agriculture

MDPI

Article

Validation of Molecular Markers of Barley Net Blotch Resistance Loci on Chromosome 3H for Marker-Assisted Selection

Olga Afanasenko [1,*], Irina Rozanova [2], Anastasiia Gofman [1], Nina Lashina [1], Fluturë Novakazi [3], Nina Mironenko [1], Olga Baranova [1] and Alexandr Zubkovich [4]

[1] All Russian Institute of Plant Protection (FSBSI VIZR), 196608 Saint Petersburg, Russia; gofman.a.v.92@gmail.com (A.G.); nlashina@mail.ru (N.L.); nmironenko@vizr.spb.ru (N.M.); obaranova@vizr.spb.ru (O.B.)
[2] N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR), 190000 Saint Petersburg, Russia; i.rozanova@vir.nw.ru
[3] Department of Plant Breeding, Swedish University of Agricultural Sciences, Sundsvägen 10, 23422 Lomma, Sweden; fluture.novakazi@slu.se
[4] Republican Unitary Enterprise, The Research and Practical Center of the National Academy of Sciences of Belarus for Arable Farming, Timiriazeva Street 1, 222160 Zhodino, Belarus; aa_zoubkovitch@mail.ru
* Correspondence: oafanasenko@vizr.spb.ru

Abstract: The most widespread and harmful disease of barley is net form of net blotch caused by the ascomycete *Pyrenophora teres* f. *teres* Drechsler (*Ptt*). A cost effective and environmentally sustainable strategy for barley protection against *Ptt* is to develop barley cultivars possessing genetic resistance. In previous GWA analysis, we identified SNP-markers associated with a resistance locus on chromosome 3H in the interval of 45.82–54.53 cM. These SNPs have been described previously in the literature to be located within the same region of chromosome 3H. The aim of the study was to validate QTL markers controlling resistance to *Ptt* on chromosome 3H in this region by KASP genotyping in four F_2 populations of crosses between the resistant cultivars, Morex, Fox, and Zolo, and the accession, Local k-21578, with the susceptible barley cv. Gesine and in a doubled haploid (DH) population of Canadian Lake Shore (CLS)/Harrington. Eleven of fifteen studied markers showed high efficacy (97.5–100%) for co-segregation with resistance to *Ptt* in the DH population, CLS/Harrington. Three of these markers located at 54.53 cM and one at 51.27 cM were effective in two F_2 populations of crosses of Morex and Fox with susceptible cv. Gesine. These markers are also located close to each other on the physical map (442,203,921–443,119,491 bp). Apparently, in cultivars, CLS, Morex, and Fox, resistance to *Ptt* is determined by the same locus. Markers JHI-Hv50k-2016-166392 (47.1 cM, 112,536,071 bp), Clone ID 3255462_1 (51.63 cM, 363,531,898 bp), and Clone ID 3255462_2 (51.63 cM, 363,531,871 bp) showed high efficacy in the DH population and in the F_2 population, Local k-21578/Gesine. Apparently, at least two loci controlling *Ptt* resistance exist in the chromosome region of 47.0–54.3 cM: one at 46.0–48.44 cM and another at 51.27–54.8 cM. These regions were found to harbor several genes involved in important plant functions, including disease response and signaling pathways. Allele-specific PCR markers were developed based on the KASP assay data and tested on six resistant, two moderately resistant, and two susceptible barley genotypes. Four markers were found to be effective to differentiate susceptible and resistant barley genotypes. The KASP and allele-specific PCR markers associated with *Ptt* resistance on chromosome 3H will be useful for pyramiding resistance QTLs in barley marker-assisted selection.

Keywords: barley; resistance; net blotch; *Pyrenophora teres* f. *teres*; SNP-markers; KASP genotyping; efficacy of markers

Citation: Afanasenko, O.; Rozanova, I.; Gofman, A.; Lashina, N.; Novakazi, F.; Mironenko, N.; Baranova, O.; Zubkovich, A. Validation of Molecular Markers of Barley Net Blotch Resistance Loci on Chromosome 3H for Marker-Assisted Selection. *Agriculture* **2022**, *12*, 439. https://doi.org/10.3390/agriculture12040439

Academic Editor: Ming Chen

Received: 17 February 2022
Accepted: 20 March 2022
Published: 22 March 2022

1. Introduction

The most widespread and harmful disease of barley is net blotch caused by the ascomycete, *Pyrenophora teres* Drechsler. It occurs in all major barley-growing regions and yield losses on susceptible cultivars can reach up to 40% [1] The main hosts of *Pyrenophora teres* are barley (*Hordeum vulare*) and some wild *Hordeum* species; however, reports of infected wheat (*Triticum aestivum*) have increased in recent years [2–6]. Based on lesion type, this species was divided into the two forms, i.e., *Pyrenophora teres* f. *teres* Drechsler and *P. teres* f. *maculate* [1,7]. The two forms are very similar in their morphology but distinguishable based on foliar symptoms [7]. *P. teres* f. *teres* (*Ptt*) produces brown longitudinal and transverse striations, which appear as net-like lesions, after which the disease is named. *P. teres* f. *maculate* (*Ptm*) symptoms appear as brown-spotted lesions [8].

Ptt is the dominant form on the European territory of Russia; however, *Ptm* was found in the southern part (Krasnodar region) in 2011 [9] but is still not prevalent in this region. Annual surveys at the state cultivar testing plots in the northwest of Russia indicate that all barley cultivars registered in the State Register of Breeding Achievements are susceptible to net blotch [10]. The disease occurs epiphytotic in different agroclimatic zones of the European part of Russia once every three to four years [11].

Pyrenophora teres reproduces sexually, which leads to a high genetic variability and a high number of pathotypes within populations [12–20]. Many of the resistance QTLs identified so far are pathotype/race specific. Hence, identification of suitable resistance sources is an on-going task. A cost effective and environmentally sustainable strategy for barley protection against *Ptt* is to develop barley cultivars possessing genetic resistance. A high level of genetic protection of cereal crops against diseases is achieved by cultivating resistant cultivars that are genetically diverse. In this regard, the study of barley genetic resources for resistance to *Ptt* is an important task to provide breeders with donors of resistance. A prime approach to find new resistance genes and QTLs to *Ptt* were bi-parental-mapping procedures: more than 50 QTLs have been identified on all barley chromosomes providing resistance at seedling and adult plant stages [11,21–35]. Recently, genome-wide association studies (GWAS) have emerged, which are based on the identification of SNPs associated with the desired trait. GWAS has significantly expanded the genetic diversity of resistance donors to *Ptt*. The first studies of mapping barley resistance genes to *Ptt* by using GWAS were published in 2017. At present, the results of GWAS for *Ptt* resistance have identified more than 60 major genes and QTLs in barley collections consisting of several hundred to one and a half thousand individuals (7139 in total) [33,36–43]. In all studies using GWAS, the highest number of QTLs determining resistance to *Ptt* was found on chromosomes 3H and 6H (reviewed in [44]. Chromosome 3H harbors the major gene, *Pt1*, which was first described by Mode and Schaller [45] and later designated *Rpt1* [46]. Cultivar Canadian Lake Shore was reported to possess two major resistance loci, *Pt3* (*Rpt3*) on chromosome 2H and *Pt2* (*Rpt1*) on chromosome 3H [45–47]. In previous studies, GWAS revealed a locus on chromosome 3H at 46.29–54.3 cM 390–443 Mbp (here and below, the genetic positions are aligned to the physical map of Masher 2021, MorexV3 genome [48]) significantly associated with resistance towards *Ptt* [41]. Additionally, in a bi-parental doubled haploid (DH) population generated from a cross between resistant cultivar, Canadian Lake Shore (CLS), and susceptible cultivar, Harrington, we identified a major QTL on chromosome 3H in the same region (46.18–53.26 cM, 364–392 Mbp) conferring resistance to *Ptt* [35]. This chromosome region was previously reported to be associated with net blotch resistance by Koladia et al. [33] (52.6–54.8 cM, 442–450 Mbp), Vatter et al. [37] (51.6 cM, physical position—NA), Wonneberger et al. [36] (−52.01–54.5 cM, 442–444 Mbp), and Rozanova et al. [40] (50.9–54.8 cM, 442 Mbp).

To date, marker-assisted selection (MAS) has been widely used in cereal crop breeding for resistance to obligate biotrophic pathogens, such as stem rust, brown and yellow rust, and powdery mildew [49–52]. Despite the achievements in the identification and mapping of resistance QTL to hemibiotrophic pathogens, the real use of the identified genes in practical breeding of cereal crops is hampered by the lack of reliable molecular markers (MM).

The use of high-density chips allows large-scale studies and, with GWAS, the identification of candidate SNPs. To validate a small number of SNPs, it is not economical to reuse multiplex technologies that generate from a hundred to over a million SNPs per cycle [53]. Kompetitive Allele Specific PCR (KASP) is one of the uniplex SNP-genotyping platforms that has evolved to be a global benchmark technology [53–57]. KASP is a homogeneous, fluorescence-based variant of polymerase chain reaction genotyping. It is based on allele-specific elongation of the oligonucleotide and transfer of fluorescence resonance energy for signal generation. It is cost-effective in applications and requires a small to moderate numbers of QTL markers [53].

The aim of the study was to validate QTL markers associated with resistance to *Ptt* on chromosome 3H in the region of 45.82–54.53 cM previously reported in the literature. In order to achieve this, known SNP markers were converted into KASP assays and validated in four segregating F_2 populations from crosses of varieties resistant and susceptible to *Ptt* and in a DH population (CLS/Harrington).

2. Materials and Methods

2.1. Plant Material

Four segregating populations from crosses of susceptible cultivar, Gesine, with *Ptt*-resistant cultivars, Morex (comprising of 95 F_2 plants), Fox (82 F_2 plants), Zolo (84 F_2 plants), and line Local (Ecuador) k-21578 (99 F_2 plants), were included in KASP genotyping. Resistance of these four cultivars was controlled by the locus on chromosome 3H in interval 46.29–54.3 cM, based on GWAS data [41]. Additionally, a DH population derived from a cross of the resistant cv. CLS and the susceptible cultivar, Harrington, in which the gene *qPttCLS* was previously mapped on chromosome 3H [35] was included as the control for KASP genotyping.

