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ORIGINAL ARTICLE

High-resolution mapping of *Rym14^{Hb}*, a wild relative resistance gene to barley yellow mosaic disease

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Abstract

Key message **We mapped the *Rym14^{Hb}* resistance locus to barley yellow mosaic disease in a 2Mbp interval. The co-segregating markers will be instrumental for marker-assisted selection in barley breeding.**

Abstract Barley yellow mosaic disease is caused by Barley yellow mosaic virus and Barley mild mosaic virus and leads to severe yield losses in barley (*Hordeum vulgare*) in Central Europe and East-Asia. Several resistance loci are used in barley breeding. However, cases of resistance-breaking viral strains are known, raising concerns about the durability of those genes. *Rym14^{Hb}* is a dominant major resistance gene on chromosome 6HS, originating from barley's secondary genepool wild relative *Hordeum bulbosum*. As such, the resistance mechanism may represent a case of non-host resistance, which could enhance its durability. A susceptible barley variety and a resistant *H. bulbosum* introgression line were crossed to produce a large F_2 mapping population ($n = 7500$), to compensate for a ten-fold reduction in recombination rate compared to intraspecific barley crosses. After high-throughput genotyping, the *Rym14^{Hb}* locus was assigned to a 2Mbp telomeric interval on chromosome 6HS. The co-segregating markers developed in this study can be used for marker-assisted introgression of this locus into barley elite germplasm with a minimum of linkage drag.

Introduction

Viruses are an increasing threat to crops worldwide. The soil-borne barley yellow mosaic disease, caused by a complex of two *Bymoviruses* (*Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV)) is one of the most important diseases of winter barley. Widespread in central Europe and East-Asia, it causes severe yield losses up to even total crop failure (Plumb et al. 1986; Jianping 2005; Kühne 2009). As chemical control of those viruses, transmitted by the plasmodiophorid *Polymyxa graminis* (Kanyuka et al. 2003), is not possible, only the use of resistant varieties can preserve yield in infected fields.

To date, 20 barley resistance genes have been identified, almost exclusively conferring recessive resistance (Jiang et al. 2020). Two of these loci have been cloned: the *EUKARYOTIC TRANSLATION INITIATION FACTOR*

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4E gene (*eIF4E*), (Stein et al. 2005) of which several allelic forms providing resistance are described, including *rym4* and *rym5*, (Hofinger et al. 2011; Perovic et al. 2014; Yang et al. 2017; Shi et al. 2019), and the *PROTEIN DISULFIDE ISOMERASE LIKE 5–1* (*PDI5-1*) gene which is also represented by a handful of alleles providing resistance, including *rym1* and *rym11* (Yang et al. 2017). The *rym4* allele provides a recessive resistance to BaMMV and to the common BaYMV pathotype BaYMV-1, but not to pathotype BaYMV-2, which emerged in Europe at the end of the 1980s (Adams et al. 1987; Huth 1989; Adams 1991; Graner and Bauer 1993; Steyer et al. 1995). The spectrum of *rym5* covers also *BaYMV*-2, however, resistance-breaking isolates of BaMMV and BaYMV have emerged (Kanyuka et al. 2004; Habekuß et al. 2008; Li et al. 2016). Facing the prospect of boom-and-bust cycles for known resistance genes (Brown and Tellier 2011), it is critical to continue searching for alternative resistance loci to underpin resistance breeding and to allow pyramiding of disease resistance loci. In particular, sources of non-host resistance, e.g. resistance exhibited from a plant species against all isolates of a pathogen which is not coevolutionary adapted, are particularly promising as they are thought to cover a larger resistance spectrum and to be more durable (Ayliffe and Sørensen 2019). Bulbous barley (*Hordeum bulbosum* L.), a perennial wild relative and representative of the secondary gene pool of cultivated barley (*Hordeum vulgare* L.), has been described as source of resistance to numerous barley pathogens, including barley leaf rust (Johnston et al. 2013; Yu et al. 2018) and barley powdery mildew (Xu and Kasha 1992; Pickering et al. 1995; Shtaya et al. 2007). So far, all *H. bulbosum* accessions investigated exhibited resistance to BaMMV and BaYMV (Ruge et al. 2003), suggesting that the species is probably a non-host to those viruses. Two major dominant resistance genes from *H. bulbosum* to both BaMMV and BaYMV have been described: $Rym14^{Hb}$ (Ruge et al. 2003) and $Rym16^{Hb}$ (Ruge-Wehling et al. 2006). $Rym14^{Hb}$ was introgressed to barley by translocation of a *H. bulbosum* segment to barley chromosome 6HS (Ruge et al. 2003). In the past, a lack of suitable markers, alongside severely reduced recombination in the target region between the barley and *H. bulbosum* fragments, rendered precise mapping of $Rym14^{Hb}$ elusive. Thanks to the development of genetic and genomic resources for *H. bulbosum* (Wendler et al. 2014, 2015), it is now possible to fine-map loci from this species in a *H. vulgare* background.

