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Evolution of wheat blast resistance gene *Rmg8* accompanied by differentiation of variants recognizing the powdery mildew fungus

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Abstract

Wheat blast, a devastating disease having spread recently from South America to Asia and Africa, is caused by *Pyricularia oryzae* (syn. *Magnaporthe oryzae*) pathotype *Triticum*, which first emerged in Brazil in 1985. *Rmg8* and *Rmg7*, genes for resistance to wheat blast found in common wheat and tetraploid wheat, respectively, recognize the same avirulence gene, *AVR-Rmg8*. Here, we show that an ancestral resistance gene, which had obtained an ability to recognize *AVR-Rmg8* before the differentiation of *Triticum* and *Aegilops*, has expanded its target pathogens. Molecular cloning revealed that *Rmg7* was an allele of *Pm4*, a gene for resistance to wheat powdery mildew on 2AL, whereas *Rmg8* was its homoeolog on 2BL ineffective against wheat powdery mildew. *Rmg8* variants with the ability to recognize *AVR-Rmg8* were distributed not only in *Triticum* spp. but also in *Aegilops speltoides*, *Ae. umbellulata,* and *Ae. comosa*. This result suggests that the origin of resistance gene(s) recognizing *AVR-Rmg8* dates back to the time before differentiation of A, B, S, U, and M genomes, that is, ~5 million years before the emergence of its current target, the wheat blast fungus. Phylogenetic analyses suggested that, in the evolutionary process thereafter, some of their variants gained the ability to recognize the wheat powdery mildew fungus and evolved into genes controlling dual resistance to wheat powdery mildew and wheat blast.

Introduction

Wheat cultivation is now threatened by an expanding pandemic disease – wheat blast[1]. Its causal agent is a subgroup of a hemibiotrophic fungus, *Pyricularia oryzae* (syn. *Magnaporthe oryzae*) pathotype *Triticum* (MoT)[2], which is specifically pathogenic on the genus *Triticum*[3]. MoT initially emerged in Brazil in 1985[4] through a host jump of *P. oryzae* pathotype *Lolium* (MoL) or its relatives[5]. It subsequently spread to neighboring countries such as Bolivia, Paraguay, and Argentina, becoming one of the most severe wheat diseases in South America. Recently, it spread to Asia and Africa, and caused severe outbreaks of wheat blast in Bangladesh (in 2016)[6-8] and Zambia (in 2018)[9]. Molecular analyses of isolates collected in these countries suggested that the outbreaks in Bangladesh and Zambia were caused by a lineage which spread from South America to Asia and Africa through independent introductions[1]. To control this devastating disease, we need resistance genes effective against MoT. The only genetic resource currently used in farmer's field against MoT is a 2NS chromosomal segment[10] introduced from *Aegilops ventricosa*[11]. However, the resistance gene on this segment has not been identified. Furthermore, the 2NS resistance has already been overcome by new MoT strains in South America[10,12].

Genes for resistance to MoT have been considered to be rarely found in the current wheat population because MoT is a new pathogen which emerged only ~40 years ago; most of current wheat accessions have not been exposed to the attack or infection pressures by MoT. However, Tagle et al.[13] identified a resistance gene in cultivated emmer wheat and designated it as *Rmg7*. Anh et al.[14] identified another resistance gene in common wheat cultivar S-615 and designated it as *Rmg8*. *Rmg7* and *Rmg8* conferred

complete resistance in an appropriate condition, and were located on distal ends of the long arms of chromosome 2A (2AL) and 2B (2BL), respectively[14]. In addition, these genes corresponded to the same avirulence gene, *AVR-Rmg8*[15]. These results suggested that they might be homoeologous genes derived from the same ancestral gene[15]. To be useful in farmer's fields, wheat blast resistance genes must be effective even at high temperature because the optimal temperature range for this disease may be 25-30°C[2]. *Rmg8* was effective at high temperature but *Rmg7* was not[15], suggesting that *Rmg8* is the only one wheat blast resistance gene identified so far as a major gene which may be useful in fields.

To find additional resistance genes, Wang et al.[16] screened 520 local landraces of common wheat collected from various countries over the world, and found 18 accessions resistant to MoT. We initially expected that several resistance genes might be found in these resistant accessions. However, all of these accessions recognized *AVR-Rmg8*, which led us to infer that they are all *Rmg8* carriers although one of them had an additional gene tentatively designated as *RmgGR119*[16]. These resistant accessions had been collected in Europe and Middle East between 1924 and 1971 (before the emergence of MoT), and should not have had interactions with MoT. Why have these accessions in Europe and Middle East maintained *Rmg8*, a gene for resistance to MoT? In the present study we isolated *Rmg8* and *Rmg7*, and found that they are a homoeolog and an allele, respectively, of *Pm4*, a gene for resistance to wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*, Bgt). In addition, we found their functional variants in *Aegilops speltoides*, *Ae. umbellulata*, and *Ae. comosa*, suggesting that the origin of resistance genes recognizing *AVR-Rmg8* dates back to the time before the differentiation of *Triticum* and *Aegilops*, that is, ~5 million years before the

emergence of its target pathogen, MoT. Based on these results, we present a model of evolutionary processes in which a resistance gene has gained new target pathogens through differentiation of variants.

Results

Rmg8 is a homoeolog of *Pm4* controlling the resistance to Bgt

To isolate *Rmg8* from common wheat, the resistant cultivar S-615 carrying *Rmg8* was crossed with a susceptible cultivar, Shin-chunaga (Sch), resulting in 165 $F_{2:3}$ lines. When inoculated with MoT isolate Br48, homozygous resistant, segregating, and homozygous susceptible lines segregated in a 1:2:1: ratio (45:87:33) as expected. Molecular markers (KM markers) for mapping were produced using high confidence genes on 2BL found in the whole genome sequence of cv. Chinese Spring in the database (International Wheat Genome Sequencing Consortium; IWGSC). Mapping with the KM markers delimited the candidate region to ~12Mb between KM25 and the telomere (Fig. 1a). However, we could not narrow down the candidate region further because all markers produced on its 8.4Mb distal region co-segregated with the *Rmg8* phenotype (Fig. 1a).

We then adopted the RaIDeN method developed by Shimizu et al.[17] with some modifications. Briefly, RNA-seq reads obtained from primary leaves of Sch and nine $F_{2:3}$ lines with homozygous susceptible genotypes were aligned to a reference sequence of a gene set which was constructed by de novo assembly of RNA-seq reads obtained from S-615 leaves (Extended Data Fig. 1a). We selected genes (i) which showed polymorphisms (presence/absence or single-nucleotide polymorphisms) between S-615

and Sch, (ii) whose Sch allele was shared by all of the nine susceptible $F_{2:3}$ lines, and (iii) which encoded NBS (nucleotide-binding site), NLR (nucleotide-binding domain and leucine-rich repeat), or RLK (receptor-like kinase). Consequently we found 10 genes that fulfilled the three requirements (Extended Data Fig. 1b). *In silico* analyses with whole genome sequences in the databases suggested that six out of the 10 genes were located on chromosome 2B of *T. durum* cv. Svevo. PCR markers designed on these 6 genes co-segregated with *Rmg8* in the $F_{2:3}$ lines derived from Sch × S-615, indicating that they are also located on 2B of common wheat. Finally, these candidate genes were subjected to an association analysis using 20 common wheat lines recognizing *AVR-Rmg8* (S-615, the 18 local landraces mentioned above, and GR341, an additional local landrace which proved to recognize *AVR-Rmg8*) and 20 common wheat cultivars that did not recognize *AVR-Rmg8*. One candidate, the Can-I gene amplified with PCR marker KM171, showed a perfect association with the *Rmg8* phenotype (Fig. 1b) whereas the others did not (Extended Data Fig. 1b). From these results, we assumed that the Can-I gene might be *Rmg8*. Intriguingly, the Can-I transcript sequence was 99% identical to that of *Pm4b_V2*, one of the two splicing variants of *Pm4b* controlling the resistance to wheat powdery mildew[18]. Further analyses revealed that transcripts from S-615 also contained another splicing variant which was almost identical to *Pm4b_V1*, the other splicing variant of *Pm4b*. These alternative splicing variants derived from S-615 were designated as *Rmg8-V2* and *Rmg8-V1*, respectively. A comparison of these transcripts with the genome sequence of S-615 revealed that the exon/intron structure was the same as *Pm4b* (Fig. 1c).

To check whether *Rmg8-V1* and *Rmg8-V2* recognize *AVR-Rmg8* and induce hypersensitive reaction, a protoplast cell death assay[19] was performed. cDNA fragments

of *Rmg8-V1*, *Rmg8-V2*, and a genomic fragment of the entire gene was inserted into pZH2Bik[20] so as to be driven by the rice ubiquitin promoter, and established as pRmg8-V1, pRmg8-V2, and pRmg8-genome, respectively. Barley protoplasts were co-transfected with these constructs, a plasmid carrying *AVR-Rmg8* (pAVR-Rmg8), and a plasmid carrying a luciferase reporter gene. As a negative control, pPWT3 with the *PWT3* avirulence gene[5] corresponding to *Rwt3* was employed instead of pAVR-Rmg8. Fluorescence was not reduced in pAVR-Rmg8/pRmg8-V1 or pAVR-Rmg8/pRmg8-V2 combinations (Fig. 1d). When protoplasts were co-transfected with pAVR-Rmg8 and a mixture of pRmg8-V1 and pRmg8-V2, however, fluorescence was significantly reduced. This reduction was also observed in the pAVR-Rmg8/pRmg8-genome combination, but was cancelled when pAVR-Rmg8 was replaced with pPWT3. These results suggest that the Can-I gene is *Rmg8* and that both of its splicing variants are required for the recognition of *AVR-Rmg8*. This is in accordance with the report[18] that both of *Pm4b_V1* and *Pm4b_V2* are required for the resistance to powdery mildew conferred by *Pm4b*.

To confirm that the Can-I gene is *Rmg8*, pRmg8-genome was introduced into *T. aestivum* cv. Fielder (susceptible to Br48) through Agrobacterium-mediated transformation. In the T_1 generation, resistant and susceptible individuals against Br48 segregated in a 3:1 ratio (Fig. 1e). Furthermore, these reactions to Br48 were perfectly concordant with the presence/absence of the transgene. By contrast, the T_1 individuals were all susceptible to Br48ΔA8 (*AVR-Rmg8* disruptant derived from Br48) irrespective of the presence/absence of the transgene. Against Br48ΔA8+eI (transformant of Br48ΔA8 carrying re-introduced *AVR-Rmg8* derived from Br48), resistant and susceptible T_1 individuals again segregated in a 3:1 ratio in concordance with the

presence/absence of the transgene. Transformants carrying pRmg8-V1 alone and those carrying pRmg8-V2 alone were all susceptible to Br48, Br48ΔA8, and Br48ΔA8+eI (Extended Data Fig. 2), supporting the observation in the protoplast assay. From these results, we concluded that we successfully isolated *Rmg8*. Sánchez-Martin et al.[18] found six "alleles" of *Pm4*, i.e., *Pm4a*, *Pm4b*, *Pm4d*, *Pm4f*, *Pm4g*, *Pm4h* in breeding lines or global collections of common wheat through PCR amplification and Sanger sequencing. The genetically identified *Pm4* alleles, i.e., *Pm4a*, *Pm4b*, and *Pm4d*, were located on 2A[21,22] while chromosomal locations of *Pm4f*, *Pm4g*, and *Pm4h* have not been determined. *Rmg8* was identical to *Pm4f* in the nucleotide sequence, but was located on 2B (Fig. 2a). From these results, we concluded that *Rmg8* is a homoeolog of *Pm4*.

Rmg7 is an allele of _Pm4_ controlling the resistance to Bgt

Rmg7 was identified in three accessions of tetraploid wheat, *T. dicoccum* KU-112 (abbreviated as St17), KU-120 (St24), and KU-122 (St25)[13] using Br48 as a test isolate. Since *Rmg7* was located on the distal end of 2AL in which the *Pm4* locus resided, we assumed that *Rmg7* might be an allele of *Pm4*. PCR amplification and sequencing revealed that these three accessions shared a gene identical to *Pm4a*. In 93 $F_{2:3}$ lines derived from St24 × Tat14 (*T. paleocolchicum* KU-156, susceptible to Br48), reactions to Br48 (conferred by *Rmg7*) perfectly co-segregated with the presence/absence of *Pm4a* (Fig. 2a) determined by KM200, another presence/absence PCR marker designed on *Rmg8-V2* (Fig. 1c, Extended Data Fig. 3).

To confirm that *Pm4a* recognize *AVR-Rmg8*, a protoplast cell death assay was performed. cDNA fragments of the two alternative splicing variants derived from *Pm4a* in St24 was inserted into pZH2Bik and established as pRmg7-V1 and pRmg7-V2,

188 respectively. Fluorescence was not reduced in pAVR-Rmg8/pRmg7-V1 or pAVR-
189 Rmg8/pRmg7-V2 combinations (Fig. 2b). When protoplasts were co-transfected with
190 pAVR-Rmg8 and a mixture of pRmg7-V1 and pRmg7-V2, however, fluorescence was
191 significantly reduced. This reduction was cancelled when pAVR-Rmg8 was replaced
192 with pPWT3. These results suggest that *Pm4a* specifically recognizes *AVR-Rmg8*, and
193 is therefore *Rmg7*.
194
195 **Distribution of *Rmg8* variants in common wheat**
196 From here, we will call genes that can be amplified with KM171 or KM200
197 (including *Rmg8*, *Rmg7*, and *Pm4* alleles reported previously) as *Rmg8* variants
198 collectively. As mentioned above, we previously screened 520 local landraces of
199 common wheat by inoculation with Br48 and found 18 accessions that recognized *AVR-*
200 *Rmg8*[16]. Although we assumed that these 18 accessions were *Rmg8* carriers, there
201 remained a possibility that some of them might be *Rmg7* carriers because *Rmg7* also
202 recognized *AVR-Rmg8*. In the present study we again screened a total of 526 local
203 landraces (the 520 accessions plus 6 additional accessions) with Br48, and found 21
204 resistant accessions (the 18 accession plus 3 additional accessions). They were all
205 susceptible or weakly/moderately resistant to Br48ΔA8 but resistant to Br48ΔA8+eI,
206 and therefore, confirmed to recognize *AVR-Rmg8* (Extended Data Table 1). Sequence
207 analysis revealed that more than half of them (12 accessions) carried *Pm4f* (Extended
208 Data Table 1). However, the other accessions were composed of one *Pm4b* carrier and
209 eight *Pm4a* carriers (Extended Data Table 1). These *Pm4a* carriers included three
210 accessions (IL92, CP71, and GR250) which had already been confirmed to carry a
211 single resistance gene at the same locus as S-615, i.e. on 2BL[23]. To further check

chromosomal locations of *Pm4a* in common wheat, we chose two *Pm4a* carriers (IL186, CP20) and crossed them with S-615. In the resulting F_2 populations, resistant and susceptible seedlings segregated in 15:1 ratios (Extended Data Table 2), suggesting that they were carriers of *Rmg7* located on 2AL. Taken together, these results suggest that the chromosomal location of the *Pm4a* sequence is not restricted to 2AL; it resides on 2AL in some accessions but on 2BL in others.

