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Title: Evolution of the wheat blast fungus through functional losses in a host specificity determinant

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Abstract: Wheat blast first emerged in Brazil in the mid-1980s and has recently caused heavy crop losses in Asia. Here, we show how this devastating pathogen evolved in Brazil. Genetic analysis of host species determinants in the blast fungus resulted in the cloning of avirulence genes *PWT3* and *PWT4*, whose gene products elicit defense in wheat cultivars containing the corresponding resistance genes *Rwt3* and *Rwt4*. Studies on avirulence and resistance gene distributions, together with historical data on wheat cultivation in Brazil, suggest that wheat blast emerged due to widespread deployment of *rwt3* wheat (susceptible to *Lolium* isolates) followed by the loss of function of *PWT3*. This implies that the *rwt3* wheat served as a springboard for the host jump to wheat.

One Sentence Summary: Loss of an avirulence gene/resistance gene interaction results in host jump of the blast fungus to common wheat

Main Text:

Host jumps of plant pathogens may cause outbreaks of new crop diseases. A recent, likely example is wheat blast caused by *Pyricularia oryzae* (*Magnaporthe oryzae*). *P. oryzae* is composed of host-specific subgroups such as *Oryza*, *Eleusine*, *Avena*, and *Lolium* pathotypes pathogenic on rice, finger millet, oat, and perennial ryegrass, respectively (1-3). Wheat blast was first reported in 1985 in Paraná state located in the southern region of Brazil, and then spread to wheat growing areas in neighboring states and countries in South America (4). The causal agent (the wheat blast pathogen) was identified as *P. oryzae* (4), but was specifically pathogenic on wheat and its wild relatives (*Triticum* spp. and *Aegilops* spp.) (3, 5), and therefore, considered to be a new host-specific subgroup (*Triticum* pathotype). In 2011 wheat blast was found in North America - at a University of Kentucky research plot (6). Though less virulent than South American isolates, the strain isolated in Kentucky was pathogenic on wheat, and inferred to have evolved from annual ryegrass pathogen in North America through host jump (6). In 2016 wheat blast suddenly appeared in Bangladesh and caused a significant loss of wheat production (7, 8). Phylogenomic analyses revealed that this outbreak was most likely caused by a wheat-infecting strain from South America (7, 8). Currently, this devastating disease has taken a major step towards becoming pandemic and poses a serious threat to wheat production worldwide. Here, we show that the wheat blast pathogen evolved through functional losses in a host specificity determinant.

Triticum isolates are most closely related to *Avena* and *Lolium* isolates (Fig. 1A). Takabayashi et al. (9) identified two genes, *PWT3* and *PWT4*, in *Avena* isolate Br58, which conditioned its avirulence on wheat. They also identified the resistance gene in wheat that

recognizes *PWT4*, and designated it as *Rmg1* (synonymous with *Rwt4*). Similarly, Vy et al. (10) identified a gene (tentatively named *A1*) playing the primary role in the avirulence of *Lolium* isolate TP2 on wheat, and its corresponding resistance gene *Rmg6*. Allelism tests revealed that *PWT3* is located at the same locus as *A1* (fig. S1) and recognized by *Rmg6* (fig. S2). These data suggest that the same gene pair, *PWT3* - *Rmg6* (syn. *Rwt3*), is involved in the incompatibility of both *Lolium* and *Avena* isolates on wheat (fig. S3).

We isolated *PWT3* from *Avena* isolate Br58 through map-based cloning (fig. S4). The *PWT3* nucleotide sequence from Br58 (designated as A-type later) was shared by all 12 *Lolium* isolates analyzed, including TP2 (table S1), supporting the status of *PWT3* as a host species specificity gene. We also isolated *PWT4* from Br58 using bulked segregant analysis coupled with whole genome sequencing (fig. S4). The predicted *PWT3* and *PWT4* proteins contained putative signal peptides (fig. S4), but lacked similarity to known proteins or protein domains.

PWT3 and *PWT4* were identified through seedling infection assays, but wheat blast is mainly a spike disease in Brazilian wheat fields. To estimate their roles during field infections, spikes of wheat cultivars Norin 4 (N4) (*Rwt3/Rwt4*), Chinese Spring (CS) (*Rwt3/rwt4*), Transfed (Tfed) (*rwt3/Rwt4*), and Hope (*rwt3/rwt4*) were inoculated at early anthesis with *Triticum* isolate Br48, and transformants carrying *PWT3* (Br48+3) and *PWT4* (Br48+4). Br48 was virulent on spikes of all four cultivars, whereas Br48+3 and Br48+4 showed specific avirulence on the *Rwt3* and *Rwt4* carriers, respectively (Fig. 2A). Thus, *PWT3-Rwt3* and *PWT4-Rwt4* interactions play critical roles in spike infection.

We performed gene disruption experiments to determine if *PWT3-Rwt3* and *PWT4-Rwt4* interactions are the only barriers preventing the non-wheat isolates from infecting *Rwt3/Rwt4* wheat. *Lolium* isolate TP2 was avirulent on *Rwt3* cultivars but virulent on *rwt3* cultivars (Fig.

2B). When *PWT3* was disrupted (fig. S5), the resulting strain TP2Δ3 gained virulence on the *Rwt3* cultivars (Fig. 2B, fig. S6). *Avena* isolate Br58 was avirulent on the *Rwt3* and/or *Rwt4* carriers and virulent only on Hope (*rwt3/rwt4*) (Fig. 2C). When *PWT3* and *PWT4* were disrupted individually (fig. S5, S7), the resulting strains Br58Δ3 and Br58Δ4 gained virulence on CS (*Rwt3/rwt4*) and Tfed (*rwt3/Rwt4*), respectively (Fig. 2C, fig S6). Furthermore, a double disruptant gained virulence on all cultivars including N4 (*Rwt3/Rwt4*). These results indicate that mutations or deletions of *PWT3* and *PWT4* would lead to a gain of virulence on a majority of wheat cultivars.

To determine if mutation or loss of *PWT3* and/or *PWT4* served as key events in the evolution of wheat blast, we screened for their presence in genome sequences from a comprehensive collection of *P. oryzae* strains. *PWT4* homologs were found in some isolates but were considered nonfunctional (Fig. 1B, fig. S8). In contrast, *PWT3* homologs were detected in all isolates tested, with the ORF sequences conforming to one of four basic types (A, B, C, and D) (Fig. 1B, fig. S9). The *Avena* and *Lolium* A-type homolog was also found in *Eleusine* isolates and some *Triticum* isolates (Fig. 1B). Upstream sequences further divided the A-type into three subtypes (Ao, Ao', and Atm). Compared with the original Br58 type (Ao), the *Eleusine* isolates' type (Ao') had one base substitution in the upstream region. Three *Triticum* isolates (Fig. 1) represented 3 distinct types: the Ao type, the Atm type carrying an insertion of the LINE-like element MGR583 (11) (fig. S10A), and the B type which had 13 SNPs causing 12 amino acid substitutions in ORF (fig. S9A). Among 19 *Triticum* isolates collected in Brazil from 1990 to 1992, six, six, and seven isolates contained the Ao, Atm, and B types, respectively (table S2).

The Atm and B carriers were virulent on the four test cultivars while the Ao carriers were avirulent on the *Rwt3* cultivars (table S2, fig. S6A). An *in planta* expression analysis revealed

that the Ao and B type genes in *Triticum* isolates were as highly expressed as the Ao type in Br58 (fig. S10B). In contrast, the expression level of the Atm type was reduced by a factor of ten (fig. S10B). These results indicate that the MGR583 insertion in the Atm type has compromised its avirulence function by reducing expression.

To determine the origins of the highly divergent *PWT3* types, we screened ~100 representative *Pyricularia* isolates from various hosts, and found that an isolate from *Brachiaria plantaginea* (Br35) collected in Brazil in 1990 carried the B type *PWT3* with the 100% nucleotide sequence identity to that of Br48. However, it seemed unlikely that such a *Brachiaria* isolate was a direct ancestor of Br48 because Br35 was phylogenetically remote from Br48 (fig. S11). Comparative analyses of whole genome sequences suggested that the direct ancestor of Br48 inherited a 1.6Mb chromosomal segment carrying the B type *PWT3* from a Br35-like *Brachiaria* isolate (fig. S12).

The *Triticum* isolates employed above were early isolates collected in 1990-1992 (Kobe University collection). To reveal population dynamics, we performed *PWT3* typing using more recent *Triticum* isolates preserved at universities/institutes in the Americas and genome sequences in public databases. Kentucky strain WBKY11-15 collected from wheat in 2011 had a novel type (Atp) composed of an A-type *PWT3* ORF and an inverted repeat transposon Pot3 (12) inserted into its upstream region (Fig. 3A). Bolivian strain B71 collected in 2012 had another type (Atc) in which the *PWT3*-ORF (with two nucleotide substitutions compared with the A-type) was disrupted by a complex insertion of transposable elements, Pyret (13) and RETRO5 (14) (Fig. 3B). All isolates characterized from Bangladesh in 2016 carried the Atc type (Fig. 3C). The Atc type was present in 1990s as a minor population in the southern states in Brazil (Fig. 3C), and became prevalent throughout Brazil in the 2000s. It spread to other countries in South

America in the 2010s, and was finally transmitted to Asia and caused the outbreak of wheat blast in Bangladesh.

A critical question is why strains with the functional Ao type *PWT3* have been isolated as “*Triticum* isolates”. To answer this question, we surveyed the distribution of *Rwt3* and *Rwt4* in common wheat (fig. S13). In 499 local landraces collected worldwide, *Rwt3* and *Rwt4* carriers accounted for 77 and 87%, respectively (Fig. 4 and table S3). Only 6.6% of the accessions lacked both genes. Such ubiquity appears to be a key characteristic that makes resistance genes conditioning the subgroup-genus specificity distinct from those conditioning race-cultivar specificity. When ~60 improved cultivars from the Americas were tested, however, we found a noteworthy change in the early 1980s. In the late 1970s and early 1980s, the most planted cultivar in Brazil was IAC-5 (15), a carrier of *Rwt3* (table S4). Around 1980 a new cultivar Anahuac was introduced to Brazil and recommended to farmers (15) because it adapted very well in nonacid soils. Anahuac was a semidwarf cultivar with high yield potential, but a non-carrier of *Rwt3* (table S4). In 1985, a few years after the release of Anahuac, the outbreak of wheat blast occurred (4). Such wide cultivation of *rwt3* cultivar(s) in Brazil would explain why *P. oryzae* strains with the Ao type *PWT3* have been isolated from common wheat in the early period of the wheat blast outbreak in Brazil (table S2) and thereafter (Fig. 3C).

Based on the results described above, we present a model for the emergence of the wheat blast fungus in Brazil (fig. S14). Wide-spread cultivation of *rwt3* cultivars in the early 1980s allowed certain *P. oryzae* strains to colonize common wheat and increase the population despite carrying the Ao type *PWT3*. Wheat cultivars possessing *Rwt3* were still cultivated nearby and imposed selection for mutation or loss of *PWT3*. As a result, *pwt3* strains arose through independent events involving: i) *de novo* transposon insertion; or ii) gain of mutated *PWT3* from

a remote strain, which would finally establish wheat pathogens as pathogenic to the entire wheat population.

