bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239145; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

1 **Authors:** Hélène Pidon[1*], Neele Wendler[2], Antje Habekuβ[3], Anja Maasberg[4], Brigitte Ruge-Wehling[5],
2 Dragan Perovic[3], Frank Ordon[3], Nils Stein[1,6*]

3 **Title:** High-resolution mapping of *Rym14[Hb]*, a wild relative resistance gene to barley yellow mosaic
4 disease

5 **Authors affiliations:**
6 [1] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), OT Gatersleben, Corrensstr. 3, D-
7 06466 Stadt Seeland, Germany

8 [2] KWS SAAT SE & Co. KGaA, Grimsehlstr. 31, D-37574 Einbeck, Germany

9 [3] Julius Kühn Institute (JKI), Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Straße
10 27, D-06484 Quedlinburg, Germany

11 [4] KWS LOCHOW GMBH, Ferdinand-von-Lochow-Straße 5, D-29303 Bergen, Germany

12 [5] Julius Kühn Institute (JKI), Institute for Breeding Research on Agricultural Crops, Groß Lüsewitz,
13 Rudolf-Schick-Platz 3a, D-18190 Sanitz, Germany

14 [6] CiBreed - Center for Integrated Breeding Research, Von Siebold Straße 8, Georg-August University,
15 D-37075 Göttingen, Germany

16 ***Corresponding authors:** pidon@ipk-gatersleben.de or stein@ipk-gatersleben.de

17 Authors ORCID:
18 Pidon H.: 0000-0002-9802-1787
19 Habekuβ A.: 0000-0001-9627-0280
20 Dragan P.: 0000-0002-0292-1693
21 Ordon F.: 0000-0002-1695-6395
22 Stein N.: 0000-0003-3011-8731

23 **Abstract**
24 Barley yellow mosaic disease is caused by Barley yellow mosaic virus and Barley mild mosaic virus,
25 and leads to severe yield losses in barley (*Hordeum vulgare*) in Central Europe and East-Asia. Several
26 resistance loci are used in barley breeding. However, cases of resistance-breaking viral strains are
27 known, raising concerns about the durability of those genes. *Rym14[Hb]* is a dominant major resistance
28 gene on chromosome 6HS, originating from barley's secondary genepool wild relative *Hordeum*
29 *bulbosum*. As such, the resistance mechanism may represent a case of non-host resistance, which could
30 enhance its durability. A susceptible barley variety and a resistant *H. bulbosum* introgression line were
31 crossed to produce a large F_2 mapping population (n=7,500), to compensate for a ten-fold reduction in
32 recombination rate compared to intraspecific barley crosses. After high-throughput genotyping, the
33 *Rym14[Hb]* locus was assigned to a 2Mbp telomeric interval on chromosome 6HS. The co-segregating
34 markers developed in this study can be used for marker-assisted introgression of this locus into barley
35 elite germplasm with a minimum of linkage drag.

36 **Keywords**
37 High-resolution mapping, Soil-borne Bymovirus, disease resistance, barley, *Hordeum bulbosum*,
38 *Rym14[Hb]*

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239145; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

39 **Declarations**

40 **Funding** This work was supported as part of the collaborative projects “TransBulb” (grant
41 0315966 from the German Federal Ministry of Education and Research (BMBF)) and “BulbOmics”
42 (grant 2818201615 from the German Federal Ministry of Food and Agriculture (BMEL)).

43 **Conflicts of interest/Competing interests** NW and AM are employed at KWS SAAT SE & Co
44 and KWS LOCHOW, respectively. The other authors declare no conflict of interest.

45 **Ethics approval** Not applicable

46 **Consent to participate** Not applicable

47 **Consent for publication** Not applicable

48 **Availability of data and material** The GBS dataset generated and analyzed in this study is
49 deposited at EMBL-ENA under the project ID PRJEB39211 (not accessible during peer-review).

50 **Code availability** Not applicable

51 **Authors' contributions** NS, FO and DP concepted the project and acquired the funding. BRW
52 and AM designed and constructed the mapping populations. HP and NW performed the genotyping
53 experiments. AH carried out the phenotyping experiments. HP processed the experimental data,
54 performed the analysis and drafted the manuscript. NS supervised the project. All authors provided
55 critical feedback and helped shape the manuscript.

56 **Acknowledgments**
57 We gratefully acknowledge the excellent technical support by Manuela Kretschmann in DNA extraction
58 and KASP genotyping, Dörte Grau in BaMMV resistance phenotyping and Susanne König in GBS
59 library preparation. We thank Axel Himmelbach for his valuable support in next generation sequencing,
60 Klaus Oldach, Viktor Korzun and Jörg Grosser for their valuable inputs and Timothy Rabanus-Wallace
61 for language editing.

62 **Key message (<30 words)**
63 We mapped the *Rym14*^{Hb} resistance locus to barley yellow mosaic disease in a 2Mbp interval. The co-
64 segregating markers will be instrumental for marker assisted selection in barley breeding.

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239145; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

65 **Introduction**

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

73 To date, 20 barley resistance genes have been identified, almost exclusively conferring recessive
74 resistance (Jiang et al. 2020). Two of these loci have been cloned: the *EUKARYOTIC TRANSLATION*
75 *INITIATION FACTOR 4E* gene (*eIF4E*), (Stein et al. 2005) of which several allelic forms providing
76 resistance are described, including *rym4* and *rym5*, (Hofinger et al. 2011; Perovic et al. 2014; Yang et
77 al. 2017; Shi et al. 2019), and the *PROTEIN DISULFIDE ISOMERASE LIKE 5-1* (*PDI5-1*) gene which
78 is also represented by a handful of alleles providing resistance, including *rym1* and *rym11* (Yang et al.
79 2017). The *rym4* allele provides resistance to BaMMV and to the common BaYMV pathotype BaYMV-
80 1, but not to pathotype BaYMV-2, which emerged in Europe at the end of the 1980s (Adams et al. 1987;
81 Huth 1989; Adams 1991; Graner and Bauer 1993; Steyer et al. 1995). The spectrum of *rym5* covers also
82 *BaYMV*-2, however, resistance-breaking isolates of BaMMV and BaYMV have emerged (Kanyuka et
83 al. 2004; Habekuß et al. 2008; Li et al. 2016). Facing the prospect of boom-and-bust cycles for known
84 resistance genes (Brown and Tellier 2011), it is critical to continue searching for alternative resistance
85 loci to underpin resistance breeding and to allow pyramiding of disease resistance loci. In particular,
86 sources of non-host resistance, e.g. resistance exhibited from a plant species against all isolates of a
87 pathogen which is not coevolutionary adapted, are particularly promising as they are thought to cover a
88 larger resistance spectrum and to be more durable (Ayliffe and Sørensen 2019). Bulbous barley
89 (*Hordeum bulbosum* L.), a wild relative and representative of the secondary gene pool of cultivated
90 barley (*Hordeum vulgare* L.), has been described as source of resistance to numerous barley pathogens,
91 including barley leaf rust (Johnston et al. 2013; Yu et al. 2018) and barley powdery mildew (Xu and
92 Kasha 1992; Pickering et al. 1995; Shtaya et al. 2007). So far, all *H. bulbosum* accessions investigated
93 exhibited resistance to BaMMV and BaYMV (Ruge et al. 2003), suggesting that the species is probably
94 a non-host to those viruses. Two major dominant resistance genes from *H. bulbosum* to both BaMMV
95 and BaYMV have been described: *Rym14*^{Hb} (Ruge et al. 2003) and *Rym16*^{Hb} (Ruge-Wehling et al. 2006).
96 *Rym14*^{Hb} was introgressed to barley by translocation of a *H. bulbosum* segment to barley chromosome
97 6HS (Ruge et al. 2003). In the past, a lack of suitable markers, alongside severely reduced recombination
98 in the target region between the barley and *H. bulbosum* fragments, rendered precise mapping of
99 *Rym14*^{Hb} elusive. Thanks to the development of genetic and genomic resources for *H. bulbosum*
100 (Wendler et al. 2014, 2015), it is now possible to fine-map loci from this species in a *H. vulgare*
101 background.