To find other *Rmg8* variants, we screened the rest of the local landraces (505 susceptible accessions) with the PCR marker KM200, and found 7 accessions carrying *Pm4g* and 3 accessions carrying a new variant tentatively designated as *PM4_h1* (Extended Data Table 1). They were considered to be ineffective against MoT (Extended Data Table 1). *Pm4d* or *Pm4h* were not detected in our collection of common wheat local landraces.

These *Rmg8* variants were plotted on maps of Europe, Middle East, and Africa (Ethiopia). *Pm4f* and *Pm4a*, which are effective against MoT, were distributed from Middle East through southern Europe (Fig. 3a). On the other hand, *Pm4g*, which is ineffective against MoT, was distributed around mid-northern areas of Europe. The *Rmg8* variants were scarcely detected in accessions collected in Asia and the Americas (Extended Data Table 3).

Distribution of *Rmg8* variants in tetraploid wheat

To trace the origin of the *Rmg8* variants, we screened tetraploid wheat composed of 46 accessions of *T. dicoccoides*, 76 accessions of *T. dicoccum*, 72 accessions of *T. durum*, and 4 accessions of *T. paleocolchicum* with KM200. For accessions positive with KM200, the entire gene was amplified and sequenced. In the wild emmer wheat (*T.*

236 *dicoccoides*) *Pm4f* was detected more frequently than *Pm4a* (Extended Data Table 3).
237 In the cultivated emmer wheat (*T. dicoccum*), however, *Pm4a* extremely predominated
238 over *Pm4f* (Extended Data Table 3). Their geographical distribution suggested that,
239 after the domestication of emmer wheat, *Pm4a* was preferentially transmitted from
240 Fertile Crescent to Spain and Ethiopia (Fig. 3b, c). A new variant designated tentatively
241 as *PM4_h2* was found in two accessions of *T. dicoccum* collected in Ethiopia. *Pm4a*
242 and *Pm4f* were also detected in *T. durum* (Extended Data Table 3).
243
244 **Distribution of *Rmg8* variants in *Aegilops* spp.**
245 To reveal the origin of the functional genes recognizing *AVR-Rmg8*, *Aegilops* spp.
246 composed of 909 accessions were screened by inoculation. Accessions resistant to Br48
247 but weakly resistant or susceptible to Br48ΔA8 were determined to be carriers of
248 functional *Rmg8* variants. Such accessions were found in *Ae. umbellulata*, *Ae.*
249 *speltoides*, and *Ae. comosa* (Extended Data Tables 4, 5). It should be noted that, in *Ae.*
250 *umbellulata*, the 27 accessions resistant to Br48 were either susceptible (26 accessions)
251 or weakly resistant (1 accession) to Br48ΔA8, suggesting that they all recognize *AVR-*
252 *Rmg8*. Geographically, they were distributed around Fertile Crescent and Turkey (Fig.
253 3d).
254 Six accessions were arbitrarily chosen from the 26 accessions mentioned above and
255 crossed with susceptible accessions. In each F_2 population resistant and susceptible
256 seedlings segregated in a 3:1 ratio (Extended Data Table 6), suggesting that the
257 resistance of each accession is controlled by a single major gene. In addition, crosses
258 among resistant accessions yielded no susceptible F_2 seedlings (Extended Data Table 6),
259 which was consistent with an assumption that they were allelic at the same locus.

Phylogenetic analysis of *Rmg8* variants

The resistance genes recognizing *AVR-Rmg8* (*Rmg8* homologs) were amplified from seven, two, and one accessions of *Ae. umbellulata*, *Ae. speltoides*, and *Ae. comosa,* respectively, and sequenced. *Rmg8* variants from these species were designated as *AeuRmg8*, *AesRmg8*, and *AecRmg8*, respectively. These nucleotide sequences were aligned with those of tetraploid and hexaploid wheat lines, and a phylogenetic tree was constructed using MEGA7 (Fig. 4a). SY-Mattis, a common wheat cultivar carrying *Pm4d*[18], was included in the materials. *AeuRmg8* and *AecRmg8* were grouped into a cluster remote from the others while *AesRmg8* was clustered with those of *Triticum* spp. and formed a subcluster with *Pm4g*. This is reasonable because the S genome in *Ae. speltoides* is close to the B genome of *Triticum* spp.[24,25]. *Rmg8* variants in *Triticum* spp. except *Pm4g* formed another subcluster. *Pm4f* was located on the basal part of this large subcluster and composed of various haplotypes, suggesting that *Pm4f* emerged earlier than the others. The topology suggested that *Pm4a*, *Pm4d*, *PM4_h1*, and *PM4_h2* originated from *Pm4f*, and that *Pm4b* originated from *Pm4d*.

Amino acid sequences of those variants are summarized in Fig. 4b with one representative from each of the *Aegilops* variants, i.e., AeuRmg8_h1 from *Ae. umbellulata* KU-4026, AesRmg8_h1 from *Ae. speltoides* KU-7707, and AecRmg8_h1 from *Ae. comosa* KU-17-2. The various haplotypes of *Pm4f* encoded the same protein with an identical amino acid sequence. Pm4a, Pm4d, PM4_h1, and PM4_h2 had a single amino acid substitution at different sites in comparison with Pm4f, suggesting that they emerged from Pm4f independently. Pm4b had two amino acid substitutions in comparison with Pm4f, but one of them was shared with Pm4d, supporting the idea that

Pm4b evolved from Pm4d. AesRmg8_h1 was very similar to the Rmg8 variants in *Triticum* spp. while AeuRmg8_h1 and AecRmg8_h1 had large indels in comparison with them (Fig. 4b).

Reactions of *Rmg8* variants to MoT and Bgt

Reactions of representative *Rmg8* variants in *Triticum* spp. to wheat blast and wheat powdery mildew were tested using three MoT strains (Br48, Br48ΔA8, and Br48ΔA8+eI) and 14 Bgt isolates collected in various locations in Japan (Extended Data Table 7). Some examples are shown in Fig. 5. For *Pm4a*, common wheat cultivar Chancellor (Cc) and its near-isogenic line carrying *Pm4a* (Cc-Pm4a) were employed. Cc-Pm4a is a line (Khapli × Cc[8]) bred for mildew resistance by Briggle[26]. If our analysis mentioned above is correct, Cc-Pm4a should recognize *AVR-Rmg8*. Indeed, Cc-Pm4a was resistant to Br48, susceptible to Br48ΔA8, and again resistant to Br48ΔA8+eI while Cc was susceptible to all of the three strains (Fig. 5). Against Bgt, Cc-Pm4a was resistant to Th2 while Cc was susceptible (Fig. 5), confirming that *Pm4a* recognize Bgt. Cc-Pm4a was susceptible to another Bgt isolate Th1, suggesting that the avirulence gene corresponding to *Pm4a* is carried by Th2 but not by Th1. St24 is a tetraploid accession in which *Rmg7* (=*Pm4a*) was first identified[13]. Against the three MoT strains, St24 showed the same reactions as Cc-Pm4a as expected (Fig. 5). In addition, St24 showed strong resistance to the Bgt isolates (Fig. 5). Taken together, we confirmed that *Pm4a* recognize both MoT and Bgt.

For *Pm4f,* common wheat cultivar Chikugoizumi (ChI) and its near-isogenic line carrying *Rmg8* (=*Pm4f*) (ChI-Rmg8)[27] were employed. ChI-Rmg8 was resistant to Br48, susceptible to Br48ΔA8, and again resistant to Br48ΔA8+eI as expected while

ChI was susceptible to all of the three strains (Fig. 5). Other *Pm4f* carriers (S-615 and IL191) showed the same reactions. By contrast, these *Pm4f* carriers were all susceptible to Th1 and Th2 (Fig. 5). Furthermore, ChI-Rmg8 were susceptible to all Japanese Bgt isolates tested (Extended Data Table 7). Taken together, we concluded that *Pm4f* is effective against MoT but ineffective against Bgt.

GR192 carrying *Pm4b* and SY-Mattis carrying *Pm4d* were resistant to Br48, susceptible to Br48ΔA8, and resistant to Br48ΔA8+eI (Fig. 5), indicating that these alleles recognize *AVR-Rmg8*. These alleles have already been shown to be effective against Bgt through a transformation assay or infection assay with multiple accessions/isolates[18]. Indeed, the carriers of these alleles (GR192 and SY-Mattis) were resistant to the Bgt isolates tested in the present study (Fig. 5). These results suggest that *Pm4b* and *Pm4d* are effective against both MoT and Bgt. On the other hand, IL16 carrying *Pm4g* was susceptible to all of the MoT strains and Bgt isolates tested (Fig. 5, Extended Data Table 7), suggesting that *Pm4g* is ineffective against both MoT and Bgt.

Ae. umbellulata KU-4026 carrying *AeuRmg8_h1* was resistant to Br48, susceptible to Br48ΔA8, and resistant to Br48ΔA8+eI (Fig. 5), confirming that *AeuRmg8_h1* recognizes *AVR-Rmg8*. When inoculated with the Th1 and Th2, primary leaves of KU-4026 became slightly chlorotic, but produced conidia enough to proceed to the next infection cycle (Fig. 5). KU-4026 showed similar reactions to all Japanese Bgt isolates tested (Extended Data Table 7). These results suggest that *AeuRmg8_h1* is effective against MoT but ineffective against Bgt.

Discussion

In wheat, 11 blast resistance genes have been identified so far (see ref. 28 for a comprehensive list of 10 genes and ref. 29 for a gene identified recently). Among them five (*Rmg2*, *Rmg3*, *Rmg7*, *Rmg8*, and *RmgGR119*) are genes against MoT while the others are genes against other pathotypes functioning as host-specificity barriers. Arora et al[30] cloned two genes in the latter category (genes against other pathotypes), i.e., *Rwt3* and *Rwt4*, and showed that they encoded a nucleotide-binding site leucine-rich repeat protein (NLR) and a tandem kinase, respectively. However, there has been no report of successful cloning of genes in the former category (genes against MoT). *Rmg2* and *Rmg3* identified in common wheat 'Thatcher'[31] were not effective in spikes or at high temperature. *Rmg7* and *RmgGR119* were effective in spikes but not at high temperature[13,15,16]. At present, therefore, *Rmg8* is the only one genetic factor that has been identified as a major gene for resistance to MoT and supposed to be effective against wheat blast in farmer's fields[27]. Its effectiveness has been confirmed in field tests in Bangladesh (Dr. Tofazzal Islam, personal communication). In the present study we isolated *Rmg8* and found that it is a non-canonical resistance gene encoding a kinase fusion protein[32]. Intriguingly, *Rmg8* was identical to *Pm4f*, which was reported to be an "allele" of *Pm4*, a gene for resistance to wheat powdery mildew[18]. *Rmg8* was located on 2BL[14,27] (Fig. 2a) while *Pm4a*, *Pm4b*, and *Pm4d* were reported to reside on 2AL[21,22]. This apparent discrepancy could be explained by considering that *Pm4f* was not a genetically identified allele but was found through PCR amplification and sequencing. We suggest that *Rmg8* is a homoeologous gene of *Pm4* alleles on 2AL. We further isolated *Rmg7* located on 2AL and found that this gene is identical to *Pm4a*. This is reasonable because *Rmg7* and *Rmg8* have been inferred to be homoeologous genes[15]. Intriguingly, the *Pm4a* sequence was also detected at the *Rmg8* locus on 2BL in some

356 accessions (Extended Data Table 1). The *Pm4a* gene in these accessions should be
357 considered to be *Rmg8* from the viewpoint of Mendelian genetics, but is identical to
358 *Rmg7* at the molecular level. O'Hara et al.[33] isolated a wheat blast resistance gene
359 through GWAS (genome-wide association study) and similarly concluded that alleles of
360 the wheat powdery mildew resistance gene *Pm4* also confers resistance to wheat blast.
361 The *Pm4* "alleles" tested were divided into three groups from the viewpoint of
362 reactions to MoT and Bgt. The first one composed of *Pm4a*, *Pm4b*, and *Pm4d* was
363 effective against both MoT and Bgt while the second one, *Pm4f*, was effective against
364 MoT but ineffective against Bgt (Fig. 5). The third one, *Pm4g*, was ineffective against
365 both MoT and Bgt (Fig. 5). *Pm4a*, *Pm4b*, and *Pm4d* have been identified as genes for
366 resistance to Bgt and used for breeding. On the other hand, *Pm4f* and *Pm4g* were
367 suggested to be susceptible "alleles" against Bgt by Sánchez-Martin et al[18]. In addition,
368 carriers of these "alleles" were susceptible to all Japanese Bgt isolates tested (Extended
369 Data Table 7). One hypothesis to explain this general susceptibility would be that they
370 had been effective against Bgt at the time of their emergence, but were later overcome
371 by newly evolved virulent races. However, this scenario implies that their
372 corresponding avirulence genes have been eliminated from the Bgt populations in both
373 Europe and Far East, and therefore requires a wide cultivation of wheat lines carrying
374 these 'resistance genes'. Considering their low frequencies in local landraces and no
375 record of wide cultivation of such cultivars, however, such perfect elimination is
376 unlikely to occur. Therefore, *Pm4f* and *Pm4g* are considered to have been ineffective
377 against Bgt from the time of their emergence. The phylogenetic tree (Fig. 4) suggested
378 that *Pm4a*, *Pm4b*, and *Pm4d* evolved from *Pm4f*. Taken together with the above
379 discussion, we suggest that these *Pm4* alleles for resistance to powdery mildew have

evolved from *Pm4f* through gaining an ability to recognize Bgt. The analysis of their geographical distribution showed that *Pm4a* was a minor allele in the wild emmer wheat in comparison with *Pm4f* but predominated over *Pm4f* in the cultivated emmer wheat (Figs. 3, 4). This may be attributable to the preferential transmission of *Pm4a* carriers from Fertile Crescent to Spain and Ethiopia by peoples who noticed the advantage of powdery mildew resistance conferred by this allele.

The gain of the ability to recognize Bgt was caused by a single amino acid substitution (Fig. 4b), and resulted in the generation of the alleles expressing resistance to both MoT and Bgt (Fig. 5). There are two additional examples suggesting close associations of recognition of *P. oryzae* and *B. graminis*. Two amino acid deletion of *Rwt4*, a gene for resistance to an *Avena* isolate of *P. oryzae* encoding a tandem kinase, resulted in a gain of resistance to Bgt[30,34]. *Mla3*, an allele at the *Mla* locus conditioning the resistance of barley to *B. graminis* f. sp. *hordei* (Bgh, the barley powdery mildew fungus), recognized *PWL2*, an avirulence gene derived from *P. oryzae* pathotype *Oryza* (the rice blast fungus)[35]. Mechanisms of such dual specificity with *P. oryzae* and *B. graminis* should be elucidated at the level of molecular structures.

