-2 at 98.1 cM on 5H (Consensus map: 170.0–170.1 cM, POPseq: 158.9–159.8 cM [25]) was found in all years of field experiments with adult plants and seedling inoculations with three different isolates. This QTL explained a considerable part of the genetic variance, and its stability throughout different environments suggests that the gene underlying this QTL confers broad-range resistance in various environments, which makes it a promising candidate for implementation in resistance breeding. For this, further investigation of this locus, e.g. by fine-mapping, and the identification of closely linked markers is needed. AL_QRptt7-2 was also associated with resistance in both seedlings and adults. These findings indicate that a part of the NB resistance in seedlings and adult plants is conferred by the same genes. AL_QRptt4-1, AL_QRptt5-1 and AL_QRptt6-1 were found in seedling inoculations with at least two isolates. They were not significant in field experiments, but the LOD curves for these traits suggest that these loci still might have a small effect on resistance under field conditions (Fig 2). The other QTL AL_QRptt3-1, AL_QRptt6-2, AL_QRptt6-3 and AL_QRptt7-1 were only significant in one environment each in field trials, so they might represent resistance against naturally occurring NB strains. Further tests under different environments or with additional isolates will be required in order to test this hypothesis and to determine how stable these QTL are.

A number of QTL found in this study have already been described in the literature, while others are putatively novel. Tamang et al. identified markers at 53.7–59.2 cM (consensus map) on chromosome 4H associated with seedling resistance against the two SFNB isolates NZKF2 and DEN.2 from New Zealand and Denmark, respectively, and this region co-locates with AL_QRptt4-1 found in this study [26]. One of the markers significantly associated with resistance to NZKF2 and DEN2.6 is also present in the AxL linkage map and showed significant association with seedling resistance to 6949B. Afanasenko et al. found seedling resistance against a Russian NB isolate in this region (marker 11_11207 at 56.7 cM on the consensus map) [27]. In an association mapping study of 1050 globally collected barley accessions, Richards et al. identified a marker-trait association at 52.7 cM on the POPseq map with seedling resistance to the isolates 6A and LDN [28]. This marker is within the AL_QRptt4-1 region. Additionally, the QRpts4 locus described by Grewal et al. conferring seedling resistance against both NFNB and SFNB isolates is located in close vicinity [13]. These results suggest that this locus is an important seedling resistance QTL which is effective against a number of both

NFNB and SFNB isolates from different regions of the world. It is not clear yet, however, if the QTL presented in these studies are identical or if this region harbors multiple resistance genes.

The QTL AL_Qrptt3-1 and AL_QRptt5-1 both co-locate with two resistance QTL described by Afanasenko et al. against the Russian isolates PL9 and PP7, respectively [27]. AL_QRptt3-1, which is at 128.5–129.6 cM on the POPseq map, is in close vicinity of the marker 11_20920 at 123.23 cM, and the marker 11_10845 at 129.44 cM on the POPseq map is within the AL-QRptt5-1 interval of 129.9–130.7 cM [27]. In addition, Afanasenko et al. found an association of the marker 11_20531 (POPseq map: 94.9 cM) with resistance to the two Russian net form isolates PP1 and PP6. This marker is located between the two QTL AL_QRptt6-2 (POPseq: 87.6–88.6 cM) and AL_QRptt6-3 (POPseq: 104.8 cM), and it remains to be elucidated whether this marker represents a separate QTL or one of the two QTL.

Due to the close relatedness of Arve and Lavrans, many of the genotyped markers were not polymorphic in the population, resulting in several gaps in the map. The LOD curve for AL_QRptt5-2 increased until the end of the map, suggesting that the true location of the QTL might be beyond the map. It will thus be useful to map this QTL in other populations in order to determine its precise location.

Potential effect of PH and DH on NB resistance

It is important to distinguish between 'true' disease severity and the possible confounding influences of other developmental factors such as PH and DH. It is conceivable that the top leaves of tall plants might remain healthy for a longer period than those of shorter plants because they can escape the fungus more easily. Lines with an early heading date develop faster and might be exposed to the pathogen for a longer time than late lines. Early maturation of plants may promote disease development of necrotrophic pathogens due to facilitated infection of senescing leaves. Additionally, in maturing plants accurate disease severity assessment may be hampered by the confusion of infected leaf tissue with naturally senescing tissue. In this study, we found that PH had a significant ($P<0.05$) effect on disease severity in two years and was thus used as a covariate in QTL mapping. Interestingly, the correlation in 2014 and 2015 was significant and positive, indicating that taller plants were more susceptible than shorter plants. In 2016, taller plants tended to be more resistant than shorter ones, although this association was not significant. A significant positive correlation was also found between PH and disease severity after seedling inoculations with two of three isolates. Since we can rule out an effect of PH on disease severity in two-week old spray-inoculated seedlings, we assume that this correlation is not caused by plant architecture but rather is of genetic nature, i.e. determined by closely linked genes or by one gene with a pleiotropic effect on both traits. Two of the loci associated with NB resistance, AL_QRptt3-1 and AL_QRptt4-1, were significantly associated with PH in this study. At both loci, low plant height was conferred by the allele from Arve, while the allele conferring resistance was from Arve on AL_QRptt3-1, and from Lavrans on AL_QRptt4-1. In an association mapping study identifying QTL for a number of agronomic traits in a global collection of spring barley, Pasam et al. identified the PH QTL QTL16_PHT associated with PH at 58.9 cM (consensus map) on 6H which co-locates with one of the PH QTL found in this study. Additionally, the authors identified the PH QTL QTL3_PHT on 4H at 69 cM on the consensus map, and this region was also significantly associated with PH in this study. Pasam et al identified this QTL as a QTL previously detected in a Harrington x Morex cross by Marquez-Cedillo et al. [29]. Previous studies reported that dwarfing and semi-dwarfing genes determining PH in barley are able to confer increased disease resistance against necrotrophic pathogens through attenuation of phytohormone pathways such as the brassinosteroid (BR) or salicylic acid and jasmonic acid pathways [30, 31]. Loci

