ORIGINAL ARTICLE

Genetic analysis of a worldwide barley collection for resistance to net form of net blotch disease (*Pyrenophora teres* f. *teres*)

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Abstract

Key message **A total of 449 barley accessions were phenotyped for *Pyrenophora teres* f. *teres* resistance at three locations and in greenhouse trials. Genome-wide association studies identified 254 marker–trait associations corresponding to 15 QTLs.**

Abstract Net form of net blotch is one of the most important diseases of barley and is present in all barley growing regions. Under optimal conditions, it causes high yield losses of 10–40% and reduces grain quality. The most cost-effective and environmentally friendly way to prevent losses is growing resistant cultivars, and markers linked to effective resistance factors can accelerate the breeding process. Here, 449 barley accessions expressing different levels of resistance comprising landraces and commercial cultivars from the centres of diversity were selected. The set was phenotyped for seedling resistance to three isolates in controlled-environment tests and for adult plant resistance at three field locations (Belarus, Germany and Australia) and genotyped with the 50 k iSelect chip. Genome-wide association studies using 33,818 markers and a compressed mixed linear model to account for population structure and kinship revealed 254 significant marker–trait associations corresponding to 15 distinct QTL regions. Four of these regions were new QTL that were not described in previous studies, while a total of seven regions influenced resistance in both seedlings and adult plants.

Communicated by Kevin Smith.

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Introduction

Net blotch caused by *Pyrenophora teres* Drechsler is one of the most important, damaging and widely distributed diseases of barley (Mathre 1997). *Pyrenophora teres* exists in two forms: *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata*, causing 'net form' net blotch (NFNB) and 'spot form' net blotch (SFNB), respectively. These two forms are similar in morphology during the sexual and asexual stages and only differ in the symptoms they cause (Lightfoot and Able 2010; Smedegård-Petersen 1971). NFNB produces brown netted lesions containing longitudinal and transverse striations, while SFNB produces brown spotted lesions (Smedegård-Petersen 1976). Under favourable conditions, NFNB causes significant reductions in both yield (Brandl and Hoffmann 1991; Kangas et al. 2005; Mathre 1997) and quality (Burleigh et al. 1988). Yield losses caused by NFNB on susceptible barley cultivars can reach up to 40% under favourable epidemic conditions (Steffenson et al. 1996). In the Northern Caucasus, the North-West and Central regions of the Non-Chernozem region, the South Ural, in the far east of Russia and in Belarus *P. teres* f. *teres* is the most

important disease in barley. In these regions, an epidemic appears every 4–5 years. Yield losses due to epidemics are estimated to be at 36–45% (Afonin et al. 2008). In Australia, *P. teres* f. *teres* is considered a major disease in barley and is estimated to cause average annual losses of $AUD 19 million (Murray and Brennan 2009). Conservation tillage is standard practice in Australian farming systems resulting in a plentiful bank of over-seasoning inoculum. Losses of up to 70% with severe lodging were recorded in 2009 in South Australia on barley cultivar 'Maritime' (Wallwork et al. 2016). Resistance to *P. teres* f. *teres* is a major priority of all barley breeding programs in Australia.

Pyrenophora teres f. *teres* is a highly variable pathogen (Khan 1982; Liu et al. 2011; Serenius 2006; Steffenson and Webster 1992; Tekauz 1990). As a result of global virulence studies, 153 pathotypes among 1162 isolates were identified in different geographic populations of *P. teres* f. *teres* originating from Europe, Syria and Canada on a set of 9 barley differential lines (Anisimova et al. 2017). One of the reasons for the high diversity of *P. teres* f. *teres* populations is the ability to reproduce both sexually and asexually (Mathre 1997). This high heterogeneity concerning virulence of the pathogen implies high genetic diversity in host resistance. In several studies, the complexity of the *P. teres* f. *teres*–barley interaction was shown to be controlled by major qualitative genes (Afanasenko et al. 1999; Cakir et al. 2003; Friesen et al. 2006; Grewal et al. 2012; Ma et al. 2004; Manninen et al. 2006) and quantitative trait loci (QTL) (Douglas and Gordon 1985; König et al. 2013, 2014; Robinson and Jalli 1997; Steffenson et al. 1996; Vatter et al. 2017).

Resistance genes and QTL against NFNB were identified on all seven barley chromosomes in bi-parental mapping populations and by association genetics studies (Afanasenko et al. 2015; Berger et al. 2013; Cakir et al. 2011; Cakir et al. 2003; Graner et al. 1996; Grewal et al. 2008, 2012; Gupta et al. 2004; Koladia et al. 2017; König et al. 2013, 2014; Ma et al. 2004; Manninen et al. 2006; O'Boyle et al. 2014; Raman et al. 2003; Richards et al. 2017; Richter et al. 1998; Steffenson et al. 1996; Wonneberger et al. 2017a, b). Several studies report that resistance genes identified in adult plants are often different from genes conferring NFNB resistance at the seedling stage (Cakir et al. 2003; Grewal et al. 2012; Steffenson et al. 1996; Wonneberger et al. 2017a). Pathotype-specific resistance QTL were found when different *P. teres* f. *teres* isolates were used for phenotyping different mapping populations (Afanasenko et al. 2015; Grewal et al. 2012; Koladia et al. 2017; Richards et al. 2017).

In recent years, genome-wide association studies (GWAS) have become popular for mapping QTL and major genes for several reasons: (1) segregating populations and the construction of own genetic linkage maps are not needed for GWAS, (2) It enables discovery of useful genetic variation in a broader portion of the genetic diversity present in a species

than bi-parental mapping approaches, and (3) it exploits historic recombination events, and by using populations including breeding lines and commercial cultivars, there is a higher probability that markers are directly transferable into current breeding programmes. Nevertheless, a limiting factor in GWAS can be population size. As shown by Wang et al. (2012), population size in GWAS approaches should be at least around 380 individuals to ensure statistically sound and consistently detectable marker–trait associations (MTAs). Another limitation is the presence of extensive linkage disequilibrium (LD) associated with natural or artificial selection, particularly in crop species, which have been subject to intense breeding. LD is the non-random association between two alleles at different loci and is affected by population size and mutation rate, but mostly by recombination rate (Flint-Garcia et al. 2003; Rafalski and Morgante 2004). In outcrossing species, such as maize, recombination rates are high and LD therefore decays within hundreds to a few thousands of base pairs (Remington et al. 2001; Yan et al. 2009). In contrast, in selfing species, which are usually homozygous, recombination is less effective (Flint-Garcia et al. 2003). The highly inbreeding model species *Arabidopsis thaliana*, for example, displays an average genome-wide LD decay of around 250 kilobases, corresponding to about 1 cM (Nordborg et al. 2002). Thus, compared to its very small genome size of roughly 130 Mb, LD extends over large blocks. In barley GWAS panels, LD was reported to be between 18 and 1.3 cM, depending on the diversity of the evaluated materials (Bellucci et al. 2017; Bengtsson et al. 2017; Burlakoti et al. 2017; Gyawali et al. 2017; Massman et al. 2010; Mitterbauer et al. 2017; Tamang et al. 2015; Vatter et al. 2017; Wehner et al. 2015; Wonneberger et al. 2017a). It was shown that LD decays faster in global populations. In other words, genetically and geographically diverse GWAS sets, which include *inter alia* landraces, have a comparatively low LD (Mohammadi et al. 2015; Nordborg et al. 2002). Low LD has the advantage of higher mapping resolution, narrowing the intervals of interesting QTL. However, this also means that a higher marker density is required (Zhu et al. 2008). Recently developed genotyping methods for barley, like the 50 k Barley iSelect SNP Chip (Bayer et al. 2017) or genotyping-by-sequencing (GBS) (Poland et al. 2012), reduce genotyping costs and ensure high marker density and coverage of the barley genome and overcome some of the limitations outlined. Based on these considerations, the main objectives of this study were (1) to screen a diverse set of barley accessions for resistance against *Pyrenophora teres* f. *teres* under greenhouse and field conditions, (2) to genotype this set with the 50 k iSelect chip, (3) to identify QTL for resistance against NFNB and (4) to compare these with previously known QTL in order to identify potentially new resistance loci along with associated markers for resistance breeding.

Materials and methods

Germplasm set

For GWAS, a set of 277 barley landraces and 172 commercial cultivars (total 449 accessions) from the N. I. Vavilov Research Institute of Plant Genetic Resources (VIR) collection were studied (Online Resource 1). This set was the result of a long-term joint research project between the All-Russian Institute of Plant Protection (VIZR), the VIR and the Institute of Resistance Research and Stress Tolerance of the Federal Research Centre for Cultivated Plants (JKI). A total of 12,000 barley accessions from different centres of barley diversity and commercial cultivars were screened for resistance to *Pyrenophora teres* f. *teres* and *Cochliobolus sativus* under greenhouse conditions and in detached leaf assays at the JKI and VIZR (Afanasenko 1995; Silvar et al. 2010; Trofimovskaya et al. 1983). Out of these, about 300 and 150 accessions with different levels of resistance to *P. teres* f. *teres* and *C. sativus* were identified, respectively, and investigated in the present study. With respect to the landraces, the set comprises 31 accessions from Ethiopia and Sudan, 56 from the Middle East (Turkey, Syria, Israel, Palestine), 20 from the Mediterranean Region (Cyprus, Italy, Spain, Crete, Greece), 59 from Central Asia (Tajikistan, Turkmenistan, Kyrgyzstan, Uzbekistan, Kazakhstan), 33 from China, Japan and Mongolia, 37 from South America (Peru, Ecuador, Bolivia), 4 from India, Korea, and Pakistan, 2 from Tunisia and Egypt, 8 from the Caucasus region (Georgia, Armenia, Dagestan) and 6 from the USA. With regard to commercial cultivars, the set includes one cultivar each from Austria, Denmark, Finland, Italy, Kyrgyzstan, Manchuria, Sardinia, Tunisia, Turkey, Turkmenistan, Uruguay and Yugoslavia. Mexico, the Netherlands, Poland, Portugal and Sweden are represented with two cultivars each, India with three and Germany and Ethiopia with four cultivars each. Six cultivars each are from China, France and Kazakhstan, eight and nine from the Czech Republic and Ukraine, respectively. Canada, and the USA and Japan are represented with 11, 12 and 18 cultivars, respectively. Thirty-one cultivars are from Australia and 33 from Russia.