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

105 **Materials and methods**

106 **Plant material**

107 A first round of low-resolution genetic mapping was performed using four F_6 families derived from F_5
108 plants heterozygous at the *Rym14*^{Hb} locus from the BAZ-4006 family of the population 'Borwina' x
109 'A42' described in Ruge et al. (2003).

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239145; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

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

115 **DNA extraction**

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

119 **Genotyping-by-sequencing and data analysis**

120 GBS libraries for the low-resolution mapping were prepared from genomic DNA digested with *Pst*I and
121 *Msp*I (New England Biolabs) as described by Wendler et al. (2015). Between 93 and 153 barcoded
122 samples were pooled in an equimolar manner per lane and sequenced on the Illumina HiSeq 2500 for
123 107 cycles, single-end reads, using a custom sequencing primer.

124 The GBS reads were processed, aligned, and used to generate variant calls as described by Milner et al.
125 (2019). Alignment was performed against the TRITEX genome assembly of barley cultivar 'Morex'
126 (Monat et al. 2019). Individual variant calls were accepted wherever the read depth exceeded four.
127 Variant sites were retained if they presented a minimum mapping quality score (based on read depth
128 ratios calculated from the total read depth and depth of the alternative allele) of 20, a maximum fraction
129 of 40% of missing data, a fraction of heterozygous calls between 30 and 70%, and between 10 and 40%
130 of each homozygous call. Individuals with more than 40% missing data were excluded.

131 **Marker development**

132 Exome capture data of the introgression line '4006/163', described in Wendler et al. (2014) (accession
133 number ERP004445), were mapped to the TRITEX genome assembly of barley cultivar Morex (Monat
134 et al. 2019) together with the exome capture data of the *H. bulbosum* genotype 'A42' and of eight barley
135 varieties: 'Bonus', 'Borwina', 'Bowman', 'Foma', 'Gull', 'Morex', 'Steptoe', and 'Vogelsanger Gold',
136 described in Mascher et al. (2013b) (accession number PRJEB1810). Read mapping and variant calling
137 were performed as described by Milner et al. (2019). The SNP matrix was filtered for the following
138 criteria: heterozygous and homozygous calls had to be covered by a minimum depth of three and five
139 reads, respectively, and have a minimum quality score of 20. SNP sites were retained if they had less
140 than 20% missing data and less than 20% heterozygous calls. SNPs that were carrying the reference call
141 in all eight barleys and the alternate call in 'A42' and '4006/163' were selected as candidates to design
142 KASP markers, either using KASP-by-design (LGC Genomics, Berlin, Germany) or 3CR Bioscience
143 (Essex, UK) free assay design service. Those markers are latter designated as KASP and PACE markers,
144 respectively. Since no suitable SNPs were identified in the first 500 kbp of chromosome 6HS on the
145 'Morex' reference genome, the exome capture data were additionally mapped to the genome assembly
146 of cultivar 'Barke' (Jayakodi et al. under revision). The SNP at coordinate 241,723 bp on chromosome
147 6H of the 'Barke' genome assembly was retrieved and used to design the telomeric marker
148 Rym14_Bar241723. Furthermore, in order to control the genetic state at the segregating *rym4* resistance
149 locus, the diagnostic SNP for the resistance conferring allele (Stein et al. 2005) was also used to design
150 a KASP marker. Further information on KASP and PACE markers is provided in supplementary tables
151 1 and 2, respectively.

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239145; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

152 **Genotyping**

153 Genotyping assays with KASP markers were carried out in a final volume of 5 µl consisting of 0.7 µl
154 genomic DNA (50-100 ng/µL), 2.5 µl of KASP V4.0 2X Master Mix High Rox (LGC Genomics,
155 Berlin), 0.07 µl KASP assay mix (KASP-by-design, LGC Genomics, Berlin) containing the primers,
156 and 2.5 µl of sterile water. PCR amplifications were performed using the Hydrocycler 16 (LGC
157 Genomics, Berlin) with cycling conditions as follows: 94 °C for 15 min, followed by a touchdown
158 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
159 26 cycles at 94 °C for 20 s and 55 °C for 1 min. Genotyping assays with PACE markers were carried
160 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
161 Mix High Rox (3cr Bioscience, Essex, United Kingdom), 0.07 µl primer mix containing the primers
162 (12 µM of each allele specific primers and 30 µM of the common reverse primer), and 2.5 µl of sterile
163 water. PCR amplifications were performed using the Hydrocycler 16 (LGC Genomics, Berlin) with
164 cycling conditions as follows: 94 °C for 15 min, followed by a touchdown profile of 10 cycles at 94 °C
165 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
166 and 57 °C for 1 min.

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

170 **Phenotyping**

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

182 **Results**

183 **Low-resolution mapping**

184 A population of 427 F_6 from the cross 'Borwina' x 'A42' was genotyped by GBS and phenotyped for
185 resistance to BaMMV. Data for 389 plants and 77 SNPs passed the quality filters (supplementary table
186 3). On chromosome 6H, 73 plants were homozygous for the 'Borwina' allele, 92 were homozygous for
187 the 'A42' allele, 220 were heterozygous and four recombined. The infection rate was low with only 10%
188 of plants infected, compared to an expected 25% when resistance is controlled by a single dominant
189 gene. However, among the 39 plants phenotyped as susceptible to BaMMV, 38 were homozygous for
190 the 'Borwina' allele and one recombined on chromosome 6H, indicating a strong association of
191 phenotype and genotype.

192 To further confirm this association, 26 lines were phenotyped on progenies of 12 to 20 plants (Figure 1,
193 supplementary table 4). These included (i) 17 lines with the susceptible genotype on chromosome 6H
194 but scored as resistant, (ii) five heterozygous lines, and (iii) the four recombinant lines. Progenies of
195 lines presenting the susceptible genotype displayed infection rates between 50 and 95%, while those of
196 heterozygous lines displayed rates between 5 and 17%.

