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

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Key message: Association mapping of resistance to *Pyrenophora teres* f. *teres* in a collection of Nordic barley germplasm at different developmental stages revealed 13 quantitative loci with mostly small effects.

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.

Author contribution statement

M. L. planned the project together with A. F., managed the project and the experiments, provided the plant material and contributed to the discussion and interpretation of the results. R. W. conducted the disease screenings and data analysis and interpretation and wrote the manuscript.

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Conflict of interest

The authors declare that there are no conflicts of interest in the reported research.

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 which 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 ESM1).

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, Br.Pteres 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 ESM3 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	5.6	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

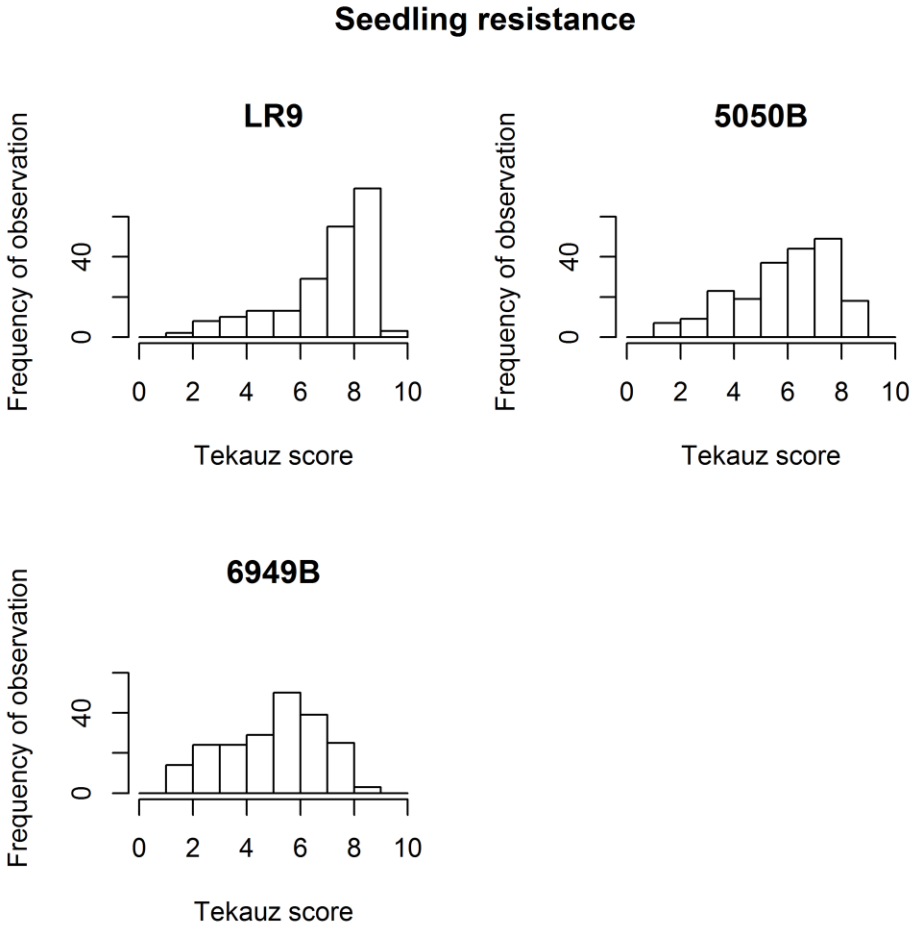


Fig. 2 Frequency distributions for adult plant disease responses under inoculated field conditions in three years