We aimed to map $Rym14^{Hb}$ at high resolution, and to provide markers for its introgression into elite barley, ideally without linkage drag, using large populations and high-throughput genotyping to overcome the lack of recombination.

Materials and methods

Plant material

A first round of low-resolution genetic mapping was performed using four F_6 families derived from F_5 plants heterozygous at the $Rym14^{Hb}$ locus from the BAZ-4006 family of the population described in Ruge et al. (2003) and obtained from the cross between the susceptible winter barley cv 'Borwina' and the resistant *H. bulbosum* accession 'A42'.

To achieve a population size suitable for fine mapping, an additional eight F_2 families were generated by crossing an $Rym14^{Hb}/Rym14^{Hb}$ F_6 plant (derived from F_5 4006/337) to either (i) var. 'KWS Orbit' or (ii) var. 'KWS Higgins', both missing the $Rym14^{Hb}$ resistance locus (-/-). In the purpose of instant pyramiding of disease resistance loci, both cultivars carry *rym4*-based resistance (*rym4*/*rym4*) to BaMMV and BaYMV.

DNA extraction

Genomic DNA of plants from the low-resolution mapping population was isolated as described by Stein et al. (2001). Genomic DNA of plants from the fine mapping population was extracted according to the guanidine isothiocyanate-based protocol described by Milner et al. (2019).

Genotyping-by-sequencing and data analysis

Genotyping-by-sequencing (GBS) libraries for the low-resolution mapping were prepared from genomic DNA digested with *Pst*I and *Msp*I (New England Biolabs) as described by Wendler et al. (2015). Between 93 and 153 barcoded samples were pooled in an equimolar manner per lane and sequenced on the Illumina HiSeq 2500 for 107 cycles, single-end reads, using a custom sequencing primer.

The GBS reads were processed, aligned, and used to generate variant calls as described by Milner et al. (2019). Alignment was performed against the TRITEX genome assembly of barley cultivar 'Morex' (Monat et al. 2019). Individual variant calls were accepted wherever the read depth exceeded four. Variant sites were retained if they presented a minimum mapping quality score (based on read depth ratios calculated from the total read depth and depth of the alternative allele) of 20, a maximum fraction of 40% of missing data, a fraction of heterozygous calls between 30 and 70%, and between 10 to 40% of each homozygous call. Individuals with more than 40% missing data were excluded.

Marker development

Exome capture data of the introgression line '4006/163', described in Wendler et al. (2014) (accession number ERP004445), were mapped to the TRITEX genome assembly of barley cultivar 'Morex' (Monat et al. 2019) together with the exome capture data of the *H. bulbosum* genotype 'A42' and of eight barley varieties: 'Bonus', 'Borwina', 'Bowman', 'Foma', 'Gull', 'Morex', 'Steptoe', and 'Vogelsanger Gold', described in Mascher et al. (2013b) (accession number PRJEB1810). Read mapping and variant calling were performed as described by Milner et al. (2019). The variant matrix was filtered for the following criteria: heterozygous and homozygous calls had to be covered by a minimum depth of three and five reads, respectively, and have a minimum quality score of 20. Single nucleotide polymorphism (SNP) sites were retained if they had less than 20% missing data and less than 20% heterozygous calls. SNPs that were carrying the reference call in all eight barleys and the alternate call in 'A42' and '4006/163' was selected as candidates to design Kompetitive Allele Specific PCR (KASP) markers, either using KASP-by-design (LGC Genomics, Berlin, Germany) or 3CR Bioscience (Essex, UK) free assay design service. Those markers are latter designated as KASP and PCR Allele Competitive Extension (PACE) markers, respectively. Since no suitable SNPs were identified in the first 500 kbp of chromosome 6HS on the 'Morex' reference genome, the exome capture data were additionally mapped to the genome assembly of cultivar 'Barke' (Jayakodi et al. in press). The SNP at coordinate 241,723 bp on chromosome 6H of the 'Barke' genome assembly was retrieved and used to design the telomeric marker Rym14_Bar241723. Furthermore, in order to control the genetic state at the segregating *rym4* resistance locus, the diagnostic SNP for the resistance conferring allele (Stein et al. 2005) was also used to design a KASP marker. Further information on KASP and PACE markers is provided in supplementary tables 1 and 2, respectively.

