May 2021

Student Report No. 53

Barley resistance to rhynchosporium: new sources and closely linked markers

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This report was produced as the final report of a 42-month project (21130048) that started in November 2017. The work was funded by AHDB Cereals & Oilseeds (£70,500) with additional funding and in-kind support from Secobra Recherches SAS, Agrii and Lantmannen.

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1. Abstract

Rhynchosporium is one of the most damaging diseases of barley worldwide, particularly in cool and wet conditions.

This project aimed to provide information on the location and sources of resistance genes/quantitative trait loci (QTL) to help barley breeders improve the rhynchosporium resistance of barley varieties.

Firstly, the development of rhynchosporium was assessed in a collection of 312 barley landraces representing the worldwide diversity of spring barley. A Genome Wide Association Study (GWAS) was carried out to localise genomic regions associated with the resistance and resulted in the detection of 21 QTLs.

Secondly, a biparental population study aimed to map, by linkage mapping, a resistance from a Middle East landrace and test its efficacy in a European genetic background. It resulted in a novel source of resistance at the *Rrs1* locus, with markers available for marker assisted selection (MAS).

2. Introduction

Rhynchosporium or leaf scald is one of the main barley diseases in the United Kingdom. It is caused by the fungal pathogen *Rhynchosporium commune* and can lead to up to 45% yield losses in untreated crops (Avrova and Knogge 2012). An important part of the management of the disease rely on fungicides, with 1 to 2 sprays recommended in the United Kingdom (AHDB 2020). These fungicides represent a cost for farmers and can also have a negative environmental impact. Moreover, repeated use of the same fungicide can lead to a decrease of efficiency of these products through the selection of fungal strains with decreased fungicide sensitivity (Hahn 2014). The use of resistant barley cultivars is an important way to manage the disease more sustainably, but very little is known on the genetic basis of the resistances, slowing the breeding of resistant cultivars, and preventing the conscious use of genetically diverse resistance genes when selecting varieties. *Rhynchosporium commune* requires cool and wet conditions to thrive and consequently the resistance to the disease has been an important trait to select for in winter cultivars. Whereas spring barley cultivars are selected more for malting quality parameters reducing drastically the diversity available in the malting spring barley germplasm, leading to cultivars more susceptible to rhynchosporium (with the exception of feed varieties). The goal of this project was to provide information on the genetic location of resistances, from the worldwide diversity of the spring barley germplasm, to speed up the breeding of cultivars resistant to rhynchosporium. In order to reach this objective, two methods were used: association mapping in a highly diverse collection, and QTL mapping by linkage mapping in a developed Nested Association Mapping (NAM) population. Association mapping in a highly diverse collection provides a very wide genetic diversity containing a high number of resistances, and with numerous historic recombination events can aid physical mapping of the resistances within short intervals (Hamblin, Buckler, and Janninck 2011). Nevertheless, association mapping is based on correlation between a trait and genotypes and can be considered as an exploratory analysis and require further genetic information for validation (Alqudah et al. 2020). Combining association mapping with QTL mapping by linkage mapping is a common practice to get certainty on the presence of causative genes for a trait (Brachi et al. 2010; Zhao et al. 2018). QTL mapping by linkage mapping in a biparental population results in large QTL intervals due to a relatively low number of recombination events. Both methods were used in this project to have a broad view of the location of resistance genes and on the number of resistances present in the worldwide spring barley germplasm, while checking a subset of these resistances using linkage mapping, in order to develop markers for use in barley breeding.

3. Materials and Methods

3.1 Association Mapping

Association mapping or genome wide association study (GWAS) is a method linking the genetic information to a phenotype in order to detect genomic regions likely to be involved in the determination of the phenotype (Yu et al. 2006).

A diverse collection of 312 genebank accessions was chosen to represent a large range of diversity among the cultivated spring barley germplasm. The accessions represent all the major barley growing areas (**Figure 1**): 108 collected from the Middle East, 87 from Europe, 52 from Asia, 29 from North Africa, 18 from Ethiopia, 10 from North America and 8 from South America. Most accessions are classified as landraces (223) and the remaining 89 categorised as old cultivars.

