*Plant Breeding*

WILEY

Plant Breeding

ORIGINAL ARTICLE OPEN ACCESS

# A Major Locus Conferring Both Fusarium Crown Rot Resistance and Drought Tolerance in Barley (*Hordeum vulgare* L.)

Zhouyang Su[1,2] | Shang Gao[3] | Haiyan Hu[4] | Sergey Shabala[5,6] | Meixue Zhou[2] | Chunji Liu[4] | Zhi Zheng[1]

[1]CSIRO Agriculture and Food, Australian Capital Territory, Australia | [2]Tasmanian Institute of Agriculture, University of Tasmania, Prospect, Tasmania, Australia | [3]Institute of Crop Sciences and State Key Laboratory of Crop Gene Resources and Breeding, Chinese Academy of Agricultural Sciences (CAAS), CIMMYT-China office, Beijing, China | [4]School of Agriculture, Henan Institute of Science and Technology, Xinxiang, Henan, China | [5]School of Biological Sciences, University of Western Australia, Crawley, Western Australia, Australia | [6]International Research Centre for Environmental Membrane Biology, Foshan University, Guangdong, China

**Correspondence:** Zhi Zheng (zhengzhi113322@gmail.com)

**Received:** 26 November 2024 | **Revised:** 10 May 2025 | **Accepted:** 20 May 2025

**Funding:** The authors received no specific funding for this work.

**Keywords:** drought tolerance | Fusarium crown rot | QTL mapping | recombinant inbred lines | wild barley

## ABSTRACT

The detrimental effects of drought on crop production are strongly influenced by interactions with other biotic and abiotic stresses, including Fusarium crown rot (FCR), a disease known to cause severe yield loss in crops experiencing drought after anthesis. This study aimed to identify loci that simultaneously confer drought tolerance and FCR resistance using a recombinant inbred line (RIL) population. The population derived from a cross between cultivated barley (*Hordeum vulgare* L.) and wild barley (*Hordeum spontaneum* C. Koch.), was evaluated under drought-stressed and non-drought-stressed conditions. Three loci conferring drought tolerance were identified on chromosome arms 2HL, 4HL and 5HS. Of these, the 2HL locus had the largest effect, influencing leaf wilting, leaf senescence and shoot dry weight, and importantly, also conferred FCR resistance. The RIL population was exposure to FCR infection and drought stress in glasshouse and controlled environment facility, respectively. Genotyping-by-sequencing (GBS) was used to develop a high-density genetic map, which facilitated QTL identification through linkage analysis. Significant correlations were observed among the assessed traits and previously collected agronomic traits. Candidate genes at the 2HL locus were identified based on its location and sequence variations between parental genotypes. This study represents the first identification of such a locus conferring both drought tolerance and FCR resistance, which may open new avenues for leveraging existing drought tolerant genes in enhancing FCR resistance by targeting pathways associated with water stress adaptation.

## 1 | Introduction

Of all environmental constraints, drought is by far the most destructive factor for agriculture, causing USD 80 billion in crop production loss worldwide (Razzaq et al. 2021). Results from some studies suggest that, during the twenty-first century, drought frequency and areas have already increased by 50 to 200%, respectively (Zhao and Dai 2017). With climate changes, drought is predicted to become more intense and frequent in many crop production regions (Trenberth et al. 2014; Chen et al. 2023). Drought stress can affect plant performance at any stage of plant ontogeny, from seed germination to maturity. Specific agronomical attributes affected by drought include plant height, flag leaf length and spike length, among others (Sallam

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2025 The Author(s). *Plant Breeding* published by Wiley-VCH GmbH.

et al. 2019). In addition to its direct impact, drought stress also affects crop production indirectly by altering tolerance and resistance to a wide range of diseases (Sallam et al. 2019; Su et al. 2021; Su et al. 2022). For these reasons, enhancing drought tolerance of major crop species is considered as a most critical element of global food security (Chen et al. 2023).

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop worldwide after wheat, rice and maize in terms of production quantity and area harvested. It is widely grown and used as food, feed, fodder as well as in brewing industries. This crop was domesticated some 10,000 years ago from the progenitor *Hordeum spontaneum* C. Koch. The cultivated and wild barley share the same genome (HH, $2n = 2x = 14$), and there are no known barriers in producing fertile progeny between them. For this reason, they are considered by some as the same species (Forster et al. 2000). Like many other crop species, domestication and selection processes have dramatically reduced genetic variation in cultivated barley. Results from previous studies showed that cultivars represent only a part of the range of genetic variation in wild barley. Some studies indicated that less than half of the alleles found in wild barley are present in cultivars (Ellis et al. 2000; Tombuloglu et al. 2015). Results from pan-genome studies also showed that large numbers of genes found in wild barley, especially those conferring tolerance and resistance to biotic and abiotic stresses, may have been lost during evolution, domestication, or breeding (Gao et al. 2019; Ma et al. 2019). In other words, wild barley genotypes do not only provide a rich source of new alleles but also novel genes for breeding programs. This highlighted the potential of wild barley for improving dual stress resistance in cultivated varieties. It is thus not surprising that wild barley has already proven to be a very fruitful source of gene resources for improving modern crop cultivars (Thomas et al. 1998), and populations derived from crosses between cultivated and wild barley have been used extensively in biological studies (Chen et al. 2023; Hu et al. 2019; Niu et al. 2022).

Fusarium crown rot (FCR) is a chronic and severe disease affecting cereal production worldwide. Severe yield loss from this disease occurs mainly in crops that suffered drought stress after anthesis (Liu and Ogbonnaya 2015). FCR can be caused by a wide range of *Fusarium* species with *F. pseudograminearum* as the most prevalent pathogen (Akinsanmi et al. 2004). Seedlings infected by FCR are characterized by brown lesions in roots and crowns. Progression of the disease is characterized by an increased degree of leaf sheath and stem discolouration (browning). Severe FCR infection can lead to seedling and plant death. Whiteheads are the pronounced visual impact of severe FCR infection especially when moisture stress occurs after anthesis, and these heads or spikes possess shrivelled or even no kernels (Chakraborty et al. 2006; Murray and Brennan 2009). Previous studies showed that *Fusarium* pathogens proliferated more rapidly under drought conditions once entering plants (Liu and Liu 2016), which explains why incorporating drought stress is effective in enhancing FCR severity in glasshouse experiments (Mitter et al. 2006; Li et al. 2008; Jiang et al. 2019; Gao, Zheng, Hu, et al. 2020). An earlier study also showed that wheat lines with greater drought tolerance could trigger a stronger defence against FCR infection likely due to lower levels of drought stress (Su et al. 2021). Recently, transcriptome analysis revealed that similar regulatory networks were activated in wheat under the two stress conditions (Su et al. 2024).

To date, three studies have reported four QTL conferring FCR resistance in barley. The first study detected *Qcrs.cpi-4H* from a population of recombinant inbred lines (RILs) whose donor parent, the wild barley genotype (*H. spontaneum* L.) originated from Iran (Chen, Liu, Ma, et al. 2013). The second study investigated the population of RILs whose donor parent is a landrace originated from Japan, identifying two loci *Qcrs.cpi-1H* and *Qcrs.cpi-3H* (Chen, Liu, Wei, et al. 2013). The third study detected *Qcrs.caf-6H* from a population of RILs whose donor parent is a landrace originated from South Korea (Gao et al. 2019). Numerous QTL for drought-related traits were detected under drought stress based on a review (Elakhdar et al. 2022) across all chromosomes. However, none of these QTL were detected from the same population conferring both FCR resistance and drought tolerance.

To enhance the understanding of relationships between drought tolerance and FCR resistance, we investigated if loci conferring drought tolerance co-locate with any of those loci conferring FCR resistance by assessing both traits in a single population generated between a domesticated barley cultivar and a wild barley accession (Zhou et al. 2021). Additionally, interactions between these two characteristics and other traits of agronomic importance were assessed. Taking advantage of the genome assemblies of the two parental genotypes, candidate genes underlying the targeted locus were identified.

## 2 | Materials and Methods

### 2.1 | Plant Materials

A population consisting of 201 RILs was assessed in this study under both FCR infection and drought stress. The population was obtained from a cross of Morex/AWCS276. Morex is a cultivar (*H. vulgare* L.) used by international barley researchers in generating a high-quality genome assembly (Mascher et al. 2017). AWCS276 is a wild genotype (*H. spontaneum* L.) with strong resistance to FCR infection (Chen, Liu, Ma, et al. 2013; Chen, Liu, Wei, et al. 2013). Genome assembly for AWCS276 is also available (Liu et al. 2020). This population has been used in mapping loci controlling a wide range of characteristics, including flag leaf thickness (FLT), flag leaf length (FLL), flag leaf width (FLW), flag leaf area (FLA), flag leaf length and width ratio (FLWR), thousand kernel weight (TKW), kernel number per spike (KNPS), fertile tiller numbers (FTN), heading date (HD), spike row type (SRT), third leaf thickness of seedling (S3LT), third leaf length of seedlings (S3LL), third leaf width of seedling (S3LW), third leaf area (S3LA), and ratios of third leaf length and width of seedlings (S3LWR). Data for these agronomic traits collected from a previous study (Zheng et al. 2022) were used for correlation analysis with those collected in the study reported here.

