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High Resolution Mapping of a *Hordeum bulbosum*-Derived Powdery Mildew Resistance Locus in Barley Using Distinct Homologous Introgression Lines

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Powdery mildew caused by *Blumeria graminis* f. sp. *hordei (Bgh)* is one of the main foliar diseases in barley (*Hordeum vulgare* L.; *Hv*). Naturally occurring resistance genes used in barley breeding are a cost effective and environmentally sustainable strategy to minimize the impact of pathogens, however, the primary gene pool of *H. vulgare* contains limited diversity owing to recent domestication bottlenecks. To ensure durable resistance against this pathogen, more genes are required that could be unraveled by investigation of secondary barley gene-pool. A large set of *Hordeum bulbosum* (*Hb*) introgression lines (ILs) harboring a diverse set of desirable resistance traits have been developed and are being routinely used as source of novel diversity in gene mapping studies. Nevertheless, this strategy is often compromised by a lack of recombination between the introgressed fragment and the orthologous chromosome of the barley genome. In this study, we fine-mapped a *Hb* gene conferring resistance to barley powdery mildew. The initial genotyping of two *Hb* ILs mapping populations with differently sized 2HS introgressions revealed severely reduced interspecific recombination in the region of the introgressed segment, preventing precise localization of the gene. To overcome this difficulty, we developed an alternative strategy, exploiting intraspecific recombination by crossing two *Hv/Hb* ILs with collinear *Hb* introgressions, one of which carries a powdery mildew resistance gene, while the other doesn't. The intraspecific recombination rate in the *Hb*-introgressed fragment of 2HS was approximately 20 times higher than it was in the initial simple ILs mapping populations. Using high-throughput genotyping-by-sequencing (GBS), we allocated the resistance gene to a 1.4 Mb interval, based on an estimate using the *Hv* genome as reference, in populations of only 103 and 146 individuals, respectively, similar to what is expected at this locus in barley. The most likely candidate resistance gene within this

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interval is part of the coiled-coil nucleotide-binding-site leucine-rich-repeat (CC-NBS-LLR) gene family, which is over-represented among genes conferring strong dominant resistance to pathogens. The reported strategy can be applied as a general strategic approach for identifying genes underlying traits of interest in crop wild relatives.

Keywords: introgression lines, *Hordeum bulbosum*, recombination, mapping, powdery mildew, resistance, crop wild relative

INTRODUCTION

The increased genetic uniformity of cultivated crops, makes them highly vulnerable to various biotic and abiotic stresses, leading to crop yield losses and serious food security issues (Hoisington et al., 1999). Disease resistance breeding is a cost effective and environmentally sustainable strategy for minimizing the damage caused by plant pathogens. Hence, plant breeders are continuously working to discover novel sources of genetic resistance. Crop wild relatives (CWRs) are a reservoir of genetic variation providing an important source of novel alleles for the genetic improvement of cultivated species. Crosses between cultivars and CWRs have been carried out in several crop species to unlock this favorable genetic diversity (Tanksley and McCouch, 1997; Feuillet et al., 2008). Some prominent examples of the introgression of favorable disease resistance alleles from CWRs are the introductions of late blight resistance into potato from the wild potato *Solanum demissum* (Rao, 1979; Prescott-Allen and Prescott-Allen, 1986), and of stem rust resistance genes *Sr21* (Chen et al., 2015) and *Sr39* (Kerber and Dyck, 1990), both effective against the race Ug99, into bread wheat from *Triticum monococcum* and *Aegilops speltoides*, respectively.

Barley (*Hordeum vulgare* L.), the fourth most important cereal crop in the world, is affected each year by up to 30% potential yield loss due to pests and diseases (Savary et al., 2012). Limitations in the availability of novel resistance genes or alleles in the primary gene pool of barley, comprising the cultivated barley *H. vulgare* spp. *vulgare* and its wild progenitor *H. vulgare* spp. *spontaneum*, has directed the focus of research toward other barley gene pools. Bulbous barley, *Hordeum bulbosum* (*Hb*), a wild self-incompatible species and the only member of the secondary gene pool of cultivated barley (von Bothmer et al., 1995) is resistant to many barley pathogens (Xu and Kasha, 1992; Walther et al., 2000). A large panel of *Hb* introgression lines (ILs) harboring a diverse spectrum of resistance traits has been developed during recent years (Pickering et al., 1995; Johnston et al., 2009). This resource comprises ILs carrying, among others, the barley leaf rust resistance gene *Rph26* on chromosome $1H^bL$ (Yu et al., 2018); barley leaf rust gene *Rph22* (Johnston et al., 2013) and barley mild mosaic virus gene $Rym16^{Hb}$ (Ruge-Wehling et al., 2006) both located on chromosome $2H^bL$; barley yellow dwarf virus resistance gene $Ryd4^{Hb}$ on chromosome $3H^bL$ (Scholz et al., 2009) as well as loci conferring powdery mildew resistance located on chromosome $2H^bS$, $2H^bL$ and $7H^bL$ in barley/*Hb* introgression lines (Xu and Kasha, 1992; Pickering et al., 1995; Shtaya et al., 2007). These ILs represent a unique genetic resource for improving barley resistance to pathogens and for scientific investigation of resistance mechanisms as they provide access to further genetic diversity out of the primary gene pool of barley (Tanksley and Nelson, 1996; Zamir, 2001; Johnston et al., 2009). Genotyping-by-sequencing (GBS) of 145 *Hv/Hb* introgression lines (Wendler et al., 2014, 2015) has provided an extensive pool of molecular markers, sequence resources and single-nucleotide polymorphisms (SNPs) information, greatly improving the efficiency of mapping *Hb* loci.

Since the identification and the extensive use of the durable and complete *mlo* resistance gene in European germplasm (Jørgensen, 1992), the threat of powdery mildew to barley has been largely mitigated. However, *mlo* is associated with yield penalties (Kjær et al., 1990) and increased susceptibility to some hemibiothrophic and necrotrophic fungi (Jarosch et al., 1999; Kumar et al., 2001; McGrann et al., 2014). Thus, the search for alternative sources of resistance to powdery mildew remains important for barley breeding (Czembor, 2002; Corrion and Day, 2015). The wild progenitor *H. vulgare* spp. *spontaneum* proved to be a great source of diversity of resistance genes (Fischbeck et al., 1976; Dreiseitl and Bockelman, 2003). However, these genes are mostly race-specific, major resistance genes. They are particularly effective, but prone to be rapidly overcome by emerging new pathotypes. Therefore, the search for non-host resistance, from plant species to which the pathogen is not coevolutionary adapted, represent a great hope to achieve a complete and durable resistance. Several *Hv*/*Hb* introgression lines carrying a locus conferring powdery mildew resistance have been described (Xu and Kasha, 1992; Pickering et al., 1995, 1998; Shtaya et al., 2007). The *Hb* accession A42 displays a dominant high resistance to powdery mildew that has been localized on the short arm of chromosome $2H^b$ in preliminary studies (Szigat and Szigat, 1991; Michel, 1996). Interestingly, several significant QTLs and major genes associated with powdery mildew resistance have repeatedly been reported in this region in cultivated barley (Backes et al., 2003; von Korff et al., 2005; Řepková et al., 2009). Moreover, a resistance gene to powdery mildew has been reported in this region in various *Hb* accessions (Pickering et al., 1995; Zhang et al., 2001; Shtaya et al., 2007).

While the potential value of untapped genetic diversity of CWR is immense, their application in breeding programs through the use of ILs is hampered by negative linkage drag, mainly caused by severely repressed genetic recombination (Wijnker and de Jong, 2008; Prohens et al., 2017), which can confer yield penalties or other unfavorable characteristics (Hospital, 2001). The degree of drag is correlated with the size of introgressed CWR chromatin segments, and thus can be mitigated by reducing the size of respective introgressions through recombination (Frisch and Melchinger, 2001). However, the efficient utilization of *Hb* germplasm in barley crop

improvement and the genetic mapping of loci contributed by *Hb* suffers from highly reduced frequency of recombination in introgressed intervals up to 14-fold compared to intraspecific barley crosses (Ruge et al., 2003; Ruge-Wehling et al., 2006; Kakeda et al., 2008; Johnston et al., 2013). Possible explanations for this phenomenon include excessive sequence diversity, structural variation among *Hordeum* genomes, and probably other unknown mechanisms (Pickering, 1991; Hoffmann et al., 2004; Wendler et al., 2017). To reduce the negative linkage drag, precise delimitation of the causal gene is required, which usually demands intensive screening of large segregating *Hv/Hb* ILs populations.

Canady et al. (2006) compared the recombination rate in crosses between cultivated tomato (*Solanum lycopersicum*) and ILs with *Solanum lycopersicoides* fragments in *S. lycopersicum* backgrounds with the recombination in crosses between ILs with *S. lycopersicoides* fragments and ILs with *Solanum pennellii* fragments in the same region. They showed that tomato ILs with overlapping fragments from closely related species exhibited increased recombination rates in those fragments. Similarly, in barley, Johnston et al. (2015) demonstrated the usefulness of intraspecific recombination between *Hb* ILs to overcome the negative linkage between genes conferring pathogen resistance and reduced yield. They crossed two recombinant ILs containing an *Hb* locus on chromosome 2HL comprising the genes *Rph22* and $Rym16^{Hb}$, together with the proximal region of the original introgression for one of them, and the distal region for the other. They obtained four lines with reduced introgressions around the locus of interest for which the yield was close to the one of the recurrent *Hv* genotype.

The current study aimed to map a dominant powdery mildew resistance locus on chromosome $2H^bS$ introgressed from the tetraploid A42 powdery mildew resistant *Hb* accession into a susceptible barley cultivar "Borwina." Mapping in populations of over 200 F7 and BC1F6 from crosses between a susceptible barley cultivar and an *Hb* IL showed severely repressed recombination between the introgressed segment and the *Hv* genome. To overcome this difficulty, we exploited intraspecific recombination instead of interspecific recombination by crossing the IL carrying the resistance locus with another *Hv/Hb* IL carrying a homologous *Hb* introgression without resistance loci.