Some of the landrace accessions date back to Nikolai I. Vavilov, who collected them on different expeditions. Accession VIR CI 3175 dates back to his first expedition in Pamir during 1916. Accessions VIR CI 7687–8378 were collected in Syria, Tunis and Cyprus during 1926, and accessions VIR CI 8515–8877 were collected in Italy, Spain and Ethiopia in 1927.

The set represents 31 morphological forms of *Hordeum vulgare* (Online Resource 1). It includes 178 two-rowed and 271 six-rowed accessions. Out of 449 accessions, 20 are winter types, 28 have black kernels, and 51 have naked kernels.

Single plant selections were made for each accession, and these selections were self-pollinated by bagging in 2013 and 2014 under field conditions at VIR (Pushkin, Russia) and in Zhodino (Belarus).

Fungal isolates

Five single-spore-derived *P. teres* f. *teres* isolates were used in this study. They were selected based on their origin, virulence and sporulation ability. Isolate *No 13* was collected in 2014 near Volosovo in the Leningrad Region, Russia, and isolate *Hoehnstedt* was collected in 2016 on infected fields close to the village of Hoehnstedt in Saxony-Anhalt (Germany). The three isolates *NFNB 50*, *NFNB 73* and *NFNB 85* are Australian isolates from Queensland, each with different virulence profiles. *NFNB 50* and *NFNB 85* were from the Gatton area, while *NFNB 73* was collected from Tansey in the South Burnett region. *NFNB 50* was used in both greenhouse and field trials, but *NFNB 73* and *NFNB 85* were used in field trials only.

Pyrenophora teres isolates *No 13* and *Hoehnstedt* were grown on V8-agar medium containing 150 ml V8 juice, 10.0 g Difco PDA, 3.0 g $CaCO_3$, 10.0 g agar and 850 ml distilled water. Petri dishes were placed in a dark chamber at room temperature for 5 to 7 days, exposed to light for 24 h and placed again in a dark chamber at 13 °C for 24 h. Conidia were then harvested by adding sterile water to the Petri dish and scraping conidia off with a sterile spatula. Conidia were counted with a haemocytometer, and the concentration was adjusted to 5000 conidia/ ml. Australian isolates were grown as described by Martin et al. (2018).

In the Australian field trials, single isolates were used for inoculation. For this, isolated blocks of highly susceptible varieties were sown in early to mid-April. Inoculum for these blocks was multiplied in the laboratory and applied to the blocks at the 4–5 leaf stage. Epidemics in these blocks were promoted by sprinkler irrigation at least twice a week when conditions for infection were favourable. These blocks provided the inoculum for the subsequent field screening (Martin et al. 2018).

Greenhouse trials

Greenhouse trials were performed at the Julius Kuehn-Institute in Quedlinburg, Germany, in 2015 and 2017 with isolates *No 13* and *Hoehnstedt*, respectively. Three seeds per accession were grown in plastic pots ($8 \times 8 \times 8$ cm) for 2–3 weeks at 16–18 °C with alternating 12 h periods of light/darkness (exposure min 5000 lx). The experiment was set up in four replications in a complete randomized block design. The NFNB differential set proposed by Afanasenko

et al. (2009) was included in each replication as a standard. When the second leaf was fully developed (BBCH 12-13), plants were spray-inoculated with the spore suspension until the inoculum was at the point of running off (approximately 0.35 mL/plant). Plants were then covered with plastic foil for 48 h to ensure 100% humidity and grown for another 10–14 days at 20–22 °C and 70% humidity until symptoms were clearly visible.

Isolate *NFNB 50* was tested at the Hermitage Research Facility in Warwick, Queensland, Australia, in 2017. Plants were grown in commercial potting mix (Searles Premium Potting Mix) in plastic maxipots (10 cm in diameter, 17 cm tall). Four to five seeds were sown at 0°, 120° and 240° around the circumference of each pot. The experiment was set up in two replications in an incomplete block design, where pots corresponded to blocks and there were three lines per block. Pots were maintained in the greenhouse at 15/27 °C for 2 weeks until the second leaf was fully expanded. Field-collected conidia were suspended in water at 3000 conidia/mL and applied from four directions using a WallWick® commercial spray gun delivering an average 3 mL/pot. Immediately after inoculation, pots were placed into a fogging chamber for 20 h at 19 °C with 14 h in the dark. After incubation, pots were returned to the greenhouse, double-spaced and bottom watered, and grown for another 8 days. Pots were fertilized twice weekly after emergence with a soluble complete fertilizer (Grow Force EX7) until notes were taken.

Infection response type was assessed on the second leaf of each plant following the scale of Tekauz (1985).

Field trials

Field trials were conducted at three locations, Belarus, Germany and Australia, during the years 2015, 2016 and 2017.

Trials in Belarus were conducted at the Research and Practical Centre of the National Academy of Sciences of Belarus for Arable Farming (Zhodino, Belarus) in 2016 and 2017. Accessions were sown in rows of 1 m with 15–20 seeds per row and a spacing of 0.3 m between rows. The trial was set up in a complete randomized block design with two replications. The cultivar 'Thorgal', susceptible to net blotch and resistant to powdery mildew, was sown around the trial as a border and after every 10th accession to support net blotch and reduce powdery mildew development in the nurseries. Additionally, 'Thorgal' was used as a spreader for net blotch. To increase infection, infected barley straw was spread in the rows of 'Thorgal' after sowing. The infected straw was harvested in the previous year at the same location. The percentage of leaf area infected was assessed at early-dough to mid-dough stages (BBCH 83–85) on the three upper leaves of the plants.

In Germany, the field trials were conducted at the Julius Kuehn-Institute (Quedlinburg, Germany) in 2015 and 2016 using the Summer Hill design developed by König et al. (2013). Accessions were sown at the beginning of August in hills with 25 seeds/hill and a spacing of 0.5 m between hills. The susceptible varieties 'Candesse' and 'Stamm 4046' were used as spreader rows and sown in rows between the hills, spacing 1.0 m between rows as described in Vatter et al. (2017). The trials were set up in two replications in a complete randomized block design. Infected barley straw was used as inoculum and was incorporated into the soil prior to sowing. Phenotyping started when symptoms were clearly visible on the susceptible standards. The percentage of leaf area infected (Moll et al. 2010) and the infection response type (Tekauz 1985) were assessed at three different time points with a period of 2 weeks between scoring dates. The area under disease progress curve (AUDPC) was calculated and used to calculate the average ordinate (AO) as described by Vatter et al. (2017).

Field trials in Australia were conducted at the Hermitage Research Facility in Warwick, Queensland in 2017 with three distinct isolates. Trials were sown as hill plots with an in-row spacing of 0.5 m and between-row spacing of 0.76 m. Two rows of datum plots were sown between five rows of spreader (0.19 m spacing). Spreaders were sown 11–19 days before the plots and were inoculated by spreading infected green plant material cut from inoculum increase blocks when the spreaders were at about BBCH 30. Epidemics were promoted with overhead sprinkler irrigation applied in the late afternoon and/or early evening so that the nurseries remained wet overnight. In the absence of rainfall, irrigation was applied two or more nights per week when conditions were favourable for infection. Infection responses were taken on a whole plot basis using a 0 to 9 scale at BBCH stages 70–73. The scale is a variant of the scale by Saari and Prescott (1975). It takes into account the plant response (infection type; IT) and the amount of disease in a plot and therefore correlates very well with the host response and the leaf area diseased (Martin et al. 2018).

Statistical analysis

Statistical analyses and analysis of variance (ANOVA) were performed using the software package SAS 9.4 (SAS Institute Inc. Cary, NC, USA) using *proc mixed* and *proc glimmix* for greenhouse trials and field trials in Australia. For field trials, the least square means (lsmeans) and for greenhouse trials the means of the infection response type were calculated and used for genome-wide association studies (GWAS). Broad sense heritability across years was calculated using the formula $h^2 = V_G/(V_G + V_{GY}/y + V_R/year)$ as described by Vatter et al. (2017), where V_G is genotypic variance, V_{GY} is genotype x year variance, V_R is residual

variance, and y and r are the number of years and replicates, respectively.

Genotyping, population structure, kinship and linkage disequilibrium

Genomic DNA was extracted from 14-day-old plants according to Stein et al. (2001). The accessions were genotyped on the Illumina iSelect 50 k Barley SNP Chip (Illumina) at Trait Genetics GmbH (Gatersleben, Germany). Physical positions of markers were taken from Bayer et al. (2017), which is based on the barley pseudo-molecule assembly by Mascher et al. (2017). SNPs having failure rates > 10%, heterozygous calls > 12.5% and a minor allele frequency (MAF) < 5% were excluded from the analyses, as well as unmapped SNPs. Thus, 33,818 SNPs were left for subsequent GWAS. In order to calculate the kinship matrix and the population structure, the markers were further filtered with the software PLINK 1.9 (www.cog-genomics.org/plink/1.9/) (Chang et al. 2015). The tool *LD prune* was used with the following parameters: indep pairwise window size 50, step 5 and an r^2 threshold 0.5 (Campoy et al. 2016). This resulted in 8533 markers for calculating the kinship and population structure. Kinship was calculated with the web-based platform Galaxy (Afgan et al. 2016) using the tool *Kinship* and the modified Roger's distance (Reif et al. 2005). Population structure was determined with the software STRUCTURE v2.3.4 (Pritchard et al. 2000). In order to identify the optimal subpopulations, an admixture model was used with a burn-in of 50,000, followed by 50,000 Monte Carlo Markov chain (MCMC) replications for $k = 1$ to $k = 10$ with 10 iterations. STRUCTURE HARVESTER (Earl and vonHoldt 2012) was used to identify the optimal k. Following this, a new STRUCTURE analysis was performed with a burn-in of 100,000 and 100,000 MCMC iterations at the optimal k value. Accessions were considered as admixed, when their membership probabilities were < 80% (Richards et al. 2017). Linkage disequilibrium (LD) was calculated as squared allele frequency correlations (R^2) between all intra-chromosomal marker pairs using the tool *linkage disequilibrium* in the web-based platform Galaxy. Genome-wide LD decay was plotted as R^2 of a marker against the corresponding genetic distance, and a Loess regression was computed. For R^2, the default settings were used (Sannemann et al. 2015).