### 2.2 | Assessment of FCR Resistance

CS3096 is a highly aggressive isolate of *F. pseudograminearum* maintained in Commonwealth Scientific and Industrial Research Organisation (CSIRO) (Akinsanmi et al. 2004), and it was utilized for FCR inoculation. Methods used in inoculum preparation and inoculation were those detailed by Li et al. (2008). Seeds of each line from the population were sterilized using 2% hypochlorite

for 5 min and then in 70% ethanol for 1 min. The sterilized seeds were rinsed three times using distilled water. Sterilized seeds were placed on three layers of filter paper saturated with water in petri dishes to germinate. FCR inoculation was carried out by immersing the germinated seedlings in the spore suspension for 1 min when coleoptile lengths reached about 1 cm. The seedlings were then planted into 56 cell-Kwik trays filled with UC (the University of California) potting mix (50% sand and 50% peat v/v). Water stress was applied to the infected seedlings to promote the disease development. Inoculated seedlings were watered only when wilt symptoms appeared by soaking the trays in containers filled with water for 5 min.

Three independent experiments were conducted against the 201 RILs, and each experiment had three replicates with 14 seedlings in each of them. Trials were carried out in a controlled environment facility (CEF) at the Saint Lucia site of CSIRO in Brisbane. The conditions of the CEF were set as follows: day/night temperature 25/16±1 °C, day/night relative humidity 65/80% ± 5% and 14 h-photoperiod with 500 mol $m^{-2} s^{-1}$ photon flux density at the level of the plant canopy. FCR severity was scored at 3 to 4 weeks after inoculation with a 0–5 scale, where 0 represents healthy plants without visible symptoms of infection (browning of seedling bases), 1–4 for plants with incrementally increased stem browning and 5 for dead plants.

## 2.3 | Assessment of Drought Tolerance

Three independent experiments were conducted, each with three replicates. Seeds of the 201 RILs were germinated in petri dishes with three layers of water-saturated filter paper. Five seeds for each line were germinated, and the best three seedlings with similar growth rates were selected for each replicate. Young seedlings (coleoptile about 1 cm in length) were planted into 30 cell-Kwik trays (Garden City Plastics, Australia) filled with UQ23 soil mix (pH 5.5–6.5, 70% composted pine bark 0–5 mm, 30% coco peat, Osmocote Exact Standard 3–4 months (~4 kg/$m^3$) and Suscon Maxi insecticide (~830 g/$m^3$). The seedling trays were placed in a glasshouse at St Lucia site of CSIRO, Brisbane. To reduce possible influence of flowering time on assessing drought tolerance, the 201 RILs were ordered based on flowering time so that lines growing in a single tray have similar flowering times. To reduce differences caused by positions where trays were placed, all trays were moved to different locations within the glasshouse every 2 to 3 days. Conditions of the glasshouse were set as follows: 25/16(±1)°C Day/night temperatures, > 50%/70% relative day/night humidity and 12 h-photoperiod with 500 mol $m^{-2} s^{-1}$ photon flux density at the level of the plant canopy.

For the control groups, seedling trays were soaked in a container filled with water for about 10 min every 2 to 3 days during the experiments. For the first time point of drought treatment, watering the treatment groups of plants was stopped at three leaf stage (collars of the second leaves became visible in over 50% of the seedlings). About 1 week following the withholding of watering, plants showed different severities of leaf wilting. Several criteria were used in assessing drought tolerance. One of these was scoring wilting severity of leaves using a scale ranging from 1 (no drought symptom) to 4 (whole plant wilted). Individual plants for each of the RILs were scored separately, and the average was calculated for further analyses. For the second time point of drought treatment, watering to the treatment groups of plants was stopped when first tillers became visible in over 50% of the seedlings. When the symptoms of drought stress became apparent, most of the RILs reached the booting stage. In addition to those assessments carried out for the first time points, the number of yellow leaves (which was not apparent among RILs at the first time point) was another criterion assessed. As few plants had 3 or more yellow leaves at this time point, a scale ranging from 0 to 3 was used for this assessment. For example, a score of 0.5 was given to a plant when about half of its first leaf was yellow and a score of 2.0 was given to a plant when both its first and second leaves were yellow.

Following drought severity assessments, plants in both groups of the controls and treatments were harvested by cutting close to the surface of the potting mix, and fresh weight for each of the RILs was measured. The harvested plants were then placed in a dehydrator at 65 °C until a constant dry weight (no change in weight in two consecutive days) was obtained. Dry weight for each of the RILs was then measured.

## 2.4 | Statistical Analysis

Genotyping was performed using genotyping-by-sequencing (GBS), and a high-density genetic map of the mapping population was constructed following the method described by Zhou et al. (2021). Briefly, total genomic DNA was extracted from young leaves of all RILs and the parental lines using the CTAB method and submitted to a commercial sequencing provider for GBS library construction and sequencing. Paired-end reads were merged into single reads using PEAR software (Zhang et al. 2014), and low-quality reads were trimmed and filtered. A population-specific allele-specific reference (psASR) was generated, and alleles were filtered based on a minimum of four reads per sample and a minor allele frequency threshold of 0.25. The resulting sequences were aligned to the Morex reference genome using GYDLE software (Gydle Inc., Quebec, Canada), with alignment criteria of ≥70% homology over ≥95% of the sequence length. Genotype calling was performed using Bayesian statistical models for codominant markers, while dominant markers for unmapped loci were assigned based on a minimum coverage of two reads. Further filtering of SNP markers was applied, retaining those with a minor allele frequency > 0.05 and ≤15% missing data. Linkage groups were constructed using MSTMap via the R/ASMap package (Taylor and Butler 2017) with default parameters, and markers were assigned to groups based on recombination frequency and LOD score thresholds.

QTL analysis was performed using MapQTL 5.0 (Van Ooijen 2004). Interval mapping (IM) was initially used to scan for QTL, and the significance of detected QTL was assessed using permutation tests (1000 permutations per trait) to determine LOD thresholds at a significance level of $p \leq 0.05$. QTL were considered significant if their LOD scores exceeded this threshold. The confidence interval for each QTL was defined using the 1.5-LOD drop method around the peak marker (Visscher et al. 1996). Flanking markers for each QTL were identified as the nearest markers on either side of the peak within the support interval. A linkage map indicating QTL positions was

visualized using MAPCHART 2.32 (Voorrips 2002). The best linear unbiased prediction (BLUP) was calculated for each of the measurements assessed in this study using lme4 package (Bates et al. 2015) in R studio. Traits characterized by ordinal variables were used directly for QTL analysis. For measurements represented by continuous variables, their distributions were assessed first using Q-Q plots and the Shapiro-Wilk test before proceeding with QTL analysis. Traits that did not follow a normal distribution were transformed using the Box–Cox method prior to analysis. Correlation analysis between traits measured in this study and those from previous work (Zheng et al. 2022) was conducted using JMP version 16.1 (SAS In stitute, Cary, North Carolina, United States).

### 2.5 | Identification of Candidate Genes for the Targeted QTL

To predict genes underlying the targeted locus, protein sequences of the two parental genotypes were compared and those with reciprocal best hits were identified using DIAMOND version 2.1.5. The physical boundaries of the targeted locus were defined by aligning the sequences of flanking markers to Morex pseudomolecules (Mascher et al. 2017). Coding and protein sequences for Morex and AWCS276 were sourced from Ensembl Plants database https://ftp.ensemblgenomes.ebi.ac.uk/pub/plants/release-56/and NCGR wild barley database https://www.ncgr.ac.cn/wild_barley (Liu et al. 2020), respectively. Orthologous gene pairs in the targeted region between Morex and AWCS276 were identified with available coding sequences and protein sequences. Only genes with sequence variations between the two parental genotypes were outputted and used for further analyses. The identification of barley orthologs genes in *Arabidopsis* and rice was performed with BioMart on Ensembl Plants https://plants.ensembl.org/index.html. SNP variations of candidate genes and their functional annotations were detected using Snippy version 4.3.6

## 3 | Results

### 3.1 | Phenotypic Data Analysis

Phenotypic evaluation of the RIL mapping population, derived from a cross between the wild barley genotype AWCS276 and the cultivated parent Morex, revealed considerable variation in traits related to FCR severity and drought tolerance. FCR severity scores ranged from 1 to 5, with an average value of 3.5 (Table 1). In addition, leaf wilting under drought stress was assessed at two time points. Drought treatment at tillering stage (W1), wilting scores ranged from 0 to 4, averaging 2.7, whereas drought treatment at booting stage (W2), scores varied from 1.5 to 4.0 with a mean of 2.9 (Table 1). The number of yellow leaves (YL) ranged from 0.2 to 2.9 among the 201 RILs, with a population average of 1.9.

Shoot dry weight under drought stress (SDW_D) varied from 0.47 to 1.34, with an average of 0.92, while under controlled conditions (SDW_C) values ranged from 0.7 to 3.5, with a mean of 1.73 (Table 1). Similarly, shoot fresh weight under drought stress (SFW_D) ranged from 1.48 to 4.93 with a mean value of 2.89, compared to shoot fresh weight under controlled conditions (SFW_C), which varied from 7.61 to 27.17 and averaged approximately 16 (Table 1).