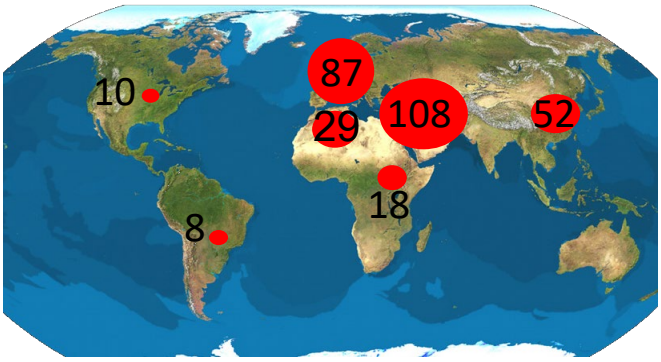

Figure 1. Map representing the origin of the barley accessions used in association mapping

Rhynchosporium was scored in each plot on a 1 to 9 scale, where 1 represents no visible symptoms to 9, where all the leaves were infected (Looseley et al. 2015). The rhynchosporium phenotyping was carried out at 4 sites in the United Kingdom and France between 2018 and 2020 growing seasons. The details of the locations and the number of field trials carried out on each site are provided in **Table 1**. Seven of the eight trials were autumn sown (end of November), and one field was sown in March at Maule in 2018. A single replicate block design of $1m^2$ was used for Dunmow, Maule and Pithiviers, and a two replicate randomized block design was used in Dundee.

Table 1. Location of the field trials

Site	Soil type	GPS coordinates	Number of field trials	Years and sowing season (A= autumn, S=spring)
Dundee (Scotland)	Sandy loam	56.45 N, -3.07 E	2	2019 (A), 2020 (A)
Dunmow (England)	Loam	51.88 N, 0.41 E	1	2019 (A)
Maule (France)	Clay loam	48.92 N, 1.82 E	3	2018 (A), 2018 (S), 2019 (A)
Pithiviers (France)	Sandy loam	48.12 N, 2.14 E	2	2018 (A), 2019 (A)

More detailed phenotyping of rhynchosporium was also carried out to assess the speed of development of symptoms on each accession. Rhynchosporium symptoms were labelled and photographed twice at Dundee in 2020, a week apart. In total 1064 photograph series were scored for the 304 accessions (an average of 3.5 photograph series/accession) in which symptoms could be observed. Photographs were scored visually to assess the speed of symptom development over a week, on a 1 to 4 scale (low to high respectively). Examples of accessions scored as low and high speed of symptom development are presented on **Figures 2 and 3** respectively.

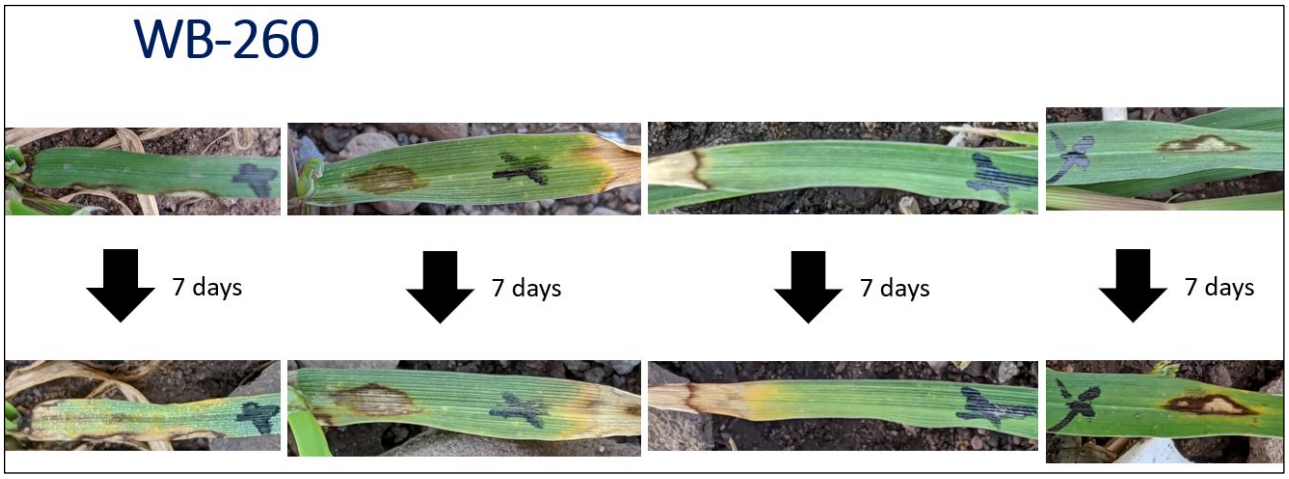

Figure 2. Pictures of symptoms a week apart, on an accession (WB-260) scored with a low speed of symptom development (for the first of the picture series on the far left of the panel, the apparent quick development of disease symptoms is due to the location of the disease lesion at the basis of the leaf, cutting the leaf from the rest of the plant)