Functional *Rmg8* variants were also detected in *Ae. umbellulata* (U genome), *Ae. speltoides* (S genome), and *Ae. comosa* (M genome). This result suggests that the prototype of *Rmg8* equipped with the function for recognizing *AVR-Rmg8* was established before the differentiation of the A, B, U, S, and M genomes in the *Triticum – Aegilops* complex, which was estimated to be 5–6 million years ago[36]. This implies that this gene has maintained the function for recognizing *AVR-Rmg8* for 5–6 million years without infection pressure exerted by MoT because MoT first emerged in 1985. However, it seems unlikely that a resistance gene has maintained its function for such a

long time under no infection pressure by pathogens. One possibility is that *Rmg8* and its variants had been interacting with *P. oryzae* before the differentiation into pathotypes, and after the differentiation, have been interacting with pathotype(s) that maintained *AVR-Rmg8*. The most probable candidate of such pathotypes is inferred to be MoL (*Lolium* pathotype) with three reasons. First, MoL is phylogenetically the closest to MoT[37]. Second, functional *AVR-Rmg8* is widely distributed in the population of MoL[38]. Third, the hosts of MoL (Italian ryegrass and perennial ryegrass) are widely distributed in Middle East and southern Europe[39-41] where functional *Rmg8* variants are frequently found (Fig. 3). Another possibility is that the *Rmg8* variants have been effective against pathogens other than the blast fungus (and the powdery mildew fungus). It should be noted that *Sr33* in wheat and *Sr50* in rye, genes for resistance to stem rust, are homologs of *Mla*, a barley gene for resistance to Bgh[42,43]. Also, an allele at the *Mla* locus, *Mla8*, was shown to be effective against wheat stripe rust[44].

In Introduction we raised a question why common wheat accessions in Europe and Middle East have maintained *Rmg8*, a gene for resistance to MoT. The present study revealed that "*Rmg8*" detected in those accessions was composed of *Pm4a*, *Pm4b*, and *Pm4f*. We suggest that about a half of them (carriers of *Pm4a* and *Pm4b*) have maintained these genes due to their effectiveness against wheat powdery mildew. The maintenance of *Pm4f* in the other accessions may be explained by the same reasoning as the *Rmg8* variants in *Aegilops* spp.; *Pm4f* may have been effective against MoL or other pathogens. It is suggestive that *Pm4f* is distributed in similar regions as the *Rmg8* variants in *Aegilops* spp., i.e., warm areas around the same latitude (Fig. 3).

The evolutionary process of *Rmg8* inferred from the present study is summarized in Fig. 6. The prototype of *Rmg8* gained an ability to recognize *AVR-Rmg8* before the

differentiation of *Triticum* and *Aegilops*. It then differentiated into variants including *Pm4g* and *Pm4f*. Some variants derived from *Pm4f* gained an ability to recognize Bgt, and evolved into *Pm4a*, *Pm4d*, and *Pm4b*, genes for resistance to wheat powdery mildew. Finally, when MoT emerged in 1985, those *Rmg8* variants appeared as genes for resistance to wheat blast because they recognized an effector encoded by *AVR-Rmg8* of MoT. This figure illustrates an evolutionary process in which a resistance gene has expanded its target pathogens.

This study offers practical recommendations for resistance breeding, focusing on challenges in Europe and North America where field assays for wheat blast resistance have been hindered by quarantine restrictions, as there has been no MoT invasion. The identification of *Rmg8* through specific markers empowers breeders to monitor its integration into elite varieties at the molecular level. Opting for *Rmg8* variants effective against both MoT and Bgt facilitates efficacy assessments through Bgt field tests, contributing to resistance development and readiness for potential MoT invasions. In addition, the present study provides more general perspectives on breeding. When a resistance gene to a known pathogen is cloned, nucleotide sequences of its "susceptible alleles" should be also clarified. If a "susceptible allele" maintaining an ORF is distributed in the crop population in a certain frequency, it may be a functional resistance gene against other pathogen(s) which is prevailing now or those which will emerge in future. Conversely, when a new disease emerges, resistance genes against its causal agent (a new pathogen) may be found among known resistance genes against currently prevailing pathogens.

Methods

452

453 **Plant materials**

454 Parental cultivars for mapping of *Rmg8*, *Triticum aestivum* cv. S-615 and cv. Shin-
455 chunaga (Sch), were provided by K. Tsunewaki, Emeritus professor at Kyoto
456 University, Japan. Parental accessions for mapping of *Rmg7*, *T. dicoccum* St24
457 (accession No. KU-120) and *T. paleocolchicum* Tat14 (KU-156), were provided by S.
458 Sakamoto, Emeritus professor at Kyoto University. *Hordeum vulgare* cv. Golden
459 Promise (GP) for protoplast assay and *T. aestivum* cv. Fielder (KT020-061) for
460 transformation were provided by K. Sato, Okayama University, Japan, and the National
461 BioResource Project –Wheat (NBRP) (https://shigen.nig.ac.jp/wheat/komugi/), Japan,
462 respectively. *T. aestivum* cv. Chancellor (Cc) and its near isogenic line Cc-Pm4a
463 carrying *Pm4a* (=Khapli × Cc[8] produced by Briggle[26]) were provided by U. Hiura,
464 Emeritus professor at Okayama University. *T. aestivum* cv. Chikugoizumi (ChI) and its
465 near-isogenic line carrying *Rmg8* (ChI-Rmg8)[27] were produced in the BRAIN project
466 (see Acknowledgments), and maintained at NARO (National Agriculture and Food
467 Research Organization), Japan. *T. aestivum* cv. SY-Mattis, one of the accessions
468 analyzed by the wheat pangenome project[45], was provided by John Innes Centre to S.
469 Nasuda and maintained at Kyoto University. The 526 local landraces of *T. aestivum*
470 used for the distribution analysis were a collection of K. Kato, Okayama University,
471 Japan. Original providers of the *T. aestivum* accessions carrying the *Rmg8* variants are
472 shown in Extended Data Table 1. The accessions of tetraploid wheat used for the
473 distribution analysis were collections of N. Mori, Kobe University, and S. Nasuda,
474 Kyoto University. The tetraploid accessions carrying the *Rmg8* variants are shown in
475 Fig. 4. Among them accessions with the prefix KU- were provided by NBRP while

476 those with the prefixes PI and Citr were provided by the U.S. National Plant Germplasm
477 System. The 909 accessions of *Aegilops* spp. used for screening for functional *Rmg8*
478 variants were provided by NBRP.
479
480 **Fungal materials**
481 Wheat blast strains used for infection assay were *Pyricularia oryzae* pathotype *Triticum*
482 wild isolate Br48 collected in 1990 in Brazil, Br48ΔA8_d6 (abbreviated as Br48ΔA8), a
483 disruptant of *AVR-Rmg8* derived from Br48[16], and Br48ΔA8+eI-3 (abbreviated as
484 Br48ΔA8+eI), a transformant of Br48ΔA8 carrying the eI type of *AVR-Rmg8*[46]. They
485 have been maintained on sterilized barley seeds at Kobe University.
486 Wheat powdery mildew strains used were wild isolates of *Blumeria graminis* f. sp.
487 *tritici* collected in various regions in Japan (Extended Data Table 7). They were purified
488 through single-conidium isolation and have been maintained at 4°C on primary leaves
489 of *T. aestivum* cv. Norin 4 through subculturing.
490
491 **Inoculation with wheat blast strains**
492 Seeds of *Triticum* and *Aegilops* spp. were pregerminated on a moistened filter paper for
493 24h. Germinated seeds of *Triticum* spp. were sown in vermiculite supplied with liquid
494 fertilizer in a seedling case (5.5 × 15 × 10 cm) and grown at 22℃ with a 12-h
495 photoperiod of fluorescent lighting for 8 days. Germinated seeds of *Aegilops* spp.
496 accessions were sown in the seedling case filled with Sakata Prime Mix soil (Sakata,
497 Japan) and grown at 22℃ with a 12-h photoperiod of fluorescent lighting for 21 days.
498 Primary leaves of eight-day-old wheat seedlings or first to third leaves of 21-day-old
499 *Aegilops* seedlings were fixed onto a plastic board with rubber bands just before

inoculation. Conidial suspensions (1×10^5 conidia/ml) prepared as described previously[13] were sprayed onto fixed leaves using an air compressor. The inoculated seedlings were incubated in a sealed box under dark and humid conditions at 22°C for 24h, then transferred to dry conditions with a 12h photoperiod of fluorescent lighting, and incubated for additional 3–5 days at 22°C. Four to six days after inoculation, symptoms were evaluated based on the color of lesions and the affected leaf area. The affected area was rated by six progressive grades from 0 to 5: 0 = no visible evidence of infection; 1 = pinhead spots; 2 = small lesions (<1.5 mm); 3 = scattered lesions of intermediate size (<3 mm); 4 = large typical blast lesions; and 5 = complete blighting of leaf blades. A disease score (infection type) was designated by combining a number which denotes the size of lesions and a letter or letters indicating the lesion color, i.e., 'B' for brown and 'G' for green. Infection types 0 to 5 with brown lesions were considered to be resistant while infection types 3G, 4G, and 5G were considered to be susceptible. Infection type 3BG accompanies by a mixture of brown and green lesions were taken as weakly resistant.

Inoculation with powdery mildew isolates

Seeds of test plants were sown in autoclaved soil in 2×30cm or 2×35 cm test tubes. Eight days after sowing, primary leaves were inoculated with conidia from eight-day-old colonies using writing brushes. The seedlings were incubated at 22 °C in a controlled-environment room with a 12-h photoperiod of fluorescent lighting. Seven to eight days after inoculation, infection types were recorded using five progressive grades from 0 to 4: 0, no mycelial growth or sporulation; 1, scant sporulation; 2, reduced sporulation; 3, slightly reduced sporulation; 4, heavy sporulation.

524

525 **Mapping of *Rmg8* and *Rmg7***

526 A total of 165 $F_{2:3}$ lines derived from a cross between S-615 and Sch were used for
527 mapping of *Rmg8*. Twenty seeds were retrieved from each $F_{2:3}$ line and subjected to
528 infection assay with Br48 for phenotyping. Another set of 20 seeds was retrieved from
529 each $F_{2:3}$ line, sown in vermiculite, and grown at 22℃ for 7 days. Seven-day-old
530 primary leaves were bulked, and used for DNA extraction by the CTAB method. For
531 detecting polymorphisms between S-615 (*Rmg8*) and Sch (*rmg8*), total RNA was
532 extracted from their primary leaves using Maxwell RSC Plant RNA Kit (Promega).
533 Sequence libraries were generated by NEBNext Ultra II Directional RNA Library Prep
534 Kit, and sequenced using Illumina Hiseq (paired-end) by sequencing service of
535 Novogene, Japan. Sequence reads of S-615 and Sch were aligned to the reference
536 genome of Chinese Spring version 1.1, 2.0, and 2.1[47,48] using HISAT2 (v2.2.1), and
537 variants were called by samtools (v1.18) to generate VCF files. Using the VCF files,
538 Cleaved Amplified Polymorphic Sequence (CAPS) and presence/absence markers were
539 developed. Marker fragments were amplified from genomic DNA of the parental
540 cultivars and the $F_{2:3}$ lines using 2× Quick Taq HS DyeMix (TOYOBO, Osaka, Japan)
541 following the manufacturer's instructions. Fragments amplified with primers for CAPS
542 markers were digested with appropriate restriction enzymes supplied by Takara Bio
543 (Kusatsu, Japan) or New England Biolabs Japan (Tokyo, Japan) (Supplementary Table
544 1). PCR products were electrophoresed in 0.7–2.0% agarose gels and stained with
545 ethidium bromide for visualization. MAPMAKER/EXP v3.0b was used for constructing
546 a genetic map[49]. The logarithm-of-odds (LOD) threshold for declaration of linkage was
547 set at 4.0. Genetic distance was calculated with the Kosambi function.

For mapping of *Rmg7*, RNA sequencing of St24 and Tat14 was performed in a similar way as mentioned above. Sequenced reads were aligned to the reference genome of *T. durum* cv. Svevo (https://plants.ensembl.org/Triticum_turgidum /Info/Index) to develop genetic markers.

Screening for an *Rmg8* transcript

To detect candidate genes for *Rmg8*, we carried out association analysis of expressed genes. First, cDNA sequences of S-615 transcripts were generated by sequencing service (GeneBay, Japan). Base calling of ONT reads was performed on FAST5 files using Guppy (v4.4.1, Oxford Nanopore Technologies). Hybrid de novo assembly was performed by rnaSPAdes (v3.15.3)[50] using ONT reads and Illumina short reads both, resulting in 161,852 transcripts. ORFs coding more than 300 amino acids in these transcripts were predicted by TransDecoder (v5.5.0)[51] and CD-HIT (v4.8.1)[52], resulting in a reference cDNA sequence set of S-615 composed of 27,205 transcripts.

Next, we chose nine $F_{2:3}$ lines susceptible to Br48 arbitrarily, extracted total RNA from three leaves of each $F_{2:3}$ line using Maxwell RSC Plant RNA Kit (Promega), prepared sequence libraries, and sequenced them using Illumina Hiseq4000 (150 bp Paired-End reads) in a similar way as mentioned above. The presence/absence analysis was carried out based on the transcripts per million (TPM) value of the transcripts for selecting genes which were expressed in S-615 but not in either Sch or the nine susceptible $F_{2:3}$ lines.

Cell death assay with barley protoplasts

The genomic sequence of *Rmg8* (*Rmg8-genome*) and the transcript variants of *Rmg8* and *Rmg7* (*Rmg8-V1*, *Rmg8-V2*, *Rmg7-V1*, and *Rmg7-V2*) were employed for protoplast cell death assays. The fragment of *Rmg8-genome* was amplified from genomic DNA of S-615. *Rmg8-V1* and *Rmg8-V2* were amplified from cDNA of S-615 while *Rmg7-V1* and *Rmg7-V2* were amplified from cDNA of St24. RNA extraction and cDNA synthesis were performed as mentioned in the previous sections. The ORFs of *PWT3* and *AVR-Rmg8* without signal peptides were amplified from genomic DNA of Br58[5] and Br48, respectively. Primers used for these PCR reactions are shown in Supplementary Table 1. All of these fragments were cloned into the *Kpn*I site of the pZH2Bik vector using In-Fusion Cloning Kit (Takara) so as to be driven by the rice ubiquitin promoter, resulting in pRmg8-genome, pRmg8-V1, pRmg8-V2, pRmg7-V1, pRmg7-V2, pPWT3, and pAVR-Rmg8. Established plasmids were extracted by NucleoBond Xtra Maxi (Macherey-Nagel, Düren, Germany). Barley cultivar GP was employed as a recipient of transgenes because barley epidermis could be peeled off more easily than wheat epidermis for releasing protoplasts. Mesophyll protoplasts were prepared from eight-day-old primary leaves of GP. Transfection assays with those plasmids were performed as described in Saur et al.[19]. Briefly, plasmids containing AVR and resistance genes were introduced into the GP protoplasts with a plasmid containing the luciferase gene (pAHC17-LUC) via the polyethylene glycol treatment. After 18 hours incubation at 20°C in the dark, the protoplasts were lysed, and luciferase activity in the resulting cell extracts was measured for 1 second/well on the Tristar 3 luminometer mode (Berthold). The measured luminescence was normalized using the negative control in which the AVR gene was substituted with the empty pZH2Bik vector. This experiment was repeated four times independently.

595

596 **Production of transgenic plants**

597 pRmg8-V1, pRmg8-V2, and pRmg8-genome were introduced into *T. aestivum* cv.
598 Fielder via the Agrobacterium-mediated transformation as described by Ishida et al.[53]
599 Insertions of transgenes were checked by PCR with the HPT primers (Supplementary
600 Table 1). We obtained three, eleven, and three T_1 lines carrying *Rmg8-V1*, *Rmg8-V2*,
601 and *Rmg8-genome*, respectively. Transgenic T_1 seedlings were inoculated with Br48,
602 Br48ΔA8, and Br48ΔA8+eI to evaluate functions of the transgenes.