The parents of the crosses presented above were used to optimize PCR conditions. Additionally, two moderate resistant accessions, Local Turkey 7689 and S-328 Mexico 28671, and the resistant variety, Harbin k-19282, were included. In these accessions, the *Ptt* resistance locus on chromosome 3H at the same interval was identified via GWAS.

2.2. Pathogen Isolates and Culture Conditions

Ten additional *Ptt* single conidia isolates of different geographical origin were used to characterize the resistance of barley accessions (Table 1).

Table 1. Origin of *P. teres* f. *teres* isolates.

Isolate	Origin	Barley Cultivar	Year
F18	Belarus, Zhodino	Fest	2017
S18	Russia, Krasnodar	Sprinter	2017
B18	Russia, Leningrad region	Tausen’	2016
V13	Russia, Leningrad region	Suzdalets	2015
Pr2	Russia, Far East region	Primorskij 207	2015
Germ7	Germany, Quedlinburg	unknown	2011
Czech11.1	Czech Republic, Lysice	unknown	2011
Can11	Canada, Alberta	Harrington	2010
SA7	South Africa, Bredasdorp	unknown	2017
Mor1	Morocco, Brachoua of Zaer region	Amalou	2017

Isolate F18 is avirulent on Morex, Fox, Zolo, and line Local (Ecuador) k-21578 and was used to study the co-segregation of resistance and molecular markers in the F_2 and DH populations.

Ptt isolates were grown on modified Chapek medium containing KCL—0.5 g, KH_2PO_4—0.5 g, $MgSO_4$—0.5 g, urea—1.2 g, lactose—20 g, agar-agar—20 g per 1 L of distilled water. Isolates were cultivated at room temperature under artificial light (exposure 3000 lx) at 16 h of light/8 h of dark for 10 days.

2.3. Fungal Preparation, Inoculation of Seedlings, and Disease Assessment

The *Ptt* cultures were flooded with distilled water containing 0.01% TWEEN 20 and conidia were harvested by adding sterile water to the Petri dish and scraping conidia with a sterile spatula. The conidia suspension was filtered through gauze. Conidia were counted with a hemocytometer, and the concentration was adjusted to 5000 conidia/mL for inoculation.

To evaluate the resistance of barley accessions to different *Ptt* isolates and for the analysis of segregation in F_2 populations, three seeds per accession or F_2 plant were grown in plastic containers (18 × 13 × 6 cm) for two to three weeks at 16–18 °C with alternating 12 h periods of light/darkness (exposure 5000 lx). Studies of resistance in barley accessions to each isolate were set up in three replications (three plants in each). When the second leaf was fully developed (BBCH 12–13), plants were spray-inoculated with the conidia suspension (approximately 0.3 mL/pot). Plants were then covered with plastic foil for 48 h to ensure 100% humidity. After incubation, plants were grown for 10–12 days at 20–22 °C and 70% humidity until symptoms were clearly visible.

Infection response type was assessed on the second leaf of each plant following the 10-point scale of Tekauz [58] in which a score of 1–4.9 corresponds to resistance and a score of 5–10 corresponds to susceptibility. Cultivars, Harrington and Gesine, were used as susceptible checks.

For KASP genotyping, 20 resistant and 20 susceptible plants of each F_2 and DH population were selected.

2.4. Development of KASP Markers

Positions of chosen markers were determined using the source BARLEYMAP (http://floresta.eead.csic.es/barleymap accessed on 16 February 2022) [59] where markers were assigned to their physical position on the current version of the MorexV3 genome [48] and their genetic position on POPSEQ_2017 map [60,61]. In total, thirteen polymorphic SNPs on chromosome 3H around the target areas in intervals 52.0–54.3 cM and 45.0–47.07 cM and three DArT-seq markers associated with net blotch resistance were selected. The localization in the genome of the identified SNPs was determined using the Ensembl Plants database (http://plants.ensembl.org/, accessed on 16 February 2022). When a marker was detected within a gene, the product encoded by this gene was determined using the UniProtKB database (https://www.uniprot.org/, accessed on 16 February 2022).

After the candidate SNPs were identified, the nucleotide sequences flanking the SNPs (50 bp on each side) were exported from the Essembl Plants database. Sequences containing candidate SNPs were used for the KASP assay primer design. Further KASP genotyping was performed by LGC Genomic (UK).

2.5. Testing the Diagnostic Value of SNP-Markers

The diagnostic value of tested co-segregating markers (%) was calculated using the following equation:

$$Diagnostic\ value = \frac{number\ of\ lines\ showing\ correct\ test\ results}{total\ number\ of\ test\ results}$$

2.6. Development of PCR Markers

For the development of a PCR marker based on the chosen SNPs, primers with an allele-specific nucleotide at the 3'end were constructed. The sequences flanking SNPs (500 bp on each side) were exported from the Ensembl Plants database. The UGENE program was used to design the primers [62].

PCR reactions were performed in a 25 µL-volume-containing reaction buffer with 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 15 pmol forward primer, 15 pmol reverse primer, 1 unit of *Taq* polymerase, and 40 ng of genomic DNA. PCR was carried out using the following cycling profiles: 4 min at 94 °C; 31 cycles of 45 s at 94 °C, 1 min at annealing

temperature (64–68 °C, optimized for each marker), and 1 min at 72 °C, with a final extension step of 5 min at 72 °C. PCR products were separated by electrophoresis in 1.5% agarose gel.

3. Results

3.1. Resistance of Barley Cultivars and Accessions to Ptt Isolates

The resistance of accessions, previously resistant to two *Ptt* isolates and field conditions at three locations [41], including the resistant parents of the F_2 and DH populations were evaluated against eight *Ptt* isolates of different origin (Table 2). Cv. Gesine and Harrington were included as susceptible parents of F_2 and DH populations. Cv. Morex was resistant to all studied isolates. Cv. CLS and Fox were susceptible to one isolate, cv. Zolo, and Harbin to two isolates, and barley accession Local k-21538 (Ecuador) to three isolates. Barley accession Local Turkey k-7689 was resistant to three isolates, moderately resistant to three isolates, and susceptible to four isolates. This data testify to the race-specific resistance of the studied cultivars and accessions. Accession S-328 Mexico k-28671 was susceptible to all but one of the isolates (Cz11.1).

Table 2. Mean infection responses for ten barley genotypes after inoculation with *P. teres* f. *teres* isolates of different origin.

N	Barley Genotypes	Infection Responses (IRs) to Isolates										
		F 18	**S 18**	**B 18**	**V 13**	**Pr2**	**Germ 7**	**Cz 11.1**	**Can11**	**SA 7**	**Mor 1**	**Mean**
1.	Gesine (susceptible) Germany	9	9	10	9	10	9	10	10	10	9	**9.6**
2.	Harrington, TR 306 (susceptible) Canada	10	9.8	10	7	10	7	9.3	9.7	8.8	7.8	**8.9**
3.	Local Turkey k-7689	7	8	8	5.8	3.7	4	5.3	5.8	4.5	6.2	**5.8**
4.	S-328 Mexico k-28671	9	7	8	7	6	6.5	3.8	7	7	7	**6.8**
5.	Morex (C.I.15773) k-26959	1.7	3.8	1	2	3	2	4	2	2	4	**2.6**
6.	Fox (C.I.9190, NFC 883) k-19182	1.7	2	1	2.1	2.8	2.8	2.5	6	5.5	3	**2.9**
7.	Zolo k-18552	1.5	3	1	1.4	3.7	6	1.7	3	2	7	**3.1**
8.	Local Ecuador k-21578	1	3	1	8	3	7	3.5	2.7	1.5	8.7	**3.9**
9.	Harbin (C.I. 4929) k-19282	4	5.3	2.1	2.1	4.3	7	1.8	2.5	2.3	3.5	**3.5**
10.	Canadian Lake Shore, (C.I. 2750) k-25282	1	2.5	3.3	3.3	3.2	5.5	1.3	2.3	2.3	2.5	**2.7**

Infection response type was assessed on the 10-point scale of Tekauz [58] IRs below five were considered resistant (R) IRs 5.0–6.9—moderately resistant (MR) and IRs seven and above were considered susceptible (S).

3.2. Segregation Analysis

Infection response for parental lines to *Ptt* isolate F18 are shown in Figure 1. The frequency distributions observed in the DH and F_2 populations after inoculation with isolate F18 are shown in Table 3 and in Supplementary Figures S1–S5. The segregation ratios detected for the DH population, CLS/Harrington, and F_2 populations of Morex/Gesine, Zolo/Gesine, and k-21578/Gesine showed segregation ratios, which fit to a 1R:1S segregation in the DH population and a 3:1 in the F_2 populations. A two complementary gene

model was found to be significant based on Chi-squared analysis in the segregating F_2 population of Fox/Gesine (Table 3).

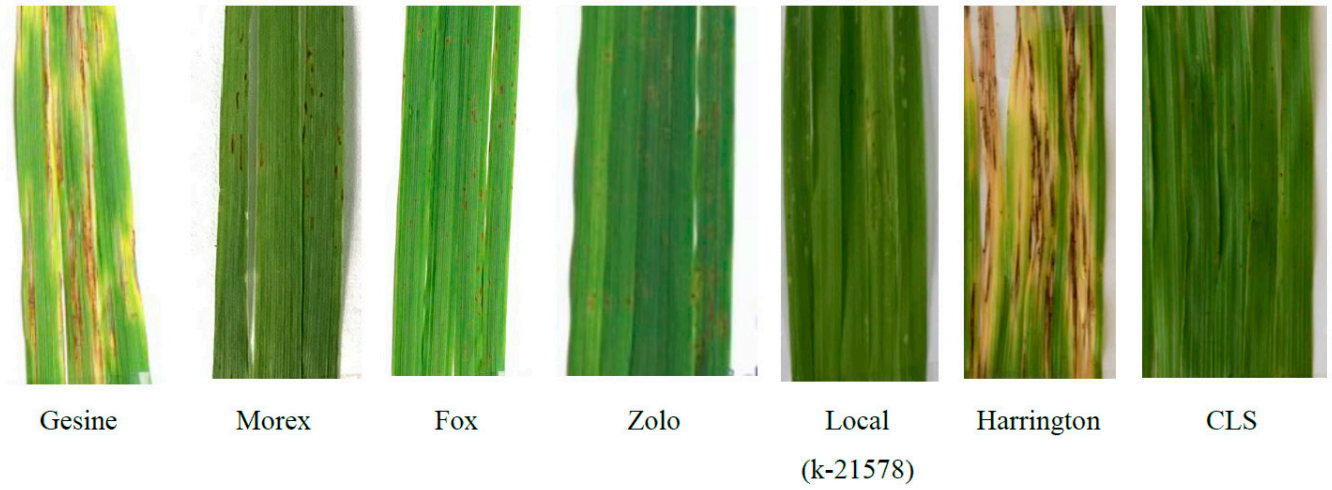

Figure 1. Infection response on 12 dpi for barley parental genotypes to *Ptt* isolate F18 scored on the second leaf of the seedling.

