Wild barley (Hordeum spontaneum) and landraces (Hordeum vulgare) from Turkey contain an abundance of novel Rhynchosporium commune resistance loci

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Abstract

Rhynchosporium commune, the causal agent of the disease scald or leaf blotch of barley is a hemibiotrophic fungal pathogen of global importance, responsible for yield losses ranging from 30-40% on susceptible varieties. To date, over 150 resistance loci have been characterized in barley. However, due to the suspected location of the *R. commune* host jump in Europe, European germplasm has been the primary source used to screen for *R. commune* resistance leaving wild (*Hordeum spontaneum*) and landrace (*H. vulgare*) barley populations from the center of origin largely underutilized. A diverse population consisting of 94 wild and 188 barley landraces from Turkey were genotyped using PCR-GBS amplicon sequencing and screened with six Turkish *R. commune* isolates. The isolates were collected from distinct geographic regions of Turkey with two from the Aegean region, two from central Turkey and two from the Fertile Crescent region. The data was utilized for association mapping analysis with a total of 21 loci identified, of which 13 were novel, indicating that these diverse primary barley gene pools contain an abundance of novel *R. commune* resistances that could be utilized for resistance breeding.

Key Message

Rhychosporium commune is a globally devastating pathogen of barley. Wild and landrace barley are underutilized; however, contain an abundance of loci that can be used as potential sources of resistance.

Introduction

Rhynchosporium commune, the causal agent of the barley (*Hordeum vulgare*) foliar disease known as scald or leaf blotch is a hemibiotrophic fungal pathogen (Avrova and Knogge 2012; Zhang et al. 2020). The disease is characterized by pale grey lesions that form distinctive brown margins that often coalesce (Fig. 1). Scald is of global importance, as it is found in the majority of barley production regions, particularly those with cool and humid environments (Zhan et al. 2008). Yield losses due to scald typically range between 30–40%, and also diminishes grain quality reducing its acceptance by end-users (Brown 1985; Zhan et al. 2008; Zhang et al. 2020). Originally isolated from rye, *R. commune* was given the *Rhynchosporium secalis* nomenclature, however Zaffarano et al. (2011) reclassified *Rhynchosporium* into three species with *R. commune* infecting *Hordeum* spp. and *Bromus diandrus*. However, *R. commune* has also been reported and isolated from *H. murinum* subsp. *glaucum, Festuca perennis* and *Avena sativa* (Seifollahi et al. 2018). Therefore, wild grass species can serve as a reservoir of potentially virulent isolates and primary hosts to complete its sexual cycle (Linde et al. 2016; Seifollahi et al. 2018), whereas the secondary host remains unidentified. In a recent publication, Crous et al. (2021) proposed renaming *R. commune* to *R. graminicola.*

An *R. commune* study of Turkish isolates found low levels of genetic diversity, supporting the hypothesis that *R. commune* did not coevolve with barley at its center of origin in the Fertile Crescent, but represented a founder population in the region (Çelik Oğuz et al. 2021). This hypothesis was also supported by the low genetic diversity of *R. commune* populations collected from Syria, Jordan and Iran (Kiros-Meles et al. 2011; Seifollahi et al. 2018). However, 14 Icelandic *R. commune* isolates phenotyped on a differential set of 15 near-isogenic lines all showed unique virulence profiles (Novakazi et al. 2021), implying that the Northwestern Europe *R. commune* populations contain considerable diversity. This study, along with other studies using the *R. commune* effector gene *NIP1* (Rohe et al. 1995; Brunner et al. 2007), restriction fragment length polymorphisms (Zaffarano et al. 2009) and microsatellites (Linde et al. 2009) implied that barley - *R. commune* host-parasite interactions co-evolved in Northwestern Europe. This high level of pathogen diversity also exemplifies the need to identify new sources of scald resistance in diverse barley populations. These efforts are important for durable resistance because of the genetic bottlenecks created in modern breeding programs (Clare et al. 2021).

Since *R. commune* was originally classified as *R. secalis*, gene designations follow the *Rrs* nomenclature for *Reaction/Resistance to Rhyncosporium secalis* (Bjørnstad et al. 2002). A total of eleven major *Rrs* genes have been mapped and the *Rrs1*, *Rrs2*, *Rrs3, Rrs4*, *Rrs12*, *Rrs13*, *Rrs14*, *Rrs15*, *Rrs16*, *Rrs17*, and *Rrs18* gene nomenclatures (Bjørnstad et al. 2002; Zhang et al. 2020). The loci *Rrs5, rrs6, rrs7, rrs8, Rrs9, Rrs10 and rrs11* (Chełkowski et al. 2003) were not mapped and may therefore represent alleles of previously mentioned *Rrs* loci. Therefore, the numbering of *Rrs* loci is not in strict sequential order due to nomenclature alterations, the unmapped *Rrs* loci mentioned and the fact *Rrs3* remains unanchored to the barley reference genome of Morex (Zhang et al. 2020). Major loci have been designated on all chromosomes except for 5H, with *Rrs14* on chromosome 1H, *Rrs17* on chromosome 2H, *Rrs1* and *Rrs4* on chromosome 3H, *Rrs3* and *Rrs16* on chromosome 4H, *Rrs13* on chromosome 6H and *Rrs2, Rrs12 and Rrs15* on chromosome 7H (Fig. 2) (Bjørnstad et al. 2002; Zhang et al. 2020). Adding to the complexity are the 27 major or minor QTL reported within the *Rrs1* locus at the centromeric region of chromosome 3HL that spans from 429 to 503 Mb on version 2 of the Morex genome assembly (Zhang et al. 2020), and two additional loci designated *Rrs1* reported within cvs. Sultan and Halcyon at 383.9 and 573.5 Mb, respectively (Fig. 2) (Genger et al. 2003; Zhang et al. 2020). *Rrs1* is therefore a complex locus in a centromeric region (Büttner et al. 2020; Zhang et al. 2020), showing similarities to the *Rpt5/Spt1* locus found at the centromeric region of chromosome 6HL conferring dominant resistance and susceptibility to *Pyrenophora teres* f. *teres* the causal agent of the disease net form net blotch of barley (Richards et al. 2016; Clare et al. 2020). The *R. commune* effector NIP1 is known to interact with *Rrs1* (Schürch et al. 2004), and species specific effectors within *R. commune* are believed to extend the length of its biotrophic phase and shortens its necrotrophic phase (Penselin et al. 2016).

Wild and landrace barley lines are excellent sources of novel disease resistance genes due to their diversity (Russell et al. 2016; Çelik Oğuz et al. 2017, 2019; Azamparsa et al. 2019). A recent study used nested association mapping in the HEB-25 wild barley population crossed to the German line 'Barke' identifying eight loci (Büttner et al. 2020). In addition, association mapping within the Scottish 'Bere' landrace barley population, used due to the suspected Northwestern Europe origin of *R. commune*, also identified eight loci (Cope et al. 2021). The work by Clare et al. (2021) recently demonstrated that despite low marker density, novel resistance/susceptibility loci against fungal pathogens with complex host-pathogen genetic interactions can be identified by association mapping using natural wild and landrace barley populations. The work reported here relied on the previously reported genotyping data of Clare et al. (2021) and identified an abundance of novel resistance loci within the barley-*R. commune* interaction from wild and landrace barley of Turkish origin. This diversity of scald resistance genes is remarkable considering that the barley accessions are from the barley origin of domestication (Fertile Crescent). The Fertile Crescent is not considered a center of *R. commune* diversity, therefore some of the resistance may be representative of non-host resistance mechanisms that may be

more prevalent in the wild population and may account for the higher level of resistance found in the wild barley population as compared to the domesticated barley population. However, it would be of interest to challenge these new sources of resistance with diverse pathogen isolates to determine the effectiveness of these novel resistances.

