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Evaluation of durability of blast resistance gene *Rmg8* in common wheat based on analyses of its corresponding avirulence gene

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The nucleotide sequence data reported are available in the DDBJ/EMBL/GenBank databases under the accession numbers, LC535796 (eL1), LC535797 (eL2), and LC535798 (eL3).

Abstract

Wheat blast caused by the *Triticum* pathotype of *Pyricularia oryzae* is a serious threat to wheat production in South America and Asia. *Rmg8* is a promising gene for resistance to the wheat blast fungus found in common wheat. To predict its durability, stability of its corresponding avirulence gene, *AVR-Rmg8*, was evaluated based on (i) a genome structure around *AVR-Rmg8* in a *Triticum* isolate, and (ii) polymorphisms of *AVR-Rmg8* in *Lolium* isolates, a close relative of *Triticum* isolates. *AVR-Rmg8* was located in a gene-rich and repeat-poor region, suggesting that *AVR-Rmg8* is not so easily lost as avirulence genes located in repeat-rich regions. This implies that *Rmg8* is relatively durable compared with resistance genes corresponding to avirulence genes located in repeat-rich regions. *AVR-Rmg8* was widely distributed in *Lolium* isolates, and divided into three types, eL1, eL2, and eL3. Complementation tests revealed that eL1 and eL2 were functional and recognized by *Rmg8*. In eL3, however, retrotransposon Pyret was inserted into the ORF of *AVR-Rmg8*. Actually, three isolates carrying eL3 were virulent on common wheat cultivar S-615 carrying *Rmg8*, indicating that the insertion of Pyret caused a loss of function of *AVR-Rmg8*. Nevertheless, the three isolates have survived in nature, suggesting that *AVR-Rmg8* is not indispensable for survival of *Pyricularia* isolates, and in turn that *Rmg8* is not likely to be completely durable.

Key words *Pyricularia oryzae*, *Magnaporthe oryzae*, ryegrass, wheat, avirulence gene

Introduction

Pyricularia oryzae (Zhang et al. 2016, syn. *Magnaporthe oryzae*), a causal agent of blast disease of gramineous plants, consists of several subgroups or pathotypes, each of which is pathogenic on a plant genus, e.g., *Oryza* (including rice), *Setaria* (including foxtail millet), or *Eleusine* (including finger millet) (Kato et al. 2000). This fungal species has a potential to cause destructive epidemics on plant genera which have not been known as its primary hosts, and has evolved new pathotypes. An example is evolution of a pathotype specific to *Lolium* spp. A new disease of perennial ryegrass (*Lolium perenne*, abbreviated as prg in this article) called gray leaf spot was first reported in Pennsylvania in 1991 (Landschoot and Hoyland 1992), and then spread to other regions in the U.S. (Tosa et al. 2007). A few years later, a similar outbreak of this disease occurred on prg in Japan (Tosa et al. 2004). Tosa et al. (2004) reported that prg isolates belonged to *Magnaporthe oryzae* (= *P. oryzae*) and that those collected in Japan were divided into two populations, i.e., isolates from west Japan (WK isolates) which were considered to be indigenous to Japan, and those from east or north Japan (TALF isolates) which were inferred to be transmitted from the U.S. to Japan via prg seeds (Tosa et al. 2007). The TALF isolates were specifically pathogenic on *Lolium* spp., and therefore designated as *Lolium* pathotype (Tosa et al. 2004).

Another example is evolution of a pathotype pathogenic on *Triticum* spp. (*Triticum* pathotype). This devastating pathogen – the wheat blast fungus - was first reported in Brazil in 1985 (Urashima et al. 1993), and then spread to surrounding countries in South America (Cruz and Valent 2017). Inoue et al. (2017) suggested that the wheat blast fungus emerged through a loss of function of *PWT3*, a host specificity determinant corresponding to *Rwt3*, in *Lolium* isolates or their relatives. In 2011, a blast lesion was found on a wheat spike in a wheat trial plot at the University of Kentucky in the U.S. Its causal agent was not similar to *Triticum* isolates found in Brazil, but was very close to isolates from annual

ryegrass (*Lolium multiflorum*) prevailing in the U.S. Farman et al. (2017) suggested that this first occurrence of wheat blast in the U.S. was most likely caused by a strain that evolved from an endemic *Lolium*-infecting pathogen. *Lolium* isolates are now considered to be closely associated with the evolution of the wheat blast fungus. This seems reasonable because *Triticum* isolates are phylogenetically very close to *Lolium* isolates (Gladieux et al. 2018).

In 2016 wheat blast suddenly occurred in Bangladesh and caused severe yield losses (Callaway 2016). This was the first report of wheat blast epidemics in Asia. Phylogenetic analysis suggested that this outbreak was caused by a wheat blast lineage from South America (Islam et al. 2016; Malaker et al. 2016). There is a possibility that Bangladesh will be a center of spread of this destructive disease to Asian countries. To mitigate the damage by this pathogen, resistance genes are needed. Resistance genes against the wheat blast fungus must be effective at the heading stage and at high temperature because this disease occurs mainly on spikes and is severe at high temperature. Tagle et al. (2015) identified a resistance gene in tetraploid accessions and named it as *Rmg7*. This gene was expressed at the heading stage, but was not so effective at high temperature (Anh et al. 2018). Subsequently, Anh et al. (2015) identified a resistance gene in a hexaploid wheat cultivar, S-615, and named it as *Rmg8*. This gene was effective at the heading stage, and also showed resistance to some degree at high temperature. Recently, Wang et al. (2018) found a highly resistant common wheat accession, GR119, in a screening of ~500 local landraces. Genetic analysis revealed that GR119 carried a new resistance gene, tentatively designated as *RmgGR119*, in addition to *Rmg8*. The strong resistance of GR119 was attributed to additive effects of these two genes.

Resistance genes that are durable are the most desirable for breeding commercial cultivars. From this viewpoint, cloning of avirulence genes is important because durability of resistance genes may be predicted by estimating stability of their corresponding avirulence genes (Tosa and Chuma 2014). Anh et al. (2018) cloned *AVR-*

Rmg8, an avirulence gene corresponding to *Rmg8*, and showed that it was also recognized by *Rmg7*. Wang et al. (2018) found three types (eI, eII, and eII') of *AVR-Rmg8* in *Triticum* isolates collected in Brazil. The *AVR-Rmg8* gene cloned by Anh et al. (2018) was the eI type. The eII and eII' types were also inferred to be functional (Wang et al. 2018). On the other hand, only the eI type was detected in nucleotide sequences of Bangladeshi isolates in databases (Wang et al. 2018). Based on this result, *Rmg8* was suggested to be effective against wheat blast in Bangladesh (Wang et al. 2018).