This model implies that *rwt3* cultivars served as “springboards” for the host jump of *P. oryzae* strains (with *PWT3;pwt4*) in Brazil. Similarly, *rwt3/rwt4* cultivars may become springboards for *Avena* isolates (*PWT3;PWT4*) to jump hosts. Taken together, it is advisable to cultivate common wheat cultivars that carry both *Rwt3* and *Rwt4* for forestalling reoccurrence of host jumps or at least for preventing wheat blast disease caused by infection with *Lolium* or *Avena* pathogens.

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Figure legends

Fig. 1. Distribution of *PWT3* and *PWT4* in *Pyricularia* spp. (A) A maximum likelihood tree of *P. oryzae* isolates constructed from SNPs in whole genome sequences. *P. grisea* (Dig41) was used as an outgroup. The numbers on the branches indicate bootstrap probability. The bar below the tree shows genetic distance per site. (B) Schematic representation of *PWT3* and *PWT4* types among *Triticum* isolates from the 1990s (Kobe University collection) and other pathotypes. The arrows and horizontal bars represent ORFs and flanking regions, respectively. In the column of *PWT3*, Ao and B are avirulent and virulent types, respectively. The A' and Atm types are identical to the Ao type except for a one-base substitution and an insertion of retrotransposon MGR583 (gray triangle) in the upstream region, respectively. In the column of *PWT4*, red, light green, and blue represent the avirulent type, the virulent type, and a truncated virulent type, respectively. Dotted lines indicate absence of homologs.

Fig. 2. *PWT3* and *PWT4* serve as the host species specificity barrier for wheat. Spikes of wheat cultivars Norin 4 (N4), Chinese Spring (CS), Transfed (Tfed), and Hope were inoculated with wild types (WT) of *Triticum* (A), *Lolium* (B), *Avena* (C) isolates, their transformants

carrying introduced *PWT3* (+3) or *PWT4* (+4), disruptants of *PWT3* ($\Delta 3$) or *PWT4* ($\Delta 4$), and double disruptant ($\Delta 3\Delta 4$). Symptoms are shown after 8 days.

Fig. 3. Dynamics of the *PWT3* types during the three decades. (A) Structure of the Atp type in WBKY11-15 (LC229726), an isolate collected from wheat in Kentucky. (B) Structure of the Atc type in B71 (LC215054), a highly aggressive *Triticum* isolate collected in Bolivia. (C) Geographical and historical distribution of the *PWT3* types in *Triticum* isolates. Each symbol represents an isolate collected in the indicated year, and color-coded according to its *PWT3* type. The Atc type appears to have expanded over time while the B type was not found beyond the 1990s.

Fig. 4. Distribution of *Rwt3* and *Rwt4* in local landraces of common wheat in the world. Pie chart size is in proportion to numbers of accessions tested. A magnified map of the region inside the dashed line is shown on its left.

Supplementary Materials:

Materials and Methods

Figures S1-S14

Tables S1-S4

References (16-31)

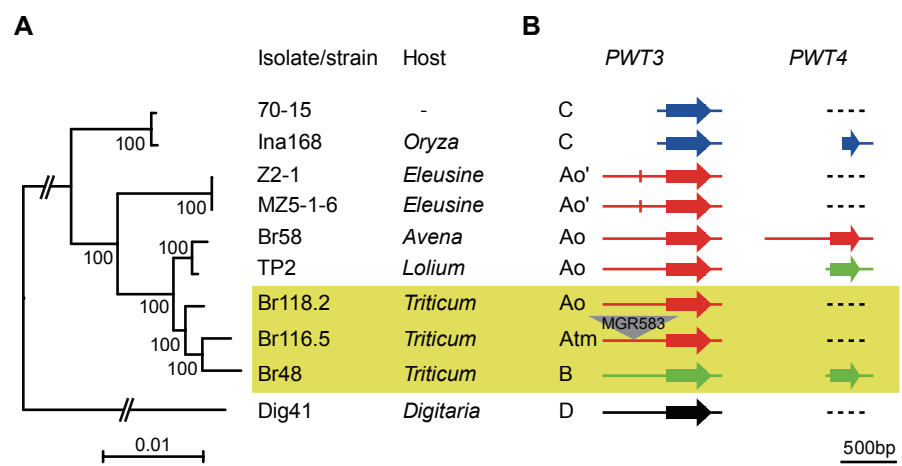


Fig. 1

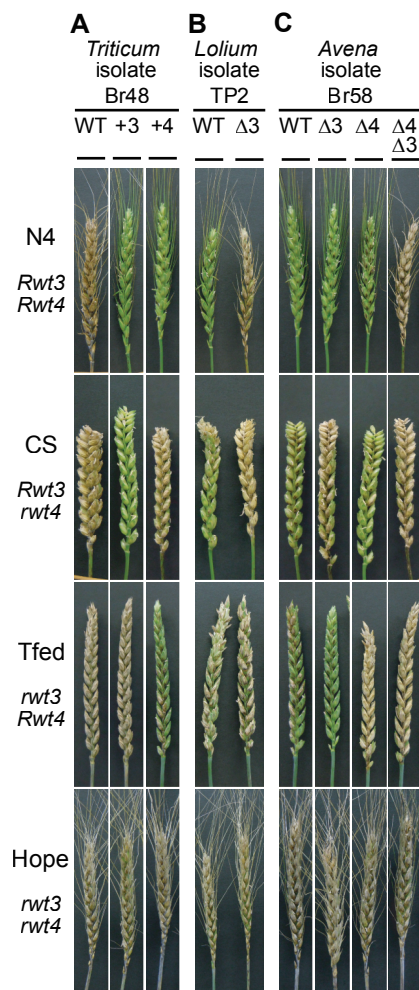


Fig. 2

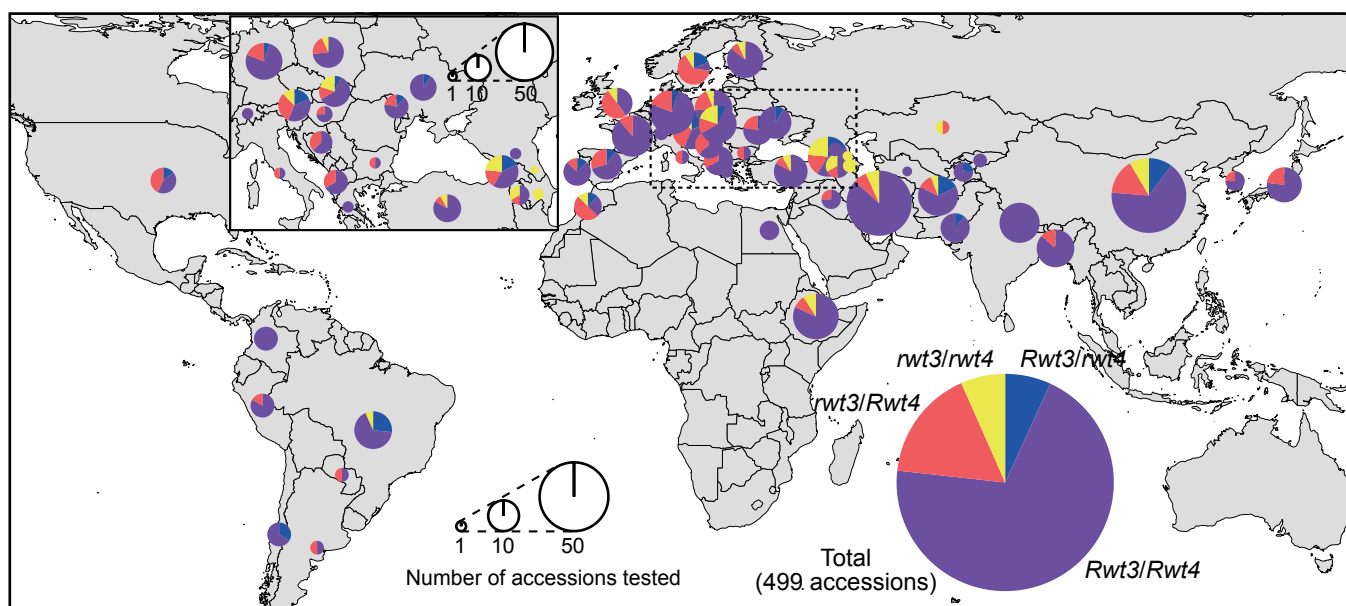


Fig. 4



Supplementary

Materials for

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Materials and Methods
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Materials and Methods

Fungal and plant materials

Pyricularia isolates used or analyzed in the present study are listed in table S1. Improved cultivars of common wheat (*Triticum aestivum*) used for the *Rwt3/Rwt4* typing were obtained from Genebank at National Agriculture and Food Research Organization, Japan. Local landraces of common wheat used for the *Rwt3/Rwt4* typing have been preserved in Okayama University, Japan, by K. Kato.

Genetic crosses of *P. oryzae*

Mapping populations were produced by crossing *P. oryzae* isolates on oatmeal agar media as described previously (16). One ascospore was isolated from each ascus so that each of resulting hybrids was derived from an independent meiotic event.

Infection assay

For seedling inoculation, 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 for 8 days. Inocula were prepared as described by Murakami et al. (16) with minor modifications. Ten milliliters of conidial suspension (1.0×10^5 conidia/mL) with 0.01 % Tween 20 were sprayed on wheat primary leaves fixed onto a hard plastic board in a seedling case with an air compressor. The cases were sealed to maintain high humidity and placed in darkness for 24 h at 22 °C. The inoculated seedlings were then transferred to a growth chamber at 22 °C with a 12-h photoperiod. Four to five days after inoculation, symptoms were evaluated based on the color and the size 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 (<1.5 mm); 3, lesions with an intermediate size (<3 mm); 4, large typical lesions; and 5, complete blighting of leaf blades (10). These lesions were classified into two categories on the basis of their color, i.e., brown (B) and green (G). Infection types were represented by the combination of lesion size scores (0–5) and color codes (B and G) (10). Each infection assay was repeated at least twice.

For infection assays at the heading stage, wheat plants were grown to maturity in greenhouse or field for 4-5 months. Wheat spikes were collected between flowering and early grain filling. Stems with spikes were cut at the base, brought back to the laboratory, trimmed to ~50 cm, and put in test tubes with water. The harvested spikes were inoculated with conidial suspensions (1×10^5 spores/ml) prepared as described above and were kept in test tubes with water. The whole set-up was covered with a plastic bag to maintain high humidity and placed in darkness at 25°C in a growth chamber for 24 h. The plastic bag was removed and the set-up was incubated further at the same temperature with a 12-h photoperiod. Six to eight days after inoculation, spike infections were evaluated. Each infection assay was repeated at least twice.

Transformation of *P. oryzae*

Plasmid clones carrying Avex210, Avex211, Avex1312, and Avex4415 were introduced into Br48 by co-transformation with pSH75 containing the hygromycin B phosphotransferase gene as described by Tosa et al. (17).