Table 3. Frequency distribution of infection responses (IRs) in the CLS/Harrington DH population and F_2 populations from crosses of resistant and susceptible barley genotypes.

DH or F_2 Population	IRs of Parents		Number of Resistant (R) and Susceptible (S) Plants		Genetic Ratio	Chi Square
	P1	P2	R	S		
DH CLS/Harrington	1.0	10	20	21	1:1	0.02
F_2 Morex/Gesine	1.7	10	71	24	3:1	0.003
F_2 Fox/Gesine	1.7	9.0	46	36	3:1 9:7	15.62 0.000
F_2 Zolo/Gesine	1.5	9.0	58	26	3:1	1.59
F_2 k-21578/Gesine	1	9.0	66	33	3:1	3.67

IRs 1–4.9—resistant, 5.0–6.9—moderately resistant, 7–10—susceptible. P5% = 3.84 at 1 df.

3.3. KASP Genotyping Results

The sequences containing SNPs were converted for KASP genotyping (Table 4). Developed KASP markers were tested on one DH and four F_2 populations to determine which markers showed the highest association with *Ptt* resistance. The results were plotted on a Cartesian plot where the *x*-axis shows the FAM signal fluorescence value for each sample associated with the first allele, and the *y*-axis shows the HEX signal fluorescence value associated with the second allele [53].

Eleven of fifteen SNPs examined in the DH population, CLS/Harrington, seven in the F_2 population, Local k-21578/Gesine, and five in F_2 populations, Fox/Gesine, Morex/Gesine, and Zolo/Gesine contained alternative allelic variants of extreme values of resistance to *Ptt* in the homozygous state (Table 5). All results of KASP-genotyping in DH and F_2 barley populations are presented in Supplementary Table S1.

Table 4. SNPs on chromosome 3H, converted into KASP-markers.

SNP	Position		Sequence Containing SNP
	cM	bp	
JHI-Hv50k-2016-164734	45.82	48,713,634	CTTTATGAGATCAACTGCTTCCTGCAGAAGTTTAGCCTTTCCCATAAGAT **[C/T]**CACCATACAGCCATAATGCTCGTGCTTGGGTTCAATCCTGTATTCCTGGA
JHI-Hv50k-2016-165152	45.82	61,409,056	TGACATTGAGCTGCTTTGCTTTGGTTCATCTCCGTTCTTCTTTTCTTTTA **[C/G]**TTTGAGCGGCAGCAGCACTGATGATGATGACGACGACGATGATGGACGGG
JHI-Hv50k-2016-166356	47.31	102,070,302	CCTCTTTACCAAGGATTCGTGTCTTTTTTGTTTAACCTTGTGAGTTCTGA **[T/C]**TGACTACTAAAAAGATCCGTGCCTGGTATCTTTCATGAAATAGCCCCATT
JHI-Hv50k-2016-166392	47.1	112,536,071	AAGACGGTTGGGTCTCCGGCTCTCCGACGCACACACGCCGCGCCGTCCAG **[T/C]**TGGTGGTTTCGTTGCTTTTTCTTTGAACTGCCCACCTTGTATAATCAATC
SCRI_RS_160464	51.56	255,019,281	CTTGTAGTCGGTCGGTGTGTGGGAAGTTGGGATGAGAATGAACAAAAAGG **[T/A]**AAAAAGAAGAAATGAAAAGGATGAAAAAAGTTGGTGAAAAAGCTTGCACT
JHI-Hv50k-2016-173670	51.56	285,191,294	TTGCACTTGTGAAACTATTTGAATGTCTAAATGGGCTAACGAATGTTGCC **[T/C]**TTGCGAACCATGGTAGCAGAAGTCCATGACAAGGATACCTAAAATTTCAG
JHI-Hv50k-2016-174303	51.56	301,082,623	AGACTGTTCTTTGCCAGATGTTGATTATCTCTACTCTCCACATGACAACT **[G/A]**TCATCCAAAACAACAACAGGTACAGGGAACCCCATAACAGGTTTACGGTT
JHI-Hv50k-2016-179690	51.63	389,686,103	GCGTGACCTCGGTAAAAAAACTTAGCCCGTCTGAAATTTTGCTTGAATCA **[G/A]**TACTTTCGCACTGAGTTAGATCTTCATTATACTTTCGACAATAGATTGTG
JHI-Hv50k-2016-183207	52.46	442,203,921	TTGCCACCAAAAGTGCTCTTGAGTTGACATGTTTATATATTGTTCTCGCC **[A/T]**ACTTGCTCCAGCATTTGCATAATAATCTGTAAACAGCTCGGACACTTCTT
JHI-Hv50k-2016-183351	54.53	442,550,473	TCTTGACGCCGGGAACCAGCATGAGAAGATATTTGAATGATGACATTTGC **[T/C]**TCTGGGATATCAATGGAATTATCACCCACCTATGCATTAATAGCAGAGAT
JHI-Hv50k-2016-202195	98.65	553,150,117	GGAAAGAAGATTGCTGCTTTCGTTCCCAATGATGGTTGCCTGAACTTCAT **[C/T]**GAGGAAAATGTATGTTCCCCATCTTGTACTTCTCAAATGTATGTTCTACA
JHI-Hv50k-2016-183463	54.53	443,119,491	TATCCATGGACCTGAAAGTGCCAAATTGTATAAGCCATATCATGTTTTTT **[T/C]**AGTACAAGCCAGATCATGCTTACAATGCTCACTTTATTCTTTCAAACATA
JHI-Hv50k-2016-183478	54.53	44,311,006	GCTAACTTTGTCACCAGCTGTGGTTCTTCTGATGTGTTTGTTTCTACCA **[C/A]**ATTTGTGTCTGTTAGTACTGGAACTTCAGAACTTTGAGGTTCGTTAGTTG
Clone ID 3272635	51.27	391,906,604	CATCAAGAAGGCTGAGTCAAAGCCACGGGAGCCTAAGAAGAGGGTATAAC **[C/G]**TGCAGCTGGTGTTATATTGAGGTCCTTATAACCTTCACCTTGCATGCTCT
Clone ID 3255462_1	51.63	363,531,898	GAAATTGGACATGTCAATCCGACCAAGAGATTCAGGAGAAATCCTCTCTA **[G/A]**GAAACCAAAATCAGATATAAGGATCTTTGCAGCGCGTTCATCCCATGCAT
Clone ID 3255462_2	51.63	363,531,871	AGATTCAGGAGAAATCCTCTCTAGGAAACCAAAATCAGATATAAGGATCT **[T/C]**TGCAGCGCGTTCATCCCATGCATGGGCAGGAATGCCGGATAATTCCAGGC

Diagnostic efficiency of SNP markers of *Ptt* resistance QTL on chromosome 3H is shown in Figure 2. Eleven of the twelve listed markers co-segregated with resistance in the DH population, CLS/Harrington, with high efficiency (97.5–100%). In the Morex/Gesine and Fox/Gesine F_2 populations, the co-segregation efficiency of markers, JHI-Hv50k-2016-183351, JHI-Hv50k-2016-183463, JHI-Hv50k-2016-183478, Clone ID 3272635, and JHI-Hv50k-2016-183207 with resistance to *Ptt*, was 81–86%. In the k-21578/Gesine F_2 population, highly efficient co-segregation with resistance was obtained for the markers, JHI-Hv50k-2016-166392, Clone ID 3255462_1, and Clone ID 3255462_2 (94%). No marker exceeded 80% efficiency in the co-segregation analysis with resistance in the Zolo/Gesine F_2 population. While for the varieties, Morex, Fox, and Zolo, heterozygous plants exhibited a parental response-resistance type, which indicates dominant resistance. In the Local k-21578/Gesine population, the mean score of the resistant parent was 2.5; the response of the heterozygous plants corresponded to susceptibility type (mean score 7.31). This indicates recessive resistance in the barley accession Local k-21578 to isolate F18.

Table 5. KASP-genotyping results in DH and F_2 progeny genotypes.

		Position			Number of Plants	
N	**SNP**	**Genetic Position (cM)**	**Physical Position (bp)**	**Allele**	**Resistant**	**Susceptible**
DH population Canadian Lake Shore/Harrington						
1	JHI-Hv50k-2016-179690	51.63	389,686,103	GG	20	0
				AG	0	0
				AA	0	21
2	Clone ID 3272635	51.27	391,906,604	GG	19	0
				CG	0	0
				CC	1	20
3	JHI-Hv50k-2016-183463	54.53	443,119,491	CC	1	21
				CT	0	0
				TT	19	0
4	JHI-Hv50k-2016-183478	54.53	443,111,006	CC	19	0
				CA	0	0
				AA	1	21
5	JHI-Hv50k-2016-183207	52.46	442,203,921	TT	19	0
				TA	0	0
				AA	1	21
6	JHI-Hv50k-2016-164734	45.82	48,713,634	CC	20	0
				CT	0	0
				TT	0	21
7	JHI-Hv50k-2016-165152	45.82	61,409,056	CC	20	0
				CG	0	0
				GG	0	21
8	JHI-Hv50k-2016-166392	47.1	112,536,071	CC	0	21
				TC	0	0
				TT	20	0
9	JHI-Hv50k-2016-183351	54.53	442,550,473	CC	1	21
				CT	0	0
				TT	19	0
10	Clone ID 3255462_1	51.63	363,531,898	GG	19	0
				AG	0	0
				AA	1	21
11	Clone ID 3255462_2	51.63	363,531,871	CC	1	21
				CT	0	0
				TT	19	0