**TABLE 1** | Distributions of measured traits among the RILs of the mapping population.

| Trial | Trait | Min | Max | Mean | SD | CV (%) |
|---|---|---|---|---|---|---|
| Trial 1 | FCR | 1.0 | 5.0 | 3.5 | 1.0 | 27.8 |
| | W1 | 1.0 | 4.0 | 2.7 | 0.8 | 29.5 |
| | W2 | 1.7 | 4.0 | 3.0 | 0.6 | 18.8 |
| | YL | 0.2 | 2.8 | 1.7 | 0.6 | 34.6 |
| | SDW_D | 0.47 | 1.34 | 0.94 | 0.16 | 17.3 |
| | SDW_C | 0.81 | 3.54 | 1.78 | 0.41 | 23.2 |
| | SFW_D | 1.48 | 4.48 | 2.84 | 0.50 | 17.5 |
| | SFW_C | 7.61 | 27.17 | 16.10 | 3.18 | 19.8 |
| Trial 2 | FCR | 1.0 | 5.0 | 3.5 | 1.0 | 27.9 |
| | W1 | 0.0 | 4.0 | 2.6 | 0.8 | 31.3 |
| | W2 | 1.5 | 4.0 | 2.9 | 0.6 | 19.7 |
| | YL | 0.3 | 2.9 | 1.9 | 0.5 | 28.9 |
| | SDW_D | 0.53 | 1.25 | 0.92 | 0.1 | 14.6 |
| | SDW_C | 0.74 | 3.30 | 1.69 | 0.4 | 22.1 |
| | SFW_D | 1.82 | 4.93 | 2.97 | 0.4 | 14.8 |
| | SFW_C | 9.28 | 24.71 | 16.25 | 2.8 | 17.0 |
| Trial 3 | FCR | 1.0 | 5.0 | 3.6 | 1.0 | 26.7 |
| | W1 | 0.0 | 4.0 | 2.7 | 0.8 | 30.2 |
| | W2 | 1.5 | 4.0 | 2.9 | 0.6 | 19.4 |
| | YL | 0.3 | 3.0 | 2.1 | 0.5 | 25.7 |
| | SDW_D | 0.52 | 1.17 | 0.90 | 0.1 | 13.5 |
| | SDW_C | 0.79 | 3.47 | 1.71 | 0.4 | 23.8 |
| | SFW_D | 1.87 | 4.21 | 2.87 | 0.4 | 14.5 |
| | SFW_C | 9.05 | 25.44 | 15.82 | 2.8 | 17.8 |
| BLUP | FCR | 1.1 | 5.0 | 3.5 | 0.9 | 26.0 |
| | W1 | 0.7 | 4.0 | 2.7 | 0.8 | 29.2 |
| | W2 | 1.6 | 4.0 | 3.00 | 0.5 | 18.1 |
| | YL | 0.4 | 2.8 | 1.9 | 0.5 | 15.9 |
| | SDW_D | 0.59 | 1.23 | 0.92 | 0.1 | 13.5 |
| | SDW_C | 0.81 | 3.42 | 1.72 | 0.4 | 22.7 |
| | SFW_D | 1.81 | 4.48 | 2.90 | 0.4 | 14.7 |
| | SFW_C | 9.27 | 22.64 | 15.80 | 2.6 | 16.1 |

[#]FCR represents for Fusarium crown rot severity, W1 for leaf wilting severity at first time point, W2 for leaf wilting severity at second time point, YL for the number of yellow leaves, SDW_D for shoot dry weight under drought stress, SDW_C for shoot dry weight under control (well-watered treatment), SFW_D for shoot fresh weight under drought stress and SFW_C for shoot fresh weight under control treatment.

### 3.2 | QTL Mapping

Composite interval mapping identified several QTL associated with the traits measured. A single QTL on chromosome arm 2HL for FCR severity (Figure 1), designated *Qcrs.caf-2H* where where 'crs' represents 'Fusarium crown rot severity and 'caf' represents 'CSIRO Agriculture & Food'. This locus exhibited LOD values ranging from 4.6 to 4.8 and explained up to 10.9% of the phenotypic variance, with the resistant allele contributed by the wild barley parent AWCS276, as further supported by BLUP analyses (Table 2).

For leaf wilting measured at the first time point (W1), two QTL were detected: one on chromosome arm 2HL (*Qw1.caf-2H*) and a second on 4HL (*Qw1.caf-4H*) (Figure 1). The QTL on 2HL had a LOD value of approximately 3.0 and accounted for up to 6.5% of the variance, while the QTL on 4HL exhibited LOD values between 3.1 and 3.8 and explained up to 8.5% of the variance (Table 2). The tolerant allele at *Qw1.caf-2H* was derived from AWCS276, whereas the allele at *Qw1.caf-4H* came from Morex. In contrast, the leaf wilting assessment at the second time point (W2) revealed a single QTL, *Qw2.caf-2H*, located on chromosome arm 2HL. This QTL had LOD values ranging from 3.2 to 4.3 and explained approximately 10.0% of the phenotypic variance, with the tolerant allele also contributed by AWCS276.

A QTL for yellow leaf number, designated *Qyl.caf-2H*, was identified on chromosome arm 2HL (Figure 1). This locus showed LOD values between 5.2 and 5.7 and explained up to 12.5% of the phenotypic variance, with the drought-tolerant allele contributed by AWCS276 (Table 2).

Analysis of shoot dry weight revealed that under drought stress (SDW_D), the QTL *Qsdw.caf_d-2H* was mapped to chromosome arm 2HL (Figure 1), exhibiting LOD values from 4.1 to 4.7 and accounting for up to 10.4% of the variance (Table 2). Under controlled conditions (SDW_C), two QTL were detected: one on chromosome arm 2HL (*Qsdw.caf_c-2H*) with LOD values between 4.7 and 5.2, explaining up to 10.5% of the variance, and another on chromosome arm 5HS (*Qsdw.caf_c-5H*) with LOD values ranging from 3.7 to 4.0, which explained up to 9.5% of the

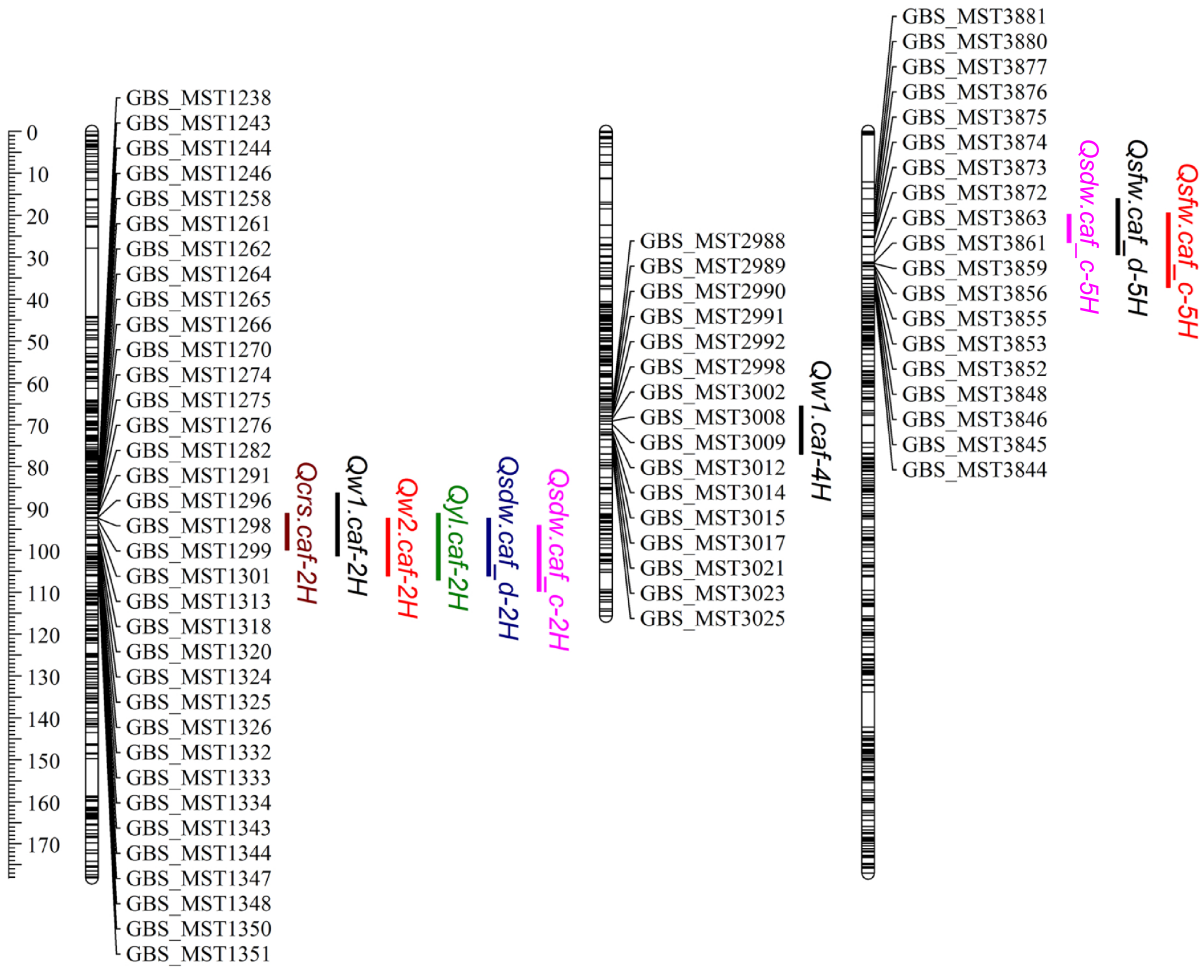



**FIGURE 1** | QTL identified in the mapping population. The locus detected for FCR (Fusarium crown rot severity) was designated as *Qcrs.caf-2H*. The loci detected for W1 (wilt severity at tillering stage) were designated as *Qw1.caf-2H* and *Qw1.caf-4H*. The locus detected for W2 (wilt severity at booting stage) was designated as *Qw2.caf-2H*. The locus detected for YL (the number of yellow leaves) was designated as *Qyl.caf-2H*. The locus detected for SDW_D (shoot dry weight under drought stress) was designated as *Qsdw.caf_d-2H*. The locus detected for SDW_D (shoot dry weight under control) was designated as *Qsdw.caf_c-2H*. The locus detected for SFW_D (shoot fresh weight under drought stress) was designated as *Qsfw.caf_d-5H*. The locus detected for SFW_C (shoot fresh weight under control) was designated as *Qsfw.caf_c-5H*. [Color figure can be viewed at wileyonlinelibrary.com]