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239145; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

197 These results support the low penetrance of the infection in this experiment, with only half of the
198 expected susceptible plants successfully infected, as well as the association of the chromosome 6H locus
199 with resistance to BaMMV. Moreover, the phenotypes of the four recombinant progenies defined
200 *Rym14^{Hb}* interval between the telomere of chromosome 6HS and the marker position at base pair
201 4,553,134.

202 **Fig. 1** Graphical genotype and phenotype of the 26 F_6 lines phenotyped on progenies. *H. vulgare*, *H.*
203 *bulbosum*, and heterozygous phenotyped are represented as orange, blue, and yellow bars, respectively.
204 Coordinates on Morex reference genome (Monat et al. 2019) of strategic markers are displayed.
205 Phenotypes are indicated as the number of infected plants out of the total of F_7 progenies phenotyped,
206 colored according to the F_6 phenotype interpreted, following the same color code as for genotypes.

207 **Fine mapping**

208 The population of 7,500 F_2 was genotyped at the *Rym14^{Hb}* locus with four KASP markers
209 (Rym14_Bar241723, Rym14_2370223, Rym14_3087282, and Rym14_5003183, supplementary table
210 1). Resistance due to segregation of the recessive resistance gene *rym4* on chromosome 3HL was
211 controlled for with the rym4_SNP KASP marker (supplementary table 1). We identified 28
212 recombination events, corresponding to a genetic distance of ~0.2 cM, between the markers
213 Rym14_Bar241723 and Rym14_5003183. These results confirmed the strongly reduced recombination
214 rate between the *H. bulbosum* and the *H. vulgare* fragments on chromosome 6HS. In cultivated barley,
215 the syntenic 5 Mbp *Rym14^{Hb}* interval on chromosome 6HS corresponds to a genetic distance of 4 cM
216 (Mascher et al. 2013a), implying a 20-fold reduction in recombination frequency between the *H.*
217 *bulbosum* and the *H. vulgare* fragment.

218 All recombinants were genotyped with seven PACE markers (supplementary tables 2 and 4). Among
219 the recombinants, ten plants were homozygous for the *rym4* allele, nine were heterozygous and the
220 remaining nine were homozygous wildtype at the *rym4* locus (supplementary table 5). As plants
221 homozygous for the *rym4* allele would be resistant to BaMMV, irrespective to their genotype at
222 *Rym14^{Hb}*, only F_3 families derived from the 18 *Rym14*-recombinants heterozygous or homozygous for
223 the susceptible allele at *rym4* were phenotyped using 30 and 20 F_3 siblings, respectively. All phenotyped
224 plants were genotyped at Rym14_Bar241723, Rym14_2370223, Rym14_5003183 and *rym4*
225 (supplementary table 6). The infection rate during this round of phenotyping was much higher than
226 during the preceding low-resolution mapping, with less than 2 % of the susceptibility control showing
227 no viral content. Five out of 86 F_3 siblings expected to be susceptible based on their genotype were not
228 infected by virus, hence producing false-negative phenotypic results.

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

231 **Fig. 2** Physical map of the *Rym14^{Hb}* locus. KASP and PACE markers are represented as black and blue
232 vertical lines, respectively. Barley chromosome 6HS is depicted as a black horizontal line and genotypes
233 of recombinant F_2 plants are indicated by horizontal bars: blue=*H. bulbosum* homozygous; orange=*H.*
234 *vulgare* homozygous; yellow=heterogygous. The number of recombinant lines corresponding to each
235 genotype pattern is indicated on the left while the phenotypes of their progeny are shown on the right
236 (R: resistant, S: susceptible, seg: segregation of resistance).

237 **Candidate genes**

238 In the absence of a genomic sequence for a *Rym14^{Hb}* plant, we cannot precisely define the genes present
239 in the *Rym14^{Hb}* interval. However, as synteny between the two *Hordeum* species is high (Wendler et al.
240 2017), it is still relevant to assess the genes annotated in the homolog interval of the *H. vulgare* reference

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239145; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

241 genome as a proxy for suggesting *Rym14*^{Hb} candidate genes. In the respective interval of the Morex V2
242 reference sequence 30 high-confidence (HC) (Table 1) and 17 low-confidence genes (Monat et al. 2019)
243 are annotated. All HC gene models were checked for homology with other genes by a BLASTx (v2.9.0,
244 default parameters) homology searches against the non-redundant protein sequence database (Camacho
245 et al. 2009) and for presence of conserved domains in NCBI conserved domains (Lu et al. 2019). Among
246 the HC genes, HORVU.MOREX.r2.6HG0448010 is annotated as a TIR-NBS-LRR gene, however, it
247 does not contain any of the major NLR domains (coiled-coil, NB-ARC and LRR), and is therefore
248 interpreted as a pseudogene. HORVU.MOREX.r2.6HG0448100, annotated as a dirigent protein, is a
249 jacalin-related lectin, while HORVU.MOREX.r2.6HG0448250, annotated as part of the protein kinase
250 protein family, displays the highest homology with a wall-associated receptor kinase, and
251 HORVU.MOREX.r2.6HG0448290 codes for a papain-like cysteine protease (PLCP). Interestingly, the
252 interval also contains no less than 14 HC genes annotated as thionins, sharing with each other at least
253 88% of their coding sequence. In addition to these annotated genes in the Morex genome, additional
254 candidate genes could be unique to the resistant genotypes.

255 **Table 1** Genes annotated with high confidence in *Rym14*^{Hb} interval on the Morex genome (Monat et al.
256 2019).

name	start	stop	gene type
HORVU.MOREX.r2.6HG0447840	195540	196334	Thionin
HORVU.MOREX.r2.6HG0447850	220610	221213	Thionin
HORVU.MOREX.r2.6HG0447860	256998	259999	Thionin
HORVU.MOREX.r2.6HG0447880	373994	438209	Thionin
HORVU.MOREX.r2.6HG0447890	460556	461157	Thionin
HORVU.MOREX.r2.6HG0447900	461856	462457	Thionin
HORVU.MOREX.r2.6HG0447910	497194	497795	Thionin
HORVU.MOREX.r2.6HG0447920	597800	598403	Thionin
HORVU.MOREX.r2.6HG0447930	625302	625905	Thionin
HORVU.MOREX.r2.6HG0447940	691184	707575	Thionin
HORVU.MOREX.r2.6HG0447950	749829	776991	Thionin
HORVU.MOREX.r2.6HG0447960	792195	827832	Thionin
HORVU.MOREX.r2.6HG0447980	958137	958736	Thionin
HORVU.MOREX.r2.6HG0447990	1004017	1004618	Thionin
HORVU.MOREX.r2.6HG0448010	1259976	1260591	TIR-NBS-LRR class disease resistance protein
HORVU.MOREX.r2.6HG0448020	1300107	1300565	Dimeric alpha-amylase inhibitor
HORVU.MOREX.r2.6HG0448100	1493250	1493945	Dirigent protein
HORVU.MOREX.r2.6HG0448110	1574160	1575749	Cytochrome P450 family protein, expressed
HORVU.MOREX.r2.6HG0448120	1578752	1580023	Aspartic proteinase nepenthesin-1
HORVU.MOREX.r2.6HG0448130	1598418	1600649	Subtilisin-like protease
HORVU.MOREX.r2.6HG0448140	1605306	1610732	Fatty acyl-CoA reductase
HORVU.MOREX.r2.6HG0448160	1753412	1756451	Glycerol-3-phosphate acyltransferase 3, putative
HORVU.MOREX.r2.6HG0448200	1792383	1794963	Transposon protein, putative, CACTA, En/Spm sub-class
HORVU.MOREX.r2.6HG0448210	1796825	1804280	O-acyltransferase WSD1
HORVU.MOREX.r2.6HG0448220	1840897	1842376	GDSL esterase/lipase
HORVU.MOREX.r2.6HG0448230	1853483	1854626	Short-chain dehydrogenase/reductase
HORVU.MOREX.r2.6HG0448250	1945996	1952442	Protein kinase family protein
HORVU.MOREX.r2.6HG0448260	1954346	1955384	zinc finger MYM-type-like protein
HORVU.MOREX.r2.6HG0448290	2061596	2062919	Cysteine protease-like protein
HORVU.MOREX.r2.6HG0448300	2066856	2067293	Proteinase inhibitor type-2