conferring resistance against other barley diseases such as Fusarium crown rot and Fusarium Head Blight have also been reported to co-locate with PH loci [32–34]. In the case of the AL_QRptt3-1 and AL_QRptt4-1 loci, however, further studies are required to investigate the interactions of resistance and plant height.

In this study, we could not find a clear association between NB resistance and DH. While there was a weak positive correlation between these traits in 2014, it was weakly negative in 2015. In 2016, the correlation was positive and significant. This might be due to different sowing times and different weather conditions during early plant development in the different years. Conversely, we found highly significant positive correlations between DH in two years and NB resistance in seedlings ($p<0.001$), which indicates either genetic linkage or pleiotropy. Pasam et al. found the DH QTL QTL16_HD on chromosome 7H, which the authors identified as the previously described HvCO1 locus [35, 36]. This locus co-locates with the QTL we identified at 46.5–48.4 cM (consensus map: 38.3–43.4 cM) [37]. HvCO1 is a regulator of flowering induction in response to photoperiod [38]. The HvCO1 locus did not have any effect on other agronomic traits [35], and it is not known if it influences disease resistance. The resistance QTL AL_QRptt4-1 and AL_QRptt7-2 were significantly associated with DH. To date, the effect of earliness on net blotch resistance has not been established. Spaner et al. identified a multi-disease resistance locus in TR306 x Harrington against net blotch, stem rust and scald in a region on chromosome 4H which is associated with DH and the authors speculate that different maturity times may constitute a disease escape mechanism, but this has not been established yet [39, 40]. In FHB infected plants, resistance is usually associated with a late heading date, and regions on chromosomes have been associated with both FHB resistance and heading date [41, 42].

Conclusions

In this study, nine QTL associated with NB resistance were found on all chromosomes except 1H and 2H in the Arve x Lavrans mapping population, suggesting that the disease is controlled by several genes, most of them with relatively moderate effects. The most significant QTL AL_QRptt5-2 was observed in all environments and developmental stages and explained up to 54.7% of the genetic variance, making it a very promising candidate for introducing stable NB resistance into barley breeding programs. Eight other QTL on 3H, 4H, 5H, 6H and 7H were present in at least one of the conditions tested. The QTL that were only found in one environment are likely to represent defense mechanisms that are only functional in certain environments or against a small number of isolates or may be attributed to naturally occurring NB isolates in the field. AL_QRPtt6-1 was race-specific and was not found in inoculations with 6949B.

Inoculations with more isolates from different regions worldwide and under different environmental conditions will clarify whether these QTL represent general or race-specific defense mechanisms. Further work will include the validation of the QTL in other populations. Combining resistance genes functional in all growth stages and against a range of isolates will be most effective in breeding for stable resistance to net blotch in Norwegian barley cultivars. Understanding the molecular background of the barley NB pathosystem will allow for more efficient resistance breeding of locally adapted cultivars that maintain yield and quality under the current climatic and environmental conditions and thus contribute to a sustainable and integrated approach to disease management.

Since the genetic map of the Arve x Lavrans population contains major gaps, additional QTL can be expected to be present in this population. All but one resistance QTL found in this study were contributed by the moderately resistant parent Lavrans, making this variety a promising candidate for further investigation.

Supporting information

S1 Fig. Frequency distributions for disease severities in adult plants in both scorings in 2015 and 2016. Vertical solid line represents the disease scores of Arve, vertical dashed lines represents disease scores of Lavrans.
(TIF)

S2 Fig. Frequency distributions for DH (top) and PH (bottom) in the Arve x Lavrans mapping population in three years. Vertical solid line represents the disease scores of Arve, vertical dashed lines represents disease scores of Lavrans.
(TIFF)

S1 Table. Correlation of marker positions between consensus map and AxL map. Included are markers mapped in both maps.
(XLSX)

S2 Table. QTL for earliness (DH) in the Arve x Lavrans mapping population.
(DOCX)

S3 Table. QTL for plant height (PH) in the Arve x Lavrans mapping population.
(DOCX)

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Conceptualization: ML AF.