**TABLE 2** | QTL for measured traits identified in the RIL population.

| Trial | Trait | QTL | LOD value | Phenotypic variation (%) | Genetic distance (cM) | Left marker | Right marker | Source of resistant or tolerant allele |
|---|---|---|---|---|---|---|---|---|
| Trial 1 | FCR | Qcrs.caf-2H | 4.8 | 10.9 | 91.2–99.9 | GBS_ MST1291 | GBS_MST1332 | AWCS276 |
| | W1 | Qw1.caf-2H | 2.9 | 6.5 | 90.9–91.2 | GBS_ MST1282 | GBS_MST1291 | AWCS276 |
| | | Qw1.caf-4H | 3.8 | 8.5 | 67.8–70.9 | GBS_ MST2992 | GBS_MST3012 | Morex |
| | W2 | Qw2. caf-2H | 4.3 | 9.7 | 94.1–100.8 | GBS_ MST1301 | GBS_MST1333 | AWCS276 |
| | YL | Qyl.caf-2H | 5.5 | 12.1 | 90.9–101.3 | GBS_ MST1282 | GBS_MST1334 | AWCS276 |
| | SDW_D | Qsdw. caf_d-2H | 4.7 | 10.4 | 98.6–106.1 | GBS_ MST1325 | GBS_MST1360 | AWCS276 |
| | SDW_C | Qsdw. caf_c-2H | 4.7 | 10.5 | 98.6–106.1 | GBS_ MST1325 | GBS_MST1360 | AWCS276 |
| | | Qsdw. caf_c-5H | 4.0 | 9.5 | 19.5–27.5 | GBS_ MST3872 | GBS_MST3880 | AWCS276 |
| | SFW_D | Qsfw. caf_d-5H | 3.4 | 8.3 | 25.3–29.4 | GBS_ MST3863 | GBS_MST3873 | AWCS276 |
| | SFW_C | Qsfw. caf_c-5H | 3.5 | 8.4 | 20.1–27.5 | GBS_ MST3872 | GBS_MST3877 | AWCS276 |
| Trial 2 | FCR | Qcrs.caf-2H | 4.6 | 10.1 | 92.4–100.3 | GBS_ MST1296 | GBS_MST1332 | AWCS276 |
| | W1 | Qw1.caf-2H | 2.7 | 6.2 | 90.9–91.2 | GBS_ MST1282 | GBS_MST1291 | AWCS276 |
| | | Qw1.caf-4H | 3.1 | 7.1 | 69.0–71.1 | GBS_ MST3008 | GBS_MST3012 | Morex |
| | W2 | Qw2. caf-2H | 3.6 | 8.2 | 95.2–98.9 | GBS_ MST1313 | GBS_MST1326 | AWCS276 |
| | YL | Qyl.caf-2H | 5.7 | 12.5 | 92.9–101.3 | GBS_ MST1299 | GBS_MST1334 | AWCS276 |
| | SDW_D | Qsdw. caf_d-2H | 4.2 | 9.3 | 98.6–104.8 | GBS_ MST1325 | GBS_MST1358 | AWCS276 |
| | SDW_C | Qsdw. caf_c-2H | 4.7 | 10.6 | 97.1–107.8 | GBS_ MST1324 | GBS_MST1363 | AWCS276 |
| | | Qsdw. caf_c-5H | 3.7 | 8.8 | 16.1–25.3 | GBS_ MST3873 | GBS_MST3881 | AWCS276 |
| | SFW_D | Qsfw. caf_d-5H | 3.4 | 8.4 | 25.0–29.4 | GBS_ MST3863 | GBS_MST3874 | AWCS276 |
| | SFW_C | Qsfw. caf_c-1H | 3.1 | 6.9 | 36.1–37.2 | GBS_MST71 | GBS_MST93 | AWCS276 |
| | | Qsfw. caf_c-2H | 3.6 | 8.1 | 102.2–105.8 | GBS_ MST1347 | GBS_MST1359 | AWCS276 |
| | | Qsfw. caf_c-5H | 3.4 | 8.0 | 20.1–25.0 | GBS_ MST3874 | GBS_MST3877 | AWCS276 |

(Continues)

**TABLE 2** | (Continued)

| Trial | Trait | QTL | LOD value | Phenotypic variation (%) | Genetic distance (cM) | Left marker | Right marker | Source of resistant or tolerant allele |
|---|---|---|---|---|---|---|---|---|
| Trial 3 | FCR | Qcrs.caf-2H | 4.7 | 10.2 | 92.4–99.9 | GBS_MST1298 | GBS_MST1332 | AWCS276 |
| | W1 | Qw1.caf-2H | 2.7 | 6.1 | 91.2–98.6 | GBS_MST1291 | GBS_MST1325 | AWCS276 |
| | | Qw1.caf-4H | 3.4 | 7.7 | 69.0–72.5 | GBS_MST3008 | GBS_MST3017 | Morex |
| | W2 | Qw2.caf-2H | 3.2 | 10.3 | 96.3–97.1 | GBS_MST1318 | GBS_MST1324 | AWCS276 |
| | YL | Qyl.caf-2H | 5.2 | 11.5 | 94.1–101.3 | GBS_MST1301 | GBS_MST1334 | AWCS276 |
| | SDW_D | Qsdw.caf_d-2H | 4.1 | 9.1 | 96.8–104.8 | GBS_MST1320 | GBS_MST1358 | AWCS276 |
| | SDW_C | Qsdw.caf_c-2H | 5.2 | 10.5 | 97.1–107.8 | GBS_MST1324 | GBS_MST1363 | AWCS276 |
| | | Qsdw.caf_c-5H | 4.0 | 9.5 | 16.1–27.5 | GBS_MST3872 | GBS_MST3881 | AWCS276 |
| | SFW_D | Qsfw.caf_d-5H | 3.9 | 9.6 | 20.1–29.4 | GBS_MST3863 | GBS_MST3877 | AWCS276 |
| | SFW_C | Qsfw.caf_c-1H | 3.0 | 6.9 | 36.1–37.2 | GBS_MST71 | GBS_MST93 | AWCS276 |
| | | Qsfw.caf_c-2H | 3.7 | 8.4 | 103.6–107.7 | GBS_MST1351 | GBS_MST1363 | AWCS276 |
| | | Qsfw.caf_c-5H | 3.8 | 8.9 | 16.1–27.5 | GBS_MST3872 | GBS_MST3881 | AWCS276 |
| BLUP | FCR | Qcrs.caf-2H | 4.8 | 10.6 | 92.1–100.3 | GBS_MST1296 | GBS_MST1332 | AWCS276 |
| | W1 | Qw1.caf-2H | 2.9 | 6.6 | 90.9–92.1 | GBS_MST1282 | GBS_MST1296 | AWCS276 |
| | | Qw1.caf-4H | 3.6 | 8.0 | 67.8–71.1 | GBS_MST2992 | GBS_MST3012 | Morex |
| | W2 | Qw2.caf-2H | 4.0 | 8.9 | 94.1–100.8 | GBS_MST1301 | GBS_MST1333 | AWCS276 |
| | YL | Qyl.caf-2H | 5.7 | 12.3 | 92.9–106.1 | GBS_MST1299 | GBS_MST1334 | AWCS276 |
| | SDW_D | Qsdw.caf_d-2H | 4.7 | 10.4 | 98.6–104.8 | GBS_MST1325 | GBS_MST1359 | AWCS276 |
| | SDW_C | Qsdw.caf_c-2H | 4.9 | 10.9 | 97.1–107.8 | GBS_MST1324 | GBS_MST1363 | AWCS276 |
| | | Qsdw.caf_c-5H | 4.0 | 9.3 | 16.1–25.0 | GBS_MST3874 | GBS_MST3881 | AWCS276 |

(Continues)

**TABLE 2** | (Continued)

| Trial | Trait | QTL | LOD value | Phenotypic variation (%) | Genetic distance (cM) | Left marker | Right marker | Source of resistant or tolerant allele |
|---|---|---|---|---|---|---|---|---|
| | SFW_D_BLUP | Qsdw.caf_c-2H | 3.6 | 8.0 | 21.8–25.0 | GBS_MST3874 | GBS_MST3876 | AWCS276 |
| | SFW_C_BLUP | Qsfw.caf_c-1H | 2.9 | 6.7 | 48.4–49.8 | GBS_MS355 | GBS_MST359 | AWCS276 |
| | | Qsfw.caf_c-2H | 3.2 | 7.5 | 104.2–105.8 | GBS_MST1355 | GBS_MST1359 | AWCS276 |
| | | Qsfw.caf_c-5H | 3.3 | 7.8 | 20.1–25.3 | GBS_MST3873 | GBS_MST3877 | AWCS276 |

[#]Genetic distance cM for genetic distance from the tip of the short arm to the peak of the QTL, FCR represents for Fusarium crown rot severity, W1 for leaf wilting severity at first time point, W2 for leaf wilting severity at second time point, YL for the number of yellow leaves, SDW_D for shoot dry weight under drought stress, SDW_C for shoot dry weight under control (well-watered treatment), SFW_D for shoot fresh weight under drought stress and SFW_C for shoot fresh weight under control treatment.

variance (Figure 1; Table 2). In both cases, the tolerant alleles were contributed by AWCS276.