257 **Discussion**

258 Resistance genes deployed in breeding and in the field are often overcome by new pathogen variants
259 after only a few years (Brown and Tellier 2011). Pyramiding several resistance genes has proven to
260 increase the resistance durability, however, this strategy requires the availability of several independent
261 resistance loci (Werner et al. 2005; Riedel et al. 2011; Kim et al. 2011). In light of these facts, non-
262 adapted resistance genes from wild crop relatives are precious, since they are assumed to confer more
263 durable resistance than genes originating from within the diversity of the cultivated species, owing to
264 co-evolution between the cultivated host and pathogen genotypes (Fonseca and Mysore 2019). Until
265 recently, the fine mapping of genes from crop wild relatives species was impractical, owing to strong
266 suppression of recombination with the cultivated species (Ruge et al. 2003; Kakeda et al. 2008; Wijnker

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239145; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

267 and de Jong 2008; Prohens et al. 2017). The results of this study demonstrate that high-throughput
268 genotyping coupled with large mapping populations can overcome this limitation, by constraining the
269 interval of the *Rym4*^{Hb} viral resistance gene to the telomeric 2 Mbp of chromosome 6HS, and providing
270 markers suitable for marker-assisted-selection.

271 While genes coding for nucleotide-binding and leucine-rich repeat domain proteins (NLR) are the usual
272 suspects for dominant resistance to pathogens, including viruses (de Ronde et al. 2014; Boualem et al.
273 2016), only a pseudogene presenting similarities with this gene family is annotated in the *Rym14*^{Hb}
274 interval on the barley reference genome. However, it is not rare that susceptible genotypes do not possess
275 a functional copy of the resistance gene. NLRs are overrepresented in regions displaying
276 presence/absence variation (Xu et al. 2012; Bush et al. 2013). Therefore, some NLR resistance genes,
277 like *RPM1* and *RPS5*, are only present in the resistant genotype (Grant et al. 1998; Henk et al. 1999). In
278 the case of wheat leaf rust resistance gene *Lr21*, it was shown that the gene is a chimera of two
279 nonfunctional alleles that probably evolved via a recombination event (Huang et al. 2009).

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

290 However, other genes in the *Rym14*^{Hb} interval, even if less likely candidates, might also play a role in
291 resistance. For example, HORVU.MOREX.r2.6HG0448290 codes for a PLCP. PLCPs are known to
292 play a major role in programmed cell death triggered by NLR genes. Interestingly, CYP1, a tomato
293 PLCP, is targeted by the Tomato yellow leaf curl virus V2 protein, suggesting that V2 could
294 downregulate CYP1 to counteract host defenses (Bar-Ziv et al. 2012). *Rcr3*, a tomato papain-like
295 cysteine protease gene, is required for the function of the resistance gene *Cf-2* to *Cladosporium fulvum*
296 (Krüger et al. 2002), while *NbCathB*, from *Nicotina benthamiana*, is requested for the HR triggered by
297 the non-host pathogens *Erwinia amylovora* and *Pseudomonas syringae* (Gilroy et al. 2007). The high
298 level of thionin duplication at this locus also raised our attention. Thionins are part of common anti-
299 bacterial and anti-fungal peptides (Bohlmann and Broekaert 1994), conferring enhanced resistance to
300 several pathogens. Thionins were also found to exhibit increased expression in resistant compared to
301 susceptible pepper genotypes during infection by the Chili leaf curl virus (Kushwaha et al. 2015),
302 suggesting a possible role in basal defense. Additionally, the cytochrome P450 superfamily has been
303 associated with resistance to the Soybean mosaic virus (Cheng et al. 2010; Yang et al. 2011). Some
304 subtilisin proteases are induced by pathogens and involved in programmed cell death (Figueiredo et al.
305 2014), and GDSL lipases were found to be either negative or positive regulators of plant defense
306 mechanisms (Hong et al. 2008; Kwon et al. 2009).

307 The feasibility of further reducing the target interval by recombination through additional fine mapping
308 is low and would require the screening of tens of thousands of additional F_2 plants for the chance of
309 finding one additional recombinant in the smallest target region. Therefore, a candidate gene approach
310 may be a more fruitful strategy for continued progress. Despite the presence of promising candidate
311 genes like HORVU.MOREX.r2.6HG0448250 and HORVU.MOREX.r2.6HG0448100 in the haplotype

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239145; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

312 of the susceptible cultivar Morex, the resistance conferring gene may be present only in the haplotype
313 of the resistant *H. bulbosum*. Therefore, deciphering the resistant haplotype, most likely though a high-
314 quality chromosome-scale genome assembly of the interval in *H. bulbosum*, is an essential prerequisite
315 to the prioritization of candidate genes for further functional testing.

316 The markers identified in this study are tightly linked to *Rym14*^{Hb} and therefore are of prime importance
317 to barley breeding. These markers will allow the reliable introgression of this resistance into barley elite
318 lines with a minimum of linkage drag compared to the previously established markers (Ruge et al. 2003).
319 This is essential for introducing this gene into new cultivars, As the prevalence of resistance-breaking
320 isolates of *rym4* and *rym5* will increase in the barley growing area in Europe and Asia (Kühne 2009),
321 introgression of *Rym14*^{Hb} into new elite varieties together with other resistance loci represents a critical
322 opportunity to improve the durability and spectrum of barley resistance to BaMMV and BaYMV.