Although the wheat blast population in Bangladesh is considered to be clonally derived from a single strain (Cruz and Valent 2017) and to be uniform at present, there is a possibility that it may differentiate into lineages with different mutations at the *AVR-Rmg8* locus in future. To predict possible modes of differentiation or mutations of *AVR-Rmg8* in future, we examined (i) a genome structure around *AVR-Rmg8* in a *Triticum* isolate, and (ii) polymorphisms of *AVR-Rmg8* in *Lolium* isolates, a close relative of *Triticum* isolates.

Materials and methods

Fungal materials

Pyricularia oryzae isolates tested were listed in Table 1. Br5, Br48, and Br116.5 were representative *Triticum* isolates carrying the eI, eII, and eII' types of *AVR-Rmg8*, respectively. They were collected in Brazil in 1990-1992. The others were *Lolium* isolates collected in Japan in 1996-1999. TALF (TP-, AK-, LW-, FI-) and WK- isolates were isolated from *Lolium perenne* (prg) (Tosa et al. 2004) while M99- and Y99- isolates were isolated from *Lolium multiflorum* (Italian ryegrass) (Kusaba et al. 2006). Br48ΔA8_d6 (Br48ΔA8 hereafter), an *AVR-Rmg8* disruptant of Br48 (Wang et al. 2018), was used as a recipient of *AVR-Rmg8* homologs cloned from *Lolium* isolates. These strain/isolates

have been maintained on sterilized barley grains under dry conditions at 4°C in Kobe University.

Plants materials

Plant materials used were *Triticum aestivum* (L.) Thell. accession GR119 (*Rmg8/RmgGR119*), cv. S-615 (*Rmg8/-*), cv. Chinese Spring (CS) (*-/-*) and cv. Hope (*-/-*). CS and Hope were used as susceptible controls carrying no resistance gene against the wheat blast fungus. GR119 and Hope were provided by K. Kato, Okayama University, and H. Tsujimoto, Tottori University, respectively, while S-615 and CS were provided by K. Tsunewaki, emeritus professor of Kyoto University.

Infection assay

Wheat seeds were sown in vermiculite supplied with liquid fertilizer in a seedling case (5.5 × 15 × 10 cm), and grown at 22°C in a controlled-environment room with a 12-h photoperiod of fluorescent lighting. Eight-day-old primary leaves were fixed onto a hard plastic board with rubber bands, and spray-inoculated with a conidial suspension (1~2 × 10⁵ conidia/ml) prepared as described by Tagle et al. (2015). The inoculated leaves were placed in humid trays and incubated in darkness at 25°C for 24 h. They were then transferred to a growth chamber with a 12 h photoperiod of fluorescent lighting, and incubated further at 25°C. Four to 5 days after inoculation, symptoms were evaluated based on the size and color of lesions. The size was rated using six progressive grades from 0 to 5: 0, no visible evidence of infection; 1, pinpoint spots; 2, small lesions (< 1.5mm); 3, lesions with an intermediate size (< 3mm); 4, large typical lesions; and 5, shriveling of leaf blades. The color was designated as B (brown) or G (green). A disease score (infection type) was represented by a combination of size scores (0-5) and color

codes (B and G). For example, infection type 2B indicated brown small lesions. Infection types 0 to 5 with brown lesions were considered resistant whereas 3G to 5G were considered susceptible. All infection assays were repeated at least 3 times.

Southern blot analysis

For Southern hybridization of genomic DNA, total Genome DNA (1 µg) extracted as described previously (Nakayashiki et al. 1999) was digested with *EcoRI* (Takara bio, Kusatsu, Japan), fractionated on a 0.7% (w/v) agarose gel in the TBE buffer, and transferred to a nylon membrane (Hybond-N+, GE Healthcare, Little Chalfont, UK). Southern hybridization was performed using the ECL Direct Nucleic Acid Labeling and Detection System (GE Healthcare) according to the manufacturer's instruction. To produce an *AVR-Rmg8* probe, a 322 bp fragment (probe A8) containing the *AVR-Rmg8* ORF was amplified from genomic DNA of *Triticum* isolate Br48 with primers, AVR-Rmg8_F2 (5'-ACCGCATCGGCTTTTCTT-3') / AVR-Rmg8_R2 (5'-TGCCTTCTAGTACCGGGAAGT-3'), and labeled. The membrane was hybridized overnight with the labeled probe in the Gold hybridization buffer (GE healthcare) containing 0.5 M NaCl at 42°C. After hybridization, the membrane was washed twice in the primary washing buffer (0.4% (w/v) sodium dodecyl sulfate and 0.5 × SSC) for 10 min at 55°C, then twice in 2 × SSC for 5 min at room temperature. Detection of target DNA was performed according to the manufacturer's instruction.

For Southern hybridization of PCR products, amplicons with the AVR-Rmg8_F2/R2 primers were run on a gel, blotted, and hybridized with the probe A8 or a Pyret fragment (638 bp) which was amplified from genomic DNA of Br48 with primers, Py_FW1 (5'-GATTTACGAACGCCGACA-3') and Py_RV1 (5'-GTCCTAATGTGGTGGGTT-3').

Sequencing of *AVR-Rmg8* homologs

Fragments containing *AVR-Rmg8* homologs (1,458 bp in expected size) were amplified from genomic DNA of *Lolium* isolates with primers, AVR-Rmg8_F1 (5'-CGGGCTGTACAACATTTTCA-3')/ AVR-Rmg8_R1 (5'-GGAGATTTTCACGATAGCAAAGC-3'). Amplicons with the expected size were directly sequenced using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Witham, MA, USA) and the ABI 3100 Genetic Analyzer following the manufacturer's instructions. Sequences obtained were converted to amino acids, aligned, and used for construction of a phylogenetic tree with the Maximum Likelihood algorithm implemented in the MEGA7 (Kumar et al. 2016).

From three *Lolium* isolates (M99A4, M99A9, M99B8), an 8.8 kb fragment was amplified with the AVR-Rmg8_F1/R1 primers. An amplicon from M99A9 was purified by a gel extraction kit (FastGene, Nippon Genetics, Tokyo, Japan), and inserted into the *EcoRV* site of Bluescript II SK (+) using DNA ligation kit ver.2 (Takara bio). The ligation products were introduced into *Escherichia coli* DH5 α . The insert of the recombinant plasmid was sequenced through primer walking.

Transformation of *P. oryzae* with *AVR-Rmg8* clones

The 1,458 bp fragments amplified from genomic DNA of representative *Lolium* isolates, TP2 and WK4-2, were purified, inserted into the *EcoRV* site of Bluescript II SK (+), and introduced into *E. coli* DH5 α as described above. The recombinant plasmid was extracted from recovered colonies with QIAprep spin mini prep kit (Qiagen, Hilden, Germany) and introduced into Br48 Δ A8 following the PEG-mediated co-transformation protocol (Tosa et al. 2005) with pII99 (carrying a geneticin resistance gene) (Namiki et al. 2001) as a selection marker.