Genotyping

Genotyping assays with KASP markers were carried out in a final volume of 5 µl consisting of 0.7 µl genomic DNA (50–100 ng/µL), 2.5 µl of KASP V4.0 2X Master Mix High Rox (LGC Genomics, Berlin), 0.07 µl KASP assay mix (KASP-by-design, LGC Genomics, Berlin) containing the primers, and 2.5 µl of sterile water. PCR amplifications were performed using the Hydrocycler 16 (LGC Genomics, Berlin) with cycling conditions as follows: 94 °C for 15 min, followed by a touchdown profile of 10 cycles at 94 °C for 20 s and 61 °C for 1 min with a 0.6 °C reduction per cycle, followed by 26 cycles at 94 °C for 20 s and 55 °C for 1 min. Genotyping assays with PACE markers were carried out in a final volume of 5 µl consisting of 0.7 µl genomic DNA (50–100 ng/µL), 2.5 µl of PACE Master Mix High Rox (3cr Bioscience, Essex, United Kingdom), 0.07 µl primer mix containing the primers (12 µM of each allele specific primers and 30 µM of the common reverse primer), and 2.5 µl of sterile water. PCR amplifications were performed using the Hydrocycler 16 (LGC Genomics, Berlin) with cycling conditions as follows: 94 °C for 15 min, followed by a touchdown profile of 10 cycles at 94 °C for 20 s and 65 °C for 1 min with a 0.8 °C reduction per cycle, followed by 30 cycles at 94 °C for 20 s and 57 °C for 1 min.

For both marker types, the genotyping results were read out using the ABI 7900HT (Applied Biosystems) using an allelic discrimination file. Readings were made before and after PCR, and the data were analyzed using SDS 2.4 Software (Applied Biosystems).

Phenotyping

Resistance to BaMMV was tested under greenhouse conditions as described by Habekuß et al. (2008). After sowing, the plants were grown in a greenhouse (16 h day/8 h night, 12 °C). The susceptible barley variety 'Maris Otter' was systematically included to monitor success of infection. At the 3-leaf stage (around 2 weeks after sowing), the plants were mechanically inoculated twice at an interval of 5–7 days with the isolate BaMMV-ASL1 (Timpe and Kühne 1994) using the leaf-sap of BaMMV-infected leaves of susceptible cv. 'Maris Otter', mixed in K_2HPO_4 buffer (1:10; 0.1 M; pH 9.1) containing silicon carbide (caborundum, mesh 400, 0.5 g/25 ml sap). Five weeks after the first inoculation, the number of infected plants with mosaic symptoms were scored, and double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with polyclonal BaMMV-specific antibodies produced by the Serum Bank of the Institute of Epidemiology and Pathogen Diagnostics (JKI Quedlinburg, Germany) was carried out in parallel according to published protocols (Clark and Adams 1977). Virus particles were estimated via extinction at 405 nm using a Dynatech MR 5000 microtiter-plate reader. Plants with an extinction $E_{405} > 0.1$ were qualitatively scored as susceptible.

Results

Low-resolution mapping

A population of 427 F_6 from the cross 'Borwina' x 'A42' was genotyped by GBS and phenotyped for resistance to BaMMV. Data for 389 plants and 77 SNPs passed the quality filters (supplementary table 3). On chromosome 6H, 73 plants were homozygous for the 'Borwina' allele, 92 were homozygous for the 'A42' allele, 220 were heterozygous,

and four recombined. The infection rate was low with only 10% of plants infected, compared to an expected 25% when resistance is controlled by a single dominant gene. Two susceptible barley cultivars were tested: 92.5% and 72.2% of Maris Otter and Igri plants, respectively, were infected. It is known that the penetrance of infection in such experiments is never complete, and that the genetic background of the plant plays a role in this phenomenon, Maris Otter being the most susceptible cultivar tested (Adams et al. 1986, 1993). Despite that, among the 39 plants phenotyped as susceptible to BaMMV, 38 were homozygous for the 'Borwina' allele and one recombined on chromosome 6H, indicating a strong association of phenotype and genotype.

To further confirm this association, 26 lines were phenotyped on progenies of 12–20 plants (Fig. 1a, Table 1, supplementary table 4). These included (i) 17 lines with the susceptible genotype on chromosome 6H but scored as resistant, (ii) five heterozygous lines, and (iii) the four recombinant lines. Progenies of lines presenting the susceptible genotype displayed infection rates between 50 and 95%, while those of heterozygous lines displayed rates between 5 and 20%. The progeny of line 5204–58 displayed an intermediate level of susceptibility, with 35% of infected plants. However, this line had been phenotyped as susceptible in F_2 generation, and was therefore classified as susceptible.

These results support the low penetrance of the infection in this experiment, with only half of the expected susceptible plants successfully infected, as well as the association of the chromosome 6H locus with resistance to BaMMV. Moreover, the phenotypes of the four recombinant progenies defined *Rym14*^{Hb} interval between the telomere of chromosome 6HS and the marker position at base pair 4,553,134.