323 **References**

324 Adams MJ (1991) The distribution of barley yellow mosaic virus (BaYMV) and barley mild mosaic
325 virus (BaMMV) in UK winter barley samples, 1987-1990. Plant Pathol 40:53–58.
326 https://doi.org/10.1111/j.1365-3059.1991.tb02292.x

327 Adams MJ, Swaby AG, Jones P (1987) Occurrence of two strains of barley yellow mosaic virus in
328 England. Plant Pathol 36:610–612. https://doi.org/10.1111/j.1365-3059.1987.tb02284.x

329 Ayliffe M, Sørensen CK (2019) Plant nonhost resistance: paradigms and new environments. Curr Opin
330 Plant Biol 50:104–113. https://doi.org/10.1016/j.pbi.2019.03.011

331 Bar-Ziv A, Levy Y, Hak H, et al (2012) The Tomato yellow leaf curl virus (TYLCV) V2 protein interacts
332 with the host papain-like cysteine protease CYP1. Plant Signal Behav 7:983–989.
333 https://doi.org/10.4161/psb.20935

334 Bohlmann H, Broekaert W (1994) The Role of Thionins in Plant Protection. CRC Crit Rev Plant Sci
335 13:1–16. https://doi.org/10.1080/07352689409701905

336 Boualem A, Dogimont C, Bendahmane A (2016) The battle for survival between viruses and their host
337 plants. Curr Opin Virol 17:32–38. https://doi.org/10.1016/j.coviro.2015.12.001

338 Brown JKM, Tellier A (2011) Plant-parasite coevolution: bridging the gap between genetics and
339 ecology. Annu Rev Phytopathol 49:345–67. https://doi.org/10.1146/annurev-phyto-072910-
340 095301

341 Bush SJ, Castillo-Morales A, Tovar-Corona JM, et al (2013) Presence–Absence Variation in A. thaliana
342 Is Primarily Associated with Genomic Signatures Consistent with Relaxed Selective Constraints.
343 Mol Biol Evol 31:59–69. https://doi.org/10.1093/molbev/mst166

344 Camacho C, Coulouris G, Avagyan V, et al (2009) BLAST+: architecture and applications. BMC
345 Bioinformatics 10:421. https://doi.org/10.1186/1471-2105-10-421

346 Cheng H, Yang H, Zhang D, et al (2010) Polymorphisms of soybean isoflavone synthase and flavanone
347 3-hydroxylase genes are associated with soybean mosaic virus resistance. Mol Breed 25:13–24.
348 https://doi.org/10.1007/s11032-009-9305-8

349 Chisholm ST, Mahajan SK, Whitham SA, et al (2000) Cloning of the Arabidopsis RTM1 gene, which
350 controls restriction of long-distance movement of tobacco etch virus. Proc Natl Acad Sci 97:489–
351 494. https://doi.org/10.1073/pnas.97.1.489

352 Citovsky V, McLean BG, Zupan JR, Zambryski P (1993) Phosphorylation of tobacco mosaic virus cell-
353 to-cell movement protein by a developmentally regulated plant cell wall-associated protein kinase.
354 Genes Dev 7:904–910. https://doi.org/10.1101/gad.7.5.904

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239145; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Clark MF, Adams AN (1977) Characteristics of the Microplate Method of Enzyme-Linked Immunosorbent Assay for the Detection of Plant Viruses. J Gen Virol 34:475–483. https://doi.org/https://doi.org/10.1099/0022-1317-34-3-475

de Ronde D, Butterbach P, Kormelink R (2014) Dominant resistance against plant viruses. Front Plant Sci 5:307. https://doi.org/10.3389/fpls.2014.00307

Dmochowska-Boguta M, Kloc Y, Zielezinski A, et al (2020) TaWAK6 encoding wall-associated kinase is involved in wheat resistance to leaf rust similar to adult plant resistance. PLoS One 15:e0227713

Figueiredo A, Monteiro F, Sebastiana M (2014) Subtilisin-like proteases in plant–pathogen recognition and immune priming: a perspective . Front. Plant Sci. 5:739

Fonseca JP, Mysore KS (2019) Genes involved in nonhost disease resistance as a key to engineer durable resistance in crops. Plant Sci 279:108–116. https://doi.org/https://doi.org/10.1016/j.plantsci.2018.07.002

Gilroy EM, Hein I, Van Der Hoorn R, et al (2007) Involvement of cathepsin B in the plant disease resistance hypersensitive response. Plant J 52:1–13. https://doi.org/10.1111/j.1365-313X.2007.03226.x

Graner A, Bauer E (1993) RFLP mapping of the ym4 virus resistance gene in barley. Theor Appl Genet 86:689–693. https://doi.org/10.1007/BF00222657

Grant MR, McDowell JM, Sharpe AG, et al (1998) Independent deletions of a pathogen-resistance gene in Brassica and Arabidopsis. Proc Natl Acad Sci 95:15843–15848. https://doi.org/10.1073/pnas.95.26.15843

Habekuß A, Kühne T, Krämer I, et al (2008) Identification of Barley mild mosaic virus Isolates in Germany Breaking rym5 Resistance*. J Phytopathol 156:36–41. https://doi.org/10.1111/j.1439-0434.2007.01324.x

Henk AD, Warren RF, Innes RW (1999) A New Ac-Like Transposon of Arabidopsis Is Associated With a Deletion of the RPS5 Disease Resistance Gene. Genetics 151:1581–1589

Hofinger BJ, Russell JR, Bass CG, et al (2011) An exceptionally high nucleotide and haplotype diversity and a signature of positive selection for the eIF4E resistance gene in barley are revealed by allele mining and phylogenetic analyses of natural populations. Mol Ecol 20:3653–3668. https://doi.org/10.1111/j.1365-294X.2011.05201.x

Hong JK, Choi HW, Hwang IS, et al (2008) Function of a novel GDSL-type pepper lipase gene, CaGLIP1, in disease susceptibility and abiotic stress tolerance. Planta 227:539–558. https://doi.org/10.1007/s00425-007-0637-5

Huang L, Brooks S, Li W, et al (2009) Evolution of New Disease Specificity at a Simple Resistance Locus in a Crop–Weed Complex: Reconstitution of the Lr21 Gene in Wheat. Genetics 182:595 LP – 602. https://doi.org/10.1534/genetics.108.099614

Huth W (1989) Ein weiterer Stamm des barley yellow mosaic virus aufgefunden. Nachrichtenbl. Dtsch Pflanzenschutzd 41:6–7

Jayakodi M, Padmarasu S, Haberer G, et al (under revision) The barley pan-genome reveals the hidden legacy of mutation breeding

Jiang C, Kan J, Ordon F, et al (2020) Bymovirus-induced yellow mosaic diseases in barley and wheat: viruses, genetic resistances and functional aspects. Theor Appl Genet 133:1623–1640. https://doi.org/10.1007/s00122-020-03555-7

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239145; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

397 Jianping C (2005) Progress and prospects of studies on Polymyxa graminis and its transmitted cereal
398 viruses in China. Prog Nat Sci 15:481–490. https://doi.org/10.1080/10020070512331342440

399 Johnston PA, Niks RE, Meiyalaghan V, et al (2013) Rph22: mapping of a novel leaf rust resistance gene
400 introgressed from the non-host Hordeum bulbosum L. into cultivated barley (Hordeum vulgare
401 L.). Theor Appl Genet 126:1613–1625. https://doi.org/10.1007/s00122-013-2078-9

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

405 Kanyuka K, McGrann G, Alhudaib K, et al (2004) Biological and sequence analysis of a novel European
406 isolate of Barley mild mosaic virus that overcomes the barley rym5 resistance gene. Arch Virol
407 149:1469–1480. https://doi.org/10.1007/s00705-004-0318-7