Material And Methods

Biological Materials

The barley populations utilized in this study consisted of 105 *Hordeum spontaneum* genotypes and 198 barley landraces previously described by Clare et al. (2021). These genotypes were obtained from the Central Research Institute for Field Crops located in Ankara, Turkey. Insufficient seeds were obtained from *H. spontaneum* genotypes numbered as 4, 15, and 41 and barley landraces numbered as 43 and 116 and were not used, thus a total of 303 genotypes were included in this association mapping study.

Six *R. commune* isolates were used for phenotyping the wild and landrace barley populations. Four isolates were the most virulent isolates (GPS71-U, 13GPS203, 13GPS207, and 13GPS109) in the study of Azamparsa and Karakaya (2020). Whereas, one isolate was the most virulent (13GPS149) and another represented the most common pathotype (E4) from Azamparsa et al. (2019). Isolates GPS71-U, 13GPS203, 13GPS207, 13GPS109, 13GPS149 and E4 were obtained from the Gaziantep-Subağı, Manisa-Kula, İzmir-Bergama, Ankara-Şereflikoçhisar, Mardin-Midyat and Eskişehir-Tepebaşı regions of Turkey, respectively. Isolates 13GPS109 and E4 were from Central Turkey. Isolates 13GPS203 and 13GPS207 were from the Aegean region of Turkey and isolates GPS71-U and 13GPS149 were from the Fertile Crescent section of Turkey (Azamparsa and Karakaya 2020).

Phenotypic Assay

To produce inoculum, each single spore isolate was grown on bean agar medium (1 L distilled water, 18 g agar, 20 g dextrose, 140 g fresh bean) for approximately 14 days before distilled water was added to the Petri dish to allow spores to be released and collected. The harvested spore suspension was cleaned using a cheesecloth and the spore concentration was adjusted to 1×10^6 spores mL^{-1} (Mert and Karakaya 2003). One drop of Tween 20 was added to each 100 mL of inoculum. Five to 10 seeds of each barley genotype were planted in 7 cm diameter plastic pots and placed in the greenhouse. Three replications were performed with inoculation performed at growth stage 11 (Zadoks et al. 1974). Following inoculation, plants were transferred to a mist chamber with 100% relative humidity at 16–17°C for 48 hours. Two days post inoculation, plants were moved to the greenhouse with a 20–25°C temperature range (Azamparsa et al. 2019). The disease assessment was made using a 0–4 scale developed by El-Ahmed (1981) 18 days post inoculation and used in subsequent association mapping. Scale values between 0–2.0 were considered resistant reactions and scale values between 2.1-4.0 were considered susceptible reactions.

Barley Genotyping, Filtering and Relatedness

Genotyping of barley lines was performed by Clare et al. (2021). In brief, two independent PCR-GBS SNP marker panels consisting of 365 and 1,272 SNPs were used to genotype lines on the Ion Torrent sequencing platform (Sharma Poudel et al. 2018; Tamang et al. 2019; Ruff et al. 2020; Clare et al. 2021). Redundant markers were eliminated and absolute marker positions were obtained from the Morex reference genome (Mascher et al. 2017). Markers without absolute positions were estimated based on the iSelect consensus map (Muñoz-Amatriaín et al. 2014). Heterozygous calls were included in the analysis due to the fact that the population was considered natural. Barley genotypes and markers with more than 30% missing data were eliminated, resulting in 94 *Hordeum spontaneum* genotypes and 188 landraces for a total of 282 genotypes. Subsequently, missing data was imputed using LinkImpute (Money et al. 2015) within TASSEL 5.2.60 (Bradbury et al. 2007) and markers within linkage disequilibrium above an R^2 threshold of 0.8 were removed resulting in 522 markers (**Supplemental Fig. 1**). Markers with a minor allele frequency above 0.05 were included in the analysis based on GAPIT best practice. Population structure was accounted for with STRUCTURE analysis (Pritchard et al. 2000) and principal component analysis within GAPIT 3 (Wang and Zhang 2021), whereas relatedness was accounted for with the efficient mixed model association (EMMA) kinship matrix (Kang et al. 2008).

Association Mapping

For association mapping, a naïve model for each isolate was constructed using only genotypic and phenotypic data in a general linear model (GLM) in GAPIT version 3 (Wang and Zhang 2021) in *R* 3.6.3. Barley row type was used as a control phenotype. To account for population structure an additional four fixed effect models were generated using STRUCTURE analysis (Q), and principal component analysis accounting for 25% (PC1), the plateau of the scree plot (PC4, **Supplemental Fig. 2**) and 50% of the phenotypic variation (PC9) using the GLM method. The kinship matrix was created with the EMMA algorithm (Kang et al. 2008) to use as the random effect component in random and mixed models based on Clare et al. (2021). In addition, up to 35 models using the random effect were generated using MLM (Yu et al. 2006), CMLM (Zhang et al. 2010), ECMLM (Li et al. 2014), SUPER (Wang et al. 2014a), MLMM (Segura et al. 2012), FarmCPU (Liu et al. 2016) and BLINK (Huang et al. 2019) algorithms in conjunction with the EMMA kindship matrix or reconstructed kinship matrices of the respective algorithm. All combinations of models mentioned resulted in a total of 40 models per isolate. To determine the best model, mean-squared deviation (MSD) of each model was calculated (Mamidi et al. 2011) and used to inform the best model based on visual inspection of the quantile-quantile (QQ) plot (Fig. 3, **Supplemental File 1**). This approach was used due to the fact that the lowest scoring MSD model would result in highly correlated observed and expected $-\log_{10}(p)$ values and zero significant markers. A threshold for significant markers was calculated at an α level of 0.01 and 0.05 for a $-\log10(p)$ threshold of 4.72 and 4.02, respectively. The final Manhattan and QQ plot figures were generated with the CMplot 3.5.1 package (https://github.com/YinLiLin/R-CMplot) in *R* 3.6.3.

Loci Identification

Absolute marker positions for significant marker trait associations (MTAs) were extracted from the version one of the Morex reference genome (Mascher et al. 2017) due to the fact that *Rrs* loci are currently only anchored in this version of the genome assembly (Zhang et al. 2020). Loci were collapsed together if the

nearest neighbor marker was not significant or if the gap to the next marker exceeded 10 Mb in physical distance if no marker was present in a similar strategy to Clare et al. (2020). Loci were deemed novel if no previously reported quantitative trait loci (QTL) were reported within approximately 10 Mb of the significant markers in a similar manner to Clare et al. (2021).