MATERIALS AND METHODS

Fungal Isolate and Phenotyping Test

The swiss powdery mildew field isolate CH4.8 was chosen based on its hypersensitive response it triggers on resistant plants and its capacity to overcome the resistance gene *MlRu2* present in Borwina, allowing a clear discrimination between resistant and susceptible plants. The resistance test was carried out in two technical replicates by detached leaf assay on the second seedling leaf sampled 14 days after sowing, as described in Hoseinzadeh et al. (2019). The inoculated detached leaves were kept for 7 days in a growth cabinet (MLR-352H, Panasonic, Japan) with LED light sources (S2 20W matt nw, ARTEKO-LED, Germany) at 20°C with 60% humidity and a 16:8 h photoperiod. Powdery mildew resistance phenotype was scored macroscopically based on percentage infection area as described by Mains and Dietz (1930). Plants without mycelial growth and sporulation but presenting necrotic flecks (infection type 1)were classified as resistant, while those having infection types similar to the susceptible parent "Borwina," with sporulation of well-developed colonies (infection types 3 and 4) were classified as susceptible.

Plant Material and Population Development

Initial genetic mapping was performed on the F7 and BC1F6 populations "5216" and "4176," respectively, both derived from crossing a tetraploid derivative of the colchicine treated German winter barley cultivar "Borwina" and the tetraploid *Hb* accession A42, which is resistant to several European barley powdery mildew isolates. Both "5216" and "4176" populations were derived from selfing of a single plant from the previous generation established as both resistant and heterozygous at the resistance locus. The crossing scheme used to generate those populations is described in **Figure 1**.

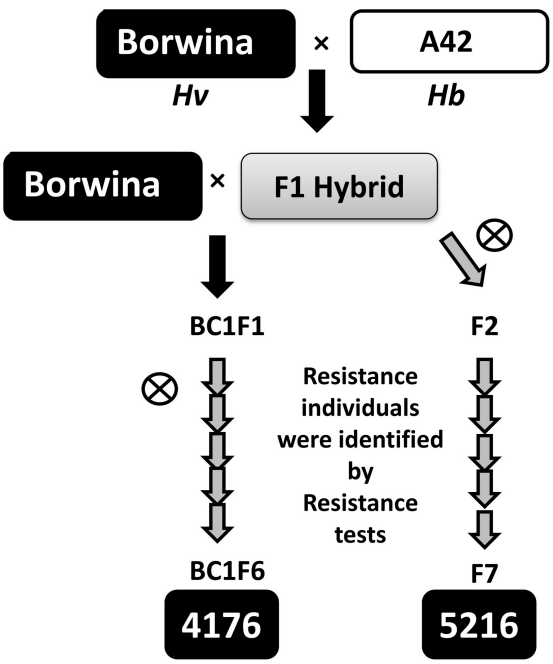

FIGURE 1 | Schematic outline of the mapping population design. The tetraploid *Hb* accession A42, resistant to the isolate CH4.8, was crossed to the tetraploid derivative of the susceptible barley cultivar "Borwina." From tetraploid F1 hybrids, two different introgression mapping populations were developed: the "4176" population was developed by backcrossing the F1 hybrid once to the parent "Borwina," followed by five generations of selfing. A gray arrow indicates a selfing generation. The "5216" population was derived from six generations of selfing from the F1 hybrid. In the course of development of populations "4176" and "5216," the generations were diploid from BC1F2 and F2, respectively. Through the population development, each new selfing generation was obtained by selecting and selfing a single resistant heterozygous plant identified by resistance test with the powdery mildew isolate CH4.8 and marker data analysis, in order to promote recombination.

We hypothesized that differences in the sequence or organization between *Hv* and *Hb* orthologous genome regions would severely reduce meiotic recombination. To test this hypothesis, we analyzed intraspecific *Hb*/*Hb* recombination in a *Hv* background. We generated two F2 populations by crossing two independent *Hv*/*Hb* ILs carrying independent but overlapping *Hb* introgressions at the terminal 2HS chromosome, thus representing different *Hb* genotypes at the resistance locus. Three introgression lines "IL 88," "IL 99," and "IL 116," developed in New Zealand Institute for Crop and Food Research, were selected that carry independent *Hb* introgressions at end of the short arm of barley chromosome 2H (Wendler et al., 2015; **Table 1**). These three ILs were phenotyped for resistance against isolate CH4.8 (**Figure 2**). Only "IL 88" displayed full susceptibility to CH4.8 and was used for further population development.

The homozygous resistant ILs 5216/4_40 and 4176/26_33 from the populations "5216" and "4176" were crossed to the susceptible "IL 88." From each cross, a single F1 plant was selfed, resulting in 103 and 146 F2 seeds, respectively. These two F2 populations were named "dIL_5216" and "dIL_4176," respectively.

Genomic DNA Extraction

Genomic DNA was extracted from third leaves of barley seedlings using a guanidine isothiocyanate-based DNA isolation method in 96-well plate format as described earlier (Milner et al., 2018). dsDNA concentration was measured by Qubit® 2.0 Fluorometer using the Qubit™ dsDNA BR (Broad Range) Assay Kit (Invitrogen, Carlsbad, CA, United States) following the manufacturer's protocol.

Marker Development

To screen the initial IL mapping populations "4176" and "5216" for recombinants, nine CAPS markers (**Supplementary Table 1**) were designed to be evenly distributed over the distal 20 cM of barley chromosome 2HS, based on the conserved interspecific SNPs identified by targeted enrichment re-sequencing of 145 *Hb* ILs (Wendler et al., 2015). This set of ILs included the F4 homozygous 2HS IL (3026) from the mapping population "5216" and its associated donor lines, plus four additional *Hv* cultivars and four *Hb* accessions (Wendler et al., 2015). Only conserved *Hv*/*Hb* SNPs with a minimum of six-fold read coverage and located in the target region on the barley draft genome (International Barley Genome Sequencing Consortium., 2012) were selected and converted into CAPS markers using SNP2CAPS software (Thiel et al., 2004). Primer design was carried out using the default settings of Primer3 v.0.4.01 (Koressaar and Remm, 2007; O'Halloran, 2015) with minor modifications: The primer length was set between 19–21 bp. Primer melting temperature (Tm) was set to minimum Tm = 58°C, optimum Tm = 59°C and maximum Tm = 60°C. The product size was defined to be between 700 and 1,000 bp and Guanine-Cytosine content (GC-content) was set within the range of 50–55%.

CAPS Genotyping

Genotyping of populations "4176" and "5216" with the described CAPS markers was performed in a 20 µl PCR reaction volume including 20 ng genomic DNA, 0.1 U of HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany), 1x PCR reaction buffer containing 15 mM $MgCl_2$ (Qiagen, Hilden, Germany), 0.2 mM of each dNTP (Fermentas, Fermentas, St. Leon-Rot, Germany), and 0.5 mM of each primer. All fragments were amplified using the following touchdown PCR profile: an initial denaturing step of 15 min at 95°C was followed by four cycles with denaturation at 95°C for 30 s, annealing at 62°C for 30 s (decreasing by 1°C per cycle), and extension at 72°C for 30 s. A final extension step was performed at 72°C for 7 min. The enzymatic digestion of the amplicons was performed in a 10 µl volume containing 5 µl of PCR product, 1× of appropriate buffer (New England Biolabs, Hitchin, United Kingdom), 1 U of enzyme (New England Biolabs, Hitchin, United Kingdom) and adjusted to final volume by adding molecular biology grade pure water. The reaction mix was incubated for 1 h at recommended incubation temperature. The digested PCR products were resolved by 1.5–2.5% gel-electrophoresis depending on amplicon size.

Genotyping-by-Sequencing and Data Analysis

GBS was used, following published procedures (Mascher et al., 2013b), to check the genetic purity and state of heterozygosity of F1 hybrid seeds derived from crosses between overlapping 2HS introgression lines as well as to genotype the whole F2 populations derived from these crosses. DNA of the progeny and parental lines were pooled per Illumina HiSeq2500 lane in an equimolar manner and sequenced for 107 cycles, single read, using a custom sequencing primer as previously described (Hoseinzadeh et al., 2019). The reads were aligned to the TRITEX genome assembly of barley cultivar Morex (Monat et al., 2019) with BWA-MEM version 0.7.12a (Li and Durbin, 2009). Variants were filtered following the protocol of Milner et al. (2018) for a minimum depth of sequencing of four to accept a genotype call, a minimum

TABLE 1 | *Hb* introgression lines containing segments overlapping with IL "4176" and IL "5216" and their observed resistance phenotype to the *Bgh* isolate CH4.8.

IPK ID	IL code (New Zealand)	Crossing scheme	Introgression location GBS	Start (Mb)	End (Mb)	Phenotype of ILs to isolate CH4.8
88	200A3/7/M1	Emir x A17/1	2HS	0	19,4	Susceptible
99	213G3/2/2/2/1	Emir x (2920/4 x Tinos16/1)	2HS, 6HS	0	22,1	Resistant
116	230H24/5/M1/M1	Morex x 2032	2HS	0	19,4	Resistant

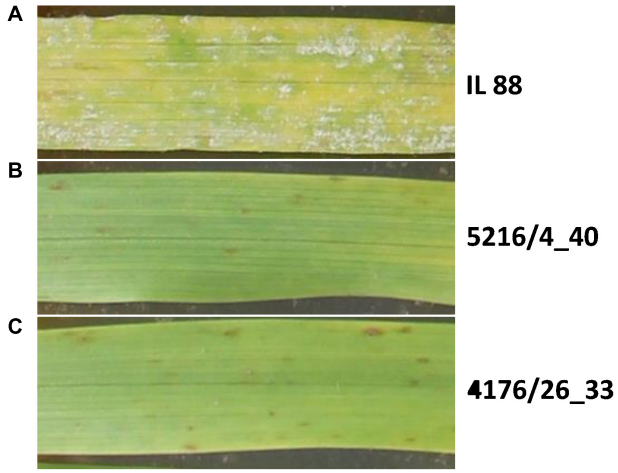

FIGURE 2 | Powdery mildew infection (isolate CH4.8) on the second leaf of seedling from the three independent *Hv*/*Hb* parental ILs, 7 days after inoculation. **(A)** Phenotype of the susceptible parental introgression line "IL 88" population "dIL_5216" and "dIL_4176." **(B)** Phenotype of the resistant parental introgression line 5216/4_40 from population "dIL_5216." **(C)** Phenotype of the resistant parental introgression line 4176/26_33 from population "dIL_4176."