408 Kanyuka K, Ward E, Adams MJ (2003) Polymyxa graminis and the cereal viruses it transmits: a research
409 challenge. Mol Plant Pathol 4:393–406. https://doi.org/10.1046/j.1364-3703.2003.00177.x

410 Kim H-S, Baek S-B, Kim D-W, et al (2011) Evaluation and Verification of Barley Genotypes with
411 Known Genes for Resistance to Barley yellow mosaic virus and Barley mild mosaic virus Under
412 Field Conditions in South Korea. Plant Pathol J 27:324–332.
413 https://doi.org/10.5423/PPJ.2011.27.4.324

414 Krüger J, Thomas CM, Golstein C, et al (2002) A Tomato Cysteine Protease Required for
415 Cf-2-Dependent Disease Resistance and Suppression of Autonecrosis.
416 Science (80-) 296:744 LP – 747. https://doi.org/10.1126/science.1069288

417 Kühne T (2009) Soil-borne viruses affecting cereals—Known for long but still a threat. Virus Res
418 141:174–183. https://doi.org/https://doi.org/10.1016/j.virusres.2008.05.019

419 Kushwaha N, Sahu PP, Prasad M, Chakraborty S (2015) Chilli leaf curl virus infection highlights the
420 differential expression of genes involved in protein homeostasis and defense in resistant chilli
421 plants. Appl Microbiol Biotechnol. https://doi.org/10.1007/s00253-015-6415-6

422 Kwon SJ, Jin HC, Lee S, et al (2009) GDSL lipase-like 1 regulates systemic resistance associated with
423 ethylene signaling in Arabidopsis. Plant J 58:235–245. https://doi.org/10.1111/j.1365-
424 313X.2008.03772.x

425 Li H, Kondo H, Kühne T, Shirako Y (2016) Barley Yellow Mosaic Virus VPg Is the Determinant Protein
426 for Breaking eIF4E-Mediated Recessive Resistance in Barley Plants . Front. Plant Sci. 7:1449

427 Li H, Zhou S-Y, Zhao W-S, et al (2009) A novel wall-associated receptor-like protein kinase gene,
428 OsWAK1, plays important roles in rice blast disease resistance. Plant Mol Biol 69:337–346.
429 https://doi.org/10.1007/s11103-008-9430-5

430 Li Q, Hu A, Qi J, et al (2020) CsWAKL08, a pathogen-induced wall-associated receptor-like kinase in
431 sweet orange, confers resistance to citrus bacterial canker via ROS control and JA signaling. Hortic
432 Res 7:42. https://doi.org/10.1038/s41438-020-0263-y

433 Lu S, Wang J, Chitsaz F, et al (2019) CDD/SPARCLE: the conserved domain database in 2020. Nucleic
434 Acids Res 48:D265–D268. https://doi.org/10.1093/nar/gkz991

435 Mascher M, Muehlbauer GJ, Rokhsar DS, et al (2013a) Anchoring and ordering NGS contig assemblies
436 by population sequencing (POPSEQ). Plant J 76:718–727. https://doi.org/10.1111/tpj.12319

437 Mascher M, Richmond TA, Gerhardt DJ, et al (2013b) Barley whole exome capture: a tool for genomic
438 research in the genus Hordeum and beyond. Plant J 76:494–505. https://doi.org/10.1111/tpj.12294

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239145; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

439 Milner S, Jost M, Taketa S, et al (2019) Genebank genomics reveals the diversity of a global barley
440 collection. Nat Genet 51:319–326. https://doi.org/10.1038/s41588-018-0266-x

441 Monat C, Padmarasu S, Lux T, et al (2019) TRITEX: chromosome-scale sequence assembly of Triticeae
442 genomes with open-source tools. Genome Biol 20:284. https://doi.org/10.1186/s13059-019-1899-
443 5

444 Perovic D, Krämer I, Habekuss A, et al (2014) Genetic analyses of BaMMV/BaYMV resistance in
445 barley accession HOR4224 result in the identification of an allele of the translation initiation factor
446 4e (Hv-eIF4E) exclusively effective against Barley mild mosaic virus (BaMMV). Theor. Appl.
447 Genet. 1–11

448 Pickering RA, Hill AM, Michel M, Timmerman-Vaughan GM (1995) The transfer of a powdery mildew
449 resistance gene from Hordeum bulbosum L to barley (H. vulgare L.) chromosome 2 (2I). Theor
450 Appl Genet 91:1288–1292. https://doi.org/10.1007/BF00220943

451 Plumb RT, Lennon EA, Gutteridge RA (1986) The effects of infection by barley yellow mosaic virus
452 on the yield and components of yield of barley. Plant Pathol 35:314–318.
453 https://doi.org/10.1111/j.1365-3059.1986.tb02020.x

454 Prohens J, Gramazio P, Plazas M, et al (2017) Introgressiomics: a new approach for using crop wild
455 relatives in breeding for adaptation to climate change. Euphytica 213:158.
456 https://doi.org/10.1007/s10681-017-1938-9

457 Riedel C, Habekuß A, Schliephake E, et al (2011) Pyramiding of Ryd2 and Ryd3 conferring tolerance
458 to a German isolate of Barley yellow dwarf virus-PAV (BYDV-PAV-ASL-1) leads to quantitative
459 resistance against this isolate. Theor Appl Genet 123:69. https://doi.org/10.1007/s00122-011-
460 1567-y

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

464 Ruge B, Linz A, Pickering R, et al (2003) Mapping of Rym14Hb, a gene introgressed from Hordeum
465 bulbosum and conferring resistance to BaMMV and BaYMV in barley. Theor Appl Genet
466 107:965–971. https://doi.org/10.1007/s00122-003-1339-4

467 Shi L, Jiang C, He Q, et al (2019) Bulked segregant RNA-sequencing (BSR-seq) identified a novel rare
468 allele of eIF4E effective against multiple isolates of BaYMV/BaMMV. Theor Appl Genet
469 132:1777–1788. https://doi.org/10.1007/s00122-019-03314-3

470 Shtaya MJY, Sillero JC, Flath K, et al (2007) The resistance to leaf rust and powdery mildew of
471 recombinant lines of barley (Hordeum vulgare L.) derived from H. vulgare × H. bulbosum crosses.
472 Plant Breed 126:259–267. https://doi.org/10.1111/j.1439-0523.2007.01328.x

473 Stein N, Herren G, Keller B (2001) A new DNA extraction method for high-throughput marker analysis
474 in a large-genome species such as Triticum aestivum. Plant Breed 120:354–356.
475 https://doi.org/10.1046/j.1439-0523.2001.00615.x

476 Stein N, Perovic D, Kumlehn J, et al (2005) The eukaryotic translation initiation factor 4E confers
477 multiallelic recessive Bymovirus resistance in Hordeum vulgare (L.). Plant J 42:912–922.
478 https://doi.org/10.1111/j.1365-313X.2005.02424.x

479 Steyer S, Kummert J, Froidmont F (1995) Characterization of a resistance-breaking BaYMV isolate
480 from Belgium. Agronomie 15:433–438

