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Title: High-resolution mapping of *Ryd4^{Hb}*, a major resistance gene to *Barley yellow dwarf virus* from *Hordeum bulbosum*

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Abstract

Virus diseases are causing high yield losses in crops worldwide. The *Barley yellow dwarf virus* (BYDV) complex is responsible for one of the most widespread and economically important viral diseases of cereals. While no complete resistance gene has been uncovered in the primary genepool of barley, sources of resistance were identified in the wild relative *Hordeum bulbosum*, representing the secondary genepool of barley. One such locus, *Ryd4^{Hb}*, has been previously introgressed into barley, and was allocated to chromosome 3H, but is tightly linked to a sublethality factor that prevents the incorporation and utilization of *Ryd4^{Hb}* in barley varieties. To solve this problem, we fine-mapped *Ryd4^{Hb}* and separated it from this negative factor. We narrowed the *Ryd4^{Hb}* locus to a 66.5 kbp physical interval in the barley 'Morex' reference genome. The region comprises one complete and one partial gene from the nucleotide-binding and leucine-rich repeat immune receptor family, typical of dominant virus resistance genes. The closest homolog to these two *Ryd4^{Hb}* candidate genes is the wheat *Sr35* stem rust resistance gene. In addition to the fine mapping, we reduced the sublethality factor interval to 600 kbp in barley. Aphid feeding experiments demonstrated that *Ryd4^{Hb}* provides a direct resistance to BYDV rather than a resistance to its vector. The presented results, including the high-throughput molecular markers, will permit a more targeted selection of the resistance in breeding, enabling the use of *Ryd4^{Hb}* in barley varieties.

Keywords
High-resolution mapping, *Barley yellow dwarf virus*, disease resistance, barley, *Hordeum bulbosum*, *Ryd4*^{Hb}

Declarations

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Conflicts of interest/Competing interests Neele Wedler, Viktor Korzun, Klaus Oldach, and Anja Maasberg-Prelle are employed at KWS SAAT SE & Co and KWS LOCHOW. The other authors declare no conflict of interest.

Availability of data and material The Exome capture sequencing datasets generated and/or analyzed in this study are deposited at EMBL-ENA under the project IDs PRJEB7909 and PRJEB65283.

Authors' contributions Brigitte Ruge-Wehling, Nils Stein, Neele Wedler, and Viktor Korzun conceived the project and acquired the funding. Brigitte Ruge-Wehling, Neele Wedler, Klaus Oldach, Anja Maasberg-Prelle, and Viktor Korzun designed and built the mapping populations. Brigitte Ruge-Wehling and Kristin Fischer performed the initial linkage mapping and broke the linkage between the resistance and the sublethality loci. Brigitte Ruge-Wehling and Antje Habekuß performed the phenotyping experiments. Neele Wedler did the exome capture experiment. Torsten Will performed and analyzed the aphid feeding experiments. Hélène Pidon performed the high-resolution mapping and the pangenome analysis and drafted the manuscript. Nils Stein supervised the project. All authors provided critical feedback and helped shape the manuscript.

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Key message (<30 words)

We mapped *Ryd4*^{Hb} in a 66.5 kpb interval in barley and dissociated it from a sublethality factor. These results will enable a targeted selection of the resistance in barley breeding.

Introduction

Virus diseases cause significant yield losses and represent an increasing threat to agricultural crop production worldwide (Oerke, 2006). Among them, *Barley yellow dwarf virus* (BYDV) complex is responsible for one of the most widespread and economically important viral diseases of cereals. Transmitted in a persistent and circulative manner by several species of aphids, BYDV causes dwarfing and leaf discoloration, leading to significant yield loss in major cereal crops, in particular barley, wheat, maize, and oats (Ali et al., 2018). In recent years, it has become increasingly important in winter barley with an incidence that could reach 70% and yield loss of up to 80% (Beoni et al., 2016; Dedryver et al., 2010; Ordon et al., 2009). As climate change scenarios predict longer and warmer autumns, which favor aphid infestations of winter crop fields, BYDV could become one of the most threatening diseases of cereal crops (Roos et al., 2011; Trebicki, 2020). Reduction of yield losses by insecticide-based vector control is possible in principle, but undesirable for ecological reasons. To ensure sustainable barley cultivation in the expanding infestation areas and thus secure yields and quality in the long term, the cultivation of virus resistant varieties would provide the best solution.

So far, three genes and some QTLs have been described as providing partial resistance or tolerance to BYDV in barley. The gene *ryd1*, providing recessive intermediate tolerance was identified by Suneson (1955) but is still not cloned (Niks et al., 2004). Its effectiveness is low and it is rarely used in breeding. *Ryd2* was identified from an Ethiopian barley landrace (Schaller et al., 1964). It provides field tolerance to the virus serotypes BYDV-PAV, BYDV-MAV, and BYDV-SGV (Baltenberger et al., 1987). Mapped close to the centromere of chromosome 3H (Collins et al., 1996), *Ryd2* is used in several breeding lines (Kosova et al., 2008) where it can reduce significantly the yield loss caused by BYDV (Beoni et al., 2016). The third gene, *Ryd3* was also identified from an Ethiopian barley landrace (Niks et al., 2004). The gene was mapped in the centromeric region of chromosome 6H but, despite fine mapping on more than 3,000 F_2 plants (Lüpken et al., 2014), the mapping interval is still large. *Ryd3* has been transferred to commercial varieties where it provides a quantitative resistance, improved when in combination with *Ryd2* (Riedel et al., 2011). QTLs on chromosomes 1H, 2H, 4H, 5H, and 7H have been reported, however, providing only a limited level of tolerance (Toojinda et al., 2000; Riedel et al., 2011; Hu et al., 2019). No complete resistance to BYDV or its aphid vectors is known in barley, and broadening the genetic basis of resistance is therefore needed to ensure a durable and stable production of winter barley fields.

The secondary genepool of barley, consisting of the species *Hordeum bulbosum*, has not yet been used to improve resistance to the BYDV complex. Michel (1996) identified resistance to BYDV in the tetraploid (2n=4x=28) *Hordeum bulbosum* accession A17 (Bu10/2) from the Botanical Garden of Montevideo, Uruguay. Plants of this accession remained ELISA-negative for BYDV after several inoculations with aphids charged with the virus isolates BYDV-PAV1 Aschersleben, BYDV-MAV1 Aschersleben, and CYDV (*Cereal yellow dwarf virus*)-RPV Dittersbach (Habekuß et al., 2004). A17 was used as a parent in interspecific crosses and backcrosses with *H. vulgare* cv. Igri to generate an *H. bulbosum* introgression to barley. Its resistance was described as complete, dominant, and monogenic, and the locus, assigned to chromosome 3H, was named $Ryd4^{Hb}$ (Scholz et al., 2009). Adversely, a recessive sublethality factor was cosegregating with $Ryd4^{Hb}$ in the respective introgression. A study revealed low aphid feeding on the *H. bulbosum* A17 accession, suggesting that resistance may not be acting directly on the virus but rather indirectly against the aphid vector (Schliephake et al., 2013).

The present study reports the fine mapping of the $Ryd4^{Hb}$ locus, the identification of candidate genes, and the description of aphids feeding behavior on susceptible and resistant introgression lines.

Material and methods

Plant material

BC_2F_5 and BC_2F_6 selected from BC_2F_4 plants from the Scholz et al. (2009) population were used for the low-resolution linkage mapping and development of an introgression line lacking the sublethality factor. This population is later named LM_Pop.

Two additional populations were generated to map *Ryd4*^{Hb} at a higher resolution. FM_Pop1 was derived from a BC_2F_7 plant from LM_Pop crossed successively with three different barley elite varieties. The pedigree of the 15 lineages that constitute FM_Pop1 is presented in supplementary table 1. The donor of resistance in the FM_Pop2 was a BC_2F_8 derived from the BC_2F_6 JKI-5215 homozygous for the *H. vulgare* (Hv)-allele in the sublethality factor locus. As for FM_Pop1, it was crossed successively with two different barley elite varieties. The pedigree of the lines of four lineages that constitute FM_Pop2 is presented in supplementary table 2. The F_1 plants from the successive crosses were checked with markers to ensure the presence of the *H. bulbosum* (Hb)-allele at the *Ryd4*^{Hb} locus and were further selfed to obtain the F_2 lineages forming FM_Pop1 and FM_Pop2.

Test of resistance to BYDV

Five to ten *Rhopalosiphum padi* aphids of the biotype R07, that were reared on BYDV-PAV1 Aschersleben (PAV1-ASL) infected plants as described by Kern et al. (2022), were placed on each one-week-old seedlings to be phenotyped in an air-conditioned greenhouse (20 °C, 16 h photoperiod, 10 klx). The aphids were killed after two days using the insecticide Confidor®WG 70 (Bayer CropScience AG, Germany). Further cultivation of the plants was carried out. Five to six weeks after inoculation, leaf samples of 50 mg from two leaves were taken and tested by a double-antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) according to Clark & Adams (1977) using custom-made antibodies. The viral content was evaluated by measuring extinction at 405 nm on a microtitre plate reader (Opsys MR, ThermoLabsystems or Tecan Sunrise, Tecan) one hour after the addition of the enzyme substrate. Based on negative controls, a extinction threshold was set in each experiment, usually at 0.1, under which a plant was classified as resistant. Phenotyping tests for gene mapping were performed on ten to 15 seeds of progenies of each genotype.

DNA extraction

Genomic DNA from the LM-Pop was either isolated following a slightly modified protocol after Stein et al. (2001) or using the Biosprint 96 DNA Plant Kit (Qiagen) and the Biosprint 96 working station (Qiagen) following the manufacturer's instructions. DNA was dissolved in TE buffer, quantified via photometric approaches (NanoQuant, Tecan, Austria) and diluted to a working concentration of 10 ng/µl. DNA extractions of plants from FM_Pop1 and FM_Pop2 were carried out according to the protocol described in Milner et al. (2019).

Marker development for low-resolution linkage mapping

The EST-derived SSR anchor markers *GBM1050* and *GBM1059* (Scholz et al., 2009; Stein et al., 2007; Thiel et al., 2003; Varshney et al., 2007) were kindly provided by Prof. Andreas Graner (Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben). The STS markers *GLMWG883* and *GLABC161* were derived from the sequence of RFLP probe *MWG883* (Rostoks et al., 2005; Szűcs et al., 2009) and barley anchor marker *ABC161* (Close et al., 2009), respectively. Orthology of the interval on the rice chromosome 1 using the mapping of the anchor marker *ABC161* at 40.43 Mbp on said chromosome allowed

153 for the development of 18 tentative consensus (TC) markers polymorphic between Hv and Hb on
154 chromosome 3HL.

155 Additional polymorphisms between the *H. bulbosum* and *H. vulgare* genomes for marker development were
156 identified by RNASeq and Massive analysis of cDNA-ends (MACE). To this end, 1,000 plants from BC_2F_5
157 of LM_Pop were screened with the TC marker *TC173485*. Among them, 200 plants homozygous for the *H.*
158 *bulbosum* allele, considered resistant, and 200 plants homozygous for the *H. vulgare* allele, considered
159 susceptible, were selected. In total, 100 plants of each category were inoculated by aphids carrying the
160 isolate BYDV-PAV1, and an equal amount of plants were infested with control aphids without virus titer in
161 a separate climate chamber. 1h, 4h, 8h, and 24h after inoculation plant material of 25 genotypes per category
162 and treatment was harvested and sent to GenXPro (Frankfurt am Main, Germany) for RNA isolation and
163 sequencing. RNASeq and MACE were performed as described in Santos et al. (2018) and Braun et al.
164 (2019), respectively. In short, the raw data was cleaned off adapter sequences using the software TagDust
165 (Lassmann et al., 2009). All RNAseq datasets were combined to create a reference library. Assembly was
166 performed using the software Trinity (Grabherr et al., 2011). The reads of the individual libraries were
167 hereafter mapped to the reference library and single nucleotide polymorphisms (SNPs) were identified using
168 the software SNVMix (Goya et al., 2010). SNPs between the *H. bulbosum* and *H. vulgare* genomes were
169 identified and sequences 100 bps up- and downstream of each SNP were determined. Annotation of the
170 SNP-containing sequences was done by using the database Swiss-Prot (Boeckmann et al., 2003).