603

604 **Sequencing of *Rmg8* variants and phylogenetic analysis**

605 In the distribution analyses in *Triticum* spp., all test accessions were screened with
606 KM171 and KM200, and those with amplicons were subjected to sequence analyses
607 irrespective of their phenotypes (resistant or susceptible). In the analysis of *Aegilops*
608 spp., all accessions were first screened by inoculation with Br48 and Br48ΔA8, and
609 those recognizing *AVR-Rmg8* were subjected to sequence analyses. Two primer pairs
610 were used to amplify two different regions of the *Rmg8* genomic fragment, one
611 encoding exons 1 to 5 and the other encoding exons 6 and 7. These fragments were
612 inserted into the *Eco*RV site of pBSIISK+, sequenced with ABI capillary sequencers,
613 and aligned with MAFFT (v7.520). Coding sequences were extracted from obtained
614 sequences and concatenated. A maximum likelihood tree was constructed using MEGA
615 7 (v7.0.26)[54] with 1,000 bootstrap replicates. Primers used in this section are listed in
616 Supplementary Table 1. *Rmg8* variants detected were plotted on a map created with
617 rworldmap (v1.3-6) in R (v4.3.1).

618

Data availability

Sequence data were deposited in the GenBank/EMBL database under the accession numbers LC779671, LC779672, LC779673, and LC779674. All plasmids, plant lines, and fungal strains used or generated in this work are available from the authors upon request. Source data are available in the Supporting Information. Any additional data supporting the findings in the present study are available from the corresponding author upon request. The databases used in the present study were the reference genome of Chinese Spring (https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies) and the reference genome of *T. durum* cv. Svevo (https://plants.ensembl.org/Triticum_turgidum /Info/Index).

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649 International Collaborative Research Grant (Type B Fostering Joint Research) (provided
650 for SA).
651

652 **Author contributions** K.M. performed molecular mapping and crossing of
653 *Aegilops* accessions. M.S. and R.T. selected transcripts of candidate genes. F.A. and
654 M.K. performed wheat transformation. C.N., Y.T., M.S., M.Y., M.I., and Z.S.
655 performed screening of germplasms and their molecular analyses. S.N., H.H., M.F.,
656 M.T., K.H., N.M., Y.M., and K.K. provided germplasms and scientific advice. S.A.
657 performed the other experiments including the protoplast assay and summarized the
658 data. S.A. and Y.T. designed the research and wrote the manuscript.
659

660 **Competing interests** The authors declare no competing interests.
661

662 **Additional information**

663 Correspondence and requests for materials should be addressed to Yukio Tosa.
664

Figure legends

Fig. 1: Cloning of *Rmg8* identified on chr. 2BL in common wheat. a, Genetic and physical maps around *Rmg8* on chr. 2BL. Numbers of recombinants between each molecular marker and *Rmg8* are shown in parentheses. Positions of high confidence genes in the Chinese Spring reference genome v2.1 are indicated by vertical lines. Genes used as molecular markers, the Can-I gene, and other genes picked up through the association analysis are highlighted with blue, red, and yellow, respectively. **b**, Association between responses to *AVR-Rmg8* and amplifications with the KM171 primers in common wheat. This experiment was repeated three times. **c**, Structure of the gene producing the Can-I transcript. Can-I was one of the two splicing variants (*Rmg8-V1* and *Rmg8-V2*) of the gene. Bold lines represent positions of presence/absence PCR markers (KM171 and KM200). **d**, Cell death assay with protoplasts. Barley protoplasts were transfected with pAHC17-LUC containing a luciferase gene, pZH2Bik containing avirulence genes (*PWT3* or *AVR-Rmg8*) or no insert (empty vector), and pZH2Bik containing *Rmg8-V1*, *Rmg8-V2*, *Rmg8-genome*, or a mixture of pRmg8-V1 and pRmg8-V2 in a 1:1 molar ratio. Luciferase activity was represented as relative activities compared with those in samples with the empty vector. The experiments were repeated four times. Results from the four replicates were represented as a boxplot with original data points. Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend to 1.5x the interquartile range from the 25th and 75th percentiles. Welch's two-sided t-test was used for the comparisons of relative luciferase activity between the two groups. No adjustments for multiple comparisons were made for indication of the significance. Double and triple asterisks indicate significant differences at the 1 and 0.1% levels,

689 respectively. NS, not significant. **e**. Validation of *Rmg8* through transformation. S-615
690 (*Rmg8*), Fielder (*rmg8*), and T_1 individuals derived from transformation of Fielder with
691 the genomic fragment (*Rmg8-genome)* were inoculated with Br48, Br48ΔA8, and
692 Br48ΔA8+eI, and incubated for five days. Presence (+) /absence (-) of the transgene
693 confirmed by PCR with the KM171 and HPT markers are shown below the panels.
694
695 **Fig. 2: Cloning of *Rmg7* identified on chr. 2AL in tetraploid wheat. a**, Genetic map
696 around *Rmg7* constructed using 93 $F_{2:3}$ lines derived from *T. dicoccum* accession St24
697 (*Rmg7*) x *T. paleocolchicum* accession Tat14 (*rmg7*). For a comparison a genetic map
698 around *Rmg8* on 2BL is shown on the right which was constructed using 91 $F_{2:3}$ lines
699 derived from *T. aestivum* cv. Shin-Chunaga (Sch, *rmg8*) x *T. aestivum* cv. S-615 (*Rmg8*).
700 KM200 and KM171, PCR markers designed on *Rmg8*, perfectly co-segregated with
701 phenotypes conferred by *Rmg7*. **b**, Cell death assay with protoplasts. Protoplasts
702 isolated from barley primary leaves were transfected with pAHC17-LUC containing a
703 luciferase gene, pZH2Bik containing avirulence genes (*PWT3* or *AVR-Rmg8* lacking
704 signal peptides) or no insert (empty vector), and pZH2Bik containing constructs of the
705 *Rmg7* candidate gene (*Rmg7-V1*, *Rmg7-V2*) or a mixture of pRmg7-V1 and pRmg7-V2
706 in a 1:1 molar ratio. Luciferase activity was determined 18-hours after transfection and
707 represented as relative activities compared with those in samples with the empty vector.
708 The experiments were repeated four times. Results from the four replicates were
709 represented as a boxplot with original data points. Center lines show the medians; box
710 limits indicate the 25th and 75th percentiles; whiskers extend to 1.5x the interquartile
711 range from the 25th and 75th percentiles. Welch's two-sided t-test was used for the
712 comparisons of relative luciferase activity between the two groups. No adjustments for

multiple comparisons were made for indication of the significance. Triple asterisks indicate significant differences at the 0.1% level. NS, not significant.

Fig. 3: Geographical distribution of *Rmg8* variants in Europe, Middle East, and Ethiopia. In common wheat (**a**), cultivated tetraploid wheat (**b**) and wild tetraploid wheat (**c**), accessions carrying *Pm4* "alleles" were plotted. The *Pm4* "alleles" were color-coded. In *Ae. umbellulata* (**d**), accessions recognizing *AVR-Rmg8* were plotted without differentiation of their *Rmg8* variants.

Fig. 4: Phylogenetic relationships among *Rmg8* variants in *Triticum* and *Aegilops*. **a**, Maximum likelihood phylogenetic tree of *Rmg8* variants constructed using nucleotide sequences of the coding region. *Rmg8* variants in *Triticum* spp. are represented by their *Pm4* "allele" symbols. *PM4_h1* and *PM4_h2* are new variants which have not been reported previously. *AesRmg8*, *AecRmg8*, and *AeuRmg8* are *Rmg8* variants found in *Ae. speltoides*, *Ae. comosa*, and *Ae. umbellulata*, respectively. Bootstrap values (more than 50) from 1,000 replications are shown at nodes. Ta, *T. aestivum*; Trm, *T. durum*; Tdm, *T. dicoccum*; Tds, *T. dicoccoides*; Aes, *Ae. speltoides*; Aec, *Ae. comosa*; Aeu, *Ae. umbellulata*. **b**, Amino acid alignments of *Rmg8* variants detected in *Triticum* and *Aegilops* spp. AesRmg8_h1, AecAmg8_h1, and AeuRmg8_h1 are representatives of AesRmg8, AecAmg8, and AeuRmg8 detected in *Ae. speltoides* KU-7707, *Ae. comosa* KU-17-2, and *Ae. umbellulata* KU-4026, respectively.

Fig. 5: Reactions of *Rmg8* variants to wheat blast (MoT) and wheat powdery mildew (Bgt) fungi. Wheat accessions carrying *Rmg8* variants were inoculated with

Br48 (wild MoT isolate), Br48ΔA8 (disruptant of *AVR-Rmg8*), and Br48ΔA8+eI (transformant of Br48ΔA8 carrying re-introduced *AVR-Rmg8*), and incubated for five days, or were inoculated with Th2 (Bgt carrying *AvrPm4a,* the putative avirulence gene corresponding to *Pm4a*) and Th1 (Bgt carrying *avrPm4a*, the non-functional allele of *AvrPm4a*), and incubated for eight days. *Rmg8* variants carried by the wheat lines are shown in parentheses. St24 and KU-4026 are a tetraploid accession and an *Ae. umbellulata* accession, respectively, and the others are common wheat lines. Cc-Pm4a is a near-isogenic line of cv. Chancellor (Cc) carrying *Pm4a* while ChI-Rmg8 is a near-isogenic line of cv. Chikugoizumi (ChI) carrying *Rmg8*.

Fig. 6: A model illustrating evolutionary processes in which resistance gene *Rmg8* has gained new target pathogens through differentiation of variants. The *Rmg8* variants painted in pink and enclosed in a blue rectangle indicate those with functions for recognizing wheat blast (MoT) and wheat powdery mildew (Bgt), respectively. The red rectangle indicates recognition as a useful gene for resistance to wheat blast. mya, million years ago.

Extended Data Fig. 1. **Search for *Rmg8* candidate genes through association analyses of expressed genes with susceptible $F_{2:3}$ lines. a**, An outline of screening of candidate genes. **b**, A list of *Rmg8* candidate genes. NBS, Nucleotide-binding site; NLR, Nucleotide-binding site and leucine-rich repeat; RLK, Receptor-like kinase.

Extended Data Fig. 2. Reactions of T_1 transformants carrying *Rmg8-V1* and *Rmg8-V2*. T_1 individuals derived from transformation of Fielder with the *Rmg8-V1* CDS

761 (Fielder+Rmg8-V1) or with *Rmg8-V2* CDS (Fielder+Rmg8-V2) were inoculated with
762 Br48, Br48ΔA8, and Br48ΔA8+eI, and incubated for five days. Presence (+)/absence (-)
763 of the transgene confirmed by PCR with the HPT primers are shown below the panels.
764
765 **Extended Data Fig. 3. PCR products amplified with KM200 primers.** Genomic
766 DNAs of common wheat (S-615, Sch) and tetraploid wheat (St24, Tat14) were
767 subjected to amplification with KM200 primers, and resulting amplicons were run on a
768 2% gel for 30 min. The 424bp fragment is amplified from both of the *Rmg8* carrier and
769 the *Rmg7* carrier, but not from the noncarriers. The 350bp fragment is amplified from
770 all cultivars/accessions irrespective of their genotypes, and can be used as an indicator
771 of successful PCR reactions.
772
773