Genome-wide association studies

Genome-wide association studies (GWAS) were performed using the Galaxy implemented tool *GAPIT*, which uses the *R* package GAPIT (Lipka et al. 2012). The model used was a compressed mixed linear model (CMLM)(Zhang et al. 2010) including the population structure (Q) and kinship (K). In order to detect significant marker–trait associations,

a Bonferroni correction was employed. For this, the reduced marker set of 8533 markers, which was used for calculating population structure and kinship, and a significance level of $P = 0.2$ was used (Muqaddasi et al. 2017; Storey and Tibshirani 2003). This resulted in a threshold of $-\log_{10}(P) \geq 4.63$. GWAS for greenhouse trials, and field trials in Australia were conducted for each isolate separately. For field trials in Zhodino and Quedlinburg, GWAS were conducted across years for each location. Manhattan plots were generated with the *R* v.3.4.4 package *qqman*.

In order to compare previously described QTL with QTL identified in the present study, the databases GrainGenes (https://wheat.pw.usda.gov/GG3/) and BARLEX (https://apex.ipk-gatersleben.de/apex/f?p=284:10) were used to obtain marker information and identify physical positions of previously published QTL studies. Where previously described QTL were identified based on iSelect markers, the physical positions were obtained from Bayer et al. (2017).

Results

Phenotypic evaluation of greenhouse trials

Phenotyping in the greenhouse with three different isolates showed a wide range of variability in the infection response type (1–10 scale) for all three isolates tested. The average infection response type (IRT) for isolate *Hoehnstedt* ranged from 1 to 9 (mean 3.96), for *No 13* from 1 to 10 (mean 5.28) and for *NFNB 50* from 1 to 10 (mean 3.6) (Fig. 1). Analysis of variance showed significant differences among the barley accessions for seedling resistance to NFNB for all isolates (Table 1). In trials with isolates *No 13* and *Hoehnstedt,* the proposed differential set by Afanasenko et al. (2009) was used as a reference. The infection scores for the differential lines can be seen in Table 2. For isolate *No 13,* the infection scores ranged from 0.75 (CI 5791) to 8 (Harrington). For *Hoehnstedt,* the lines showed less variance and the scores ranged from 2.63 (Harbin) to 4.89 (CLS 25282). For isolate *NFNB 50,* the lines used as references and their respective infection scores can be obtained from Table 3. The scores ranged from 0.8 (Beecher) to 9.1 (Grimmett).

Phenotypic evaluation of field trials

A wide range of disease severity was observed for all three locations (Fig. 2; Table 1).

In Germany in both years, infection pressure for NFNB was high in Summer Hill trials. Disease severity scores ranged on average between 4.1 and 31.5% (mean 11.5%). The frequency distribution was slightly right skewed with 184 accessions showing disease severity of < 10% and 7

Fig. 1 Frequency distribution for *Pyrenophora teres* f. *teres* reaction after inoculation with isolates Hoehnstedt, No 13 and NFNB 50
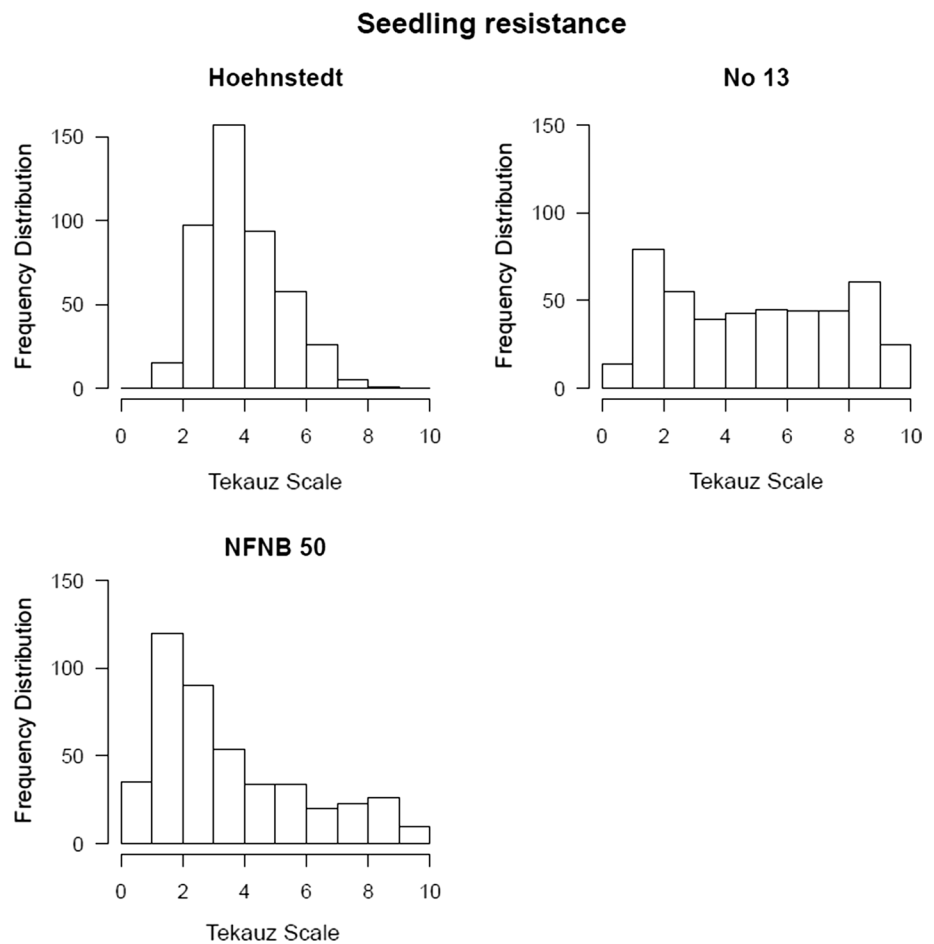
Seedling resistance

Table 1 Analysis of variance (ANOVA) for net form of net blotch (NFNB) severity for 449 barley genotypes evaluated under greenhouse and field conditions

Isolate/location	Effect	F value	P value
Hoehnstedt	Genotype	4.66	< 0.0001
No 13	Genotype	19.12	< 0.0001
NFNB 50 (greenhouse)	Genotype	22.15	< 0.0001
Quedlinburg	Genotype	3.68	< 0.0001
Zhodino	Genotype	4.63	< 0.0001
NFNB 50 (field)	Genotype	11.9	< 0.0001
NFNB 73	Genotype	12.29	< 0.0001
NFNB 85	Genotype	12.61	< 0.0001

Table 2 Disease severities of differential lines (Afanasenko et al. 2009) used in field trials in Belarus and Germany, and in greenhouse trials with isolates *No 13* and *Hoehnstedt*

Differential line	Trial			
	Belarus[a]	Germany[a]	No 13[b]	Hoehnstedt[b]
Harrington	19.75	12.20	8.00	4.75
Skiff	13.5	7.49	6.13	3.22
Prior	3	5.52	7.63	–
CI 9825	1.5	10.27	1.56	2.44
Harbin	3	11.79	1.38	2.63
K 20019	3	10.19	1.63	2.89
CI 5791	0.75	14.51	0.75	2.86
CLS 25282	0.75	14.11	1.17	4.89
K 8755	1	14.06	1.88	3.38

[a] % of leaf area infected

[b] infection response type based on Tekauz (1985), 1 to 9 scale

accessions showing scores of > 25%. The heritability for this location was estimated at $h^2 = 0.73$.

In Belarus in 2017, conditions were unfavourable for NFNB yet favourable for powdery mildew, which did not allow any more than two assessments of net blotch. Hence, for this location the disease score based on the respective last scoring date was used to calculate the mean disease severity across years. Disease severity scores ranged on average between 0.1 and 60% (mean 7.7%). The frequency distribution for this location is right-skewed with 201 accessions showing a disease severity of < 5% and 5 accessions showing scores of 40% and higher. Heritability for

Table 3 Disease severity of reference lines used in Australian field trials and in greenhouse trials with isolate *NFNB50* (0 to 9 scale based on Saari and Prescott 1975)

Reference line	Trial			
	NFNB50 (GH)	NFNB50	NFNB73	NFNB85
Commander		4.9	5.3	6.6
Beecher	0.8			
BS89-4-3		8.8	8.6	7.6
Corvette		4.7	5.2	8.0
Fleet	3.7			
Grimmett	9.1			
Kaputar	4.8			
Prior	2.7	2.6	2.2	8.8
QB15127		7.7	6.0	5.2
Rojo		2.1	2.0	2.3
Schooner		5.1	4.0	3.8
Shepherd	4.0	4.9	7.9	4.5
Skiff	8.0	8.7	5.7	3.1
WPG8412-9-2-1	2.2	2.2	1.5	1.7

field trials in Belarus was $h^2 = 0.79$. In field trials in Germany and Belarus again the differential set by Afanasenko et al. (2009) was scored as a reference (Table 2). In Germany, the disease severity among the differentials ranged from 5.52 (Prior) to 14.51% (CI 5791). In Belarus, the disease severity ranged from 0.75 (CI 5791, CLS 25282) to 19.75% (Harrington).