Results

Phenotypic Analysis

In all cases, landrace barley was statistically (Wilcoxon rank sum test) more susceptible to the *R. commune* isolates than wild barley at a significance of less than 0.001 (Fig. 4). On average, approximately 18.4% of landraces were resistant to the six *R. commune* isolates, whereas 66.1% of wild barley lines were resistant (Table 1). The mean phenotypic score for all isolates was 3.2 on the landraces and 1.6 on wild barley. The isolate GPS71-U was the most virulent overall with a mean phenotypic score of 3.4 on the barley landraces and 2.6 on wild barley (Table 1).

Association Panel and Population Structure

As previously reported by Clare et al. (2021), a total of 282 barley genotypes were used in the association mapping analysis after removing genotypes that exceeded 30% missing data or not deemed unique using a similarity of individual matrix. In addition, 522 SNP markers were used after removing markers that exceeded 30% missing data or an LD R^2 threshold of 0.8. The population structure of the wild and landrace barley was sourced from Clare et al. (2021). In brief, two subpopulations were identified, with subpopulation one consisting of 89 wild genotypes and subpopulation two consisted of 118 landraces. A total of 75 genotypes consisting of landrace or wild barley were considered admixture due to their population membership probabilities of less than 0.8 (Richards et al. 2017; Clare et al. 2021). Principal component analysis was also used to infer population structure. Principal components explaining 25% (PC1) and 50% (PC9) of the genotypic variation as well as the plateau of the scree plot (PC4, **Supplemental Fig. 1**) were used. The first four principal components explained 29.4, 6.4, 3.4 and 2.4% of the genotypic variation, respectively.

Association Mapping

Across all models and isolates, a total of 151 MTA were identified, resulting in the identification of 62 unique QTL, of which 36 are suspected of being novel and 26 previously reported and consolidated by Zhang et al. (2020) and more recent publications (Büttner et al. 2020; Cope et al. 2021; Hautsalo et al. 2021). However, when selecting only the best model for each isolate, a total of 31 MTA (28 unique SNP makers) were identified, resulting in 21 unique QTL, of which 13 are novel and nine have been previously reported and consolidated by Zhang et al. (2020). The BLINK algorithm model was selected as the best model for four isolates (GPS71-U, 13GPS149, 13GPS203 and 13GPS207), whereas the FarmCPU algorithm model was selected as the best for the remaining two isolates (E4 and 13GPS109). Using only the best model, a total of one, four, four, three, six, and three QTL were identified on barley chromosomes 1H, 2H, 3H, 4H, 5H and 7H, respectively (Fig. 4; Table 2). There was one QTL identified on the “unassigned” chromosome and no QTL identified on barley chromosome 6H, which was surprising given that chromosome 5H is typically depleted for QTL in barley - *R. commune* interactions. Barley row type was used as a control phenotype with most significant SNP marker (SSM) SCRI_RS_13565 on chromosome 2H at position 655,112,232 corresponding to the known row type *vrs1* locus (**Supplemental Figs. 3 & 4**).

Isolate E4

The best model for isolate E4 was the mixed model using the FarmCPU algorithm and one principal component. Seven unique QTL were identified at the 0.01 significance threshold and an additional four at the 0.05 significance threshold, of which five are hypothesized to be novel (Table 2). Three QTL were identified on chromosome 2H, two QTL on chromosome 3H, three QTL on chromosome 5H and two QTL on chromosome 7H (Fig. 2). The loci *QRrs-2H.1, QRrs-2H.2, QRrs-5H.2* and *QRrs-7H.3* were all novel. *QRrs-2H.1* and *QRrs-2H.2* were identified on chromosome 2H at positions 579,016,838 – 579,380,380 with SSMs 12_30724 and 11_21166 and position 622,777,603 (SSM 11_20947), respectively. *QRrs-5H.2* was identified on chromosome 5H at position 610,172,204 (SSM 12_10633). The last novel locus *QRrs-7H.3*, was identified on chromosome 7H at position 573,627,082 (SSM SCRI_RS_200020) approximately 14 Mb proximal to the locus *qS271_7* (Coulter et al. 2019), but there are three insignificant markers in between *QRrs-7H.3* and *qS271_7* (Table 2). The first previously reported locus is *QRrs-2H.3* identified on chromosome 2H at position 655,112,232 (SSM SCRI_RS_13565), approximately 3.4 Mb distal of *QSc.*TxFr-2H (Li and Zhou 2011), 1.5 Mb proximal of *QA2* (Looseley et al. 2018). The second previously reported locus is *QRrs-3H.2* identified on chromosome 3H at position 493,556,092 (SSM 12_30829), approximately 6.1 Mb distal of *qC174_3* (Coulter et al. 2019), 9.9 Mb proximal of *QTLSR-3H-2015* (Zhang et al. 2019), and within the *Qsc_3H_2* locus (Hautsalo et al. 2021). The third previously identified locus is *QRrs-3H.3* located on chromosome 3H at position 574,441,946 (SSM SCRI_RS_162539) located approximately 0.9 Mb distal of *Rrs1, QTLSHN1999, QTLSHS2000* and *QTLSHG2001* (Genger et al. 2003; Read et al. 2003), and may colocalize with the undesignated 3H locus reported in the HEB-25 association mapping (Büttner et al. 2020). The locus *QRrs-5H.4* located on chromosome 5H at position 557,014,868 (SSM 11_20549) is at the same position as *QTLCW5H.1* (Looseley et al. 2012) and an unnamed locus reported in the Bere landrace association mapping study using the isolate L77 (Cope et al. 2021). The locus *QRrs-5H.6* was identified on chromosome 5H at location 640,002,824 (SSM SCRI_RS_155322) and is within the *Qsc_5H_1* locus (Hautsalo et al. 2021). The locus *QRrs-7H.2* was identified on chromosome 7H at position 66,021,760 (SSM 12_30181) located approximately 3.9 Mb proximal to *QTLSR-7H-2017* (Zhang et al. 2019).

Isolate GPS71-U

The best model for isolate GPS71-U was the random model using the BLINK algorithm and nine principal components. A total of five QTL were identified at the 0.01 significance threshold, with one additional QTL at the 0.05 significance threshold, with a total of three out of the six being novel QTL. One QTL was identified on chromosome 3H, one QTL on chromosome 4H, three QTL on 5H and one locus that has not been assigned to a chromosome (Fig. 2). The loci *QRrs-3H.1, QRrs-5H.1*, and *QRrs-Un.1* were all novel and identified on chromosomes 1H, 5H, and the unassigned chromosome at positions 169,952,332 (SSM 11_20866), 459,971,602 (SSM 11_21344), and 75,893,225 (SSM SCRI_RS_139563), respectively (Table 2). The first previously identified locus is *QRrs-4H.1*

located at position 7,870,312 (SSM 12_30540), 6.1 Mb distal of *Rrs16 Hb1, Rrs16 Hb2* (Pickering et al. 2006), *QSc.VlWa.4H.2* (Wang et al. 2014b), *QTLVB4H.1*, and *QTLVB4H.3* (Wallwork et al. 2014), 6.4 Mb distal from *Qsc_4H_1* (Hautsalo et al. 2021), and 2.2 Mb proximal to *QA6* (Looseley et al. 2018). The second previously reported locus *QRrs-5H.4*, was also identified with isolate E4 as described above. The last previously identified locus was *QRrs5H.6* identified on chromosome 5H at position 642,241,089 (SSM 12_30566) embedded within the *Qsc_5H_1* locus (Hautsalo et al. 2021).