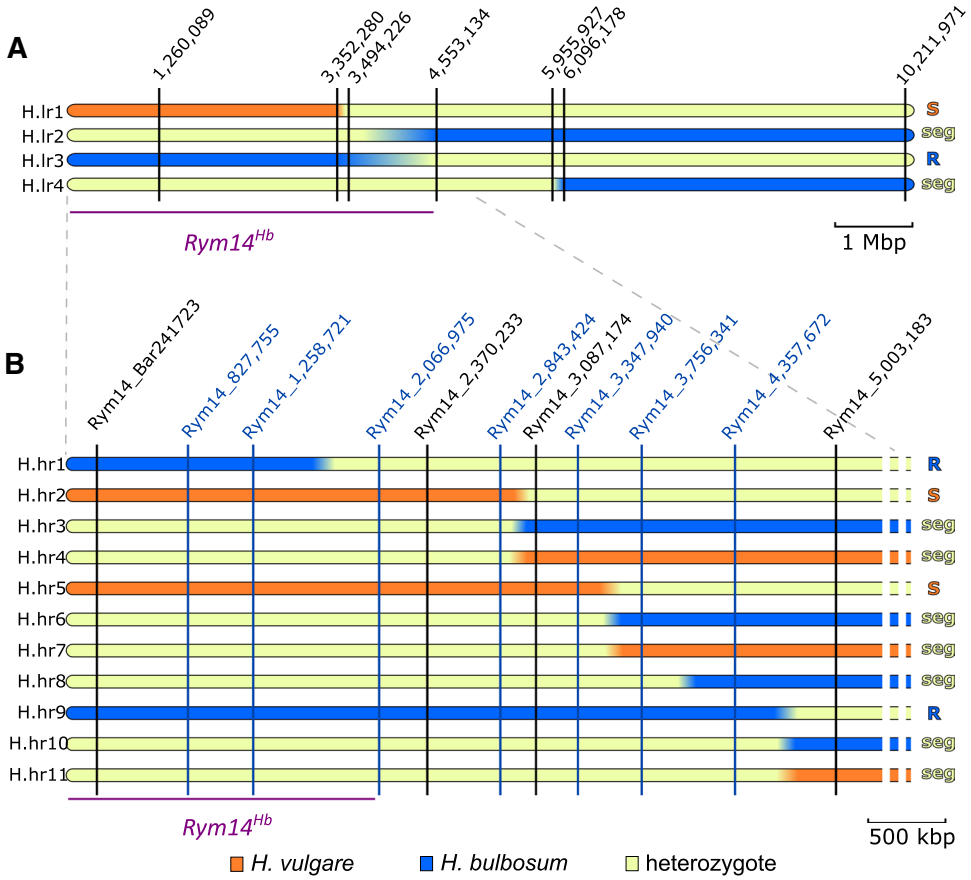

Fig. 1 Physical map of the *Rym14*^{Hb} locus. **a** Low-resolution mapping of the *Rym14*^{Hb} locus. Graphical genotype and phenotype of the four recombinant F_6 lines. *H. vulgare*, *H. bulbosum*, and heterozygous allelic states are represented as orange, blue, and yellow bars, respectively. Coordinates on 'Morex' reference genome (Monat et al. 2019) of strategic markers are displayed. Names of the haplotypes are displayed on the left, and phenotypes deduced from the phenotyped F_7 progenies are shown on the right (*R* resistant, *S* susceptible, *seg* segregation of resistance). **b** High-resolution mapping of the *Rym14*^{Hb} locus. KASP and PACE markers are represented as black and blue vertical lines, respectively, and the 11 recombinant haplotypes found in F_2 plants are indicated by horizontal bars: blue = *H. bulbosum* homozygous; orange = *H. vulgare* homozygous; yellow = heterogygous. The haplotype name is indicated on the left while the phenotypes of their progeny are shown on the right (*R* resistant, *S* susceptible, *seg* segregation of resistance)

Table 1 Phenotype of 26 F_2 lines from the cross 'Borwina' x 'A42' and of their F_3 progenies