171 Additional polymorphisms were retrieved from exome capture sequencing, performed according to Wendler
172 et al. (2014) on the *H. bulbosum* parent A17 (Wendler et al., 2015) and the BC_2F_4 plant 5194/5, homozygous
173 for the 3HL-*H. bulbosum* introgression and mapped on the first barley genome assembly (International
174 Barley Genome Sequencing Consortium, 2012). Single nucleotide variants between *H. vulgare* and *H.*
175 *bulbosum* were called with samtools (SNP call score <200). Variants located within 200 bp of the end of a
176 reference sequence contig or supported by less than fivefold sequence read coverage were excluded from
177 further evaluation. The flanking sequences (50–60 bp) of variant positions were used for marker assay
178 development.

179 The primer design for PCR markers was carried out using Primer 3 (Untergasser et al., 2012). Conversion
180 of SNPs in CAPS markers was done by using SNP2CAPS (Thiel et al., 2004). All markers used for the low-
181 resolution linkage mapping are described in supplementary table 3.

182 **Marker development for high-resolution linkage mapping**

183 Exome capture data of the *H. bulbosum* A17 parent and of the BC_2F_4 plant 5194/5 was remapped onto the
184 barley reference genome MorexV3 (Mascher et al., 2021) together with the exome capture data of 13 barley
185 varieties from (Russell et al., 2016) (cultivars 'Barke', 'Bonus', 'Borwina', 'Bowman', 'Foma', 'Gull',
186 'Harrington', 'Haruna-Nijo', 'Igri', 'Kindred', 'Morex', 'Steptoe', and 'Vogelsanger Gold'). Reads
187 mapping and variant calling were performed as described in Milner et al., (2019). The SNP matrix was
188 filtered on the following criteria: heterozygous and homozygous calls had to have a minimum mapping
189 quality score of three and five, respectively, and be supported by a minimum of ten reads. SNP sites were
190 retained if they had less than 20% missing data and less than 20% heterozygous calls. SNPs that were within
191 the $Ryd4^{Hb}$ 20 Mbp interval defined by the low-resolution linkage mapping, homozygous for one allele in
192 all barley varieties and for the other allele in A17 and the BC_2F_4 introgression line, were retained.

193 For six SNPs, a 100 bp sequence containing the SNP in its center was provided to LGC genomics (Berlin,
194 Germany) for KASPar marker production (Supplementary table 4). Within the sublethality factor interval,

195 ten more SNPs were retrieved and sequences of 100 bp around each one were sent to 3CR Bioscience
196 (Welwyn Garden City, UK) for PACE assay design (Supplementary table 5). Primers were ordered from
197 Metabion (Germany) and mixed according to 3CR Bioscience (Welwyn Garden City, UK)
198 recommendations.

199 Thirteen CAPS markers (Supplementary table 6) were developed using NEBcutter (Vincze et al., 2003) to
200 identify the cutting enzyme and Primer 3 (Untergasser et al., 2012) to design the PCR primers.

201 **Genotyping assays**

202 For PCR of SSR, STS, and CAPS markers were carried out in a volume of 10µL containing 50-100ng of
203 DNA, 1X PCR buffer (Qiagen), 0.5 mM of each primer, 0.5 U of Taq DNA polymerase (Qiagen), and 0.2
204 mM of dNTPs. PCR amplification was carried out with an initial 10 min step at 95°C, followed by a
205 touchdown profile of ten cycles at 95°C for 30 seconds, 60°C for 30 seconds with a 0.5°C reduction per
206 cycle, and 72°C for 1 minute, followed by 35 cycles at 95°C for 30 seconds, 55°C for 30 seconds then 72°C
207 for 1 minute, and a last step of 7 minutes at 72°C. For CAPS markers, a 5 µl aliquot of the PCR product was
208 digested in 10 µL with 1 U of restriction enzyme and 1x of the appropriate digestion buffer at the temperature
209 recommended by the manufacturer. Pre- and post-digestion PCR products were separated on 2.5 % agarose
210 gels followed by ethidium bromide staining or in 10% polyacrylamide gels followed by silver nitrate
211 staining according to Budowle et al. (1991).

212 Detection of SNPs as genetic markers were performed by high-resolution melt analysis (HRM) by using the
213 Rotor Gene Technology (Qiagen). PCR was carried out in 20 µl volume containing 20 ng template DNA, 1
214 x buffer (Promega), 2.5 mM $MgCl_2$, 0.8 mM dNTP mix, 0.5 µM of each primer, 1 x EvaGreen Dye
215 (Biotium, Inc.) and 0.3 U *Taq* DNA polymerase (Promega). A touchdown PCR protocol was conducted
216 with a temperature gradient from 60–50°C. The melt curve analysis was conducted by ramping from 65 C
217 to 95 C with a 0.1 C decrease per capture.

218 Genotyping assays with KASPar and PACE markers were carried out as described in Pidon et al. (2020).

219 **Genetic linkage analysis**

220 LM_Pop was genotyped with 4 EST-derived SSR anchor markers, 18 TC-markers, 6 MACE-SNP, 13 STS-
221 markers derived from MACE (denoted as MACE_b), 9 markers derived from RNA-seq experiment, and 3
222 markers derived from exome capture. Linkage analysis was performed using the JoinMap® 4.1 software
223 (Van Ooijen, 2006). Genetic maps were displayed and edited in MapChart2.2 (Voorrips, 2002).

224 **Pangenome comparison**

225 The flanking markers and the *Ryd4*^{Hb} interval in the MorexV3 genome were searched on the 19 other
226 assemblies of the barley pangenome (Jayakodi et al., 2020) using BLAST+ (Camacho et al., 2009). The
227 resulting intervals were extracted and reannotated through a combination of alignments of the Morex
228 candidate genes to them, the search for the presence of conserved domains using NCBI *conserved domains*
229 (S. Lu et al., 2019), and NLRs annotation with NLR-Annotator (Steuernagel et al., 2020). The interval
230 structures were compared using Easyfig with blastn (Sullivan et al., 2011).

231 **Aphids feeding experiment**

232 In order to test if *Ryd4*^{Hb} provides resistance to aphids, a resistant and a susceptible progeny of the
233 heterozygous lines at *Ryd4Hb* locus EP_16-271_3_206 and EP_16-460_5_228 were selected. The
234 susceptible progenies EP_16-271_3_206_2 and EP_16-460_5_228_2 were carrying Hv alleles at both
235 Ryd4_CAPS19 and Ryd4_CAPS24, while the resistant lines EP_16-271_3_206_4 and EP_16-

236 460_5_228_6 were recombining in the interval and carrying a Hb allele at Ryd4_CAPS24 or
237 Ryd4_CAPS19, respectively. The feeding behavior of 12 to 16 adult apterouse non-viruliferous *R. padi*
238 (clone R07) of random age was observed on an individual healthy plant of the respective genotypes per
239 aphid by using the electrical penetration graph (EPG) technique (Tjallingii, 1978). Plants for EPG
240 experiments were reared in a greenhouse and were used at a 3-4 leaf stage where aphids were placed on the
241 lower side of the second leaf. Aphids were reared as described before (Kern et al., 2022). Aphids starved
242 for 1 hour before they were placed on the leaf. The recording started when all aphids were prepared. The
243 observation period was set to 8 hours and recording was started after all aphids were placed. For data
244 acquisition, the GIGA-8 EPG amplifier and EPG stylet software (EPG Systems, Wageningen, The
245 Netherlands) were used and data were analyzed with the EPG stylet analysis module. Waveforms were
246 annotated according to Tjallingii (1978) and Tjallingii & Esch (1993). Subsequently, selected parameters
247 were analyzed by using an Excel workbook (Alvarez et al., 2021). Recordings of aphids that fell from the
248 leaf or escaped during the experiment were not used.

249 **Results**

250 **Low-resolution linkage mapping of *Ryd4^{Hb}***

251 From LM_Pop, 1,125 BC_2F_5 and BC_2F_6 were used for the low-resolution linkage mapping of *Ryd4^{Hb}*.
252 Phenotyping of progenies revealed 570 resistant, 276 susceptible plants, and 279 plants that died at early
253 stages. This high mortality rate was expected due to the recessively inherited sublethality factor very closely
254 linked to *Ryd4^{Hb}*. This locus prevents normal plant development, resulting in plant death (Figure 1). Because
255 of the close linkage between the two loci, we considered that all plants that died in the BC_2F_5 and BC_2F_6
256 families could be defined as homozygous resistant, while the resistant plants that survived would be
257 heterozygous at *Ryd4^{Hb}*. The phenotype distribution would indeed fit the expected 1:2:1 ratio of homozygous
258 resistant, heterozygous resistant, and homozygous susceptible genotypes for a dominant monogenic
259 inheritance of *Ryd4^{Hb}* resistance (Chi-Square Goodness of Fit Test: χ^2= 0.216, p=0.90), confirming the
260 previous observation for that locus on the BC_2F_4 generation (Scholz et al., 2009).

Fig. 1 Five months-old homozygous resistant plants either carrying a sublethality factor (left) or vital (right). The growth of plants homozygous at the sublethality factor is greatly reduced compared to plants carrying the resistance locus without the sublethality factor. They died before reaching heading.

The population was genotyped with 53 co-dominant markers and the linkage mapping was performed on the 1,014 individuals with a low amount of missing data. The introgression size was estimated to be 18.7 cM limited distally by the marker EXCAP_16 and proximally by the marker MACE_b_79 (Figure 2). The resistance locus *Ryd4*^{Hb} is flanked by the MACE marker Mace_b_53 and the TC marker TC262452 with a genetic distance of 0.3 cM proximally and 0.5 cM distally, respectively. Moreover, the 200 BC_2F_5 plants screened with TC262452 and phenotyped by DAS-ELISA showed a perfect cosegregation of the TC262452 marker data with the resistance: the 100 plants carrying at least one Hb allele at the TC173485 marker had an extinction <0.1 whereas the 100 plants homozygous for the Hv allele had an extinction >0.5.