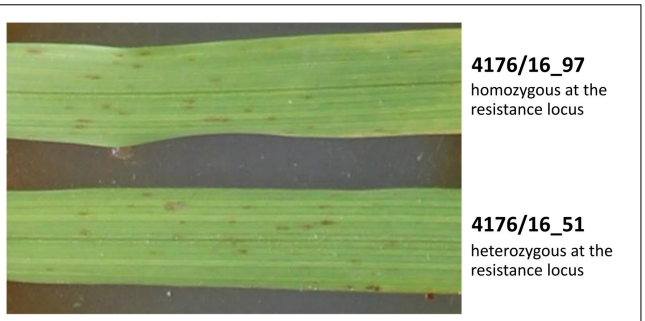

FIGURE 3 | Powdery mildew infection (isolate CH4.8) on the second leaf of seedling from two BC2F6 ILs from population "4176," 7 days after inoculation. 4176/16_97 is homozygous at the resistance locus whereas 4176/16_51 is heterozygous. Their resistance phenotype is identical and present necrotic lesions characteristic of HR.

mapping quality score of the SNPs (based on read depth ratios calculated from the total read depth and depth of the alternative allele) of six, a maximum fraction of heterozygous call of 70% and a maximum fraction of 25% of missing data. The resulting tables of polymorphisms are provided in **Supplementary Tables 2**, **3**.

RESULTS

Inheritance of the Resistance Contributed by *Hb*

The susceptible parental *Hv* genotype "Borwina" consistently displayed a leaf infection area of ≥80% in all experiments. The scoring rates of all susceptible individuals of both populations "4176" and "5216" was similar to the susceptible parent and did not significantly vary between phenotyping experiments, indicating high infection efficiency and reproducibility in all phenotyping experiments. The resistance phenotype to the CH4.8 powdery mildew isolate of plants carrying the *Hb* introgressed segment in a heterozygous state was identical to that of plants homozygous for the *Hb* fragment (**Figure 3**). The resistance phenotype was invariably accompanied with a hypersensitive response (HR) forming a necrotic lesion.

Phenotypic segregation for powdery mildew resistance against CH4.8 isolate was consistent with a 3:1 ratio (resistant/susceptible, R/S, $P < 0.05$) in all mapping populations, indicating the control of resistance by a single dominant resistance gene (**Table 2**). We propose the temporary name *Mlhb.A42* for this locus, based on previous naming of *Hb* powdery mildew resistance genes (Pickering et al., 1995; Steffenson, 1998).

TABLE 2 | Phenotypic segregation of powdery mildew resistance in each of 2HS IL mapping populations.

Mapping population	Number of resistant lines	Number of susceptible lines	χ^2
"4176" (BC1F6)	199	67	0,005**
"5216" (F7)	150	53	0,13**
"dIL_5216" (F2)	81	22	0.73**
"dIL_4176" (F2)	114	32	0.74*

χ^2 indicates the result of the Chi squared test performed to determine the goodness to fit a 3R:1S ratio, expected for a dominant monogenic inheritance. Asterisks indicate significance of the test with a p-value inferior to 1% (**) and 5% (*).

Recombination Frequency and Mapping of *Mlhb.A42* in Single-Introgression Line Mapping Populations

Genotyping of the mapping populations employed nine CAPS markers designed based on existing exome capture re-sequencing data (**Supplementary Table 1**). This showed that the BC1F6 population "4176" carried a longer introgressed segment compared to the F7 population "5612" (**Figure 4**). The genotyping results for population "5612" confirmed the extent of the introgressed segment, previously detected by exome capture in the F6 generation (Wendler et al., 2015).

Genotyping of 266 and 203 individuals in the initial IL mapping populations "4176" and "5612," uncovered only three and two recombinants within the introgressed segments, respectively (**Figure 4**). The introgressed segment therefore has a genetic length of approximately 1 centiMorgan (cM). Yet, the segment of population "5612" represents 10 cM on the barley POPSEQ map (Mascher et al., 2013a; Wendler et al., 2015). This confirms the anticipated reduced recombination between the *Hv* genome and the introgressed *Hb* segment. The resulting genetic interval for *Mlhb.A42* is flanked by markers CAPS02 and CAPS11, corresponding to a 9.5 Mbp interval between bp positions 2,269,761 and 11,819,688 on chromosome 2HS.

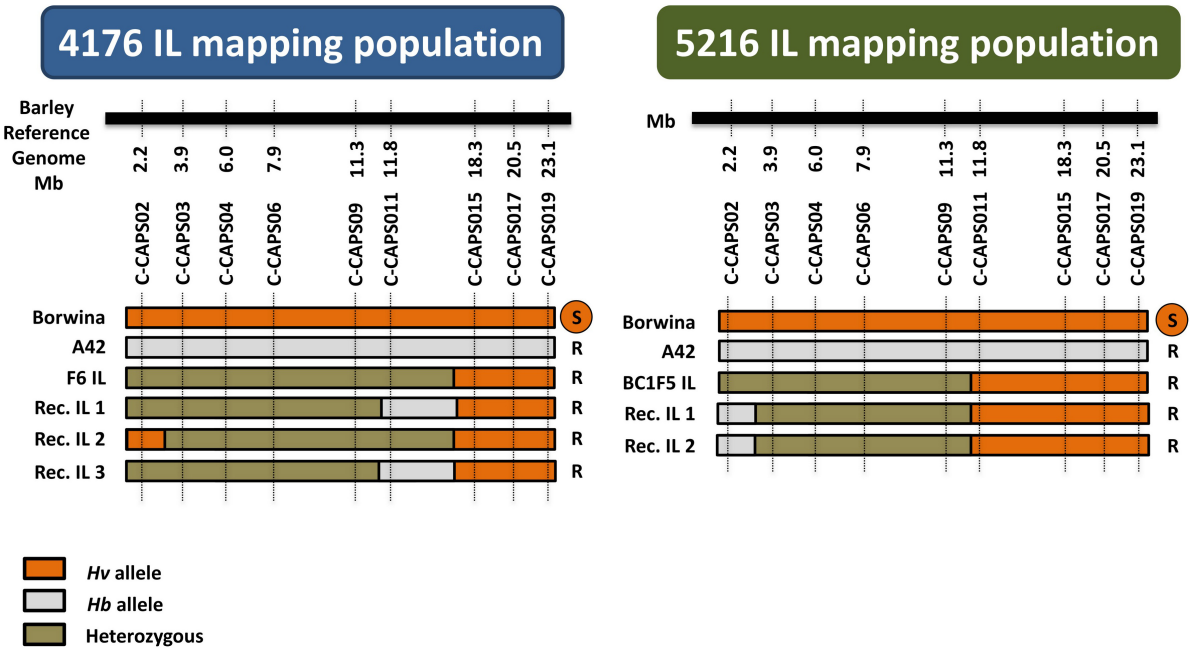

FIGURE 4 | Graphical genotypes and phenotypes of the recombinant *Hv/Hb* ILs from the two IL mapping populations. "Borwina" and "A42" are the original susceptible and resistant parents of the populations. "F6 IL" and "BC1F5 IL" represent the plant that was selfed in the previous generation to obtain populations "4176" and "5216," respectively. "Rec. IL" 1 to 3 are the identified recombinant plants. Nine CAPS markers named C-CAPS02 to C-CAPS019, were developed based on conserved *Hv/Hb* SNP loci described in Wendler et al. (2014), spanning the terminal 23 Mbp of barley chromosome 2HS. The black horizontal bars represent schematically the barley reference genome. The physical position of each selected SNPs on the barley reference genome is written below the black line. The homozygous state of the alleles from susceptible and resistant parents is shown as orange and gray colors, respectively, whereas heterozygous state is shown in green. The phenotype of each recombinant is indicated on the right of their genotype (R = resistant; S = susceptible). The number of recombinants identified through screening with the developed markers was only three and two in IL mapping population "4176" and "5216," respectively.

Recombination and Mapping of *Mlhb.A42* in Double Introgression Lines

The 103 and 146 F2 plants from the two double introgression populations "dIL_5216" and "dIL_4176," respectively, were genotyped by GBS and phenotyped for resistance against CH4.8 isolate. Based on obtained SNP variants across candidate interval for *Mlhb.A42* defined in the initial mapping populations, 14 and 36 recombinants were obtained in "dIL_5216" and "dIL_4176," respectively (**Figure 5**). The phenotypes of all non-recombinant plants corresponded to their genotype in this interval. Association between the phenotypes and genotypes of the recombinants defined overlapping 1.7 Mbp (between 7,943,409 and 9,595,691 bp) and 1.4 Mbp (between 8,193,151 and 9,595,691 bp) intervals on barley chromosome 2HS (Monat et al., 2019) in "dIL_5216" and "dIL_4176," respectively (**Figure 5**).

Candidate Genes Within the Target Interval

Based on the annotated barley reference genome sequence (Monat et al., 2019), 46 high confidence (HC) genes (**Table 3**) are located within the 1.4 Mbp *Mlhb.A42* interval as delimited in the "dIL_4176" population. Those genes with a putative functional annotation included a nucleotide-binding-site leucine-rich-repeat class of gene (NBS-LLR), an HR-like lesion-inducing protein-coding gene, a lectin receptor kinase (LecRK) and a Heat shock protein 90, all gene functions that could be directly or indirectly implied with plant resistance to biotic and/or abiotic stresses and therefore, qualify as candidate genes for *Mlhb.A42*. HORVU.MOREX.r2.2HG0082250 is annotated as a LecRK. However, analysis of its protein sequence with InterPro (Mitchell et al., 2018), showed that, like LecRKs, it is composed of an extracellular legume (L-type) lectin domain and a transmembrane domain. However, its cytoplasmic domain is only 23 amino-acid long and does not bear a kinase domain, making this gene a probable lectin receptor-like protein (LecRLP).