Isolate 13GPS109

The best model for isolate 13GPS109 was the mixed model using the FarmCPU algorithm and Q population structure. Similar to isolate GPS71-U, a total of six QTL were identified using isolate 13GPS109 at the 0.01 significance threshold and one additional QTL at the 0.05 significance threshold, with three being novel. One QTL was identified on chromosomes 1H, 2H, 3H, 4H, 5H and 7H (Fig. 2). The novel loci *QRrs-1H.1, QRrs-3H.4*, and *QRrs-4H.3* were identified on chromosomes 1H, 3H, and 4H at positions 508,780,596 (SSM 11_10433), 667,790,880 (SSM 12_31161), and 617,971,329 (SSM 12_10824), respectively (Table 2). The first previously identified locus *QRrs-2H.3* was identified on chromosome 2H at position 655,324,614 (SSM SCRI_RS_3376), approximately 3.6 Mb distal of *QSc.TxFr-2H* (Li and Zhou 2011) and 1.3 Mb proximal of *QA2* (Looseley et al. 2018). The second previously identified locus *QRrs5H.6* was identified on chromosome 7H at position 638,951,179 (SSM 12_30162) embedded within the *Qsc_5H_1* locus (Hautsalo et al. 2021). The third previously identified locus *QRrs-7H.1* was identified on chromosome 7H at position 15,857,316 (SSM SCRI_RS_139563) approximately 7.1 Mb distal from the boundary of *Qsc_7H_1* (Hautsalo et al. 2021), 5 Mb distal of *Rh2* (Schweizer et al. 1995) and 0.2 Mb proximal to *Rrs12* (Abbott et al. 1991; Genger et al. 2003).

Isolate 13GPS149

The best model for isolate 13GPS149 was the mixed model using the BLINK algorithm and four principal components. A total of two QTL were identified at the 0.01 significance threshold, with one additional QTL at the 0.05 significance threshold, with two of the three being novel QTL. These QTL were identified on chromosomes 2H, 3H and 4H (Fig. 2). The novel loci *QRrs-*2H.1 and *QRrs-4H.2* were identified on chromosomes 2H and 4H at positions 567,016,838 (SSM 12_30724) and 27,327,299 (SSM 12_10860), respectively (Table 2). The previously identified locus *QRrs-3H.2* was also identified using the isolate E4 on chromosome 3H at position 493,556,092 (SSM 12_30829), approximately 6.1 Mb distal of *qC174_3* (Coulter et al. 2019) and 9.9 Mb proximal of *QTLSR-3H-2015* (Zhang et al. 2019), and embedded within the *Qsc_3H_2* locus (Hautsalo et al. 2021).

Isolate 13GPS203

The best model for isolate 13GPS203 was the mixed model using the BLINK algorithm and nine principal components. A total of three QTL were identified at the 0.01 significance threshold, of which two were novel and identified on chromosome 5H (Fig. 2). No additional QTL were detected at the 0.05 significance threshold. The novel loci *QRrs-5H.3* and *QRrs-5H.5*, and *QRrs-5H.6* were identified on chromosome 5H at positions 528,355,024 (SSM 12_31427), and 599,128,110 (SSM 12_30930), respectively (Table 2). The locus *QRrs-5H.6* at position 638,951,179 (SSM 12_30162) was previously identified and embedded within the locus *Qsc_5H_1* (Hautsalo et al. 2021).

Isolate 13GPS207

The best model for isolate 13GPS207 was the mixed model using the BLINK algorithm and four principal components. One QTL was identified at the 0.01 significance threshold and one additional QTL at the 0.05 significance threshold, with no novel loci. The first previously identified locus *QRrs-5H.6* was identified on chromosome 5H at position 649,232,960 (SSM 11_10600) and embedded within the *Qsc_5H_1* locus (Hautsalo et al. 2021). The previously identified locus *QRrs-7H.1* was identified on chromosome 7H at position 22,774,581 (SSM 11_20495) approximately 6.7 Mb distal of *Rrs12* (Abbott et al. 1991; Genger et al. 2003).

Enrichment Analysis

The percentage of resistant alleles for all SSMs were calculated for wild and landrace barley (Fig. 5). Wild barley was enriched for resistance SSMs in 13 cases, whereas landrace barley was enriched for resistance SSMs in 15 cases. A total of two loci were fixed within the different barley classes, with *QRrs-2H.3* (SSM SCRI_RS_13565) fixed in landrace barley and *QRrs-4H.1* (12_20540) fixed in wild barley. The locus *QRrs-3H.1* showed the largest difference with 1% wild barley containing the resistance allele and 81% containing the resistance allele in landrace barley.

Discussion

Barley scald is regarded as one of the most economically important disease of barley worldwide (Zhang et al. 2020). Yet, important information pertaining to host - pathogen interactions remain elusive in the pathosystem such as the identity of the sexual host/s (Penselin et al. 2016; Holtz 2021). Over 150 resistance loci have been described in barley against *R. commune* (Büttner et al. 2020; Zhang et al. 2020; Cope et al. 2021; Hautsalo et al. 2021), and an additional 13 novel loci were identified in this study out of a total of 21 significant loci detected. Of the major known scald resistance loci, only *Rrs1, Rrs2, Rrs12*, and *Rrs16* were identified in our study indicating that these Turkish populations are a rich resource of diverse untapped scald resistance genes. In addition, our study identified six unique loci (four novel) on chromosome 5H, where there are no formally designated genes and only eight of over 150 previously described loci have been located (Looseley et al. 2012; Coulter et al. 2019; Zantinge et al. 2019; Daba et al. 2019; Büttner et al. 2020; Zhang et al. 2020; Cope et al. 2021), increasing the number of loci described on chromosome 5H by 50%. Interestingly, the isolate 13GPS203 had three QTL exclusively identified on chromosome 5H.

Loci of particular interest include the locus *QRrs-2H.1*, identified using isolates E4 and 13GPS149 with marker 12_30724. However, the variation of virulence for isolate E4 also showed a significant marker trait association with SNP 11_21166 approximately 12 Mb from 12_30724, with no marker in between, suggesting that this locus is complex, with different haplotypes and possibly distinct genes providing resistance or susceptibility to the specific isolates. Other loci identified across multiple isolates include *QRrs-2H.3* with isolate E4 and 13GPS109, *QRrs-3H.2* with isolate E4 and 13GPS149, *QRrs-5H.4* with isolate E4 and GPS71-U, and *QRrs-7H.1* with isolates 13GPS109 and 13GPS207 (Fig. 1, Table 2). The *QRrs-5H.6* locus, which was only previously reported once

(Hautsalo et al. 2021), was identified for all isolates except 13GPS149, suggesting that it represents broader-spectrum resistance to the Turkish *R. commune* population. Using enrichment analysis within the *QRrs-5H.6* locus, SSMs SCRI_RS_155322 (landrace 4% versus wild 38%) and 12_30162 (6% versus 20%) were more prevalent in wild accessions and SSMs 12_30566 (90% versus 46%) and 11_10600 (95% versus 83%) were more prevalent in the barley landraces, suggesting that this locus is complex with multiple resistance genes sourced from both wild barley and landraces.