For shoot fresh weight, a QTL under drought stress (SFW_D), designated Qsfw.caf_d-5H, was located on chromosome arm 5HL (Figure 1) and exhibited LOD values as high as 3.9, explaining up to 9.6% of the phenotypic variance (Table 2). Under controlled conditions (SFW_C), a corresponding QTL (*Qsfw.caf_c-5H*) was detected on the same chromosome arm with LOD values ranging from 3.5 to 6.0, accounting for up to 10.5% of the variance (Figure 1; Table 2). The drought-tolerant allele for both SFW-related QTL was derived from AWCS276.

### 3.3 | Correlations Among FCR Resistance, Drought Tolerance and Agronomic Traits

FCR severity displayed significant and positive correlation ($p < 0.05$) with several of the measurements for drought tolerance and other characteristics including W1, S3LL S3LWR, FLWR and TKW (Figure 2). FCR was negatively correlated with KNPS and SRT. Traits used in measuring drought tolerance showed significant correlations with several characteristics assessed in this study. For example, YL was positively correlated with TKW, FTN, S3LL, S3LA, S3LWR, and it was negatively correlated with KNPS and SRT (Figure 2).

### 3.4 | Candidate Genes Underlying the Major Locus on Chromosome arm 2HL

As *Qyl.caf-2H* exhibited the most significant effects on both drought tolerance and FCR resistance, an attempt of identifying candidate genes for this QTL was made. The physical positions of flanking markers for this locus covered a range from 634.8 to 658.6 Mbp on chromosome arm 2HL. Based on the genome assembly of Morex, a total of 597 genes were detected in this region. Among the 249 genes with non-synonymous SNPs, candidate genes were prioritized based on their known associations with drought tolerance or disease resistance in Arabidopsis and rice orthologs (Table S1). Based on reported functions of their orthologs in rice and A*rabidopsis*, at least 10 of these genes are associated with disease defence, six with drought tolerance, and one associated with both biotic and abiotic stresses (Table 3).

## 4 | Discussion

In this study, we identified a novel QTL on chromosome arm 2HL, conferring both FCR resistance and drought tolerance underscores the potential for genetic locus that mediate dual stress responses in barley (Liu and Ogbonnaya 2015; Gao et al. 2019). The co-localization of these traits suggests possible pleiotropy or tight linkage, which aligns with emerging research on the genetic interplay between biotic and abiotic stress tolerance in cereals (Satish et al. 2009; Suanum et al. 2016). The contribution of beneficial alleles from the wild barley parent further reinforces the value of wild germplasm in breeding programs aimed at enhancing stress resilience (Araus et al. 2008; Capasso et al. 2021). Notably, the distinct expression patterns of drought tolerance QTL on 4HL and 5HS indicate that multiple physiological pathways regulate drought response, emphasizing the complexity of this trait. The identification of candidate genes within the 2HL region provides a foundation for future functional studies to dissect the underlying molecular mechanisms, offering promising avenues for the development of barley varieties with enhanced resilience to both disease and water-limited conditions.

Pleiotropic effects are a common phenomenon (Stearns 2010; Wagner and Zhang 2011; Chen et al. 2016), which could interfere with the identification of QTL conferring a trait of interest. One example is the influence of plant height on FCR resistance (Li et al. 2008). A large effect QTL conferring FCR resistance on chromosome arm 3HL was detected. Similar to work on other loci conferring FCR resistance (Ma et al. 2012; Habib et al. 2016; Gao et al. 2019; Su et al. 2024), we also tried to generate NILs for the 3HL locus but without success. As the QTL co-located with the widely used gene conferring plant height (Li et al. 2009), we conducted an analysis on NILs for various genes controlling

plant height and realized that the genes controlling plant height all significantly affect FCR resistance in wheat (Liu et al. 2010). Similar results showing that plant height affects FCR resistance were also demonstrated in barley (Chen et al. 2014). However, the locus conferring FCR resistance and drought tolerance on 2HL does not seem to be affected by any obvious traits of agronomic importance as RILs with very similar morphology (including plant height, flowering time, developmental stage, tiller and leaf numbers, and spike row-type) but with contrasting response to drought stress were found in the mapping population (Figure 3).

Interestingly, the 2HL locus was detected by measuring dry weight in both the control (drought stress not imposed) and the drought stressed treatments. Similar result was also reported for loci conferring salt tolerance (Forster et al. 1997). One possible explanation for such phenomena is that gene action was induced by the stress in concern in some cases, or that normal activity was sufficiently reduced to reveal the action of other loci (Forster et al. 2000). In any case, dry weight from non-stressed experiments provides a convenient method to detect this locus.

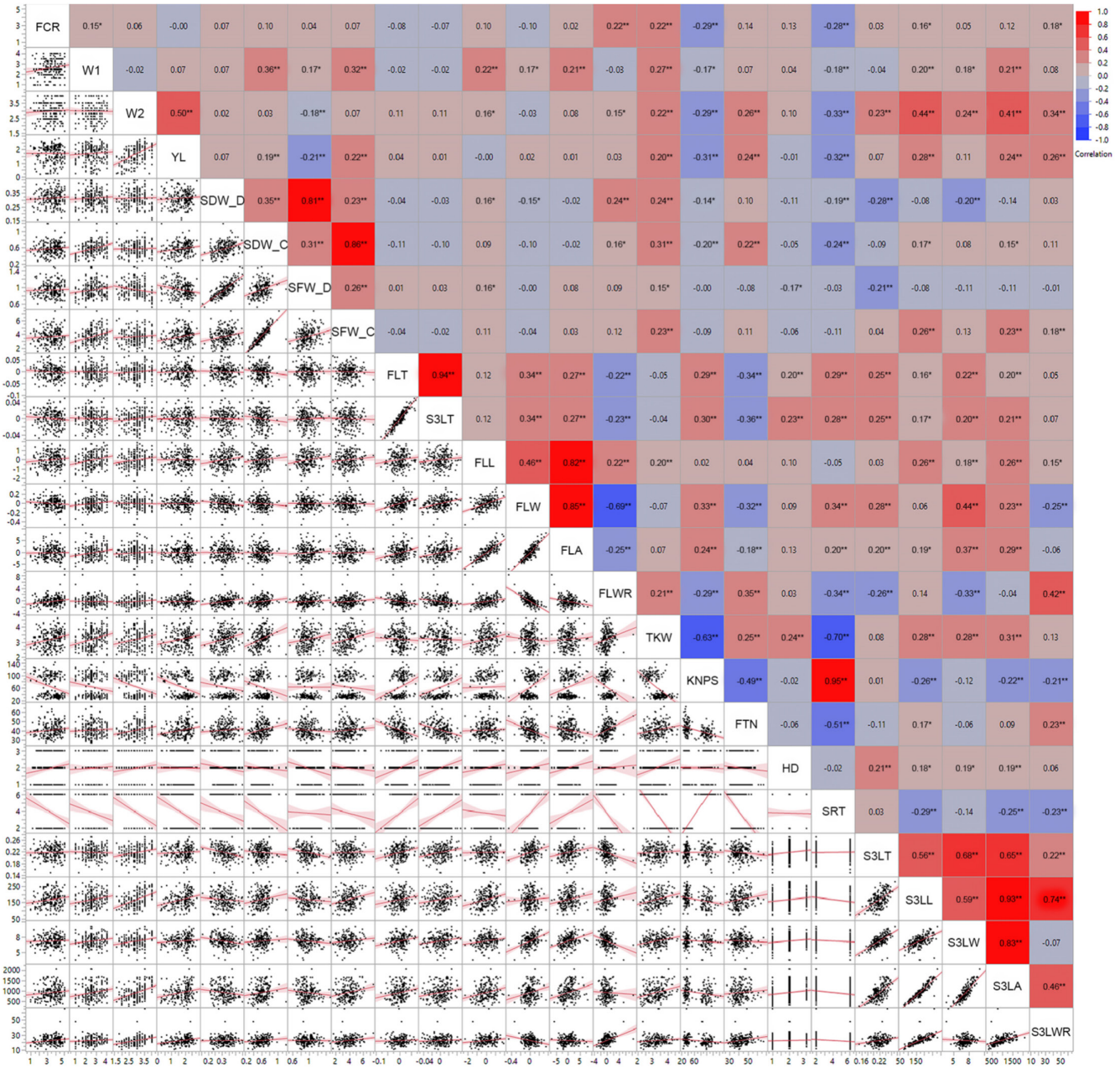



**FIGURE 2** | Correlation matrix among different measurements and traits in the mapping population. FCR represents for Fusarium crown rot severity, W1 for wilt severity at first time point, W2 for wilt severity at second time point, YL for the number of yellow leaves, SDW_D for shoot dry weight under drought stress, SDW_C for shoot dry weight under control (well-watered treatment), SFW_D for shoot fresh weight under drought stress, SFW_C for shoot fresh weight under control treatment, FLT for flag leaf thickness, S3LT for 3rd leaf thickness from seedling, FLL for flag leaf length, FLW for flag leaf width, FLA for flag leaf area, FLWR for flag leaf length and width ratio, TKW for thousand kernel weight, KNPS for kernel number per spike, FTN for fertile tiller number, HD for heading date, SRT for spike row type, S3LT for 3rd leaf thickness from seedling, S3LL for 3rd leaf length from seedling, S3LW for 3rd leaf width from seedling, S3LA for 3rd leaf area, and S3LWR for 3rd leaf length and width ratio from seedling. [Color figure can be viewed at wileyonlinelibrary.com]