Table 5. *Cont.*

		Position			Number of Plants	
N	**SNP**	**Genetic Position (cM)**	**Physical Position (bp)**	**Allele**	**Resistant**	**Susceptible**
Population F_2 Fox/Gesine						
12	Clone ID 3272635	51.27	391,906,604	GG	9	2
				CG	10	4
				CC	0	11
13	JHI-Hv50k-2016-183463	54.53	443,119,491	CC	0	12
				CT	7	4
				TT	9	2
14	JHI-Hv50k-2016-183478	54.53	443,111,006	CC	10	2
				CA	9	4
				AA	0	12
15	JHI-Hv50k-2016-183207	52.46	442,203,921	TT	10	1
				TA	8	4
				AA	0	12
16	JHI-Hv50k-2016-183351	54.53	442,550,473	CC	0	12
				CT	9	4
				TT	10	2
Population F_2 Morex/Gesine						
17	Clone ID 3272635	51.27	391,906,604	GG	6	1
				CG	11	4
				CC	2	19
18	JHI-Hv50k-2016-183463	54.53	443,119,491	CC	2	18
				CT	11	6
				TT	6	0
19	JHI-Hv50k-2016-183478	54.53	443,111,006	CC	6	0
				CA	11	6
				AA	2	19
20	JHI-Hv50k-2016-183207	52.46	442,203,921	TT	6	0
				TA	11	5
				AA	2	20
Population F_2 Zolo/Gesine						
21	Clone ID 3272635	51.27	391,906,604	GG	9	2
				CG	10	4
				CC	0	11
22	JHI-Hv50k-2016-183463	54.53	443,119,491	CC	0	12
				CT	7	4
				TT	9	2

Table 5. *Cont.*

		Position			Number of Plants	
N	**SNP**	**Genetic Position (cM)**	**Physical Position (bp)**	**Allele**	**Resistant**	**Susceptible**
Population F_2 Zolo/Gesine						
23	JHI-Hv50k-2016-183478	54.53	443,111,006	CC	10	2
				CA	9	4
				AA	0	12
24	JHI-Hv50k-2016-183207	52.46	442,203,921	TT	10	1
				TA	8	4
				AA	0	12
25	JHI-Hv50k-2016-183351	54.53	442,550,473	CC	0	12
				CT	9	4
				TT	10	2
Population F_2 Local k-21578/Gesine						
26	JHI-Hv50k-2016-179690	51.63	389,686,103	GG	18	0
				AG	1	8
				AA	1	9
27	JHI-Hv50k-2016-183463	54.53	443,119,491	CC	1	11
				CT	10	7
				TT	9	0
28	JHI-Hv50k-2016-183478	54.53	443,111,006	CC	9	0
				CA	10	7
				AA	1	11
29	JHI-Hv50k-2016-183207	52.46	442,203,921	TT	9	0
				TA	10	7
				AA	1	11
30	JHI-Hv50k-2016-166392	47.1	112,536,071	CC	1	10
				TC	1	8
				TT	18	0
31	JHI-Hv50k-2016-183351	54.53	442,550,473	CC	1	11
				CT	10	7
				TT	9	0

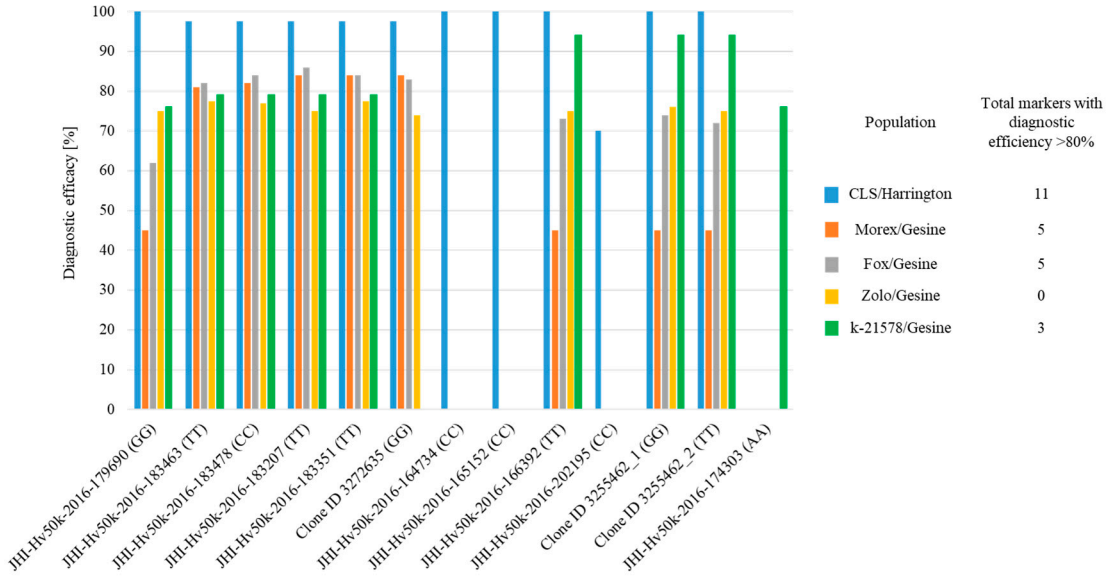

Figure 2. The diagnostic value of SNP-markers co-segregated with resistance to *Ptt* on chromosome 3H.

3.4. Allele-Specific PCR Markers Test

Allele-specific PCR markers were developed based on the KASP assay data (Table S2). To optimize PCR conditions, the annealing temperature (from 60 °C to 68 °C) was varied with four primers and DNA of 10 barley varieties, presented in Table S3. The presence of the amplification product in DNA of the cv. CLS and absence of amplification in the susceptible cv. Harrington and Gesine were the benchmarks. Of the six markers studied, four met the control values: Clone ID 3272635, JHI-Hv50k-2016-183463, JHI-Hv50k-2016-183351, and JHI-Hv50k-2016-183478 (Figure 3, Supplementary Figures S6–S9). For markers JHI-Hv50k-2016-183478 and JHI-Hv50k-2016-183351, there were no amplification products from Morex DNA, and for markers JHI-Hv50k-2016-183463 and Clone ID 3272635, there were no amplicons in Local (Ecuador) k-21578 DNA samples. Amplification products for all studied markers were found for the accession S-328 Mexico, which was susceptible to most isolates tested in the present study.

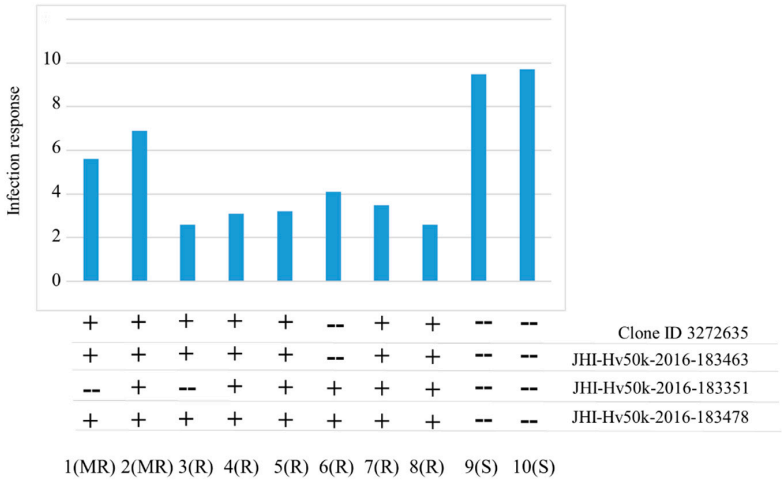

Figure 3. Mean infection response to ten *Ptt* isolates of barley genotypes and data of molecular marker analysis. 1—Local Turkey (k-7689), 2—S328 Mexico (k-28671), 3—Morex (C.I.15773), 4—Fox (C.I.9190), 5—Zolo Morocco, 6—Local Ecuador (k-21578), 7—Harbin (C.I.4929), 8—Canadian Lake Shore (C.I.2750), 9—Gesine, 10—Harrington. Resistance groups: R—resistant, MR—moderately resistant, S—susceptible. «+»—fragment presents, «−»—fragment absents.

4. Discussion

Eleven out of fifteen studied markers showed high efficacy (97.5−100%) for co-segregation with resistance in the DH population CLS/Harrington, including the markers Clone ID 3255462 and 3272635 previously identified in this DH population for the *qPttCLS* locus in interval 51.27–51.63 cM [35]. Three markers at 54.53 cM (JHI-Hv50k-2016-183463, JHI-Hv50k-2016-183478, and JHI-Hv50k-2016-183351) and one at 51.27 cM (Clone ID 3272635) were effective in the DH population and in two F_2 populations from crosses from Morex and Fox with susceptible cv. Gesine. Moreover, these markers are closely located to each other on the physical map [48]. The distance between the outermost markers is 915 kb between 442,203,921 and 443,119,491 bp on chromosome 3H. However, the distance between neighboring markers is 500 kb or less. We assume that they belong to the same genomic region. Results indicate that resistance to *Ptt* in cvs. CLS, Morex, and Fox is determined by the same locus. The studied cultivars and accessions show race-specific resistance to *Ptt* (Table 2). In GWA studies, the resistance of certain SNP haplotypes of these barley genotypes was associated with a locus on chromosome 3H [26,41]. At the same time, there were differences in the responses of these barley genotypes to inoculation with *Ptt* isolates of different origin. The data in Table 2 does not contradict the conclusion that resistance to *Ptt* in cvs. CLS, Morex, and Fox is determined by the same locus because IR to all studied isolates was approximately the same: cv. Morex was resistant to all *Ptt* isolates, cv. CLS and Fox were resistant to all isolates except one. Infection response of cv. CLS to isolate Germ 7 and cv. Fox to isolate SA 7 was 5.5 (MR). Local Ecuador k-21578 was susceptible to isolate V 13 to which all other cultivars were resistant; also, it was susceptible to isolate Mor 1 to which Fox and Morex were resistant (Table 2). Three SNP-markers co-segregated with resistance in the cross with the barley accession Local Ecuador k-21578 with high efficiency (94%) (Figure 2). These markers were not effective for crosses with Morex and Fox. Apparently, different QTLs on chromosome 3H controlled resistance of Local Ecuador k-21578 and CLS, Fox, and Morex.