DISCUSSION

We report the fine mapping of *Mlhb.A42*, a dominant powdery mildew resistance locus originating from *Hb*, using mapping populations derived by crossing two independent, resistant and susceptible, *Hv*/*Hb* ILs. The strong suppression of recombination between homeologous genomic segments in *Hv*/*Hb* introgression lines, which typically results in severe linkage drag, previously represented a barrier to the efficient utilization of *Hb* germplasm in barley crop improvement, and to the isolation of disease resistance genes introgressed from *Hb*.

The genomic resource created by the GBS study of Wendler et al. (2015) on 145 *Hv/Hb* ILs proved to be a useful tool

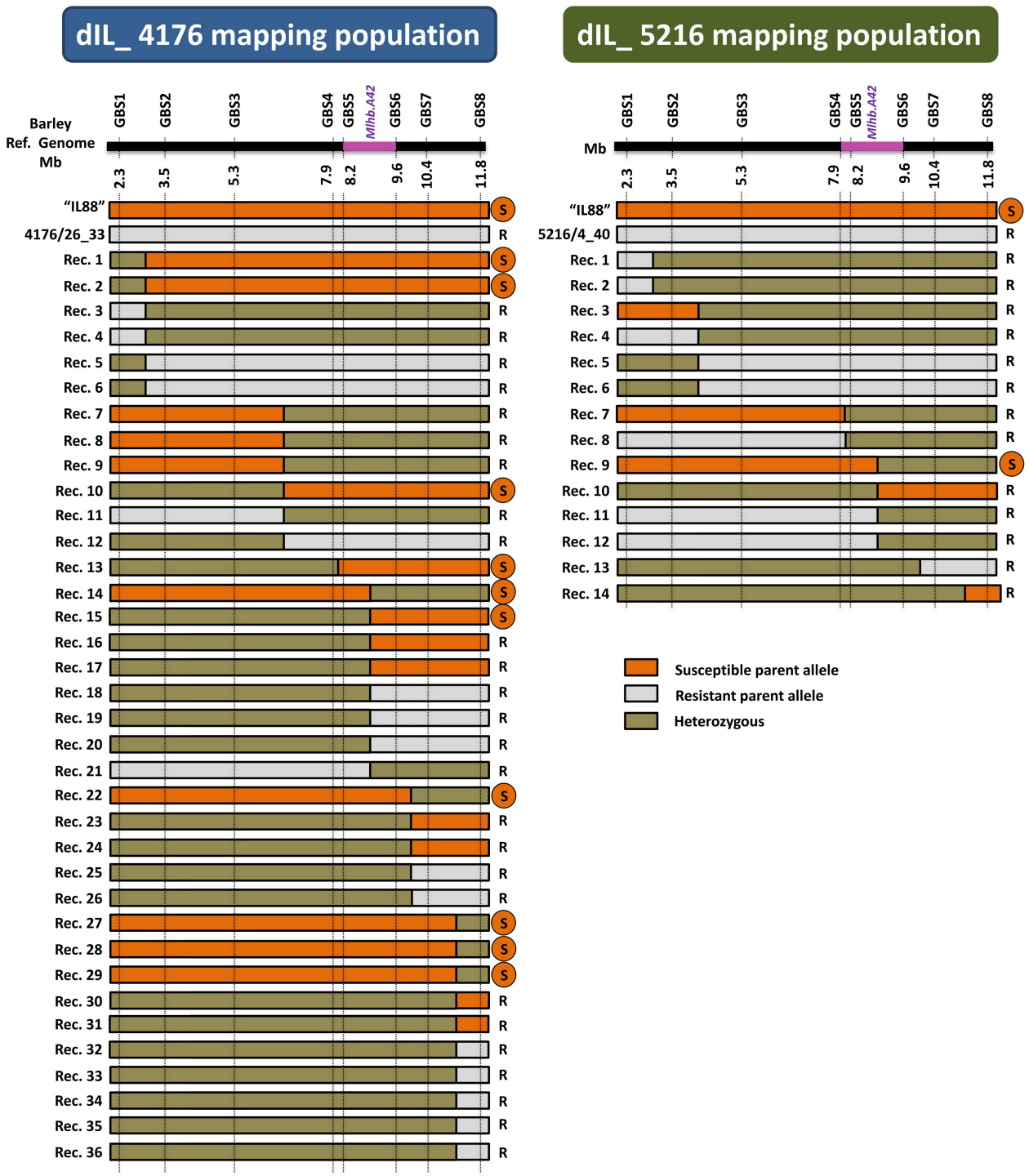

FIGURE 5 | Intraspecific mapping of the powdery mildew resistance locus *Mlhb.A42* in F2 populations derived from two different 2HS *Hv/Hb* introgression lines. The horizontal black bar schematically represents barley chromosome 2HS. The physical coordinates of each GBS marker are indicated below it. The genomic region containing the *Mlhb.A42* locus on barley chromosome 2HS is shown in purple. The graphical genotypes of recombinants are indicated as horizontal bars. The genotype of the susceptible parent "IL88" is represented in orange, the one of the resistant parents 4176/26_33 and 5216/4_40 is represented in gray, and heterozygous state is shown in olive green. The phenotype of each recombinant plant is indicated on the right of its genotype (R = resistant; S = susceptible). The recombination rates in "dIL_4176" and "dIL_5216" are 24.7 and 13.6%, respectively, corresponding to a 20-fold increase compared to single introgression lines. The *Mlhb.A42* gene was allocated to a 1.4 Mb interval, based on an estimate using the *Hv* genome as reference.

TABLE 3 | High confidence (HC) genes based on automated annotation of barley reference genome.

Gene name	Start[1]	End[1]	Annotation
HORVU.MOREX.r2.2HG0081650	8223803	8224706	Serine/threonine-protein phosphatase 7 long form-like protein
HORVU.MOREX.r2.2HG0081660	8227095	8233246	NBS-LRR-like resistance protein
HORVU.MOREX.r2.2HG0081670	8278638	8280547	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein, putative
HORVU.MOREX.r2.2HG0081680	8302525	8304486	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein, putative
HORVU.MOREX.r2.2HG0081690	8320263	8321485	12-oxophytodienoate reductase-like protein
HORVU.MOREX.r2.2HG0081700	8328715	8329968	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein, putative
HORVU.MOREX.r2.2HG0081720	8364720	8365206	NADH-ubiquinone oxidoreductase chain 1
HORVU.MOREX.r2.2HG0081740	8381983	8383512	ATP synthase subunit alpha
HORVU.MOREX.r2.2HG0081760	8470715	8473118	HR-like lesion-inducing protein-related protein
HORVU.MOREX.r2.2HG0081770	8474573	8487616	Actin-related protein
HORVU.MOREX.r2.2HG0081780	8511271	8512941	Cytochrome P450
HORVU.MOREX.r2.2HG0081810	8576680	8577147	Cytochrome P450
HORVU.MOREX.r2.2HG0081820	8607940	8608750	Cytochrome P450
HORVU.MOREX.r2.2HG0081830	8615323	8617046	Cytochrome P450
HORVU.MOREX.r2.2HG0081840	8664852	8679924	Cytochrome P450
HORVU.MOREX.r2.2HG0081850	8686994	8691803	Kaurene synthase
HORVU.MOREX.r2.2HG0081860	8729062	8731108	Cytochrome P450, putative
HORVU.MOREX.r2.2HG0081880	8896440	8898414	Copalyl diphosphate synthase
HORVU.MOREX.r2.2HG0081890	8905788	8907365	Cytochrome P450
HORVU.MOREX.r2.2HG0081900	8930477	8930938	Isoaspartyl peptidase/L-asparaginase
HORVU.MOREX.r2.2HG0081920	8961925	8964012	Copalyl diphosphate synthase
HORVU.MOREX.r2.2HG0081930	8965503	8967761	Cytochrome P450
HORVU.MOREX.r2.2HG0081980	9008320	9009897	Cytochrome P450
HORVU.MOREX.r2.2HG0082000	9064624	9068933	Copalyl diphosphate synthase
HORVU.MOREX.r2.2HG0082010	9095159	9096736	Cytochrome P450
HORVU.MOREX.r2.2HG0082020	9253167	9258032	Agenet domain, putative
HORVU.MOREX.r2.2HG0082050	9310583	9312095	Chalcone synthase
HORVU.MOREX.r2.2HG0082060	9337837	9339296	O-methyltransferase family protein
HORVU.MOREX.r2.2HG0082080	9357711	9359256	Glycosyltransferase
HORVU.MOREX.r2.2HG0082090	9360337	9378163	ABC transporter B family protein
HORVU.MOREX.r2.2HG0082100	9379916	9381655	Glycosyltransferase
HORVU.MOREX.r2.2HG0082140	9435844	9439807	Transcription factor
HORVU.MOREX.r2.2HG0082150	9441094	9442595	Serine/threonine-protein kinase
HORVU.MOREX.r2.2HG0082160	9462767	9463051	TTF-type zinc finger protein with HAT dimerization domain-containing protein
HORVU.MOREX.r2.2HG0082180	9468781	9469636	F-box protein PP2-A13
HORVU.MOREX.r2.2HG0082190	9472177	9476645	ATP sulfurylase (Sulfate adenylyltransferase)
HORVU.MOREX.r2.2HG0082200	9478069	9482961	Zinc finger family protein
HORVU.MOREX.r2.2HG0082210	9488137	9491244	carbohydrate esterase, putative (DUF303)
HORVU.MOREX.r2.2HG0082220	9498236	9499725	Serpin
HORVU.MOREX.r2.2HG0082240	9505214	9505813	Maternal effect embryo arrest protein
HORVU.MOREX.r2.2HG0082250	9538406	9539521	Lectin receptor kinase
HORVU.MOREX.r2.2HG0082260	9543886	9546930	Carboxymethylenebutenolidase-like protein
HORVU.MOREX.r2.2HG0082270	9550542	9550993	NAD(P)-binding rossmann-fold protein
HORVU.MOREX.r2.2HG0082280	9555561	9557307	Nicotianamine synthase
HORVU.MOREX.r2.2HG0082310	9591121	9593401	Heat shock protein 90
HORVU.MOREX.r2.2HG0082320	9595584	9597283	caspase-6 protein

[1] *Coordinates based on TRITEX Morex assembly (Monat et al., 2019).*

to identify suitable partners for the development of double ILs populations. The exploitation of intraspecific recombination allowed us to overcome the barrier to recombination usually observed in IL populations. Recombination rates in the region carrying the introgressed fragment were estimated to be 24.7% ("dIL_4176") and 13.6% ("dIL_5216"), comparable to the corresponding 10% rate observed in pure *Hv/Hv* mapping populations [e.g. POPSEQ map (Mascher et al., 2013a)]. The rate of recombination was exceeded by a factor of 20-fold as compared to the F7 and BC1F6 single IL populations "5216" and "4176"

(approximately 1%). The polymorphisms and markers identified in this study can be converted into KASP assays which would enable for rapid and high-throughput screening of large breeding population for the purpose of introgression of *Mlhb.A42* into barley cultivars.