**TABLE 3** | Candidate genes associated with disease resistance or drought tolerance in the targeted region.

| Candidate gene | Species | Homologue | Functions | Reference |
|---|---|---|---|---|
| *HORVU.MOREX.r3.2HG0205130* | *Arabidopsis* | *NHO1* | Required for resistance against non-host and avirulent Pseudomonas strains and against the necrotrophic fungal pathogen *Botrytis cinerea* A significant component of the immune system in Arabidopsis | Kámán-Tóth et al. (2019) |
| *HORVU.MOREX.r3.2HG0205210* | *Arabidopsis* | *T1N6_22* | An important role in resistance to necrotrophic fungi Enhanced salt tolerance | Xing et al. (2012) |
| *HORVU.MOREX.r3.2HG0205380* | *Arabidopsis* | *AT1G15290* | Drought stress response gene | Lasky et al. (2014) |
| *HORVU.MOREX.r3.2HG0206960* | *Arabidopsis* | *TGA7* | Regulation of disease resistance | Kesarwani et al. (2007) |
| *HORVU.MOREX.r3.2HG0207170* | *Arabidopsis* | *GAUT10* | Regulation of root meristem and in response to auxin Essential for stomatal behaviour and drought performance Required for root cell differentiation and elongation | Pu et al. (2019) Guo et al. (2021) Dash et al. (2023) |
| *HORVU.MOREX.r3.2HG0209080* | *Arabidopsis* | *MVK* | A key enzyme in the mevalonate pathway and plays a critical role in plant disease defence | Cho et al. (2022) |
| *HORVU.MOREX.r3.2HG0209180* | *Arabidopsis* | *ALN* | Regulation of concentration of allantoin and impacted on drought and osmotic shock | Watanabe et al. (2014) |
| *HORVU.MOREX.r3.2HG0213220* | *Arabidopsis* | *ACBP2* | Promoting ABA signalling in germination, seedling development and the drought response | Du et al. (2013) |
| *HORVU.MOREX.r3.2HG0213240* | *Arabidopsis* | *ARP6* | Regulating inflorescence and flower development Response to salinity stress through regulating PP2C genes Enhanced resistance to Pst DC3000 | Deal et al. (2005) Do et al. (2023) Jakada et al. (2023) |
| ***HORVU.MOREX.r3.2HG0213610*** | *Arabidopsis* | *PUB22* | Negative regulation of plant immunity Respond to drought stress | Yi et al. (2024) |
| *HORVU.MOREX.r3.2HG0214130* | *Arabidopsis* *Rice* | *AAE3* | Involved in seed development and fungal pathogens defence Negative regulator in rice blast resistance | Foster et al. (2012) Liu et al. (2017) |

(Continues)

**TABLE 3** | (Continued)

| Candidate gene | Species | Homologue | Functions | Reference |
|---|---|---|---|---|
| ***HORVU.MOREX.r3.2HG0214490*** | *Arabidopsis*<br>*Rice* | *KEA2*<br>*OsMYB6* | Mediation of seedling primary root growth<br>Involved in drought response<br>Regulation of drought and salt response | Sánchez-McSweeney et al. (2021)<br>Tang et al. (2019) |
| *HORVU.MOREX.r3.2HG0206550* | *Rice* | *OsSK3* | Contributed to the defence response and to floral organ development | Kasai et al. (2005) |
| *HORVU.MOREX.r3.2HG0207660* | *Rice* | *OsBIDK1* | Regulation of disease defence | Huang et al. (2017) |
| *HORVU.MOREX.r3.2HG0211940* | *Rice* | *OsCP1* | Modulating blast resistance | Li et al. (2022) |
| *HORVU.MOREX.r3.2HG0214090* | *Rice* | *OsFTIP6* | Regulation of drought response | Yang et al. (2022) |
| *HORVU.MOREX.r3.2HG0214130* | *Rice* | *OsAAE3* | A negative regulator in rice blast resistance, floret development and lignin biosynthesis | Liu et al. (2017) |



**FIGURE 3** | Two recombinant inbred lines (three plant each) with similar morphology (in plant height, flowering time, developmental stage, tiller and leaf numbers, and spike row-type) but with contrasting number of yellow leaves under drought stress. [Color figure can be viewed at wileyonlinelibrary.com]

Using yellow leaves post drought stress induction as an indicator, genes for leaf senescence were detected on five of the seven barley chromosomes. One of them was located on chromosome 2HL, and it was positioned at 58.1 cM based on POPSEQ map (IBSC 2012). The length of the DNA sequence given for the gene (GenBank ID AK367894.1) was 2375 bp and the corresponding contig in the reference genome of Morex was hv-36,476 (Wehner et al. 2016). However, no hit for this sequence could be found in the current reference genome of Morex with an acceptable e-value threshold. Two of the possible reasons which might explain the discrepancy include (a) the sequence differences between the genotype used in the previous study and the reference genotype of Morex as it is known that dispensable genome accounts for a large portion of the genes in barley (Ma et al. 2019; Gao, Wu, Stiller, et al. 2020), and (b) the poor quality of sequences available to the authors in those days.

Both parental genotypes of the mapping population used in this study have high-quality genome assemblies, facilitating the identification of candidate genes in targeted chromosomal regions. Based on functional analysis of their orthologs in rice and *Arabidopsis*, at least 18 of the genes located in the interval of the targeted QTL were associated with resistance or tolerance to disease infection and drought stress (Table 3). One of them, *HORVU.MOREX.r3.2HG0213610* is orthologous to *PUB22* in *Arabidopsis*, which would form our primary target. In addition to responding to drought stress (Cho et al. 2008), *PUB22* also negatively regulates plant immunity (Yi et al. 2024). Another one is *HORVU.MOREX.r3.2HG0214490*. The ortholog of the gene in rice is *OsMYB6* which functions as a stress-responsive transcription factor and is known to enhance tolerance to both drought and salinity stress (Tang et al. 2019). Its ortholog in *Arabidopsis* is *KEA2* which involves in drought response (Sánchez-McSweeney et al. 2021).

There is little doubt that orthologous and gene expression analyses help a more focused effort to further characterize the candidate genes. However, results from such analyses cannot be used to rule out any of those genes with non-synonymous variations between the two parental genotypes in the targeted interval. Developing and analysing NILs (Ma et al. 2012; Chen et al. 2014; Habib et al. 2018), as well as NIL-derived populations, have been used effectively in refining a targeted locus thus reducing the numbers of candidate genes (Zheng et al. 2015; Jiang et al. 2019; Gao, Zheng, Hu, et al. 2020; Gao et al. 2023). Further efforts in identifying gene(s) underlying the targeted locus require the exploitation of more reliable methods in determining gene functions such as NIL development, gene editing and transformation.

### Author Contributions

C.L., M.Z., S.S., Z.Z. designed the study reported here. Z.S., S.G. gathered and analysed the data. Z.S., S.G., H.H., Z.Z., S.S., M.Z. and C.L. contributed to preparing the manuscript. All authors read and approved the submitted manuscript.


### Acknowledgements

Zhouyang Su is grateful to the University of Tasmania (UTAS) and Commonwealth Scientific and Industrial Research Organisation (CSIRO) for support during the tenure of her PhD studentship.


### Ethics Statement

The authors have nothing to report.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### References


Akinsanmi, O. A., V. Mitter, S. Simpfendorfer, D. Backhouse, and S. Chakraborty. 2004. "Identity and Pathogenicity of *Fusarium* Spp. Isolated From Wheat Fields in Queensland and Northern New South Wales." *Australian Journal of Agricultural Research* 55: 97–107.

Araus, J. L., G. A. Slafer, C. Royo, and M. D. Serret. 2008. "Breeding for Yield Potential and Stress Adaptation in Cereals." *Critical Reviews in Plant Sciences* 27: 377–412.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software* 67: 1–48.

Capasso, G., G. Santini, M. Petraretti, S. Esposito, and S. Landi. 2021. "Wild and Traditional Barley Genomic Resources as a Tool for Abiotic Stress Tolerance and Biotic Relations." *Agriculture* 11: 1102.

Chakraborty, S., C. Liu, V. Mitter, et al. 2006. "Pathogen Population Structure and Epidemiology Are Keys to Wheat Crown Rot and Fusarium Head Blight Management." *Australasian Plant Pathology* 35: 643–655.

Chen, G., Y. Liu, J. Ma, et al. 2013. "A Novel and Major Quantitative Trait Locus for Fusarium Crown Rot Resistance in a Genotype of Wild Barley (*Hordeum spontaneum* L.)." *PLoS ONE* 8: e58040.