The locus *QRrs-7H.1* is positioned between loci identified using isolates L2A and 13–13, which they postulate as *Rrs12* (Abbott et al. 1991) and *Rrs2* (Hanemann et al. 2009). The *locus QRrs-4H.1* may colocalize with a locus reported against isolate L2A, and correlates with *Rrs16* (Pickering et al. 2006), however no marker position was provided (Cope et al. 2021). The remaining loci identified in the Scottish Bere landrace association mapping (Cope et al. 2021) and potentially all but one locus from HEB-25 wild barley population (Büttner et al. 2020) show no overlap with our study showing that geographically distinct barley populations and their interactions with local isolates harbor different loci involved in *R. commune* interactions.

The fact that the wild barley population was more resistant to all six *R. commune* isolates compared to the landrace population suggests that this wild population harbors more effective alleles or a greater diversity of resistance genes (Fig. 4). Although there are several possible explanations for these findings it is probable that this study may not have had the diversity within the isolates utilized to identify individuals with virulence on the wild barley genotypes. Thus, it would be prudent to screen these populations with a greater diversity of isolates to identify additional resistance sources and identify those with broad resistance that could be more valuable to breeding programs.

Interestingly, three loci colocalize with genes associated with different post-domestication barley market classes. The locus *QRrs-2H.3* is approximately 3.1 Mb distal to *vrs1*, and the locus *QRrs-5H.4* is approximately 4 Mb distal from *vrs2.* Both *vrs1* and *vrs2* control spikelet morphology and the switch from wildtype two-row barley to the mutant six-row phenotype (Zwirek et al. 2019). The majority of six-row barley cultivars are controlled by the presence of natural recessive *vrs1* alleles and accompanied by a natural *vrs5* allele that improves lateral spikelet grain fill (Zwirek et al. 2019). Therefore, the fact that *vrs1* is the most significant marker in our row type association and the *vrs2* locus was not identified (**Supplemental Figs. 3 & 4**) is not surprising given that *vrs2, vrs3* and *vrs4* are not prevalent in six-row barley (Zwirek et al. 2019). The *vrs1* and *QRrs-2H.3* locus were identified with the same SSM SCRI_RS_13565, with six row barley carrying the allele that increases resistance and thus is more prevalent in the landrace barley lines. This is surprising given that in general, wild barley is more resistant than landrace barley, however this provides the opportunity to break this linkage and stack resistance genes from both landrace and wild barley into breeding material. The *QRrs-2H.3* locus was previously reported in three previous studies, one of which noted the close proximity to *vrs1* (Li and Zhou 2011; Looseley et al. 2018; Fériani et al. 2020). Interestingly, *QRrs-2H.3* and *QRrs-5H.4* were only identified on FarmCPU models. The last locus *QRrs-5H.5* colocalizes with *vrn-H1*, which is responsible for the spring and winter growth habit, with 99% of wild lines having the allele, whereas 49% of landrace barley having the same allele. The fact that 99% of wild barley have the same allele for *QRrs-5H.5* may be due to the linkage drag from the close proximity to *vrn-H1* and the fact that all wild barley are of winter growth habit (Fernández-Calleja et al. 2021).

Barley scald remains a global issue, being one of the most destructive diseases that hampers barley production (Zhan et al. 2008). Compared to other barley diseases such as net blotch or leaf rust, relatively few resistance loci have been identified that can be used in breeding programs (Dracatos et al. 2019; Clare et al. 2020; Zhang et al. 2020). Worldwide, barley is predominantly grown as a feed crop (IBGSC 2012), however, barley is the precursor to malt, a vital component within the brewing and distilling industry that cannot be replaced by other cereal grains (Olaniran et al. 2017). In addition, barley is gaining traction as a food crop because of its high nutritional value and heart healthy attributes (Zeng et al. 2020). In the United States, barley is primarily grown for malt, as malt commands a premium within the multi-billion dollar brewing and distilling industry. However, within the United States, barley has seen significant acreage drops and has been forced off premium agricultural land by more profitable crops such as corn and soybeans (Brueggeman et al. 2020). Along with the impacts of climate change, there could be worldwide malt shortages in the near future that are beginning to be witnessed with stagnating yields seen in southern Europe (Dawson et al. 2015; Xie et al. 2018). Therefore, identification of sources of resistance that can be used to maintain yield increases and minimize inputs to barley production against important pathogens such as *R. commune* are paramount. Here we report the identification of 21 distinct loci encompassing 13 novel loci from wild and landrace barley from the origin of domestication, rather than the initial location of barley - *R. commune* co-evolution, that could be introgressed into elite barley cultivars.

Declarations

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Competing Interesting

The authors declare that there is no conflict of interest.

Author Contributions

A.Ç.O., A.K., and R.S.B. conceived the study. M.R.A., A.Ç.O. and A.K. carried out phenotyping and DNA extractions. K.E. and S.C. carried out genotyping. S.C. performed the analysis and wrote the manuscript with contributions from A.Ç.O., K.E., A.K., and R.B. All authors approved the final manuscript.

Data Availability

Isolates are available upon request. The authors ensure that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables. File SF1 contains phenotyping data. File SF2 contains the mean-squared deviations of each association mapping model tested. Genotyping data, marker positions, population structure and EMMA kinship matrix can be found as File SF1, SF2, SF4, and SF5, respectively at figshare: https://doi.org/10.25387/g3.14725311 of Clare *et al.* (2021).

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Tables

Table 1. Phenotypic responses of landrace and wild barley to six *Rhynchosporium commune* isolates with absolute and percentage of accessions in each resistant or susceptible class. The mean phenotypic score of each barley class to the respective isolate is also provided.

	Landrace			Wild		
Isolate	Resistant	Susceptible	Mean	Resistant	Susceptible	Mean
E4	17 (9%)	171 (91%)	3.6	64 (68.1%)	30 (31.9%)	1.3
GPS71-U	26 (13.9%)	161 (86.10%)	3.4	35 (37.2%)	59 (62.8%)	2.6
13GPS109	19 (10.1%)	169 (89.9%)	3.4	71 (75.5%)	23 (24.5%)	1.3
13GPS149	18 (9.6%)	170 (90.4%)	3.5	72 (76.6%)	22 (23.4%)	1.3
13GPS203	87 (46.3%)	101 (53.7%)	2.6	65 (69.1%)	29 (30.9%)	1.7
13GPS207	40 (21.3%)	148 (78.7%)	2.9	66 (70.2%)	28 (29.8%)	1.3
Mean	34.5 (18.4%)	153.3 (81.6%)	3.2	62.2 (66.1%)	31.8 (33.9%)	1.6

Table 2. Significant marker trait associations identified from genome wide association analysis order by isolate, followed by chromosome and base pair positions. The loci designations are provided as well as overlapping previously identified loci (corresponding loci), the model used to identify the marker, reference and alternative alleles, logarithm of odds score and minor allele frequency.