Sample name	Genotype at *Rym14*^{Hb} locus[a]	Phenotype on F_2[b]	F_3 progenies			
			Non-infected plants	Infected plants	% of infected plants	Deduced phenotype[b]
Sample_5204_58	Recombinant (G.lr1)	S	9	5	36	S
Sample_5204_79	Recombinant (G.lr2)	R	17	2	11	seg
Sample_5214_89	Recombinant (G.lr3)	R	19	0	0	R
Sample_5218_43	Recombinant (G.lr4)	R	12	2	14	seg
Sample_5220_18	Heterozygous	R	15	3	17	seg
Sample_5220_4	Heterozygous	R	17	2	11	seg
Sample_5220_5	Heterozygous	R	17	2	11	seg
Sample_5220_81	Heterozygous	R	12	3	20	seg
Sample_5214_30	Heterozygous	R	19	1	5	seg
Sample_5204_102	*H. vulgare*	R	7	7	50	S
Sample_5204_56	*H. vulgare*	R	9	10	53	S
Sample_5204_67	*H. vulgare*	R	7	12	63	S
Sample_5204_78	*H. vulgare*	R	8	11	58	S
Sample_5204_98	*H. vulgare*	R	4	16	80	S
Sample_5214_10	*H. vulgare*	R	3	13	81	S
Sample_5214_100	*H. vulgare*	R	1	16	94	S
Sample_5214_132	*H. vulgare*	R	1	18	95	S
Sample_5214_133	*H. vulgare*	R	4	15	79	S
Sample_5214_146	*H. vulgare*	R	4	16	80	S
Sample_5214_147	*H. vulgare*	R	7	10	59	S
Sample_5214_23	*H. vulgare*	R	3	17	85	S
Sample_5214_33	*H. vulgare*	R	3	11	79	S
Sample_5214_90	*H. vulgare*	R	4	16	80	S
Sample_5214_91	*H. vulgare*	R	3	9	75	S
Sample_5218_6	*H. vulgare*	R	6	12	67	S
Sample_5219_29	*H. vulgare*	R	5	15	75	S
Maris Otter (susceptible control)	*H. vulgare*	S	2	22	92	S

[a]Either the recombinant haplotype name in Fig. 1, or, for non-recombinant lines, 'heterozygous' or '*H. vulgare*'

[b]Resistant (R), susceptible (S) or segregating (seg)

Fine mapping

The population of 7500 F_2 was genotyped at the *Rym14*^{Hb} locus with four KASP markers (Rym14_Bar241723, Rym14_2370223, Rym14_3087282, and Rym14_5003183, supplementary table 1). The recessive resistance gene *rym4* to BaMMV and BaYMV-1, located on chromosome 3HL, also segregated in the population. Therefore, in order to properly assign the resistance to the control of *Rym14*^{Hb}, the segregation of *rym4* was monitored with the rym4_SNP KASP marker (supplementary table1). We identified 28 recombination events, corresponding to a genetic distance of ~ 0.2 cM, between the markers Rym14_Bar241723 and Rym14_5003183. These results confirmed the strongly reduced recombination rate between the *H. bulbosum* and the *H. vulgare* fragments on chromosome 6HS. In cultivated barley, the syntenic 5 Mbp *Rym14*^{Hb} interval on chromosome 6HS corresponds to a genetic distance of 4 cM (Mascher et al. 2013a), implying a 20-fold reduction in recombination frequency between the *H. bulbosum* and the *H. vulgare* fragment.

All recombinants were genotyped with seven PACE markers (Fig. 1b, supplementary tables 2 and 4). Among the recombinants, ten plants were homozygous for the *rym4* allele, nine were heterozygous, and the remaining nine were homozygous wildtype at the *rym4* locus (supplementary table 5). As plants homozygous for the *rym4* allele would be resistant to BaMMV, irrespective to their genotype at *Rym14*^{Hb}, only F_3 families derived from the 18 *Rym14*-recombinants heterozygous or homozygous for the susceptible allele at *rym4* were phenotyped using 30 and 20 F_3 siblings, respectively (Table 2). The infection rate during

Table 2 Phenotype of the progeny that are not homozygous for the *rym4* allele of the 18 F_2 recombinants from high-resolution mapping population. F_3 plants that are homozygous for *rym4* allele were excluded from this table

Sample name	Genotype at *Rym14*^{Hb} locus[a]	Non-infected plants	Infected plants	% of infected plants	*p*-value χ^2 test 3R:1S ratio[b]	Deduced phenotype[c]
Rym14_48/9_261	Recombinant (G.hr1)	23	0	0	0.0056**	R
Rym14_49/9_372	Recombinant (G.hr2)	2	18	90	–	S
Rym14_48/2_188	Recombinant (G.hr3)	15	5	25	1	seg
Rym14_48/6_178	Recombinant (G.hr3)	16	4	20	0.61	seg
Rym14_49/6_174	Recombinant (G.hr3)	16	4	20	0.61	seg
Rym14_49/8_179	Recombinant (G.hr3)	17	5	23	0.81	seg
Rym14_49/7_69	Recombinant (G.hr4)	14	4	22	0.79	seg
Rym14_48/8_187	Recombinant (G.hr4)	14	10	42	0.59	seg
Rym14_49/1_73	Recombinant (G.hr5)	0	18	100	–	S
Rym14_49/7_149	Recombinant (G.hr6)	22	1	4	0.02*	seg
Rym14_49/10_125	Recombinant (G.hr6)	17	2	11	0.15	seg
Rym14_49/3_301	Recombinant (G.hr7)	17	3	15	0.36	seg
Rym14_48/1_328	Recombinant (G.hr7)	17	2	11	0.15	seg
Rym14_49/2_527	Recombinant (G.hr8)	14	1	7	0.10	seg
Rym14_48/8_389	Recombinant (G.hr9)	20	0	0	0.0098**	R
Rym14_49/3_58	Recombinant (G.hr9)	23	0	0	0.0056**	R
Rym14_48/4_354	Recombinant (G.hr10)	17	4	19	0.53	seg
Rym14_48/3_381	Recombinant (G.hr11)	18	2	10	0.12	seg
Maris otter (susceptible control)	*H. vulgare*	1	51	98	–	S

