High-resolution mapping of Rym14Hb, a wild relative resistance gene to barley yellow mosaic disease

Hélène Pidon1 · Neele Wendler2 · Antje Habekuß3 · Anja Maasberg4 · Brigitte Ruge-Wehling5 · Dragan Perovic3 · Frank Ordon3 · Nils Stein1,6

Received: 6 August 2020 / Accepted: 18 November 2020 / Published online: 2 December 2020
© The Author(s) 2020

Abstract
Key message We mapped the Rym14Hb 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. Rym14Hb is a dominant major resistance gene on chromosome 6HS, originating from barley’s secondary gene-pool 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 F2 mapping population (n = 7500), to compensate for a ten-fold reduction in recombination rate compared to intraspecific barley crosses. After high-throughput genotyping, the Rym14Hb 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

Communicated by Kevin Smith.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00122-020-03733-7) contains supplementary material, which is available to authorized users.

1 Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Corrensstr. 3, 06466 Seeland, Germany
2 KWS SAAT SE & Co. KGaA, Grimsehlstr. 31, 37574 Einbeck, Germany
3 Institute for Resistance Research and Stress Tolerance, Julius Kühn Institute (JKI), Erwin-Baur-Straße 27, 06484 Quedlinburg, Germany
4 KWS LOCHOW GMBH, Ferdinand-von-Lochow-Straße 5, 29303 Bergen, Germany
5 Institute for Breeding Research On Agricultural Crops, Julius Kühn Institute (JKI), Groß Lüsewitz, Rudolf-Schick-Platz 3a, 18190 Sanitz, Germany
6 Center for Integrated Breeding Research (CiBreed), Georg-August University, Von Siebold Straße 8, 37075 Göttingen, Germany
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: Rym14Hb (Ruge et al. 2003) and Rym16Hb (Ruge-Wehling et al. 2006). Rym14Hb 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 Rym14Hb 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 Rym14Hb 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 F2 families derived from F1 plants heterozygous at the Rym14Hb 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 F2 families were generated by crossing an Rym14Hb/Rym14Hb F1 plant (derived from F1 4006/337) to either (i) var. ‘KWS Orbit’ or (ii) var. ‘KWS Higgins’, both missing the Rym14Hb 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 PstI and MspI (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 PRJEB18110). 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 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 K2HPO4 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 E405 > 0.1 were qualitatively scored as susceptible.

Results

Low-resolution mapping

A population of 427 F6 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, 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 F2 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 $Rym_{14}^{Hb}$ interval between the telomere of chromosome 6HS and the marker position at base pair 4,553,134.

---

**Fig. 1** Physical map of the $Rym_{14}^{Hb}$ locus. a Low-resolution mapping of the $Rym_{14}^{Hb}$ locus. Graphical genotype and phenotype of the four recombinant F2 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 F2 progenies are shown on the right ($R$ resistant, $S$ susceptible, seg segregation of resistance). b High-resolution mapping of the $Rym_{14}^{Hb}$ locus. KASP and PACE markers are represented as black and blue vertical lines, respectively, and the 11 recombinant haplotypes found in F2 plants are indicated by horizontal bars: blue $= H. bulbosum$ homozygous; orange $= H. vulgare$ homozygous; yellow $=$ heterozygous. 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)
Fine mapping

The population of 7500 F2 was genotyped at the Rym14Hb 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 table 1). 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 Rym14Hb 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 Rym14Hb, only F3 families derived from the 18 Rym14-recombinants heterozygous or homozygous for the susceptible allele at rym4 were phenotyped using 30 and 20 F3 siblings, respectively (Table 2). The infection rate during

### Table 1: Phenotype of 26 F2 lines from the cross ‘Borwina’ x ‘A42’ and of their F3 progenies

| Sample name       | Genotype at Rym14Hb locus | Phenotype on F2 | F3 progenies | Non-infected plants | Infected plants | % of infected plants | Deduced phenotype |
|-------------------|---------------------------|----------------|--------------|---------------------|-----------------|----------------------|------------------|
| 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       | H. vulgare                | S              | 2            | 22                  | 92              | S                    |

aEither the recombinant haplotype name in Fig. 1, or, for non-recombinant lines, ‘heterozygous’ or ‘H. vulgare’
bResistant (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 times, 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 \textit{rym4} (supplementary table 6). The Rym14\textsuperscript{HB} phenotypes of the recombinant lines were deduced from the segregation of infection in F\textsubscript{3} progenies that does not carry \textit{rym4} at homozygous state, controlled by a $\chi^2$ 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).