Results

Structure around *AVR-Rmg8* in a *Triticum* isolate

The first checkpoint for predicting the durability of a resistance gene is the genomic structure around its corresponding avirulence gene. To examine the structure around *AVR-Rmg8*, the whole genome sequence of B71 (Peng et al. 2019), a *Triticum* isolate collected in Bolivia in 2012, was downloaded because it was almost identical to Bangladeshi isolates (Gladieux et al. 2018). Its analysis revealed that *AVR-Rmg8* is located in a gene-rich and repeat-poor region (Fig. 1), suggesting that the *AVR-Rmg8* locus is in a relatively stable region of the genome.

Distribution of *AVR-Rmg8* in *Lolium* isolates

The second checkpoint for predicting the durability of a resistance gene is distribution and polymorphisms of its corresponding avirulence gene in the fungal population. All *Triticum* isolates tested by Wang et al. (2018), as well as those in the databases, were carriers of *AVR-Rmg8* (eI, eII, and derivatives of eII). In addition, Bangladeshi isolates (as far as in the databases) were all carriers of the eI type (data not shown). This is reasonable because it has been only four years since the clonal propagation of the Bangladeshi population started in 2016 (Cruz and Valent 2017). To obtain clues to a long term dynamics of *AVR-Rmg8*, we examined populations of *Lolium* isolates, close relatives of *Triticum* isolates (Gladieux et al. 2018).

Genomic DNAs of 27 *Lolium* isolates were run on a gel and hybridized with the probe A8. All *Lolium* isolates carried *AVR-Rmg8* homologs (Fig. 2). Based on the size of signals, these homologs were grouped into three types. The first type designated as eL1

was 6.9 kb in size and shared by the TALF isolates. The second type designated as eL2 was 7.9 kb in size and shared by WK-, Y99-, and three M99- isolates. The third type designated as eL3 was 5.9 kb in size and shared by three M99- isolates, i.e., M99A4, M99A9, and M99B8.

Analysis of the eL1 and eL2 types

When the *AVR-Rmg8* region was amplified from the eL1 and eL2 carriers, fragments with the expected size (322 bp with the AVR-Rmg8_F2/R2 primers and 1,458 bp with the AVR-Rmg8_F1/R1 primers) were obtained. Sequence analysis revealed that amplicons from the carriers of each type (16 with eL1 and 8 with eL2) shared a 100% identical nucleotide sequence (DDBJ/EMBL/GenBank accession numbers, LC535796 for eL1 and LC535797 for eL2). These two types had one or two amino acid substitutions compared with the eII type in *Triticum* isolates (Supplementary Fig. 1). Based on these amino acid sequences, a phylogenetic tree was constructed using a sequence of an *AVR-Rmg8* homolog in a *P. grisea* isolate (Dig41) from *Digitaria sanguinalis* (Yoshida et al. 2016) as an outgroup (Fig. 3). The eL1 and eL2 types were more similar to the eII type than to the eI type.

To check whether these new types were recognized by *Rmg8*, fragments containing eL1 and eL2 were amplified with the AVR-Rmg8_F1/R1 primers from genomic DNA of TP2 and WK4-2, respectively, and introduced into Br48ΔA8, an *AVR-Rmg8* disruptant derived from Br48. Seven and 15 colonies were recovered from geneticin-containing media in the transformation with eL1 and eL2, respectively. Among them, 4 and 6 colonies were PCR-positive, i.e., carriers of the eL1 and eL2 transgenes, respectively (Fig. 4a). All these positive strains gained avirulence on S-615 (carrying *Rmg8*) without losing virulence on Hope (lacking *Rmg8*) (Fig. 4). These results indicate that both of the eL1 and eL2 types are functional and recognized by *Rmg8*.

Analysis of the eL3 type

From genomic DNA of the three eL3 carriers (M99A4, M99A9, M99B8), larger fragments were amplified. Their sizes were 8.8 kb with the AVR-Rmg8_F1/R1 primers and 7.7 kb with the AVR-Rmg8_F2/R2 primers (Fig. 5). Sequence analysis revealed that this fragment contained a Ty3/Gypsy retrotransposon, Pyret (Nakayashiki et al. 2001). The Pyret element (7,382 bp in size) was inserted into ~35 bp downstream from the start of the *AVR-Rmg8* ORF (DDBJ/EMBL/GenBank accession number, LC535798). The *AVR-Rmg8* portion of eL3 was similar to the eI type. Southern hybridization of the PCR products confirmed that the 7.7 kb amplicon contained both *AVR-Rmg8* and Pyret (Fig. 5).

Pathogenicity of *Lolium* isolates

Pathogenicity of the 27 *Lolium* isolates was checked on four common wheat cultivars (Table 1). They were all avirulent on CS, which does not carry any resistance gene against *Triticum* isolates but carries *Rwt3*, a resistance gene corresponding to avirulence gene *PWT3* commonly found in *Lolium*, *Avena*, and *Eleusine* isolates (Asuke et al. 2020; Inoue et al. 2017). A half of these *Lolium* isolates were virulent on Hope, which does not carry resistance genes against *Triticum* isolates nor *Rwt3*. The three isolates carrying the eL3 type (M99A4, M99A9, and M99B8) were virulent on S-615 carrying *Rmg8*, indicating that they lack functional *AVR-Rmg8* as expected. GR119 carrying *Rmg8* and *RmgGR119* showed strong resistance even against these three isolates.

Discussion

Recent accumulation of whole genome sequences of fungal pathogens led to the “two speed genome” model in which pathogen genomes have a bipartite architecture (Dong et al. 2015), that is, gene-rich and repeat-poor regions which evolve slowly and gene-poor, repeat-rich regions which evolve rapidly (Seidl and Thomma 2017). Abundant repetitive sequences in the latter compartment cause frequent chromosomal rearrangements such as translocation, duplication, or deletion of genes (Seidl and Thomma 2017), or increased levels of local mutagenesis through repeat-induced point (RIP) mutations (Dong et al. 2015). For example, repetitive elements flanking *AVR-Pita* (an avirulence gene of the rice blast fungus) are inferred to mediate its frequent loss, which enabled quick adaptation of the pathogen to *Pita*, its corresponding resistance gene in rice (Chuma et al. 2011). Generally speaking, effector genes tend to be located in the compartment enriched in repetitive sequences and transposable elements (Dong et al. 2015) as with *AVR-Pita*. However, the present study showed that *AVR-Rmg8* is located in a repeat-poor region (Fig. 1). This result suggests that *AVR-Rmg8* is not so easily lost as *AVR-Pita* or other avirulence genes located in repeat-rich regions. This implies that *Rmg8* would be relatively durable compared with *Pita* or other resistance genes corresponding to avirulence genes located in repeat-rich regions.