[a]Specified as the recombinant (rec) haplotype name in Fig. 1

[b]Resulting *P-value* from a χ^2 test of goodness to fit the expected ratio of 3R:1S (three resistant plants for one susceptible plant expected for a dominant locus)

[c]Resistant (R), susceptible (S) or segregating (seg)

this round of phenotyping was much higher than during the preceding low-resolution mapping, with only one susceptible control showing no viral content. The inoculation of this round of phenotyping and the one carried out for low-resolution mapping occurred at very different time, and with a different batch of inoculum on plants with different genetic backgrounds. A small difference in inoculum concentration, environment or intensity of inoculation could explain the difference observed, as could the genetic background of the populations.

All phenotyped plants were genotyped at Rym14_Bar241723, Rym14_2370223, Rym14_5003183 and *rym4* (supplementary table 6). The *Rym14*^{Hb} phenotypes of the recombinant lines were deduced from the segregation of infection in F_3 progenies that does not carry *rym4* at homozygous state, controlled by a χ^2 test of goodness to fit the expected ratio of 3R:1S (three resistant plants for one susceptible plant expected for a dominant locus) for segregation of a single dominant resistance gene (Table 2). The cosegregation of the phenotype with the genotype at the three markers in the F_3 progenies largely confirmed the analysis (supplementary table 6). The only discrepancy was the significant χ^2 result for line Rym14_49/7_149, that was explained by a distortion of segregation of the susceptible allele in the small number of plants tested: a single plant was homozygous for *H. vulgare* allele in *Rym14*^{Hb} interval.

Based on this analysis, the *Rym14*^{Hb} target region was reduced to a 2 Mbp interval on the 'Morex' reference genome, between the telomere of chromosome 6HS and Rym14_2066975 (Fig. 1b, Table 2).

Candidate genes

In the absence of a genomic sequence for a *Rym14*^{Hb} plant, we cannot precisely define the genes present in the *Rym14*^{Hb} interval. However, as synteny between the two *Hordeum* species is high (Wendler et al. 2017), it is still relevant to assess the genes annotated in the orthologous interval of the *H. vulgare* reference genome as a proxy for suggesting *Rym14*^{Hb} candidate genes. In the respective interval of the 'Morex' V2 reference sequence, 30 high-confidence (HC) (Table 3) and 17 low-confidence genes (Monat et al. 2019) are annotated. In addition, all HC gene models were checked for homology with other genes by a BLASTx (v2.9.0, default parameters) homology searches against the non-redundant protein sequence database (Camacho et al. 2009) and for

Table 3 Genes annotated with high confidence in *Rym14*^{Hb} interval on the 'Morex' genome (Monat et al. 2019)

Name	Start	Stop	Gene type
HORVU.MOREX.r2.6HG0447840	195,540	196,334	Thionin
HORVU.MOREX.r2.6HG0447850	220,610	221,213	Thionin
HORVU.MOREX.r2.6HG0447860	256,998	259,999	Thionin
HORVU.MOREX.r2.6HG0447880	373,994	438,209	Thionin
HORVU.MOREX.r2.6HG0447890	460,556	461,157	Thionin
HORVU.MOREX.r2.6HG0447900	461,856	462,457	Thionin
HORVU.MOREX.r2.6HG0447910	497,194	497,795	Thionin
HORVU.MOREX.r2.6HG0447920	597,800	598,403	Thionin
HORVU.MOREX.r2.6HG0447930	625,302	625,905	Thionin
HORVU.MOREX.r2.6HG0447940	691,184	707,575	Thionin
HORVU.MOREX.r2.6HG0447950	749,829	776,991	Thionin
HORVU.MOREX.r2.6HG0447960	792,195	827,832	Thionin
HORVU.MOREX.r2.6HG0447980	958,137	958,736	Thionin
HORVU.MOREX.r2.6HG0447990	1,004,017	1,004,618	Thionin
HORVU.MOREX.r2.6HG0448010	1,259,976	1,260,591	TIR-NBS-LRR class disease resistance protein
HORVU.MOREX.r2.6HG0448020	1,300,107	1,300,565	Dimeric alpha-amylase inhibitor
HORVU.MOREX.r2.6HG0448100	1,493,250	1,493,945	Dirigent protein
HORVU.MOREX.r2.6HG0448110	1,574,160	1,575,749	Cytochrome P450 family protein, expressed
HORVU.MOREX.r2.6HG0448120	1,578,752	1,580,023	Aspartic proteinase nepenthesin-1
HORVU.MOREX.r2.6HG0448130	1,598,418	1,600,649	Subtilisin-like protease
HORVU.MOREX.r2.6HG0448140	1,605,306	1,610,732	Fatty acyl-CoA reductase
HORVU.MOREX.r2.6HG0448160	1,753,412	1,756,451	Glycerol-3-phosphate acyltransferase 3, putative
HORVU.MOREX.r2.6HG0448200	1,792,383	1,794,963	Transposon protein, putative, CACTA, En/Spm sub-class
HORVU.MOREX.r2.6HG0448210	1,796,825	1,804,280	O-acyltransferase WSD1
HORVU.MOREX.r2.6HG0448220	1,840,897	1,842,376	GDSL esterase/lipase
HORVU.MOREX.r2.6HG0448230	1,853,483	1,854,626	Short-chain dehydrogenase/reductase
HORVU.MOREX.r2.6HG0448250	1,945,996	1,952,442	Protein kinase family protein
HORVU.MOREX.r2.6HG0448260	1,954,346	1,955,384	zinc finger MYM-type-like protein
HORVU.MOREX.r2.6HG0448290	2,061,596	2,062,919	Cysteine protease-like protein
HORVU.MOREX.r2.6HG0448300	2,066,856	2,067,293	Proteinase inhibitor type-2