Several other GWA studies have reported resistance loci in this genome region (Table 6). The data in Table 6 indicate that there are probably three *Ptt* resistance loci in the intervals: 45.82–47.61 cM [38,41,63,64], 51.27–52.46 cM [33,35,37,40,41], and 54.53 cM [36,41].

In the first GWA study on *Ptt* resistance, Richards et al. (2017) mapped resistance QTL *QRptts-3HL* towards the isolates LDN (45.82 cM) and 15A (47.61 cM) from North Dakota and California, respectively. Novakazi et al. (2019) [41] found a *Ptt* resistance QTL in the same position—45.82 cM. In a bi-parental DH population of Ushi/HHOR3073 a QTL named *QTLUH-3H* in interval 45–51 cM was determined by König et al. (2013) [64], conferring adult plant resistance. Yun et al. (2006) [63] found QTL *Rpt-3H-4* in the same position (46.0 cM) in the H. vulgare subsp. spontaneum accession OUH602 (Table 6).

Three markers (11_10728, SCRI_RS_152172, and SCRI_RS_186102) of QTL *NBP_QRptt3-2* (52.76–53.26 cM) were significantly associated with seedling and adult plant *Ptt* resistance in a collection of Nordic barley germplasm [36] (Table 6). Apparently, the same QTL on chromosome 3H at POPSEQ position 54.53 cM was significantly associated with seedling and adult plant resistance to isolate NFNB 50 [41]. In a QTL-mapping study in a bi-parental population, a QTL in interval 51.56–52.46 was determined in different genetic backgrounds in a RIL population of CIho5791/Tifang (52.01 cM) by Koladia et al. [33] and in a DH population of CLS/Harrington (51.27–51.63 cM) by Dinglasan et al. (2019) [35]. Novakazi et al. (2019) [41] and Rozanova et al. (2019) [40] identified in GWA studies, QTL associated with seedling resistance to different *Ptt* isolates in the same position (51.2–51.56 cM) in a worldwide barley collection of landraces and commercial cultivars from the centers of diversity and in Siberian barley germplasm, respectively [40,41]. *QPt.3H-3* was determine by Vatter et al. (2017) [37] in a nested association mapping population also at position 51.63 cM.

Table 6. Summary of QTL associated with resistance to *Ptt* on chromosome 3H in the target interval.

QTL	Genetic Position (cM) *	Physical Position (bp) **	Markers	Literature	Note
Rpt-3H-4	46.0	NA	Bmag0828-Bmac0067	[63]	OUH602/Harrington RIL
	45.82	NA	11_20356	[38]	GWAS Isolate LDN
	45.82	48,713,634	JHI-Hv50 k-2016-164734	[41]	GWAS
	47.1–47.31	102,070,302 112,536,071	JHI-Hv50 k-2016-166356 JHI-Hv50 k-2016-166392	[41]	GWAS
QTLUH-3H	45–51	NA	HVM33	[64]	DH Uschi/HHOR3073 Adult
QRptts-3HL	47.61	NA	12_30721	[38]	GWAS Isolate 15A
	54.53	442,550,473	JHI-Hv50 k-2016-183351	[41]	GWAS
qPttCLS	51.27–51.63	398,203,862–435,526,243	3255462, 3257991, 3272635, 4190028	[35]	DH CLS/Harrington DArTseq markers
NBP_QRPtt3-2	52.01–54.53	NA 443,115,672 443,551,729	11_10728 SCRI_RS_152172 SCRI_RS_186102	[36]	GWAS
	51.2–52.46	189,518,077 184,635,059 442,203,921	JHI-Hv50k-2016-169338, SCRI_RS_186341, JHI-Hv50k-2016-183207	[40]	GWAS A2.6.0
QPt.3H-3	51.63	NA	i_11_10966	[37]	Wild barley NAM population HEB-25
	52.01	442,185,927	SCRI_RS_221644	[33]	RIL CIho 5791/Tifang F_6
	51.56	255,019,281 285,191,294 301,082,623	SCRI_RS_160464 JHI-Hv50 k-2016-173670 JHI-Hv50 k-2016-174303	[41]	GWAS

*—Marker position based on POPseq map by Mascher et al. [60], **—Marker position based on physical map MorexV3 (released in 2021) [48].

Markers, JHI-Hv50k-2016-166392, Clone ID 3255462_1, and Clone ID 3255462_2, in the interval between 47.1–51.63 cM showed high efficacy in the DH population, CLS/Harrington, and in the F_2 population, Local (Ecuador) k-21578/Gesine. In the present study, at least two loci controlling *Ptt* resistance were determined in the chromosome region spanning from 47.0–54.3 cM: one in chromosome fragment 46.0–48.44 cM (markers JHI_Hv50k-2016-164734, JHI_Hv50k-2016-165152, and JHI_Hv50k-2016-166392) and another in fragment 51.27–54.8 cM (markers JHI_Hv50k-2016-174303, JHI_Hv50k-2016-179690, Clon ID 3272635, Clon ID 3255462_1, and Clon ID 3255462_2) (Figures 2 and 4).

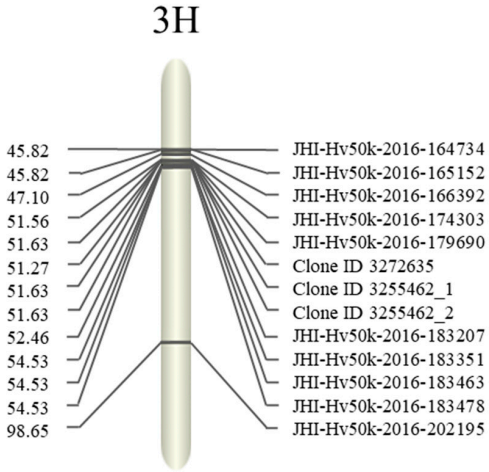

Figure 4. Markers of resistance to *Ptt* on chromosome 3H.

The studied cultivars and barley accession Local (Ecuador) k-21578 express race-specific resistance; thus, the identified markers are of interest in pyramiding QTL with isolate-specific resistance.

Some SNPs were located in protein-coding regions of genes. SNP JHI-Hv50k-2016-183207 was in a gene-encoding, receptor-like protein kinase that participates in abiotic stress response in plants [65] (Table 7). SNP JHI-Hv50k-2016-183351 was located in a gene encoding DNA repair helicase which can provide stress tolerance to plants [66]. SNPs, JHI-Hv50k-2016-183478 and JHI-Hv50k-2016-179690, were located in a gene-encoding DUF1645 family protein. This protein was first identified to enhance drought tolerance in transgenic rice and Arabidopsis [67]. Later, data indicated that genes encoding DUF1645 domain-containing proteins perform a conserved function in regulating stress-tolerance [68]. Located within 150 kb of marker JHI-Hv50k-2016-179690 are genes encoding receptor-like kinase and serine/threonine protein phosphatase and ethylene-responsive subfamily genes that are involved in plant defense responses [69,70]. Ethylene-responsive subfamily genes mainly work to mediate pathogen- and disease-related stimuli by activating multiple signaling pathways, such as jasmonic acid, ethylene, and salicylic acid pathways [71]. This genome region can be considered promising for further studies.

SNP JHI-Hv50k-2016-183463 was located in a gene-encoding RING/U-box superfamily protein. Sharma et al. [72] reported that this protein is involved in plant response to stress. Genes located within 150 kb of the identified markers were identified as encoding kinase family proteins involved in plant immunity against fungal infection [73] (Supplementary Table S4).

Clone ID 3255462_2 (363 531 871 bp) and Clone ID 3255462_1 (363 531 898 bp) are closely linked markers. These SNPs were selected from DArT marker sequences [35]. According to the Ensembl Plants database, both SNPs are located in gene BART1_0-u20880 (non-translating CDS). Genes located within 150 kb from these SNPs encode products relevant to retrotransposons. The regulation of retrotransposon expression in plants is not fully understood. Most of these active plant elements characterized to date are largely dormant during normal development but can be induced by biotic and/or abiotic stresses, including pathogen defense [74].

The other six markers are sufficiently separated that they belong to different genomic loci.

The marker JHI-Hv50k-2016-164734 was located downstream of the gene HORVU.MOREX.r3.3HG0236970 encoding pentatricopeptide repeat-containing protein (PPR). It is one of the largest protein families in embryophytes. A typical PPR protein is targeted to mitochondria or chloroplasts, binds one or several organellar transcripts, and influences their expression by altering RNA sequence, turnover, processing, or translation [75]. Nearby, less than 2 kb away from this marker is a gene-encoding serine carboxypeptidase-like protein (SCPL) (Supplementary Table S3). The SCPL family plays a vital role in stress response, growth, development and pathogen defense [76].

The marker JHI-Hv50k-2016-165152 is located in the gene HORVU.MOREX.r3.3HG0238960 encoding DUF581 family protein. DUF581 is a zf-FCS type zinc finger or FCS-like zinc finger (FLZ). It was found that it is highly conserved in sequence and structure and involved in protein–protein interactions [77]. Nearby, at 150 kb, there is another gene encoding the DUF581 family protein, as well as a gene encoding the LURP-one-like protein, which mediates resistance by coordinated transcriptional upregulation of plant defense genes [78].

The marker JHI-Hv50k-2016-166392 is located upstream of the gene HORVU.MOREX.r3.3HG0246080, which encodes DUF538. This protein belongs to a super family that includes a number of plant proteins, but their role is not yet clear. Analysis of genes located within 150 kb revealed no genes involved in the response to stress.

Table 7. Significant SNPs associated with *Ptt* resistance and descriptions of genes located in the target interval on chromosome 3H.