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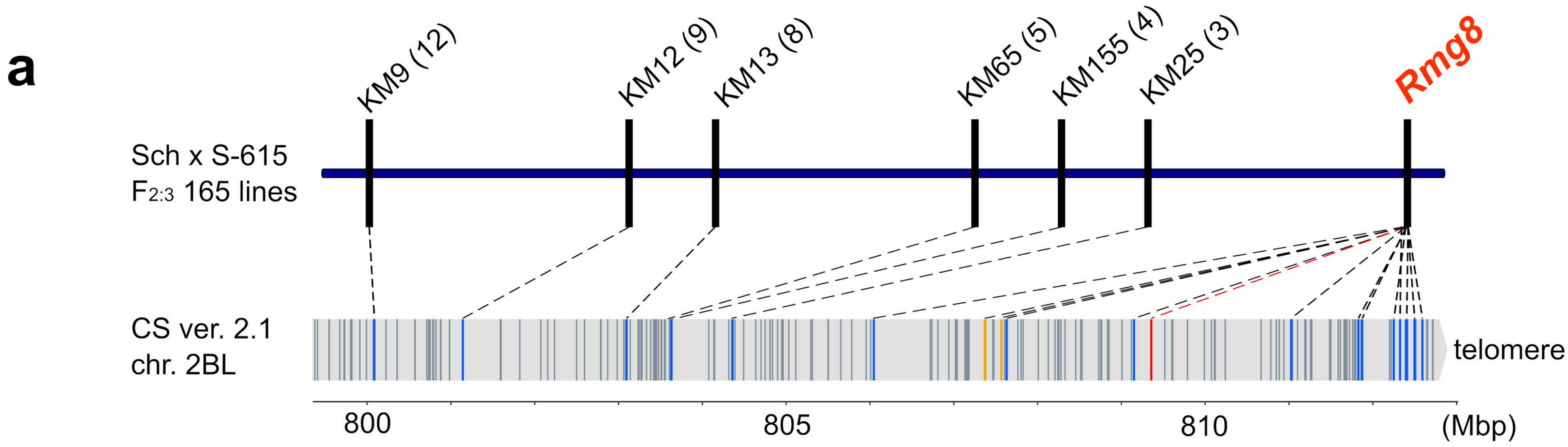
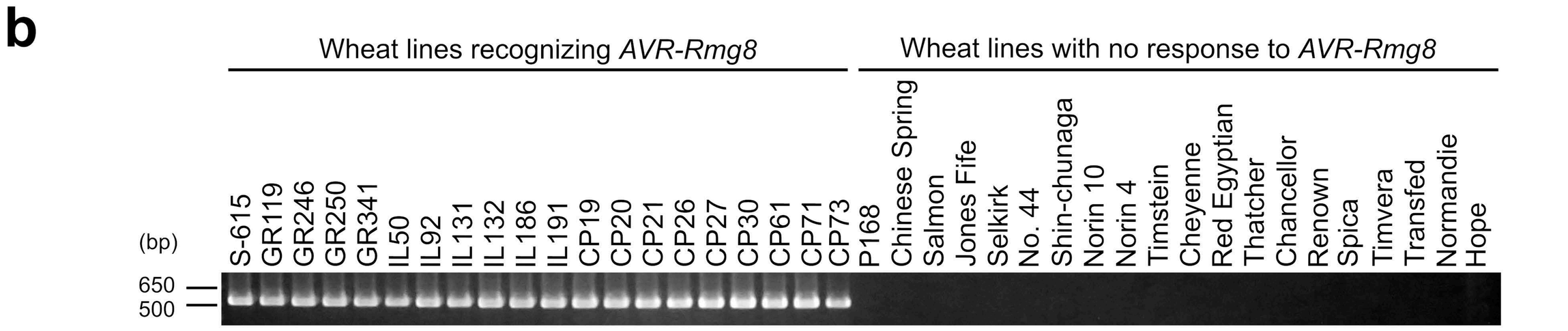
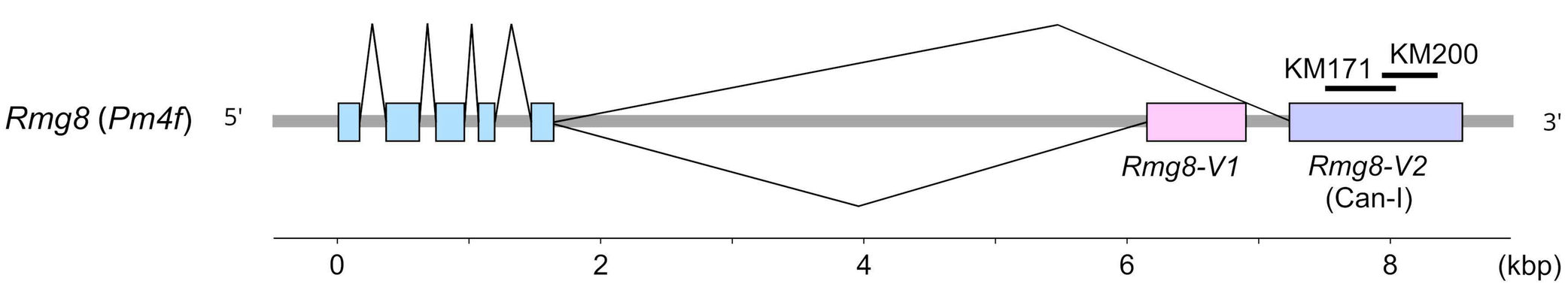
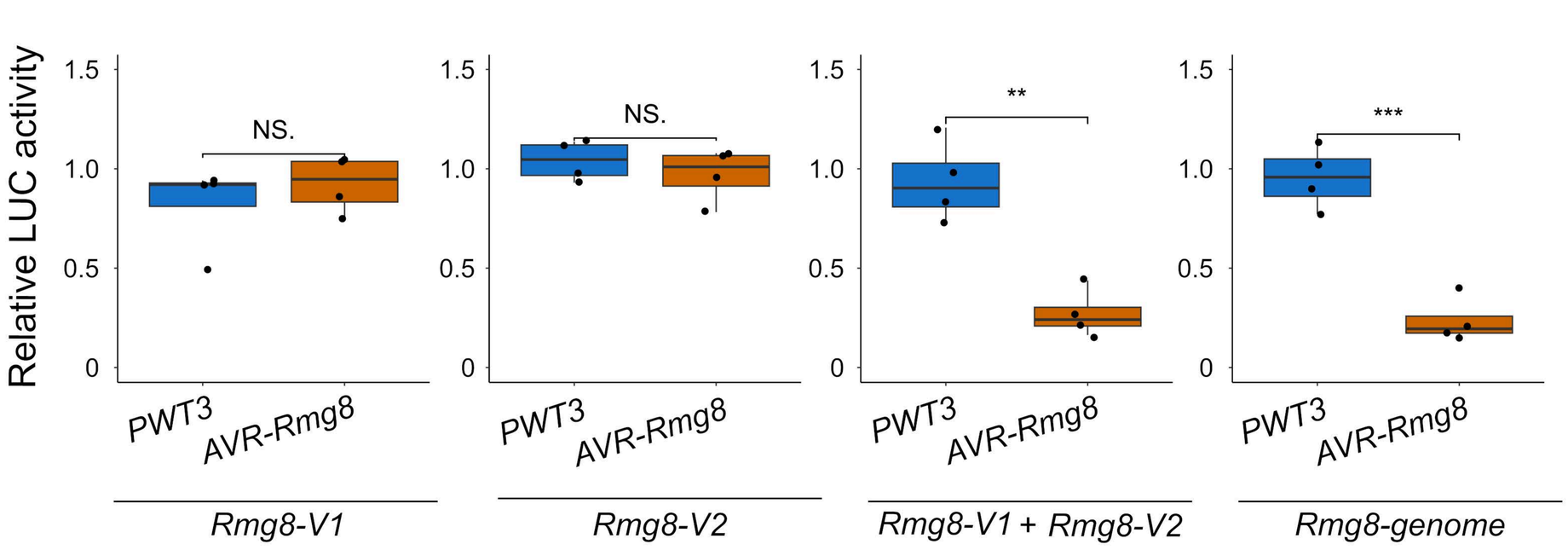
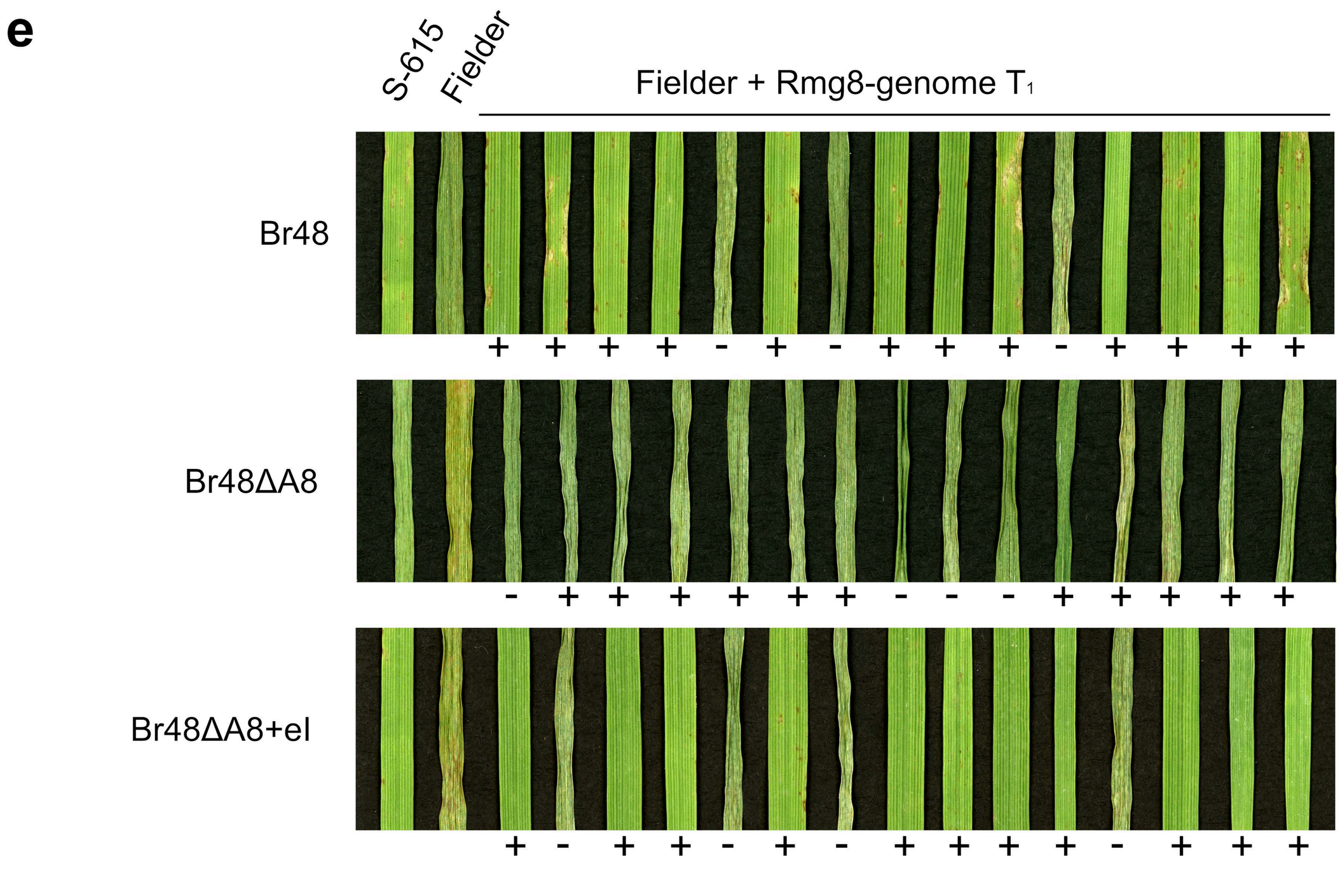

a
KM9 (12)
KM12 (9)
KM13 (8)
KM65 (5)
KM155 (4)
KM25 (3)
Rmg8
Sch x S-615
F2:3 165 lines
CS ver. 2.1
chr. 2BL
telomere
800
805
810
(Mbp)


b
Wheat lines recognizing AVR-Rmg8
Wheat lines with no response to AVR-Rmg8
(bp)
650
500
S-615
GR119
GR246
GR250
GR341
IL50
IL92
IL131
IL132
IL186
IL191
CP19
CP20
CP21
CP26
CP27
CP30
CP61
CP71
CP73
P168
Chinese Spring
Salmon
Jones Fife
Selkirk
No. 44
Shin-chunaga
Norin 10
Norin 4
Timstein
Cheyenne
Red Egyptian
Thatcher
Chancellor
Renown
Spica
Timvera
Transfed
Normandie
Hope


c
Rmg8 (Pm4f)
5'
3'
KM171
KM200
Rmg8-V1
Rmg8-V2
(Can-I)
0
2
4
6
8
(kbp)


d
Relative LUC activity
1.5
1.0
0.5
0
NS.
NS.
**
***
PWT3
AVR-Rmg8
Rmg8-V1
Rmg8-V2
Rmg8-V1 + Rmg8-V2
Rmg8-genome


e
S-615
Fielder
Fielder + Rmg8-genome T1
Br48
Br48ΔA8
Br48ΔA8+el
+ + + + - + - + + + - + + + +
- + + + + + + - - - + + + + +
+ - + + - + - + + + + - + + +

a

St24 (*Rmg7*) x Tat14 (*rmg7*)

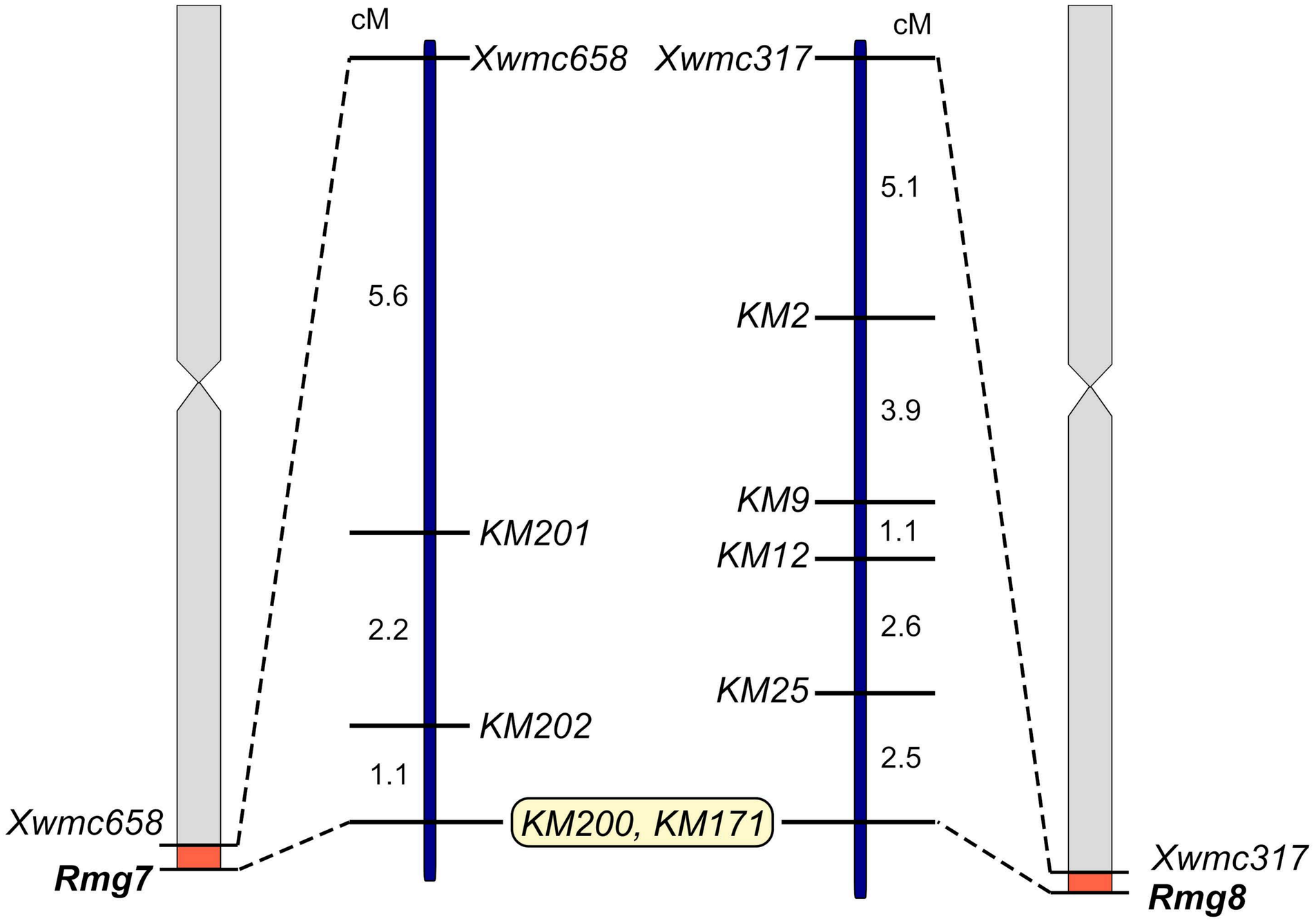
Tetraploid wheat

Chr. 2A

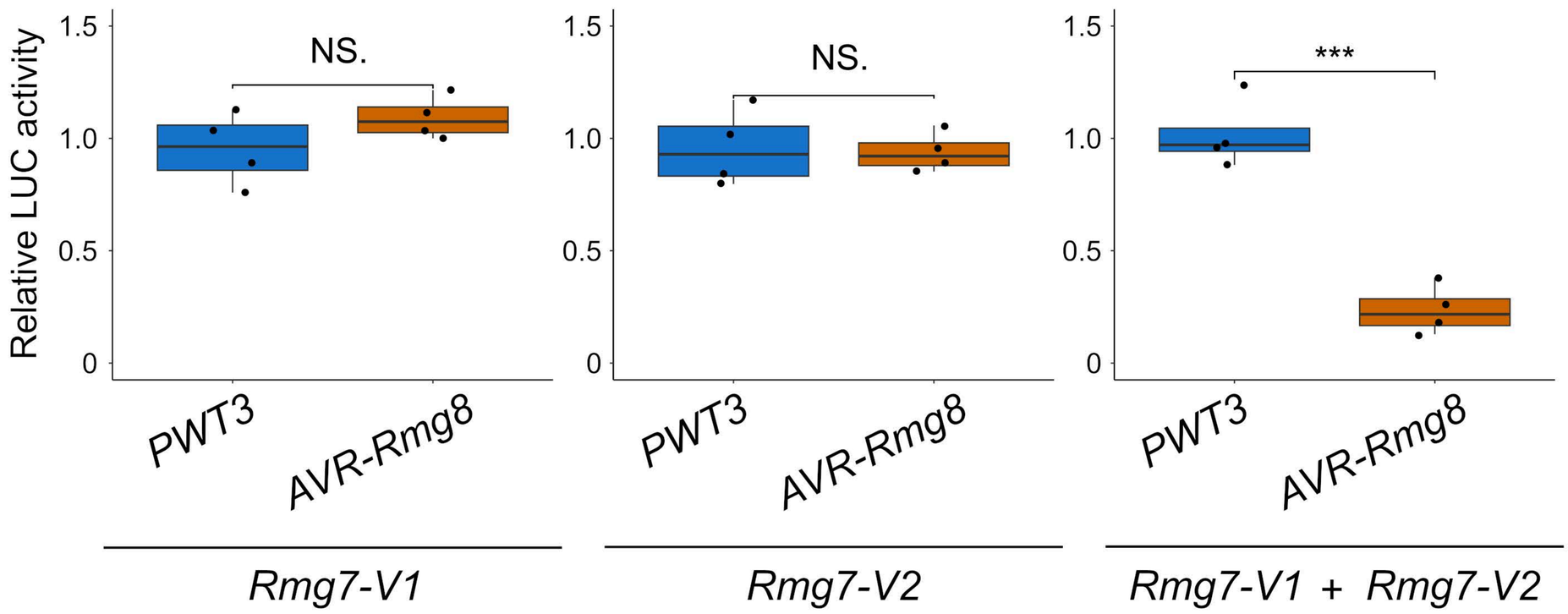
Sch (*rmg8*) x S-615 (*Rmg8*)