Analysis of *AVR-Rmg8* distribution showed that all *Lolium* isolates carried its homologs (Fig. 2). The phylogenetic analysis suggested that the eL1 and eL2 types in *Lolium* isolates were close to the eII type in *Triticum* isolate (Fig. 3). The complementation test with the transformants carrying the eL1 and eL2 transgenes indicated that both of them were functional. We suggest that *AVR-Rmg8* is a relatively conserved gene widely distributed in populations of *Triticum* and *Lolium* isolates.

Completely durable resistance genes should be those corresponding to effector genes that are indispensable for pathogen’s survival. Regarding *Rmg8*, however, this was not the case. We found three isolates virulent on S-615 in a population of indigenous *Lolium* isolates (Table 1). *AVR-Rmg8* in these isolates was disrupted by an insertion of Pyret (Fig.

5). Nevertheless, these isolates have survived in nature. Taken together, there is a possibility that, in future, *Rmg8* will be defeated by a new race of the wheat blast fungus that lost function of *AVR-Rmg8*. In the breeding for resistance to wheat blast, therefore, it is advisable to introduce *Rmg8* together with other genes such as *RmgGR119*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Anh VL, Anh NT, Tagle AG, Vy TTP, Inoue Y, Takumi S, Chuma I, Tosa Y. (2015) *Rmg8*, a new gene for resistance to *Triticum* isolates of *Pyricularia oryzae* in hexaploid wheat. *Phytopathology* 105:1568–1572
- Anh VL, Inoue Y, Asuke S, Vy TTP, Anh NT, Wang S, Chuma I, Tosa Y. (2018) *Rmg8* and *Rmg7*, wheat genes for resistance to the wheat blast fungus, recognize the same avirulence gene *AVR-Rmg8*. *Mol Plant Pathol* 19:1252–1256
- Asuke S, Nishimi S, Tosa Y. (2020) At least five major genes are involved in the avirulence of an *Eleusine* isolate of *Pyricularia oryzae* on common wheat. *Phytopathology* 110:465–471
- Callaway E. (2016) Devastating wheat fungus appears in Asia for first time. *Nature* 532: 421–422
- Chuma I, Isobe C, Hotta Y, Ibaragi K, Futamata N, Kusaba M, Yoshida K, Terauchi R, Fujita Y, Nakayashiki H, Valent B, Tosa Y. (2011) Multiple translocation of the *AVR-Pita* effector gene among chromosomes of the rice blast fungus *Magnaporthe oryzae* and related species. *PLoS Pathog* 7: e1002147
- Cruz CD, Valent B. (2017) Wheat blast disease: danger on the move. *Trop Plant Pathol* 42:210–222
- Dong S, Raffaele S, Kamoun S. (2015) The two-speed genomes of filamentous pathogens: waltz with plants. *Curr Opin Genet Dev* 35:57–65
- Farman M, Peterson G, Chen L, Starnes J, Valent B, Bachi P, Murdock L, Hershman D, Pedley K, Fernandes JM, Bavaresco J. (2017) The *Lolium* pathotype of *Magnaporthe oryzae* recovered from a single blasted wheat plant in the United States. *Plant Dis* 101:684–692
- Gladieux P, Condon B, Ravel S, Soanes D, Maciel JLN, Nhani Jr A, Chen L, Terauchi R, Lebrun M –H, Tharreau D, Mitchell T, Pedley KF, Valent B, Talbot NJ, Farman M,

- 359 Fournier E. (2018) Gene flow between divergent cereal- and grass-specific lineages
360 of the rice blast fungus *Magnaporthe oryzae*. mBio 9:e01219-17
- 361 Inoue Y, Vy TTP, Yoshida K, Asano H, Mitsuoaka C, Asuke S, Anh VL, Cumagun CJR,
362 Chuma I, Terauchi R, Kato K, Mitchell T, Valent B, Farman M, Tosa Y. (2017)
363 Evolution of the wheat blast fungus through functional losses in a host specificity
364 determinant. Science 357:80–83
- 365 Islam MT, Croll D, Gladieux P, Soanes DM, Persoons A, Bhattacharjee P, Hossain MS,
366 Gupta DR, Rahman MM, Mahboob MG, Cook N, Salam MU, Surovy MZ, Sancho
367 VB, Maciel JLN, Nhani Júnior A, Castroagudín VL, de Assis Reges JT, Ceresini
368 PC, Ravel S, Kellner R, Fournier E, Tharreau D, Lebrun M-H, McDonald BA, Stitt
369 T, Swan D, Talbot NJ, Saunders DGO, Win J, Kamoun S. (2016) Emergence of
370 wheat blast in Bangladesh was caused by a South American lineage of
371 *Magnaporthe oryzae*. BMC Biol. 14:84.
- 372 Kato H, Yamamoto M, Yamaguchi-Ozaki T, Kadouchi H, Iwamoto Y, Nakayashiki H,
373 Tosa Y, Mayama S, Mori N. (2000) Pathogenicity, mating ability and DNA
374 restriction fragment length polymorphisms of *Pyricularia* populations isolated from
375 Gramineae, Bambusideae and Zingiberaceae plants. J Gen Plant Pathol 66:30–47
- 376 Kumar S, Stecher G, Tamura K. (2016) MEGA7: molecular evolutionary genetics analysis
377 version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874
- 378 Kusaba M, Hirata K, Sumida Y, Yamagashira A, Konagai-Urata H, Yaegashi H. (2006)
379 Molecular genetic characterization and host specificity of *Pyricularia* isolates from
380 annual ryegrass in Japan. Plant Path J 5:72–79
- 381 Landschoot PJ, Hoyland BF. (1992) Gray leaf spot of perennial ryegrass turf in
382 Pennsylvania. Plant Dis 76:1280–1282
- 383 Malaker PK, Barma NCD, Tiwari TP, Collis WJ, Duveiller E, Singh PK, Joshi AK, Singh
384 RP, Braun H-J, Peterson GL, Pedley KF, Farman ML, Valent B. (2016) First report

385 of wheat blast caused by *Magnaporthe oryzae* pathotype *triticum* in Bangladesh.
386 Plant Dis 100:2330

387 Nakayashiki H, Kiyotomi K, Tosa Y, Mayama S. (1999) Transposition of the
388 retrotransposon MAGGY in heterologous species of filamentous fungi. Genetics
389 153:693–703

390 Nakayashiki H, Matsuo H, Chuma I, Ikeda K, Betsuyaku S, Kusaba M, Tosa Y, Mayama
391 S. (2001) Pyret, a Ty3/Gypsy retrotransposon in *Magnaporthe grisea* contains an
392 extra domain between the nucleocapsid and protease domains. Nucl Acid Res
393 29:4106–4113

394 Namiki F, Matsunaga M, Okuda M, Inoue I, Nishi K, Fujita Y, Tsuge T. (2001) Mutation
395 of an arginine biosynthesis gene causes reduced pathogenicity in *Fusarium*
396 *oxysporum* f. sp. *melonis*. Mol Plant-Microbe Interact 14:580–584