presence of conserved domains in NCBI conserved domains (Lu et al. 2019). Among the HC genes, HORVU.MOREX.r2.6HG0448010 is annotated as a TIR-NBS-LRR gene in Monat et al. (2019), however, our analysis reveals that it does not contain any of the major domains of nucleotide-binding and leucine-rich repeat domain (NLR) genes (TIR or coiled-coil, NB-ARC and LRR), and monocotyledons so far have not been shown to contain TIR-NLR genes (Jacob et al. 2013). This gene is therefore interpreted as a pseudogene. HORVU.MOREX.r2.6HG0448100, annotated as a dirigent protein, is a jacalin-related lectin, while HORVU.MOREX.r2.6HG0448250, annotated as part of the protein kinase protein family, displays the highest homology with a wall-associated receptor kinase, and HORVU.MOREX.r2.6HG0448290 codes for a papain-like cysteine protease (PLCP). Interestingly, the interval also contains no less than 14 HC genes annotated as thionins, sharing with each other at least 88% of their coding sequence. In addition to these annotated genes in the 'Morex' genome, additional candidate genes could be unique to the resistant genotypes.

Discussion

Resistance genes deployed in breeding and in the field are often overcome by new pathogen variants after only a few years (Brown and Tellier 2011). Pyramiding several resistance genes has proven to increase the resistance durability, however, this strategy requires the availability of several independent resistance loci (Werner et al. 2005; Riedel et al. 2011; Kim et al. 2011). In light of these facts, non-adapted resistance genes from wild crop relatives are precious, since they are assumed to confer more durable resistance than genes originating from within the diversity of the cultivated species, owing to co-evolution between the cultivated host and pathogen genotypes (Fonseca and Mysore 2019). Until

recently, the fine mapping of genes from crop wild relative species was impractical, owing to strong suppression of recombination with the cultivated species (Ruge et al. 2003; Kakeda et al. 2008; Wijnker and de Jong 2008; Prohens et al. 2017). The results of this study demonstrate that high-throughput genotyping coupled with large mapping populations can overcome this limitation, by constraining the interval of the *Rym14*^{Hb} viral resistance gene to the telomeric 2 Mbp of chromosome 6HS, and providing markers suitable for marker-assisted-selection.

Rym14^{Hb} was described as providing resistance against both BaMMV and BaYMV (Ruge et al. 2003). However, phenotyping for resistance to BaYMV is only feasible in infested fields, and is not well adapted to gene mapping. Therefore, in this study, we only mapped BaMMV resistance. Among cloned by movirus resistance genes, the resistance alleles *rym4* and *rym5* of the eIF4E gene, and the alleles *rym1* and *rym11* of PDI5-1 gene provide resistance against isolates of both virus species (Kanyuka et al. 2004; Stein et al. 2005; Ordon et al. 2005; Habekuss et al. 2008). Thus, the two viruses are genetically similar enough for a gene to provide resistance against isolates of both viruses. But the possibility of the described *Rym14*^{Hb} BaYMV resistance being provided by a closely associated, but distinct, locus cannot be excluded at this point and will require further testing.