Targeted gene knockout in *P. oryzae*

Split marker strategy was employed for targeted gene knockout. For disruption of *PWT3* (fig. S5), a 1.2kb DNA fragment upstream of *PWT3* ORF was amplified from genomic DNA of Br58 with primers, Br58_scaf36_210_LFu_XhoI (5'-

TTTctcgagTTTCTGTGGGAAGGCTCAAC-3') and Br58_scaf36_210_LFd_HindIII (5'-TTTaaagcttGGCGGAATTTTGCAGCTTTC-3'), digested with *Xho*I and *Hind*III, and cloned into pSP72-HPH (18) digested with *Xho*I and *Hind*III, to produce pSP72-PWT3LF-HPH. A 0.6 kb DNA fragment downstream of *PWT3* was amplified from genomic DNA of Br58 with primers, Br58_scaf36_210_RFu_BamHI (5'-TTggatccGGGCATGAGGAGGTTTTTCAG-3') and Br58_scaf36_210_RFd_SacI (5'-TTgagctcCAACACCGTGATTGACTTGG-3'), digested with *Bam*HI and *Sac*I, and cloned into pSP72-HPH digested with *Bam*HI and *Sac*I, to produce pSP72-HPH-PWT3RF. Then, a fragment carrying 1.2kb *PWT3* upstream region fused to 5' part of HPH cassette was PCR-amplified with primers, Br58_scaf36_210_LFu_XhoI and HY (5'-GGATGCCTCCGCTCGAAGTA-3'), using pSP72-PWT3LF-HPH as a template. A fragment carrying 3' part of HPH cassette fused to 0.6kb *PWT3* downstream region was PCR-amplified with primers, YG (5'-CGTTGCAAGACCTGCCTGAA-3') and Br58_scaf36_210_RFd_SacI, using pSP72-HPH-PWT3RF as a template. Two PCR amplified fragments were mixed and used for transformation of *P. oryzae*.

For disruption of *PWT4* (fig. S7), a 0.5 kb DNA fragment upstream of *PWT4* was amplified from genomic DNA of Br58 with primers, ORF2_LFu1_BglII (5'-TTTtagatctTGGCACATTATATATCGCAGTGGTT -3') and ORF2_LFd1_EcoRI (5'-AAGaattcGGCAAAGGAAATCTGTGGGT-3'), digested with *Bgl*II and *Eco*RI, and cloned into pII99 (19) digested with *Bgl*II and *Eco*RI, to produce pII99-PWT4LF-NPTII. A 0.9 kb DNA fragment downstream of *PWT4* was amplified from genomic DNA of Br58 with primers, ORF2_RFu2_XbaI (5'-TAtctagaGATGGGGTTTTAGTACGTGTGG -3') and ORF2_RFd2_HindIII (5'-

ATTaagcttCGTGACAGTTGGTGAAGAAGA -3'), digested with *Xba*I and *Hind*III, and cloned into pII99 digested with *Xba*I and *Hind*III, to produce pII99-NPTII-PWT4RF. Then, a fragment carrying 0.5 kb *PWT4* upstream region fused to 5' part of NPTII cassette was PCR-amplified with primers, ORF2_LFu1_BglII and NP (5'-CTGATGCTCTTCGTCCAGATCA-3'), using pII99-PWT4LF-NPTII as a template. A fragment carrying 3' part of NPTII cassette fused to 0.9 kb *PWT4* downstream region was PCR-amplified with primers, PT (5'-TTGGGTGGAGAGGCTATTCG-3') and ORF2_RFd2_HindIII, using pII99-NPTII-PWT4RF as a template. Two PCR-amplified fragments were mixed and used for transformation of *P. oryzae*.

One disruptant was arbitrarily chosen from each of 13 (Br58 Δ PWT3), 7 (Br58 Δ PWT4), 6 (Br58 Δ PWT3 Δ PWT4), 14 (TP2 Δ PWT3), and 7 (Br118.2 Δ PWT3) independent disruptants obtained from the transformation, and used for infection assay. One ectopic mutant obtained from each transformation was also used for infection assay.

DNA extraction, sequencing, and construction of phylogenetic trees

Whole genomes of *P. oryzae* isolates Ina168, Z2-1, Br58, and Br48 and a *P. grisea* isolate Dig41 were sequenced by Yoshida et al. (20). Those of *P. oryzae* isolates Br116.5, Br118.2, TP2, MZ5-1-6, and Br35 were sequenced in the present study by Illumina MiSeq. Total DNAs were prepared from mycelia of *P. oryzae* using Plant Genomic DNA Extraction Miniprep System (Viogene) or DNeasy Plant Mini kit (QIAGEN). Sequence libraries were prepared with NEBNext DNA Library Prep Master Mix Set for Illumina (New England Biolabs) and NEBNext Multiplex Oligos for Illumina (New England Biolabs) and sequenced with MiSeq Reagent Kit v3 (Illumina). A mate-

pair library for Br58 was prepared with Mate Pair Library Preparation Kit (Illumina) and sequenced with Illumina Genome Analyzer IIX.

Raw sequence reads were subjected to adapter and quality trimming by using Trimmomatic-0.32 (21). A maximum likelihood tree was constructed by RealPhy v1.12 (22) that automatically performs alignment of sequencing reads, SNP calling and construction of phylogenetic tree. In this tool, bowtie2 (23) was used for mapping filtered paired-end reads to 70-15 strain (MG8) reference sequence, and RAxML (24) was used for building the ML tree. MEGA7 (25) was used for building the NJ tree with p-distance method.

De novo assembly and gene predictions

Br58 WT genome contigs were constructed by *de novo* assembly of paired-end reads with CLC (<http://www.clcbio.com>) software. The contigs were used to generate scaffolds using mate-pair sequence reads of Br58 with SSPACE (26). Gene predictions were performed with fgenesh (Softberry) trained for *Magnaporthe*. In addition, ORFs longer than 150bp were picked up by 6-frame translation. Putative secreted proteins were predicted using signalP-4.1 (27) with D-value cutoff of 0.34.

Sequencing of bulked DNA from backcross progeny and selection of *PWT4* candidate genes

A paired-end library for a bulked DNA sample was prepared with TruSeq DNA LT Sample Prep kit (Illumina) and sequenced with Illumina Genome Analyzer IIX. Raw sequence reads were subjected to adapter and quality trimming as described above. Trimmed reads were mapped to a Br58 reference genome with BWA (28). Bam files were converted to sam and pileup files by samtools-0.1.8 (29). SNP calling was

performed by Coval (30). SNPindex (31) for SNP sites and average read depth for contigs were calculated from pileup file. Contigs with average SNPindex lower than 0.1 with *PWT4* bulk sample were considered to be tightly linked to *PWT4*. For contigs without SNPs, average read depths (ARD) with Br58, Br48, and *PWT4* bulk sample were compared for each contig. Contigs with significantly higher (fisher's exact test, $P < 0.01$) Br58 ARD than Br48 ARD were picked up as Br58-specific contigs. For each Br58-specific contig, if *PWT4* bulk sample ARD was significantly higher (fisher's exact test, $P < 0.01$) than Br48 ARD, the contig was considered to represent a genomic region tightly linked to *PWT4*. Putative secreted protein genes on Br58 contigs tightly linked to *PWT4* were picked up as *PWT4* candidate genes.

In planta expression analysis of avirulence genes

Susceptible barley cv. Nigrate was grown for 9 days as described in wheat seedling inoculation assay. *P. oryzae* conidia were prepared as described in infection assay and sprayed on 9-day-old seedlings. At 48 hours post inoculation, twenty inoculated barley primary leaves were collected, pooled, and subjected to RNA extraction. Total RNA was extracted with Sepasol-RNA I Super G (Nacalai Tesque) and treated with DNase I, Amplification Grade (Invitrogen). cDNA was synthesized using PrimeScript RT reagent Kit (Perfect Real Time) (Takara) and subjected to qRT-PCR analysis using FastStart SYBR Green Master (Roche) and Thermal Cycler Dice Real Time System (Takara). The primer sequences used in the qRT-PCR analysis were 5'-CTACTACGAATTGAAAGCGGAAC-3' and 5'-CCATCATCAGGTTTCATAAGTTCA-3' for *PWT3* and 5'-GCGGTTACACCTCTCTACCAC-3' and 5'-AGTCTGGATCTCCTGCTCAAAG-3' for actin gene (MGG_03982).

PWT3 typing of isolates preserved in Americas or in database

PWT3 types of isolates preserved/sequenced in University of Kentucky, Kansas State University, or Ohio State University (6), were inferred from sequences of the *PWT3* ORF and positions of insertion of transposable elements in their whole genome sequences. Whole genome sequences available for the public were also analyzed similarly. Genome sequences of wheat blast isolates PY36.1, PY86.1, PY0925, PY5003, PY5010, PY6017, PY6025, PY6045 (7) were accessed from openwheatblast.org. These isolates were collected by Joao Leodato Nunes Maciel, Embrapa, Passo Fundo, Brazil, sequenced and analysed by Darren Soanes and Nicholas J. Talbot, University of Exeter, and Antonio Nhani Jr, Embrapa.

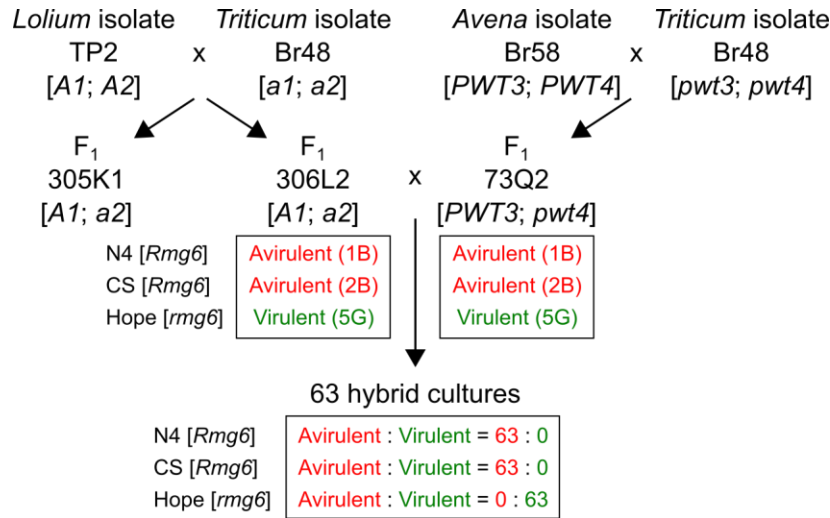


Fig. S1. Allelism test of avirulence genes, *A1* and *PWT3*. The avirulence of *Lolium* isolate TP2 on wheat is controlled by *A1* with a major effect and *A2* with a very minor effect negligible in conducive conditions (10) while the avirulence of *Avena* isolate Br58 on wheat is controlled by *PWT3* and *PWT4* (9). 306L2 (an F_1 strain derived from TP2 x *Triticum* isolate Br48) carrying *A1* alone was crossed with 73Q2 (an F_1 strain derived from Br58 x Br48) carrying *PWT3* alone. The resulting 63 hybrid cultures were all avirulent on *Triticum aestivum* cv. Norin 4 (N4) and cv. Chinese Spring (CS) carrying *Rmg6* while they were all virulent on cv. Hope lacking *Rmg6*, suggesting that *A1* and *PWT3* are located at the same locus. Infection types of these cultivars with the parental F_1 strains are shown in parentheses.