The 1.4 Mbp identified target interval is containing 46 annotated HC genes on the most recent chromosome-scale genome assembly of cultivar "Morex" (Monat et al., 2019). The powdery mildew resistance conferred by this *Hb* locus is dominantly inherited, displaying chlorotic/necrotic flecks of HR with collapsed hyphae at inoculation sites, suggesting a salicylic acid (SA)-independent resistance pathway. Genes from the NBS-LLR or the receptor-like-kinase (RLK) families are over-represented among genes conferring this type of strong dominant resistance to pathogens (Kourelis and van der Hoorn, 2018), making genes from these families likely candidates in the context of this study. HORVU.MOREX.r2.2HG0081660, one of the annotated genes in the interval, is a coiled-coil (CC)-NBS-LRR gene and therefore the most likely candidate gene. However, HORVU.MOREX.r2.2HG0082250 is annotated as a LecRK and could also be a good candidate. LecRKs are a type of RLK characterized by an extracellular lectin motif (Wu and Zhou, 2013). They have been described as implicated in biotic stress resistance, mostly to bacteria and fungi (Singh and Zimmerli, 2013). This type of gene has been identified in resistance to oomycetes (Wang et al., 2015a,b; Balagué et al., 2017) and fungi (Huang et al., 2013; Wang et al., 2014a) in *Arabidopsis thaliana* and to wheat powdery mildew in *Haynaldia villosa* (Wang et al., 2018). According to InterPro (Mitchell et al., 2018), HORVU.MOREX.r2.2HG0082250 does not bear a kinase domain and is likely to be a L-type LecRLP. So far, the only LecRLPs described have a lectin-like Lysin-motif (LysM)-type lectin domain. Two LysM-LecRLPs from *A. thaliana* and three from rice have been identified reported in context of disease resistance through interaction with the LysM-LecRK CERK1. The rice LysM-LecRLP CEBiP recognizes chitin and, through a direct interaction with CERK1, confers pattern-triggered immunity against fungi (Shimizu et al., 2010). Similarly, LYP4 and LYP6 both perceive peptidoglycan and chitin and interact with CERK1 (Liu et al., 2012). In *A. thaliana*, LYM1 and LYM3 sense pectidoglycan and trigger immunity, through CERK1, to bacterial infection (Willmann et al., 2011). *A. thaliana* contains only four L-type LecRLPs (Bellande et al., 2017) but, so far, no L-type LecRLPs have been functionally described. However, a mode of action of HORVU.MOREX.r2.2HG0082250 similar to the one of LysM-LecRLPs is a possibility for further investigation.

Almost all the genes annotated in the delimited target interval might be directly or indirectly involved in resistance to biotic and abiotic stresses and should be considered tentative candidates. NADH-ubiquinone oxidoreductase is involved in intracellular ROS production that can prevent pathogen infection (van der Merwe and Dubery, 2006). Cytochrome P450 and O-methyltransferase proteins are responsible for production of several molecules that can play a role in resistance to pathogens or pests (He and Dixon, 2000; Dixon, 2001; Noordermeer et al., 2001). Some glycosyl-transferase genes have been identified as necessary for the HR (Langlois-Meurinne et al., 2005). The rice gene *OsHRL* encodes for a HR-like lesion inducing protein and has been shown to be associated with resistance to bacterial blight (Park et al., 2010). Copalyl-diphosphate synthases are implicated in the biosynthesis of phytohormones including gibberellic acid or phytoalexins, which contribute to defensive secondary metabolism (Prisic et al., 2004; Harris et al., 2005). Finally, HORVU.MOREX.r2.2HG0082310 is annotated as a Heat shock protein 90 (Hsp90), which are involved in stress resistance, in particular disease resistance, mediating signal transduction for HR (Xu et al., 2012). Notably, it has been shown by virus-induced gene silencing that an *Hsp90* gene is required for *Mla13* resistance of barley to powdery mildew (Hein et al., 2005).

In addition to the annotated genes, other genes, e.g. members of NBS-LRR and RLK families, might be present in the interval of the resistant *Hb* parent but missing in the respective interval of the "Morex" reference sequence. Indeed, *Hv* and *Hb* diverged 6 million years ago, accumulating structural variations since then (Jakob and Blattner, 2006). Therefore, this *Hb* resistance to powdery mildew could be due to a gene absent from the barley reference genome. In particular, NBS-LRR genes are subject to frequent duplication (Flagel and Wendel, 2009), and resistances conferred by NBS-LRRs genes are frequently due to presence/absence variation of such genes (Grant et al., 1998; Griffiths et al., 1999; Henk et al., 1999; Stahl et al., 1999; Tian et al., 2002). The wheat powdery mildew-resistance gene *Pm21* (Xing et al., 2018) originates from the wheat/*Dasypyrum villosum* translocation line T6AL.6VS and is localized in a region presenting a high level of synteny with the *Mlhb.A42* locus. This gene confers broad spectrum dominant resistance against wheat powdery mildew isolates and encodes a RPP13-like NBS-LRR gene (He et al., 2017; Xing et al., 2018). The protein sequence of *Pm21* only shares 34% identity with the translated nucleotide sequence of HORVU.MOREX.r2.2HG0081660. However, its localization in a syntenic region to the resistance conferring *Mlhb.A42* locus provides a tempting working hypothesis that *Pm21* and *Mlhb.A42* could represent orthologous genes or members of the same locally evolved gene family.

A resequencing strategy of the *Mlhb.A42* locus in both resistant and susceptible *Hb* will be necessary to ascertain the structure of the locus and the number and nature of the candidate genes. With the development of new sequencing methods this could be achieved by Cas9-guided enrichment of the locus (Wang et al., 2014b) or sorting and sequencing for assembly of the chromosome 2H in one of the two introgression lines (Thind et al., 2017). Moreover, a locus of resistance to powdery mildew has been identified on chromosome 2HS in the *Hb* accessions S1 – where it was named *Mlhb1.a* – (Pickering et al., 1995; Shtaya et al., 2007), 2032 (Zhang et al., 2001; Shtaya et al., 2007), and A17 (Shtaya et al., 2007). Allelism tests are required to check whether the same locus is involved in the resistance from those four accessions or not. Xu and Kasha (1992) identified another *Hb* powdery mildew resistance gene in the accession GBC141. However, the causal dominant gene, designated as *Mlhb2.b*, was

located by Kasha et al. (1996) on chromosome 2HL and therefore not allelic to *Mlhb1.a* or *Mlhb.A42*.

Durability and spectrum of a resistance gene are two major criteria to assess its application potential (Mundt, 2014). Durability of resistance genes can become a major concern as deployed resistance genes are experiencing a boom-and-bust phenomenon (Gladieux et al., 2015). The spectrum of *Mlhb.A42* was not evaluated in this study and should be tested in order to ascertain its potential for field resistance. Durability is difficult to estimate under laboratory scenarios. The durability of orthologs genes can be used as a proxy, yet quite imperfect. As discussed earlier, the wheat resistance gene *Pm21*could be orthologous to *Mlhb.A42*. Varieties carrying *Pm21* have increasingly been cultivated in China in the recent years (Bie et al., 2015) and its durability can therefore be evaluated in real conditions. Unfortunately, in some wheat fields, new *Bgt* isolates, virulent against *Pm21* have been identified (Shi et al., 2009; Yang et al., 2009). However, resistance based on this gene persisted close to 40 years (Tang et al., 2018) and is still effective against more than a thousand of field isolates in China (Zeng et al., 2014) and Poland (Czembor et al., 2014). To counteract the risk of isolates breaking the resistance provided by a single locus it is of ongoing importance to identify new resistance genes and to pyramid new loci with existing sources of resistance to increase the durability of resistance in the system (Wu et al., 2019). Moreover, exploiting CWR resistances could be a way to unravel even more effective resistance genes. Indeed, Wang et al. (2019) showed that the *Hb* LecRLK gene of resistance to leaf rust *Rph22* confers a stronger resistance to leaf rust adapted to *Hv* than its *Hv* ortholog *Rphq2*. The hypothesis is that crop receptors have a lower recognition of crop-adapted pathogens than the CWR receptors because of adaptation of the pathogens during centuries of coevolution with their host plant.

In the current study we report genetic mapping of *Mlhb.A42*, a dominant resistance locus introgressed to cultivated barley from *Hb*. This work is a proof-of-concept study for establishing the basic steps of map-based cloning of genes present in *Hv/Hb* IL collections by exploiting double ILs mapping populations. Using this strategy, we circumvented the limitation of repressed meiotic recombination which was frequently observed in attempts of genetic mapping employing populations derived between *Hv*/*Hb* introgression lines and pure barley cultivars. Here, we observed similar or even higher recombination rates as expected in *Hv* and thus providing a major step toward facilitated exploitation of secondary gene pool-derived resistance genes in barley crop improvement.

DATA AVAILABILITY STATEMENT

The GBS datasets generated and analyzed in this study are deposited at e!DAL PGP repository (Arend et al., 2016) under the doi: 10.5447/IPK/2020/2.