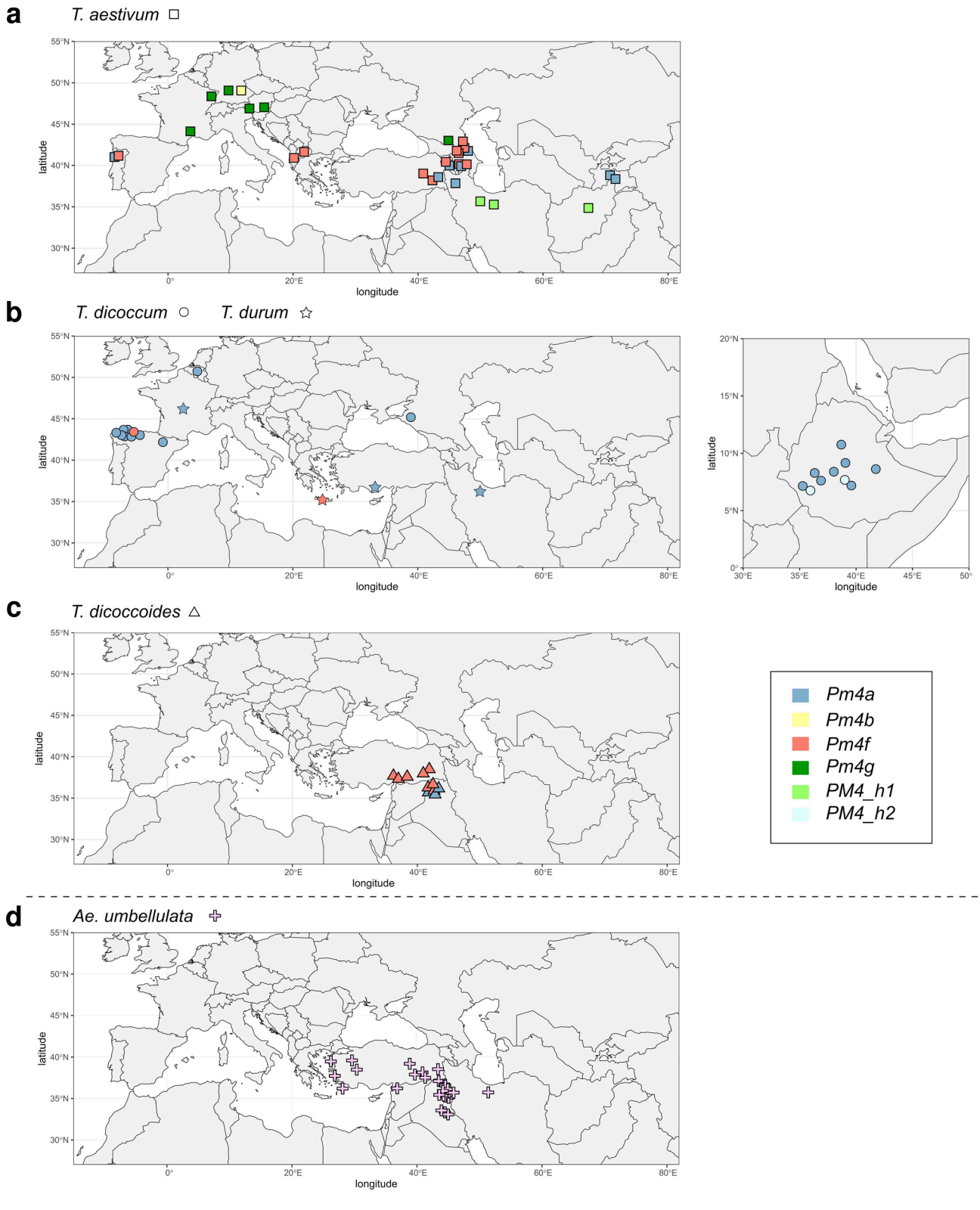
Hexaploid wheat

Chr. 2B

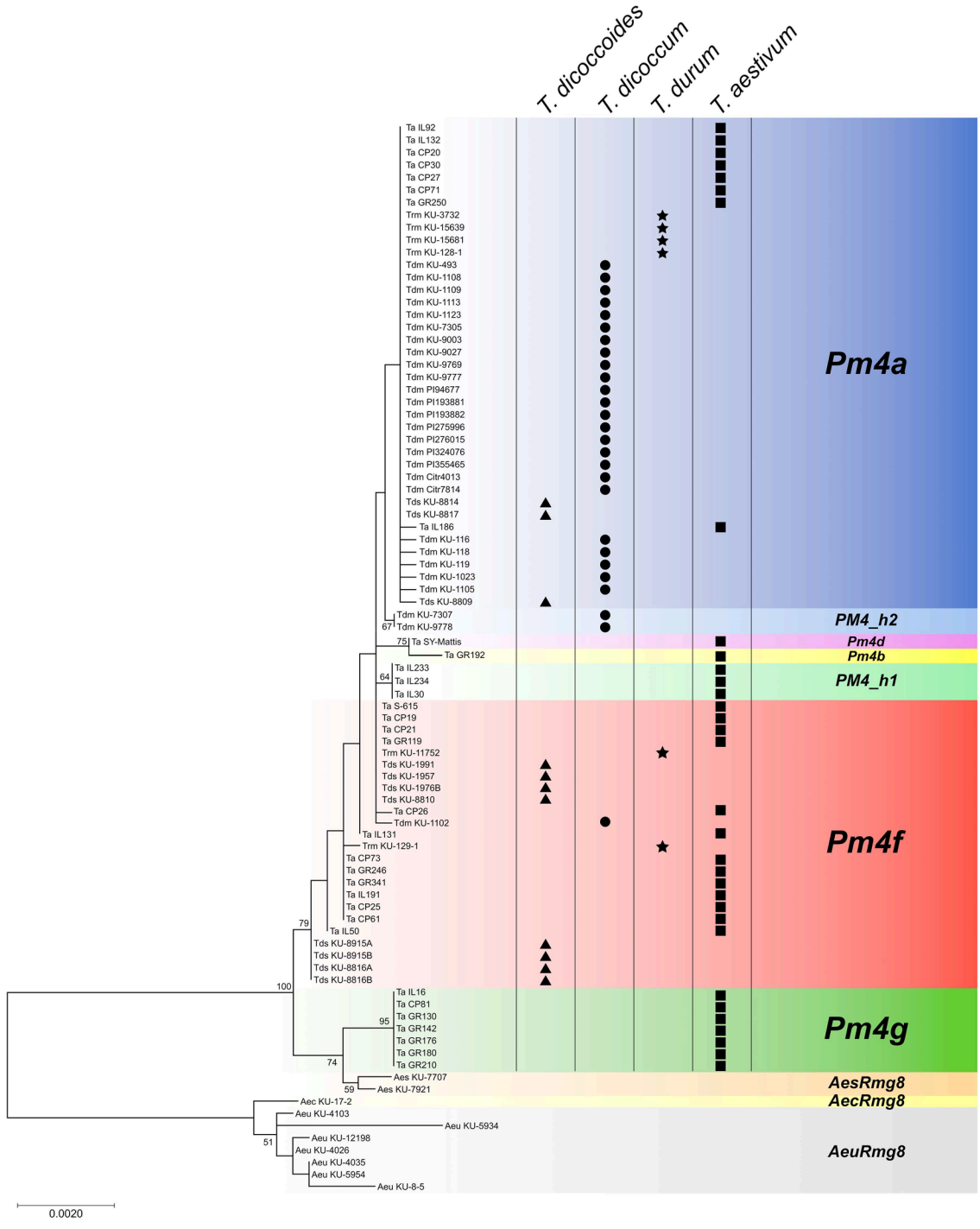


b




a
T. aestivum □
b
T. dicoccum ○
T. durum ☆
c
T. dicoccoides △
d
Ae. umbellulata
latitude
longitude
55°N
50°N
45°N
40°N
35°N
30°N
0°
20°E
40°E
60°E
80°E
20°N
15°N
10°N
5°N
30°E
35°E
40°E
45°E
50°
Pm4a
Pm4b
Pm4f
Pm4g
PM4_h1
PM4_h2