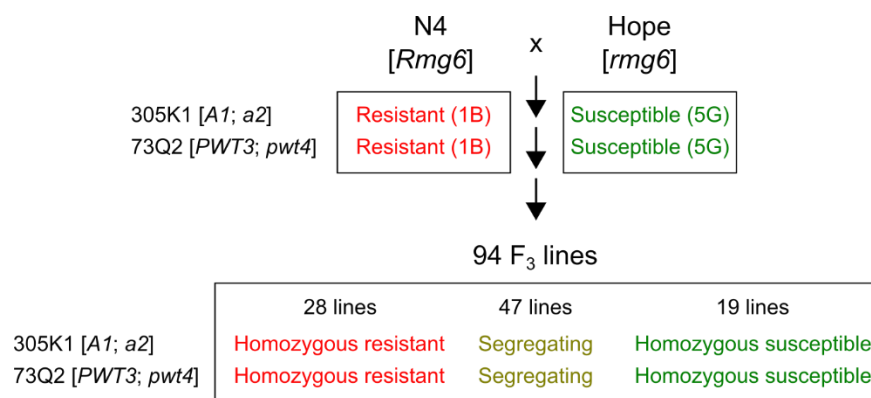


Fig. S2. Allelism test of resistance genes corresponding to *A1* and *PWT3*. The resistance of N4 to 305K1 carrying *A1* alone (fig. S1) is controlled by *Rmg6* corresponding to *A1* (10). When 94 F₃ lines derived from N4 (*Rmg6*) x Hope (*rmg6*) were inoculated with 305K1, homozygous resistant, segregating, and homozygous susceptible lines segregated in a 1:2:1 ratio ($\chi^2 = 1.7$, $P = 0.4$). This is the monofactorial segregation conferred by *Rmg6*. When inoculated with 73Q2 carrying *PWT3* alone (fig. S1), these F₃ lines showed perfectly concordant segregation, suggesting that *Rmg6* recognizes *PWT3*. Infection types of the parental cultivars with the F₁ strains are shown in parentheses.

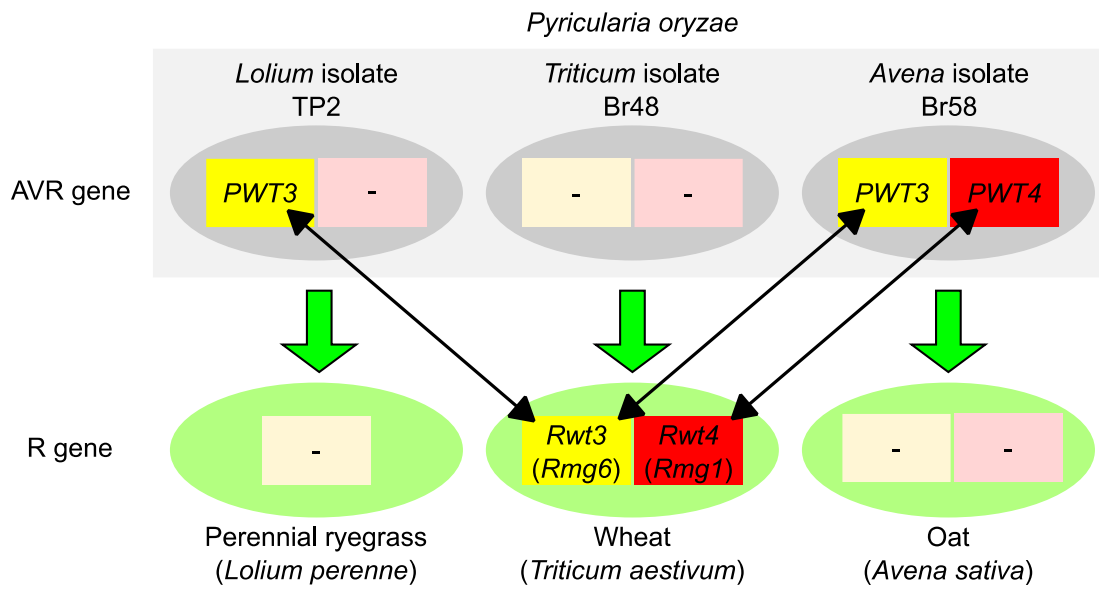


Fig. S3. Gene-for-gene interactions between *P. oryzae* isolates and gramineous plants. Avirulence gene - resistance gene pairs are indicated by bidirectional black arrows. Green arrows indicate compatible interactions. Hyphens indicate non-functional alleles.

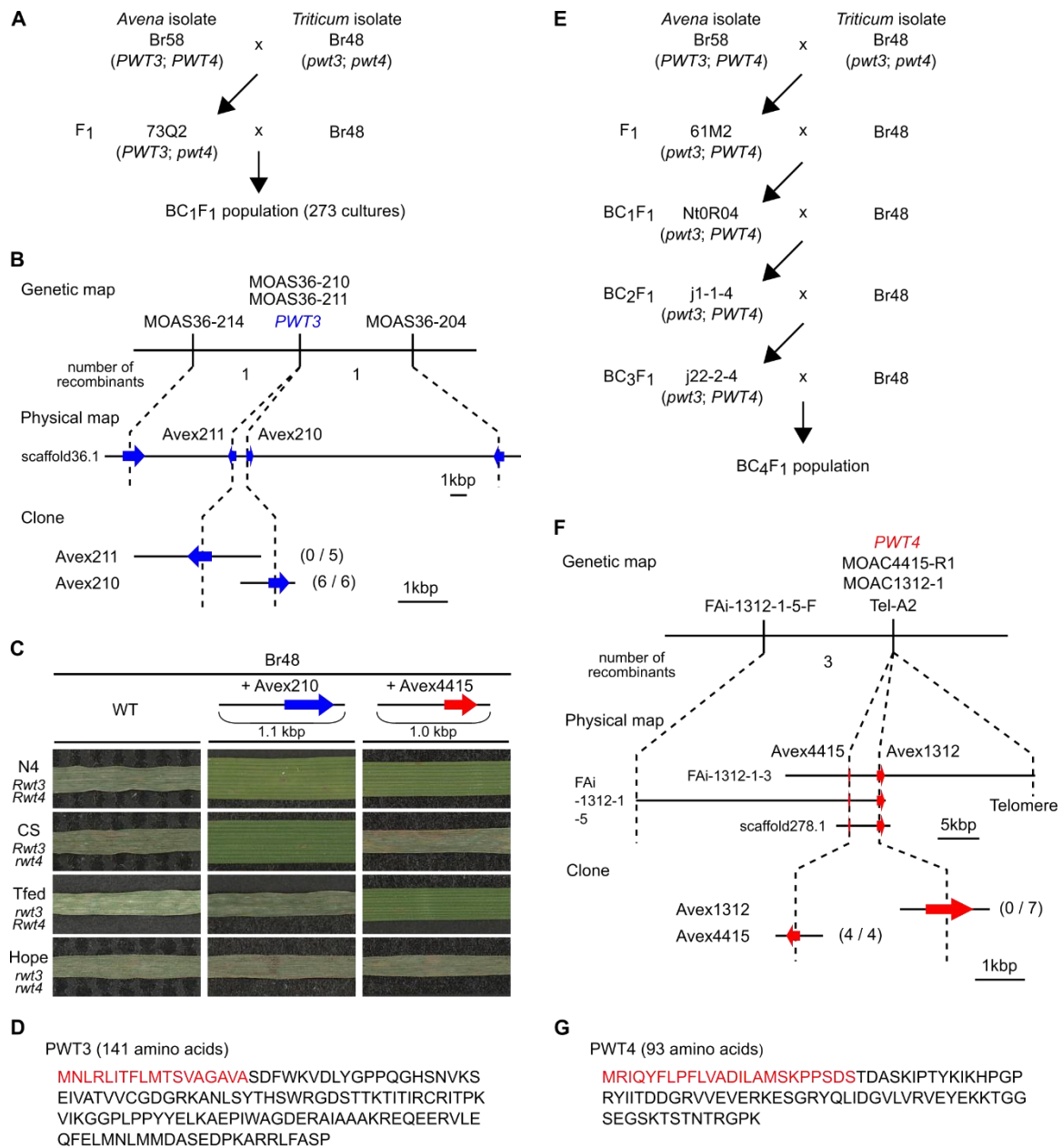


Fig. S4. Molecular cloning of *PWT3* (A, B, C, D) and *PWT4* (E, F, C, G) from *Avena* isolate Br58. Br58 (*PWT3*; *PWT4*) was crossed with *Triticum* isolate Br48 (*pwt3*; *pwt4*), and an F₁ strain carrying *PWT3* alone (73Q2) was backcrossed with Br48, resulting in 273 BC₁F₁ progeny among which *PWT3* segregated in a 1:1 ratio (A). Linkage mapping using the 273 progeny located *PWT3* between the two molecular markers MOAS36-214

and MOAS36-204, encompassing a 22kbp region containing two putative secreted protein-encoding genes, Avex210 and Avex211 (B). Each gene was introduced into Br48 through co-transformation with pSH75, a plasmid carrying a hygromycin resistance gene. The resulting transformants were inoculated onto seedlings of wheat cultivars N4 carrying both *Rwt3* and *Rwt4*, CS carrying *Rwt3* alone, Transfed (Tfed) carrying *Rwt4* alone, and Hope carrying neither gene. No transformants carrying Avex211 were avirulent on the *Rwt3* carriers (N4 and CS) whereas all transformants carrying Avex210 showed specific avirulence on the *Rwt3* carriers (B, C), indicating that Avex210 is *PWT3*. *PWT3* encoded a small secreted protein composed of 141 amino acids (LC202650) (D). For cloning of *PWT4*, F₁ strain 61M2 carrying *PWT4* alone was selected from the F₁ progeny derived from Br58 x Br48, and backcrossed with Br48 four times, resulting in the creation of a BC₄F₁ population (E). Genomic DNAs from 48 cultures (40 BC₄F₁ and 8 BC₁F₁) carrying *PWT4* were pooled and subjected to illumina sequencing to identify genomic regions tightly linked to *PWT4*. Among 27 scaffolds identified, only one (scaffold278.1; 6,555bp in size) showed perfect association with the *PWT4* phenotype in the BC₄F₁ population (F). This scaffold contained two candidate ORFs, Avex1312 and Avex4415 (F). When introduced into Br48, Avex4415 conferred specific avirulence on seedlings of the wheat cultivars carrying *Rwt4* (N4 and Tfed), but Avex1312 did not (F, C), indicating that Avex4415 is *PWT4*. *PWT4* encoded a small secreted protein composed of 93 amino acids (LC202655) (G). In B and F, shown in parentheses are number of transformants avirulent on carriers of corresponding resistance genes / number of transformants tested. In D and G predicted signal peptides are shown in red.

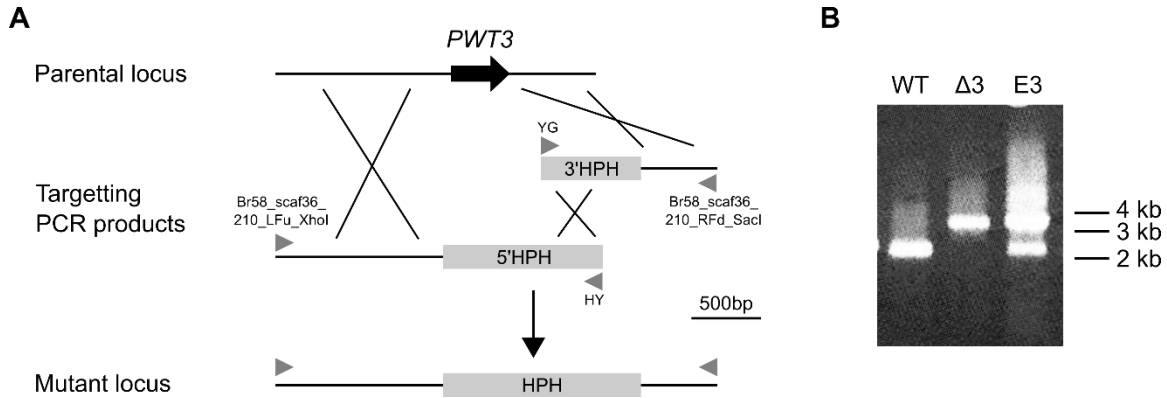


Fig. S5. Targeted gene disruption of *PWT3*. (A) A split marker strategy was used for the deletion of *PWT3*. Regions demarked by crossover lines represent the regions used in the targeting vector to facilitate homologous recombination. Primers used to amplify the targeting fragments are indicated. The truncated or complete HPH cassettes are shown as gray boxes named 5'HPH, 3'HPH or HPH. The length of the overlapping region was 466 bp. (B) Verification of *PWT3* deletion by PCR using primers indicated in A (Br58_scaf36_210_LFu_XhoI and Br58_scaf36_210_RFd_SacI). PCR fragments of 2309 and 3184 bp were predicted to be amplified from the wild type and mutant genomic DNA, respectively. An ectopic recombination would show both bands. Marker sizes are shown to the right. WT, wild-type Br58; Δ3, a Δ*PWT3* mutant; E3, an ectopic mutant.