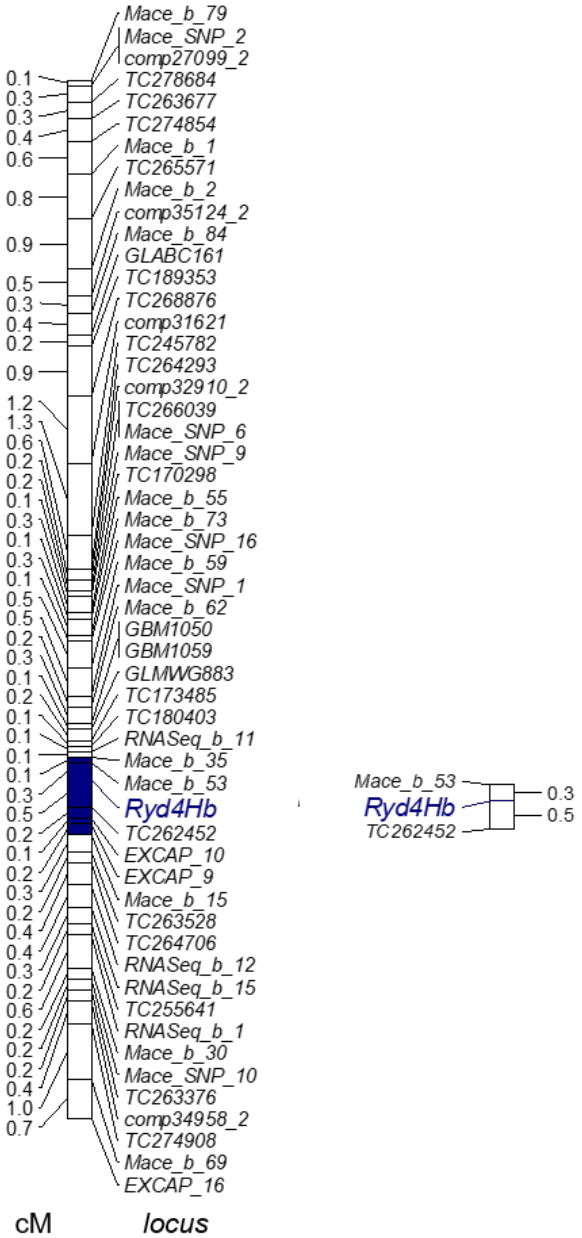


Fig. 2 Linkage map of chromosome 3HL carrying *Ryd4*^{Hb}. The extent of the *Ryd4*^{Hb} locus is represented in blue (1), marker bracket around *Ryd4*^{Hb} displaying the flanking markers (2)

Development of an introgression line without sublethality factor

The exploitation of *Ryd4*^{Hb} for breeding programs requires the selection of homozygous resistant and vital (by opposition with sublethal) plants. To select recombinants lacking the sublethality factor, 12,133 BC_2F_6 plants of LM_Pop were screened with markers TC262452 and Mace_b_53. Among those, 3,103 plants were homozygous for Hb-alleles, 6,020 heterozygous (Hb/Hv), and 3,010 homozygous for *H. vulgare* (Hv) at *Ryd4*^{Hb} locus, confirming the 1:2:1 segregation (Chi-Square Goodness of Fit Test: χ^2= 2.14, p=0.34). The 3,103 plants homozygous for Hb-alleles were propagated and the progenies were checked for resistance. A single progeny, denoted as JKI-5215, was both completely resistant and vital. Marker analysis revealed that this population was segregating for Hb- and Hv-alleles distally from *Ryd4*^{Hb}.

The JKI-5215 population, made of 43 BC_2F_7 plants from JKI-5215 progeny, was genotyped with all 16 markers that mapped distally from *Ryd4*^{Hb}. Markers TC262452 to RNASeq_b_1 were homozygous for Hb-alleles whereas Mace_b_30 and all the remaining markers distally located were segregating in a 1:2:1 fashion (Figure 3). The recombination occurred within the initial 3HL introgression and resulted in a reduced Hb-segment of 3.4 cM and all progenies are completely vital. A BC_2F_8 from the family JKI-5215 homozygous for the Hv-segment in the sublethality factor interval was selfed and used as the resistant donor for the FM_Pop2.

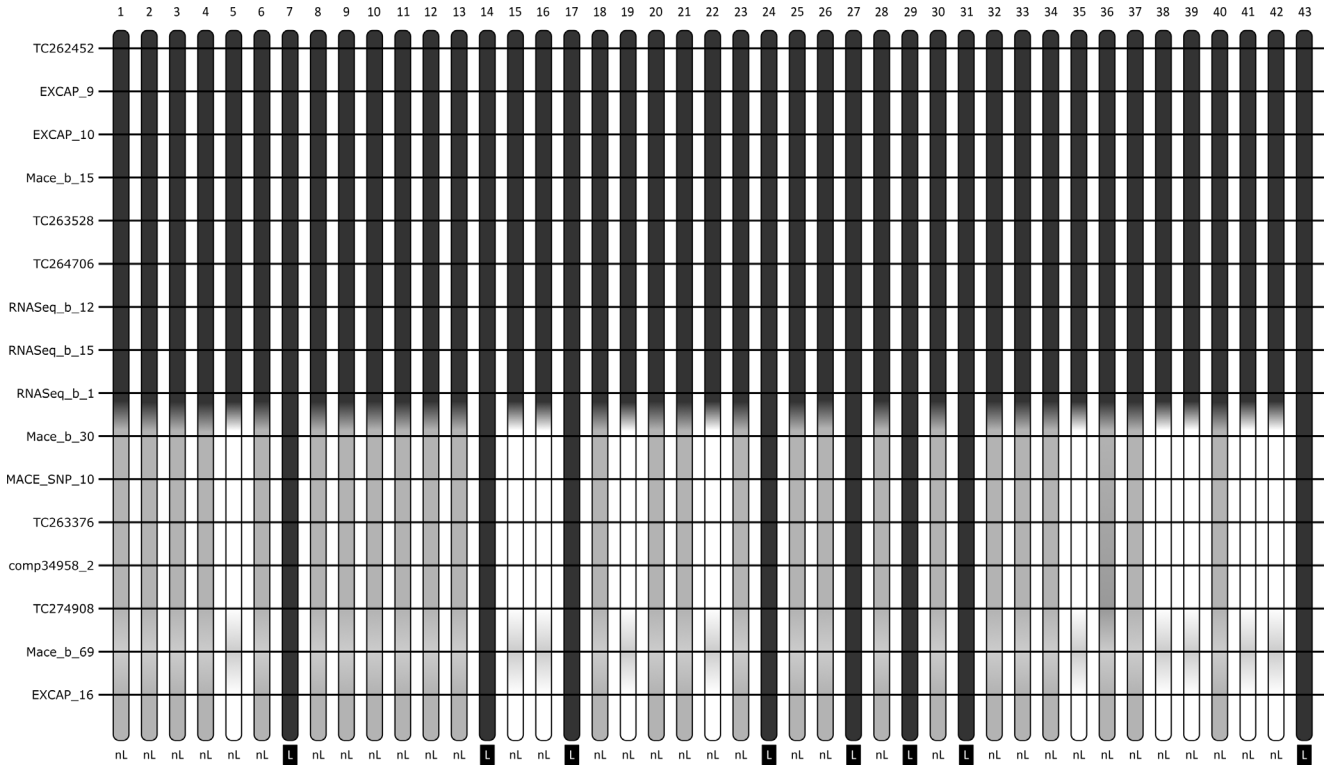


Fig. 3 Characterization of 43 plants from the family JKI-5215 characterized by reduced introgressed Hb-fragment without sublethality factor. Each vertical bar represents one offspring from JKI-5215. White, black, and grey fragments represent Hb fragments, Hv fragments, and heterozygous genotypes at the markers indicated on the right side, respectively. The light grey fragments at the dominant marker Mace_b_69 are either Hv or heterozygous genotypes. The phenotype is indicated below the figure as 'nL' for vital plants and 'L' for sublethal ones.

High-resolution mapping of *Ryd4*^{Hb} in two F_2 populations

To precisely map *Ryd4*^{Hb}, 5,589 F_2 plants from FM_Pop1 and 10,155 F_2 plants from FM_Pop2 were genotyped with two KASPar markers to identify recombination at the locus. Plants from FM_Pop1 were screened with Ryd4_KASP1 and Ryd4_KASP4, and plants from FM_Pop2 with Ryd4_KASP1 and Ryd4_KASP5. Indeed, Ryd4_KASP4 is located in the sublethality factor interval and was segregating in FM_Pop1, but not in FM_Pop2 where it was fixed Hv in its resistant donor. We identified 46 and 68 recombinant plants in FM_Pop1 and FM_Pop2, corresponding to 0.82% and 0.67% of recombination, respectively. The positions of the SNPs of the markers Ryd4_KASP1, Ryd4_KASP4 and Ryd4_KASP5 were identified in the GBS data from 92 recombinant inbred lines of the cross 'Barke' x 'Morex' (Mascher et al., 2013). A distance of 15 cM was observed between the markers Ryd4_KASP1 and Ryd4_KASP4, and of 12.6 cM between Ryd4_KASP1 and Ryd4_KASP5, indicating by definition a 15% and 12.6% probability of recombination in these intervals, respectively. The observed rate of recombination at the *Ryd4*^{Hb} locus is

310 therefore about 20-times lower than expected for the same genetic interval in a pure intraspecific barley
311 cross.

312 Recombinants were phenotyped on 15 offsprings and genotyped with the 13 CAPS markers. The resulting
313 interval for *Ryd4*^{Hb} was 66.5 kbp-long in MorexV3 genome between the coordinates 592,685,940 and
314 592,752,329 flanked by CAPS19_2 and CAPS24, describing an interval comprising six recombination
315 events (Figure 4, Supplementary table 7). Four genes are annotated with high confidence on the MorexV3
316 genome in that interval: HORVU.MOREX.r3.3HG0318400, an S-formylglutathione hydrolase,
317 HORVU.MOREX.r3.3HG0318420, a partial nucleotide-binding and leucine-rich repeat immune receptors
318 (NLR) with a coiled-coil domain (CNL) lacking LRR domain which is likely a pseudogene,
319 HORVU.MOREX.r3.3HG0318450, a complete CNL, and HORVU.MOREX.r3.3HG0318470, an ankyrin-
320 repeat-containing gene. Those genes will later be referred to as *Ryd4_SFGH*, *Ryd4_pCNL1*, *Ryd4_CNL2*,
321 and *Ryd4_ANK*, respectively. The genes' homology revealed that locus *Ryd4*^{Hb} is syntenic to the *Triticum*
322 *monococcum* locus *Sr35,* conferring resistance to wheat stem rust (Saintenac et al., 2013). The candidate
323 genes present a high similarity to one of the *Sr35* candidate genes. In particular, the translation of the
324 complete *Ryd4_CNL2* gene sequence from the MorexV3 reference genome shows 83% identity with the
325 SR35 protein while the respective coding sequence shows 88.7% nucleotide identity. The *Ryd4*^{Hb} interval
326 also overlaps almost completely the ones of the *Rph13* leaf rust resistance gene from the *H. vulgare* ssp.
327 s*pontaneum* Hs2986 (Jost et al., 2020) and of the *Jmv2* resistance gene to the *Japanese soil-borne wheat*
328 *mosaic virus* from the barley cultivar 'Sukai Golden' (Okada et al., 2022). *Rph13* is located on chr3H
329 between the coordinates 592,658,337 and 592,786,929 on MovexV3 (128.6 kbp). Comparing the number
330 of recombinants, the size of the interval in which they occurred, and the size of the mapping population for
331 *Rph13* and *Ryd4*^{Hb} (4 recombinant in 128.6 kbp out of 719 plants and 6 recombinants plants in 66.5 kbp out
332 of 15,774, respectively), the recombination rate observed in the *Ryd4*^{hb} populations is 7.5 times lower than
333 observed in the intraspecific cross used to map *Rph13*.