Isolate	Loci	Marker	Chr[1]	Position[1]	Corresponding Loci	Models Identified	Allele[2]	L(s(
E4	*QRrs-2H.1*	12_30724	2	567016838	Novel	PC1 + K (FarmCPU)	G/A	4. ((
	QRrs-2H.1	11_21166	2	579380380	Novel		A/C	6. ((
	QRrs-2H.2	11_20947	2	622777603	Novel		T/C	5. ((
	QRrs-2H.3	SCRI_RS_13565	2	655112232	*QSc.TxFr-2H/QA2*		A/C	1: ((
	QRrs-3H.2	12_30829	3	493556092	*qC174_3/QTLSR-3H-2015/Qsc_3H_2*		A/T	5. ((
	QRrs-3H.3	SCRI_RS_162539	3	574441946	*Rrs1/QTLSHN1999/QTLSHS2000/QTLSHG2001*		G/A	6. ((
	QRrs-5H.2	12_10633	5	510172204	Novel		G/C	4. ((
	QRrs-5H.4	11_20549	5	557014868	*QTLCW5H.1/Cope.5H*		C/A	4. ((
	QRrs-5H.6	SCRI_RS_155322	5	640002824	*Qsc_5H_1*		A/G	8. ((
	QRrs-7H.2	12_30181	7	66021760	*QTLSR-7H-2017*		A/G	4. ((
	QRrs-7H.3	SCRI_RS_200020	7	573627082	Novel		G/A	7. ((
GPS71-U	*QRrs-3H.1*	11_20866	3	169952332	Novel	PC9 + K (BLINK)	A/G	4. ((
	QRrs-4H.1	12_30540	4	7870312	*Rrs16* Hb1/2*/QSc.VIWa.4H.2/QTLVB4H.1/3/Qsc_4H_1//QA6*		A/G	5. ((
	QRrs-5H.1	11_21344	5	459971602	Novel		C/T	5. ((
	QRrs-5H.4	SCRI_RS_129893	5	568196561	*QTLCW5H.1/Cope.5H*		C/T	5. ((
	QRrs-5H.6	12_30566	5	642241089	*Qsc_5H_1*		G/A	8. ((
	QRrs-Un.1	SCRI_RS_160332	Un	75893225	Novel		A/G	6. ((
13GPS109	*QRrs-1H.1*	11_10433	1	508780596	Novel	Q + K (FarmCPU)	G/A	6. ((
	QRrs-2H.3	SCRI_RS_3376	2	655324614	*QSc.TxFr-2H/QA2*		A/G	6. ((
	QRrs-3H.4	12_31161	3	667790880	Novel		G/A	4. ((
	QRrs-4H.3	12_10824	4	617971329	Novel		C/T	5. ((
	QRrs-5H.6	12_30162	5	638951179	*Qsc_5H_1*		C/G	6. ((
	QRrs-7H.1	SCRI_RS_139563	7	15857316	*Qsc_7H_1/Rh2//Rrs12*		A/G	5. ((
13GPS149	*QRrs-2H.1*	12_30724	2	567016838	Novel	PC4 + K (BLINK)	G/A	9. ((
	QRrs-3H.2	12_30829	3	493556092	*qC174_3/Qsc_3H_2/QTLSR-3H-2015*		A/T	4. ((
	QRrs-4H.2	12_10860	4	27327299	Novel		C/T	5. ((
13GPS203	*QRrs-5H.3*	12_31427	5	528355024	Novel	PC9 + K (BLINK)	C/G	5. ((
	QRrs-5H.5	12_30930	5	599128110	Novel		G/C	8. ((
	QRrs-	12_30162	5	638951179	*Qsc_5H_1*		C/G	4.

	5H.6							(0
13GPS207	*QRrs-5H.6*	11_10600	5	649232960	*Qsc_5H_1*	PC4 + K (BLINK)	G/A	7. (0
	QRrs-7H.1	11_20495	7	22774581	*Rrs12*		C/G	4. (0

[1], Location based on the first version of the Morex assembly (Mascher et al., 2017).

[2], Reference/alternative allele

[3], LOD scores based on best model with significance threshold in brackets

[4], Minor allele frequency

Figures

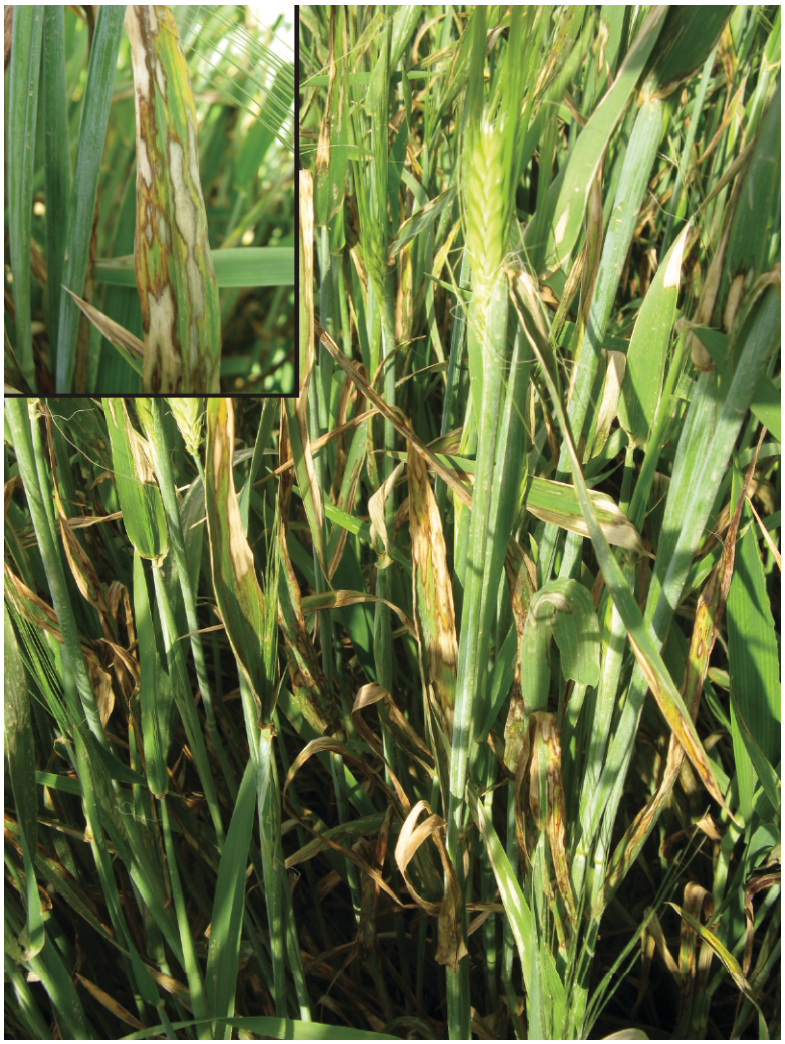

Figure 1

Photograph of a barley field infected with *Rhynchosporium commune* with close up inset in the upper left corner showing a typical susceptible scald reaction with coalescing lesions.