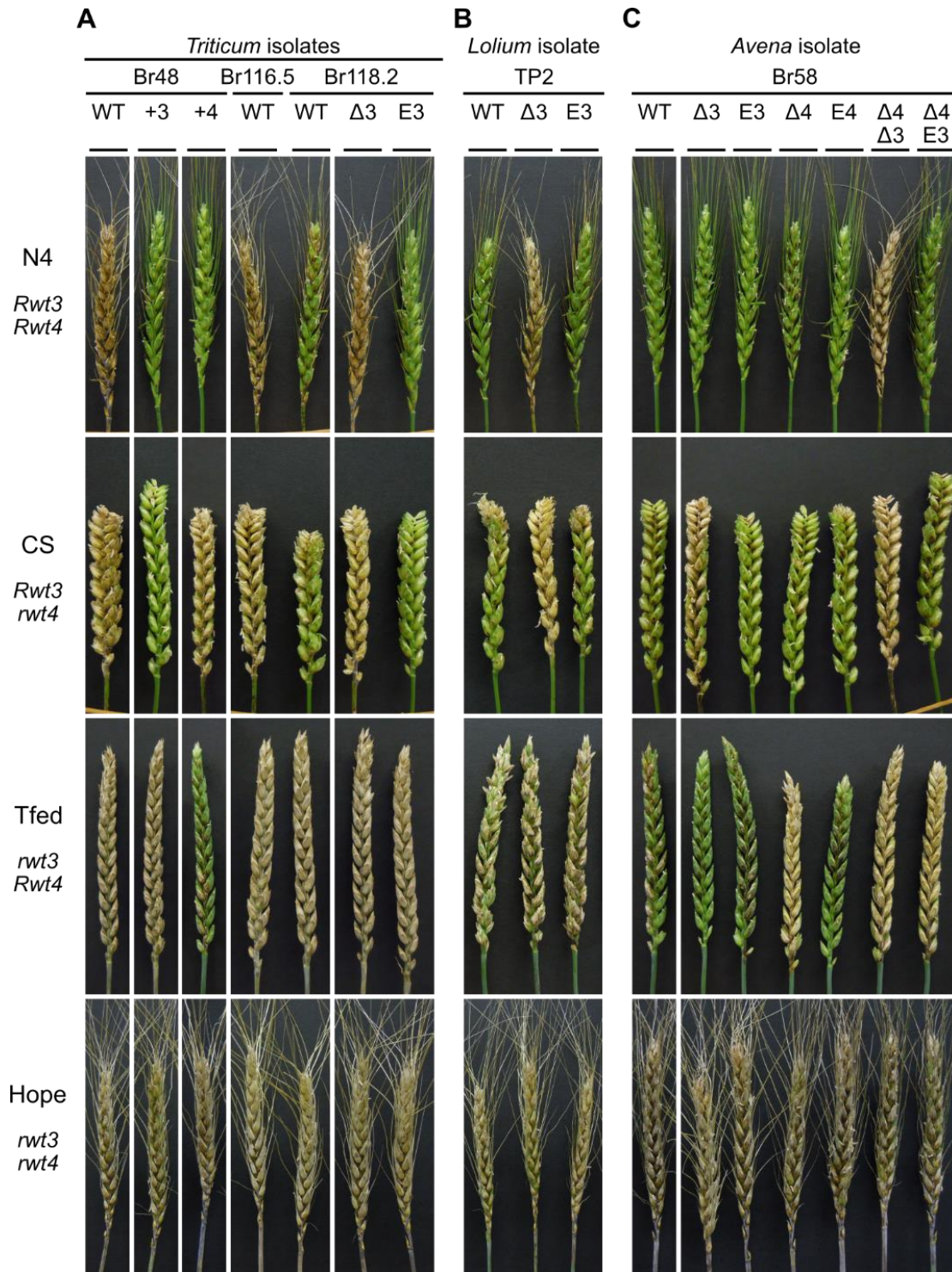


Fig. S6. Pathogenicity of *P. oryzae* isolates and their transformants on wheat spikes. Spikes of four common wheat cultivars (N4, CS, Tfed, and Hope) were inoculated with

wild types (WT) of *Triticum* isolates (A), *Lolium* isolates (B), *Avena* isolate (C), and their transformants carrying introduced *PWT3* (+3) or *PWT4* (+4), disruptants of *PWT3* ($\Delta 3$) or *PWT4* ($\Delta 4$), transformants with ectopic integration of the *PWT3* disruption vector (E3) or *PWT4* disruption vector (E4), and a double disruptant of *PWT3* and *PWT4* ($\Delta 3\Delta 4$), and then incubated for 8 days. The three *Triticum* isolates shown in (A), Br48, Br116.5 and Br118.2, are representatives of the three *PWT3* types, B, Atm, and Ao, respectively. Pictures of representative spikes extracted from this figure are also shown in Fig. 2.

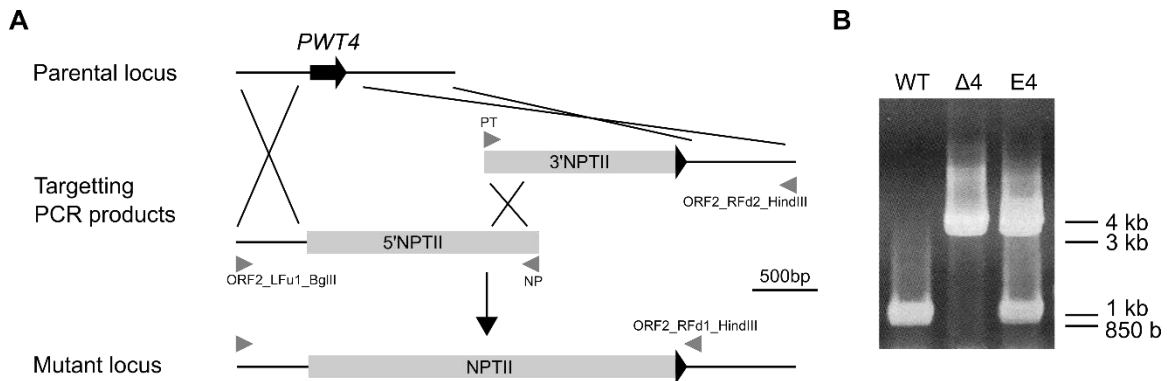


Fig. S7. Targeted gene disruption of *PWT4*. (A) A split marker strategy was used for the deletion of *PWT4*. Regions demarked by crossover lines represent the regions used in the targeting vector to facilitate homologous recombination. Primers used to amplify the targeting fragments are indicated. The truncated or complete NPTII cassettes are shown as gray boxes named 5'NPTII, 3'NPTII or NPTII. The length of the overlapping region was 448 bp. (B) Verification of *PWT4* deletion by PCR using primers indicated in A (ORF2_LFu1_BglII and ORF2_RFd1_HindIII). PCR fragments of 956 and 3542 bp were predicted to be amplified from the wild type and mutant genomic DNA, respectively. An ectopic recombination would show both bands. Marker sizes are shown to the right. WT, wild-type Br58; Δ4, a Δ*PWT4* mutant; E4, an ectopic mutant.

A

Br58 type	1	MRIQYFLPFLVADILAMSKPPSDSTDASKIPTYKIKHPGPRYITDDGRV
Br48 type	1	MRIQYFLPSLVAGILAMPKPPSSNSLNSPEVPTYKIGHPGPRYIRYNDGEI
Ina168 type	1	-----IRYNDGEI
Br58 type	51	VEVERKESGRYQLIDGVLRVEYEKKTGGSEGSKTSTNTRGPK
Br48 type	51	HEVERKESGRYRVDDGGLIREEYEKKTQDSEGSKTPT-TRGPK
Ina168 type	9	HEVERKESGRYRVDDGGLIREEYEKKTQDSEGSKTST-TRGPK

B

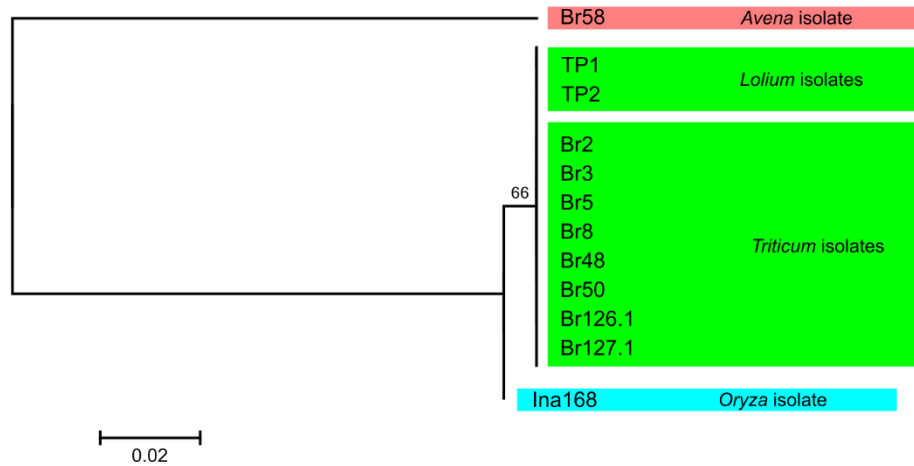


Fig. S8. *PWT4* homologs in *P. oryzae* isolates. (A) Amino acid sequence alignment of predicted *PWT4* proteins of three *PWT4* homolog types (Br58 type, LC202655; Br48 type, LC202656; Ina168 type, LC202657). (B) Phylogenetic relationships of *PWT4* homologs of *Pyricularia* isolates from various hosts. A maximum likelihood (ML) tree based on ORF nucleotide sequences is shown. Isolates sharing the same *PWT4* type are labeled with the same color. The bar below the tree shows nucleotide substitutions per site. The numbers on the branches indicate bootstrap probability. *Lolium* isolates (including TP2) and several *Triticum* isolate (including Br48) shared an identical homolog, but its nucleotide sequence was highly divergent (82% identity) from that of the functional *PWT4* allele of *Avena* isolate Br58. This homolog was considered to have lost its avirulence function because Br48 and TP2 were virulent on Tfed (*rwt3/Rwt4*)

(Fig. 2). A homolog found in the *Oryza* isolate Ina168 was truncated. *PWT4* homologs were not detected in the other *Triticum* isolates listed in table S2.