### Table 2

Phenotype of the progeny that are not homozygous for the \textit{rym4} allele of the 18 F\textsubscript{2} recombinants from high-resolution mapping population. F\textsubscript{3} plants that are homozygous for \textit{rym4} allele were excluded from this table.

| Sample name  | Genotype at Rym14\textsuperscript{HB} locus\textsuperscript{a} | Non-infected plants | Infected plants | % of infected plants | $p$-value \textsuperscript{b} | $\chi^2$ test 3R:1S ratio\textsuperscript{b} | Deduced phenotype\textsuperscript{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) | \textit{H. vulgare} | 1 | 51 | 98 | – | S |

\textsuperscript{a}Specified as the recombinant (rec) haplotype name in Fig. 1

\textsuperscript{b}Resulting $P$-value from a $\chi^2$ test of goodness to fit the expected ratio of 3R:1S (three resistant plants for one susceptible plant expected for a dominant locus)

\textsuperscript{c}Resistant (R), susceptible (S) or segregating (seg)

In the absence of a genomic sequence for a Rym14\textsuperscript{HB} plant, we cannot precisely define the genes present in the Rym14\textsuperscript{HB} interval. However, as synten between the two \textit{Hordeum} species is high (Wendler et al. 2017), it is still relevant to assess the genes annotated in the orthologous interval of \textit{H. vulgare} as a proxy for suggesting Rym14\textsuperscript{HB} candidate genes. In the respective interval of the ‘Morex’ V2 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\textsuperscript{HB} plant, the candidate resistance gene was homologous to the \textit{rym4} allele in the small number of plants tested: a single plant was homologous for \textit{H. vulgare} allele in the Rym14\textsuperscript{HB} interval.

Based on this analysis, the Rym14\textsuperscript{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).
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 $rmys$ of the eIF4E gene, and the alleles $rmy1$ and $rmy11$ of PDI1-1 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 down-regulate 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 anti-fungal 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 F2 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 Rym1d 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.

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

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

Funding Open Access funding enabled and organized by Projekt DEAL. This work was supported as part of the collaborative projects “TransBulb” (grant 0315966 from the German Federal Ministry of Education and Research (BMBF)) and “BulbOmics” (grant 2818201615 from the German Federal Ministry of Food and Agriculture (BMEL)).

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.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

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

Adams MJ, Swaby AG, Macfarlane I (1986) The susceptibility of barley cultivars to barley yellow mosaic virus (BaYMV) and its fungal vector, Polymyxa graminis. Ann Appl Biol 109:561–572. https://doi.org/10.1111/j.1744-7348.1986.tb03213.x

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

Adams MJ, Jones DR, O’Neill TM, Hill SA (1993) The effect of cereal break crops on barley mild mosaic virus. Ann Appl Biol 123:37–45. https://doi.org/10.1111/j.1744-7348.1993.tb04070.x

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

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

Bohlmann H, Broekaert W (1994) The role of thionins in plant protection. CRC Crit Rev Plant Sci 13:1–16. https://doi.org/10.1080/0270732X.1994.11759905

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

Brown JKM, Tellier A (2011) Plant-parasite coevolution: bridging the gap between genetics and ecology. Annu Rev Phytopathol 49:345–367. https://doi.org/10.1146/annurev-phyto-072910-095301

Bush SJ, Castillo-Morales A, Tovar-Corona JM et al. (2013) Presence-absence variation in A. thaliana is primarily associated with genomic signatures consistent with relaxed selective constraints. Mol Biol Evol 31:59–69. https://doi.org/10.1093/molbev/mst166

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

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

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

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

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/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

Springer
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/10.1016/j.plantsci.2018.07.002

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

Granner 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 RPP5 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 <em>emm</em>-<em>Lr21</em> gene in wheat. Genetics 182:395–602. https://doi.org/10.1534 genetics.108.099614

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

Jacob F, Vernaldi S, Mackawa T (2013) Evolution and conservation of plant NLR functions. Front Immunol 4:1–16. https://doi.org/10.3389/fimmu.2013.00297

Jayakodi M, Padmaraasu S, Haberer G et al (2020) The barley pan-genome reveals the hidden legacy of mutation breeding. Nature. https://doi.org/10.1038/s41586-020-2947-8

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

Jianping C (2005) Progress and prospects of studies on Polymyxa graminis and its transmitted cereal viruses in China. Prog Nat Sci 15:481–490. https://doi.org/10.1007/s10354-005-1323-31342440

Johnston PA, Niks RE, Meijalahagian V et al (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. https://doi.org/10.1007/s00122-013-2078-9

Kakeda K, Ibiti T, Suzuki J 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 Genom 280:509–519. https://doi.org/10.1007/s00438-008-0383-9