In Australia, three individual isolates were tested in the field. For all three isolates, disease scores from 1 to 9 were observed. Frequency distributions for isolates *NFNB 50* and *NFNB 73* were right-skewed towards resistance with 316 and 292 accessions showing disease scores ≤3, respectively. For isolate *NFNB 85* only 149 accessions showed disease scores ≤3. The reference lines used in field trials in Australia are shown in Table 3. The infection scores ranged from 1.5 (WPG8412-9-2-1) to 8.8 (BS89-4-3). Line WPG8412-9-2-1 was resistant against all isolates used. So far no isolate found in Australia has been virulent on this line.

Population structure and linkage disequilibrium

STRUCTURE analysis identified an optimal *k* value of 3, with 58, 139 and 91 individuals belonging to subpopulation one, two and three, respectively (Online Resource 2; Online Resource 3). Out of the 449 accessions, 161 showed membership probabilities of less than 0.8 and were considered as admixed. Accessions belonging to subpopulations one and two were mainly 6-rowed types, population three comprised mainly 2-rowed accessions. Genome-wide linkage disequilibrium (LD) decay was estimated at 167 kb.

Genome-wide association mapping

Seedling resistance

For isolate *Hoehnstedt,* eight significant marker–trait associations (MTAs) were detected on chromosome 6H (Fig. 3). Their $-\log_{10}(p)$ ranged from 4.63 to 6.3 (Table 4, Online Resource 4). The MTAs corresponded to two regions. The first region spanned from 128 to 165 Mbp (53.52 cM), including seven markers, with the peak marker at 140 Mbp with a $-\log_{10}(p) = 6.3$ (JHI-Hv50 k-2016-391848), which explained 4.9% of the phenotypic variance. In addition, a second region comprising one significant marker (JHI-Hv50 k-2016-399702) was identified at 370 Mbp with a $-\log_{10}(p) = 5.2$ and an R^2 of 3.9%.

GWAS for isolate *No 13* revealed 12 significant MTAs with $-\log_{10}(p)$ from 4.71 to 6.92 (Table 4; Fig. 3). Five markers associated with resistance were located on chromosome 4H between 64 and 70 Mbp with a peak marker at 70 Mbp with a $-\log_{10}(p) = 6.92$ (JHI-Hv50 k-2016-237924), explaining 2.8% of the phenotypic variance. Additional MTAs were detected on chromosomes 4H (JHI-Hv50 k-2016-241935) and 6H (SCRI_RS_176650) at 352 Mbp and 373 Mbp, respectively. In addition, five MTAs were detected on chromosome 7H at 645 Mbp explaining 1.8–2.2% of the phenotypic variance.

For isolate *NFNB 50* 32, significant MTAs were detected (Fig. 3). The $-\log_{10}(p)$ ranged from 4.64 to 9.24 (Table 4). On chromosome 3H, 15 MTAs were detected, corresponding to three regions. One marker was located at 73 Mbp (46.29 cM, $R^2 = 2.0\%$) (JHI-Hv50 k-2016-165152), and three were located between 119 and 138 Mbp (46.68 cM), explaining 2.2–2.6% of the phenotypic variance (Online Resource 4). The third region spanned from 490 to 492 Mbp (48.44–48.63 cM) and included 11 markers, with the peak marker (JHI-Hv50 k-2016-183207) located at 490 Mbp ($-\log_{10}(p) = 9.24$), which explained 4.4% of the phenotypic variance. On chromosome 6H, MTAs were detected in three regions, i.e. eight MTAs between 64 and 72 Mbp (52.73 cM, $R^2 = 2.0$ to 2.6%), six MTAs between 133 and 140 Mbp (53.52 cM, $R^2 = 2.1\%$ per marker) and two MTAs at 373 Mpb (SCRI_RS_188243 and SCRI_RS_195914), each explaining 2.1% of the phenotypic variance. One single MTA was detected on chromosome 7H (JHI-Hv50 k-2016-440870) at 5 Mbp with an R^2 of 2.4%.

Adult plant resistance

GWAS based on the data of the field trials in Quedlinburg revealed nine MTAs on chromosome 6H with $-\log_{10}(p)$ between 4.78 and 6.62 (Table 4; Fig. 4, Online Resource 5). Five markers were located between 44 and 47 Mbp with three peak markers at 47 Mbp ($-\log_{10}(p) = 4.95$), explaining

Fig. 2 Frequency distribution of resistance to *Pyrenophora teres* f. *teres* for field trials in Quedlinburg (Germany), Zhodino (Belarus) and Warwick (Australia) with isolates NFNB 50, NFNB 73 and NFNB 85
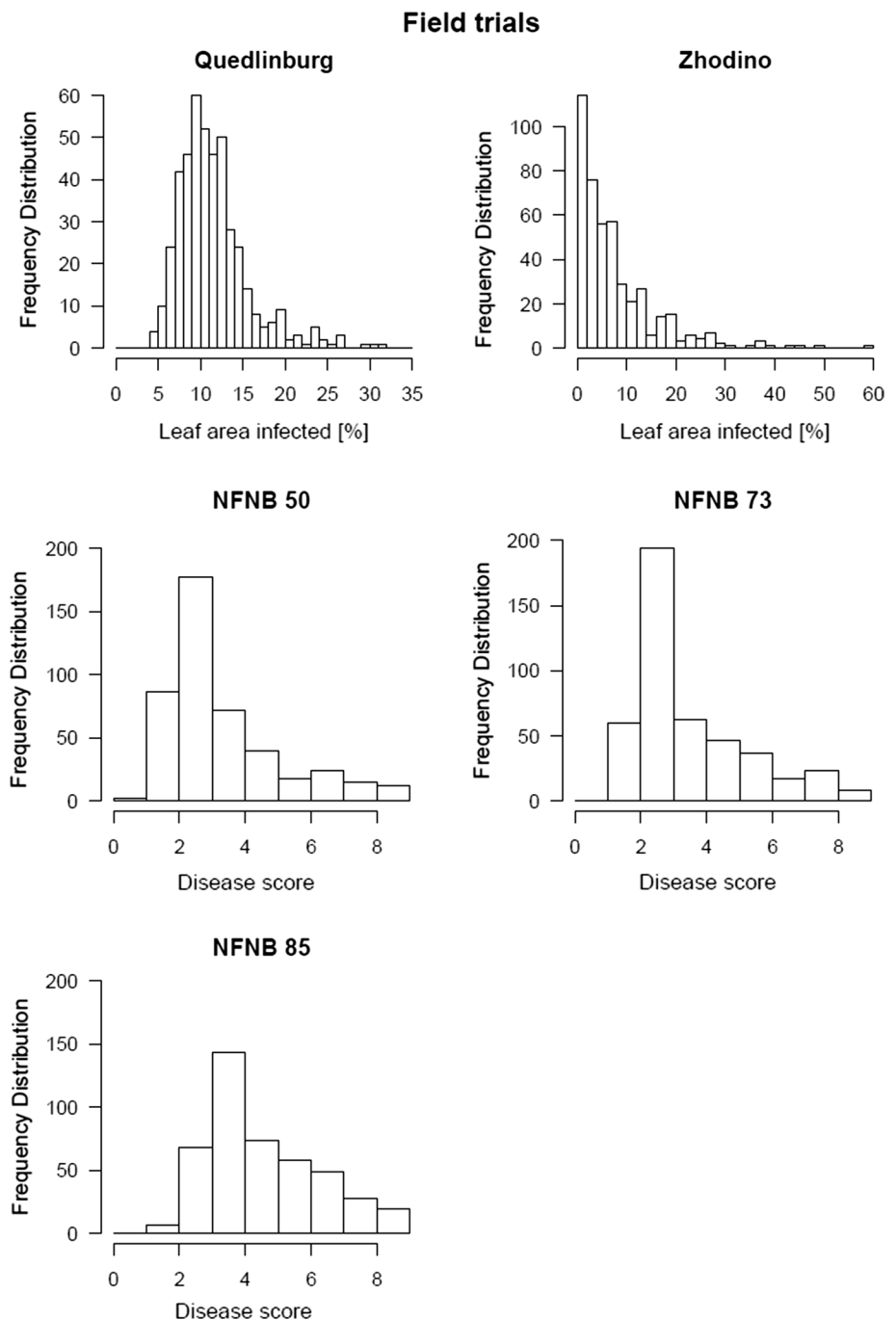
2.8% of the phenotypic variance, each. The remaining four markers were located at 406–410 Mbp with the peak marker (JHI-Hv50 k-2016-403424) at 406 Mbp explaining 3.9% of the phenotypic variance.