SNP	Physical Map (bp)	Gene	Type of Mutation	Amino Acid Submission	Polymorphism		Protein Product *
					Resistant	Susceptible	
JHI-Hv50k-2016-164734	48,713,634	HORVU.MOREX.r3.3HG0236970	E	C/T	CC	TT	Pentatricopeptide repeat-containing protein
JHI-Hv50k-2016-165152	61,409,056	HORVU.MOREX.r3.3HG0238960	D	G/C	CC	GG	DUF581 family protein
JHI-Hv50k-2016-166392	112,536,071	HORVU.MOREX.r3.3HG0246080	U	C/T	TT	CC	plant/protein (protein of unknown function, DUF538)
	301,082,623	HORVU.MOREX.r3.3HG0265210	E	G/A	AA	GG	Tudor/PWWP/MBT superfamily protein
Clone ID 3255462_2	363,531,871	BART1_0-u20880		T/C	TT	CC	Nontranslating CDS
Clone ID 3255462_1	363,531,898	BART1_0-u20880		A/G	GG	AA	Nontranslating CDS
JHI-Hv50k-2016-179690	389,686,103	HORVU.MOREX.r3.3HG0274650	I	A/G	GG	AA	DUF1645 family protein
Clone ID 3272635	391,906,604	HORVU.MOREX.r3.3HG0275090	I	G/C	GG	CC	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like protein 1
JHI-Hv50k-2016-183207	442,203,921	HORVU.MOREX.r3.3HG0281340	D	A/T	TT	AA	Receptor-like protein kinase
JHI-Hv50k-2016-183351	442,550,473	HORVU.MOREX.r3.3HG0281430	E	C/T	TT	CC	DNA repair helicase
JHI-Hv50k-2016-183478	443,111,006	HORVU.MOREX.r3.3HG0281530	E	C/A	CC	AA	DUF1645 family protein
JHI-Hv50k-2016-183463	443,119,491	HORVU.MOREX.r3.3HG0281540	I	T/C	TT	CC	RING/U-box superfamily protein

Date of access to database 1 February 2022; Intron—I, Exon—E, Downstream—D, Upstream—U, *—data from Barleymap.

The marker JHI-Hv50k-2016-174303 is located in the exon of gene HORVU.MOREX.r3.3HG0265210, encoding a Tudor/PWWP/MBT superfamily protein. These are conserved proteins found both in embryophytes and in their unicellular ancestors. It was suggested that they have been transmitted through evolution as conserved linear arrangements ('cassettes') [79]. Genes-encoding, leucine-rich repeat protein kinase family proteins are located in the regions closest to this locus. Plant receptor-like kinases (RLKs) are an important class of proteins acting in plant defense responses. RLKs have been identified to be involved in broad-spectrum, elicitor-initiated defense responses and as dominant resistance (R) genes in race-specific pathogen defense. Most defense-related RLKs are of the leucine-rich repeat (LRR) subclass [80].

SNP Clone ID 3272635 is in the intron of the HORVU.MOREX.r3.3HG0275090 gene encoding the SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily A-like protein involved in DNA replication [81] and is involved in the heat stress response in Arabidopsis [82].

5. Conclusions

Diagnostic efficiency of SNP markers for resistance QTL towards *Ptt* on chromosome 3H in the interval 45.82–54.53 cM was determined by KASP genotyping. Eleven markers showed high efficacy (97.5–100%) for co-segregation with resistance to *Ptt* in the DH population, CLS/Harrington. Three markers closely located to each other on the genetic and physical map were effective in two F_2 populations of crosses between cvs. Morex and Fox with susceptible cv. Gesine. Apparently, in cultivars CLS, Morex, and Fox, resistance to *Ptt* is determined by the same locus. Three markers showed high efficacy in the DH population and in the F_2 population, Local (Ecuador) k-21578/Gesine. Data suggests, at least two loci controlling *Ptt* resistance exist in the 47.0–54.3 cM chromosome region: one between 46.0 and 48.44 cM, and another between 51.27 and 54.8 cM. These regions were found to contain several genes involved in important plant functions, including disease response and signaling pathways.

The effectiveness of KASP genotyping for the validation of SNP markers related to Ptt barley resistance has been shown. Four allele-specific PCR markers were developed based on the KASP assay data and found to be effective to differentiate susceptible and resistant barley genotypes. The KASP and allele-specific PCR markers associated with *Ptt* resistance on chromosome 3H will be useful for pyramiding resistance QTLs in marker-assisted selection in barley-breeding programs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12040439/s1, Figure S1: DH Lines CLS/Harrington 12 dpi with *Ptt* isolate F18 scored on the second leaf of the seedling; Figure S2: Segregation on resistance to *Ptt* isolate F18 12 dpi in F_2 population Local k-21578/Gesine scored on the second leaf of the seedling; Figure S3: Segregation on resistance to *Ptt* isolate F18 12 dpi in F2 population Morex/Gesine scored on the second leaf of the seedling; Figure S4: Segregation on resistance to *Ptt* isolate F18 12 dpi in F_2 population Zolo/Gesine scored on the second leaf of the seedling; Figure S5: Segregation on resistance to *Ptt* isolate F18 12 dpi in F_2 population Fox/Gesine scored on the second leaf of the seedling; Table S1: KASP-genotyping results on DH and hybrid barley populations; Table S2: Allele-specific PCR markers of the QTL localized on chromosome 3H; Table S3: Resistance of barley genotypes to *Ptt* and data of molecular marker analysis; Figure S6: Results of PCR with primer 635 (marker Clone ID 3272635); Figure S7: Results of PCR with primer 351 (marker JHI-Hv50k-2016-183351), haplotypes T/C and with primer 463 (marker JHI-Hv50k-2016-183463), haplotypes T/G; Figure S8: Results of PCR with primer 478 (marker JHI-Hv50k-2016-183478) with different annealing temperature; Figure S9: Results of PCR with primer 478 (marker JHI-Hv50k-2016-183478); Table S4: Genes detected within 150 kb of the markers.

Author Contributions: O.A. and I.R. conceptualized and designed the experiments. N.L., A.Z., A.G., N.M. and O.B. performed the experiments. O.A., F.N. and I.R. analyzed the data and contributed to the writing and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Russian Fund of Basic Research (20-516-00007) and by the Belarus Fund of Basic Research B20R-131 20200504.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data supporting the findings and conclusions are contained within the article.

Acknowledgments: We gratefully acknowledge the financial support for this research by the Russian Fund of Basic Research and the Belarus Fund of Basic Research.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mathre, D.E. (Ed.) *Compendium of Barley Diseases*, 2nd ed.; American Phytopathological Society Press: St. Paul, MN, USA, 1997; ISBN 089054218X.
2. Brown, M.P.; Steffenson, B.J.; Webster, R.K. Host range of *Pyrenophora teres* f. *teres* isolates from California. *Plant Dis.* **1993**, *77*, 942–947.
3. Garozi, R.; Angelotti, P.; Feksa, H.R.; Tessmann, D.J. First Report of *Pyrenophora teres* f. *teres* Causing Leaf Spot on Wheat in Brazil. *Plant Dis.* **2020**, *104*, 3254. [CrossRef]
4. Perelló, A.E.; Couretot, L.; Curti, A.; Uranga, J.P.; Consolo, V.F. First report of spot lesion of wheat caused by *Pyrenophora teres* f. sp *maculata* observed in Argentina. *Crop Prot.* **2019**, *122*, 19–22. [CrossRef]
5. Mikhailova, L.A.; Ternyuk, I.G.; Mironenko, N.V. *Pyrenophora teres*, an agent causing wheat leaf spot. *Microbiology* **2010**, *79*, 561–565. [CrossRef]
6. Tóth, B.; Csősz, M.; Kopahnke, D.; Varga, J. First report on *Pyrenophora teres* causing lesions of wheat leaves in Hungary. *Plant Pathol.* **2008**, *57*, 385. [CrossRef]
7. Smedegård-Petersen, V. Pyrenophora teres f. *maculata* f. *nov.* and *Pyrenophora teres* f. teres on barley in Denmark. In *Yearbook of the Royal Veterinary and Agricultural University*; Royal Veterinary and Agricultural University: Copenhagen, Denmark, 1971; pp. 124–144.
8. Smedegård-Petersen, V. *Pathogenesis and Genetics of Net-Spot Blotch and Leaf Stripe of Barley Caused by Pyrenophora teres and Pyrenophora graminea, DSR Forlgag*; Royal Veterinary and Agricultural University: Copenhagen, Denmark, 1976; 176p.
9. Anisimova, A.V.; Mironenko, N.V.; Levshtanov, S.A. The first finding of the fungus *Pyrenophora teres* f. maculata in the Krasnodar region. *Plant Prot. News* **2011**, *3*, 53–56.
10. Lashina, N.M.; Afanasenko, O.S. Susceptibility to leaf blights of commercial barley cultivars in North-Western region of Russia. *Plant Prot. News* **2019**, *2*, 23–28. [CrossRef]
11. Afanasenko, O.S.; Koziakov, A.V.; Hedlay, P.E.; Lashina, N.M.; Anisimova, A.V.; Manninen, O.; Jalli, M.; Potokina, E.K. Mapping of the loci controlling the resistance to *Pyrenophora teres* f. *teres* and *Cochliobolus sativus* in two double haploid barley populations. *Russ. J. Genet. Appl. Res.* **2015**, *5*, 242–253. [CrossRef]
12. Afanasenko, O.S.; Jalli, M.; Pinnschmidt, H.O.; Filatova, O.; Platz, G.J. Development of an international standard set of barley differential genotypes for *Pyrenophora teres* f. *teres*. *Plant Pathol.* **2009**, *58*, 665–676. [CrossRef]
13. Tekauz, A. Characterization and distribution of pathogenic variation in *Pyrenophora teres* f. *teres* and *P. teres* f. maculata from western Canada. *Can. J. Plant Pathol.* **1990**, *12*, 141–148. [CrossRef]
14. Liu, Z.H.; Zhong, S.; Stasko, A.K.; Edwards, M.C.; Friesen, T.L. Virulence Profile and Genetic Structure of a North Dakota Population of *Pyrenophora teres* f. *teres*, the Causal Agent of Net Form Net Blotch of Barley. *Phytopathology* **2012**, *102*, 539–546. [CrossRef] [PubMed]
15. Steffenson, B.J.; Webster, R. Pathotype diversity of *Pyrenophora teres* f. *teres* on Barley. *Phytopatholoy* **1992**, *82*, 170–177. [CrossRef]
16. Oğuz, A.Ç.; Karakaya, A. Pathotypes of *Pyrenophora teres* on barley in Turkey. *Phytopathol. Mediterr.* **2017**, *56*, 224–234. [CrossRef]
17. Bouajila, A.; Zoghlami, N.; Al Ahmed, M.; Baum, M.; Ghorbel, A.; Nazari, K. Comparative virulence of *Pyrenophora teres* f. *teres* from Syria and Tunisia and screening for resistance sources in barley: Implications for breeding. *Lett. Appl. Microbiol.* **2011**, *53*, 489–502. [CrossRef]
18. Boungab, K.; Belabid, L.; Fortas, Z.; Bayaa, B. Pathotype diversity among Algerian isolates of *Pyrenophora teres* f. *teres*. *Phytopathol. Mediterr.* **2012**, *51*, 577–586. [CrossRef]
19. Jonsson, R.; Bryngelsson, T.; Gustafsson, M. Viulence studies of Swedish net blotch isolates (Drechslera teres) and identification of resistant barley lines. *Euphytica* **1997**, *94*, 209–218. [CrossRef]
20. Novakazi, F.; Göransson, M.; Stefánsson, T.S.; Hokka, M.; Jalli, M.; Hallson, J.H. Virulence of Icelandic *Pyrenophora teres* f. *teres* populations and resistance of Icelandic spring barley lines. *J. Plant Pathol.* **2022**, *104*, 205–213. [CrossRef]
21. Steffenson, B.J.; Hayes, P.M.; Kleinhofs, A. Genetics of seedling and adult plant resistance to net blotch (*Pyrenophora teres* f. *teres*) and spot blotch (*Cochliobolus sativus*) in barley. *Theor. Appl. Genet.* **1996**, *92*, 552–558. [CrossRef]