AUTHOR CONTRIBUTIONS

PH performed the experimental work. PH and HP performed the data analysis and wrote the manuscript. BR-W provided seed material and sample information for two IL mapping populations "4176" and "5216." PS supervised the phenotyping. NS designed the study, supervised the experimental work, and contributed to the writing of the manuscript. All authors read, corrected, and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.00225/full#supplementary-material

REFERENCES

Arend, D., Junker, A., Scholz, U., Schüler, D., Wylie, J., and Lange, M. (2016). PGP repository: a plant phenomics and genomics data publication infrastructure. *Database* 2016:baw033. doi: 10.1093/database/baw033

Backes, G., Madsen, L., Jaiser, H., Stougaard, J., Herz, M., Mohler, V., et al. (2003). Localisation of genes for resistance against *Blumeria graminis* f. sp. hordei and *Puccinia graminis* in a cross between a barley cultivar and a wild barley (*Hordeum vulgare* ssp. spontaneum) line. *Theor. Appl. Genet.* 106, 353–362. doi: 10.1007/s00122-002-1148-1

Balagué, C., Gouget, A., Bouchez, O., Souriac, C., Haget, N., Boutet-Mercey, S., et al. (2017). The *Arabidopsis thaliana* lectin receptor kinase LecRK-I.9 is required for full resistance to *Pseudomonas syringae* and affects jasmonate signalling. *Mol. Plant Pathol.* 18, 937–948. doi: 10.1111/mpp.12457

Bellande, K., Bono, J.-J., Savelli, B., Jamet, E., and Canut, H. (2017). Plant lectins and lectin receptor-like kinases: how do they sense the outside? *Int. J. Mol. Sci.* 18:1164. doi: 10.3390/ijms18061164

Bie, T., Zhao, R., Jiang, Z., Gao, D., Zhang, B., and He, H. (2015). Efficient marker-assisted screening of structural changes involving *Haynaldia villosa* chromosome 6V using a double-distal-marker strategy. *Mol. Breed.* 35:34. doi: 10.1007/s11032-015-0211-y

Canady, M. A., Ji, Y., and Chetelat, R. T. (2006). Homeologous recombination in *Solanum lycopersicoides* introgression lines of cultivated tomato. *Genetics* 174, 1775–1788. doi: 10.1534/genetics.106.065144

Chen, S., Rouse, M. N., Zhang, W., Jin, Y., Akhunov, E., Wei, Y., et al. (2015). Fine mapping and characterization of *Sr21*, a temperature-sensitive diploid wheat resistance gene effective against the *Puccinia graminis* f. sp. tritici Ug99 race group. *Theor. Appl. Genet.* 128, 645–656. doi: 10.1007/s00122-015-2460-x

Corrion, A., and Day, B. (2015). *"Pathogen Resistance Signalling in Plants," in eLS*. New York, NY: American Cancer Society, 1–14. doi: 10.1002/9780470015902.a0020119.pub2

Czembor, H. J., Domeradzka, O., Czembor, J. H., and Mańkowski, D. R. (2014). Virulence structure of the powdery mildew (*Blumeria graminis*) population occurring on triticale (x Triticosecale) in poland. *J. Phytopathol.* 162, 499–512. doi: 10.1111/jph.12225

Czembor, J. H. (2002). Resistance to powdery mildew in selections from Moroccan barley landraces. *Euphytica* 125, 397–409. doi: 10.1023/A:1016061508160

Dixon, R. A. (2001). Natural products and plant disease resistance. *Nature* 411, 843–847. doi: 10.1038/35081178

Dreiseitl, A., and Bockelman, H. E. (2003). Sources of powdery mildew resistance in a wild barley collection. *Genet. Resour. Crop. Evol.* 50, 345–350. doi: 10.1023/A:1023953819787

Feuillet, C., Langridge, P., and Waugh, R. (2008). Cereal breeding takes a walk on the wild side. *Trends Genet.* 24, 24–32. doi: 10.1016/j.tig.2007.11.001

Fischbeck, G., Schwarzbach, E., Sobel, Z., and Wahl, I. (1976). Mildew resistance in Israeli populations of 2-rowed wild barley (*Hordeum spontaneum*). *Z. Pflanzenzüchtung* 76, 163–166.

Flagel, L. E., and Wendel, J. F. (2009). Gene duplication and evolutionary novelty in plants. *New Phytol.* 183, 557–564. doi: 10.1111/j.1469-8137.2009.02923.x

Frisch, M., and Melchinger, A. E. (2001). The length of the intact donor chromosome segment around a target gene in marker-assisted backcrossing. *Genetics* 157, 1343–1356.

Gladieux, P., Feurtey, A., Hood, M. E., Snirc, A., Clavel, J., Dutech, C., et al. (2015). The population biology of fungal invasions. *Mol. Ecol.* 24, 1969–1986. doi: 10.1111/mec.13028

Grant, M. R., McDowell, J. M., Sharpe, A. G., de Torres Zabala, M., Lydiate, D. J., and Dangl, J. L. (1998). Independent deletions of a pathogen-resistance gene in *Brassica* and *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 95, 15843–15848. doi: 10.1073/pnas.95.26.15843

Griffiths, A. J. F., Gelbart, W. M., Miller, J. H., and Lewontin, R. C. (eds). (1999). "Chromosomal rearrangements," in *Modern Genetic Analysis* (New York, NY: W. H. Freeman).

Harris, L. J., Saparno, A., Johnston, A., Prisic, S., Xu, M., Allard, S., et al. (2005). The Maize *An2* gene is induced by *Fusarium* attack and encodesan ent-copalyl diphosphate synthase. *Plant Mol. Biol.* 59, 881–894. doi: 10.1007/s11103-005-1674-8

He, H., Zhu, S., Ji, Y., Jiang, Z., Zhao, R., and Bie, T. (2017). Map-based cloning of the gene *Pm21* that confers broad spectrum resistance to wheat powdery mildew. *bioRxiv* [preprint]. doi: 10.1101/177857

He, X.-Z., and Dixon, R. A. (2000). Genetic manipulation of isoflavone 7-O-methyltransferase enhances biosynthesis of 4'-O-Methylated isoflavonoid phytoalexins and disease resistance in alfalfa. *Plant Cell* 12, 1689–1702. doi: 10.1105/tpc.12.9.1689

Hein, I., Barciszewska-Pacak, M., Hrubikova, K., Williamson, S., Dinesen, M., Soenderby, I. E., et al. (2005). Virus-induced gene silencing-based functional characterization of genes associated with powdery mildew resistance in barley. *Plant Physiol.* 138, 2155–2164. doi: 10.1104/pp.105.062810

Henk, A. D., Warren, R. F., and Innes, R. W. (1999). A new ac-like transposon of *Arabidopsis* Is associated with a deletion of the *RPS5* disease resistance gene. *Genetics* 151, 1581–1589.

Hoffmann, A. A., Sgrò, C. M., and Weeks, A. R. (2004). Chromosomal inversion polymorphisms and adaptation. *Trends Ecol. Evol.* 19, 482–488. doi: 10.1016/j.tree.2004.06.013

Hoisington, D., Khairallah, M., Reeves, T., Ribaut, J.-M., Skovmand, B., Taba, S., et al. (1999). Plant genetic resources: what can they contribute toward increased crop productivity? *Proc. Natl. Acad. Sci. U.S.A.* 96, 5937–5943. doi: 10.1073/pnas.96.11.5937

Hoseinzadeh, P., Zhou, R., Mascher, M., Himmelbach, A., Niks, R. E., Schweizer, P., et al. (2019). High resolution genetic and physical mapping of a major powdery mildew resistance locus in barley. *Front. Plant Sci.* 10:146. doi: 10.3389/fpls.2019.00146

Hospital, F. (2001). Size of donor chromosome segments around introgressed loci and reduction of linkage drag in marker-assisted backcross programs. *Genetics* 158, 1363–1379.

Huang, P., Ju, H.-W., Min, J.-H., Zhang, X., Kim, S.-H., Yang, K.-Y., et al. (2013). Overexpression of L-type lectin-like protein kinase 1 confers pathogen resistance and regulates salinity response in *Arabidopsis thaliana*. *Plant Sci.* 20, 98–106. doi: 10.1016/j.plantsci.2012.12.019

International Barley Genome Sequencing Consortium. (2012). A physical, genetic and functional sequence assembly of the barley genome. *Nature* 491, 711–716. doi: 10.1038/nature11543

Jakob, S. S., and Blattner, F. R. (2006). A chloroplast genealogy of *Hordeum* (Poaceae): long-term persisting haplotypes, incomplete lineage sorting, regional extinction, and the consequences for phylogenetic inference. *Mol. Biol. Evol.* 23, 1602–1612. doi: 10.1093/molbev/msl018

Jarosch, B., Kogel, K.-H., and Schaffrath, U. (1999). The Ambivalence of the barley *Mlo* locus: mutations conferring resistance against powdery mildew (*Blumeria graminis* f. sp. hordei) Enhance Susceptibility to the Rice Blast Fungus Magnaporthe grisea. *Mol. Plant-Microbe Interact.* 12, 508–514. doi: 10.1094/MPMI.1999.12.6.508

Johnston, P. A., Meiyalaghan, V., Forbes, M. E., Habekuß, A., Butler, R. C., and Pickering, R. (2015). Marker assisted separation of resistance genes *Rph22* and *Rym16Hb* from an associated yield penalty in a barley: *Hordeum bulbosum* introgression line. *Theor. Appl. Genet.* 128, 1137–1149. doi: 10.1007/s00122-015-2495-z

Johnston, P. A., Niks, R. E., Meiyalaghan, V., Blanchet, E., and Pickering, R. (2013). Rph22: mapping of a novel leaf rust resistance gene introgressed from the non-host *Hordeum bulbosum* L. into cultivated barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* 126, 1613–1625. doi: 10.1007/s00122-013-2078-9

Johnston, P. A., Timmerman-Vaughan, G. M., Farnden, K. J. F., and Pickering, R. (2009). Marker development and characterisation of *Hordeum bulbosum* introgression lines: a resource for barley improvement. *Theor. Appl. Genet.* 118, 1429–1437. doi: 10.1007/s00122-009-0992-7

Jørgensen, I. H. (1992). Discovery, characterization and exploitation of *Mlo* powdery mildew resistance in barley. *Euphytica* 63, 141–152. doi: 10.1007/BF00023919

Kakeda, K., Ibuki, T., Suzuki, J., Tadano, H., Kurita, Y., Hanai, Y., et al. (2008). Molecular and genetic characterization of the S locus in *Hordeum bulbosum* L., a wild self-incompatible species related to cultivated barley. *Mol. Genet. Genomics* 280, 509–519. doi: 10.1007/s00438-008-0383-9

Kasha, K. J., Pickering, R. A., William, H. M., Hill, M. A., Oro, R., Reader, S., et al. (1996). "GISH and RFLP facilitated identification of a barley chromosome carrying powdery mildew resistance from Hordeum bulbosum," in *Proceedings 7th International Barley Genetics Symposium, Saskatoon*, Canada.