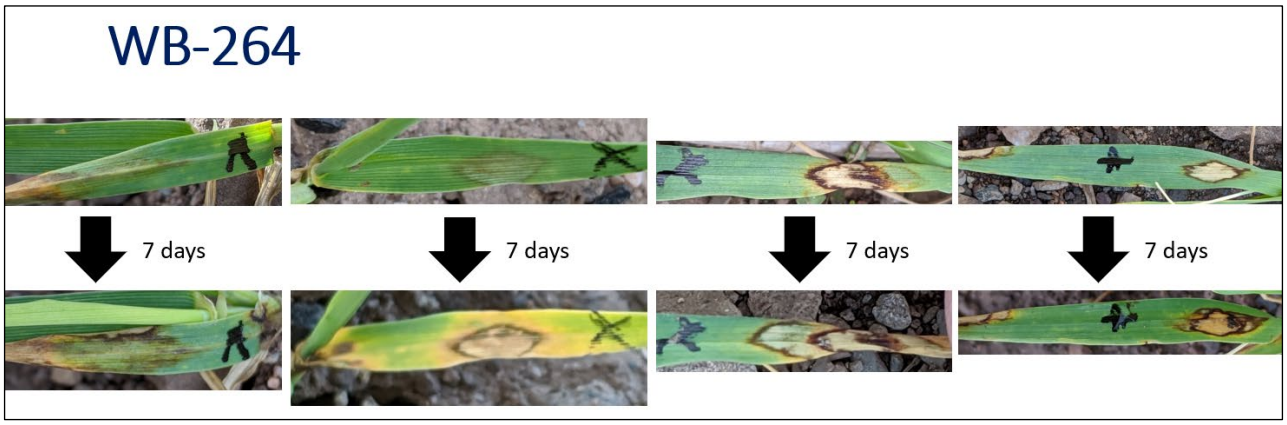

Figure 3. Pictures of symptoms a week apart, on an accession (WB-264) scored with a high speed of symptom development, all the symptoms have progressed substantially over 7 days

Plant height was also measured as this morphological trait is already known to impact rhynchosporium spread in the field (Zhan et al. 2008). In addition, the presence of the *Rrs1*$_{Rh4}$ resistance gene was determined using the diagnostic SNP chr3H_490243586 (Looseley et al. 2020).

The genotyping data came from 2 different sources depending on the accession. Most of the genotypes (229) had exome capture sequencing data already available (Bustos-Korts et al. 2019). For the rest of the collection, RNA was extracted with the Qiagen RNeasy kit, following the manufacturer's instructions. The corresponding cDNA was sequenced using Illumina paired-end sequencing. The reads were then aligned to the reference genome (Mascher et al. 2017) and the SNP (Single Nucleotide Polymorphism) calling was carried out with GATK (Brouard et al. 2019). It resulted in a dataset of 115,172 SNPs available for all the accessions.
The GWAS was carried out using the EMMAX method (Kang et al. 2010), and the FASTmrMLM algorithm, more adapted to traits determined by multiple genes (Wang et al. 2016).

3.1. QTL mapping in a biparental population

3.2.1 Experimental setup

A Syrian landrace, WB-340 in the WHEALBI collection (Bustos-Korts et al. 2019), also named BCC 0187 from the International Barley Core Collection, was crossed with the French spring barley elite cultivars RGT Planet and the F1 of this cross was backcrossed (BC) with RGT Planet. The second cross was repeated to derive lines from numerous BC1F1 seeds, in order to minimize the population structure. A total of 44 BC1F1 seeds were used in Single Seed Descent (SSD) to develop 152 BC1F6 lines (up to 4 per initial BC1F1 seed).
A total of 93 lines from the BC1F6 generation was genotyped with the barley 50K iSelect array (Bayer et al. 2017) using DNA from 4 week old seedling leaves grown in a glasshouse.

The BC1F5 generation was phenotyped in 2020 growing season, in the field in Dundee, Scotland and where enough seeds were available, in Maule, France (62 lines from the cross plus the parents). The plots were $0.5m^2$ and corresponded to seeds descended from single BC1F4 plants. The phenotyping was conducted for rhynchosporium and plant height as described above.