A

A type	1	MNLRLITFLMTSVAGAVASDFWKVDLYGPPQGH	SNVKSEIVATVVCGDGR
B type	1	MNLRLITFLMTSVAGAVARD	DFWKVDLYGPPQGYSNVKSEIVATVVCGDGR
C type	1	MNLRLITFLMTSVAGAVASDFWKVDLYGPPQGY	SNVKSEIVATVVCGDGR
D type	1	MDLKVITFLMAFVASAVAKDY	YKVELYEVQPLYSTTPAKVLTKVCGGDGD

A type	51	KANLSYTHSWRGDSTTKTITIRCR-	ITPKVIKGGPLPPYELKAEP	IWAG
B type	51	KANLSYTHSWRGDSRTETITIRCR-	ITPKVIEGGPLPPYELKAES	IPAG
C type	51	KANLSYTHSWRGDSTTNTITIRCR-	ITPKVIKGGPLPPNYELKAEP	IWAG
D type	51	RATLTITVGRLGNRASKTITIRCR	GKNPRIIDGGPLPSNYKLRA	DPISAD

A type	100	DERAIAAAKREQEERVLEQFELMNLMM	DASEDPKARRLFASP
B type	100	DERAIAAAKREQEERFHEQFELVNLMM	DASADPKARRLFASP
C type	100	DERAIAAAKREQEERFHEQFELMNLMM	DASADPKARRLFASP
D type	101	DEALTAAKETSDYRSYEALQLMNK	FMDVTDHPEKWRKYGMQ

B

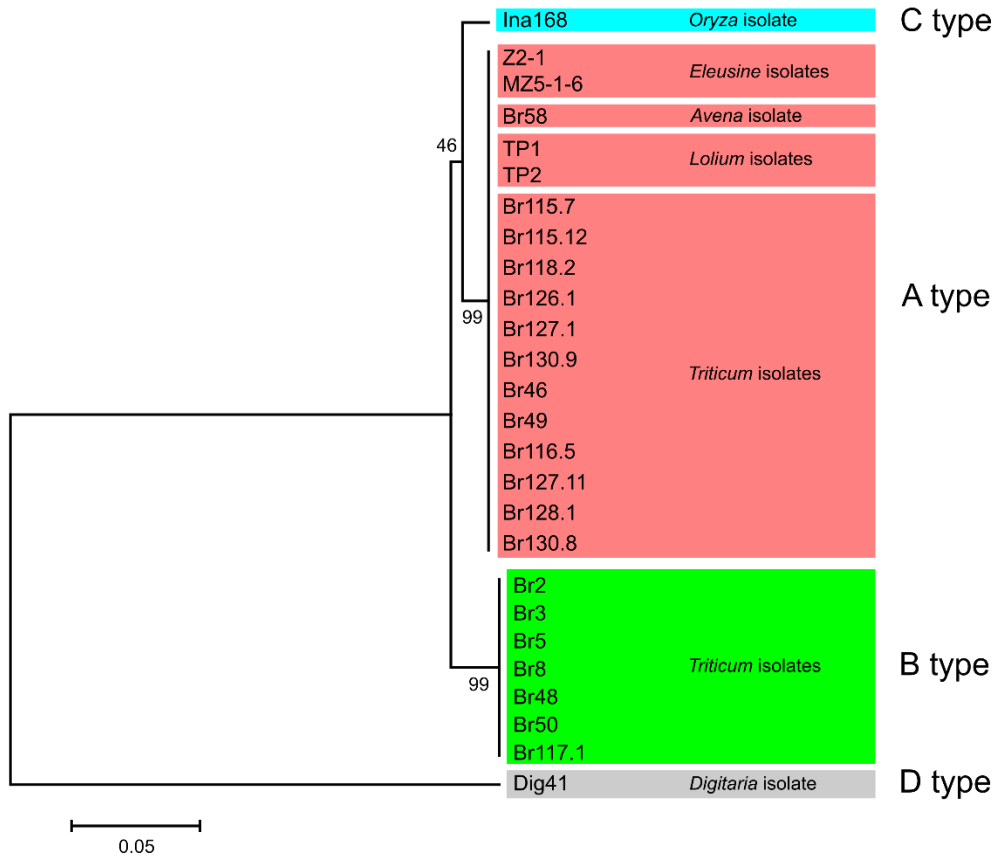


Fig. S9. *PWT3* homologs in *P. oryzae* and *P. grisea* isolates. (A) Amino acid sequence alignment of predicted *PWT3* proteins of four *PWT3* homolog types (A-type from Br58, LC202650; B type from Br48, LC202652; C type from Ina168, LC202653; D type from

Dig41, LC202654). (B) Phylogenetic relationships of *PWT3* homologs of *P. grisea* (Dig41) and *P. oryzae* isolates from various hosts. A maximum likelihood (ML) tree based on ORF nucleotide sequences is shown. Isolates sharing the same *PWT3* type are labeled with the same color. The bar below the tree shows nucleotide substitutions per site. The numbers on the branches indicate bootstrap probability.

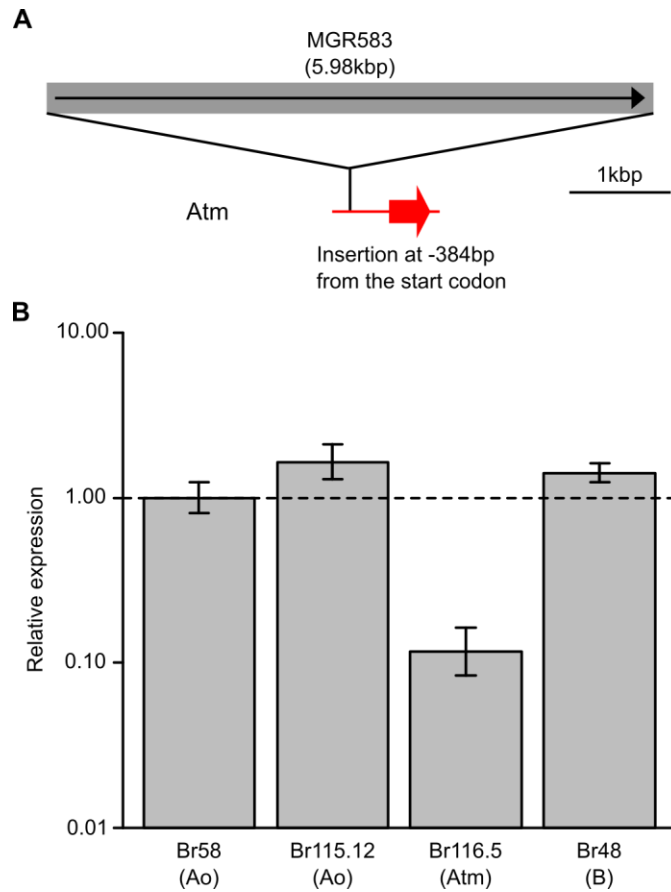


Fig. S10. Insertion of a transposable element into the *PWT3* upstream region leads to the reduction of *PWT3* gene expression. (A) Organization of the *Atm* type *PWT3* in Br116.5 (LC202651), a *Triticum* isolate collected in Brazil. MGR583, a transposable element inserted into the *PWT3* upstream region, is represented by a box with an arrow indicating its orientation. (B) *In planta* expression of *PWT3* gene at 48 hours post inoculation. Twenty inoculated barley primary leaves were collected, pooled, and subjected to RNA extraction. *PWT3* expression level relative to actin gene (MGG_03982) was measured by quantitative RT-PCR. Letters in parenthesis indicate *PWT3* types of each isolate. Bars indicate standard deviation of three technical replicates.

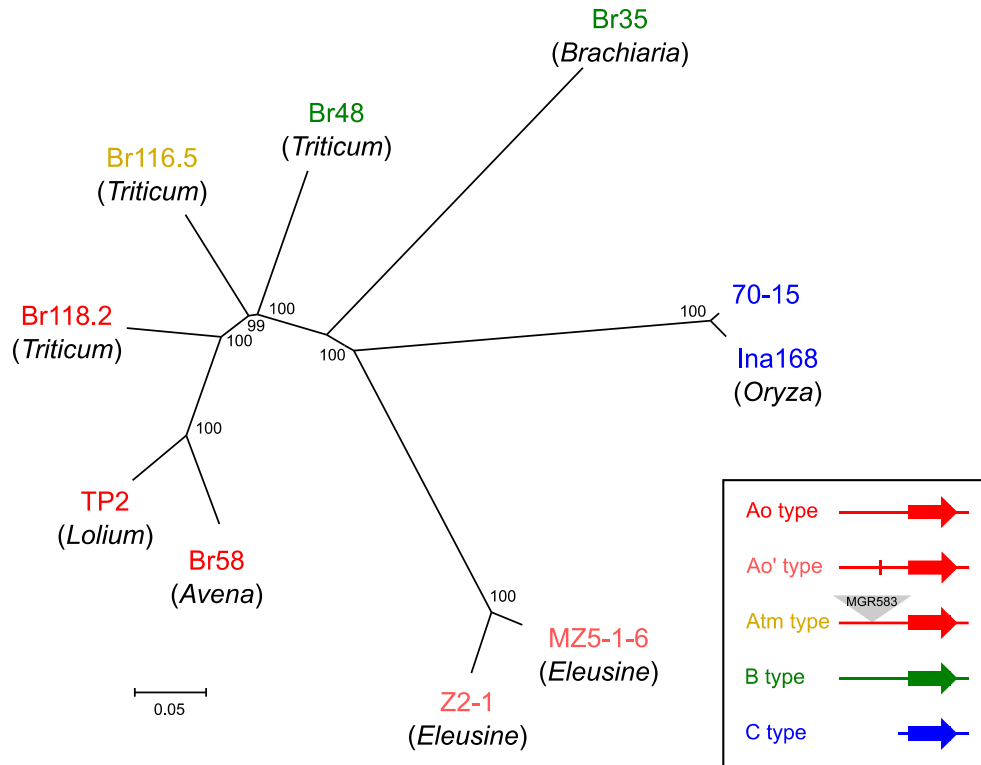


Fig. S11. *Triticum* isolate Br48 and *Brachiaria* isolate Br35 share identical B type *PWT3* genes but they are phylogenetically separated from each other. A neighbor joining (NJ) tree of *Pyricularia* isolates from various gramineous hosts was constructed from SNPs in whole genome sequences. Names of isolates are color-coded according to their *PWT3* types. Their original host genera are shown in parentheses. The numbers on the branches indicate bootstrap probability. The bar below the tree shows genetic distance per site.