Kerber, E. R., and Dyck, P. L. (1990). Transfer to hexaploid wheat of linked genes for adult-plant leaf rust and seedling stem rust resistance from an amphiploid of *Aegilops speltoides* × *Triticum monococcum*. *Genome* 33, 530–537. doi: 10.1139/g90-079

Kjær, B., Jensen, H. P., Jensen, J., and Jørgensen, J. H. (1990). Associations between three *ml-o* powdery mildew resistance genes and agronomic traits in barley. *Euphytica* 46, 185–193. doi: 10.1007/BF00027217

Koressaar, T., and Remm, M. (2007). Enhancements and modifications of primer design program Primer3. *Bioinformatics* 23, 1289–1291. doi: 10.1093/bioinformatics/btm091

Kourelis, J., and van der Hoorn, R. A. L. (2018). Defended to the nines: 25 years of resistance gene cloning identifies nine mechanisms for R protein function. *Plant Cell* 30, 285–299. doi: 10.1105/tpc.17.00579

Kumar, J., Hückelhoven, R., Beckhove, U., Nagarajan, S., and Kogel, K.-H. (2001). A compromised mlo pathway affects the response of barley to the necrotrophic fungus *Bipolaris sorokiniana* (*Teleomorph*: *Cochliobolus sativus*) and its toxins. *Phytopathology* 91, 127–133. doi: 10.1094/PHYTO.2001.91.2.127

Langlois-Meurinne, M., Gachon, C. M. M., and Saindrenan, P. (2005). Pathogen-responsive expression of glycosyltransferase genes *UGT73B3* and *UGT73B5* is necessary for resistance to *Pseudomonas syringae* pv tomato in *Arabidopsis*. *Plant Physiol.* 139, 1890–1901. doi: 10.1104/pp.105.067223

Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 25, 1754–1760. doi: 10.1093/bioinformatics/btp324

Liu, B., Li, J.-F., Ao, Y., Qu, J., Li, Z., Su, J., et al. (2012). Lysin Motif6Containing Proteins LYP4 and LYP6 play dual roles in peptidoglycan and chitin perception in rice innate immunity. *Plant Cell* 24, 3406–3419. doi: 10.1105/tpc.112.102475

Mains, E. B., and Dietz, S. M. (1930). Physiologic forms of barley mildew. *Erysiphe graminis hordei*. *Phytopathology* 20, 229–239.

Mascher, M., Muehlbauer, G. J., Rokhsar, D. S., Chapman, J., Schmutz, J., Barry, K., et al. (2013a). Anchoring and ordering NGS contig assemblies by population sequencing (POPSEQ). *Plant J.* 76, 718–727. doi: 10.1111/tpj.12319

Mascher, M., Wu, S., Amand, P. St, Stein, N., and Poland, J. (2013b). Application of genotyping-by-sequencing on semiconductor sequencing platforms: a comparison of genetic and reference-based marker ordering in barley. *PLoS One* 8:e076925. doi: 10.1371/journal.pone.0076925

McGrann, G. R. D., Stavrinides, A., Russell, J., Corbitt, M. M., Booth, A., Chartrain, L., et al. (2014). A trade off between mlo resistance to powdery mildew and increased susceptibility of barley to a newly important disease. *Ramularia leaf spot*. *J. Exp. Bot.* 65, 1025–1037. doi: 10.1093/jxb/ert452

Michel, M. (1996). *Untersuchungen zur Übertragung von Resistenzgenen aus der Wildart Hordeum bulbosum L. in die Kulturgerste Hordeum vulgare L. Diss.* Munich: TU München.

Milner, S., Jost, M., Taketa, S., Mazón, E., Himmelbach, A., Oppermann, M., et al. (2018). Genebank genomics reveals the diversity of a global barley collection. *Nat. Genet. Forthcomin.* 51, 319–326. doi: 10.1038/s41588-018-0266-x

Mitchell, A. L., Attwood, T. K., Babbitt, P. C., Blum, M., Bork, P., Bridge, A., et al. (2018). InterPro in 2019: improving coverage, classification and access to protein sequence annotations. *Nucleic Acids Res.* 47, D351–D360. doi: 10.1093/nar/gky1100

Monat, C., Padmarasu, S., Lux, T., Wicker, T., Gundlach, H., Himmelbach, A., et al. (2019). TRITEX: chromosome-scale sequence assembly of Triticeae genomes with open-source tools. *bioRxiv* [preprint]. doi: 10.1101/631648

Mundt, C. C. (2014). Durable resistance: a key to sustainable management of pathogens and pests. *Infect. Genet. Evol.* 27, 446–455. doi: 10.1016/j.meegid.2014.01.011

Noordermeer, M. A., Veldink, G. A., and Vliegenthart, J. F. G. (2001). Fatty acid hydroperoxide lyase: a plant cytochrome P450 enzyme involved in wound healing and pest resistance. *ChemBioChem* 2, 494–504. doi: 10.1002/1439-7633(20010803)2:7/8<494::aid-cbic494>3.0.co;2-1

O'Halloran, D. M. (2015). PrimerView: high-throughput primer design and visualization. *Source Code Biol. Med.* 10:8. doi: 10.1186/s13029-015-0038-2

Park, S.-R., Moon, S.-J., Shin, D.-J., Kim, M.-G., Hwang, D.-J., Bae, S.-C., et al. (2010). Isolation and characterization of rice *OsHRL* gene related to bacterial blight resistance. *Plant Pathol. J.* 26, 417–420. doi: 10.5423/PPJ.2010.26.4.417

Pickering, R. A. (1991). Comparison of crossover frequencies in barley (*Hordeum vulgare*) and *H. vulgare* × *H. bulbosum hybrids* using a paracentric inversion. *Genome* 34, 666–673. doi: 10.1139/g91-102

Pickering, R. A., Hill, A. M., Michel, M., and Timmerman-Vaughan, G. M. (1995). The transfer of a powdery mildew resistance gene from *Hordeum bulbosum* L to barley (*H. vulgare* L.) chromosome 2 (2I). *Theor. Appl. Genet.* 91, 1288–1292. doi: 10.1007/BF00220943

Pickering, R. A., Steeffenson, B. J., Hill, A. M., and Borovkova, I. (1998). Association of leaf rust and powdery mildew resistance in a recombinant derived from a *Hordeum vulgare* × *Hordeum bulbosum* hybrid. *Plant Breed* 117, 83–84. doi: 10.1111/j.1439-0523.1998.tb01453.x

Prescott-Allen, C., and Prescott-Allen, R. (1986). *The First Resource.* New Haven, CT: Yale University Press.

Prisic, S., Xu, M., Wilderman, P. R., and Peters, R. J. (2004). Rice contains two disparate ent-copalyl diphosphate synthases with distinct metabolic functions. *Plant Physiol.* 136, 4228–4236. doi: 10.1104/pp.104.050567

Prohens, J., Gramazio, P., Plazas, M., Dempewolf, H., Kilian, B., Díez, M. J., et al. (2017). Introgressiomics: a new approach for using crop wild relatives in breeding for adaptation to climate change. *Euphytica* 213:158. doi: 10.1007/s10681-017-1938-9

Rao, M. V. P. (1979). *The Transfer of Alien Genes for Stem rust Resistance to Durum Wheat*, ed. S. Ramanujam (New Delhi: Indian Society of Genetics & Plant Breeding), 338–341.

Řepková, J., Teturová, K., Dreiseitl, A., and Soldánová, M. (2009). Characterization and chromosomal location of powdery mildew resistance genes from wild barley PI282605. *J. Plant Dis. Prot.* 116, 257–259. doi: 10.1007/BF03356319

Ruge, B., Linz, A., Pickering, R., Proeseler, G., Greif, P., and Wehling, P. (2003). Mapping of *Rym14Hb*, a gene introgressed from *Hordeum bulbosum* and conferring resistance to BaMMV and BaYMV in barley. *Theor. Appl. Genet.* 107, 965–971. doi: 10.1007/s00122-003-1339-4

Ruge-Wehling, B., Linz, A., Habekuß, A., and Wehling, P. (2006). Mapping of *Rym16Hb*, the second soil-borne virus-resistance gene introgressed from *Hordeum bulbosum*. *Theor. Appl. Genet.* 113, 867–873. doi: 10.1007/s00122-006-0345-8

Savary, S., Ficke, A., Aubertot, J.-N., and Hollier, C. (2012). Crop losses due to diseases and their implications for global food production losses and food security. *Food Secur.* 4, 519–537. doi: 10.1007/s12571-012-0200-5

Scholz, M., Ruge-Wehling, B., Habekuß, A., Schrader, O., Pendinen, G., Fischer, K., et al. (2009). *Ryd4Hb*: a novel resistance gene introgressed from Hordeum bulbosum into barley and conferring complete and dominant resistance to the barley yellow dwarf virus. *Theor. Appl. Genet.* 119, 837–849. doi: 10.1007/s00122-009-1093-3

Shi, Y., Wang, B., Li, Q., Wu, X., Wang, F., Liu, H., et al. (2009). Analysis of the virulent genes of *Erysiphe graminis* f. sp. tritici and the resistance genes of wheat commercial cultivars in Shaanxi Province. *J. Triticeae Crop.* 29, 706–711.