For the location Zhodino, a total of 61 significant MTAs were detected; 58 of these were located on chromosome 3H (Fig. 4, Online Resource 5). The 58 markers can be divided into five regions based on the physical map, and genetically these regions are less than 1 cM apart (Table 4). The first region comprised two markers located at 58 (46.29 cM)

(JHI-Hv50 k-2016-164734) and 101 Mbp (46.29 cM) (JHI-Hv50 k-2016-166000) with $-\log_{10}(p) = 4.81$ and 4.74, respectively. The second region comprised two markers located at 119 (46.68 cM) (JHI-Hv50 k-2016-166356) and 130 Mbp (46.68 cM) (JHI-Hv50 k-2016-166392) with $-\log_{10}(p) = 5.84$. The third region spanned from 233 to 350 Mbp and included 50 markers with $-\log_{10}(p) = 4.83$ to 5.24. The explained phenotypic variance ranged between 2.3 and 2.6% per marker. One marker mapped at 428 Mbp (47.07 cM), and the remaining three markers mapped
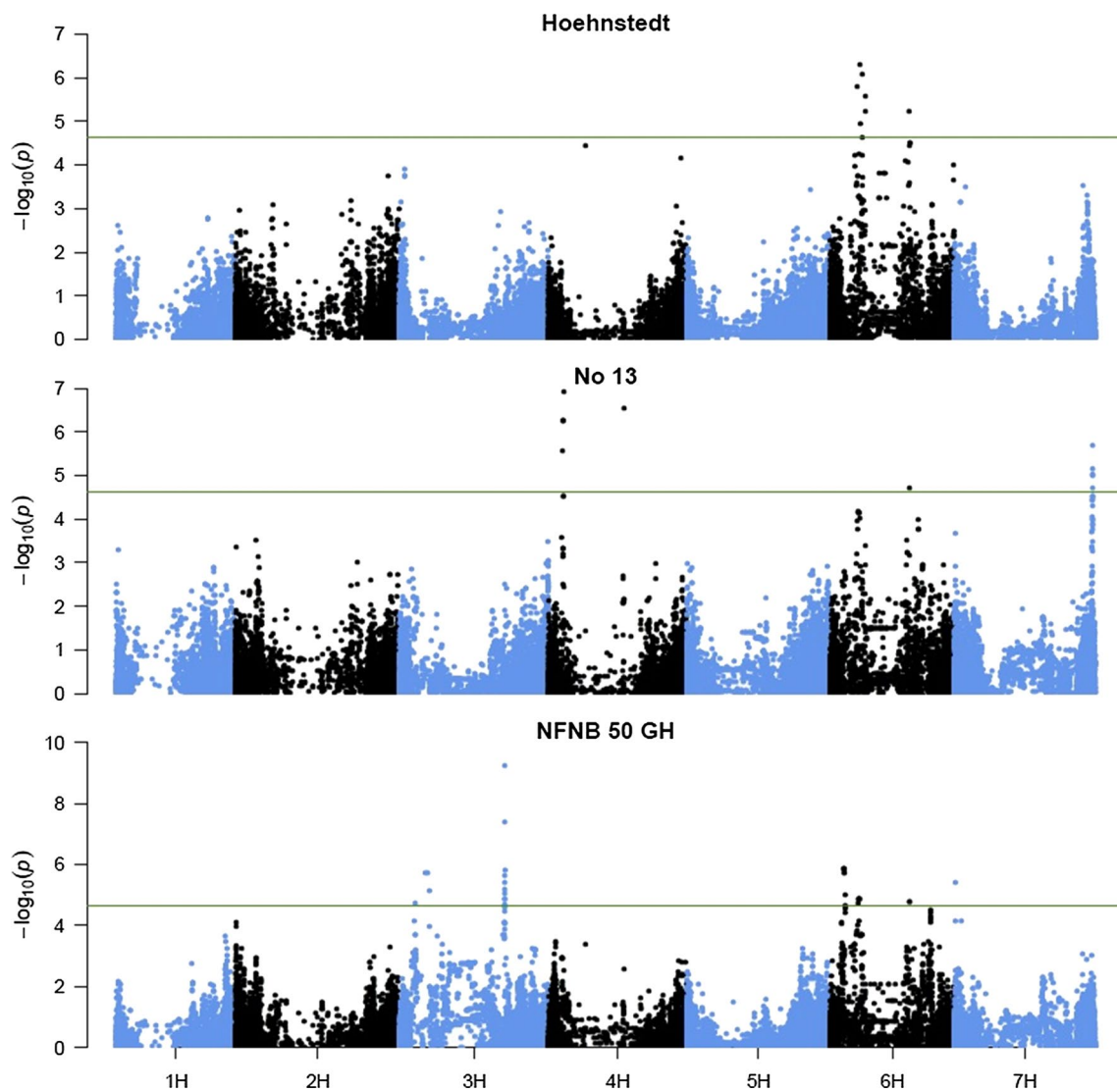
Fig. 3 Genome-wide association analyses of resistance to *Pyrenophora teres* f. *teres* isolates Hoehnstedt, No 13 and NFNB 50 tested under greenhouse conditions. The *x-axis* shows the seven barley chromosomes, positions are based on the physical map, and the $-\log10(p)$ value is displayed on the *y-axis*. The *green horizontal line* represents the significance threshold of $-\log10(p) = 4.63$

to 621 Mbp. On chromosomes 4H, 5H and 6H one MTA was identified on each, located at 33, 634 and 140 Mbp, respectively.

For field trials conducted with isolate *NFNB 50*, eleven MTAs were detected (Fig. 5), four of which were located on chromosome 3H at 446 to 490 Mbp (47.46–48.44 cM) with the peak marker located at 490 Mbp ($-\log_{10}(p) = 6.08$; JHI-Hv50 k-2016-183351) explaining 3.4% of the phenotypic variance. The remaining MTAs were located on chromosome 6H in the interval 133–135 Mbp (53.52 cM), 368 and 373 Mbp, R^2 values ranged between 2.5 and 4% (Table 4, Online Resource 6).

GWAS for isolate *NFNB 73* revealed 65 significant MTAs (Fig. 5, Online Resource 6). One marker was located on chromosome 5H at 579 Mbp ($-\log_{10}(p) = 4.65$;

JHI-Hv50 k-2016-326506) and one on chromosome 6H at 72 Mbp (JHI-Hv50 k-2016-388677). 51 MTAs mapped to chromosome 6H between 123 and 344 Mbp (53.52–53.91 cM) with $-\log_{10}(p)$ between 4.8 and 8.18, explaining 2.3 to 4% of the phenotypic variance (Table 4). The remaining 12 MTAs were also located on chromosome 6H in an interval from 356 to 373 Mbp with the peak marker at 373 Mbp with a $-\log_{10}(p) = 20.07$ and a R^2 value of 11.1% (SCRI_RS_188243).

A total of 56 markers, all located on chromosome 6H, were detected for isolate *NFNB 85*, corresponding to two regions (Fig. 5, Online Resource 6). The first region spanned from 37 to 76 Mbp with three peak markers at 47 Mbp ($-\log_{10}(p) = 10.22$), explaining 6.8% per marker (JHI-Hv50 k-2016-385826, JHI-Hv50 k-2016-385857,

Table 4 Chromosomal regions significantly associated with disease resistance/susceptibility towards *Pyrenophora teres* f. *teres*. Seedling resistance was tested using isolates *Hoehnstedt*, *No 13* and *NFNB 50*. Adult plant resistance was tested under field conditions in Quedlinburg (Germany), Zhodino (Belarus) and Warwick (Australia, with isolates *NFNB 50*, *NFNB 73* and *NFNB 85*). The complete lists of significant marker–trait associations can be found in Online Resources 4, 5 and 6

Isolate/location	Chr	Position (MB)[a]	cM[b]	−log 10 (*p* value)	R^{2c}	Peak marker
Hoehnstedt	6H	128.978649–165.696981	53.52	4.629–6.314	0.034–0.049	JHI-Hv50 k-2016-391848
	6H	370.400702	N/A	5.223	0.039	JHI-Hv50 k-2016-399702
No 13	4H	64.213185–70.916854	N/A	5.565–6.921	0.022–0.028	JHI-Hv50 k-2016-237924
	4H	352.904766	N/A	6.530	0.026	JHI-Hv50 k-2016-241935
	6H	373.424916	N/A	4.726	0.018	SCRI_RS_176650
	7H	645.343981–645.821472	N/A	4.714–5.674	0.018–0.022	JHI-Hv50 k-2016-514022
NFNB 50	3H	73.225203	46.29	4.724	0.020	JHI-Hv50 k-2016-165152
(seedling)	3H	119.62783–138.756589	46.68	5.122–5.735	0.022–0.026	JHI-Hv50 k-2016-166356
						JHI-Hv50 k-2016-166392
	3H	490.244247–492.773583	48.44–48.63	4.633–9.243	0.020–0.044	JHI-Hv50 k-2016-183207
	6H	64.21999–72.704287	52.73	4.640–5.848	0.020–0.026	JHI-Hv50 k-2016-387864
						JHI-Hv50 k-2016-387926
						JHI-Hv50 k-2016-388164
	6H	133.169988–140.843412	53.52	4.740–4.851	0.021	JHI-Hv50 k-2016-391664
						JHI-Hv50 k-2016-391711
						JHI-Hv50 k-2016-391719
						JHI-Hv50 k-2016-391721
	6H	373.423645–373.61703	N/A	4.772–4.796	0.021	SCRI_RS_188243
	7H	5.165127	N/A	5.416	0.024	JHI-Hv50 k-2016-440870
Quedlinburg	6H	44.246648–47.371815	N/A	4.775–4.947	0.027–0.028	JHI-Hv50 k-2016-385826
						JHI-Hv50 k-2016-385857
						JHI-Hv50 k-2016-385944
	6H	406.693351–410.500947	N/A	5.007–6.620	0.029–0.039	JHI-Hv50 k-2016-403424
Zhodino	3H	58.922007–101.184493	46.29	4.742–4.812	0.023	JHI-Hv50 k-2016-164734
	3H	119.62783–130.79036	46.68	5.839	0.029	JHI-Hv50 k-2016-166356
						JHI-Hv50 k-2016-166392
	3H	233.011291–350.511777	N/A	4.827–5.243	0.023–0.026	SCRI_RS_160464
						JHI-Hv50 k-2016-173670
						JHI-Hv50 k-2016-174303
	3H	428.370730	47.07	4.695	0.023	JHI-Hv50 k-2016-179690
	3H	621.113001–621.116747	N/A	4.654	0.022	JHI-Hv50 k-2016-202195
	4H	33.367057	47.27	4.836	0.024	JHI-Hv50 k-2016-233404
	5H	634.732801	N/A	4.733	0.023	SCRI_RS_236545
	6H	140.843412	53.52	5.726	0.029	JHI-Hv50 k-2016-391848
NFNB 50	3H	446.058505–490.798579	47.46–48.44	4.703–6.077	0.025–0.034	JHI-Hv50 k-2016-183351
(adult plant)	6H	133.169988–135.378543	53.52	4.728–5.250	0.025–0.029	JHI-Hv50 k-2016-391636
	6H	368.968887–373.423645	N/A	4.921–7.121	0.026–0.040	SCRI_RS_188243
NFNB 73	5H	579.069274	N/A	4.646	0.021	JHI-Hv50 k-2016-326506
	6H	72.039115	N/A	4.924	0.023	JHI-Hv50 k-2016-388677
	6H	123.046959–344.799609	53.52–53.91	4.796–8.181	0.022–0.040	JHI-Hv50 k-2016-394438
						JHI-Hv50 k-2016-394846
						JHI-Hv50 k-2016-395883
						JHI-Hv50 k-2016-397733
	6H	356.025431–373.424916	N/A	4.738–20.065	0.022–0.111	SCRI_RS_188243
NFNB 85	6H	37.571424–76.621753	N/A	4.629–10.221	0.028–0.068	JHI-Hv50 k-2016-385826
						JHI-Hv50 k-2016-385857
						JHI-Hv50 k-2016-385944
	6H	355.018439–379.784111	N/A	4.688–8.710	0.028–0.057	JHI-Hv50 k-2016-399838