3.2.2 Data analysis

A genetic map was built with the R package ASMap (Taylor and Butler 2017) with the Haldane method. The QTL mapping was carried out using a mixed model, implemented in R/qtl2 (Broman et al. 2019). Pseudomarkers were inserted every cM into the genetic map, and alleles probabilities for each pseudomarker were calculated using a hidden Markov model as implemented by the calc_genoprob function from R/qtl2 (Broman et al. 2019). The kinship matrix was generated with this grid of pseudomarkers and not the real SNP positions to avoid overrepresentation of areas with numerous genetically close markers with the calc_kinship function using the R package qtl2 (Broman et al. 2019). It was calculated for each chromosome, leaving out one chromosome at a time (Listgarten et al. 2012). This was to ensure that the chromosome analysed was not

contributing as a covariate in the QTL mapping. A mixed linear model was then used for the QTL mapping (Malosetti et al. 2011). The model can be written as:

$$Y_i = \mu + \sum \beta_j X_i + U_i + \varepsilon_{ij}$$

where Y_i is the phenotype for an individual i, μ is the average for the trait, β_j is the effect of the j^{th} marker and X_j is a vector of the alleles at the j^{th} marker, U_i is the effect of the genetic background for the individual i, and ε_{ij} is the residual.

The statistical significance of the QTL mapping results was assessed by a thousand permutation test for each trait (Churchill and Doerge 1994; Malosetti et al. 2011). This step consists of shuffling the phenotypes and genotypes to determine the maximum LOD score that can occur by chance. A false discovery rate of 5% was considered as the significance threshold.

4. Results

4.1 Association Mapping

4.1.1 Phenotyping of the landraces collection

Rhynchosporium infection levels are presented in **Figure 4**. The highest infection levels were achieved in Maule 2018, Pithiviers 2018, Dundee 2019 and Pithiviers 2019 field trials. The shape of the distribution varied for each scoring, reflecting different weather pattern, inoculum or other environmental conditions.

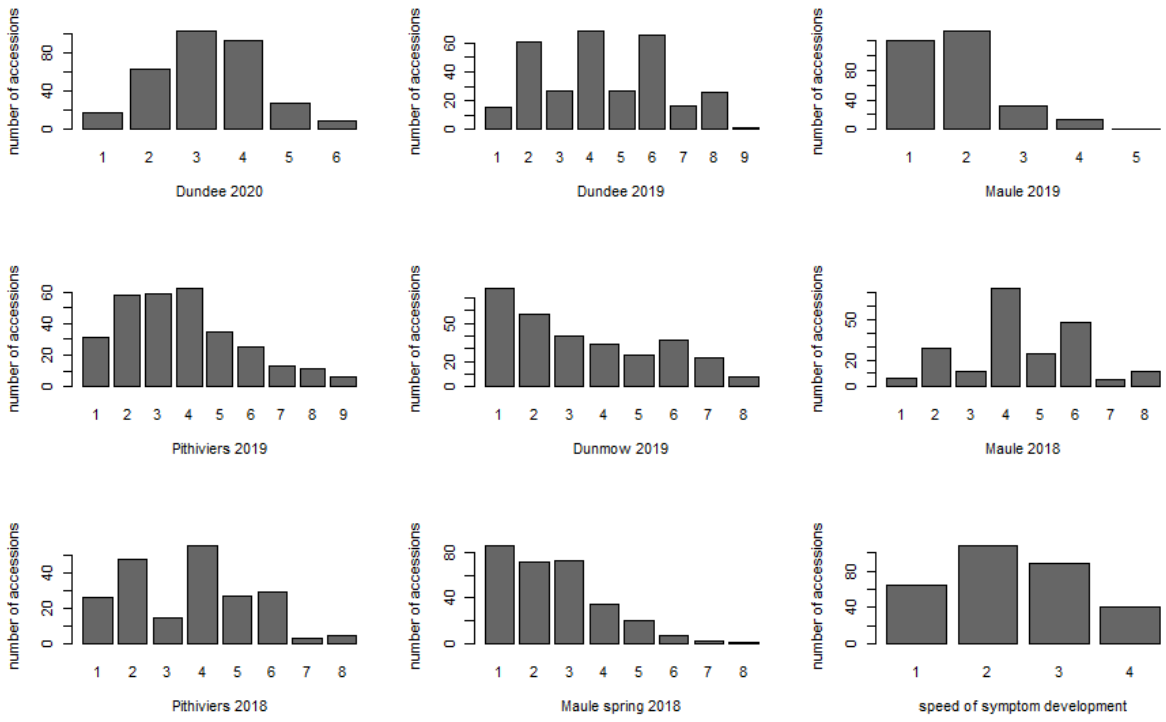

Figure 4. Distribution of the accessions for the rhynchosporium scores with each phenotyping dataset