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Investigation: RW.

Methodology: ML AF RW.

Project administration: ML RW AF.

Resources: ML AF.

Supervision: ML AF RW.

Validation: RW ML.

Visualization: RW.

Writing – original draft: RW.

Writing – review & editing: RW ML AF.

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Errata

Page number	Paragraph	Change from	Change to
IV	Line 2-3	43 significant marker-trait associations corresponding to 15 QTL	35 significant marker-trait associations corresponding to 13 QTL
2	Table 1		The first two numbers of the table need to be switched. The world production was 144.5 million metric tons on 49.4 million hectares
21	Line 8	*Ptt* isolate 6A and 15A	*Ptt* isolates 6A and 15A
29	Table 4		The table heading should include: (Sundgren et al. 2013; Åssveen 2014a, b, 2015; Åssveen et al. 2005; Åssveen et al. 2003; Åssveen et al. 2013; Åssveen and Tangsveen 2010, 2011, 2012; Åssveen et al. 2006, 2010, 2011, 2012, 2015; Åssveen et al. 2009; Åssveen et al. 2008; Åssveen et al. 2007a, b; Åssveen et al. 2016, 2017)
33	Line 5		Remove: “Fig. 4”
51			The following references should be added: Sundgren T, Åssveen M, Stabbetorp H (2013) Sorter og sortsprøving 2012. Bioforsk FOKUS 8:52-79 Åssveen M (2014a) Prøving av bygg- og havresorter på Sør-Vestlandet. NIBIO Bok 2:82-92 Åssveen M (2014b) Prøving av bygg- og havresorter på Sør-Vestlandet. Bioforsk FOKUS 9:70-79 Åssveen M (2015) Prøving av bygg- og havresorter på Sør-Vestlandet. Bioforsk FOKUS 10:67-77 Åssveen M, Linnerud H, Bergjord AK, Weiseth L (2005) Sorter og sortsprøving. Grønn kunnskap 8:60-108 Åssveen M, Linnerud H, Enger F, Bergjord AK, Weiseth L (2003) Sorter

og sortsprøving. Planteforsk Grønn forskning 1:24-55
Åssveen M, Sundgren T, Stabbetorp H (2013) Prøving av bygg- og havresorter på Sør-Vestlandet. Bioforsk FOKUS 8:82-90
Åssveen M, Tangsveen J (2010) Prøving av bygg- og havresorter på Sør-Vestlandet. Bioforsk FOKUS 5:93-99
Åssveen M, Tangsveen J (2011) Prøving av bygg- og havresorter på Sør-Vestlandet. Bioforsk FOKUS 6:54-59
Åssveen M, Tangsveen J (2012) Prøving av bygg- og havresorter på Sør-Vestlandet. Bioforsk FOKUS 7:64-72
Åssveen M, Tangsveen J, Bergjord AK, Weiseth L (2006) Sorter og sortsprøving. Grønn kunnskap 9:72-114
Åssveen M, Tangsveen J, Bergjord AK, Weiseth L (2010) Sorter og sortsprøving 2009. Bioforsk FOKUS 5:70-92
Åssveen M, Tangsveen J, Bergjord AK, Weiseth L (2011) Sorter og sortsprøving 2010. Bioforsk FOKUS 6:26-48
Åssveen M, Tangsveen J, Bergjord AK, Weiseth L (2012) Sorter og sortsprøving 2011. Bioforsk FOKUS 7:34-63
Åssveen M, Tangsveen J, Bergjord AK, Weiseth L (2015) Sorter og sortsprøving 2013. Bioforsk FOKUS 9:38-69
Åssveen M, Tangsveen J, Hedum I, Bergjord AK, Weiseth L (2009) Sorter og sortsprøving 2008. Bioforsk FOKUS 4:54-80
Åssveen M, Tangsveen J, Lundon AR, Bergjord AK, Weiseth L (2008) Sorter og sortsprøving 2007. Bioforsk FOKUS 3:24-47

			Åssveen M, Tangsveen J, Olberg E, Bergjord AK, Weiseth L (2007a) Sorter og sortsprøving 2005. Bioforsk FOKUS 1:23-52 Åssveen M, Tangsveen J, Olberg E, Bergjord AK, Weiseth L (2007b) Sorter og sortsprøving 2006. Bioforsk FOKUS 2:49-80
Paper I, p. 13	Second last line	Linkage disequilibrium	Linkage equilibrium
Paper I, p. 19	Last line	As Helium show	As Helium shows
Paper II, p. 14	l. 6	*NBP_QRptt6-1*	*NBP_QRptt6-1* QTL
Paper II, p. 14	L. 13	inoculated and inoculated	inoculated and uninoculated
Paper II, p. 14	L. 14	In chromosome	On chromosome
Paper II, p. 14	L. 14	*NBPQRptt5-2*	*NBP_QRptt5-2*
Paper II, p. 14	L. 15	*NBP_QRPtt1-2*	*NBP_QRptt1-2*