[a]physical positions based on Bayer et al. (2017)

[b]genetic positions based on RIL population of Golden Promise × Morex by Bayer et al. (2017)

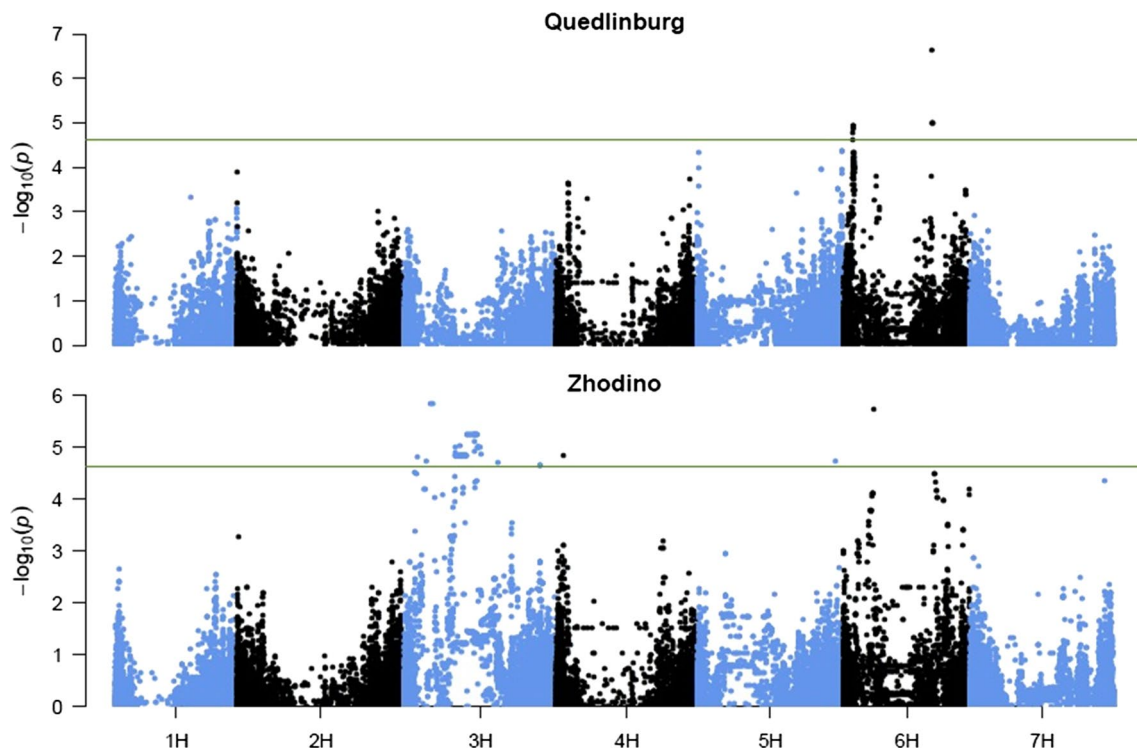[c]explained phenotypic variance per marker

Fig. 4 Genome-wide association analyses of resistance to *Pyrenophora teres* f. *teres* under field conditions in Quedlinburg (Germany) and Zhodino (Belarus). The *x-axis* shows the seven barley chromosomes, positions are based on the physical map, and the $-\log10(p)$ value is displayed on the y-*axis*. The *green horizontal line* represents the significance threshold of $-\log10(p) = 4.63$

JHI-Hv50 k-2016-385944) (Table 4). The second region was located at 355 to 379 Mbp with the peak marker at 373 Mbp and a $-\log_{10}(p) = 8.71$ (JHI-Hv50 k-2016-399838), which explained 5.7% of the phenotypic variance.

Discussion

The net form of net blotch (NFNB) is a threat to barley producing regions all over the world. The high variability of this pathogen (Khan 1982; Liu et al. 2011; Serenius 2006; Steffenson and Webster 1992; Tekauz 1990) makes it a difficult task for breeders to identify and successfully introduce new resistance genes and QTL into current breeding material. Via bi-parental mapping approaches, several QTL for resistance on all seven barley chromosomes have been identified (König et al. 2014; Liu et al. 2011; Martin et al. 2018; Vatter et al. 2017). In recent years, GWAS has become a prominent approach to identify QTL and major genes for agronomically important traits, e.g. yield, abiotic and biotic stress (Gurung et al. 2011; Lex et al. 2014; Rode et al. 2011; Simmonds et al. 2014; Wehner et al. 2015). So far, only three studies were published showing the use of GWAS to identify NFNB resistance in barley (Amezrou et al. 2018; Richards et al.

2017; Wonneberger et al. 2017a) along with one study using nested association mapping (NAM)(Vatter et al. 2017). All of these previous studies used the low-density 9 k iSelect SNP Chip as a genotyping platform and applied genetic linkage maps for approximation of QTL positions. To our knowledge, the present study represents the first study on NFNB in barley to employ the 50 k iSelect barley SNP chip in association with physical marker positions (Bayer et al. 2017) based on the pseudo-molecule genome assembly of Mascher et al. (2017).

Phenotypic results of all experiments showed a high variability of the accessions towards the pathogen. Heritability ranged from $h^2 = 0.67$ to 0.79 and was in good accordance with previously published studies, reporting on h^2 for this trait ranging between 0.62 and 0.99 (Grewal et al. 2012; König et al. 2013; Richards et al. 2017; Vatter et al. 2017; Wonneberger et al. 2017a).

Overall, 254 significant marker–trait associations (MTAs) were detected, which corresponded to 15 distinct regions. Three of them were identified conferring seedling resistance, five conferring adult plant resistance and seven were active at both growth stages. The regions were located on barley chromosomes 3H, 4H, 5H, 6H and 7H.

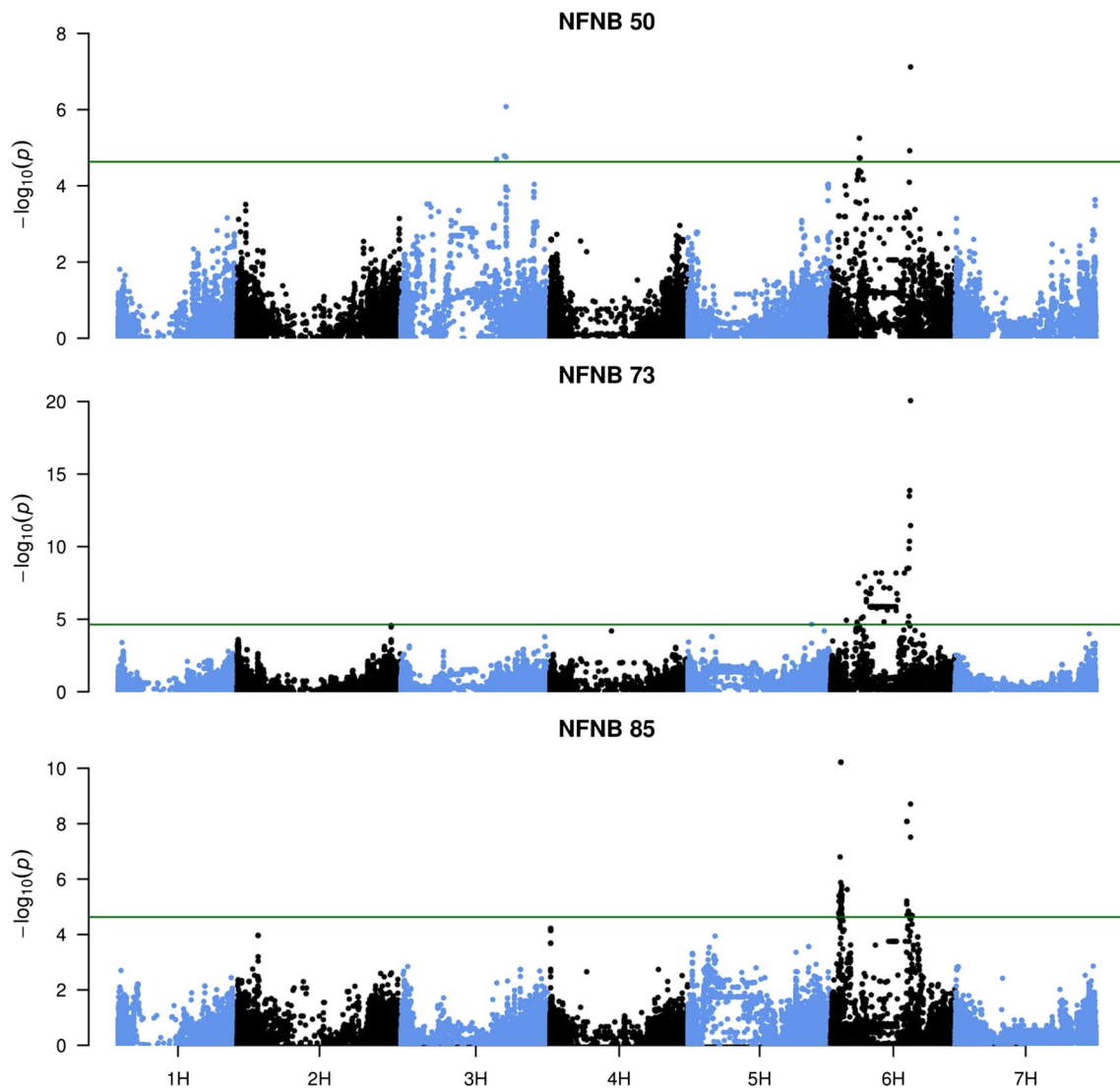On chromosome 3H, five regions were identified associated with NFNB resistance (Fig. 6). The first two regions