Table 1. Correlation coefficients (Pearson) between the rhynchosporium phenotypes (Pval<0.01 for all)

	Dundee 2020	Dundee 2019	Maule 2019	Pithiviers 2019	Dunmow 2019	Maule 2018	Pithiviers 2018	Maule 2018 spring sowing	Speed of symptom development
Dundee 2020	1								
Dundee 2019	0.80	1							
Maule 2019	0.57	0.58	1						
Pithiviers 2019	0.58	0.61	0.55	1					
Dunmow 2019	0.47	0.53	0.49	0.47	1				
Maule 2018	0.74	0.74	0.57	0.59	0.54	1			
Pithiviers 2018	0.63	0.73	0.55	0.60	0.59	0.78	1		
Maule 2018 spring sowing	0.40	0.43	0.40	0.33	0.39	0.47	0.45	1	
Speed of symptom development	0.50	0.52	0.31	0.31	0.31	0.44	0.38	0.27	1

The highest correlation coefficients were between the rhynchosporium phenotypes in two Dundee trials (80%) and between Maule 2018 and Pithiviers 2018 trials (78%), followed by correlation between Maule 2018 and both Dundee trials (74%), and Pithiviers 2018 and Dundee 2019 (73%) (**Table 2**). The correlation coefficients between the remaining trials were moderate with the lowest correlation (33-47%) between Maule 2018 spring sowing and all the autumn sown trials. The speed of symptoms development also showed modest correlation with the rest of the trials which were scored based on disease incidence (**Table 2**).

The effect of height and the presence of the resistance gene *Rrs1*$_{Rh4}$ were shown to affect rhynchosporium scores. An example of these effect is shown in **Figure 5** for Pithiviers 2018, with a highly significant negative correlation the rhynchosporium score and plant height, and an effect of *Rrs1*$_{Rh4}$ reducing the rhynchosporium score by two compared to other genotypes with similar plant height.

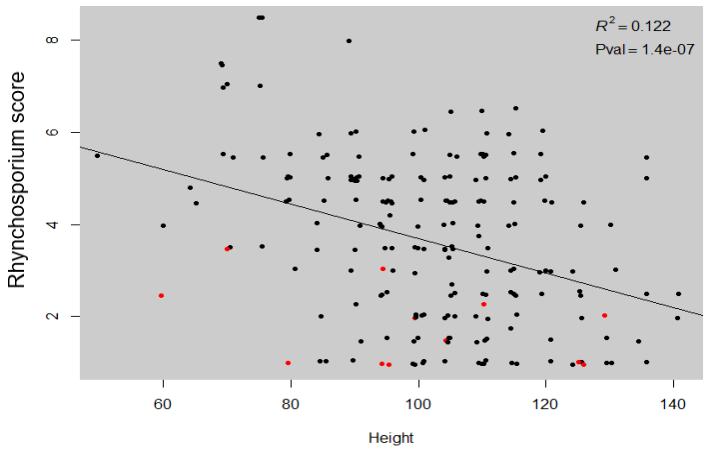

Figure 5. Rhynchosporium (1 to 9 scale where 1 means fully resistant, and 9 highly susceptible) at Pithiviers 2018 depending on plant height (cm), *Rrs1*$_{Rh4}$ carrying accessions are in red.

4.1.2 GWAS

The SNPs detected in the GWAS were grouped based on Linkage Disequilibrium (LD). The local LD decay for these SNPs was on average 14 Mb (from 0 to 263 Mb for R^2=0.2). The distance to reach an LD decay of 0.2 was considered as the confidence interval for each QTL obtained by clustering the SNPs detected in the GWAS (Alqudah et al. 2020). The 21 QTLs detected by association mapping are presented on **Figure 6**, along with already known rhynchosporium resistance genes/QTLs. Among these QTL detected by association mapping, the two main resistance genes *Rrs1* (Hofmann et al. 2013) and *Rrs2* (Hanemann et al. 2009) were detected, giving confidence in the GWAS results.