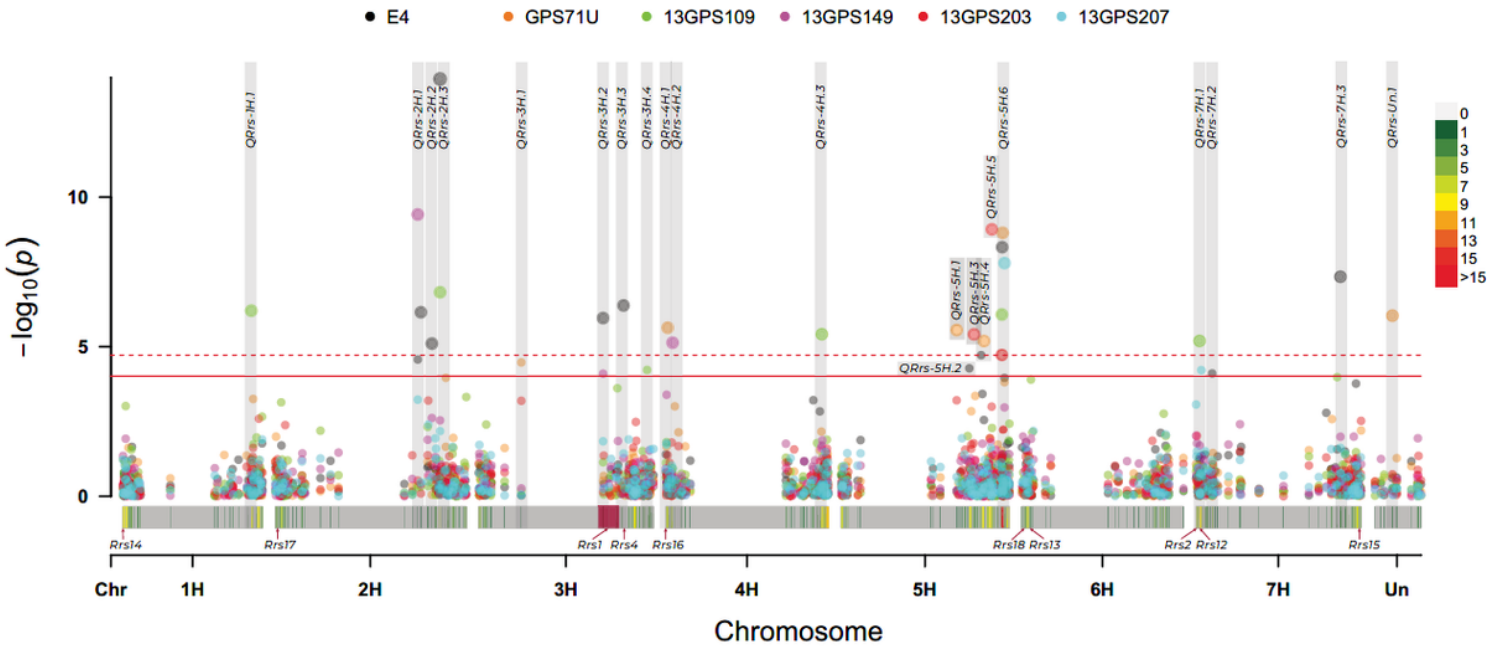

Figure 2

Manhattan plot for the best model for each color coded *Rhychosporium commune* isolate including E4 (black), GPS71-U (orange), 13GPS109 (green), 13GPS149 (magenta), 13GPS203 (red) and 13GPS207 (cyan). Chromosomes and marker density are indicated along the x axis along with formally designated *Rrs* loci. LOD score is on the y axis with thresholds indicated by the solid (α level 0.05) and dashed (α level 0.01) red lines.

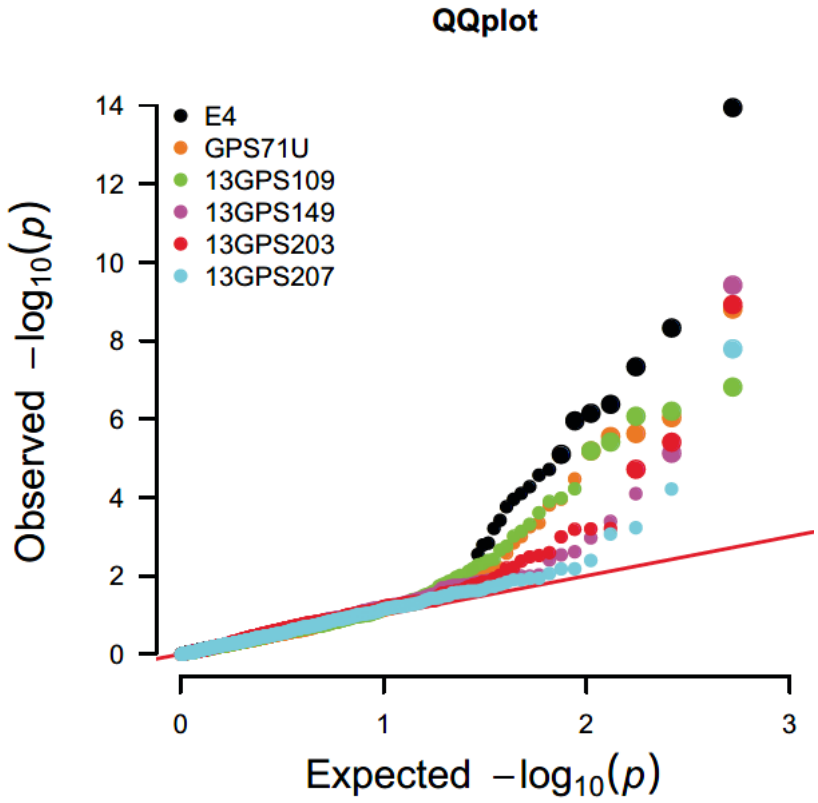

Figure 3

Quantile-quantile plot for all six *Rhychosporium commune* isolates including E4 (black), GPS71-U (orange), 13GPS109 (green), 13GPS149 (magenta), 13GPS203 (red) and 13GPS207 (cyan). Expected LOD score is along the x axis and observed LOD score along the y axis.