481 Timpe U, Kühne T (1994) The complete nucleotide sequence of RNA2 of barley mild mosaic virus
482 (BaMMV). Eur J Plant Pathol 100:233–241. https://doi.org/10.1007/BF01876238

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239145; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

483 Waigmann E, Chen M-H, Bachmaier R, et al (2000) Regulation of plasmodesmal transport by
484 phosphorylation of tobacco mosaic virus cell-to-cell movement protein. EMBO J 19:4875–4884.
485 https://doi.org/10.1093/emboj/19.18.4875

486 Wendler N, Mascher M, Himmelbach A, et al (2015) Bulbosum to Go: A Toolbox to Utilize Hordeum
487 vulgare/bulbosum Introgressions for Breeding and Beyond. Mol Plant 8:1507–1519.
488 https://doi.org/https://doi.org/10.1016/j.molp.2015.05.004

489 Wendler N, Mascher M, Himmelbach A, et al (2017) A High-Density, Sequence-Enriched Genetic Map
490 of Hordeum bulbosum and Its Collinearity to H. vulgare. Plant Genome 10:.
491 https://doi.org/10.3835/plantgenome2017.06.0049

492 Wendler N, Mascher M, Nöh C, et al (2014) Unlocking the secondary gene-pool of barley with next-
493 generation sequencing. Plant Biotechnol J 12:1122–1131. https://doi.org/10.1111/pbi.12219

494 Werner K, Friedt W, Ordon F (2005) Strategies for Pyramiding Resistance Genes Against the Barley
495 Yellow Mosaic Virus Complex (BaMMV, BaYMV, BaYMV-2). Mol Breed 16:45–55.
496 https://doi.org/10.1007/s11032-005-3445-2

497 Wijnker E, de Jong H (2008) Managing meiotic recombination in plant breeding. Trends Plant Sci
498 13:640–646. https://doi.org/https://doi.org/10.1016/j.tplants.2008.09.004

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

502 Xu X, Liu X, Ge S, et al (2012) Resequencing 50 accessions of cultivated and wild rice yields markers
503 for identifying agronomically important genes. Nat Biotechnol 30:105–111.
504 https://doi.org/10.1038/nbt.2050

505 Yamaji Y, Maejima K, Komatsu K, et al (2012) Lectin-Mediated Resistance Impairs Plant Virus
506 Infection at the Cellular Level. Plant Cell 24:778 LP – 793. https://doi.org/10.1105/tpc.111.093658

507 Yang H, Huang Y, Zhi H, Yu D (2011) Proteomics-based analysis of novel genes involved in response
508 toward soybean mosaic virus infection. Mol Biol Rep 38:511–521.
509 https://doi.org/10.1007/s11033-010-0135-x

510 Yang P, Habekuß A, Hofinger BJ, et al (2017) Sequence diversification in recessive alleles of two host
511 factor genes suggests adaptive selection for bymovirus resistance in cultivated barley from East
512 Asia. Theor Appl Genet 130:331–344. https://doi.org/10.1007/s00122-016-2814-z

513 Yu X, Kong HY, Meiyalaghan V, et al (2018) Genetic mapping of a barley leaf rust resistance gene
514 Rph26 introgressed from Hordeum bulbosum. Theor Appl Genet 131:2567–2580.
515 https://doi.org/10.1007/s00122-018-3173-8

516 **Supplementary material**

517 **Table S1** KASP markers developed for *Rym14*^{Hb} fine mapping. The indicated coordinates of the
518 genotyped SNP is respective to Morex V2 genome (Monat et al. 2019), except for Rym14_Bar241723
519 which it is based on Barke assembly (Jayakodi et al. under revision). The target SNP is identified in the
520 sequence by square brackets.

521 **Table S2** PACE markers developed for *Rym14*^{Hb} fine mapping. The indicated coordinates of the
522 genotyped SNP is respective to Morex V2 genome (Monat et al. 2019). The target SNP is identified in
523 the sequence by square brackets.

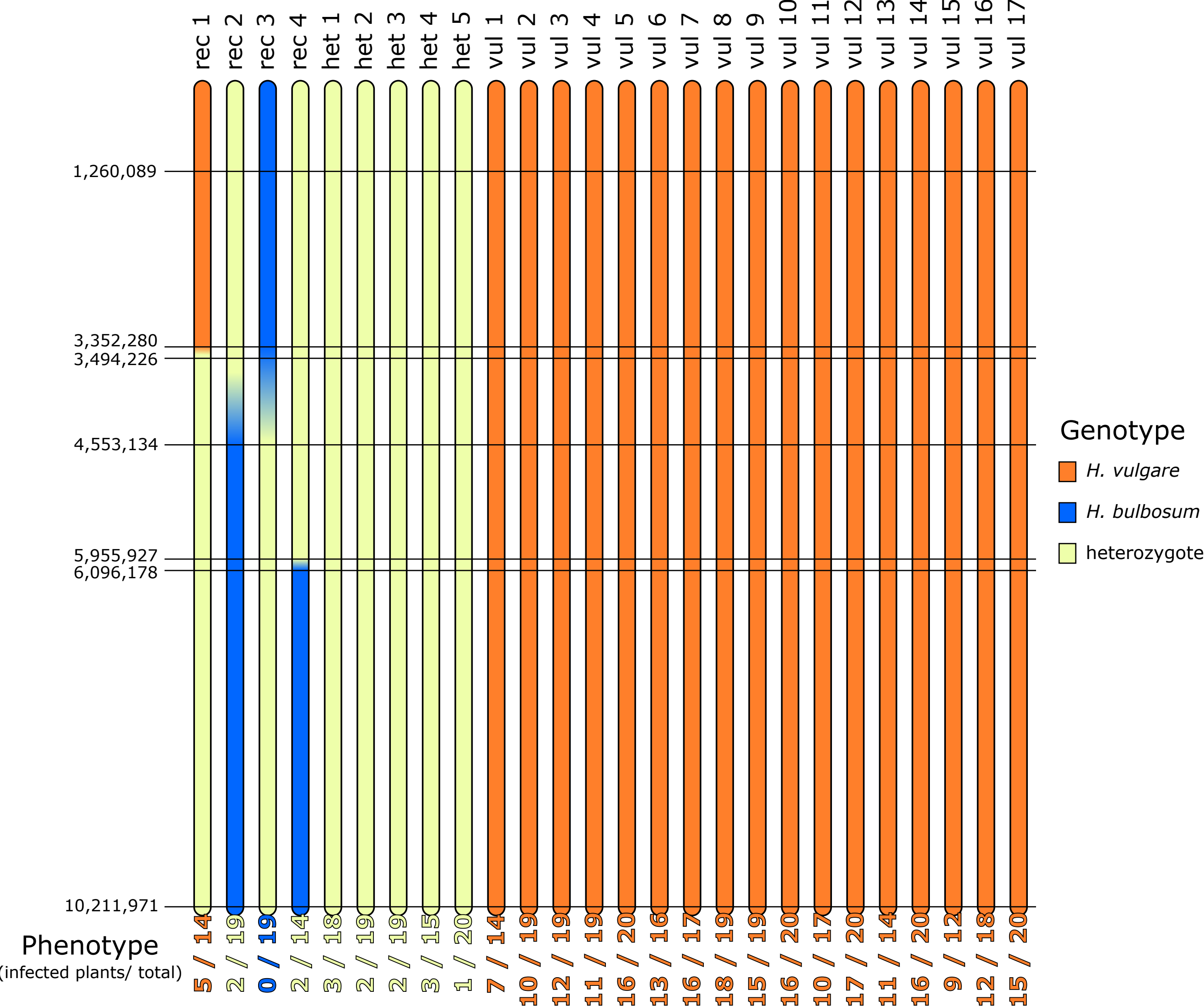
bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239145; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