Chen, G., Y. Liu, Y. Wei, et al. 2013. "Major QTL for Fusarium Crown Rot Resistance in a Barley Landrace." *Theoretical and Applied Genetics* 126: 2511–2520.

Chen, G., W. Yan, Y. Liu, et al. 2014. "The Non-Gibberellic Acid-Responsive Semi-Dwarfing Gene Uzu Affects Fusarium Crown Rot Resistance in Barley." *BMC Plant Biology* 14: 1–8.

Chen, G., H. Li, Y. Wei, Y.-L. Zheng, M. Zhou, and C. Liu. 2016. "Pleiotropic Effects of the Semi-Dwarfing Gene Uzu in Barley." *Euphytica* 209: 749–755.

Chen, X., C. Zhao, P. Yun, et al. 2023. "Climate-Resilient Crops: Lessons from Xerophytes." *Plant Journal* 117: 1815–1835.

Cho, S.-H., K. Tóth, D. Kim, et al. 2022. "Activation of the Plant Mevalonate Pathway by Extracellular ATP." *Nature Communications* 13: 450.

Cho, S. K., M. Y. Ryu, C. Song, J. M. Kwak, and W. T. Kim. 2008. "Arabidopsis PUB22 and PUB23 Are Homologous U-Box E3 Ubiquitin Ligases That Play Combinatory Roles in Response to Drought Stress." *The Plant Cell* 20: 1899–1914.

Dash, L, S. Swaminathan, J. Simura, et al. 2023. *GAUT10* Is Required for *Arabidopsis* Root Cell Differentiation and Elongation. bioRxiv:2023.2002.2007.527497

Deal, R. B., M. K. Kandasamy, E. C. McKinney, and R. B. Meagher. 2005. "The Nuclear Actin-Related Protein ARP6 Is a Pleiotropic Developmental Regulator Required for the Maintenance of FLOWERING LOCUS C Expression and Repression of Flowering in *Arabidopsis*." *Plant Cell* 17, no. 10: 2633–2646. https://doi.org/10.1105/tpc.105.035196.

Do, B. H., N. T. Hiep, T. D. Lao, and N. H. Nguyen. 2023. "Loss-Of-Function Mutation of ACTIN-RELATED PROTEIN 6 (*ARP6*) Impairs Root Growth in Response to Salinity Stress." *Molecular Biotechnology* 65: 1–7.

Du, Z. Y., M. X. Chen, Q. F. Chen, S. Xiao, and M. L. Chye. 2013. "Overexpression of Arabidopsis Acyl-CoA-Binding Protein ACBP2 Enhances Drought Tolerance." *Plant, Cell & Environment* 36: 300–314.

Elakhdar, A., S. Solanki, T. Kubo, et al. 2022. "Barley with Improved Drought Tolerance: Challenges and Perspectives." *Environmental and Experimental Botany* 201: 104965.

Ellis, R. P., B. P. Forster, D. Robinson, et al. 2000. "Wild Barley: A Source of Genes for Crop Improvement in the 21st Century?" *Journal of Experimental Botany* 51: 9–17.

Forster, B., R. Ellis, W. Thomas, et al. 2000. "The Development and Application of Molecular Markers for Abiotic Stress Tolerance in Barley." *Journal of Experimental Botany* 51: 19–27.

Forster, B., J. Russell, R. Ellis, et al. 1997. "Locating Genotypes and Genes for Abiotic Stress Tolerance in Barley: A Strategy Using Maps, Markers and the Wild Species." *New Phytologist* 137: 141–147.

Foster, J., H. U. Kim, P. A. Nakata, and J. Browse. 2012. "A Previously Unknown Oxalyl-CoA Synthetase Is Important for Oxalate Catabolism in *Arabidopsis*." *Plant Cell* 24: 1217–1229.

Gao, S., Y. Jiang, H. Zhou, et al. 2023. "Fine Mapping of a Fusarium Crown Rot Resistant Locus on Chromosome arm 6HL in Barley by Exploiting Near Isogenic Lines, Transcriptome Profiling, and a Large Near Isogenic Line-Derived Population." *Theoretical and Applied Genetics* 136: 137.

Gao, S., J. Wu, J. Stiller, et al. 2020. "Identifying Barley pan-Genome Sequence Anchors Using Genetic Mapping and Machine Learning." *Theoretical and Applied Genetics* 133: 2535–2544.

Gao, S., Z. Zheng, H. Hu, et al. 2020. "Delineating a Locus Conferring Fusarium Crown Rot Resistance on Chromosome Arm 1HL in Barley by Developing and Analysing a Large Population Derived From Near Isogenic Lines." *Crop Journal* 8: 1002–1010.

Gao, S., Z. Zheng, H. Hu, et al. 2019. "A Novel QTL Conferring Fusarium Crown rot Resistance Located on Chromosome Arm 6HL in Barley." *Frontiers in Plant Science* 10: 1206.

Guo, H., C. Xiao, Q. Liu, et al. 2021. "Two Galacturonosyltransferases Function in Plant Growth, Stomatal Development, and Dynamics." *Plant Physiology* 187: 2820–2836.

Habib, A., J. J. Powell, J. Stiller, et al. 2018. "A Multiple Near Isogenic Line (Multi-NIL) RNA-Seq Approach to Identify Candidate Genes Underpinning QTL." *Theoretical and Applied Genetics* 131: 613–624.

Habib, A., S. Shabala, L. Shabala, M. Zhou, and C. Liu. 2016. "Near-Isogenic Lines Developed for a Major QTL on Chromosome Arm 4HL Conferring Fusarium Crown Rot Resistance in Barley." *Euphytica* 209: 555–563.

Hu, H., I. Ahmed, S. Choudhury, et al. 2019. "Wild Barley Shows a Wider Diversity in Genes Regulating Heading Date Compared with Cultivated Barley." *Euphytica* 215: 1–14.

Huang, H., T. Nguyen Thi Thu, X. He, et al. 2017. "Increase of Fungal Pathogenicity and Role of Plant Glutamine in Nitrogen-Induced Susceptibility (NIS) to Rice Blast." *Frontiers in Plant Science* 8: 265. https://doi.org/10.3389/fpls.2017.00265.

IBSC. 2012. "A Physical, Genetic and Functional Sequence Assembly of the Barley Genome." *Nature* 491, no. 7426: 711–716. https://doi.org/10.1038/nature11543.

Jakada, B. H., B. Fakher, L.-A. Yao, X. Wang, M. Aslam, and Y. Qin. 2023. "Ectopic Expression of Pineapple Actin-Related Protein 6 (*AcARP6*) Regulates Flowering and Stress Responses in *Arabidopsis*." *Journal of Plant Growth Regulation* 42: 6461–6473.

Jiang, Y., A. Habib, Z. Zheng, et al. 2019. "Development of Tightly Linked Markers and Identification of Candidate Genes for Fusarium Crown Rot Resistance in Barley by Exploiting a Near-Isogenic Line-Derived Population." *Theoretical and Applied Genetics* 132: 217–225.

Kámán-Tóth, E., T. Dankó, G. Gullner, Z. Bozsó, L. Palkovics, and M. Pogány. 2019. "Contribution of Cell Wall Peroxidase-and NADPH Oxidase-Derived Reactive Oxygen Species to *Alternaria brassicicola*-Induced Oxidative Burst in *Arabidopsis*." *Molecular Plant Pathology* 20: 485–499.

Kasai, K., T. Kanno, M. Akita, Y. Ikejiri-Kanno, K. Wakasa, and Y. Tozawa. 2005. "Identification of Three Shikimate Kinase Genes in Rice: Characterization of Their Differential Expression During Panicle Development and of the Enzymatic Activities of the Encoded Proteins." *Planta* 222: 438–447.

Kesarwani, M., J. Yoo, and X. Dong. 2007. "Genetic Interactions of TGA Transcription Factors in the Regulation of Pathogenesis-Related Genes and Disease Resistance in *Arabidopsis*." *Plant Physiology* 144: 336–346.

Lasky, J. R., D. L. Des Marais, D. B. Lowry, et al. 2014. "Natural Variation in Abiotic Stress Responsive Gene Expression and Local Adaptation to Climate in *Arabidopsis Thaliana*." *Molecular Biology and Evolution* 31, no. 9: 2283–2296. https://doi.org/10.1093/molbev/msu170.

Li, X., C. Liu, S. Chakraborty, J. M. Manners, and K. Kazan. 2008. "A Simple Method for the Assessment of Crown Rot Disease Severity in Wheat Seedlings Inoculated With *Fusarium pseudograminearum*." *Journal of Phytopathology* 156: 751–754.

Li, Y., P. Liu, L. Mei, et al. 2022. "Knockout of a Papain-Like Cysteine Protease Gene OCP Enhances Blast Resistance in Rice." *Frontiers in Plant Science* 13: 1065253.

Li, H., M. Zhou, and C. Liu. 2009. "A Major QTL Conferring Crown Rot Resistance in Barley and Its Association with Plant Height." *Theoretical and Applied Genetics* 118: 903–910.

Liu, Y., X. Yang, J. Ma, et al. 2010. "Plant Height Affects Fusarium Crown Rot Severity in Wheat." *Phytopathology* 100: 1276–1281.

Liu, C., and F. C. Ogbonnaya. 2015. "Resistance to *Fusarium* Crown Rot in Wheat and Barley: A Review." *Plant Breeding* 134: 365–372.