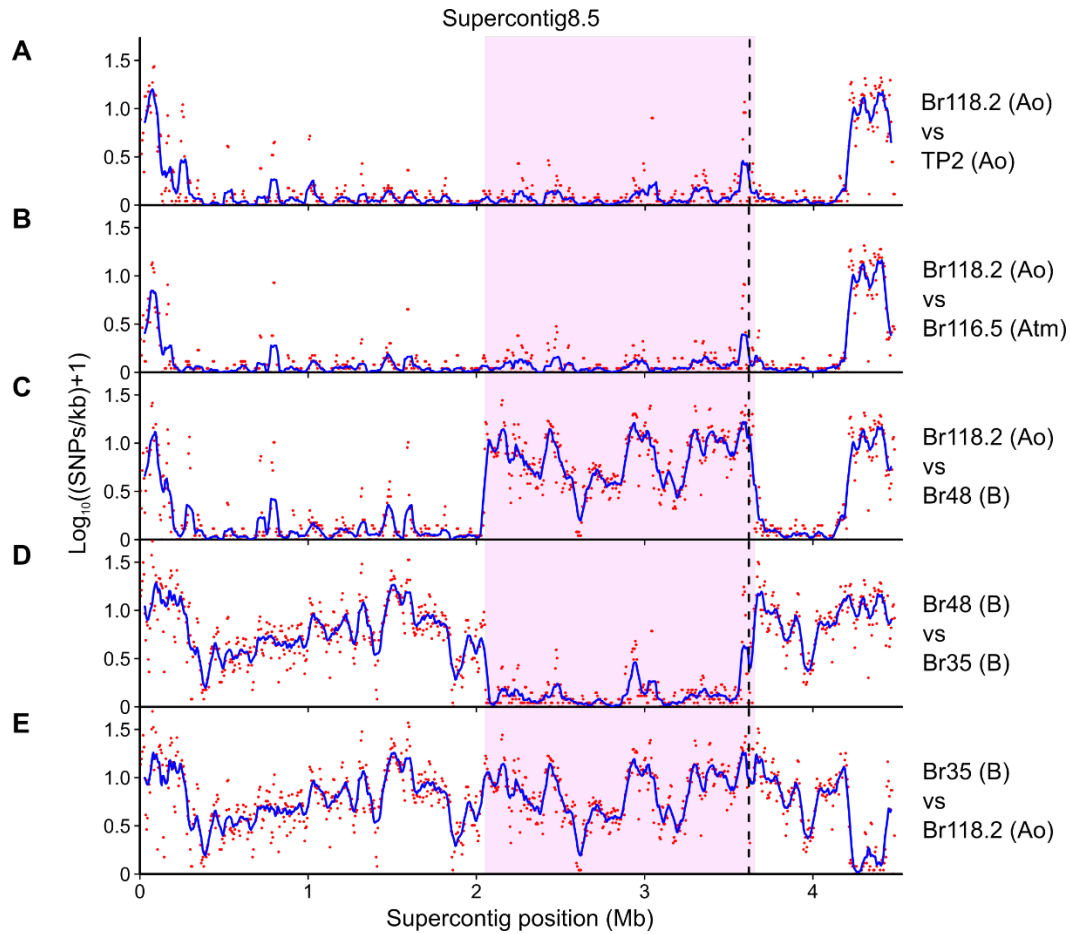


Fig. S12. *Triticum* isolate Br48 appears to have inherited a 1.6Mb genomic segment from a Br35-like *Brachiaria* isolate. Frequencies of single nucleotide polymorphisms (SNPs) among TP2 (*Lolium* isolate with Ao), Br118.2 (*Triticum* isolate with Ao), Br116.5 (*Triticum* isolate with Atm), Br48 (*Triticum* isolate with B) and Br35 (*Brachiaria* isolate with B) were calculated based on a moving window of 10kb and shifting the window 5kb at a time, and plotted along the supercontig8.5. X- and Y-axis represent the position in supercontig8.5 and the SNP frequency, respectively. The vertical dashed line indicates the location of *PWT3*. The sequences of TP2, Br118.2, and Br116.5 were similar with very low SNP frequencies (A, B) while those of Br118.2 and Br35 were quite different throughout this supercontig (E). The sequence of Br48 was chimeric; its central 1.6Mb segment (shown in pink) carrying the *PWT3* locus was different from Br118.2 (C) but similar to Br35 (D).

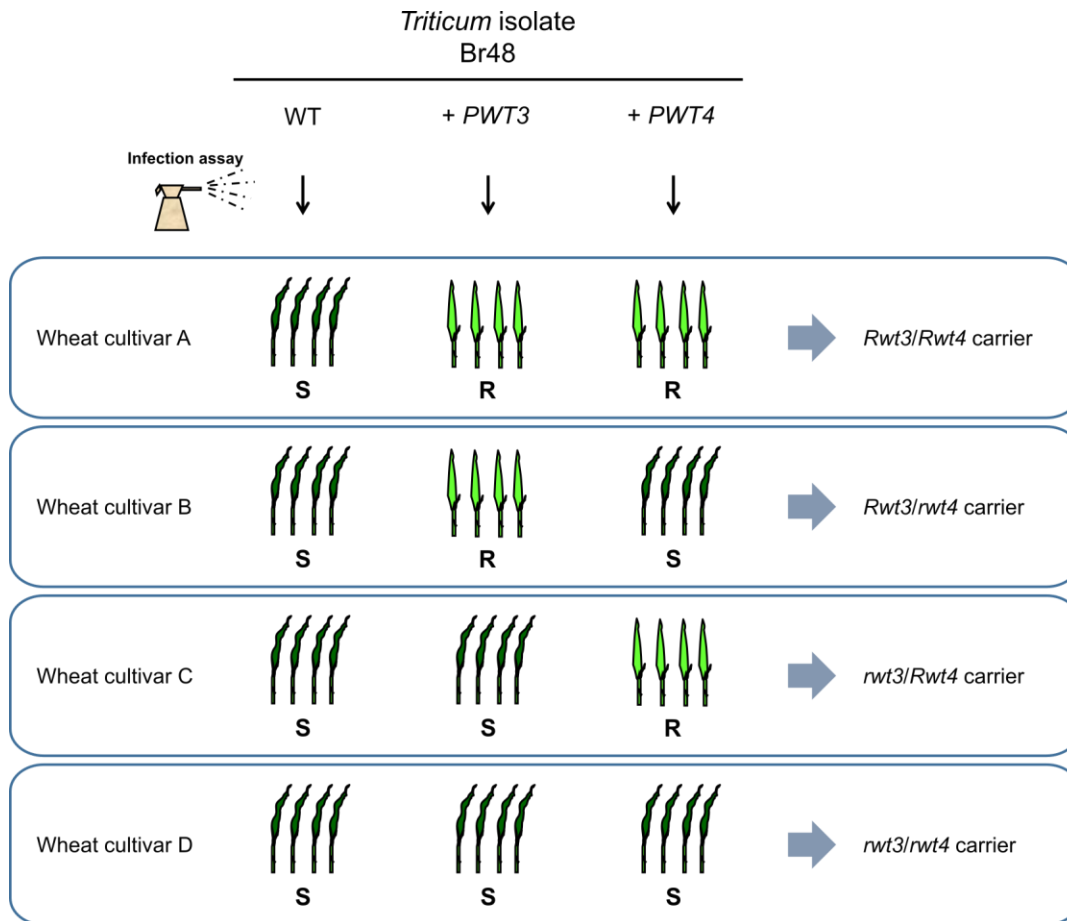


Fig. S13. Rapid detection of *Rwt3* and *Rwt4* in common wheat accessions by infection assay with transformants carrying corresponding avirulence genes. Test cultivars are inoculated with *Triticum* isolate Br48 (WT) and its transformants carrying *PWT3* or *PWT4*. If an accession is susceptible to Br48 but resistant to the transformant with *PWT3* and/or the transformant with *PWT4*, the accession should carry the corresponding resistance gene, *Rwt3* and/or *Rwt4*.

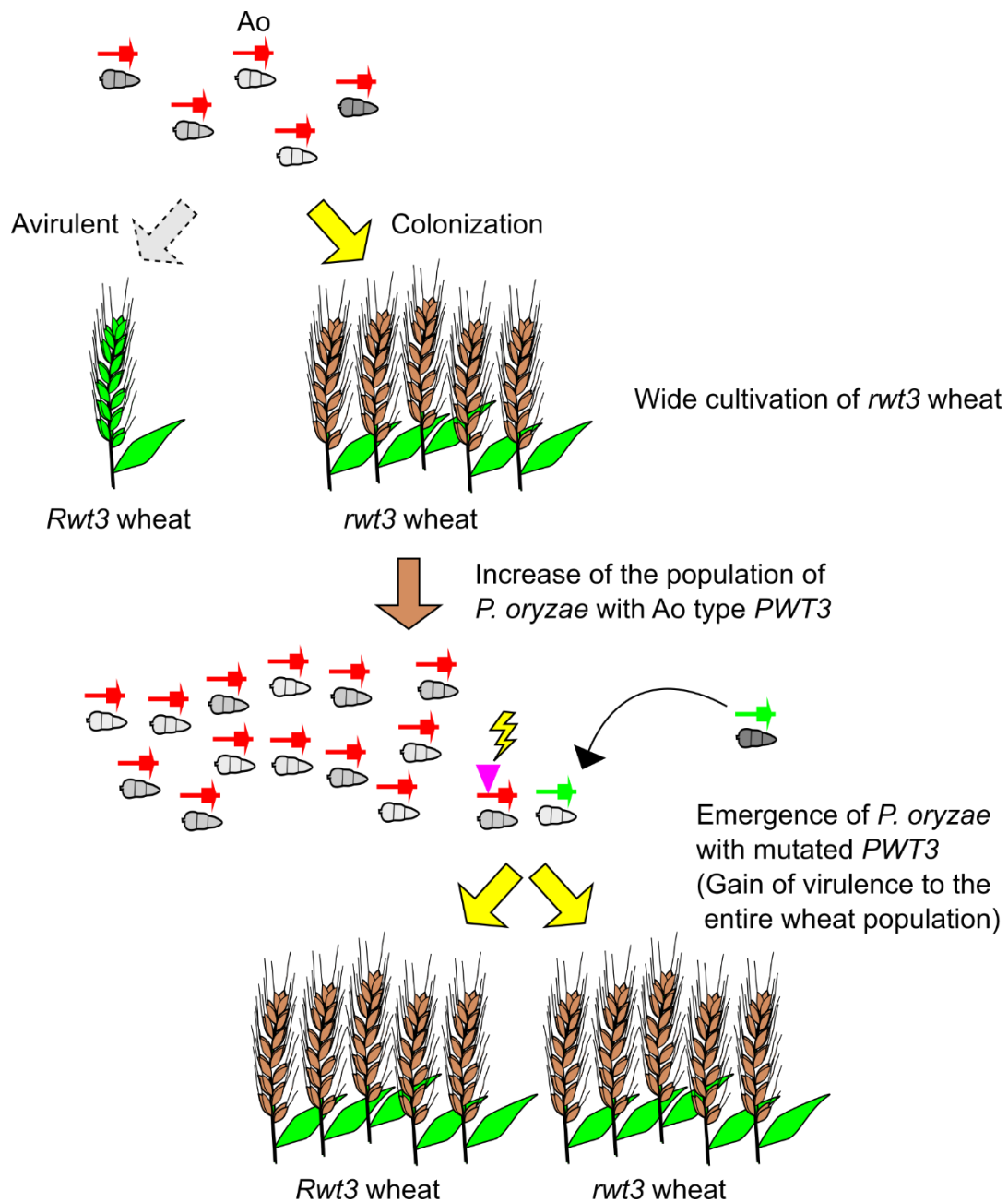


Fig. S14. A model of wheat blast emergence in Brazil. Wide cultivation of *rwt3* wheat provided an opportunity for *P. oryzae* strains with the Ao type *PWT3* to colonize its spikes. A mutation in the Ao type *PWT3* within an increased *P. oryzae* population or a gain of a nonfunctional *PWT3* homolog from a remote strain caused the host range expansion to *Rwt3* wheat.

Table S1. *Pyricularia* isolates used and their PWT3 types revealed in the present study.