397 Peng Z, Oliveira-Garcia E, Lin G, Hu Y, Dalby M, Migeon P, Tang H, Farman M, Cook
398 D, White F, Valent B, Liu S (2019) Effector gene reshuffling involves dispensable
399 mini-chromosomes in the wheat blast fungus. PLoS Genet 15:e1008272

400 Seidl MF, Thomma BPHJ. (2017) Transposable elements direct the coevolution between
401 plants and microbes. Trend Genet 33:842–851

402 Tagle AG, Chuma I, Tosa Y. (2015) *Rmg7*, a new gene for resistance to *Triticum* isolates
403 of *Pyricularia oryzae* identified in tetraploid wheat. Phytopathology 105:495–499

404 Tosa Y, Chuma I. (2014) Classification and parasitic specialization of blast fungi. J Gen
405 Plant Pathol 80:202–209

406 Tosa Y, Hirata K, Tamba H, Nakagawa S, Chuma I, Isobe C, Osue J, Urashima AS, Don
407 LD, Kusaba M, Nakayashiki H, Tanaka A, Tani T, Mori N, and Mayama S. (2004)
408 Genetic constitution and pathogenicity of *Lolium* isolates of *Magnaporthe oryzae*
409 in comparison with host species-specific pathotypes of the blast fungus.
410 Phytopathology 94:454–462

- 411 Tosa Y, Osue J, Eto Y, Oh HS, Nakayashiki H, Mayama S, Leong SA. (2005) Evolution
412 of an avirulence gene, *AVR1-CO39*, concomitant with the evolution and
413 differentiation of *Magnaporthe oryzae*. *Mol Plant Microbe Interact* 18:1148–1160
- 414 Tosa Y, Uddin W, Viji G, Kang S, Mayama S. (2007) Comparative genetic analysis of
415 *Magnaporthe oryzae* isolates causing gray leaf spot of perennial ryegrass turf in the
416 United States and Japan. *Plant Dis* 91:517–524
- 417 Urashima AS, Igarashi S, Kato H. (1993) Host range, mating type, and fertility of
418 *Pyricularia grisea* from wheat in Brazil. *Plant Dis* 77:1211–1216
- 419 Wang S, Asuke S, Vy TTP, Inoue Y, Chuma I, Win J, Kato K, Tosa Y. (2018) A new
420 resistance gene in combination with *Rmg8* confers strong resistance against
421 *Triticum* isolates of *Pyricularia oryzae* in a common wheat landrace.
422 *Phytopathology* 108:1299–1306.
- 423 Yoshida K, Saunders DGO, Mitsuoka C, Natsume S, Kosugi S, Saitoh H, Inoue Y, Chuma
424 I, Tosa Y, Cano LM, Kamoun S, Terauchi R. (2016) Host specialization of the blast
425 fungus *Magnaporthe oryzae* is associated with dynamic gain and loss of genes
426 linked with transposable elements. *BMC Genomics* 17:370
- 427 Zhang N, Luo J, Rossman AY, Aoki T, Chuma I, Crous PW, Dean R, de Vries RP,
428 Donofrio N, Hyde KD, Lebrun M-H, Talbot NJ, Tharreau D, Tosa Y, Valent B,
429 Wang Z, Xu J-R. (2016) Generic names in Magnaporthales. *IMA fungus* 7:155–
430 159.
- 431

Figure legends

Fig. 1. Structure around *AVR-Rmg8* extracted from a whole genome sequence

(MQUD01000000) of B71, a *Triticum* isolate collected in Bolivia.

Fig. 2. Distribution of *AVR-Rmg8* in *Triticum* and *Lolium* isolates. Genomic DNA was digested with *EcoRI*, run on a gel, blotted, and hybridized with the probe A8.

Original hosts of the isolates are shown on the top of the panel.

Fig. 3. Phylogenetic relationships of *AVR-Rmg8* homologs in *Lolium* and *Triticum*

isolates of *Pyricularia oryzae*. The tree was constructed with the Maximum

Likelihood algorithm implemented in the MEGA7 using an alignment of amino

acid sequences. A homolog in a *Digitaria* isolate of *P. grisea* was used as an

outgroup. The numbers at branches refer to the bootstrap values (%) in 1,000

replications. The scale bar indicates the number of substitutions per site.

Fig. 4. Functional analysis of *AVR-Rmg8* homologs derived from TP2 (eL1) and WK4-

2 (eL2). (a) PCR analysis of *Pyricularia oryzae* Br48 (wild isolate), Br48ΔA8

(*AVR-Rmg8*-disruptant of Br48), and transformants of Br48ΔA8 with an empty

vector (Br48ΔA8+EV), eL1 (Br48ΔA8+eL1), and eL2 (Br48ΔA8+eL2). *AVR-*

Rmg8 homologs were amplified with the *AVR-Rmg8_F2/R2* primers. Infection

types on wheat cultivar S-615 carrying *Rmg8* are shown below the panel. Red,

avirulent; green, virulent. (b) Reactions of primary leaves of wheat cultivars S-

615 (*Rmg8*) and Hope (*rmg8*) inoculated with Br48 and its derivatives, 4 days

after inoculation.

Fig. 5. PCR-Southern analyses of *AVR-Rmg8* homologs. *AVR-Rmg8* homologs were

amplified from genomic DNA of Br48 (eI), Br5 (eII), Br116.5 (eII'), TP1, TP2

(eL1), WK4-2, M99A7 (eL2), and M99A4, M99A7, M99B8 (eL3) with the

AVR-Rmg8_F2/R2 primers, run on a gel (a), and hybridized with the probe A8

(b) or the Pyret probe (c).

Table. 1. *Pyricularia oryzae* isolates tested, their infection types with wheat cultivars at 25°C, and *AVR-Rmg8* genotypes.