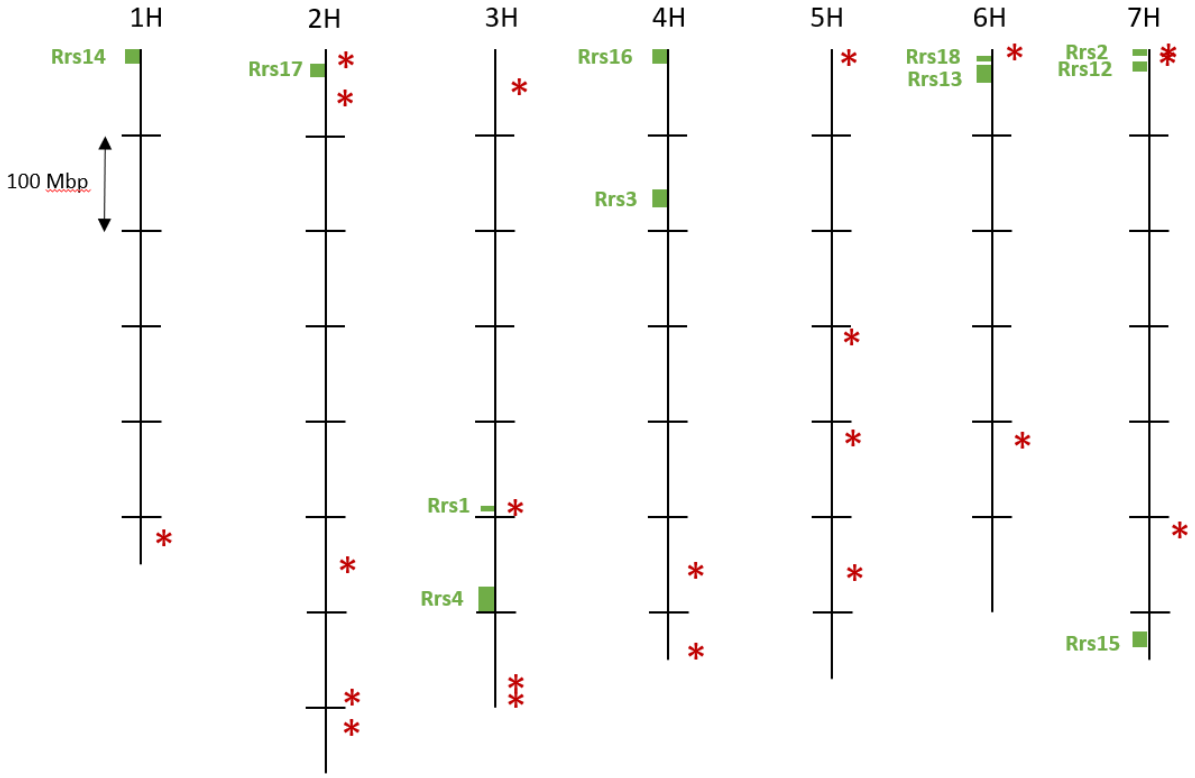

Figure 6. Location of the QTLs detected by GWAS (red stars) on the barley physical map (Mascher et al. 2017), and previously published rhynchosporium resistance QTLs (green).

4.1.3 Validation of the effect of the most detected gene in the GWAS (HvAHD-1)

At 58 Mbp on chromosome 2H, 9 SNPs detected in the GWAS presented before are in the coding region of a gene annotated as *HvADH-1* in Morex 2017 genome assembly. This gene has been previously shown to be involved in powdery mildew susceptibility and involved in chitin induced systemic resistance (Pathuri et al. 2011; Kasbauer et al. 2018). Transgenic lines overexpressing (OE 346-E13) or silenced (RNAi 344-E12) for this gene developed by Kasbauer et al. (2018) were studied by detached leaf assays {Newton, 2001 #126} with the rhynchosporium strains L43D and T-R214-GFP from the James Hutton Institute rhynchosporium strains collection. The results using L43D strain are presented on **Figure 7**.

