

| Project title  | Barley resistance to rhynchosporium: new sources and closely linked markers |                      |             |
|----------------|---|----------------------|-------------|
| Project number | 21130048  | Final Project Report | SR53        |
| Start date     | 27 November 2017  | End date             | 30 May 2021 |
| AHDB funding   | £70,500   | Total cost           | £90,300     |

#### What was the challenge/demand for the work?

Rhynchosporium is by far the most damaging barley disease in the UK, with estimated yield losses of £10.2 M per year (despite treatment). Sustainable management of this disease can be achieved by breeding more resistant cultivars. Landraces and genebank accessions are a source of diversity for barley breeding and a potential source of resistances to rhynchosporium. However, the lack of knowledge of which genetic regions contain novel candidate genes responsible for resistance has hampered their use and introgression into the elite barley germplasm. The main goal of this work was to identify resistance sources (from barley landraces) to rhynchosporium and develop markers to enable marker assisted selection.

#### How did the project address this?

The project used a two-step approach:

#### 1. Association mapping or Genome Wide Association Study (GWAS)

A collection of 312 landraces/old varieties, representing the worldwide diversity of barley (Figure 1), was studied in the field over three years at four locations (United Kingdom and France). High-density genotyping (exome capture technique) was used to provide information on the gene versions (alleles) present in each landrace. The association mapping approach aims to identify genetic regions correlated with a trait of interest, in this case rhynchosporium resistance.



Figure 1. Map showing the origin of the 312 barley landraces or old varieties studied

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law, the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document. Reference herein to trade names and proprietary products without stating that they are protected does not imply that they may be regarded as unprotected and thus free for general use. No endorsement of named products is intended, nor is any criticism implied of other alternative, but unnamed, products.



#### 2. Biparental mapping

During the first year of the project, eight landraces resistant to rhynchosporium were identified. These were crossed with a relatively susceptible elite malting spring barley cultivar, RGT Planet. A total of 736 Recombinant Inbred Lines (RILs) were developed for the eight biparental populations and grown in field trials (Dundee). A subset of 444 lines were grown in Maule. The RILs were scored for disease symptoms (phenotypic data).

For the QTL (Quantitative Trait Loci) analysis, one population, showing the greatest distribution of disease symptoms, was genotyped using the 50K barley genotyping array – 50,000 Single Nucleotide Polymorphism (SNP) markers, providing 50,000 datapoints along the barley genome. For this population, the resistant parent landrace was BCC 0187, a Syrian landrace from the Barley Core Collection (IPK, Germany), also named WB-340 in the WHEALBI collection. 15,249 SNPs were segregating between the two parents. A genetic linkage map was generated, using the phenotypic data, to identify regions of the QTL linked to rhynchosporium. These resistances are expected to be efficient in elite genetic background, as one of the parents was an elite variety.

#### What outputs has the project delivered?

#### 1. Association mapping

Association mapping resulted in 21 QTLs. These were localised on the seven barley chromosomes, with 17 novel QTLs for barley resistance to rhynchosporium. The two main resistance genes to rhynchosporium, *Rrs1* (chromosome 3H) and *Rrs2* (chromosome 7H), were detected. However, these results confirm the existence of many more resistances (Figure 2).



Chromosomes

Figure 2. Manhattan plot presenting the association mapping results for a multi-environments rhynchosporium score (BLUP). Each dot is a SNP marker. The higher the dot on the y axis, the greater the likelihood of resistance in its genomic area.

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law, the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document. Reference herein to trade names and proprietary products without stating that they are protected does not imply that they may be regarded as unprotected and thus free for general use. No endorsement of named products is intended, nor is any criticism implied of other alternative, but unnamed, products.



Flanking markers for these QTLs can be used for barley breeding or a further identification of candidate genes in the QTL intervals. However, the association mapping relies on correlation between a trait and genetic marker data, and some of these correlations can occur by chance. The results need to be checked by other methods (e.g. QTL mapping in biparental populations and reverse genetic) to be certain about the presence of these resistances.

The most detected QTL across all the trials and analysis methods was the region of the *HvADH-1* gene at 58 Mbp on chromosome 2H. This gene has been recently shown to be involved in powdery mildew resistance. Assays on transgenic lines silenced or overexpressed for the *HvADH-1* gene were performed to validate whether the gene was involved in rhynchosporium response. The results showed that the silenced line developed significantly less rhynchosporium compared to the overexpressed line (Figure 3), confirming the involvement of this gene in the quantitative resistance to the disease.



**Figure 3.** Rhynchosporium symptoms length on detached leaf assays on barley *HvADH-1* silenced or overexpressed lines (using the rhynchosporium L43D strain, 21 days post inoculation)

## 2. Biparental mapping

The biparental population RGT Planet x WB-340 resulted in the mapping of a QTL on chromosome 3H (Figure 4). A major resistance at this locus comes from WB-340. This resistance gene corresponds to the *Rrs1* region. However, the version of the gene present in WB-340 does not carry the commonly used  $Rrs1_{Rh4}$  resistance gene. This work confirmed the presence of allelic variation at this gene. An output of this project is flanking markers for this *Rrs1* allele and a set of RILs with this alternative *Rrs1* allele, which can be used in barley breeding.

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law, the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document. Reference herein to trade names and proprietary products without stating that they are protected does not imply that they may be regarded as unprotected and thus free for general use. No endorsement of named products is intended, nor is any criticism implied of other alternative, but unnamed, products.





Figure 4. QTL mapping for rhynchosporium in the RGT Planet x WB-340 population, Dundee 2020 (with 95 lines, genotyped with the 50K array)

## Who will benefit from this project and why?

This project will benefit the whole barley industry:

- Plant breeders now have improved genetic information and tools (e.g. markers)
- Ultimately, farmers will be able to reduce associated yield losses and reliance on fungicides
- A higher quality harvest rhynchosporium has an impact on grain size and homogeneity will benefit the malting and brewing industry
- A reduction of fungicide use will also be valued by consumers and benefit the environment

#### If the challenge has not been specifically met, state why and how this could be overcome

The challenge has been met through the identification of novel resistance QTL and associated markers for the barley breeding community. More broadly, one key parameter for the adaptation and spread of diseases is the genetic homogeneity of a crop. The particularly low genetic diversity of malting spring barley varieties contributes to the adaptation and spread of diseases, including rhynchosporium, and growers would benefit from more diverse varieties for a long-term disease management.

| Lead partner        | Anna Avrova and Joanne Russell, The James Hutton Institute |  |
|---------------------|--|--|
| Scientific partners | University of Dundee                                       |  |
| Industry partners   | Secobra Recherches, Agrii, Lantmannen                      |  |
| Government sponsor  | N/A  |  |

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law, the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document. Reference herein to trade names and proprietary products without stating that they are protected does not imply that they may be regarded as unprotected and thus free for general use. No endorsement of named products is intended, nor is any criticism implied of other alternative, but unnamed, products.