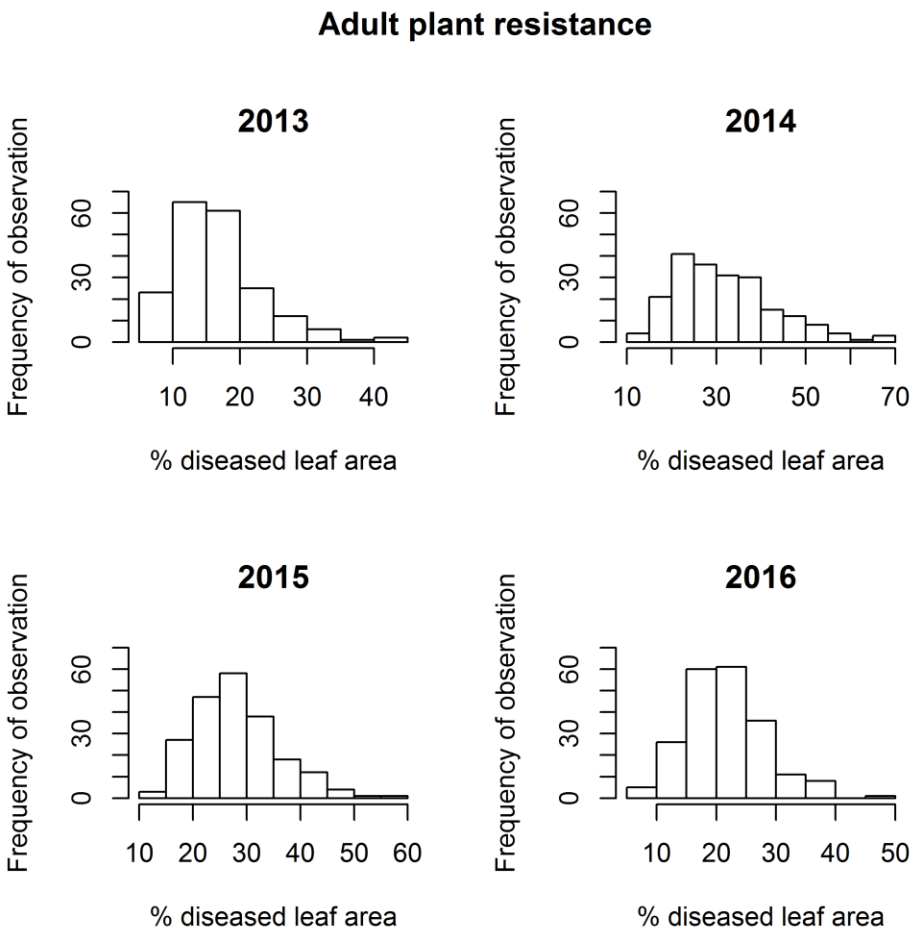
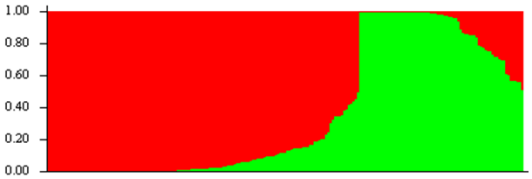


Fig. 3 Population structure in the AM panel. Bayesian clustering divided the panel mainly into two subgroups corresponding to two-rowed (green) and six-rowed (red) barleys



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

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

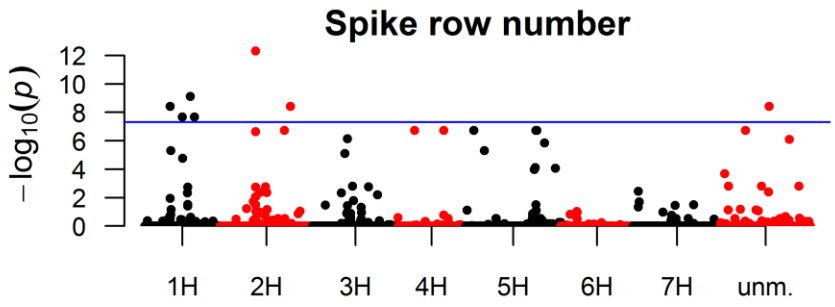


Fig. 5 Association mapping of net blotch scorings in seedlings using the MLM + K + Q model. Vertical axes show the –log(10) value of MTA p-values. Dots above the horizontal lines represent MTAs with a p-value within the 0.1 percentile quantile and are considered significant. Green vertical bars indicate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