Fig. 5 Genome-wide association analyses of resistance to *Pyrenophora teres* f. *teres* isolates NFNB 50, NFNB 73 and NFNB 85 tested under field conditions in Warwick (Australia). The *x-axis* shows the seven barley chromosomes, positions are based on the physical map, and the –log10(*p*) value is displayed on the *y-axis*. The *green horizontal line* represents the significance threshold of –log10(*p*) = 4.63

identified based on phenotypic data from the field trials at Zhodino and greenhouse trials using isolate *NFNB 50*, were located at 58–101 Mbp and 119–130 Mbp corresponding genetically to 46.29 and 46.68 cM, respectively. These MTAs are assumed to contribute resistance at seedling and adult plant stages. König et al. (2014) identified several regions on the short arm of chromosome 3H (QTLuнs-3H, QTLuнs-3H-1 and QTLuнs-3H-2), but with the data available it is not possible to determine whether it corresponds to our regions. The third region on chromosome 3H was mapped to 233–350 Mbp for data based on field trials in Zhodino. Physically, this appears to be a large interval, and Bayer et al. (2017) were not able to anchor markers located in this interval into a genetic map of their

RIL population. Physically, there is a gap between the two markers JHI-Hv50 k-2016-169770 (222.344564 Mbp) and JHI-Hv50 k-2016-175163 (352.146586 Mbp); however, genetically both markers are located at 47.07 cM (Bayer et al. 2017). The region is located near or in the centromere, where little recombination occurs, which explains the large physical interval. Graner et al. (1996) identified a gene on the long arm of chromosome 3H conferring resistance to NFNB designated *Pt,a*. In our study, two regions on chromosome 3HL were identified. The first spanned from 428 to 492 Mbp and was based on data from the field trials in Zhodino and the field and greenhouse trials with isolate *NFNB 50*, hence contributing to seedling and adult plant resistance. One of the significant associated markers in our studies was
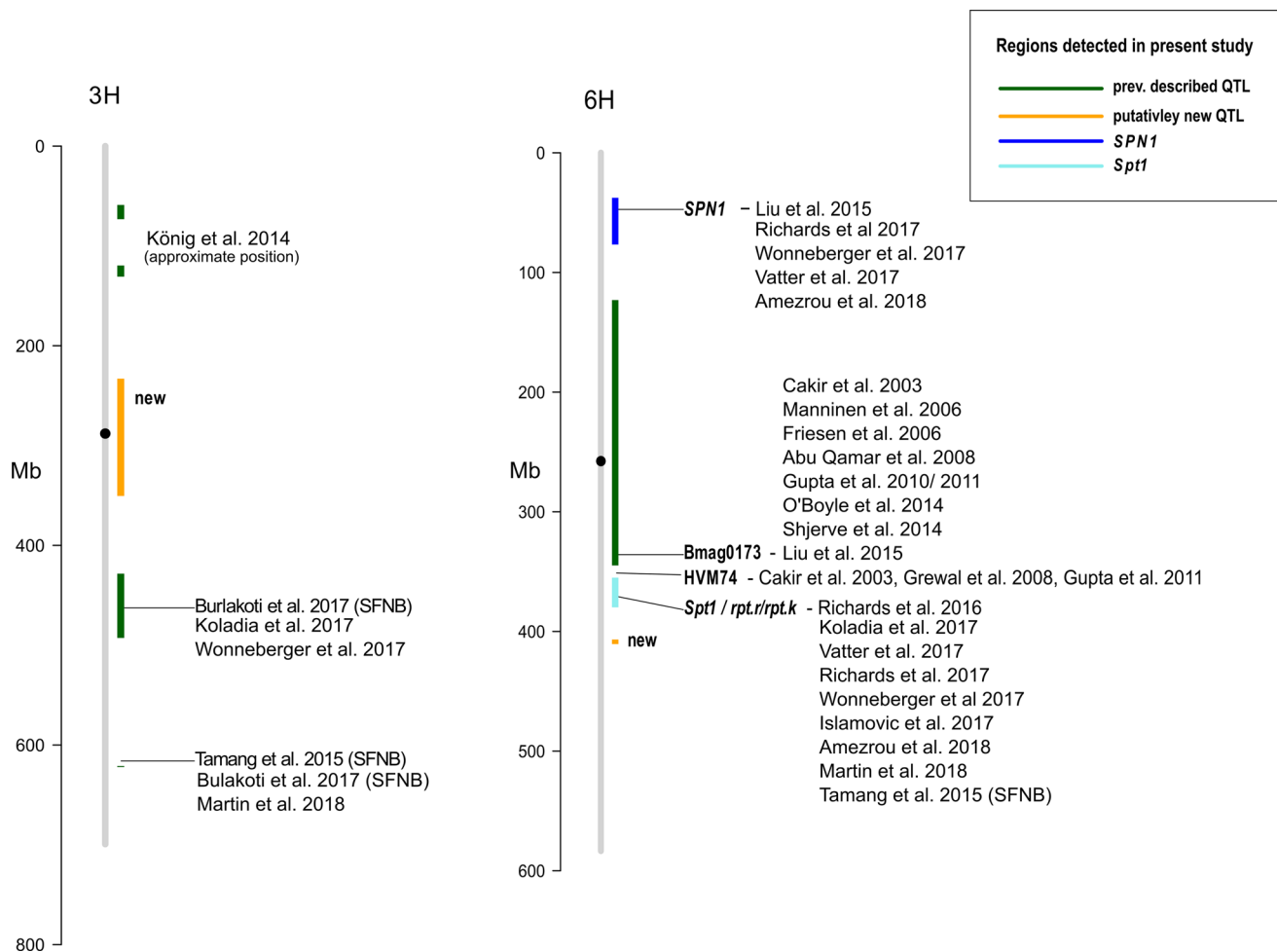
Fig. 6 QTLs for *P. teres* f. *teres* resistance in barley on chromosomes 3H and 6H. QTLs detected in the present study are represented by vertical bars. Previous studies reporting overlapping QTLs are shown next to the respective region

SCRI_RS_152172, which was also significantly associated with NFNB resistance in the study by Wonneberger et al. (2017a). Koladia et al. (2017) evaluated nine NFNB isolates and detected a QTL, which was significant for all these isolates, located at 490 Mbp with the peak marker being SCRI_RS_221644. Burlakoti et al. (2017) also found a QTL, which corresponds to our region. Interestingly, they conducted GWAS on resistance for the SFNB, indicating that even though both forms are genetically distinct, this region harbours resistance against both forms and is not isolate specific. The same holds true for the second region identified on chromosome 3HL located at 621 Mbp and identified from field data from Zhodino. Burlakoti et al. (2017) and Tamang et al. (2015), who both worked with SFNB, identified QTL on chromosome 3HL at 625 Mbp and 616–619 Mbp, respectively. Martin et al. (2018), also detected a QTL for NFNB resistance located at 622 Mbp by mapping a DH-population (UVC8 x SABBI Erica) and developed a PCR-based KASP marker (USQ3_1329) for use in breeding programmes.

On chromosome 4H, two regions harbouring NFNB resistance were identified. The first was located on the short arm of chromosome 4H at 33–70 Mbp and was detected from field trials at Zhodino and greenhouse trials with isolate *No 13*. This region corresponded to a QTL found by Islamovic et al. (2017), which was flanked by markers 4544-461 (46 Mbp) and 1944-1901 (76 Mbp) and was significantly associated with resistance for all four isolates tested. One of the isolates they used was *NFNB 50,* which was also tested in our study, but did not reveal any marker–trait association at this chromosomal region. The second region on chromosome 4H was located near the centromere at 352 Mbp. This region was found for seedling tests using isolate *No 13*. Wonneberger et al. (2017a) detected a QTL at 350 Mbp for seedling resistance and Steffenson et al. (1996) located a major QTL on chromosome 4H near the centromere for seedling resistance, which explained 31% of the phenotypic variance.

Two regions for NFNB resistance at the adult plant stage were detected on chromosome 5HL. The first region was detected using isolate *NFNB 73* and was located at 579 Mb. For this region, no overlap with previously published QTL was found. Based on the data from field trials in Zhodino, an association located at 634 Mbp was detected. The resistance locus *AL_QRptt5-2*, identified in a study using a DH-population of the cross Arve x Lavrans, and in a GWA study (Wonneberger et al. 2017a, b), is located in the same region (648–652 Mbp). In addition, Amezrou et al. (2018) detected a MTA at 624–629 Mbp and Grewal et al. (2012) mapped a QTL to the telomeric region of chromosome 5HL (199.4–206.3 cM).