Isolate ^a	Original host	Locality	Year	Infection type ^b on				<i>AVR-Rmg8</i> type
				S-615	CS	Hope	GR119	
Br48	<i>Triticum aestivum</i>	Mato Grosso do Sul, Brazil	1992	1B	5G	5G	0	eI
Br5	<i>T. aestivum</i>	Parana, Brazil	1990	2B	5G	5G	0-1B	eII
Br116.5	<i>T. aestivum</i>	Parana, Brazil	1992	1-2B	5G	5G	0-1B	eII'
TP1	<i>Lolium perenne</i>	Tochigi, Japan	1997	0-1B	1B	2B	0	eL1
TP2	<i>L. perenne</i>	Tochigi, Japan	1997	0-1B	2-3B	5G	0	eL1
TP3	<i>L. perenne</i>	Tochigi, Japan	1997	1B	2-3B	5G	0	eL1
TP4	<i>L. perenne</i>	Tochigi, Japan	1997	0	2B	4G	0	eL1
TP5	<i>L. perenne</i>	Tochigi, Japan	1997	0	0	0	0	eL1
AK2	<i>L. perenne</i>	Akita, Japan	1998	0-1B	0-1B	0-1B	0	eL1
AK3	<i>L. perenne</i>	Akita, Japan	1998	0	0	1B	0	eL1
AK4	<i>L. perenne</i>	Akita, Japan	1998	0	0	0	0	eL1
AK5	<i>L. perenne</i>	Akita, Japan	1998	1B	0	0-1B	0	eL1
LW1	<i>L. perenne</i>	Yamanashi, Japan	1999	0	0	0	0	eL1
LW2	<i>L. perenne</i>	Yamanashi, Japan	1999	0	0-1B	0	0	eL1
LW3	<i>L. perenne</i>	Yamanashi, Japan	1999	0	0	0	0	eL1
FI2	<i>L. perenne</i>	Chiba, Japan	1998	0	0	0	0	eL1
FI3	<i>L. perenne</i>	Chiba, Japan	1998	0	0	0	0	eL1
FI4	<i>L. perenne</i>	Chiba, Japan	1998	0	0	0	0	eL1
FI5	<i>L. perenne</i>	Chiba, Japan	1998	0	0	0	0	eL1
WK4-1	<i>L. perenne</i>	Yamaguchi, Japan	1996	0-1B	1B	2B	0	eL2
WK4-2	<i>L. perenne</i>	Yamaguchi, Japan	1996	1B	1B	4-5G	0	eL2
WK4-3	<i>L. perenne</i>	Yamaguchi, Japan	1996	0-1B	2-3B	4-5G	0	eL2
M99A4	<i>L. multiflorum</i>	Miyazaki, Japan	1999	5G	2B	5G	0-1B	eL3
M99A7	<i>L. multiflorum</i>	Miyazaki, Japan	1999	2B	2B	5G	0	eL2
M99A8	<i>L. multiflorum</i>	Miyazaki, Japan	1999	0-1B	1B	5G	0	eL2
M99A9	<i>L. multiflorum</i>	Miyazaki, Japan	1999	5G	1B	4-5G	0-1B	eL3
M99B4	<i>L. multiflorum</i>	Miyazaki, Japan	1999	2B	2B	5G	0	eL2
M99B8	<i>L. multiflorum</i>	Miyazaki, Japan	1999	5G	2B	5G	1B	eL3
Y99I-7	<i>L. multiflorum</i>	Yamaguchi, Japan	1999	1B	2B	5G	0	eL2
Y99I-11	<i>L. multiflorum</i>	Yamaguchi, Japan	1999	2B	3B	5G	0	eL2

^a Refer to Tosa et al. (2004) and Kusaba et al. (2006) for details of *L. perenne* isolates and *L. multiflorum* isolates, respectively.

^b Compatible reactions are shown in bold.

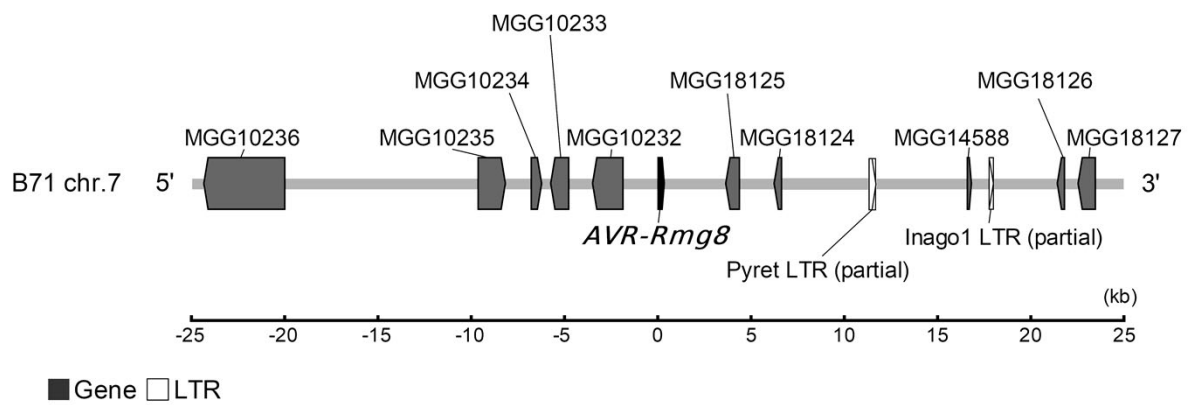


Fig. 1. Structure around *AVR-Rmg8* extracted from a whole genome sequence (MQUD01000000) of B71, a *Triticum* isolate collected in Bolivia.

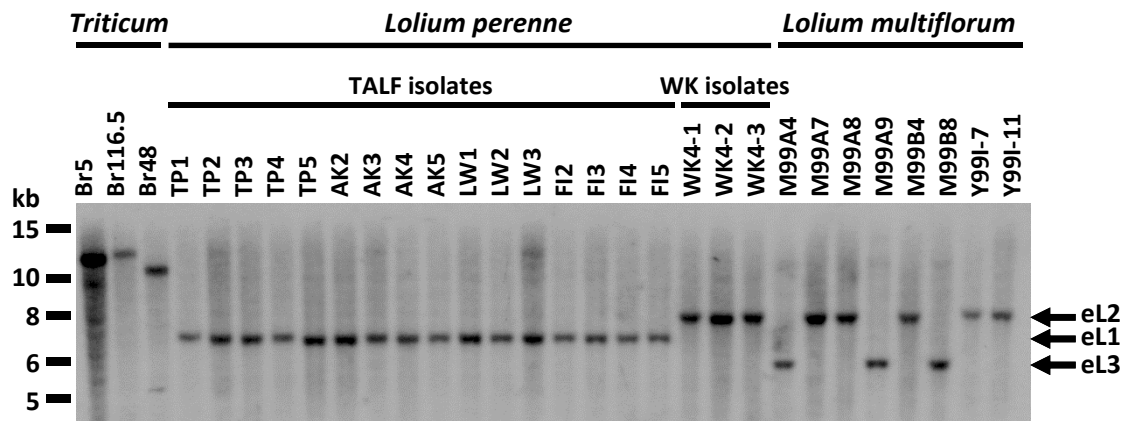


Fig. 2. Distribution of *AVR-Rmg8* in *Triticum* and *Lolium* isolates. Genomic DNA was digested with *EcoRI*, run on a gel, blotted, and hybridized with the probe A8. Original hosts of the isolates are shown on the top of the panel.