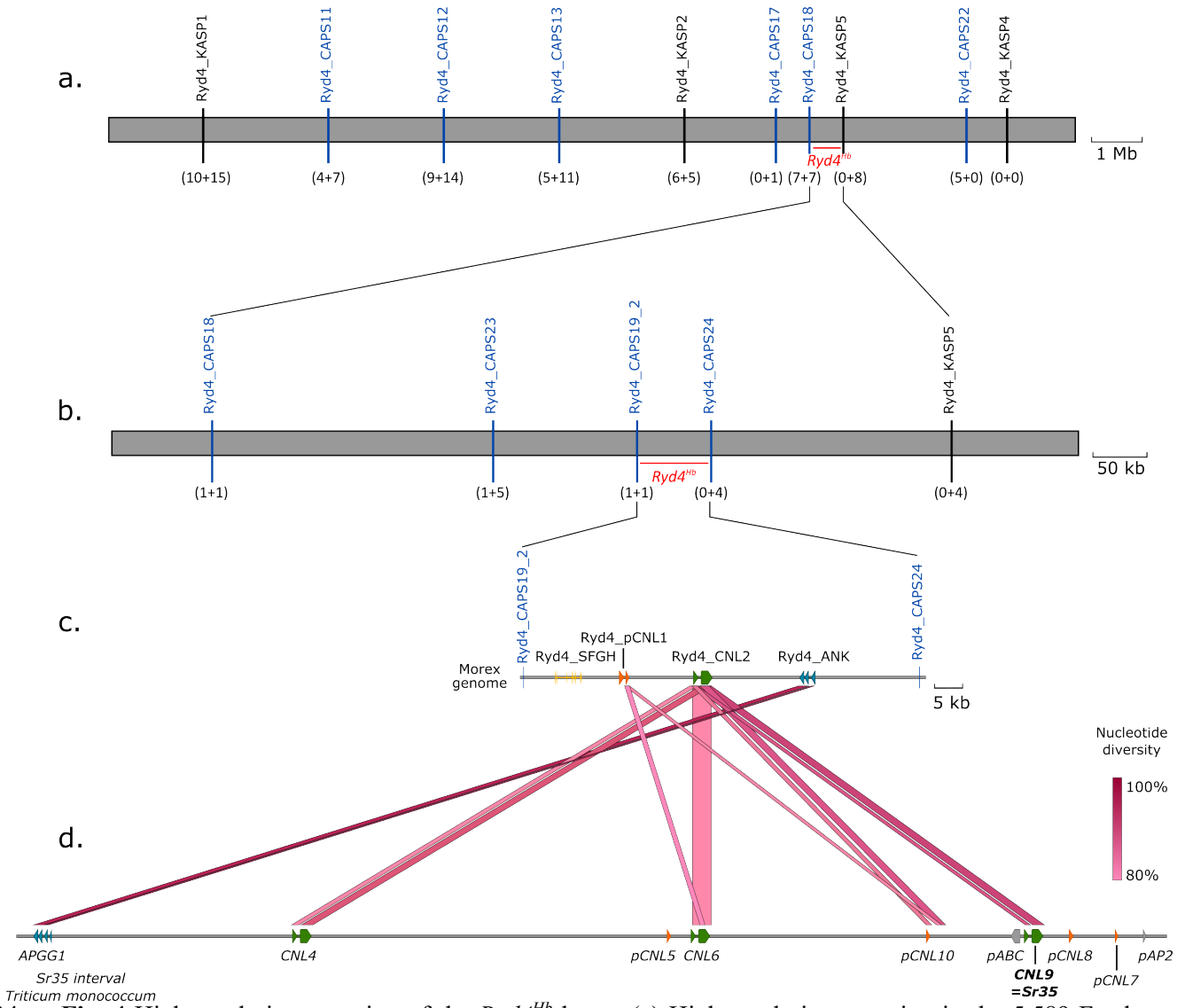
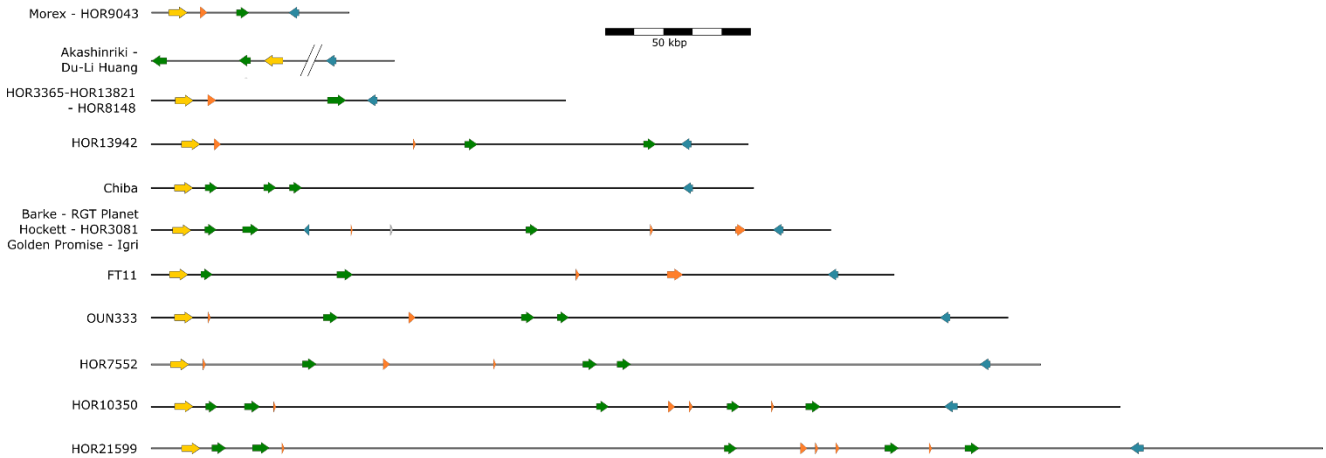


Fig. 4 High-resolution mapping of the *Ryd4*^{Hb} locus. (a) High-resolution mapping in the 5,589 F_2 plants from FM_Pop1 and 10,155 plants of FM_Pop2. The numbers in brackets show the sum of individual recombinants between the resistance locus and the corresponding marker in FM_Pop1 and FM_Pop2, respectively. (b) Marker saturation of the 18 recombinant plants at *Ryd4*^{Hb} locus. Recombinants from FM_Pop1 that died are excluded. (c) Candidate genes in the 66.5 kbp final *Ryd4*^{Hb} interval. (d) Comparison of *H. vulgare* 'Morex' and *T. monococcum* DV92 orthologous genes (only fragments with more than 80% nucleotide identity are shown).

Ryd4^{Hb} locus diversity in the barley pangenome

The orthologous intervals of the MorexV3 *Ryd4*^{Hb} region were retrieved from 19 additional diverse genome assemblies of the barley pangenome (Jayakodi et al., 2020) (supplementary table 8). We then annotated them using a combination of methods: mapping the 'Morex' genes, searching for NLR genes with NLR-Annotator (Steuernagel et al., 2020), and confirmation of the absence of additional conserved domains with NCBI conserved domains (S. Lu et al., 2019). The analysis revealed a very large divergence of the *Ryd4*^{Hb} interval in the different genotypes (Figure 5, supplementary figure 1). The shortest orthologous interval is the one of MorexV3. The largest is the one of the accession 'HOR 21599', 406 kbp-long and containing 10 NLRs, of which five are complete. The interval in the cultivars 'Akashinriki' and 'Du-Li Huang' is affected

350 by a large inversion of around 600 kbp. The observed diversity between haplotypes is mainly explained by
351 the presence of different repetitive elements and duplications. The degree of divergence in the *H. vulgare*
352 genepool in this interval suggests that an even greater diversity and divergence may be anticipated for the
353 corresponding region in the *H. bulbosum* genome.



354 **Fig. 5** Graphical representation of the haplotype size and gene composition in the barley pangenome. Grey
355 horizontal bars represent the dimension of the interval; forward slashes represent the breakpoint due to the
356 large inversion in Akashinriki and Du-Li Huang. The interval between them is around 400 kbp-long and is
357 not homologous to 'Morex' interval. Arrows represent genes; yellow genes are S-formylglutathione
358 hydrolase, orange genes are partial NLRs, green genes are complete NLRs, and blue genes are ankyrin-
359 repeats-containing genes.

360 **Mapping of the sublethality factor**

361 To better understand why the *H. bulbosum* locus is causing the sublethality, we used recombinants identified
362 in the frame of *Ryd4*^{Hb} mapping to pinpoint the responsible factor more precisely. The low-resolution linkage
363 mapping located it distally from the marker *RNASeq_b_1*, which corresponds to position 594,019,595 on
364 chromosome 3H of the MorexV3 genome. To precise its interval, ten PACE markers were designed between
365 Ryd4_KASP18 and Ryd4_KASP22 (supplementary table 5), and used to genotype plants recombining in
366 the interval. Six recombination events were available: the one of JKI-5215 that we mapped using 24 non-
367 recombinant F_2 plants from lineage EP_16-460 of FM_Pop2, and the ones of five F_2 plants from FM_Pop1
368 recombining between Ryd4_KASP5 and Ryd4_C22, of which four vital plant and the sublethal plant
369 KW15_231/340_48 which died before heading (figure 5). The genotyping of 24 non-recombinant FM_Pop2
370 F_2 plants confirmed that the JKI-5215 recombination event occurred between Ryd4_KASP5 and
371 Ryd4_CAPS22 (Figure 5), and more precisely between markers Ryd4_leth2 and Ryd4_leth3 (594,034,042
372 to 594,290,776 bp). The genotyping and phenotyping of thirty-two F_3 plants from each of the four vital
373 plants recombining between Ryd4_KASP5 and Ryd4_C22 placed the sublethality factor proximally of
374 marker Ryd4_leth7. This was confirmed by the genotyping of the sublethal plant KW15_231/340_48 from
375 FM_Pop1 which was identified as recombinant between Ryd4_leth6 and Ryd4_leth7 (Figure 6). The
376 sublethality factor could therefore be assigned to a 483 kbp-interval between markers Ryd4_leth3 and
377 Ryd4_leth7 (594,290,776-594,773,972 bp on chromosome 3H of MorexV3). This interval is annotated with
378 15 high-confidence genes described in table 1. Among those genes, one or several could be essential genes
379 for plant development with no orthologs in the corresponding region of the *H. bulbosum* genome.

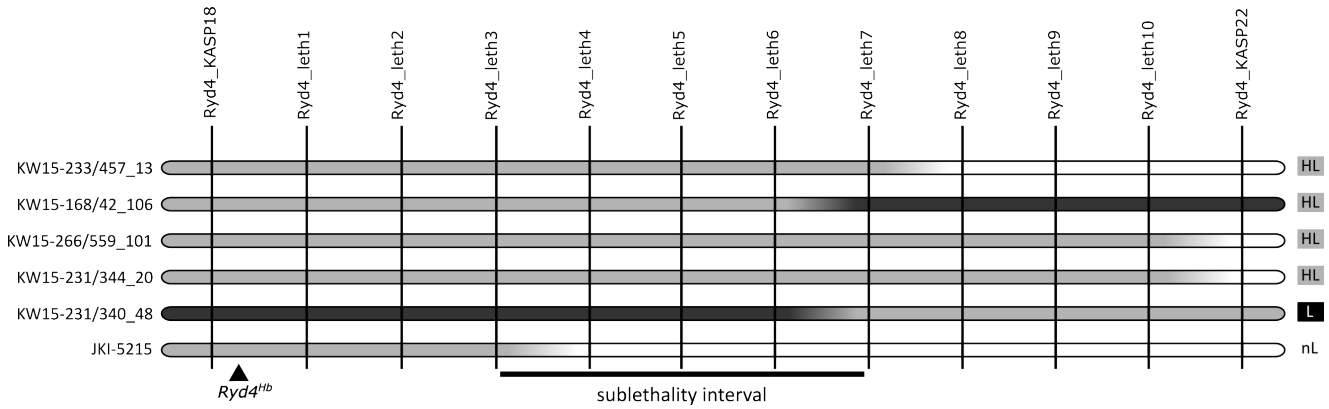


Fig. 6 Graphical representation of the genotype of lines recombining in the sublethality interval. Genotypes of KW15-233/457_13, KW15-168/41_106, KW15-266/59_101, and KW15-231/344_20 are inferred from those of 32 of their offsprings. The genotype of JKI-5215 is reconstructed from those of 24 F_2 plants of FM_Pop2 from the lineage EP_16-460. Markers are depicted as vertical black lines and genotypes as horizontal bars. White, black, and grey segments represent Hv, Hb, and heterozygotes genotypes, respectively. Phenotypes are described on the right as sublethal (L), sublethality segregating in the progeny (HL), and vital (nL). For better readability, marker positions are not to scale.