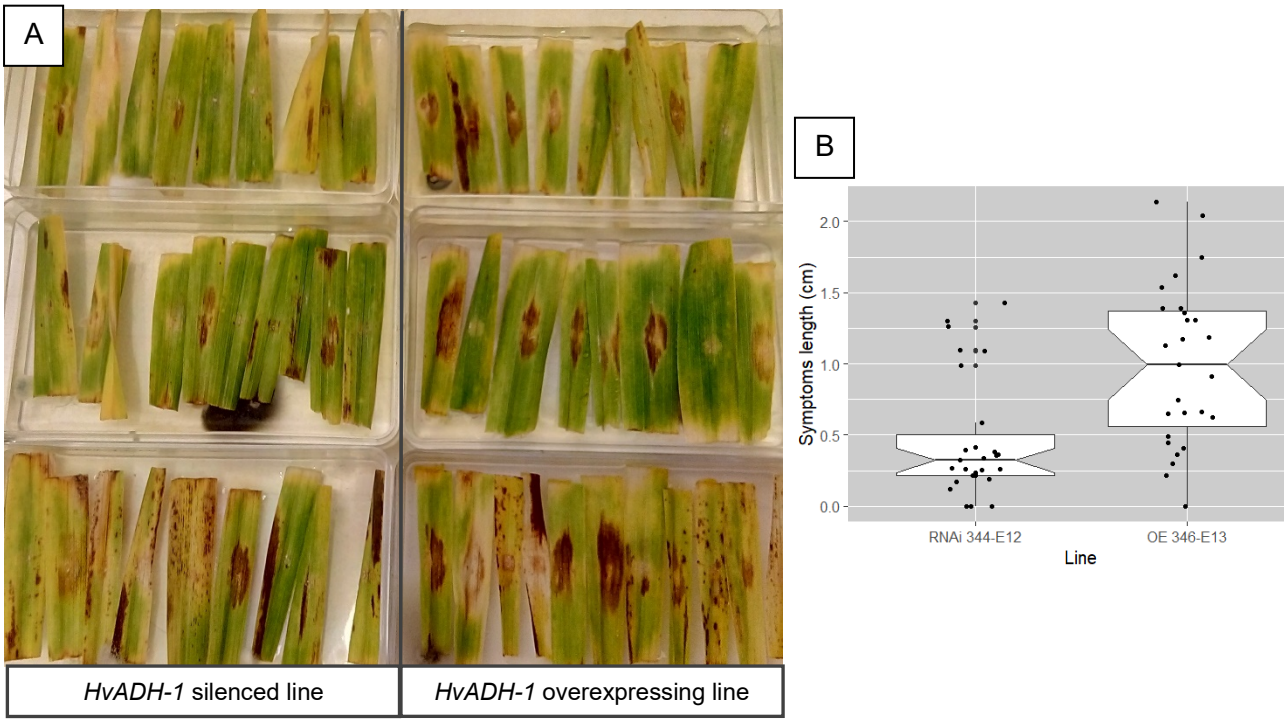

Figure 7. Detached leaf assay of the *HvADH-1* silenced and overexpressing lines using the R. commune strain L43D. A) Picture of the inoculated leaf segments 21dpi, B) Boxplot representing the length of the rhynchosporium symptoms (p-value=2.9 x 10^{-4})

A significance difference was observed with both rhynchosporium strains between the lines overexpressing and silenced for *HvADH-1*. The overexpressing line had larger symptoms and was relatively more susceptible to rhynchosporium than the silenced line.

4.2 QTL mapping in a biparental population

The QTL mapping for Dundee and Maule field trials resulted in a major QTL for rhynchosporium resistance corresponding to a 92 Mb interval on chromosome 3H (**Figure 8**), from JHI-Hv50k-2016-178998 to JHI-Hv50k-2016-188022, with the resistance allele from WB-340. The second QTL on chromosome 5H is also detected for heading date (data not shown), with the early and resistant allele originating from RGT Planet, and likely to be involved in an escape mechanism to the disease.