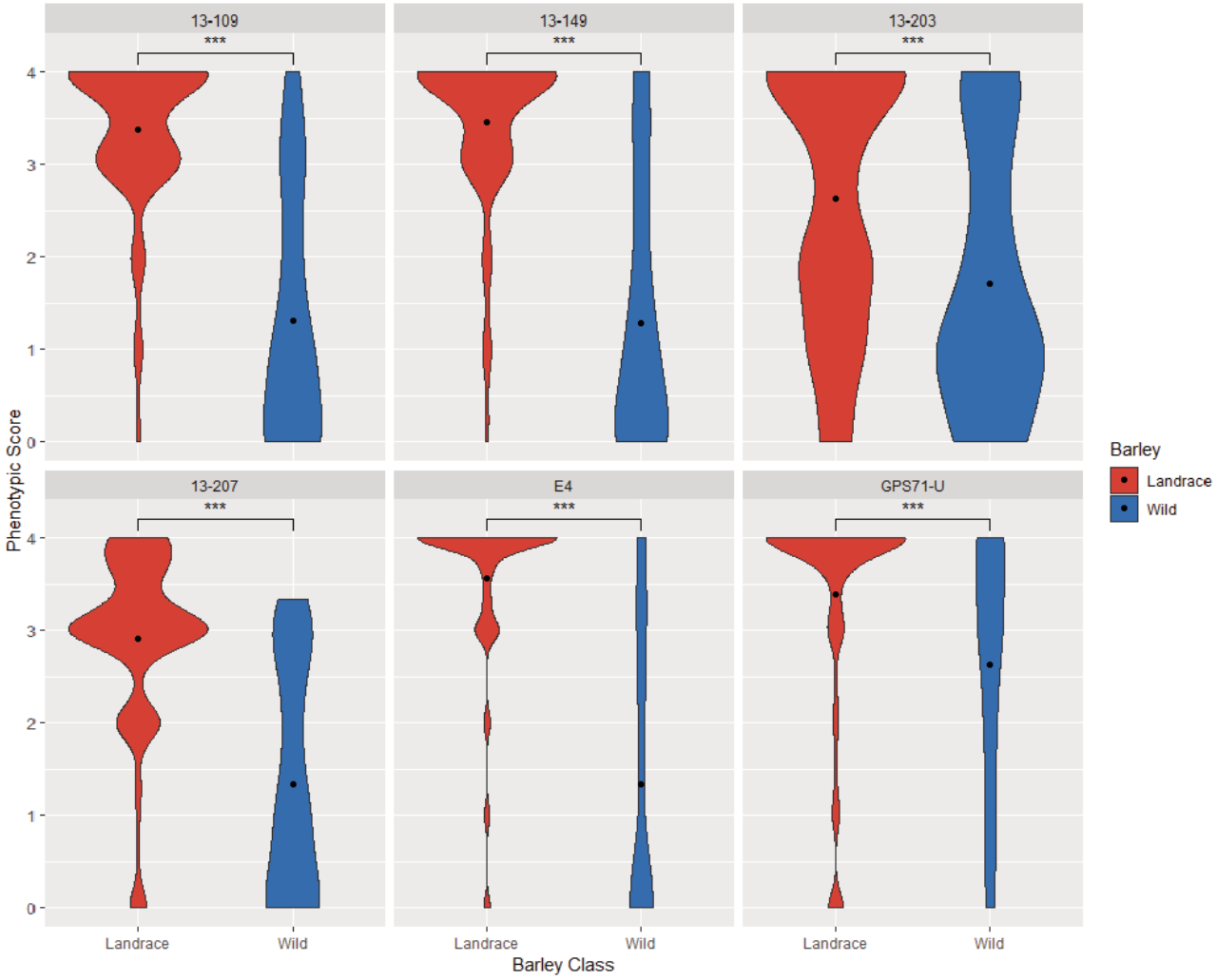

Figure 4

Violin plots of phenotypic distribution of landrace (red) and wild (blue) barley to each *Rhynchosporium commune* isolate. The width of the violin indicates the relative maximum of accessions with the respective phenotypic score and the black dot represents the barley class mean. The asterisks above each plot indicates the significance of a Wilcoxon test.

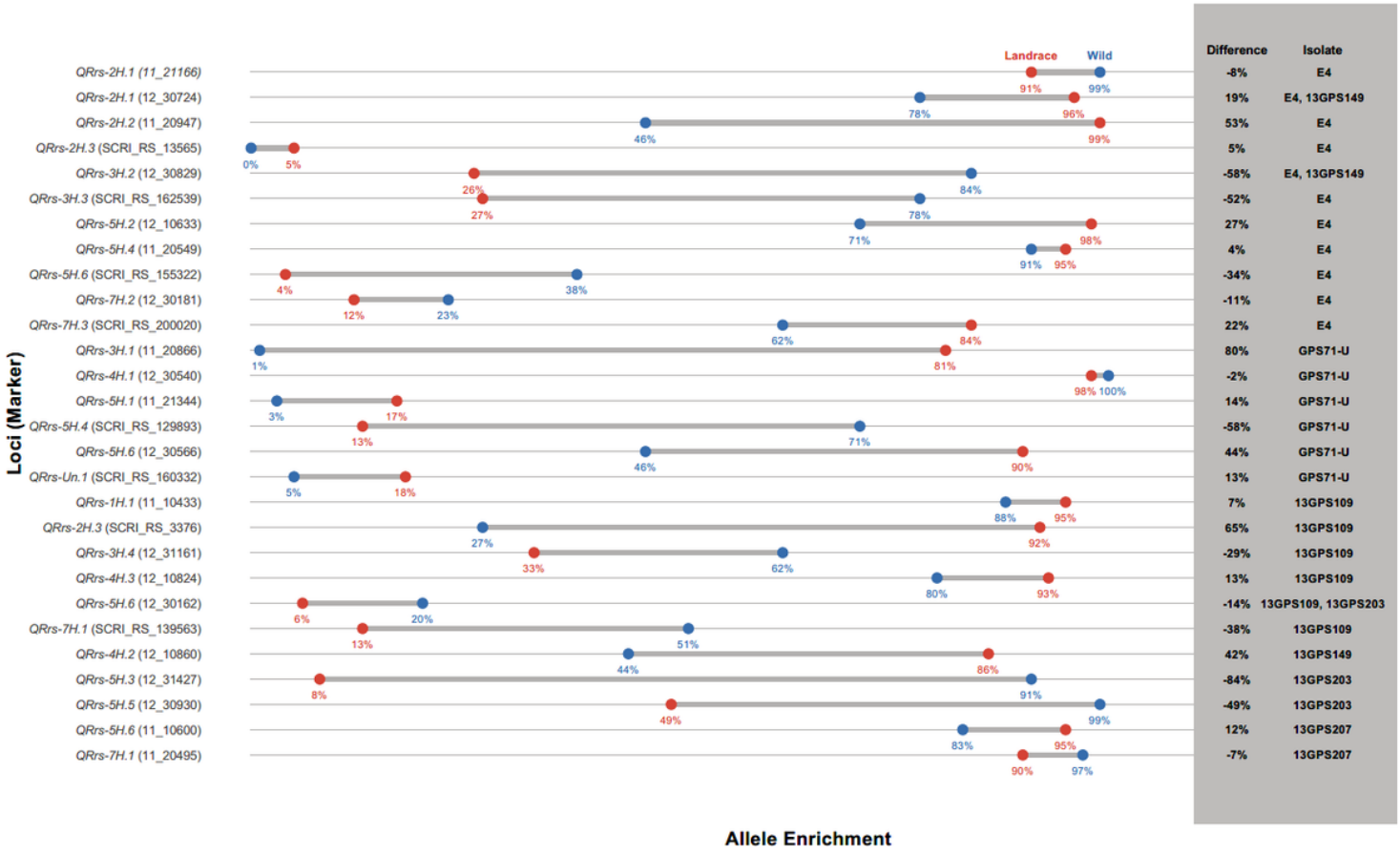

Figure 5

Dumbbell plot indicating the percentage of landrace (red) and wild (blue) barley harboring the SNP within significant loci associated with resistance. The percentage is indicated under each point and the difference between landrace and wild barley is indicated at the end of the row along with the isolates the marker was identified with.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalFigure1DensityPlot.eps
- SupplementalFigure2Scree.eps
- SupplementalFigure3Manhattan.eps
- SupplementalFigure4QQ.eps
- SupplementalFile1Pheno.txt
- SupplementalFile2MSD.txt