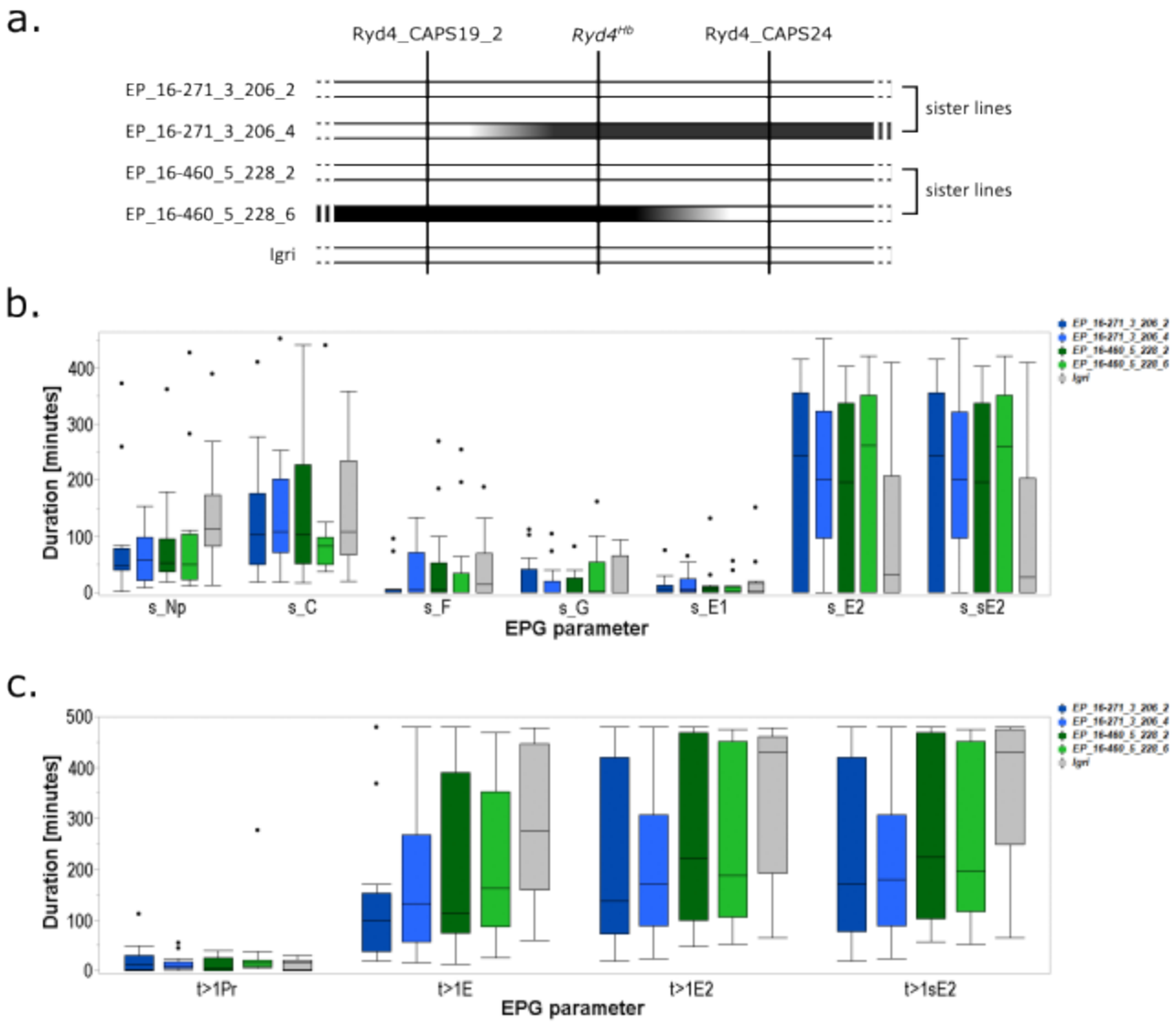
Gene name	Start	Stop	Annotation
HORVU.MOREX.r3.3HG0319200	594,288,404	594,295,050	B3 domain-containing protein
HORVU.MOREX.r3.3HG0319210	594,376,497	594,377,229	B3 domain-containing protein
HORVU.MOREX.r3.3HG0319220	594,404,110	594,405,348	Werner Syndrome-like exonuclease
HORVU.MOREX.r3.3HG0319240	594,551,247	594,553,562	B3 domain-containing protein
HORVU.MOREX.r3.3HG0319250	594,556,937	594,557,242	Ultraviolet-B-repressible protein
HORVU.MOREX.r3.3HG0319270	594,615,615	594,620,408	B3 domain-containing protein
HORVU.MOREX.r3.3HG0319280	594,622,773	594,626,954	Beta-1,3-glucanase
HORVU.MOREX.r3.3HG0319300	594,699,354	594,701,563	Beta-1,3-glucanase
HORVU.MOREX.r3.3HG0319310	594,712,025	594,712,888	F-box protein
HORVU.MOREX.r3.3HG0319320	594,721,495	594,725,844	RecA
HORVU.MOREX.r3.3HG0319330	594,731,099	594,738,322	Sentrin-specific protease
HORVU.MOREX.r3.3HG0319340	594,744,042	594,747,817	voltage-dependent L-type calcium channel subunit
HORVU.MOREX.r3.3HG0319350	594,748,418	594,749,859	Annexin
HORVU.MOREX.r3.3HG0319360	594,764,653	594,766,566	Amino acid permease
HORVU.MOREX.r3.3HG0319370	594,772,628	594,783,366	Acetyl-CoA carboxylase

Table 1 High-confidence genes annotated in the sublethality interval on MorexV3 reference genome. Coordinates refer to chromosome 3H.

Ryd4^{Hb} does not prevent aphid feeding

Resistance to insect-transmitted viruses can either be a direct resistance to the virus or an indirect one, through resistance to the vector. To test if *Ryd4^{Hb}* provides resistance to its aphid vector, we monitored the feeding of 12 to 16 aphids by EPG on five lines: two susceptible F_4 lines (EP_16-271_3_206_2 and EP_16_460_5_228_2), their two resistant sister lines (EP_16-271_3_206_4 and EP_16_460_5_228_6) and the susceptible barley cultivar 'Igri' which was the susceptible parent of LM-Pop and JKI-5215 (Figure 7a). As none of the selected EPG parameters showed a normal distribution according to a Shapiro-Wilk test with a p-value threshold of 0.05, we selected the Kruskal-Wallis test for multiple comparison. No significant differences between the lines were observed for the selected parameters s_Np (χ^2=6.78, df=4, p=0.148), s_C (χ^2=2.35, df=4, p=0.671), s_F (χ^2=4.64, df=4, p=0.327), s_G (χ^2=2.96, df=4, p=0.565), s_E1 (χ^2=1.35, df=4, p=0.854), s_E2 (χ^2=3.16, df=4, p=0.534) and s_sE2 (χ^2=3.52, df=4, p=0.474) (Figure 7b,

400 supplementary table 9). The most divergent line was Igri, with an increased median duration for s_Np and
401 decreased median durations for s_E2 and s_sE2, probably due to differences in the genetic background with
402 the other lines.



403 **Fig. 7** Aphid feeding behaviour on resistant and susceptible lines **a.** Graphical representation of the lines
404 used to test the aphid feeding behaviour on resistant and susceptible lines. Loci are depicted as vertical black
405 lines and genotypes as horizontal bars. White and black, segments represent Hv and Hb genotypes,
406 respectively. **b.** Duration of the different feeding events on the five lines summed up by type. s_Np: total
407 duration of all non-probing events. s_C: total duration of pathway phase. s_F: total duration of penetration
408 problems. s_G: total duration of xylem drinking. s_E1: total duration of secretion of watery saliva. s_E2:
409 total duration of phloem sap ingestion. s_sE2: total duration of sustained phloem sap ingestion. **c.** Duration
410 ... Lines in the box plots indicate the median, and whiskers show the upper and lower 1,5xIQR (interquartile
411 range) with dots indicating ouliers.
412
413 In addition to these general parameters, parameters associated with epidermis (t>1Pr) and mesophyll located
414 (t>1E) but also sieve element located (t>1E2, t>1sE2) defense responses were inspected. Igri showed the

415 longest median durations for reaching the sieve elements (t>1E) and to reach ingestion (t>1E2 and t>1sE2).
416 However, none of the parameters t>1Pr (χ^2=3.83, df=4, p=0.43), t>1E (χ^2=6.32, df=4, p=0.176), t>1E2
417 (χ^2=3.43, df=4, p=0.489) and t>sE2 (χ^2=5.42, df=4, p=0.247) differs significantly between the tested lines
418 (Figure 7c, supplementary table 9).

419 **Discussion**

420 BYDV is a major threat to barley cultivation that is expected to increase in the following years, as autumns
421 become longer and warmer in Northern Europe (Roos et al., 2011; Trebicki, 2020), thus breeding for BYDV
422 resistance will become an even higher priority. So far only partial resistance has been discovered in the *H.*
423 *vulgare* primary genepool (Baltenberger et al., 1987; Collins et al., 1996; Lüpken et al., 2014; Niks et al.,
424 2004; Schaller et al., 1964; Suneson, 1955). Here, we report the high-resolution mapping of *Ryd4*^{Hb}, the first
425 resistance gene to BYDV in barley, originating from the wild relative and secondary genepool species *H.*
426 *bulbosum*. We mapped the gene in a 66.5 kbp-interval of the MorexV3 barley reference genome despite the
427 linkage of the resistance locus to a sublethality factor and the reduced recombination between *H. vulgare*
428 and *H. bulbosum* genomes.

429 At the *Ryd4*^{Hb} locus, four genes are annotated with high confidence on the MorexV3 genome, including two
430 genes from the CNL family, one pseudogene, and one likely to be functional. CNL genes are part of the
431 larger NLR family which are the most common class of resistance genes to biotic stress. They are coding
432 for intracellular proteins that form complexes (Wang et al., 2019) recognizing, directly or indirectly,
433 pathogen effector molecules and inducing local cell death in response. More than 30 NLR genes conferring
434 resistance to viruses have been cloned so far (Boualem et al., 2016; Sett et al., 2022) and more are candidates.
435 An *H. bulbosum* homolog of *Ryd4_pCNL1* or *Ryd4_CNL2* is therefore a very likely candidate for *Ryd4*^{Hb}.
436 The *Ryd4*^{Hb} locus is orthologous to the *Sr35* resistance locus from the wheat wild relative *Triticum*
437 *monococcum* (Saintenac et al., 2013). *Sr35* is coding for a CNL protein that shares 83% identity with the
438 translated *H. vulgare* sequence of *Ryd4_CNL2* and provides resistance to the fungal pathogen *Puccinia*
439 *graminis* f. sp. *tritici* causing wheat stem rust. This interval was also identified as candidate for the *Rph13*
440 resistance gene to leaf rust in the *H. vulgare* spp. *spontaneum* accession 'PI 531849' (Jost et al., 2020), and,
441 overlaps with the large interval of the *Jmv2* resistance gene to the *Japanese soil-borne wheat mosaic virus*
442 from the barley cultivar 'Sukai Golden' (Okada et al., 2022). Interestingly, the best homolog of *Sr35* in rice
443 is LOC_Os11g43700, which was identified as a resistance gene to the *Rice yellow mottle virus* in the African
444 rice species *Oryza glaberrima* (Pidon et al., 2017; Bonnamy et al., 2023). It is not rare that closely related
445 NLRs provide resistance to different classes of pathogens. A good example is the potato NLRs genes GPA2
446 and RX1 which provide resistance against the nematode *Globodera pallida* and potato virus X, respectively,
447 and share 88% of their amino acid sequence (Van Der Vossen et al., 2000). The comparison of the *Ryd4*^{Hb}
448 interval in the barley pangenome (Jayakodi et al., 2020) demonstrated a very large diversity at this locus,
449 including NLR duplications. NLRs genes are indeed frequently under diversifying selection and tend to
450 evolve and duplicate by interallelic recombination between orthologs and by unequal crossing-over (Baggs
451 et al., 2017; Chen et al., 2010; Ding et al., 2007; Guo et al., 2011; Li et al., 2010; Michelmore & Meyers,
452 1998; Zhou et al., 2004). The *Sr35/Ryd4*^{Hb} locus is one of those very diverse and dynamic loci that could be
453 described as R gene factories. Together with the homology with other resistance loci, this locus' NLR
454 diversity pleads for *Ryd4*^{Hb} to be a CNL. However, in addition to the CNL genes, an *H. bulbosum* ortholog
455 of the *Ryd4_ANK* could also be a good candidate. Indeed, the structure of the encoded protein is close to the
456 one of *Arabidopsis* ACCELERATED CELL DEATH 6 (ACD6) protein. ACD6 confers enhanced resistance
457 to bacterial pathogens, including *Pseudomonas syringae*, by increasing the level of salicylic acid and

458 inducing spontaneous cell death (Rate et al., 1999; Dong, 2004; H. Lu et al., 2003, 2005). We also cannot
459 exclude that *Ryd4*^{Hb} resistance is due to presence/absence variation of a gene in the primary and the
460 secondary genepool of barley, thus the resistance gene from *H. bulbosum* may have no ortholog in the *H.*
461 *vulgare* interval. Cloning of *Ryd4*^{Hb} would therefore most likely require a *de novo* genome assembly of the
462 *Ryd4*^{Hb} interval in a resistant genotype (introgression line of *H. vulgare* or resistance donor genotype of
463 *H.bulbosum*).