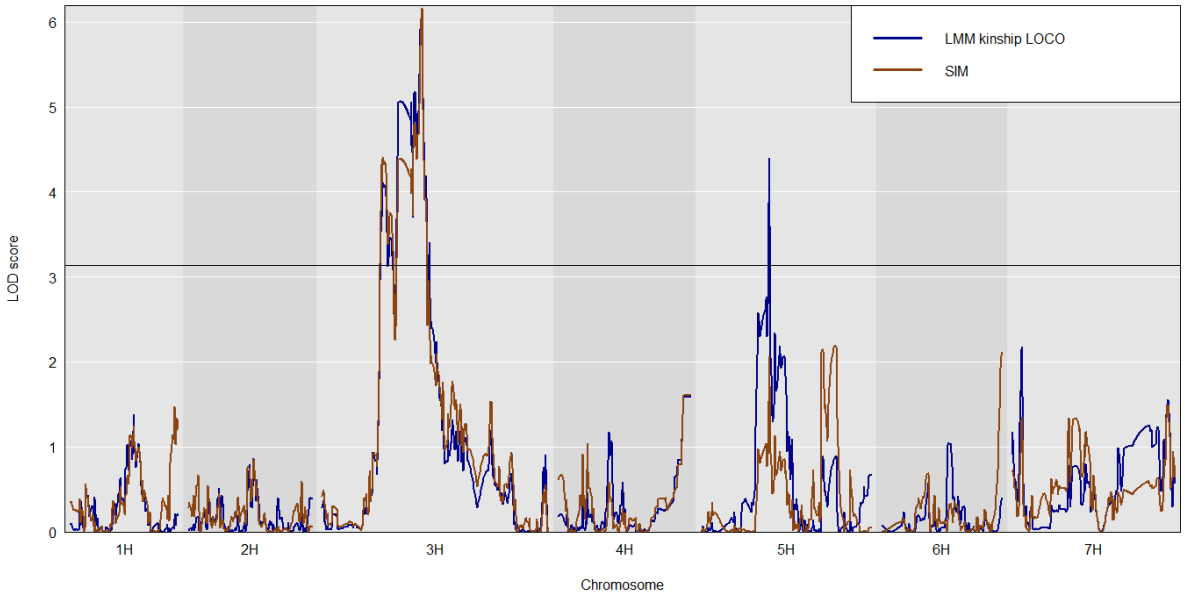

Figure 8. QTL mapping for rhynchosporium, Dundee 2020 (using 95 genotypes, with the 50K array)

5. Discussion

The association mapping resulted in 21 QTLs detected. The 2 main rhynchosporium resistance QTLs, *Rrs1* and *Rrs2,* were detected which confirms the reliability of the results. But many still unpublished regions were identified among the results. This suggests the presence of many genes involved in rhynchosporium resistance and will provide many sources of resistance for barley breeders. However, association mapping is limited to identifying regions likely to contain QTLs and validation is required, such as the use of biparental or mutant populations or transgenic and gene edited lines. A validation of the most detected gene across the field trials was carried out using existing transgenic lines overexpressing or silenced for this gene. It showed the involvement of the *HvADH-1* gene in rhynchosporium resistance/susceptibility and gives confidence on the reliability of the remaining QTLs detected by GWAS.

Because of the considerable variation detected in the landraces assembled for this project, the most obvious validation approach was to develop multi-parent and bi-parent populations, which would also serve as pre-breeding germplasm that could be exploited by breeders. Using one of the most phenotypically variable population: (WB-340 x RGT Planet) x RGT Planet resulted in an unexpected result. RGT Planet already carries the $Rrs1_{Rh4}$ resistance allele (Looseley et al. 2020). However, a resistance gene was detected in the *Rrs1* region originating from the Syrian landrace parent (WB-340), confirming the presence of several resistance genes/alleles of *Rrs1* (Bjornstad et al. 2002). Moreover, the mainstream use of the $Rrs1_{Rh4}$ resistance (Looseley et al. 2018) is likely to be resulting in a loss of efficiency of this gene, which resulted in its association with susceptibility to rhynchosporium in the biparental population. This work shows that a more effective version of *Rrs1* could be used as an alternative resistance to rhynchosporium than the commonly used $Rrs1_{Rh4}$.

The use of several resistance genes/alleles of *Rrs1* is likely to be more durable on the long term than only using $Rrs1_{Rh4}$ and will be of interest for barley breeding.

The biparental population presented here is part of a set of 8 biparental populations sharing the same elite parent RGT Planet (enabling the data from these populations to be analysed as a Nested Association Mapping or NAM population). The NAM population rhynchosporium resistant landrace founder parents represent a wide diversity from Germany, Russia, Syria, Iran, Iraq, Mexico and China and are likely to contain different rhynchosporium resistance genes. Only the biparental population presented in this report was genotyped due to the cost of the 50K array genotyping, but phenotypic data was also collected for the rest of the NAM population and could be used to map more rhynchosporium resistance genes in the future. In addition, the wide diversity present in this population could be of use to study the genetic determinism of many traits, plant and root architecture, grain and straw colour, grain skinning, spike morphology, grain quality, etc.

The high susceptibility of modern European malting spring barley varieties to rhynchosporium is surprising comparing to the landraces. The vast majority of the landraces (90%) studied in the association mapping collection were more resistant to rhynchosporium than the malting spring barley controls, providing a great opportunity to use this germplasm for the improvement of rhynchosporium resistance in modern elite varieties. The rapid adaptation of the pathogen, and probable loss of efficiency of $Rrs1_{Rh4}$ comparing to *Rrs1* from the Syrian landrace WB-340 is likely due to the very low genetic diversity present in this germplasm, and more diverse spring barley varieties could result in a more sustainable management of rhynchosporium in the long term. To conclude, this work provides a starting point to reintroduce resistances to rhynchosporium into modern European barley germplasm.

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