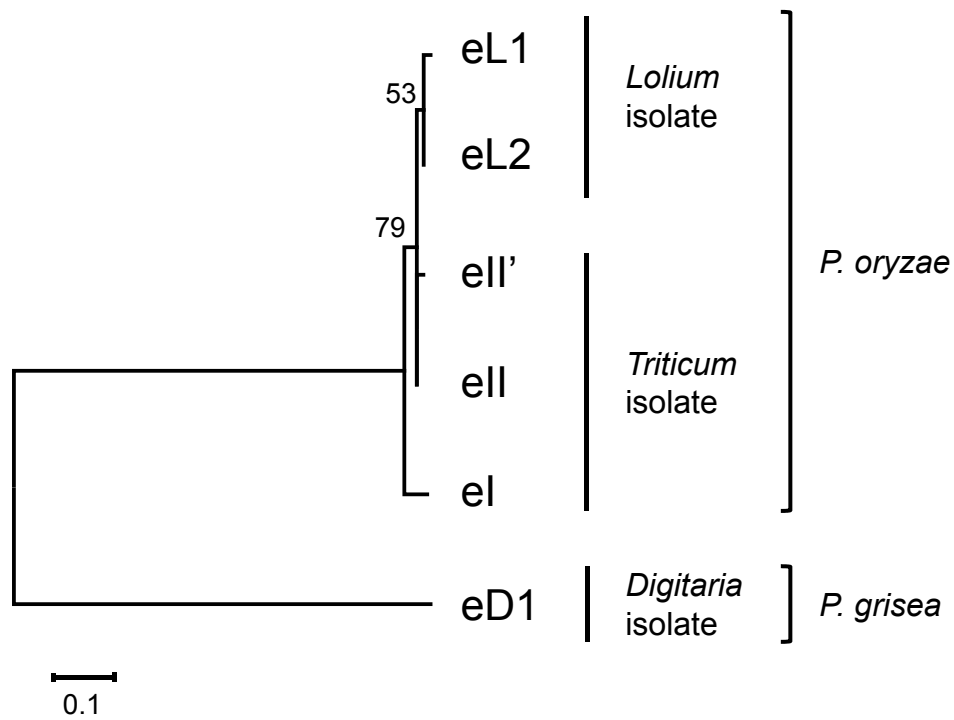


Fig. 3. Phylogenetic relationships of AVR-Rmg8 homologs in *Lolium* and *Triticum* isolates of *P. oryzae*. The tree was constructed with the Maximum Likelihood algorithm implemented in the MEGA7 using an alignment of amino acid sequences. A homolog in a *Digitaria* isolate of *P. grisea* was used as an outgroup. The numbers at branches refer to the bootstrap values in 1000 replications. The scale bar indicates the number of substitutions per site.

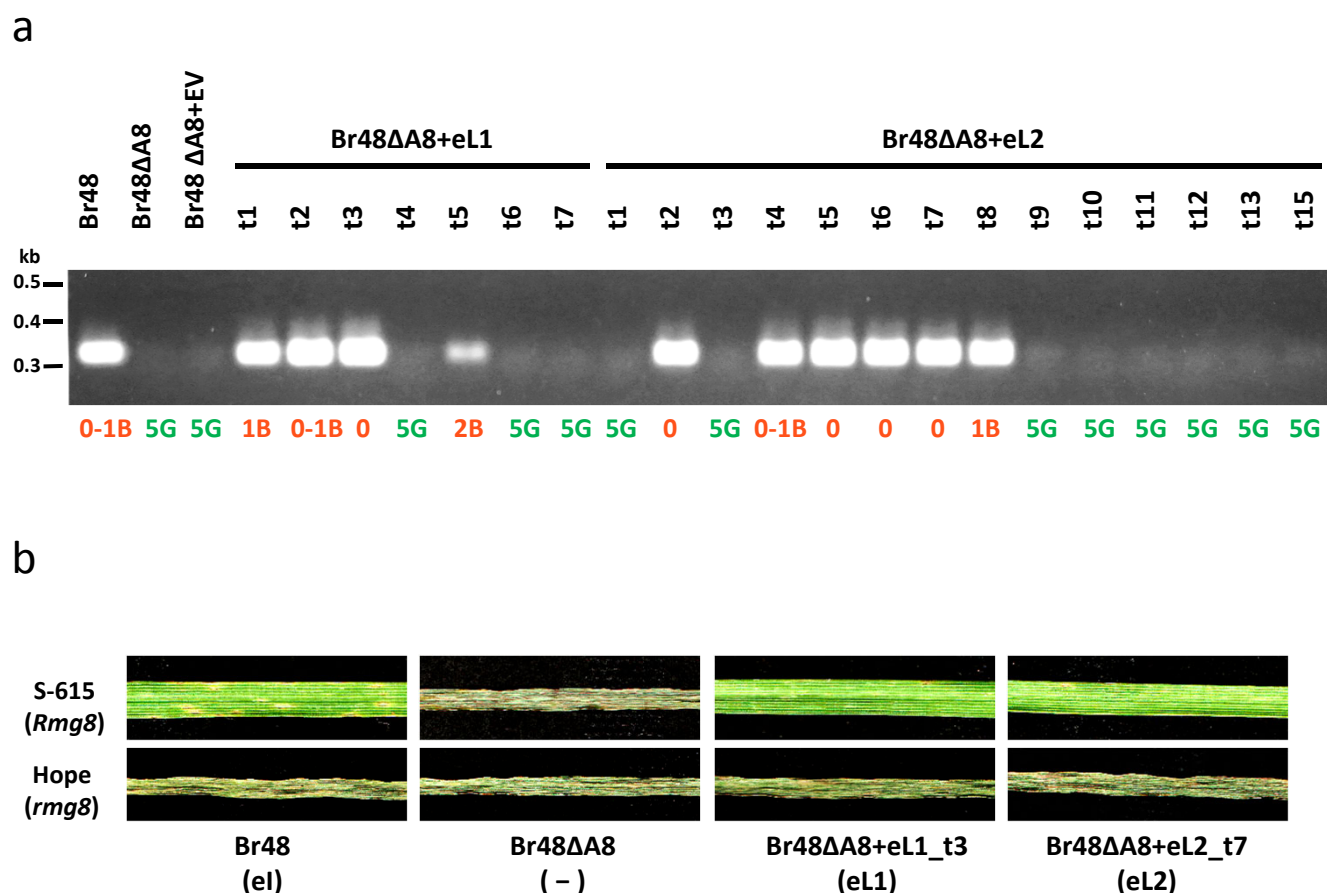


Fig. 4. Functional analysis of *AVR-Rmg8* homologs derived from TP2 (eL1) and WK4-2 (eL2). (a) PCR analysis of *P. oryzae* Br48 (wild isolate), Br48ΔA8 (*AVR-Rmg8*-disruptant of Br48), and transformants of Br48ΔA8 with an empty vector (Br48ΔA8+EV), eL1 (Br48ΔA8+eL1), and eL2 (Br48ΔA8+eL2). *AVR-Rmg8* homologs were amplified with the *AVR-Rmg8_F2/R2* primers. Infection types on wheat cultivar S-615 carrying *Rmg8* are shown below the panel. Red, avirulent; green, virulent. (b) Reactions of primary leaves of wheat cultivars S-615 (*Rmg8*) and Hope (*rmg8*) inoculated with Br48 and its derivatives, 4 days after inoculation.