464 Resistance to insect-transmitted viruses, like the one provided by *Ryd4*^{Hb}, can either be a direct resistance
465 to the virus or a resistance to the vector, which would in effect prevent infection and therefore provide
466 indirect virus resistance. The melon NLR *Vat* resistance gene is the model of this indirect resistance. VAT
467 provides resistance to *Aphis gossypii* and to all the viruses it transmits tested so far, including the *Cucumber*
468 *mosaic virus* (Boissot et al., 2016). It recognizes an effector from *A. gossypii* and triggers the hypersensitive
469 response, stopping at the same time any viral infection that may have occurred. BYDV cannot be inoculated
470 to barley mechanically, so only resistance to the aphid vector was tested. A previous study showed that *R.*
471 *padi* aphids were feeding less and having a shorter salivation time on the *Ryd4*^{Hb} *H. bulbosum* resistance
472 donor A17 compared to the BYDV-susceptible *H. bulbosum* line A21, suggesting that this could be the
473 reason for A17 BYDV resistance (Schliephake et al., 2013). However, our study showed no differences in
474 aphid feeding patterns on closely related resistant and susceptible lines, accompanied by an absence of
475 BYDV infection in the resistant lines, suggesting that the preliminary observation on the *H. bulbosum* donor
476 was probably due to A17 genetic background rather than *Ryd4*^{Hb}. *Ryd4*^{Hb}, therefore, provides direct
477 resistance to BYDV.

478 *Ryd4*^{Hb} is a prime example of the importance of crop wild relatives serving as genetic resources and gene
479 donors in breeding schemes to achieve efficient and durable disease resistance. The advantage of using a
480 crop wild relative in prebreeding schemes as a unique source of resistance, however, comes at a cost:
481 reduced frequency of recombination and/or hybrid incompatibility leading to fertility or lethality problems.
482 In the case of the *Ryd4*^{Hb} locus, recombination is 7.5 times less than in the intraspecific barley cross used to
483 map *Rph13*. To fine map the gene despite this handicap, we screened very large mapping populations with
484 high throughput genotyping technologies. At the *Ryd4*^{Hb} locus, this negative linkage drag was strongly
485 materialized by a sublethality factor characterized by the reduced growth and early death of introgression
486 lines carrying the Hb allele at homozygous state at this locus. By screening a large number of plants for
487 recombination, we managed to break the linkage, producing a resistance donor without the sublethality
488 factor, that could be included in breeding schemes. We mapped the sublethality factor to a 600 kbp interval
489 on MorexV3 genome. The observed phenotype suggested that sublethal plants are possibly lacking an
490 essential gene, or a few genes, for development, therefore that one of the genes of the *H. vulgare* interval
491 has no ortholog in the donor *H. bulbosum* genome. Among the 15 genes annotated with high confidence in
492 the interval, B3 domain-containing proteins are part of a large transcription factor superfamily whose
493 members are playing key roles in various stages of plant development, from embryogenesis to seed
494 maturation (Swaminathan et al., 2008). F-box containing proteins are central part of the ubiquitin–26S
495 proteasome system and are thus key for different processes like phytohormone signaling, plant development,
496 cell cycle, or self-incompatibility (Stefanowicz et al., 2015). RecA proteins are maintaining DNA integrity
497 during meiosis by initiating double-strand break repair (Emmenecker et al., 2023). Annexins are widely
498 involved in regulating plant processes, from growth and development to responses to stresses (Wu et al.,
499 2022). One of the corresponding genes in the MorexV3 interval could be missing in the A17 haplotype and
500 explain the observed phenotype.

501 The results of this study would be helpful to breed barley varieties with an effective resistance to BYDV.
502 We identified recombinants with a strongly reduced *H. bulbosum* fragment that can be used in breeding
503 schemes, removing almost completely the negative linkage drag. The markers closely linked to the
504 resistance can then be used in marker-assisted and genomic selection, postponing the tedious resistance
505 evaluation to the last breeding step. Knowing that $Ryd4^{Hb}$ is a direct resistance gene to BYDV would also
506 make it possible to establish the best strategy to avoid resistance breaking. Such a strategy could be
507 pyramiding it with partial resistance or tolerance sources like *Ryd2* and *Ryd3*. Such resistant varieties would
508 make a major contribution to sustainable barley cultivation.

509

510 **Supplementary figure 1** Comparison of $Ryd4^{Hb}$ in *H. vulgare* pangenome modify from Easyfig 2.2.5
511 output. Only fragments with more than 80% nucleotide identity are shown.

512

513 **References**

514 Ali, M., Anwar, S., Shuja, M. N., Tripathi, R. K., & Singh, J. (2018). The genus Luteovirus from infection
515 to disease. *European Journal of Plant Pathology*, *151*(4), 841-860. https://doi.org/10.1007/s10658-
516 018-1425-8

517 Baggs, E., Dagdas, G., & Krasileva, K. (2017). NLR diversity, helpers and integrated domains : Making
518 sense of the NLR IDentity. *Current Opinion in Plant Biology*, *38*, 59-67.
519 https://doi.org/10.1016/j.pbi.2017.04.012

520 Baltenberger, D. E., Ohm, H. W., & Foster, J. E. (1987). Reactions of Oat, Barley, and Wheat to Infection
521 with Barley Yellow Dwarf Virus Isolates1. *Crop Science*, *27*(2),
522 cropsci1987.0011183X002700020010x.
523 https://doi.org/10.2135/cropsci1987.0011183X002700020010x

524 Beoni, E., Chrpová, J., Jarošová, J., & Kundu, J. K. (2016). Survey of Barley yellow dwarf virus incidence
525 in winter cereal crops, and assessment of wheat and barley resistance to the virus. *Crop and Pasture*
526 *Science*, *67*(10), 1054-1063.

527 Boeckmann, B., Bairoch, A., Apweiler, R., Blatter, M.-C., Estreicher, A., Gasteiger, E., Martin, M. J.,
528 Michoud, K., O'Donovan, C., Phan, I., Pilbout, S., & Schneider, M. (2003). The SWISS-PROT
529 protein knowledgebase and its supplement TrEMBL in 2003. *Nucleic Acids Research*, *31*(1),
530 365-370. https://doi.org/10.1093/nar/gkg095

531 Boissot, N., Thomas, S., Chovelon, V., & Lecoq, H. (2016). NBS-LRR-mediated resistance triggered by
532 aphids : Viruses do not adapt; aphids adapt via different mechanisms. *BMC Plant Biology*, *16*(1),
533 25. https://doi.org/10.1186/s12870-016-0708-5
534 Bonnamy, M., Pinel-Galzi, A., Gorgues, L., Chalvon, V., Hébrard, E., Chéron, S., Nguyen, T. H., Poulicard,
535 N., Sabot, F., Pidon, H., Champion, A., Césari, S., Kroj, T., & Albar, L. (2023). Rapid evolution of
536 an RNA virus to escape recognition by a rice nucleotide-binding and leucine-rich repeat domain
537 immune receptor. *New Phytologist*, *237*(3), 900-913. https://doi.org/10.1111/nph.18532
538 Boualem, A., Dogimont, C., & Bendahmane, A. (2016). The battle for survival between viruses and their
539 host plants. *Current Opinion in Virology*, *17*, 32-38. https://doi.org/10.1016/j.coviro.2015.12.001
540 Braun, E.-M., Tsvetkova, N., Rotter, B., Siekmann, D., Schwefel, K., Krezdorn, N., Plieske, J., Winter, P.,
541 Melz, G., Voylokov, A. V., & Hackauf, B. (2019). Gene Expression Profiling and Fine Mapping
542 Identifies a Gibberellin 2-Oxidase Gene Co-segregating With the Dominant Dwarfing Gene Ddw1
543 in Rye (Secale cereale L.). *Frontiers in Plant Science*, *10*.
544 https://www.frontiersin.org/articles/10.3389/fpls.2019.00857
545 Budowle, B., Chakraborty, R., Giusti, A. M., Eisenberg, A. J., & Allen, R. C. (1991). Analysis of the VNTR
546 locus D1S80 by the PCR followed by high-resolution PAGE. *American Journal of Human Genetics*,
547 *48*(1), 137-144.
548 Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009).
549 BLAST+ : Architecture and applications. *BMC Bioinformatics*, *10*(1), 421.
550 https://doi.org/10.1186/1471-2105-10-421
551 Chen, Q., Han, Z., Jiang, H., Tian, D., & Yang, S. (2010). Strong Positive Selection Drives Rapid
552 Diversification of R-Genes in Arabidopsis Relatives. *Journal of Molecular Evolution*, *70*(2),
553 137-148. https://doi.org/10.1007/s00239-009-9316-4
554 Clark, M. F., & Adams, A. N. (1977). Characteristics of the Microplate Method of Enzyme-Linked
555 Immunosorbent Assay for the Detection of Plant Viruses. *Journal of General Virology*, *34*(3),
556 475-483. https://doi.org/10.1099/0022-1317-34-3-475