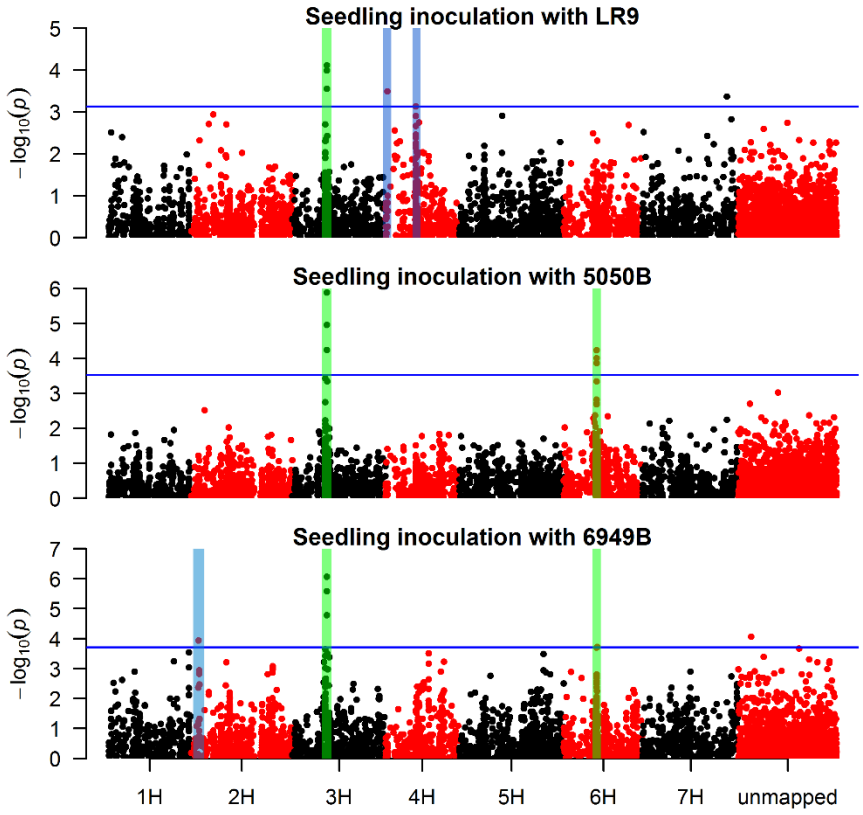


Fig. 6 Association mapping of net blotch scorings in adult plants using the MLM + K + Q model. Vertical axes show the –log(10) value of MTA p-values. Dots above the horizontal lines represent MTAs with a p-value within the 0.1 percentile quantile and are considered significant. Green vertical bars indicate 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