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

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

Kim H-S, Baek S-B, Kim D-W et al (2011) Evaluation and verification of barley genotypes with known genes for resistance to barley yellow mosaic virus and barley mild mosaic virus under field conditions in South Korea. Plant Pathol J 27:324–332. https://doi.org/10.5423/PPJ.2011.27.4.324

Krüger J, Thomas CM, Golstein C et al (2002) A tomato cysteine protease required for <em>em-2</em>-dependent disease resistance and suppression of autonecrosis. Science 296:744–747. https://doi.org/10.1126/science.1069288

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

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

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

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

Li H, Kondo H, Kühne T, Shirako Y (2016) Barley yellow mosaic virus VPg is the determinant protein for breaking eIF4E-mediated recessive resistance in barley plants. Front Plant Sci 7:1449

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

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

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

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

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

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

Ordon F, Ahlemeyer J, Werner K et al (2005) Molecular assessment of genetic diversity in barley and its use in breeding. Euphytica 146:21–28. https://doi.org/10.1007/s10681-005-5192-1

Perovic D, Krämer I, Habekuss A et al (2014) Genetic analyses of BaMMV/BaYMV resistance in barley accession HOR4224 result in the identification of an allele of the translation initiation factor 4e (Hv-eIF4E) exclusively effective against barley mild mosaic virus (BaMMV). Theor Appl Genet 127:1061–1071. https://doi.org/10.1007/s00122-014-2279-x
Pickering RA, Hill AM, Michel M, Timmerman-Vaughan GM (1995) The transfer of a powdery mildew resistance gene from *Hordeum bulbosum* L. to barley (*H. vulgare* L.) chromosome 2 (21). Theor Appl Genet 91:1288–1292. https://doi.org/10.1007/BF0022943

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

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

Riedel C, Habekuß A, Schliepake E et al (2011) Pyramiding of Ryd2 and Ryd3 conferring tolerance to a German isolate of barley yellow dwarf virus-PAV (BYDV-PAV-ASL-1) leads to quantitative resistance against this isolate. Theor Appl Genet 123:69. https://doi.org/10.1007/s00126-011-1567-y

Ruge B, Linz A, Pickering RA et al (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. https://doi.org/10.1007/s00126-003-1339-4

Ruge-Wehling B, Linz A, Habekuß A, Wehling P (2006) Mapping of Rym16Hb, the second soil-borne virus-resistance gene introgressed from *Hordeum bulbosum*. Theor Appl Genet 113:867–873. https://doi.org/10.1007/s00126-006-0345-8

Shi L, Jiang C, He Q et al (2019) Bulked segregant RNA-seq (BSR-seq) identified a novel rare allele of eIF4E effective against multiple isolates of BaYMV/BaMMV. Theor Appl Genet 132:1777–1788. https://doi.org/10.1007/s00126-019-03314-3

Shtaya MJY, Sillero JC, Flath K et al (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. https://doi.org/10.1111/j.1439-0523.2007.01328.x

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

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

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

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

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

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

Wendler N, Mascher M, Himmelbach A et al (2015) Bulbosum to go: a toolbox to utilize hordeum vulgare/bulbosum introgressions for breeding and beyond. Mol Plant 8:1507–1519. https://doi.org/10.1016/j.molp.2015.05.004

Wendler N, Mascher M, Himmelbach A et al (2017) A high-density, sequence-enriched genetic map of hordeum bulbosum and its collinearity to *H. vulgare*. Plant. Genome. https://doi.org/10.1038/nbt.2050

Werner K, Friedl W, Ordon F (2005) Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2). Mol Breed 16:45–55. https://doi.org/10.1007/s11032-005-3445-2

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

Xu J, Kashka KJ (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. https://doi.org/10.1007/BF00227383

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

Yamaji Y, Macjima K, Komatsu K et al (2012) Lectin-mediated resistance impairs plant virus infection at the cellular level. Plant Cell 24:778–793. https://doi.org/10.1105/tpc.111.093658

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

Yang P, Habekuß A, Hofinger BJ et al (2017) Sequence diversification in recessive alleles of two host factor genes suggests adaptive selection for bymovirus resistance in cultivated barley from East Asia. Theor Appl Genet 130:331–344. https://doi.org/10.1007/s00126-016-2814-z

Yu X, Kong HY, Meiyalaghan V et al (2018) Genetic mapping of a barley leaf rust resistance gene Rph26 introgressed from *Hordeum bulbosum*. Theor Appl Genet 131:2567–2580. https://doi.org/10.1007/s00126-018-1773-8

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.