557 Close, T. J., Bhat, P. R., Lonardi, S., Wu, Y., Rostoks, N., Ramsay, L., Druka, A., Stein, N., Svensson, J.
558 T., Wanamaker, S., Bozdag, S., Roose, M. L., Moscou, M. J., Chao, S., Varshney, R. K., Szűcs, P.,
559 Sato, K., Hayes, P. M., Matthews, D. E., … Waugh, R. (2009). Development and implementation
560 of high-throughput SNP genotyping in barley. *BMC Genomics*, *10*(1), 582.
561 https://doi.org/10.1186/1471-2164-10-582
562 Collins, N. C., Paltridge, N. G., Ford, C. M., & Symons, R. H. (1996). The Yd2 gene for barley yellow
563 dwarf virus resistance maps close to the centromere on the long arm of barley chromosome 3.
564 *Theoretical and Applied Genetics*, *92*(7), 858-864. https://doi.org/10.1007/BF00221898
565 Dedryver, C.-A., Le Ralec, A., & Fabre, F. (2010). The conflicting relationships between aphids and men :
566 A review of aphid damage and control strategies. *Comptes Rendus Biologies*, *333*(6), 539-553.
567 https://doi.org/10.1016/j.crvi.2010.03.009
568 Ding, J., Cheng, H., Jin, X., Araki, H., Yang, Y., & Tian, D. (2007). Contrasting patterns of evolution
569 between allelic groups at a single locus in Arabidopsis. *Genetica*, *129*(3), 235-242.
570 https://doi.org/10.1007/s10709-006-0002-9
571 Dong, X. (2004). The Role of Membrane-Bound Ankyrin-Repeat Protein ACD6 in Programmed Cell Death
572 and Plant Defense. *Science's STKE*, *2004*(221), pe6-pe6. https://doi.org/10.1126/stke.2212004pe6
573 Emmenecker, C., Mézard, C., & Kumar, R. (2023). Repair of DNA double-strand breaks in plant meiosis :
574 Role of eukaryotic RecA recombinases and their modulators. *Plant Reproduction*, *36*(1), 17-41.
575 https://doi.org/10.1007/s00497-022-00443-6
576 Goya, R., Sun, M. G. F., Morin, R. D., Leung, G., Ha, G., Wiegand, K. C., Senz, J., Crisan, A., Marra, M.
577 A., Hirst, M., Huntsman, D., Murphy, K. P., Aparicio, S., & Shah, S. P. (2010). SNVMix :
578 Predicting single nucleotide variants from next-generation sequencing of tumors. *Bioinformatics*,
579 *26*(6), 730-736. https://doi.org/10.1093/bioinformatics/btq040
580 Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., Adiconis, X., Fan, L.,
581 Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma,
582 F., Birren, B. W., Nusbaum, C., Lindblad-Toh, K., … Regev, A. (2011). Trinity : Reconstructing a

full-length transcriptome without a genome from RNA-Seq data. *Nature biotechnology*, *29*(7), 644-652. https://doi.org/10.1038/nbt.1883

Guo, Y.-L., Fitz, J., Schneeberger, K., Ossowski, S., Cao, J., & Weigel, D. (2011). Genome-Wide Comparison of Nucleotide-Binding Site-Leucine-Rich Repeat-Encoding Genes in Arabidopsis. *Plant Physiology*, *157*(2), 757-769. https://doi.org/10.1104/pp.111.181990

Habekuß, A., Schliephake, E., & Ehrig, F. (2004). Hordeum bulbosum—A source for BYDV resistance. *Proceedings of the 9th international barley genetics symposium*, 787-791.

Hu, H., Choudhury, S., Shabala, S., Gupta, S., & Zhou, M. (2019). Genomic regions on chromosome 5H containing a novel QTL conferring barley yellow dwarf virus-PAV (BYDV-PAV) tolerance in barley. *Scientific Reports*, *9*(1), Article 1. https://doi.org/10.1038/s41598-019-47820-2

International Barley Genome Sequencing Consortium. (2012). A physical, genetic and functional sequence assembly of the barley genome. *Nature*, *491*(7426), 711-716. https://doi.org/10.1038/nature11543

Jayakodi, M., Padmarasu, S., Haberer, G., Bonthala, V. S., Gundlach, H., Monat, C., Lux, T., Kamal, N., Lang, D., Himmelbach, A., Ens, J., Zhang, X.-Q., Angessa, T. T., Zhou, G., Tan, C., Hill, C., Wang, P., Schreiber, M., Boston, L. B., … Stein, N. (2020). The barley pan-genome reveals the hidden legacy of mutation breeding. *Nature*, *588*(7837), 284-289. https://doi.org/10.1038/s41586-020-2947-8

Jost, M., Singh, D., Lagudah, E., Park, R. F., & Dracatos, P. (2020). Fine mapping of leaf rust resistance gene Rph13 from wild barley. *Theoretical and Applied Genetics*, *133*(6), 1887-1895. https://doi.org/10.1007/s00122-020-03564-6

Kern, M., Meiners, T., Schliephake, · Edgar, Habekuss, A., Ordon, · Frank, & Will, T. (2022). Infection of susceptible/tolerant barley genotypes with Barley yellow dwarf virus alters the host plant preference of Rhopalosiphum padi clones depending upon their ability to transmit BYDV. *Journal of Pest Science*, *95*, 215-229. https://doi.org/10.1007/s10340-021-01367-2

Kosova, K., Chrpová, J., & Šíp, V. (2008). Recent advances in breeding of cereals for resistance to barley yellow dwarf virus. *Czech Journal of Genetics and Plant Breeding*, *44*(1), 1-10.

609 Lassmann, T., Hayashizaki, Y., & Daub, C. O. (2009). TagDust—A program to eliminate artifacts from
610 next generation sequencing data. *Bioinformatics*, *25*(21), 2839-2840.
611 https://doi.org/10.1093/bioinformatics/btp527
612 Li, J., Ding, J., Zhang, W., Zhang, Y., Tang, P., Chen, J.-Q., Tian, D., & Yang, S. (2010). Unique
613 evolutionary pattern of numbers of gramineous NBS–LRR genes. *Molecular Genetics and*
614 *Genomics*, *283*(5), 427-438. https://doi.org/10.1007/s00438-010-0527-6
615 Lu, H., Liu, Y., & Greenberg, J. T. (2005). Structure–function analysis of the plasma membrane- localized
616 Arabidopsis defense component ACD6. *The Plant Journal*, *44*(5), 798-809.
617 https://doi.org/10.1111/j.1365-313X.2005.02567.x
618 Lu, H., Rate, D. N., Song, J. T., & Greenberg, J. T. (2003). ACD6, a Novel Ankyrin Protein, Is a Regulator
619 and an Effector of Salicylic Acid Signaling in the Arabidopsis Defense Response. *The Plant Cell*,
620 *15*(10), 2408-2420. https://doi.org/10.1105/tpc.015412
621 Lu, S., Wang, J., Chitsaz, F., Derbyshire, M. K., Geer, R. C., Gonzales, N. R., Gwadz, M., Hurwitz, D. I.,
622 Marchler, G. H., Song, J. S., Thanki, N., Yamashita, R. A., Yang, M., Zhang, D., Zheng, C.,
623 Lanczycki, C. J., & Marchler-Bauer, A. (2019). CDD/SPARCLE: the conserved domain database
624 in 2020. *Nucleic Acids Research*, *48*(D1), D265-D268. https://doi.org/10.1093/nar/gkz991
625 Lüpken, T., Stein, N., Perovic, D., Habekuß, A., Serfling, A., Krämer, I., Hähnel, U., Steuernagel, B.,
626 Scholz, U., Ariyadasa, R., Martis, M., Mayer, K., Niks, R. E., Collins, N. C., Friedt, W., & Ordon,
627 F. (2014). High-resolution mapping of the barley Ryd3 locus controlling tolerance to BYDV.
628 *Molecular Breeding*, *33*(2), 477-488. https://doi.org/10.1007/s11032-013-9966-1
629 Mascher, M., Muehlbauer, G. J., Rokhsar, D. S., Chapman, J., Schmutz, J., Barry, K., Muñoz-Amatriaín,
630 M., Close, T. J., Wise, R. P., Schulman, A. H., Himmelbach, A., Mayer, K. F. X., Scholz, U.,
631 Poland, J. A., Stein, N., & Waugh, R. (2013). Anchoring and ordering NGS contig assemblies by
632 population sequencing (POPSEQ). *The Plant Journal*, *76*(4), 718-727.
633 https://doi.org/10.1111/tpj.12319

634 Mascher, M., Wicker, T., Jenkins, J., Plott, C., Lux, T., Koh, C. S., Ens, J., Gundlach, H., Boston, L. B.,
635 Tulpová, Z., Holden, S., Hernández-Pinzón, I., Scholz, U., Mayer, K. F. X., Spannagl, M., Pozniak,
636 C. J., Sharpe, A. G., Šimková, H., Moscou, M. J., … Stein, N. (2021). Long-read sequence
637 assembly : A technical evaluation in barley. *The Plant cell*, *accepted*.
638 Michel, M. (1996). *Untersuchungen zur Übertragung von Resistenzgenen aus der Wildart Hordeam*
639 *bulbosum L. in die Kulturgerste Hordeum vulgare L. Diss., TU München, Munich, Germany*.
640 Michelmore, R. W., & Meyers, B. C. (1998). Clusters of Resistance Genes in Plants Evolve by Divergent
641 Selection and a Birth-and-Death Process. *Genome Research*, *8*(11), 1113-1130.
642 https://doi.org/10.1101/gr.8.11.1113
643 Milner, S. G., Jost, M., Taketa, S., Mazón, E. R., Himmelbach, A., Oppermann, M., Weise, S., Knüpffer,
644 H., Basterrechea, M., König, P., Schüler, D., Sharma, R., Pasam, R. K., Rutten, T., Guo, G., Xu,
645 D., Zhang, J., Herren, G., Müller, T., … Stein, N. (2019). Genebank genomics highlights the
646 diversity of a global barley collection. *Nature Genetics*, *51*(2), 319-326.
647 https://doi.org/10.1038/s41588-018-0266-x
648 Niks, R. E., Habekuß, A., Bekele, B., & Ordon, F. (2004). A novel major gene on chromosome 6H for
649 resistance of barley against the barley yellow dwarf virus. *Theoretical and Applied Genetics*, *109*(7),
650 1536-1543. https://doi.org/10.1007/s00122-004-1777-7
651 Oerke, E.-C. (2006). Crop losses to pests. *The Journal of Agricultural Science*, *144*(1), 31-43.
652 https://doi.org/DOI: 10.1017/S0021859605005708
653 Okada, K., Tanaka, T., Fukuoka, S., Oono, Y., Mishina, K., Oikawa, T., Sato, K., Kato, T., Komatsuda, T.,
654 & Namai, K. (2022). Two dominant genes in barley (Hordeum vulgare L.) complementarily encode
655 perfect resistance to Japanese soil-borne wheat mosaic virus. *Breeding Science*, *72*(5), 372-382.
656 https://doi.org/10.1270/jsbbs.22046
657 Ordon, F., Habekuss, A., Kastirr, U., Rabenstein, F., & Kühne, T. (2009). Virus resistance in cereals :
658 Sources of resistance, genetics and breeding. *Journal of Phytopathology*, *157*(9), 535-545.
659 https://doi.org/10.1111/j.1439-0434.2009.01540.x

660 Pidon, H., Ghesquière, A., Chéron, S., Issaka, S., Hébrard, E., Sabot, F., Kolade, O., Silué, D., & Albar, L.
661 (2017). Fine mapping of RYMV3 : A new resistance gene to Rice yellow mottle virus from Oryza
662 glaberrima. *Theoretical and Applied Genetics*, *130*(4), 807-818. https://doi.org/10.1007/s00122-
663 017-2853-0
664 Pidon, H., Wendler, N., Habekuβ, A., Maasberg, A., Ruge-Wehling, B., Perovic, D., Ordon, F., & Stein, N.
665 (2020). High-resolution mapping of Rym14Hb, a wild relative resistance gene to barley yellow
666 mosaic disease. *Theoretical and Applied Genetics*. https://doi.org/10.1007/s00122-020-03733-7
667 Rate, D. N., Cuenca, J. V., Bowman, G. R., Guttman, D. S., & Greenberg, J. T. (1999). The Gain-of-
668 Function Arabidopsis acd6 Mutant Reveals Novel Regulation and Function of the Salicylic Acid
669 Signaling Pathway in Controlling Cell Death, Defenses, and Cell Growth. *The Plant Cell*, *11*(9),
670 1695-1708. https://doi.org/10.1105/tpc.11.9.1695
671 Riedel, C., Habekuß, A., Schliephake, E., Niks, R., Broer, I., & Ordon, F. (2011). Pyramiding of Ryd2 and
672 Ryd3 conferring tolerance to a German isolate of Barley yellow dwarf virus-PAV (BYDV-PAV-
673 ASL-1) leads to quantitative resistance against this isolate. *Theoretical and Applied Genetics*,
674 *123*(1), 69. https://doi.org/10.1007/s00122-011-1567-y
675 Roos, J., Hopkins, R., Kvarnheden, A., & Dixelius, C. (2011). The impact of global warming on plant
676 diseases and insect vectors in Sweden. *European Journal of Plant Pathology*, *129*(1), 9-19.
677 https://doi.org/10.1007/s10658-010-9692-z
678 Rostoks, N., Borevitz, J. O., Hedley, P. E., Russell, J., Mudie, S., Morris, J., Cardle, L., Marshall, D. F., &
679 Waugh, R. (2005). Single-feature polymorphism discovery in the barley transcriptome. *Genome*
680 *Biology*, *6*(6), R54. https://doi.org/10.1186/gb-2005-6-6-r54
681 Russell, J., Mascher, M., Dawson, I. K., Kyriakidis, S., Calixto, C., Freund, F., Bayer, M., Milne, I.,
682 Marshall-Griffiths, T., Heinen, S., Hofstad, A., Sharma, R., Himmelbach, A., Knauft, M.,
683 van Zonneveld, M., Brown, J. W. S., Schmid, K., Kilian, B., Muehlbauer, G. J., … Waugh, R.
684 (2016). Exome sequencing of geographically diverse barley landraces and wild relatives gives