While genes coding for NLR are the usual suspects for dominant resistance to pathogens, including viruses (de Ronde et al. 2014; Boualem et al. 2016), only a pseudogene presenting similarities with this gene family is annotated in the *Rym14*^{Hb} interval on the barley reference genome. However, it is not rare that susceptible genotypes do not possess a functional copy of the resistance gene. NLRs are overrepresented in regions displaying presence/absence variation (Xu et al. 2012; Bush et al. 2013). Therefore, some NLR resistance genes, like *RPM1* and *RPS5*, are only present in the resistant genotype (Grant et al. 1998; Henk et al. 1999). In the case of wheat leaf rust resistance gene *Lr21*, it was shown that the gene is a chimera of two non-functional alleles that probably evolved via a recombination event (Huang et al. 2009).

Among the other annotated genes at the *Rym14*^{Hb} locus, two are very good candidates. Wall-associated protein kinase-like HORVU.MOREX.r2.6HG0448250 is described resistance genes in plant-bacteria and plant-fungus pathosystems (Li et al. 2009, 2020; Dmochowska-Boguta et al. 2020). Their role in plant-virus pathosystems is less clear but it has been suggested that a cell wall-associated protein kinase was involved in the repression of plasmodesmal transport of the Tobacco mosaic virus by phosphorylating its movement protein (Citovsky et al. 1993; Waigmann et al. 2000). A second promising candidate is HORVU.MOREX.r2.6HG0448100. It codes for a jacalin-related lectin and is thus part of the family that includes the *Arabidopsis thaliana* genes *RTM1* and *JAX1* that provide dominant major resistance against poty- and potexviruses, respectively (Chisholm et al. 2000; Yamaji et al. 2012).

However, other genes in the *Rym14*^{Hb} interval, even if less likely candidates, might also play a role in resistance. For example, HORVU.MOREX.r2.6HG0448290 codes for a PLCP. PLCPs are known to play a major role in programmed cell death triggered by NLR genes. Interestingly, CYP1, a tomato PLCP, is targeted by the Tomato yellow leaf curl virus V2 protein, suggesting that V2 could downregulate *CYP1* to counteract host defenses (Bar-Ziv et al. 2012). *Rcr3*, a tomato papain-like cysteine protease gene, is required for the function of the resistance gene *Cf-2* to *Cladosporium fulvum* (Krüger et al. 2002), while *NbCathB*, from *Nicotina benthamiana*, is requested for the HR triggered by the non-host pathogens *Erwinia amylovora* and *Pseudomonas syringae* (Gilroy et al. 2007). The high level of thionin duplication at this locus also raised our attention. Thionins are part of common anti-bacterial and antifungal peptides (Bohlmann and Broekaert 1994), conferring enhanced resistance to several pathogens. Thionins were also found to exhibit increased expression in resistant compared to susceptible pepper genotypes during infection by the Chili leaf curl virus (Kushwaha et al. 2015), suggesting a possible role in basal defense. Additionally, the cytochrome P450 superfamily has been associated with resistance to the Soybean mosaic virus (Cheng et al. 2010; Yang et al. 2011). Some subtilisin proteases are induced by pathogens and involved in programmed cell death (Figueiredo et al. 2014), and GDSL lipases were found to be either negative or positive regulators of plant defense mechanisms (Hong et al. 2008; Kwon et al. 2009).

The feasibility of further reducing the target interval by recombination through additional fine mapping is low and would require the screening of tens of thousands of additional F_2 plants for the chance of finding one additional recombinant in the smallest target region. Therefore, a candidate gene approach may be a more fruitful strategy for continued progress. Despite the presence of promising candidate genes like HORVU.MOREX.r2.6HG0448250 and HORVU.MOREX.r2.6HG0448100 in the haplotype of the susceptible cultivar 'Morex', the resistance conferring gene may be present only in the haplotype of the resistant *H. bulbosum*. Therefore, deciphering the resistant haplotype, most likely though a high-quality chromosome-scale genome assembly of the interval in *H. bulbosum*, is an essential prerequisite to the prioritization of candidate genes for further functional testing.

The markers identified in this study are tightly linked to *Rym14*^{Hb} and therefore are of prime importance to barley breeding. These markers will allow the reliable introgression of this resistance into barley elite lines with a minimum of

linkage drag compared to the previously established markers (Ruge et al. 2003). This is essential for introducing this gene into new cultivars. As the prevalence of resistance-breaking isolates of *rym4* and *rym5* will increase in the barley growing area in Europe and Asia (Kühne 2009), introgression of $Rym14^{Hb}$ into new elite varieties together with other resistance loci represents a critical opportunity to improve the durability and spectrum of barley resistance to BaMMV and BaYMV.

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Availability of data and material The GBS dataset generated and analyzed in this study is deposited at EMBL-ENA under the project ID PRJEB39211.

Code availability Not applicable.

Compliance with ethical standards

Conflict of interest NW and AM are employed at KWS SAAT SE & Co and KWS LOCHOW, respectively. The other authors declare no conflict of interest.

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