22. Raman, H.; Platz, G.J.; Chalmers, K.; Raman, R.; Read, B.J.; Barr, A.R.; Moody, D.B. Mapping of genetic regions associated with net form of net blotch resistance in barley. *Aust. J. Agric. Res.* **2003**, *54*, 1359–1367. [CrossRef]
23. Ma, Z.; Lapitan, N.L.; Steffenson, B. QTL mapping of net blotch resistance genes in a doubled-haploid population of six-rowed barley. *Euphytica* **2004**, *137*, 291–296. [CrossRef]
24. Cakir, M.; Gupta, S.; Platz, G.J.; Ablett, G.A.; Loughman, R.; Embiri, L.C.; Poulsen, D.; Li, C.D.; Lance, R.C.M.; Galwey, N.W.; et al. Mapping and validation of the genes for resistance to *Pyrenophora teres* f. *teres* in barley (*Hordeum vulgare* L.). *Aust. J. Agric. Res.* **2003**, *54*, 1369–1377. [CrossRef]
25. Cakir, M.; Gupta, S.; Li, C.; Hayden, M.; Mather, D.E.; Ablett, G.A.; Platz, G.J.; Broughton, S.; Chalmers, K.J.; Loughman, R.; et al. Genetic mapping and QTL analysis of disease resistance traits in the barley population Baudin × AC Metcalfe. *Crop Pasture Sci.* **2011**, *62*, 152–161. [CrossRef]
26. Friesen, T.L.; Faris, J.D.; Lai, Z.; Steffenson, B.J. Identification and chromosomal location of major genes for resistance to *Pyrenophora teres* in a doubled-haploid barley population. *Genome* **2006**, *49*, 855–859. [CrossRef] [PubMed]
27. Grewal, T.S.; Rossnagel, B.G.; Pozniak, C.J.; Scoles, G.J. Mapping quantitative trait loci associated with barley net blotch resistance. *Theor. Appl. Genet.* **2008**, *116*, 529–539. [CrossRef] [PubMed]
28. Grewal, T.S.; Rossnagel, B.G.; Scoles, G.J. Mapping quantitative trait loci associated with spot blotch and net blotch resistance in a doubled-haploid barley population. *Mol. Breed.* **2012**, *30*, 267–279. [CrossRef]
29. Lehmensiek, A.; Platz, G.J.; Mace, E.; Poulsen, D.; Sutherland, M.W. Mapping of adult plant resistance to net form of net blotch in three Australian barley populations. *Aust. J. Agric. Res.* **2007**, *58*, 1191–1197. [CrossRef]
30. Pierre, S.S.; Gustus, C.; Steffenson, B.J.; Dill-Macky, R.; Smith, K.P. Mapping net form net blotch and Septoria speckled leaf blotch resistance loci in barley. *Phytopathology* **2010**, *100*, 80–84. [CrossRef]
31. Manninen, O.M.; Jalli, M.; Kalendar, R.; Schulman, A.; Afanasenko, O.; Robinson, J. Mapping of major spot-type and net-type net blotch resistance genes in the Ethiopian barley (Hordeum vulgare) line CI 9819. *Genome* **2006**, *49*, 1564–1571. [CrossRef]
32. König, J.; Perovic, D.; Kopahnke, D.; Ordon, F. Mapping seedling resistance to net form of net blotch (*Pyrenophora teres* f. *teres*) in barley using detached leaf assay. *Plant Breed.* **2014**, *133*, 356–365. [CrossRef]
33. Koladia, V.M.; Faris, J.D.; Brueggeman, J.K.; Richards, R.S.; Chao, S.; Friesen, T.L. Genetic analysis of net form net blotch resistance in barley lines CIho 5791 and Tifang against a global collection of P. *teres* f. *teres* isolates. *Theor. Appl. Genet.* **2017**, *130*, 163–173. [CrossRef]
34. Martin, A.; Platz, G.J.; de Klerk, D.; Fowler, R.A.; Smit, F.; Potgieter, F.G.; Prins, R. Identification and mapping of net form of net blotch resistance in south African barley. *Mol. Breed.* **2018**, *38*, 53. [CrossRef]
35. Dinglasan, E.; Hickey, L.; Ziems, L.; Fowler, R.; Anisimova, A.; Baranova, O.; Lashina, N.; Afanasenko, O. Genetic characterization of resistance to *Pyrenophora teres* f. *teres* in the International Barley Differential Canadian Lake Shore. *Front. Plant Sci.* **2019**, *10*, 326. [CrossRef] [PubMed]
36. Wonneberger, R.; Ficke, A.; Lillemo, M. Identification of quantitative trait loci associated with resistance to net form net blotch in a collection of Nordic barley germplasm. *Theor. Appl. Genet.* **2017**, *130*, 2025–2043. [CrossRef] [PubMed]
37. Vatter, T.; Maurer, A.; Kopahnke, D.; Perovic, D.; Ordon, F.; Pillen, K. A nested association mapping population identifies multiple small effect QTL conferring resistance against net blotch (*Pyrenophora teres* f. *teres*) in wild barley. *PLoS ONE* **2017**, *12*, e0186803. [CrossRef] [PubMed]
38. Richards, J.K.; Friesen, T.L.; Brueggeman, R.S. Association mapping utilizing diverse barley lines reveals net form net blotch seedling resistance/susceptibility loci. *Theor. Appl. Genet.* **2017**, *130*, 915–927. [CrossRef]
39. Amezrou, R.; Verma, R.P.S.; Chao, S.; Brueggeman, R.S.; Belgadi, L.; Arbaoui, M.; Rehman, S.; Gyawali, S. Genome-wide association studies of net form of net blotch resistance at seedling and adult plant stages in spring barley collection. *Mol. Breed.* **2018**, *38*, 58. [CrossRef]
40. Rozanova, I.V.; Lashina, N.M.; Mustafin, Z.S.; Gorobets, S.A.; Efimov, V.M.; Afanasenko, O.S.; Khlestkina, E.K. SNPs associated with barley resistance to isolates of *Pyrenophora teres* f. *teres*. *BMC Genom.* **2019**, *20*, 292. [CrossRef]
41. Novakazi, F.; Afanasenko, O.; Anisimova, A.; Platz, J.; ·Snowdon, R.; Kovaleva, O.; Zubkovich, A.; Ordon, F. Genetic analysis of a worldwide barley collection for resistance to net form of net blotch disease (*Pyrenophora teres* f. *teres*). *Theor. Appl. Genet.* **2019**, *132*, 2633–2650. [CrossRef]
42. Adhikari, A.; Stefenson, B.J.; Smith, K.P.; Smith, M.; Dill-Macky, R. Identification of quantitative trait loci for net form net blotch resistance in contemporary barley breeding germplasm from the USA using genome-wide association mapping. *Theor. Appl. Genet.* **2020**, *133*, 1019–1037. [CrossRef]
43. Daba, S.D.; Horsley, R.D.; Brueggeman, R.; Chao, S.; Mohammadi, M. Genome-623 wide association studies and candidate gene identification for leaf scald and net blotch in 624 barley (*Hordeum vulgare* L.). *Plant Dis.* **2019**, *103*, 880–889. [CrossRef]
44. Clare, S.J.; Wyatt, N.A.; Brueggeman, R.S.; Friesen, T.L. Research advances in the *Pyrenophora teres*—Barley interaction. *Mol. Plant Pathol.* **2020**, *21*, 272–288. [CrossRef] [PubMed]
45. Mode, C.J.; Schaller, C.W. Two additional factors for host resistance to net blotch in barley. *Agron. J.* **1958**, *50*, 15–18. [CrossRef]
46. Bockelman, H.E.; Sharp, E.L.; Eslick, R.F. Trisomic analysis of genes for resistance to scald and net blotch in several barley cultivars. *Can. J. Bot.* **1977**, *55*, 2142–2148. [CrossRef]
47. Fowler, R.A.; Platz, G.J.; Bell, K.L.; Fletcher, S.E.H.; Franckowiak, J.D.; Hickey, L.T. Pathogenic variation of *Pyrenophora teres* f. *teres* in Australia. *Austral. Plant Pathol.* **2017**, *46*, 115–128. [CrossRef]