685 insights into environmental adaptation. *Nature Genetics*, *48*(9), 1024-1030.
686 https://doi.org/10.1038/ng.3612
687 Saintenac, C., Zhang, W., Salcedo, A., Rouse, M. N., Trick, H. N., Akhunov, E., & Dubcovsky, J. (2013).
688 Identification of Wheat Gene Sr35 That Confers Resistance to Ug99 Stem Rust Race Group.
689 *Science*, *341*(6147), 783-786. https://doi.org/10.1126/science.1239022
690 Santos, E., Benito, C., Silva-Navas, J., Gallego, F. J., Figueiras, A. M., Pinto-Carnide, O., & Matos, M.
691 (2018). Characterization, genetic diversity, phylogenetic relationships, and expression of the
692 aluminum tolerance MATE1 gene in Secale species. *Biologia Plantarum*, *62*(1), 109-120.
693 https://doi.org/10.1007/s10535-017-0749-0
694 Schaller, C. W., Qualset, C. O., & Rutger, J. N. (1964). Inheritance and Linkage of the Yd2 Gene
695 Conditioning Resistance to the Barley Yellow Dwarf Virus Disease in Barley1. *Crop Science*, *4*(5),
696 cropsci1964.0011183X000400050034x.
697 https://doi.org/10.2135/cropsci1964.0011183X000400050034x
698 Schliephake, E., Habekuss, A., Scholz, M., & Ordon, F. (2013). Barley yellow dwarf virus transmission and
699 feeding behaviour of Rhopalosiphum padi on Hordeum bulbosum clones. *Entomologia*
700 *Experimentalis et Applicata*, *146*(3), 347-356. https://doi.org/10.1111/eea.12033
701 Scholz, M., Ruge-Wehling, B., Habekuß, A., Schrader, O., Pendinen, G., Fischer, K., & Wehling, P. (2009).
702 Ryd4Hb : A novel resistance gene introgressed from Hordeumbulbosum into barley and conferring
703 complete and dominant resistance to the barley yellow dwarf virus. *Theoretical and Applied*
704 *Genetics*, *119*(5), 837-849. https://doi.org/10.1007/s00122-009-1093-3
705 Sett, S., Prasad, A., & Prasad, M. (2022). Resistance genes on the verge of plant–virus interaction. *Trends*
706 *in Plant Science*, *27*(12), 1242-1252. https://doi.org/10.1016/j.tplants.2022.07.003
707 Stefanowicz, K., Lannoo, N., & Van Damme, E. J. M. (2015). Plant F-box Proteins – Judges between Life
708 and Death. *Critical Reviews in Plant Sciences*, *34*(6), 523-552.
709 https://doi.org/10.1080/07352689.2015.1024566

710 Stein, N., Herren, G., & Keller, B. (2001). A new DNA extraction method for high-throughput marker
711 analysis in a large-genome species such as Triticum aestivum. *Plant Breeding*, *120*(4), 354-356.
712 https://doi.org/10.1046/j.1439-0523.2001.00615.x
713 Stein, N., Prasad, M., Scholz, U., Thiel, T., Zhang, H., Wolf, M., Kota, R., Varshney, R. K., Perovic, D.,
714 Grosse, I., & Graner, A. (2007). A 1,000-loci transcript map of the barley genome : New anchoring
715 points for integrative grass genomics. *Theoretical and Applied Genetics*, *114*(5), 823-839.
716 https://doi.org/10.1007/s00122-006-0480-2
717 Steuernagel, B., Witek, K., Krattinger, S. G., Ramirez-Gonzalez, R. H., Schoonbeek, H., Yu, G., Baggs, E.,
718 Witek, A. I., Yadav, I., Krasileva, K. V., Jones, J. D. G., Uauy, C., Keller, B., Ridout, C. J., & Wulff,
719 B. B. H. (2020). The NLR-Annotator Tool Enables Annotation of the Intracellular Immune
720 Receptor Repertoire1 [OPEN]. *Plant Physiology*, *183*(2), 468-482.
721 https://doi.org/10.1104/pp.19.01273
722 Sullivan, M. J., Petty, N. K., & Beatson, S. A. (2011). Easyfig : A genome comparison visualizer.
723 *Bioinformatics (Oxford, England)*, *27*(7), 1009-1010. https://doi.org/10.1093/bioinformatics/btr039
724 Suneson, C. A. (1955). Breeding for Resistance to Yellow Dwarf Virus in Barley. *Agronomy Journal*, *47*(6),
725 283-283. https://doi.org/10.2134/agronj1955.00021962004700060014x
726 Swaminathan, K., Peterson, K., & Jack, T. (2008). The plant B3 superfamily. *Trends in Plant Science*,
727 *13*(12), 647-655. https://doi.org/10.1016/j.tplants.2008.09.006
728 Szűcs, P., Blake, V. C., Bhat, P. R., Chao, S., Close, T. J., Cuesta-Marcos, A., Muehlbauer, G. J., Ramsay,
729 L., Waugh, R., & Hayes, P. M. (2009). An Integrated Resource for Barley Linkage Map and Malting
730 Quality QTL Alignment. *The Plant Genome*, *2*(2).
731 https://doi.org/10.3835/plantgenome2008.01.0005
732 Thiel, T., Kota, R., Grosse, I., Stein, N., & Graner, A. (2004). SNP2CAPS: a SNP and INDEL analysis tool
733 for CAPS marker development. *Nucleic Acids Research*, *32*(1), e5-e5.
734 https://doi.org/10.1093/nar/gnh006

735 Thiel, T., Michalek, W., Varshney, R., & Graner, A. (2003). Exploiting EST databases for the development
736 and characterization of gene-derived SSR-markers in barley (Hordeum vulgare L.). *Theoretical and*
737 *Applied Genetics*, *106*(3), 411-422. https://doi.org/10.1007/s00122-002-1031-0
738 Tjallingii, W. F. (1978). Electronic Recording of Penetration Behaviour by Aphids. *Entomologia*
739 *Experimentalis et Applicata*, *24*(3), 721-730. https://doi.org/10.1111/j.1570-7458.1978.tb02836.x
740 Tjallingii, W. F., & Esch, Th. H. (1993). Fine structure of aphid stylet routes in plant tissues in correlation
741 with EPG signals. *Physiological Entomology*, *18*(3), 317-328. https://doi.org/10.1111/j.1365-
742 3032.1993.tb00604.x
743 Toojinda, T., Broers, L. H., Chen, X. M., Hayes, P. M., Kleinhofs, A., Korte, J., Kudrna, D., Leung, H.,
744 Line, R. F., Powell, W., Ramsay, L., Vivar, H., & Waugh, R. (2000). Mapping quantitative and
745 qualitative disease resistance genes in a doubled haploid population of barley (Hordeum vulgare).
746 *Theoretical and Applied Genetics*, *101*(4), 580-589. https://doi.org/10.1007/s001220051519
747 Trebicki, P. (2020). Climate change and plant virus epidemiology. *Virus Research*, *286*, 198059.
748 https://doi.org/10.1016/j.virusres.2020.198059
749 Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012).
750 Primer3—New capabilities and interfaces. *Nucleic Acids Research*, *40*(15), e115.
751 https://doi.org/10.1093/nar/gks596
752 Van Der Vossen, E. A. G., Van Der Voort, J. N. A. M. R., Kanyuka, K., Bendahmane, A., Sandbrink, H.,
753 Baulcombe, D. C., Bakker, J., Stiekema, W. J., & Klein-Lankhorst, R. M. (2000). Homologues of
754 a single resistance-gene cluster in potato confer resistance to distinct pathogens : A virus and a
755 nematode. *The Plant Journal*, *23*(5), 567-576. https://doi.org/10.1046/j.1365-313x.2000.00814.x
756 Van Ooijen, J. W. (2006). JoinMap 4 : Software for the calculation of genetic linkage maps in experimental
757 populations of diploid species. *Wageningen, Netherlands: Plant Research International BV and*
758 *Kayazma BV*.
759 Varshney, R. K., Marcel, T. C., Ramsay, L., Russell, J., Röder, M. S., Stein, N., Waugh, R., Langridge, P.,
760 Niks, R. E., & Graner, A. (2007). A high density barley microsatellite consensus map with 775 SSR

761 loci. *Theoretical and Applied Genetics*, *114*(6), 1091-1103. https://doi.org/10.1007/s00122-007-
762 0503-7
763 Vincze, T., Posfai, J., & Roberts, R. J. (2003). NEBcutter : A program to cleave DNA with restriction
764 enzymes. *Nucleic Acids Research*, *31*(13), 3688-3691. https://doi.org/10.1093/nar/gkg526
765 Voorrips, R. E. (2002). MapChart : Software for the Graphical Presentation of Linkage Maps and QTLs.
766 *Journal of Heredity*, *93*(1), 77-78. https://doi.org/10.1093/jhered/93.1.77
767 Wang, J., Hu, M., Wang, J., Qi, J., Han, Z., Wang, G., Qi, Y., Wang, H.-W., Zhou, J.-M., & Chai, J. (2019).
768 Reconstitution and structure of a plant NLR resistosome conferring immunity. *Science*, *364*(6435),
769 eaav5870. https://doi.org/10.1126/science.aav5870
770 Wendler, N., Mascher, M., Himmelbach, A., Johnston, P., Pickering, R., & Stein, N. (2015). Bulbosum to
771 Go : A Toolbox to Utilize Hordeum vulgare/bulbosum Introgressions for Breeding and Beyond.
772 *Molecular Plant*, *8*(10), 1507-1519. https://doi.org/10.1016/j.molp.2015.05.004
773 Wendler, N., Mascher, M., Nöh, C., Himmelbach, A., Scholz, U., Ruge-Wehling, B., & Stein, N. (2014).
774 Unlocking the secondary gene-pool of barley with next-generation sequencing. *Plant Biotechnology*
775 *Journal*, *12*(8), 1122-1131. https://doi.org/10.1111/pbi.12219
776 Wu, X., Wang, Y., Bian, Y., Ren, Y., Xu, X., Zhou, F., & Ding, H. (2022). A critical review on plant
777 annexin : Structure, function, and mechanism. *Plant Physiology and Biochemistry*, *190*, 81-89.
778 https://doi.org/10.1016/j.plaphy.2022.08.019
779 Zhou, T., Wang, Y., Chen, J.-Q., Araki, H., Jing, Z., Jiang, K., Shen, J., & Tian, D. (2004). Genome-wide
780 identification of NBS genes in japonica rice reveals significant expansion of divergent non-TIR
781 NBS-LRR genes. *Molecular Genetics and Genomics*, *271*(4), 402-415.
782 https://doi.org/10.1007/s00438-004-0990-z
783