Liu, H., Z. Guo, F. Gu, et al. 2017. "4-Coumarate-CoA Ligase-Like Gene *OsAAE3* Negatively Mediates the Rice Blast Resistance, Floret Development and Lignin Biosynthesis." *Frontiers in Plant Science* 7, no. 2041.

Liu, M., Y. Li, Y. Ma, et al. 2020. "The Draft Genome of a Wild Barley Genotype Reveals Its Enrichment in Genes Related to Biotic and Abiotic Stresses Compared to Cultivated Barley." *Plant Biotechnology Journal* 18: 443–456.

Liu, X., and C. Liu. 2016. "Effects of Drought-Stress on Fusarium Crown rot Development in Barley." *PLoS ONE* 11: e0167304.

Ma, Y., M. Liu, J. Stiller, and C. Liu. 2019. "A Pan-Transcriptome Analysis Shows That Disease Resistance Genes Have Undergone More Selection Pressure During Barley Domestication." *BMC Genomics* 20: 1–11.

Ma, J., G. Yan, and C. Liu. 2012. "Development of Near-Isogenic Lines for a Major QTL on 3BL Conferring Fusarium Crown Rot Resistance in Hexaploid Wheat." *Euphytica* 183: 147–152.

Mascher, M., H. Gundlach, A. Himmelbach, et al. 2017. "A Chromosome Conformation Capture Ordered Sequence of the Barley Genome." *Nature* 544: 427–433.

Mitter, V., M. C. Zhang, C. J. Liu, R. Ghosh, M. Ghosh, and S. Chakraborty. 2006. "A High-Throughput Glasshouse Bioassay to Detect Crown Rot Resistance in Wheat Germplasm." *Plant Pathol* 55, no. 3: 433–41.

Murray, G. M., and J. P. Brennan. 2009. "Estimating Disease Losses to the Australian Wheat Industry." *Australasian Plant Pathology* 38: 558–570.

Niu, Y., T. Chen, Z. Zheng, et al. 2022. "A New Major QTL for Flag Leaf Thickness in Barley (*Hordeum vulgare* L.)." *BMC Plant Biology* 22: 305.

Pu, Y., J. W. Walley, Z. Shen, et al. 2019. "Quantitative Early Auxin Root Proteomics Identifies *GAUT10*, a Galacturonosyltransferase, as a Novel Regulator of Root Meristem Maintenance S." *Molecular & Cellular Proteomics* 18: 1157–1170.

Razzaq, A., S. H. Wani, F. Saleem, M. Yu, M. Zhou, and S. Shabala. 2021. "Rewilding Crops for Climate Resilience: Economic Analysis and de Novo Domestication Strategies." *Journal of Experimental Botany* 72: 6123–6139.

Sallam, A., A. M. Alqudah, M. F. Dawood, P. S. Baenziger, and A. Börner. 2019. "Drought Stress Tolerance in Wheat and Barley: Advances in Physiology, Breeding and Genetics Research." *International Journal of Molecular Sciences* 20: 3137.

Sánchez-McSweeney, A., S. González-Gordo, M. N. Aranda-Sicilia, et al. 2021. "Loss of Function of the Chloroplast Membrane $K^+/H^+$ Antiporters *AtKEA1* and *AtKEA2* Alters the ROS and NO Metabolism but Promotes Drought Stress Resilience." *Plant Physiology and Biochemistry* 160: 106–119.

Satish, K., G. Srinivas, R. Madhusudhana, et al. 2009. "Identification of Quantitative Trait Loci for Resistance to Shoot Fly in Sorghum [*Sorghum bicolor* (L.) Moench]." *Theoretical and Applied Genetics* 119: 1425–1439.

Stearns, F. W. 2010. "One Hundred Years of Pleiotropy: A Retrospective." *Genetics* 186: 767–773.

Su, Z., S. Gao, Z. Zheng, et al. 2024. "Transcriptomic Insights Into Shared Responses to Fusarium Crown Rot Infection and Drought Stresses in Bread Wheat (*Triticum Aestivum* L.)." *Theoretical and Applied Genetics* 137, no. 2: 34. https://doi.org/10.1007/s00122-023-04537-1.

Su, Z., J. Powell, S. Gao, M. Zhou, and C. Liu. 2021. "Comparing Transcriptional Responses to Fusarium Crown Rot in Wheat and Barley Identified an Important Relationship Between Disease Resistance and Drought Tolerance." *BMC Plant Biology* 21: 1–11.

Su, Z., Z. Zheng, M. Zhou, S. Shabala, and C. Liu. 2022. "Tissue-Specific Responses of Cereals to Two Fusarium Diseases and Effects of Plant Height and Drought Stress on Their Susceptibility." *Agronomy* 12: 1108.

Suanum, W., P. Somta, A. Kongjaimun, et al. 2016. "Co-Localization of QTLs for Pod Fiber Content and Pod Shattering in F2 and Backcross Populations Between Yardlong Bean and Wild Cowpea." *Molecular Breeding* 36: 1–11.

Tang, Y., X. Bao, Y. Zhi, et al. 2019. "Overexpression of a MYB Family Gene, *OsMYB6*, Increases Drought and Salinity Stress Tolerance in Transgenic Rice." *Frontiers in Plant Science* 10, no. 168: 168. https://doi.org/10.3389/fpls.2019.00168.

Taylor, J., and D. Butler. 2017. "R Package ASMap: Efficient Genetic Linkage Map Construction and Diagnosis." *Journal of Statistical Software* 79, no. 6: 1–29. https://doi.org/10.18637/jss.v079.i06.

Thomas, W., E. Baird, J. Fuller, et al. 1998. "Identification of a QTL Decreasing Yield in Barley Linked to Mlo Powdery Mildew Resistance." *Molecular Plant Breeding* 4: 381–393.

Tombuloglu, G., H. Tombuloglu, M. S. Sakcali, and T. Unver. 2015. "High-Throughput Transcriptome Analysis of Barley (*Hordeum vulgare*) Exposed to Excessive Boron." *Gene* 557: 71–81.

Trenberth, K. E., A. Dai, G. Van Der Schrier, et al. 2014. "Global Warming and Changes in Drought." *Nature Climate Change* 4: 17–22.

Visscher, P. M., R. Thompson, and C. S. Haley. 1996. "Confidence Intervals in QTL Mapping by Bootstrapping." *Genetics* 143: 1013–1020.

Van Ooijen, J. 2004. MapQTL® 5, Software for the Mapping of Quantitative Trait Loci in Experimental Populations. Kyazma BV, Wageningen 63

Voorrips, R. 2002. "MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs." *Journal of Heredity* 93: 77–78.

Wagner, G. P., and J. Zhang. 2011. "The Pleiotropic Structure of the Genotype–Phenotype Map: The Evolvability of Complex Organisms." *Nature Reviews. Genetics* 12: 204–213.

Watanabe, S., M. Matsumoto, Y. Hakomori, H. Takagi, H. Shimada, and A. Sakamoto. 2014. "The Purine Metabolite Allantoin Enhances Abiotic Stress Tolerance Through Synergistic Activation of Abscisic Acid Metabolism." *Plant, Cell & Environment* 37: 1022–1036.

Wehner, G., C. Balko, K. Humbeck, E. Zyprian, and F. Ordon. 2016. "Expression Profiling of Genes Involved in Drought Stress and Leaf Senescence in Juvenile Barley." *BMC Plant Biology* 16: 1–12.

Xing, J., Q. Weng, C. Hao, et al. 2012. "*T1N6 _ 22* Gene Is Required for Biotic and Abiotic Stress Responses in *Arabidopsis*." *Russian Journal of Genetics* 48: 1191–1198.

Yang, L., Y. Chen, L. Xu, et al. 2022. "The *OsFTIP6-OsHB22-OsMYBR57* Module Regulates Drought Response in Rice." *Molecular Plant Pathology* 15: 1227–1242.

Yi, S. Y., V. Nekrasov, K. Ichimura, S.-Y. Kang, and K. Shirasu. 2024. "Plant U-Box E3 Ligases *PUB20* and *PUB21* Negatively Regulate Pattern-Triggered Immunity in Arabidopsis." *Plant Molecular Biology* 114: 1–14.

Zhang, J., K. Kobert, T. Flouri, and A. Stamatakis. 2014. "PEAR: A Fast and Accurate Illumina Paired-End reAd mergeR." *Bioinformatics* 30: 614–620.

Zhao, T., and A. Dai. 2017. "Uncertainties in Historical Changes and Future Projections of Drought. Part II: Model-Simulated Historical and Future Drought Changes." *Climatic Change* 144: 535–548.

Zheng, Z., J. Ma, J. Stiller, et al. 2015. "Fine Mapping a Major QTL Conferring Fusarium Crown Rot Resistance on the Long Arm of Chromosome 3B in Hexaploid Wheat." *BMC Genom* 16: 850. https://doi.org/10.1186/s12864-015-2105-0.

Zheng, Z., H. Hu, S. Gao, et al. 2022. "Leaf Thickness of Barley: Genetic Dissection, Candidate Genes Prediction and Its Relationship With Yield-Related Traits." *Theoretical and Applied Genetics* 135: 1843–1854.

Zhou, H., W. Luo, S. Gao, et al. 2021. "Identification of Loci and Candidate Genes Controlling Kernel Weight in Barley Based on a Population for Which Whole Genome Assemblies Are Available for Both Parents." *Crop Journal* 9: 854–861.

**Supporting Information**

Additional supporting information can be found online in the Supporting Information section.