Shimizu, T., Nakano, T., Takamizawa, D., Desaki, Y., Ishii-Minami, N., Nishizawa, Y., et al. (2010). Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. *Plant J.* 64, 204–214. doi: 10.1111/j.1365-313X.2010.04324.x

Shtaya, M. J. Y., Sillero, J. C., Flath, K., Pickering, R., and Rubiales, D. (2007). The resistance to leaf rust and powdery mildew of recombinant lines of barley (*Hordeum vulgare* L.) derived from *H. vulgare* × *H. bulbosum* crosses. *Plant Breed* 126, 259–267. doi: 10.1111/j.1439-0523.2007.01328.x

Singh, P., and Zimmerli, L. (2013). Lectin receptor kinases in plant innate immunity. *Front. Plant Sci.* 4:124. doi: 10.3389/fpls.2013.00124

Stahl, E. A., Dwyer, G., Mauricio, R., Kreitman, M., and Bergelson, J. (1999). Dynamics of disease resistance polymorphism at the Rpm1 locus of *Arabidopsis*. *Nature* 400, 667–671. doi: 10.1038/23260

Steffenson, B. J. (1998). Coordinators reports: disease and pest resistance genes. *Barley Genet. newslette*

Szigat, G., and Szigat, G. (1991). Amphidiploid hybrids between *Hordeum vulgare* and *H. bulbosum*-basis for the development of new initial material for winter barley breeding. *Vor. Pflanzenzüchtg* 20, 34–39.

Tang, S., Hu, Y., Zhong, S., and Luo, P. (2018). The potential role of powdery mildew-resistance Gene *Pm40* in Chinese wheat-breeding programs in the Post-*Pm21* Era. *Engineering* 4, 500–506. doi: 10.1016/j.eng.2018.06.004

Tanksley, S. D., and McCouch, S. R. (1997). Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* 277, 1063–1066. doi: 10.1126/science.277.5329.1063

Tanksley, S. D., and Nelson, J. C. (1996). Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theor. Appl. Genet.* 92, 191–203. doi: 10.1007/BF00223376

Thiel, T., Kota, R., Grosse, I., Stein, N., and Graner, A. (2004). SNP2CAPS: a SNP and INDEL analysis tool for CAPS marker development. *Nucleic Acids Res.* 32, e5–e5. doi: 10.1093/nar/gnh006

Thind, A. K., Wicker, T., Šimková, H., Fossati, D., Moullet, O., Brabant, C., et al. (2017). Rapid cloning of genes in hexaploid wheat using cultivar-specific long-range chromosome assembly. *Nat. Biotechnol.* 35, 793–796. doi: 10.1038/nbt.3877

Tian, D., Araki, H., Stahl, E., Bergelson, J., and Kreitman, M. (2002). Signature of balancing selection in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 99, 11525–11530. doi: 10.1073/pnas.172203599

van der Merwe, J. A., and Dubery, I. A. (2006). Benzothiadiazole inhibits mitochondrial NADH: ubiquinone oxidoreductase in tobacco. *J. Plant Physiol.* 163, 877–882. doi: 10.1016/j.jplph.2005.08.016

von Bothmer, R., Jacobsen, N., Baden, C., Jorgensen, R. B., and Linde-Laursen, I. (1995). "An ecogeographical study of the genus Hor-deum," in *Systematic and Ecogeographic Studies on Crop Gene Pools 7*, (Rome: International Plant Genetic Resources Institute).

von Korff, M., Wang, H., Léon, J., and Pillen, K. (2005). AB-QTL analysis in spring barley. I. Detection of resistance genes against powdery mildew, leaf rust and scald introgressed from wild barley. *Theor. Appl. Genet.* 111, 583–590. doi: 10.1007/s00122-005-2049-x

Walther, U., Rapke, H., Proeseler, G., and Szigat, G. (2000). *Hordeum bulbosum*-a new source of disease resistance-transfer of resistance to leaf rust and mosaic viruses from *H. bulbosum* into winter barley. *Plant Breed* 119, 215–218. doi: 10.1046/j.1439-0523.2000.00475.x

Wang, Y., Bouwmeester, K., Beseh, P., Shan, W., and Govers, F. (2014a). Phenotypic analyses of *Arabidopsis* T-DNA insertion lines and expression profiling reveal that multiple L-Type lectin receptor kinases are involved in plant immunity. *Mol. Plant-Microbe Interact.* 27, 1390–1402. doi: 10.1094/MPMI-06-14-0191-R

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., et al. (2014b). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nat. Biotechnol.* 32, 947–951. doi: 10.1038/nbt.2969

Wang, Y., Cordewener, J. H. G., America, A. H. P., Shan, W., Bouwmeester, K., and Govers, F. (2015a). *Arabidopsis* lectin receptor kinases LecRK-IX.1 and *LecRK-IX*.2 Are functional analogs in regulating phytophthora resistance and plant cell death. *Mol. Plant-Microbe Interact* 28, 1032–1048. doi: 10.1094/MPMI-02-15-0025-R

Wang, Y., Subedi, S., de Vries, H., Doornenbal, P., Vels, A., Hensel, G., et al. (2019). Orthologous receptor kinases quantitatively affect the host status of barley to leaf rust fungi. *Nat. Plants* 5, 1129–1135. doi: 10.1038/s41477-019-0545-2

Wang, Y., Weide, R., Govers, F., and Bouwmeester, K. (2015b). L-type lectin receptor kinases in *Nicotiana benthamiana* and tomato and their role in *Phytophthora* resistance. *J. Exp. Bot.* 66, 6731–6743. doi: 10.1093/jxb/erv379

Wang, Z., Cheng, J., Fan, A., Zhao, J., Yu, Z., Li, Y., et al. (2018). LecRK-V, an L-type lectin receptor kinase in *Haynaldia villosa*, plays positive role in resistance to wheat powdery mildew. *Plant Biotechnol. J.* 16, 50–62. doi: 10.1111/pbi.12748

Wendler, N., Mascher, M., Himmelbach, A., Johnston, P., Pickering, R., and Stein, N. (2015). Bulbosum to Go: a toolbox to utilize *Hordeum vulgare*/*bulbosum* Introgressions for Breeding and Beyond. *Mol. Plant* 8, 1507–1519. doi: 10.1016/j.molp.2015.05.004

Wendler, N., Mascher, M., Nöh, C., Himmelbach, A., Scholz, U., Ruge-Wehling, B., et al. (2014). Unlocking the secondary gene-pool of barley with next-generation sequencing. *Plant Biotechnol. J.* 12, 1122–1131. doi: 10.1111/pbi.12219

Wendler, N., Mascher, M., Himmelbach, A., Bini, F., Kumlehn, J., and Stein, N. (2017). A High-density, sequence-enriched genetic map of *Hordeum bulbosum* and Its collinearity to *H. vulgare*. *Plant Genome* 10, 1–11. doi: 10.3835/plantgenome2017.06.0049

Wijnker, E., and de Jong, H. (2008). Managing meiotic recombination in plant breeding. *Trends Plant Sci.* 13, 640–646. doi: 10.1016/j.tplants.2008.09.004

Willmann, R., Lajunen, H. M., Erbs, G., Newman, M.-A., Kolb, D., Tsuda, K., et al. (2011). *Arabidopsis* lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. *Proc. Natl. Acad. Sci. U.S.A.* 108, 19824–19829. doi: 10.1073/pnas.1112862108

Wu, X. X., Xu, X. F., Ma, D. X., Chen, R. Z., Li, T. Y., and Cao, Y. Y. (2019). Virulence structure and its genetic diversity analyses of *Blumeria graminis* f. sp. tritici isolates in China. *BMC Evol. Biol.* 19:183. doi: 10.1186/s12862-019-1511-3

Wu, Y., and Zhou, J.-M. (2013). Receptor-Like Kinases in Plant Innate Immunity. *J. Integr. Plant Biol.* 55, 1271–1286. doi: 10.1111/jipb.12123

Xing, L., Hu, P., Liu, J., Witek, K., Zhou, S., Xu, J., et al. (2018). *Pm21* from *Haynaldia villosa* Encodes a CC-NBS-LRR protein conferring powdery mildew resistance in wheat. *Mol. Plant* 11, 874–878. doi: 10.1016/j.molp.2018.02.013

Xu, J., and Kasha, K. J. (1992). Transfer of a dominant gene for powdery mildew resistance and DNA from *Hordeum bulbosum* into cultivated barley (*H. vulgare*). *Theor. Appl. Genet.* 84, 771–777. doi: 10.1007/BF00227383

Xu, Z.-S., Li, Z.-Y., Chen, Y., Chen, M., Li, L.-C., and Ma, Y.-Z. (2012). Heat shock protein 90 in plants: molecular mechanisms and roles in stress responses. *Int. J. Mol. Sci.* 13, 15706–15723. doi: 10.3390/ijms131215706

Yang, L., Xiang, L., Zeng, F., Wang, H., Shi, W., and Yu, D. (2009). Virulence gene structure analysis of *Blumeria graminis* f. sp. tritici in Hubei. *Plant Prot* 35, 76–79.

Yu, X., Kong, H. Y., Meiyalaghan, V., Casonato, S., Chng, S., Jones, E. E., et al. (2018). Genetic mapping of a barley leaf rust resistance gene *Rph26* introgressed from *Hordeum bulbosum*. *Theor. Appl. Genet.* 131, 2567–2580. doi: 10.1007/s00122-018-3173-8

Zamir, D. (2001). Improving plant breeding with exotic genetic libraries. *Nat. Rev. Genet.* 2, 983–989. doi: 10.1038/35103590

Zeng, F., Yang, L., Gong, S., Shi, W., Zhang, X., Wang, H., et al. (2014). Virulence and Diversity of *Blumeria graminis* f. sp. tritici Populations in China. *J. Integr. Agric.* 13, 2424–2437. doi: 10.1016/S2095-3119(13)60669-3

Zhang, L., Pickering, R. A., and Murray, B. G. (2001). Hordeum vulgare × *H. bulbosum tetraploid* hybrid provides useful agronomic introgression lines for breeders. *New Zeal. J. Crop Hortic. Sci.* 29, 239–246. doi: 10.1080/01140671.2001.9514185

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