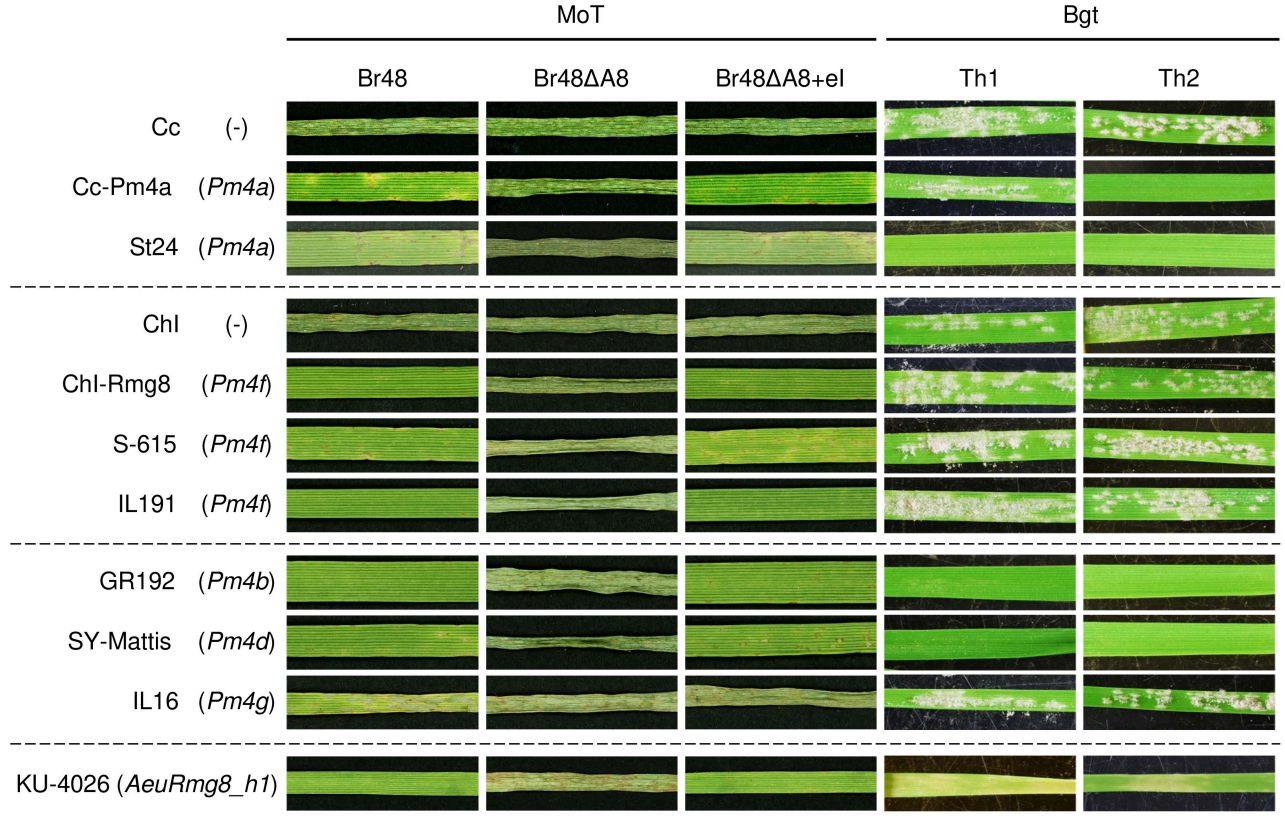
a

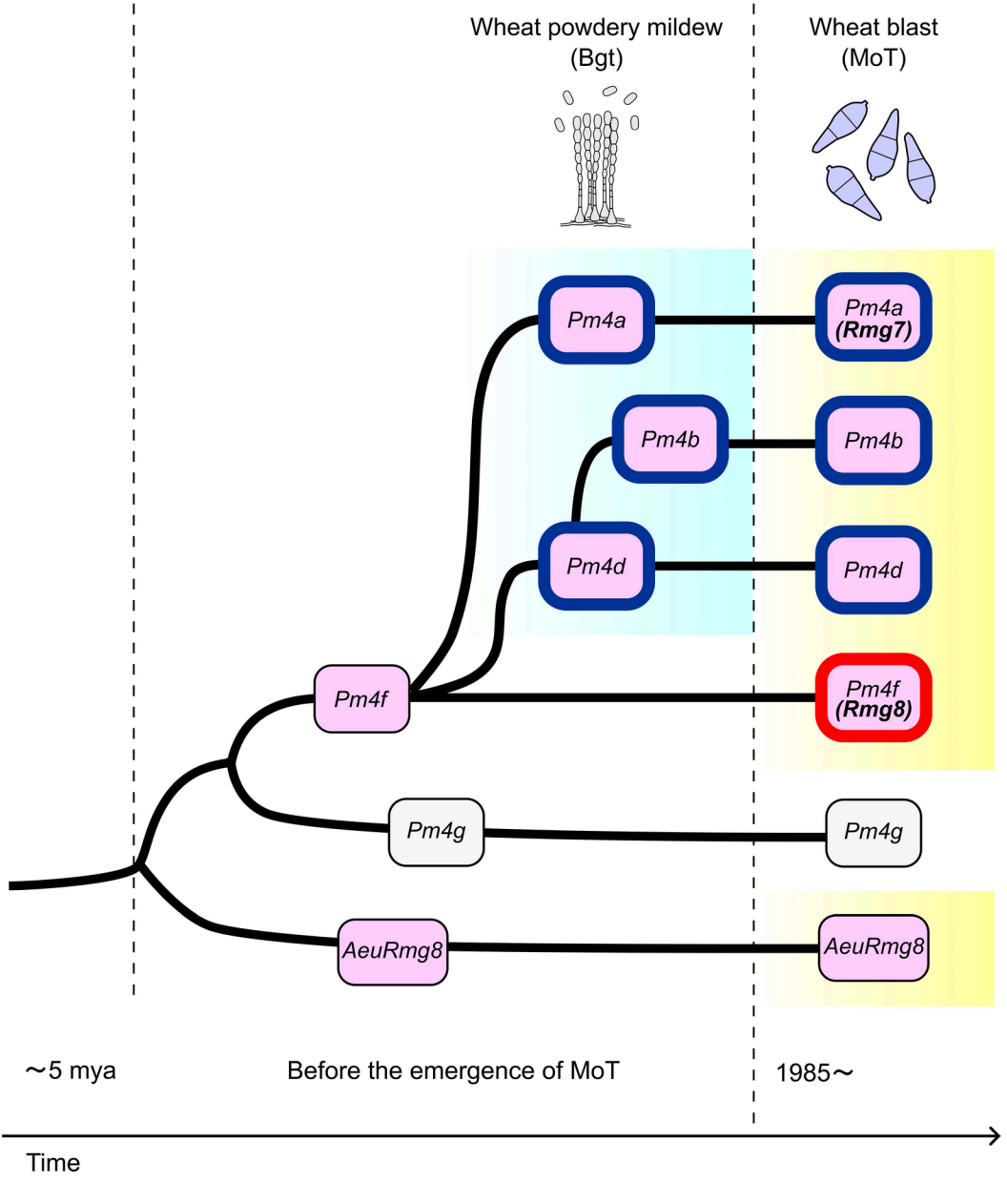


b

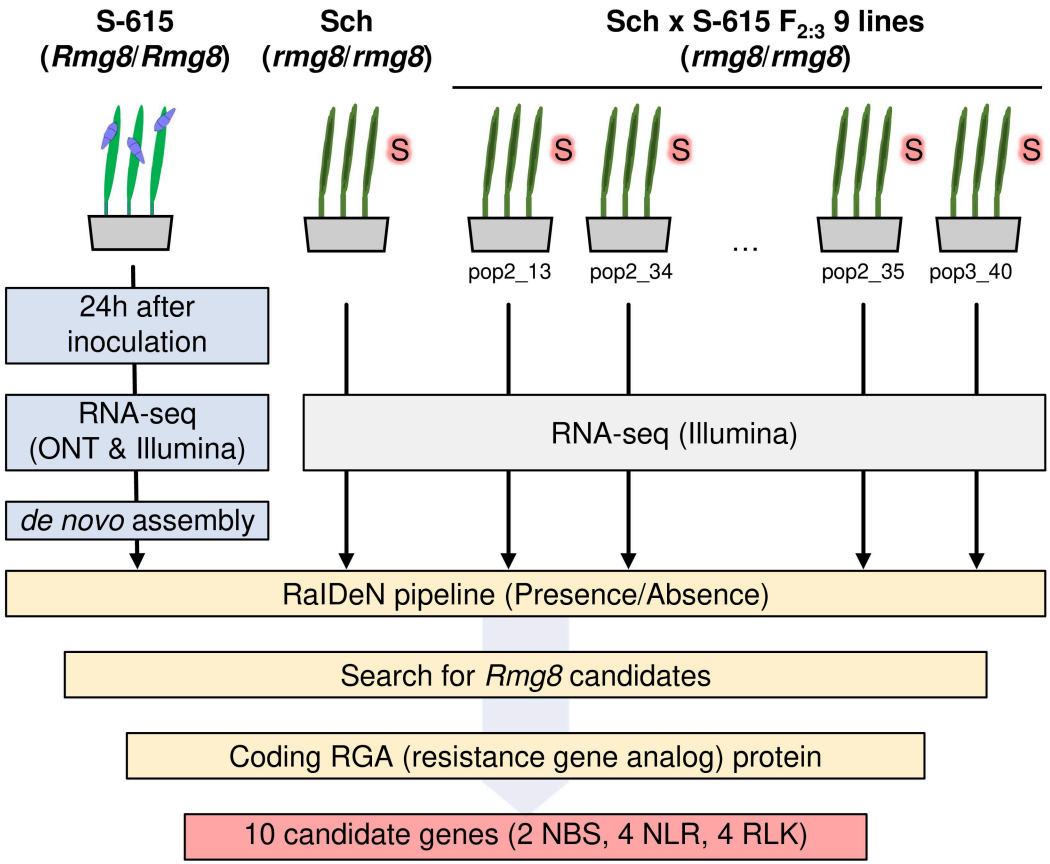
	Kinase				V1																						V2									
	1	2	2	2	3	3	3							3	3	3	3	3	3	3	4	4	4	5	5	5	5	5	5	3	3	4	4	4	5	5	6	6	7
	2	0	0	5	2	3	3	352 - 353						6	6	7	7	7	7	8	0	2	7	0	0	1	3	4	4	5	6	6	6	6	9	2	2	8	8	1
	6	5	8	5	1	2	5							2	9	0	1	2	3	4	1	0	1	3	6	7	2	7	8	9	7	3	9	2	5	6	2	8	9	2	6	3
Pm4f	E	K	L	R	V	T	S	–	–	–	–	–	–	V	M	K	L	R	L	P	G	Y	L	D	–	–	V	S	H	L	L	Q	L	D	–	P	P	A	L	S	V	A
Pm4a	.	.	W	.	.	.	.	–	–	–	–	–	–	.	.	.	.	.	.	.	.	.	.	.	–	–	.	.	.	.	.	.	.	.	–	.	.	.	.	.	.	.
Pm4b	.	E	.	.	.	.	.	–	–	–	–	–	–	.	.	.	.	.	.	.	.	.	.	.	–	–	.	.	.	.	.	.	.	.	–	.	.	.	.	.	.	G
Pm4d	.	E	.	.	.	.	.	–	–	–	–	–	–	.	.	.	.	.	.	.	.	.	.	.	–	–	.	.	.	.	.	.	.	.	–	.	.	.	.	.	.	.
Pm4g	K	.	.	.	.	.	.	–	–	–	–	–	–	.	.	.	.	.	.	.	.	.	.	.	–	–	.	.	.	.	.	.	.	.	–	.	.	.	V	.	A	.
PM4_h1	.	.	.	.	.	.	.	–	–	–	–	–	–	.	.	.	.	.	.	.	.	P	.	.	–	–	.	.	.	.	.	.	.	.	–	.	.	.	.	.	.	.
PM4_h2	.	.	S	.	.	.	.	–	–	–	–	–	–	.	.	.	.	.	.	.	.	.	.	.	–	–	.	.	.	.	.	.	.	.	–	.	.	.	.	.	.	.
AesRmg8_h1	.	.	.	.	.	.	.	–	–	–	–	–	–	.	.	.	.	.	.	.	.	.	.	.	–	–	.	.	R	.	.	.	.	.	–	.	.	.	V	.	.	.
AecRmg8_h1	.	.	.	K	A	S	T	D	P	P	E	M	V	I	L	–	–	–	–	–	–	A	H	.	E	C	D	E	P	R	.	.	H	H	H	A	A	.	–	–	T	.
AeuRmg8_h1	.	.	.	K	A	S	T	D	P	P	E	M	V	I	L	–	–	–	–	–	–	A	H	.	E	C	D	E	P	R	.	V	H	H	.	A	A	.	G	V	T	.

b


MoT
Bgt
Br48
Br48ΔA8
Br48ΔA8+el
Th1
Th2
Cc (-)
Cc-Pm4a (Pm4a)
St24 (Pm4a)
ChI (-)
ChI-Rmg8 (Pm4f)
S-615 (Pm4f)
IL191 (Pm4f)
GR192 (Pm4b)
SY-Mattis (Pm4d)
IL16 (Pm4g)
KU-4026 (AeuRmg8_h1)


Wheat powdery mildew
(Bgt)
Wheat blast
(MoT)
Pm4a
Pm4a
(Rmg7)
Pm4b
Pm4b
Pm4d
Pm4d
Pm4f
Pm4f
(Rmg8)
Pm4g
Pm4g
AeuRmg8
AeuRmg8
~5 mya
Before the emergence of MoT
1985~
Time

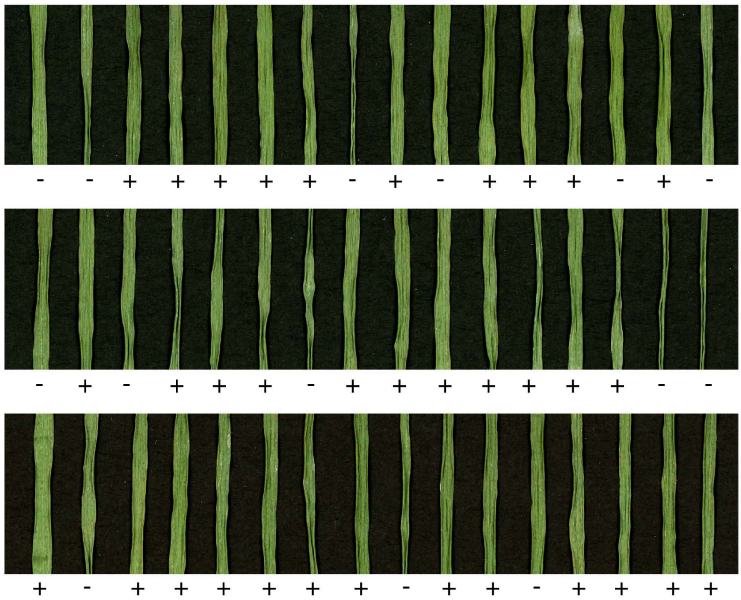
a



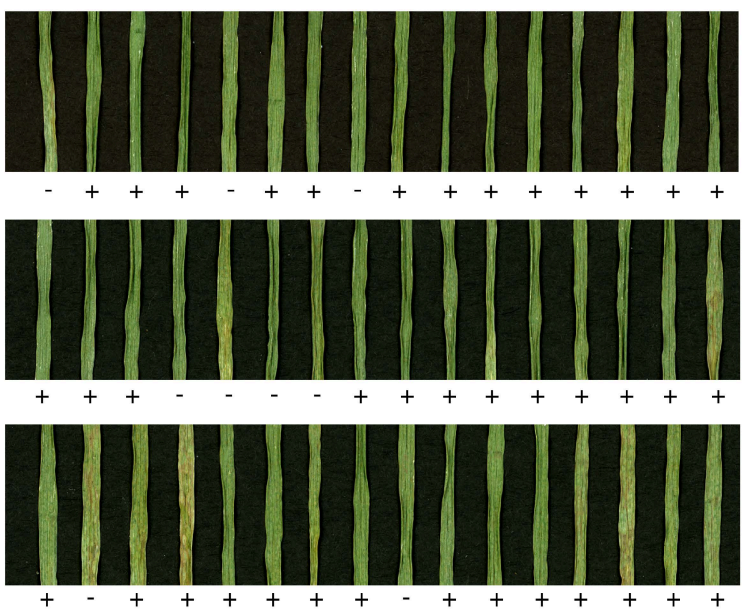
b

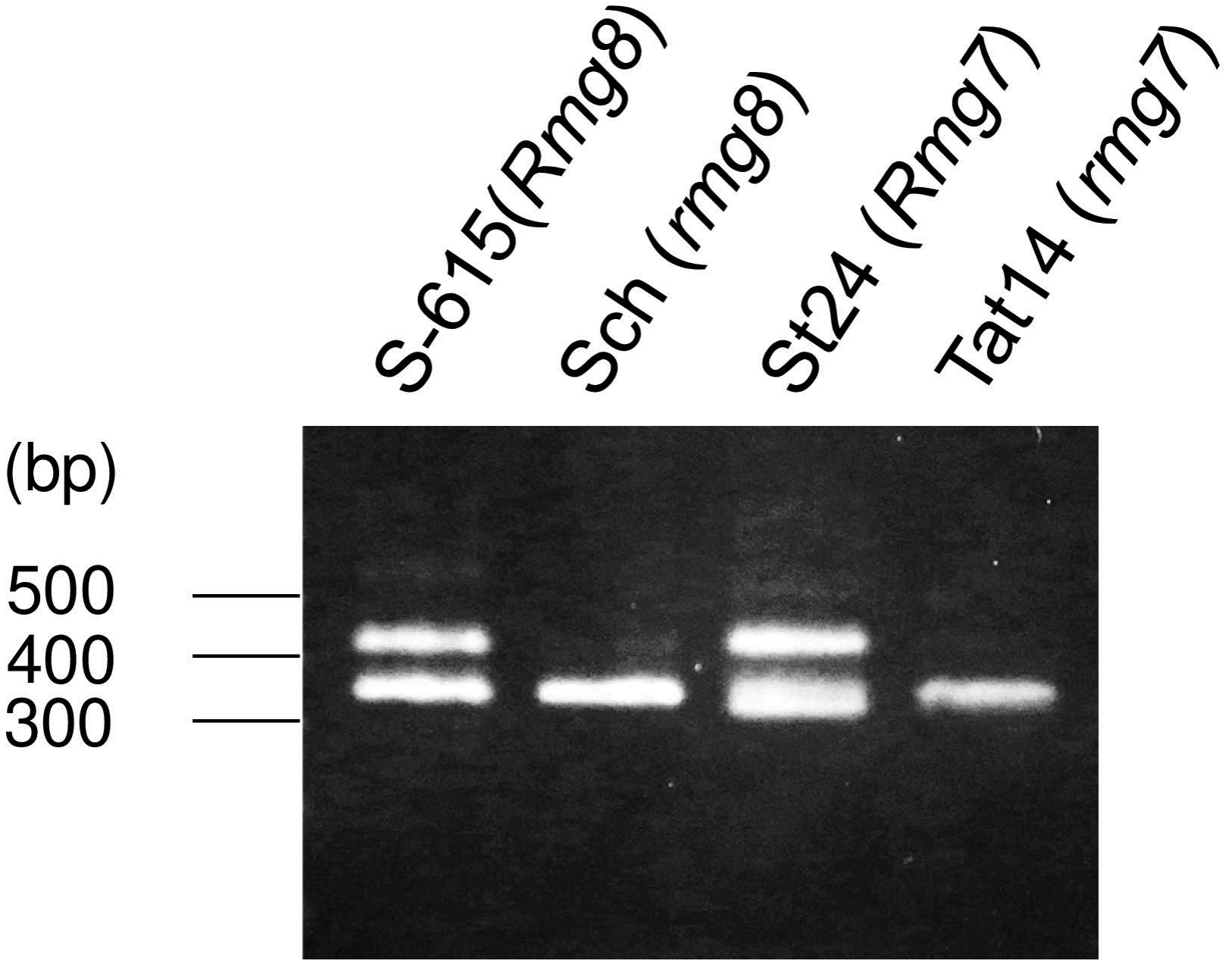
ID	ORF name	Domain prediction	Peptide homolog in *T. turgidum* cv. Svevo	Chromosome	Mapping with Sch × S-615 $F_{2:3}$		Association
					Marker	Co-segregation with *Rmg8*	
Can-A	NODE_34172	NBS	TRITD2Bv1G265560	2B	KM30, KM109	Yes	No
Can-B	NODE_47471	NBS	TRITD2Bv1G264480	2B	KM27	Yes	No
Can-C	NODE_2794	NLR	TRITD2Bv1G265560	2B	KM30, KM109	Yes	No
Can-D	NODE_43629	NLR	TRITD2Bv1G264480	2B	KM27	Yes	No
Can-E	NODE_44374	NLR	TRITD6Bv1G223090	6B	-	-	-
Can-F	NODE_13794	NLR	TRITD2Bv1G264470	2B	KM138, KM140	Yes	No
Can-G	NODE_6206	RLK	TRITD4Av1G219830	4A	-	-	-
Can-H	NODE_12802	RLK	TRITD3Av1G028450	3A	-	-	-
Can-I	**NODE_16006**	**RLK**	**TRITD2Bv1G265720**	**2B**	**KM171**	**Yes**	**Yes**
Can-J	NODE_8088	RLK	TRITD3Av1G247380	3A	-	-	-