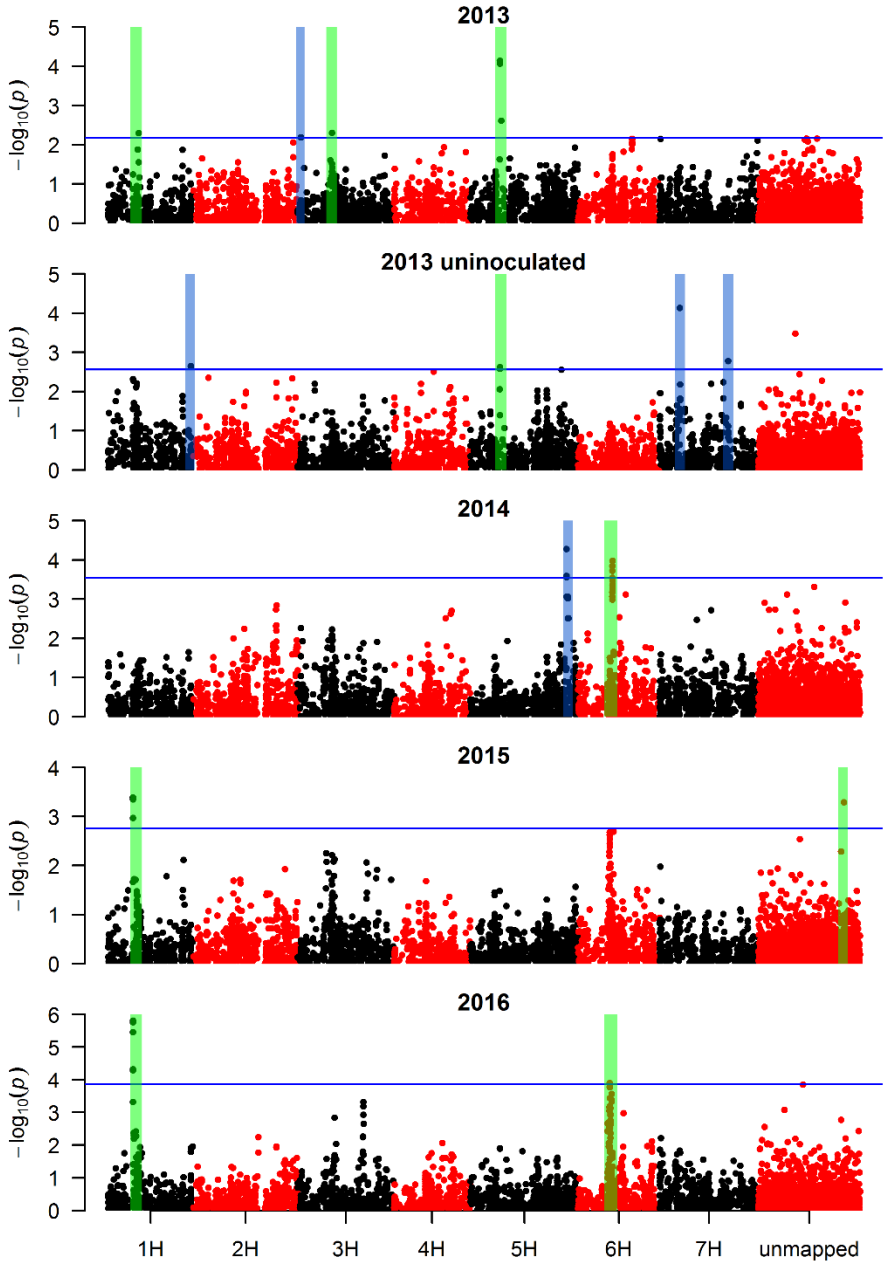


Fig. 7 Effect of QTL number on adult plant stage disease response

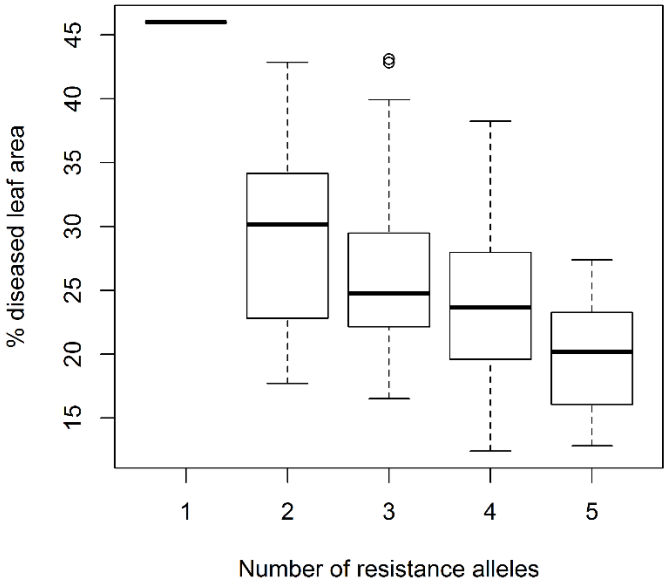


Fig. 8 Association mapping of PH using the MLM + K + Q model. Vertical axes show the –log(10) value of MTA p-values. Dots above the horizontal lines represent MTAs with a p-value within the 0.1 percentile quantile and are considered significant