Chromosome 6H is widely known to harbour several QTL for resistance/susceptibility to NFNB. In this study, four regions associated with resistance were detected (Fig. 6). The first region is located on chromosome 6HS between 37 and 76 Mbp and was detected for field trials in Quedlinburg, field trials using isolates *NFNB 73* and *NFNB 85* and for greenhouse trials using isolate *NFNB 50*. This region mapped to the sensitivity locus *SPN1*, which is flanked by the markers 4191-268 (47.261684 Mbp) and ABC08769-1-1-205 (91.140417 Mbp) (Liu et al. 2015). In that study, Liu et al. screened a RIL-population of Hector x NDB 112. The latter is a highly resistant, North American landrace, which was also included in our study and showed low infection responses for all experiments. Vatter et al. (2017) and Wonneberger et al. (2017a) both found associations at 46 Mbp, while Richards et al. (2017) identified MTAs for all isolates tested at between 42 and 66 Mbp. Interestingly, five of the markers identified in their study (SCRI_RS_162581, SCRI_RS_119674, SCRI_RS_142506, SCRI_RS_168111 and 12_30658), also revealed a significant MTA in our study. Finally, Amezrou et al. (2018) also identified a MTA at 66 Mbp. Furthermore, a large region spanning from 123 to 344 Mbp was detected based on the data for field trials in Zhodino, field trials using isolates *NFNB 50* and *NFNB 73*, and greenhouse trials using isolates *NFNB 50* and *Hoehnstedt*. Genetically, this region spanned from 53.52 to 53.91 cM (Bayer et al. 2017). Wonneberger et al. (2017a) and Islamovic et al. (2017) detected significant QTL at 120–164 Mbp and 340 Mbp (marker 5497-661), respectively. Martin et al. (2018) developed several PCR-based KASP markers for the identification of NFNB resistance QTL. Their marker USQ2_0799 is located at 335.741625 Mbp (Anke Martin, Centre for Crop Health, University of Southern Queensland, Toowoomba, QLD 4350, Australia, unpublished data) and co-located with the microsatellite marker Bmag0173 (67.4 cM). This microsatellite marker has long been associated with NFNB resistance and was used in several studies in the past (Abu Qamar et al. 2008; Cakir et al. 2003; Friesen et al. 2006; Gupta et al. 2010; Gupta et al. 2011; Liu et al. 2015; Manninen et al. 2006; O'Boyle et al. 2014;

Shjerve et al. 2014). The third region on chromosome 6H is located near the centromere at 355–379 Mbp and turned out to be significantly associated with NFNB resistance in all experiments, except field trials in Germany and Zhodino. Markers located in this interval could not be anchored genetically in the RIL population by Bayer et al. (2017). Similar to chromosome 3H, there is a gap on chromosome 6H in the region between markers JHI-Hv50 k-2016-397733 (344.799609 Mbp) and JHI-Hv50 k-2016-401495 (387.092035 Mbp). Genetically, they are located at 53.91 and 54.30 cM, respectively (Bayer et al. 2017). Nevertheless, this region corresponds to the major susceptibility locus *Spt1*, formerly named *rpt.r/ rpt.k* (Abu Qamar et al. 2008), and was fine-mapped by Richards et al. (2016). In their study, the markers rpt-M12, rpt-M13 and rpt-M20 co-segregated with the *Spt1* locus and corresponded to the Morex WGS contigs morex_contig_43862, morex_contig_64570 and morex_contig_37494, respectively. The contig morex_contig_64570 includes the iSelect marker SCRI_RS_176650, which is positioned at 373.424916 Mbp and significantly associated with resistance to isolates *No 13*, *NFNB 73* and *NFNB 85*. The gene is flanked by the markers rpt-M8 and SCRI_RS_165041 (384.412678 Mbp). Rpt-M8 corresponds to morex_contig_1573477, which includes the SNP markers SCRI_RS_171997 (370.428440 Mbp) and SCRI_RS_186193 (370.429069 Mbp). Hence, the locus is positioned between 370 and 384 Mbp. According to Richards et al. (2016) *Spt1* is delimited to 0.24 cM. This region contains 49 genes including 39 high-confidence genes with 6 genes encoding immunity receptor-like proteins. Moreover, this region was also identified via GWAS by Richards et al. (2017), Wonneberger et al. (2017a), Vatter et al. (2017) and Martin et al. (2018). In the latter study, PCR-based KASP markers were developed, namely USQ1_1140 (352 Mbp) and USQ3_0144 (384 Mb). The marker USQ1_1140 co-located with the microsatellite marker HVM 74 (68.0 cM), which was reported in several previous studies to be associated with resistance to NFNB (Cakir et al. 2003; Grewal et al. 2008; Gupta et al. 2011). In a study with SFNB, Tamang et al. (2015) identified significant MTAs at 370–373 Mbp for all isolates tested. One of the significant markers was the above-mentioned iSelect marker SCRI_RS_176650 (373.424916 Mbp). This suggests that *Spt1* does not only confer susceptibility to NFNB but also to SFNB and that this SNP (SCRI_RS_176650) can be very valuable for identifying resistant or susceptible genotypes. The last region on 6H is located at 406–410 Mbp and was identified from field trials in Quedlinburg. Amezrou et al. (2018) and Koladia et al. (2017) both reported a QTL at 390 Mbp and in both studies the marker SCRI_RS_140091 was one of the peak markers. Using the genetic map of Bayer et al. (2017), our region is located at 54.69 cM, while the region reported in the other two studies is located at 54.3 cM. Based on the

available data, it is not possible to determine whether the regions belong to one QTL or if they have to be considered as individual QTLs.

On chromosome 7H, two regions contributing to seedling resistance were identified. The first region was identified from greenhouse trials using isolate *NFNB 50* and was located on chromosome 7HS at 5 Mbp and consisted of one MTA (marker JHI_Hv50 k-2016-440870). Vatter et al. (2017) found significant associations at 2 Mbp. The second region was located on the long arm of chromosome 7H, consisted of five MTAs at 645 Mbp, and was based on greenhouse trials with isolate *No 13*. Richards et al. (2017) reported a QTL at 643 Mbp against all isolates tested. In a study by Martin et al. (2018), a QTL at 655 Mbp was reported from field trials using isolates *NFNB 50* and *NFNB 85*. These isolates were used in the present study as well, yet we did not detect any significant associations from our trials using these two isolates.

The set of barley accessions used in this study showed a genome-wide LD decay of 167 kb. Notably, for an inbreeding crop such as barley, this can be considered comparably low. LD decay in previous GWA studies in barley were estimated at 18 to 1.3 cM (Bellucci et al. 2017; Bengtsson et al. 2017; Burlakoti et al. 2017; Gyawali et al. 2017; Massman et al. 2010; Mitterbauer et al. 2017; Tamang et al. 2015; Vatter et al. 2017; Wehner et al. 2015; Wonneberger et al. 2017a). Burlakoti et al. (2017) evaluated a barley set of 376 advanced breeding lines from four breeding programmes from the Upper Midwest and reported LD decays between 10 and 18 cM. Wonneberger et al. (2017a) used a set comprising landraces and breeding lines predominantly originated from Norway, Sweden, Denmark and Finland and reported LD decay to be 13 cM. Vatter et al. (2017) used a nested association mapping (NAM) population (Maurer et al. 2015), where the spring barley cultivar Barke was crossed with 25 wild barley accessions originated from the Fertile Crescent. This population showed a LD decay of 8 cM. LD decays between 4 and 8 cM were also reported by Massman et al. (2010), Bellucci et al. (2017) and Tamang et al. (2015), who evaluated 768 advanced breeding lines from the Upper Midwest, 112 cultivated winter barleys from eleven European countries and 2062 geographically diverse barley cultivars, breeding lines and landraces, respectively. Mitterbauer et al. (2017) evaluated a set of 98 winter barleys comprising gene bank accessions and European cultivars released in different years and estimated LD decay at 1.3 cM. This shows that LD varies greatly between studies and is highly dependent on the material investigated. However, it gives an insight into the diversity of the genotypes involved. The barley accessions in the present study originated from almost 50 different countries from all over the world. The high diversity of the set was observed in the phenotypic reactions towards infection with NFNB, but is also reflected in the low LD. A low LD enables a high mapping resolution, which requires a high marker saturation (Zhu et al. 2008). With the 50 k iSelect chip (Bayer et al. 2017), this hurdle can be overcome. The chip comprises 44,040 SNP markers, of which 33,818 SNP markers were informative for the barley set investigated in the present study. This means, with a genome size of 5.1 Gb in barley, there was on average a marker every 150 kb. In combination with the physical map, it will enable researchers to define QTL more accurately. The centromeric region of chromosome 6H is a good example for this. This region has long been known to harbour several resistance/susceptibility QTL and has been described in many studies (Abu Qamar et al. 2008; Friesen et al. 2006; Gupta et al. 2010; Gupta et al. 2011; Liu et al. 2015; Martin et al. 2018; O'Boyle et al. 2014; Richards et al. 2016, 2017; Shjerve et al. 2014; Vatter et al. 2017; Wonneberger et al. 2017a). Two markers located in this region are the SSR markers Bmag0173 and HVM 74. These two markers were repeatedly reported to map to the same region. Martin et al. (2018) mapped them only 0.6 cM apart (Bmag0173: 67.4 cM, HVM 74: 68 cM). However, in the present study it was shown that they can be assigned to two different regions, located at 123–344 Mbp and 356–379 Mbp.

The aim of this study was to identify QTL for resistance against NFNB in a diverse barley set. The MTAs detected corresponded to 15 distinct regions and were located on barley chromosomes 3H, 4H, 5H, 6H and 7H. Eleven of these regions corresponded to QTL already described in previous studies, and seven regions were identified at the seedling and adult plant stage. The four putatively new QTL were located on chromosomes 3H at 233 to 350 Mbp, 5H at 579 Mbp, 6H at 406 to 410 Mbp and on chromosome 7H at 5 Mbp. Most regions were identified across several isolates and/or locations tested, which makes them interesting for breeding purposes, since they do not seem to be race-specific.

Compliance with ethical standards

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

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