Fielder + Rmg8-V1 T_1



Fielder + Rmg8-V2 T_1




S-615(Rmg8)
Sch (rmg8)
St24 (Rmg7)
Tat14 (rmg7)
(bp)
500
400
300

Extended Data Table 1. *Rmg8* variants detected in local landraces of common wheat

Accession[a]	Origin	Donor[b]	Accession No.	*Rmg8* variant	Infection type[c] with Br48	Br48ΔA8	Br48ΔA8+eI	Recognition of *AVR-Rmg8*
GR119*	Albania	JIC	W7880	*Pm4f*	0	2B	0	+
IL191*	Armenia	NBRP	KU-1649	*Pm4f*	0	5G	0	+
CP19*	Azerbaijan	VIR	WIR-39310	*Pm4f*	1B	5G	0	+
CP21*	Azerbaijan	VIR	WIR-39844	*Pm4f*	0-1B	5G	0-1B	+
CP25	Azerbaijan	VIR	WIR-39934	*Pm4f*	1B	5G	0-1B	+
CP26*	Azerbaijan	VIR	WIR-40184	*Pm4f*	1B	5G	0	+
CP61*	Daghestan, Russia	VIR	WIR-10124	*Pm4f*	1-2B	5G	0	+
CP73*	Daghestan, Russia	VIR	WIR-23924	*Pm4f*	1B	5G	0	+
GR246*	Portugal	CGN	WAG6097	*Pm4f*	1B	4-5G	0	+
IL50*	Turkey	NBRP	KU-10302	*Pm4f*	0	5G	0	+
IL131*	Turkey	NBRP	KU-10316	*Pm4f*	0	4G	0	+
GR341	North Macedonia	CGN	CGN04172	*Pm4f*	1B	2-3BG	0-1B	+
IL186*	Armenia	NBRP	KU-1588	*Pm4a*	1B	5G	0	+
CP20*	Azerbaijan	VIR	WIR-39311	*Pm4a*	0	4G	0	+
CP71*	Daghestan, Russia	VIR	WIR-23900	*Pm4a*	1B	5G	0	+
IL92*	Iran	NBRP	KU-3282	*Pm4a*	0-1B	5G	0	+
GR250*	Portugal	CGN	CGN06373	*Pm4a*	1B	4G	0-1B	+
CP27*	Tajikistan	VIR	WIR-24592	*Pm4a*	0	4G	0	+
CP30*	Tajikistan	VIR	WIR-24599	*Pm4a*	0-1B	3-4G	0	+
IL132*	Turkey	NBRP	KU-10337	*Pm4a*	1B	4G	0-1B	+
GR192	Germany	CRI	01C010671	*Pm4b*	1B	5G	0-1B	+
GR130	Austria	CGN	CGN05497	*Pm4g*	3G	3G	3G	-
GR142	Austria	CGN	CGN14905	*Pm4g*	5G	5G	5G	-
GR176	France	CGN	CGN04348	*Pm4g*	5G	5G	5G	-
GR180	France	CGN	CGN05365	*Pm4g*	3G	3G	3G	-
GR210	Germany	CGN	WAG5659	*Pm4g*	3-4G	3-4G	3-4G	-
CP81	North Ossetia, Russia	VIR	WIR-14683	*Pm4g*	4G	4G	4G	-
IL16	USA	NBRP	KU-306	*Pm4g*	3-4G	4G	4G	-
IL30	Afghanistan	NBRP	KU-3074	*PM4_h1*	5G	5G	5G	-
IL233	Iran	NBRP	KU-4315	*PM4_h1*	3G	3-4G	3-4G	-
IL234	Iran	NBRP	KU-4335B	*PM4_h1*	3G	3-4G	3-4G	-

[a] Dark gray: accessions reported to carry a single resistance gene at the *Rmg8* locus on 2BL through F_2 segregation analyses by Inoue et al.[23]
Light gray: accessions subjected to F_2 segregation analyses in the present study. (See Extended Data Table 2.)
Asterisk: The 18 accessions reported to recognize *AVR-Rmg8* by Wang et al.[16]

[b] NBRP, National BioResource Project –Wheat, Japan (https://shigen.nig.ac.jp/wheat/komugi/strains/queryFormNbrp.jsp);
VIR, The N.I.Vavilov All-Russian Institute of Plant Genetic Resources (http://91.151.189.38/virdb/maindb);
JIC, John Innes Centre (https://www.seedstor.ac.uk/index.php);
CGN, Centre for Genetic Resources, the Netherlands (https://cgngenis.wur.nl/SearchDetails).
CRI, Crop Research Institute, Czech Republic

[c] 0 = no visible infection; 1 = pinhead spots; 2 = small lesions (<1.5 mm); 3 = scattered lesions of intermediate size (<3 mm); 4 = large typical lesions; and 5 = complete blighting of leaf blades. B and G represents brown and green lesions, respectively.
0-2BG, resistant; 2-3BG, moderately or weakly resistant; 3G-5G, susceptible.

Extended Data Table 2. Segregation of reactions to Br48 in F_2 populations derived from crosses between wheat lines.

Cross[a]	Number of F_2 seedlings			$\chi 2$ (15:1)	P
	Resistant	Susceptible	Total		
S-615 (*Pm4f*) x IL186 (*Pm4a*)	183	9	192	0.80	0.37
S-615 (*Pm4f*) x CP20 (*Pm4a*)	164	11	175	0.00	0.98

[a] *Rmg8* variants carried by the wheat lines are shown in parentheses.

Extended Data Table 3. Distribution of *Rmg8* variants in *Triticum* spp.

Species	Region	Number of accessions							
		Pm4a	*Pm4b*	*Pm4f*	*Pm4g*	*PM4_h1*	*PM4_h2*	Total of carriers	Total of accessions tested
T. aestivum	Total	8 [1.5%]	1 [0.2%]	12 [2.3%]	7 [1.3%]	3 [0.6%]	0 [0%]	31 [5.9%]	526
	Europe, Middle East, and Africa	7	1	12	6	3	0	29	336
	Central Asia and Eastern Asia	1	0	0	0	0	0	1	145
	Americas	0	0	0	1	0	0	1	45
T. durum	Total	4 [5.6%]	0 [0%]	2 [2.8%]	0 [0%]	0 [0%]	0 [0%]	6 [8.3%]	72
	Europe	1	0	1	0	0	0	2	29
	Middle East	2	0	0	0	0	0	2	28
	Ethiopia	0	0	0	0	0	0	0	6
	Central Asia and Eastern Asia	1	0	0	0	0	0	1	5
	Unknown	0	0	1	0	0	0	1	4
T. dicoccum	Total	24 [31.6%]	0 [0%]	1 [1.3%]	0 [0%]	0 [0%]	2 [2.6%]	27 [35.5%]	76
	Europe Spain	8	0	1	0	0	0	9	16
	Others [a]	2	0	0	0	0	0	2	25
	Middle East	0	0	0	0	0	0	0	12
	Ethiopia	8	0	0	0	0	2	10	12
	India	3	0	0	0	0	0	3	5
	Unknown	3	0	0	0	0	0	3	6
T. paleocolchicum	Total	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	4
T. dicoccoides	Total [b]	3 [6.5%]	0 [0%]	8 [17.4%]	0 [0%]	0 [0%]	0 [0%]	11 [23.9%]	46

[a] Including accessions from Portugal, Italy, Belgium, Germany, Czech, Austria, Hungary, Bosnia and Herzegovina, Yugoslavia, Bulgaria, Romania, Russia, Armenia, Georgia, and Morocco.

[b] All accessions are from Fertile Crescent.

Extended Data Table 4. Reactions of representative accessions of *Ae. umbellulata*, *Ae. comosa*, and *Ae. speltoides* to Br48 and its *AVR-Rmg8* disruptant (Br48ΔA8)

Species	Accession[a]	Infection type[b] with		Recognition of
		Br48	Br48ΔA8	*AVR-Rmg8*
Ae. umbellulata	KU-8-4	0-1B	5G	+
	KU-8-5	0	5G	+
	KU-2737	1B	5G	+
	KU-2738	0	5G	+
	KU-4002	1B	5G	+
	KU-4005	1B	5G	+
	KU-4006	1B	5G	+
	KU-4009	0	5G	+
	KU-4013	0	5G	+
	KU-4014	0	5G	+
	KU-4018	0	5G	+
	KU-4026	0	5G	+
	KU-4035	0	5G	+
	KU-4036	0	5G	+
	KU-4040	0-1B	3BG	+
	KU-4054	0	5G	+
	KU-4056	1B	5G	+
	KU-4061	1B	5G	+
	KU-4072	0	5G	+
	KU-4077	0	5G	+
	KU-4085	0-1B	5G	+
	KU-4103	0	5G	+
	KU-4108	1B	5G	+
	KU-5954	0	5G	+
	KU-12198	0	5G	+
	KU-12204	0	5G	+
	KU-12207a	1B	5G	+
	KU-4020	5G	5G	-
	KU-4045	5G	5G	-
	KU-12182	5G	5G	-
	KU-12191	5G	5G	-
Ae. comosa	KU-17-2	1B	5G	+
Ae. speltoides	KU-2246	1B	3G	+
	KU-2247	2B	4G	+
	KU-2251	1B	3G	+
	KU-7707	2B	4G	+
	KU-7915	3BG	5G	+
	KU-7921	1B	5G	+
	KU-12962	2BG	5G	+
	KU-2248	1B	1B	-
	KU-2259	1B	1B	-
	KU-12016	2B	2B	-

[a] All accessions that recognized *AVR-Rmg8* are shown with some examples that did not recognize *AVR-Rmg8*, i.e., four susceptible accessions of *Ae. umbellulata* used for crossing in Extended Data Table 6 and three resistant accessions of *Ae. speltoides* whose resistance was not impaired by the disruption of *AVR-Rmg8*.

[b] Refer to Extended Data Table 1. 0-2BG, resistant; 3BG, weakly resistant; 3G-5G, susceptible.

Extended Data Table 5. Distribution of *Rmg8* variants in *Aegilops* spp.

Species	Genome	Number of accessions		
		Resistant to Br48	Recognizing *AVR-Rmg8*	Total
Ae. umbellulata	UU	27	27	204
Ae. comosa	MM	1	1	4
Ae. speltoides	SS	18	7[a]	139
Ae. tauschii	DD	13	0	204
Ae. caudata	CC	2	0	247
Ae. uniaristata	NN	0	0	11
Ae. longissima	S^lS^l	2	0	33
Ae. sharonensis	$S^{sh}S^{sh}$	4	0	15
Ae. searsii	S^sS^s	0	0	19
Ae. bicornis	S^bS^b	0	0	33
Total	-	67	35	909

[a] Among the 18 accessions resistant to Br48, eight accesssions have not been tested with Br48ΔA8 because sufficient seeds were not available. The seven accessions recognizing *AVR-Rmg8* were detected in the other 10 accessions with sufficient seeds.

Extended Data Table 6. Segregation of reactions to Br48 in F_2 populations derived from crosses between *Aegilops umbellulata* accessions.

Cross[a]	Number of F_2 seedlings			χ^2 (3:1)	P
	Resistant	Susceptible	Total		
KU-4013 (R) x KU-12182 (S)	29	9	38	0.04	0.85
KU-4013 (R) x KU-4045 (S)	116	34	150	0.44	0.51
KU-4014 (R) x KU-12182 (S)	32	8	40	0.53	0.47
KU-4014 (R) x KU-4045 (S)	81	31	112	0.43	0.51
KU-4036 (R) x KU-12182 (S)	26	12	38	0.88	0.35
KU-4036 (R) x KU-4045 (S)	30	8	38	0.32	0.57
KU-2737 (R) x KU-4020 (S)	28	8	36	0.15	0.70
KU-4054 (R) x KU-4020 (S)	34	6	40	2.13	0.14
KU-4085 (R) x KU-12191 (S)	29	8	37	0.23	0.64
KU-4013 (R) x KU-4014 (R)	70	0	70	–	–
KU-4036 (R) x KU-4014 (R)	73	0	73	–	–
KU-2737 (R) x KU-4054 (R)	39	0	39	–	–
KU-4054 (R) x KU-4085 (R)	33	0	33	–	–

[a] Reactions of parental accessions to Br48 are shown in parentheses. R, resistant; S, susceptible.

Extended Data Table 7. Reactions of *Triticum aestivum* lines and *Aegilops umbellulata* accessions to wheat powdery mildew isolates collected in Japan.

			Infection type[a] on									
			T. aestivum[b]							*Ae. umbellulata*[b]		
			N4	ChI	**ChI-Rmg8**	Cc	**Cc-Pm4**	IL16	GR142	**KU-4026**	**KU-4013**	KU-4020	KU-4043
Isolates	Year	Location	(-)	(-)	(*Pm4f*)	(-)	(*Pm4a*)	(*Pm4g*)	(*Pm4g*)	(*AeuRmg8_h1*)	(nd)	(nd)	(nd)
Th1	2005	Matsusaka City, Mie	4	4	4	4	4	4	4	4	4	4	4
Th2	2021	Kyoto City, Kyoto	4	4	4	4	0	4	4	4	3	4	4
Th3	2021	Chikugo City, Fukuoka	4	4	4	4	4	3	3	4	3	4	4
Th4	2021	Chikugo City, Fukuoka	4	4	4	4	4	4	4	4	3	4	4
Th5	2021	Ichikawa-cho, Hyogo	4	4	4	4	4	4	4	4	3	4	4
Th6	2021	Kasai City, Hyogo	4	4	4	4	4	4	4	3	3	3	3
Th7	2021	Tsukuba City, Ibaraki	4	4	4	4	4	4	4	4	3	4	4
Th8	2021	Tsukuba City, Ibaraki	4	4	4	4	4	4	4	4	3	4	4
Th9	2021	Tsukuba City, Ibaraki	4	4	4	4	4	4	4	4	4	4	4
Th10	2021	Tsukuba City, Ibaraki	4	4	4	4	4	4	3	4	4	4	4
Th12	2021	Kobe City, Hyogo	4	4	4	4	4	4	4	4	3	4	4
Th13	2021	Muko City, Kyoto	4	4	4	4	4	4	4	3	3	3	4
Th14	2021	Muko City, Kyoto	4	4	4	4	4	4	4	4	4	4	4
Th15	2021	Obihiro City, Hokkaido	4	4	4	4	0	4	4	4	4	4	4

[a] 0, no mycelial growth or sporulation; 1, scant sporulation; 2, reduced sporulation; 3, slightly reduced sporulation; 4, heavy sporulation.

[b] Lines/accessions resistant to MoT isolate Br48 are shown in bold. *Rmg8* variants carried by them are shown in parentheses. nd, not determined.