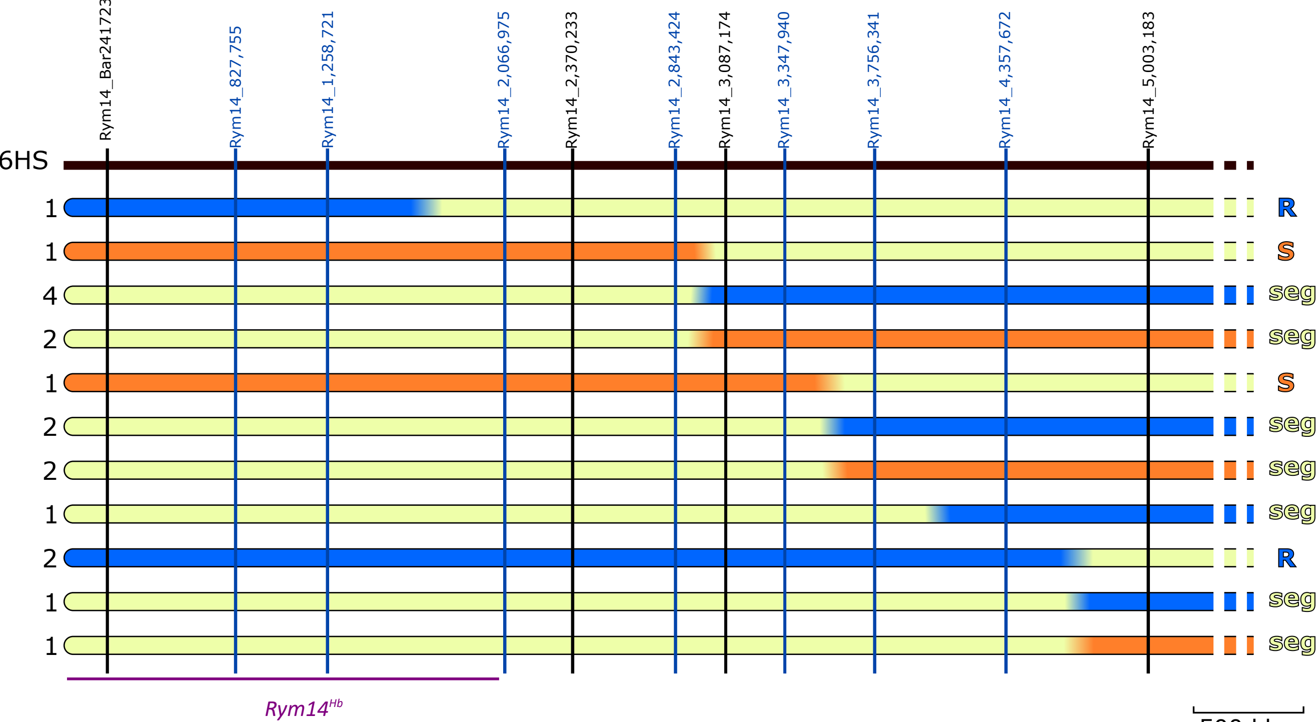
524 **Table S3** Phenotype and filtered GBS genotype of 389 F_6 plants from the cross Borwina x A42.
525 Phenotype is either resistant (R) or susceptible (S). For each SNP, the genotype is indicated as
526 homozygous *H. bulbosum* (B), homozygous *H. vulgare* (V) or heterozygous (H) and missing (-).

527 **Table S4** Phenotype on F_2, phenotype on progenies and filtered GBS genotype of 26 lines from the cross
528 Borwina x A42. Phenotype is either resistant (R) or susceptible (S). The number of susceptible plants
529 out of the total number phenotyped for each progeny is specified. For each SNP, the genotype is
530 indicated as homozygous *H. bulbosum* (B), homozygous *H. vulgare* (V) or heterozygous (H) and
531 missing (-).

532 **Table S5** Genotyping of the 28 F_2 recombinants with PACE and KASP markers. For each *Rym14^{Hb}*
533 marker, the genotype is indicated as homozygous *H. bulbosum* (B), homozygous *H. vulgare* (V) or
534 heterozygous (H). Genotype at *rym4* locus is classified as homozygous *rym4* (rym4_R), homozygous
535 for the susceptible allele (rym4_S) and heterozygous (rym4_H). Additionally, the number of susceptible
536 and resistant plants in the phenotyped progenies is specified.

537 **Table S6** Phenotype and genotyped of the F_3 progenies recombining at the *Rym14^{Hb}* locus. The
538 phenotype is given as the DAS-ELISA extinction at 405 nm. Plants with absorbance > 0.1 were scored
539 qualitatively as being susceptible. For each *Rym14^{Hb}* marker, the genotype is indicated as homozygous
540 *H. bulbosum* (B), homozygous *H. vulgare* (V) or heterozygous (H). Genotype at *rym4* locus is classified
541 as homozygous *rym4* (rym4_R), homozygous for the susceptible allele (rym4_S) and heterozygous
542 (rym4_H).


rec 1
rec 2
rec 3
rec 4
het 1
het 2
het 3
het 4
het 5
vul 1
vul 2
vul 3
vul 4
vul 5
vul 6
vul 7
vul 8
vul 9
vul 10
vul 11
vul 12
vul 13
vul 14
vul 15
vul 16
vul 17
1,260,089
3,352,280
3,494,226
4,553,134
5,955,927
6,096,178
10,211,971
Genotype
H. vulgare
H. bulbosum
heterozygote
Phenotype
(infected plants/ total)
5 / 14
2 / 19
0 / 19
2 / 14
3 / 18
2 / 19
2 / 19
3 / 15
1 / 20
7 / 14
10 / 19
12 / 19
11 / 19
16 / 20
13 / 16
16 / 17
18 / 19
15 / 19
16 / 20
10 / 17
17 / 20
11 / 14
16 / 20
9 / 12
12 / 18
15 / 20



Rym14_Bar241723
Rym14_827,755
Rym14_1,258,721
Rym14_2,066,975
Rym14_2,370,233
Rym14_2,843,424
Rym14_3,087,174
Rym14_3,347,940
Rym14_3,756,341
Rym14_4,357,672
Rym14_5,003,183
6HS
1
1
4
2
1
2
2
1
2
1
1
R
S
seg
seg
S
seg
seg
seg
R
seg
seg
Rym14^Hb
500 kb