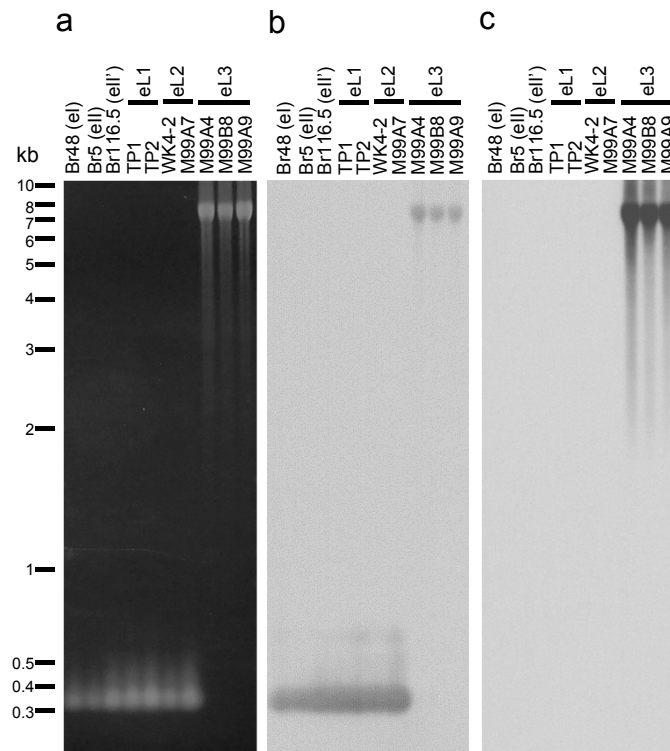


Fig 5. PCR-Southern analyses of *AVR-Rmg8* homologs. *AVR-Rmg8* homologs were amplified from genomic DNA of Br48 (eI), Br5 (eII), Br116.5 (eII'), TP1, TP2 (eL1), WK4-2, M99A7 (eL2), and M99A4, M99A7, M99B8 (eL3) with the *AVR-Rmg8_F2/R2* primers, run on a gel (a) and hybridized with the probe A8 (b) or the Pyret probe (c).

A

Br48	eI	TCCGGGCCAGATCTACGGGCAATCAATGGTCGCATATGGCTGAATGGAAAAGCAGAGGAAGCATACCTGAGAGTAATAGC	[80]
Br5	eII		[80]
Br116.5	eII'		[80]
TP2	eL1		[80]
WK4-2	eL2		[80]
Br48	eI	ATAGAGGCCAGCGTTTCATCGCTCTTGTTCTTGGGAAGCTGGCTGGTCCCATGGTTCCTTCCATCGTTTTCGCGCGGTCCC	[160]
Br5	eII		[160]
Br116.5	eII'		[160]
TP2	eL1		[160]
WK4-2	eL2		[160]
Br48	eI	GGAGCAAAAATCTCCTCGATCACACAACCTGGAGTAAAAGTTGCGGTGGGATCCCATGTCAAATTAGACTTTTACTATACT	[240]
Br5	eII		[240]
Br116.5	eII'		[240]
TP2	eL1		[240]
WK4-2	eL2		[240]
Br48	eI	GAACAAGTATATAAGCAGCCTAGCCACTGAAGAGACCGGTACACTTTCTAGTTGCATTTTCTCACTCCACTCAGCGTCTT	[320]
Br5	eII		[320]
Br116.5	eII'		[320]
TP2	eL1		[320]
WK4-2	eL2		[320]
Br48	eI	CACCTTCTTTTGTACTTTGCTTCAATTCAATTCCTTTGGCCTTTTCTCTGACCGAGCCTTTTGTCTGTCAGTTTGTACATA	[400]
Br5	eII		[400]
Br116.5	eII'		[400]
TP2	eL1		[400]
WK4-2	eL2		[400]
Br48	eI	CCTTTCGTTTTCAAGATTATCTTTGCGTCAGTTGCTTTGATTTCACCAATGACACCGCATCGGCTTTTCTTCCCTATCC	[480]
Br5	eII		[480]
Br116.5	eII'		[480]
TP2	eL1		[480]
WK4-2	eL2		[480]
Br48	eI	TATCGCTGGCGCGATGGCGCTGCCTGCGCGCAGCCTATGCCCCGAGTAGGCCAGGACAGGGAGGAAGGGCGGAAAC	[560]
Br5	eII		[560]
Br116.5	eII'		[560]
TP2	eL1		[560]
WK4-2	eL2		[560]
Br48	eI	GGCGGAAGAGGACCGGCGGACCACCCAGCAATACGAGGAGCCTGTCCCGTACCACCAACGGCGGCGGCAGCCTC	[640]
Br5	eII		[640]
Br116.5	eII'		[640]
TP2	eL1		[640]
WK4-2	eL2		[640]
Br48	eI	GCAGCCTTACCCGGGACACGTGCCAGGCGGACAACGGCCGACTGAACACTCGGAGCTTATACCAGATGACTATCCTCAAT	[720]
Br5	eII		[720]
Br116.5	eII'		[720]
TP2	eL1		[720]
WK4-2	eL2		[720]
Br48	eI	TGTCAAGGACTACGATACCTACTTTTTTGGTGGACTTCCCGGTACTAGAAGGCATAGGCAGTCTACAAGATCACTTTT	[800]
Br5	eII		[800]
Br116.5	eII'		[800]
TP2	eL1		[800]
WK4-2	eL2		[800]
Br48	eI	ATCGACGATTGGGAGAGCCAAAAACGTTAAACGGCCTATGCAACAGAAATCACATGGGCTGGTGGAGCTGTCTCTCAA	[880]
Br5	eII		[880]
Br116.5	eII'		[880]
TP2	eL1		[880]
WK4-2	eL2		[880]
Br48	eI	GCTTTATCCAGATTTTAAAAACTGGTTATCTTGACTGGCCACGCTGACAGGCGAGATTGTTCTAGTACTA	[950]
Br5	eII		[950]
Br116.5	eII'		[950]
TP2	eL1		[950]
WK4-2	eL2		[950]

B

Br48	eI	MHRIGFFFPILIAGAMALPAPQPMPPSRPGQGGRGGNGRGPGGPPPPQQYEEFVPYHQTAANAQPPYPGHVPGGQRPTEHSELIPDDYPQFVKDYDTYFFGGLPGTRRQ
Br5	eII	..C.....L.....GG.S.....
Br116.5	eII'	..C.....L.....GG.S.....
TP2	eL1	L...V.....GG.S.....
WK4-2	eL2	..C.....L...V.....GG.S.....

Supplementary Fig. 1. Comparison of *AVR-Rmg8* homologs in *Triticum* and *Lolium* isolates. (A) Nucleotide sequences around *AVR-Rmg8* homologs. The ORF is shown in red. (B) Amino acid sequences of *AVR-Rmg8* homologs. The predicted signal peptide is shown in blue.