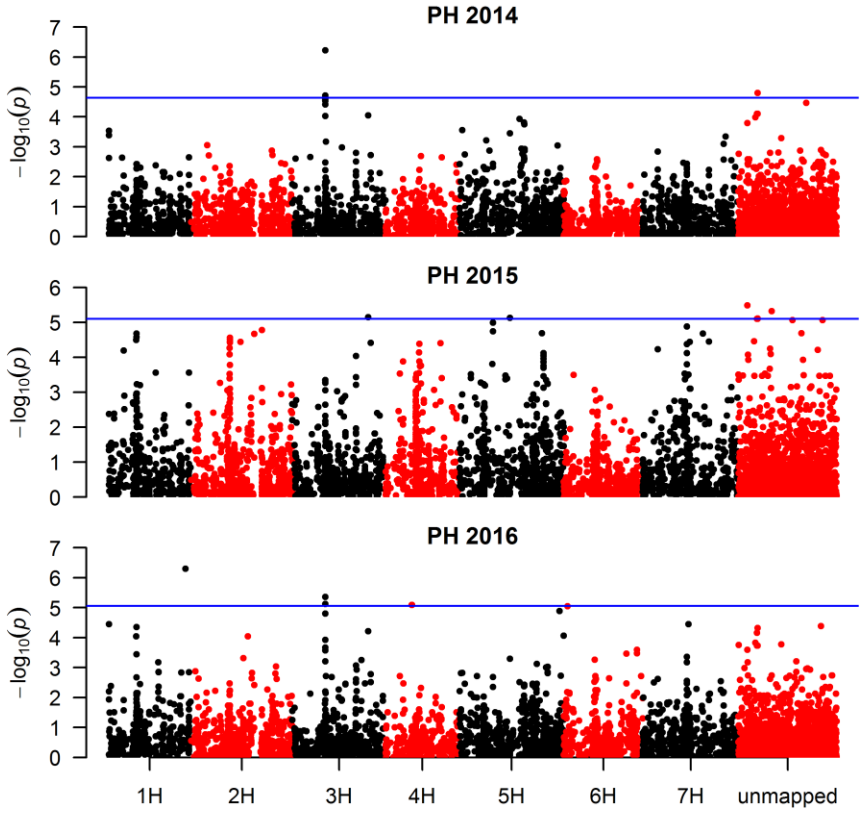
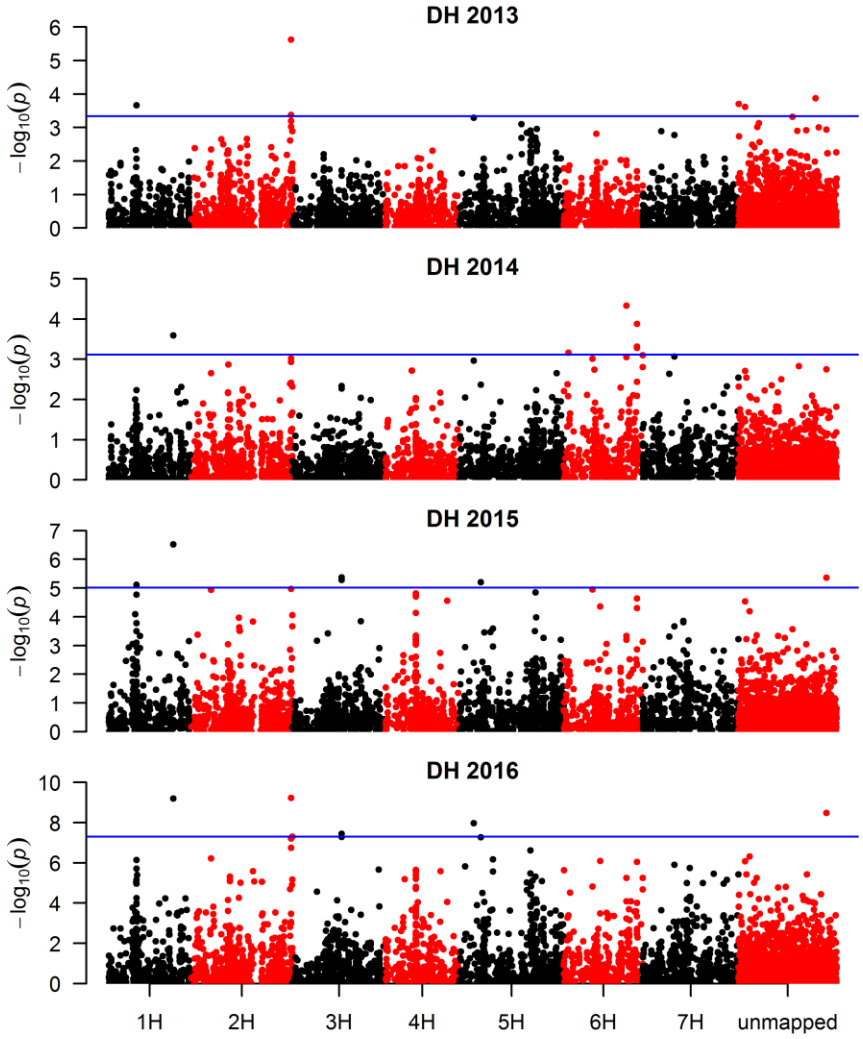


Fig. 9 Association mapping of DH using the MLM + K + Q model. Vertical axes show the –log(10) value of MTA p-values. Dots above the horizontal lines represent MTAs with a p-value within the 0.1 percentile quantile and are considered significant



ESM1 Details about the barley lines used in this study

ESM2 Properties of the Nordic association mapping panel used in this study

ESM3 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

ESM4 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)

ESM5 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

ESM6 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

ESM7 SNP markers significantly associated with adult NB resistance under field conditions in all scorings different years

ESM8 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)

ESM9 Frequency distributions for DH (a) and PH (b) in the AM panel

ESM10 Overview of numbers of mapped and unmapped markers passing different quality checks

ESM11 SNP coverage and distribution across all chromosomes after filtering

ESM12 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)

ESM13 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.

ESM14 Membership coefficients for subpopulation 1 (Q1) and 2 (Q2) based on which the individual lines were assigned to a subpopulation

ESM15 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

ESM16 SNP markers significantly associated with spike row number