48. Mascher, M.; Wicker, T.; Jenkins, J.; Plott, C.; Lux, T.; Koh, C.S.; Ens, J.; Gundlach, H.; Boston, L.B.; Tulpová, Z.; et al. Long-read sequence assembly: A technical evaluation in barley. *Plant Cell* **2021**, *33*, 1888–1906. [CrossRef]
49. Vida, G.; Gál, M.; Uhrin, A.; Veisz, O.; Syed, N.H.; Flavell, A.J.; Wang, Z.; Bedő, Z. Molecular markers for the identification of resistance genes and marker-assisted selection in breeding wheat for leaf rust resistance. *Euphytica* **2009**, *170*, 67–76. [CrossRef]
50. Gupta, S.; Li, C.D.; Loughman, R.; Cakir, M.; Platz, G.; Westcott, S.; Bradley, J.; Broughton, S.; Lance, R. Quantitative trait loci and epistatic interactions in barley conferring resistance to net type net blotch (*Pyrenophora teres* f. *teres*) isolates. *Plant Breed.* **2010**, *129*, 362–368. [CrossRef]
51. Bespalova, L.A.; Vasilyev, A.V.; Karlov, G.I.; Soloviev, A.A.; Afanasenko, O.S.; Davoyan, R.O.; Davoyan, E.R.; Divashuk, M.G.; Koshkin, V.A.; Potokina, E.K.; et al. Application of molecular markers in winter wheat and triticale breeding in Krasnodar Lukyanenko Research Institute of Agriculture. In Proceedings of the International Conferece of Wheat Genetic Resources and Genomics (WGRG), Novosibirsk, Russia, 28 August–1 September 2011; p. 48. Available online: http://www.bionet.nsc.ru/wgrg/tezis.pdf (accessed on 16 February 2022).
52. Lüders, T.; Ahlemeyer, J.; Förster, J.; Weyen, J.; Roßa, E.; Korzun, V.; Lex, J.; Friedt, W.; Ordon, F. Verification of marker-trait associations in bi-parental winter barley (*Hordeum vulgare* L.) DH populations. *Mol. Breed.* **2016**, *36*, 14. [CrossRef]
53. Semagn, K.; Babu, R.; Hearne, S.; Olsen, M. Single nucleotide polymorphism genotyping using Kompetitive allele specific PCR (KASP): Overview of the technology and its application in crop improvement. *Mol. Bred.* **2014**, *33*, 1–14. [CrossRef]
54. He, C.; Holme, J.; Anthony, J. SNP genotyping: The KASP assay. *Methods Mol. Biol.* **2014**, *1145*, 75–86. [CrossRef]
55. Ertiro, B.T.; Ogugo, V.; Worku, M.; Das, B.; Olsen, M.; Labuschagne, M.; Semagn, K. Comparison of Kompetitive Allele Specific PCR (KASP) and genotyping by sequencing (GBS) for quality control analysis in maize. *BMC Genom.* **2015**, *16*, 908. [CrossRef] [PubMed]
56. Rasheed, A.; Wen, W.; Gao, F.; Zhai, S.; Jin, H.; Liu, J.; Guo, Q.; Zhang, Y.; Dreisigacker, S.; Xia, X.; et al. Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. *Theor. Appl. Genet.* **2016**, *129*, 1843–1860. [CrossRef]
57. Tan, C.-T.; Yu, H.; Yang, Y.; Xu, X.; Chen, M.; Rudd, J.C.; Xue, Q.; Ibrahim, A.M.H.; Garza, L.; Wang, S.; et al. Development and validation of KASP markers for the greenbug resistance gene Gb7 and the Hessian fly resistance gene H32 in wheat. *Theor. Appl. Genet.* **2017**, *130*, 1867–1884. [CrossRef] [PubMed]
58. Tekauz, A. A numerical scale to classify reactions of barley to Pyrenophora teres. *Can. J. Plant Pathol.* **1985**, *7*, 181–183. [CrossRef]
59. Cantalapiedra, C.P.; Boudiar, R.; Casas, A.M.; Igartua, E.; Contreras-Moreira, B. BARLEYMAP: Physical and genetic mapping of nucleotide sequences and annotation of surrounding loci in barley. *Mol. Breed.* **2015**, *35*, 13. [CrossRef]
60. Mascher, M.; Gundlach, H.; Himmelbach, A.; Stein, N. A chromosome conformation capture ordered sequence of the barley genome. *Nature* **2017**, *544*, 427–433. [CrossRef] [PubMed]
61. Mascher, M.; Muehlbauer, G.J.; Rokhsar, D.S.; Chapman, J.; Schmutz, J.; Barry, K.; Muñoz-Amatriaín, M.; Close, T.J.; Wise, R.P.; Schulman, A.H.; et al. Anchoring and ordering NGS contig assemblies by population sequencing (POPSEQ). *Plant J.* **2013**, *76*, 718–727. [CrossRef] [PubMed]
62. Okonechnikov, K.; Golosova, O.; Fursov, M.; Ugene Team. Unipro UGENE: A unified bioinformatics toolkit. *Bioinformatics* **2012**, *28*, 1166–1167. [CrossRef]
63. Yun, S.J.; Gyenis, L.; Bossolini, E.; Hayes, P.M.; Matus, I.; Smith, K.P.; Steffenson, B.J.; Tuberosa, R.; Muehlbauer, G.J. Validation of Quantitative Trait Loci for Multiple Disease Resistance in Barley Using Advanced Backcross Lines Developed with a Wild Barley. *Crop Sci.* **2006**, *46*, 1179–1186. [CrossRef]
64. König, J.; Perovic, D.; Kopahnke, D.; Ordon, F. Development of an efficient method for assessing resistance to the net type of net blotch (*Pyrenophora teres* f. *teres*) in winter barley and mapping of quantitative trait loci for resistance. *Mol. Breed.* **2013**, *32*, 641–650. [CrossRef]
65. Ye, Y.; Ding, Y.; Jiang, Q.; Wang, F.; Sun, J.; Zhu, C. The role of receptor-like protein kinases (RLKs) in abiotic stress response in plants. *Plant Cell Rep.* **2017**, *36*, 235–242. [CrossRef] [PubMed]
66. Vashisht, A.A.; Tuteja, N. Stress responsive DEAD-box helicases: A new pathway to engineer plant stress tolerance. *J. Photochem. Photobiol. B Biol.* **2006**, *84*, 150–160. [CrossRef] [PubMed]
67. Cui, Y.; Wang, M.; Zhou, H.; Li, M.; Huang, L.; Yin, X.; Zhao, G.; Lin, F.; Xia, X.; Xu, G. OsSGL, a novel DUF1645 domain-containing protein, confers enhanced drought tolerance in transgenic rice and Arabidopsis. *Front. Plant Sci.* **2016**, *7*, 2001. [CrossRef] [PubMed]
68. Liu, K.; Li, M.; Zhang, B.; Cui, Y.; Yin, X.; Xia, X. Poaceae Orthologs of Rice OsSGL, DUF1645 Domain-Containing Genes, Positively Regulate Drought Tolerance, Grain Length and Weight in Rice. *Rice Sci.* **2022**, *29*, 1–21.
69. Yang, H.; Wang, H.; Jiang, J.; Du, M.; Li, J. The Sm Gene Conferring Resistance to Gray Leaf Spot Disease Encodes a NBS-LRR Plant Resistance Protein in Tomato. *Theor. Appl. Genet.* **2022**, 1–10. [CrossRef]
70. Faccini, N.; Delbono, S.; Çelik Oğuz, A.; Cattivelli, L.; Valè, G.; Tondelli, A. Resistance of European Spring 2-Row Barley Cultivars to Pyrenophora graminea and Detection of Associated Loci. *Agronomy* **2021**, *11*, 374. [CrossRef]
71. Rehman, S.; Mahmood, T. Functional role of DREB and ERF transcription factors: Regulating stress-responsive network in plants. *Acta Physiol. Plant.* **2015**, *37*, 178. [CrossRef]
72. Sharma, M.; Pandey, A.; Pandey, G.K. Role of plant U-BOX (PUB) protein in stress and development. *Plant Stress* **2013**, *7*, 1–9.

73. Liebrand, T.W.; van den Berg, G.C.; Zhang, Z.; Smit, P.; Cordewener, J.H.; America, A.H.; Sklenar, J.; Jones, A.M.; Tameling, W.I.; Robatzek, S.; et al. Receptor-like kinase SOBIR1/EVR interacts with receptor-like proteins in plant immunity against fungal infection. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 10010–10015. [CrossRef]
74. Todorovska, E. Retrotransposons and their role in plant—genome evolution. *Biotechnol. Biotechnol. Equip.* **2007**, *21*, 294–305. [CrossRef]
75. Barkan, A.; Small, I. Pentatricopeptide repeat proteins in plants. *Annu. Rev. Plant Biol.* **2014**, *65*, 415–442. [CrossRef] [PubMed]
76. Xu, X.; Zhang, L.; Zhao, W.; Fu, L.; Han, Y.; Wang, K.; Yan, L.; Li, Y.; Zhang, X.-H.; Min, D.-H. Genome-wide analysis of the serine carboxypeptidase-like protein family in Triticum aestivum reveals TaSCPL184-6D is involved in abiotic stress response. *BMC Genom.* **2021**, *22*, 350. [CrossRef] [PubMed]
77. Laxmi, A. DUF581 is plant specific FCS-like zinc finger involved in protein-protein interaction. *PLoS ONE* **2014**, *9*, e99074. [CrossRef]
78. Knoth, C.; Salus, M.S.; Girke, T.; Eulgem, T. The synthetic elicitor 3,5-dichloroanthranilic acid induces NPR1-dependent and npr1-independent mechanisms of disease resistance in arabidopsis. *Plant Physiol.* **2009**, *150*, 333–347. [CrossRef]
79. Alvarez-Venegas, R.; Avramova, Z. Evolution of the PWWP-domain encoding genes in the plant and animal lineages. *BMC Evol. Biol.* **2012**, *12*, 101. [CrossRef] [PubMed]
80. Goff, K.E.; Ramonell, K.M. The role and regulation of receptor-like kinases in plant defense. *Gene Regul. Syst. Biol.* **2007**, *1*, 167–175. [CrossRef]
81. Song, Z.T.; Liu, J.X.; Han, J.J. Chromatin remodeling factors regulate environmental stress responses in plants. *J. Integr. Plant Biol.* **2021**, *63*, 438–450. [CrossRef]
82. Buszewicz, D.; Archacki, R.; Palusiński, A.; Kotliński, M.; Fogtman, A.; Iwanicka-Nowicka, R.; Sosnowska, K.; Kuciński, J.; Pupel, P.; Olędzki, J.; et al. HD2C histone deacetylase and a SWI/SNF chromatin remodelling complex interact and both are involved in mediating the heat stress response in Arabidopsis. *Plant Cell Environ.* **2016**, *39*, 2108–2122. [CrossRef]