						PWT3 type	
Species	Isolate	Original host	Locality	Year	Collector or source	ORF ^a	Allele
Isolates analyzed in Kobe University							
<i>P. grisea</i>	Dig41	<i>Digitaria sanguinalis</i>	Japan (Hyogo)	1990	Y. Iwamoto	D	D
<i>P. oryzae</i>	Ina168	<i>Oryza sativa</i>	Japan (Aichi)	1958	S. Kiyosawa	C	C
<i>P. oryzae</i>	Z2-1	<i>Eleusine coracana</i>	Japan (Kagawa)	1977	S. Itoi	A	Ao'
<i>P. oryzae</i>	MZ5-1-6	<i>Eleusine coracana</i>	Japan (Miyazaki)	1976	H. Kato	A	Ao'
<i>P. oryzae</i>	Br58	<i>Avena sativa</i>	Brazil (Parana)	1990	S. Igarashi	A	Ao
<i>P. oryzae</i>	TP1	<i>Lolium perenne</i>	Japan (Tochigi)	1997	A. Tanaka, T. Tani	A	Ao
<i>P. oryzae</i>	TP2	<i>Lolium perenne</i>	Japan (Tochigi)	1997	A. Tanaka, T. Tani	A	Ao
<i>P. oryzae</i>	Br35	<i>Brachiaria plantaginea</i>	Brazil (Parana)	1990	S. Igarashi	B	B
<i>P. oryzae</i>	Br2	<i>Triticum aestivum</i>	Brazil (Parana)	1990	S. Igarashi	B	B
<i>P. oryzae</i>	Br3	<i>Triticum aestivum</i>	Brazil (Parana)	1990	S. Igarashi	B	B
<i>P. oryzae</i>	Br5	<i>Triticum aestivum</i>	Brazil (Parana)	1990	S. Igarashi	B	B
<i>P. oryzae</i>	Br8	<i>Triticum aestivum</i>	Brazil (Parana)	1990	S. Igarashi	B	B
<i>P. oryzae</i>	Br46	<i>Triticum aestivum</i>	Brazil (Mato Grosso do Sul)	1990	S. Igarashi	A	Atm
<i>P. oryzae</i>	Br48	<i>Triticum aestivum</i>	Brazil (Mato Grosso do Sul)	1990	S. Igarashi	B	B
<i>P. oryzae</i>	Br49	<i>Triticum aestivum</i>	Brazil (Mato Grosso do Sul)	1990	S. Igarashi	A	Atm
<i>P. oryzae</i>	Br50	<i>Triticum aestivum</i>	Brazil (Mato Grosso do Sul)	1990	S. Igarashi	B	B
<i>P. oryzae</i>	Br115.7	<i>Triticum aestivum</i>	Brazil (Parana)	1992	A.S. Urashima	A	Ao
<i>P. oryzae</i>	Br115.12	<i>Triticum aestivum</i>	Brazil (Parana)	1992	A.S. Urashima	A	Ao
<i>P. oryzae</i>	Br116.5	<i>Triticum aestivum</i>	Brazil (Parana)	1992	A.S. Urashima	A	Atm
<i>P. oryzae</i>	Br117.1	<i>Triticum aestivum</i>	Brazil (Parana)	1992	A.S. Urashima	B	B
<i>P. oryzae</i>	Br118.2	<i>Triticum aestivum</i>	Brazil (Parana)	1992	A.S. Urashima	A	Ao
<i>P. oryzae</i>	Br126.1	<i>Triticum aestivum</i>	Brazil (Parana)	1992	A.S. Urashima	A	Ao
<i>P. oryzae</i>	Br127.1	<i>Triticum aestivum</i>	Brazil (Parana)	1992	A.S. Urashima	A	Ao
<i>P. oryzae</i>	Br127.11	<i>Triticum aestivum</i>	Brazil (Parana)	1992	A.S. Urashima	A	Atm
<i>P. oryzae</i>	Br128.1	<i>Triticum aestivum</i>	Brazil (Parana)	1992	A.S. Urashima	A	Atm
<i>P. oryzae</i>	Br130.8	<i>Triticum aestivum</i>	Brazil (Parana)	1992	A.S. Urashima	A	Atm
<i>P. oryzae</i>	Br130.9	<i>Triticum aestivum</i>	Brazil (Parana)	1992	A.S. Urashima	A	Ao
Isolates analyzed in University of Kentucky, Kansas State University, or The Ohio State University							
<i>P. oryzae</i>	CHRF	<i>Lolium perenne</i>	U.S.A. (Maryland)	1996	M. Farman	A	Ao
<i>P. oryzae</i>	CHW	<i>Lolium perenne</i>	U.S.A. (Maryland)	1996	M. Farman	A	Ao
<i>P. oryzae</i>	FH	<i>Lolium perenne</i>	U.S.A. (Maryland)	1997	Farman et al. (2017) (6)	A	Ao
<i>P. oryzae</i>	GG11	<i>Lolium perenne</i>	U.S.A. (Kentucky)	1997	M. Farman	A	Ao
<i>P. oryzae</i>	HO	<i>Lolium perenne</i>	U.S.A. (Pennsylvania)	1996	M. Farman	A	Ao
<i>P. oryzae</i>	LpKY97-1	<i>Lolium perenne</i>	U.S.A. (Kentucky)	1997	Farman et al. (2017) (6)	A	Ao
<i>P. oryzae</i>	PgKY	<i>Lolium perenne</i>	U.S.A. (Kentucky)	2000	D. Soanes, N. Talbot	A	Ao
<i>P. oryzae</i>	PgPA	<i>Lolium perenne</i>	U.S.A. (Pennsylvania)	1998	D. Soanes, N. Talbot	A	Ao
<i>P. oryzae</i>	PL2-1	<i>Lolium multiflorum</i>	U.S.A. (Kentucky)	2002	M. Farman	A	Ao
<i>P. oryzae</i>	PL3-1	<i>Lolium multiflorum</i>	U.S.A. (Kentucky)	2002	Farman et al. (2017) (6)	A	Ao
<i>P. oryzae</i>	T25	<i>Triticum aestivum</i>	Brazil (Parana)	1988	S. Igarashi	B	B
<i>P. oryzae</i>	Br7	<i>Triticum aestivum</i>	Brazil (Parana)	1990	S. Igarashi	B	B
<i>P. oryzae</i>	Br130	<i>Triticum aestivum</i>	Brazil (Mato Grosso do Sul)	1990	A.S. Urashima, T. Mitchell	A	Ao
<i>P. oryzae</i>	Br32	<i>Triticum aestivum</i>	Brazil	1991	D. Tharreau	A	Atm
<i>P. oryzae</i>	Br80	<i>Triticum aestivum</i>	Brazil	1991	D. Tharreau	A*	Atc
<i>P. oryzae</i>	WBSS	<i>Triticum aestivum</i>	Brazil (Parana)	1992	A.S. Urashima	A*	Atc
<i>P. oryzae</i>	PY5003	<i>Triticum aestivum</i>	Brazil (Parana)	2005	J. Maciel, N. Talbot, D. Soanes	A	Atm
<i>P. oryzae</i>	PY5010	<i>Triticum aestivum</i>	Brazil (Parana)	2005	J. Maciel, N. Talbot, D. Soanes	A	Ao
<i>P. oryzae</i>	PY6017	<i>Triticum aestivum</i>	Brazil (Minas Gerais)	2006	J. Maciel, N. Talbot, D. Soanes	A*	Atc
<i>P. oryzae</i>	PY6025	<i>Triticum aestivum</i>	Brazil (Minas Gerais)	2006	J. Maciel, N. Talbot, D. Soanes	A*	Atc
<i>P. oryzae</i>	PY6045	<i>Triticum aestivum</i>	Brazil (Goias)	2006	J. Maciel, N. Talbot, D. Soanes	A*	Atc
<i>P. oryzae</i>	PY36.1	<i>Triticum aestivum</i>	Brazil (Brasilia)	2007	J. Maciel, N. Talbot, D. Soanes	A*	Atc
<i>P. oryzae</i>	PY86.1	<i>Triticum aestivum</i>	Brazil (Parana)	2008	J. Maciel, N. Talbot, D. Soanes	A	Ao
<i>P. oryzae</i>	PY0925	<i>Triticum aestivum</i>	Brazil (Minas Gerais)	2009	J. Maciel, N. Talbot, D. Soanes	A*	Atc
<i>P. oryzae</i>	WBKY11-15	<i>Triticum aestivum</i>	U.S.A. (Kentucky)	2011	L. Murdock	A	Atp
<i>P. oryzae</i>	B2	<i>Triticum aestivum</i>	Bolivia	2011	J. Stack, E. Guzman	A	Atm
<i>P. oryzae</i>	B71	<i>Triticum aestivum</i>	Bolivia	2012	J. Stack, C. Cruz	A*	Atc
<i>P. oryzae</i>	P3	<i>Triticum durum</i>	Paraguay (Canindeyu)	2012	G.L. Peterson, M. Kohli	A	Atm
<i>P. oryzae</i>	BdBar (P161)	<i>Triticum aestivum</i>	Bangladesh (Barisal)	2016	P. Malaker	A*	Atc
<i>P. oryzae</i>	BdJes (P162)	<i>Triticum aestivum</i>	Bangladesh (Jessore)	2016	P. Malaker	A*	Atc
<i>P. oryzae</i>	BdMeh (P163)	<i>Triticum aestivum</i>	Bangladesh (Mehepur)	2016	P. Malaker	A*	Atc
<i>P. oryzae</i>	BdKus	<i>Triticum aestivum</i>	Bangladesh (Kushtia)	2016	P. Malaker	A*	Atc
<i>P. oryzae</i>	BdMag	<i>Triticum aestivum</i>	Bangladesh (Magura)	2016	P. Malaker	A*	Atc

^a Refer to fig. S9. "A" is the functional type detected in *Avena* isolate Br58. A* indicates A type with two nucleotide substitutions (See Fig. 3B).

Table S2. *PWT3* types of wheat isolates collected in Brazil during 1990-1992 and their pathogenicity to wheat seedlings

Isolate	<i>PWT3</i> type ^a	Infection type ^b on wheat cultivars ^c			
		N4	CS	Tfed	Hope
Br115.7	Ao	1B	0-1B	4G-5G	4-5G
Br115.12	Ao	2B	1B	5G	5G
Br118.2	Ao	2B-3B	1B-2B	4G-5G	5G
Br126.1	Ao	2B	1B	5G	5G
Br127.1	Ao	2B-3B	1B-2B	4G-5G	5G
Br130.9	Ao	2B	1B	4G-5G	4G
Br46	Atm	3G-5G	4G	3G-5G	4G-5G
Br49	Atm	4G-5G	3G	4G-5G	5G
Br116.5	Atm	4G	3G	4G-5G	5G
Br127.11	Atm	5G	4G	3G-5G	4G-5G
Br128.1	Atm	3G-5G	3G	3G-5G	4G-5G
Br130.8	Atm	3G-4G	3G	4G-5G	4G-5G
Br2	B	4G-5G	3G-5G	5G	5G
Br3	B	4G-5G	3G-5G	4G-5G	5G
Br5	B	4G-5G	3G	3G-4G	5G
Br8	B	4G-5G	3G-5G	5G	4G-5G
Br48	B	5G	4G-5G	5G	5G
Br50	B	4G-5G	3G-5G	4G-5G	5G
Br117.1	B	4G-5G	3G	4G-5G	5G

^a Refer to Fig. 1.

^b Refer to Materials and Methods. Avirulent reactions are shown in red.

^c Norin 4 (N4) (*Rwt3/Rwt4*), Chinese Spring (CS) (*Rwt3/rwt4*), Transfed (Tfed) (*rwt3/Rwt4*), and Hope (*rwt3/rwt4*).

Table S3. Distribution of *Rwt3* and *Rwt4* in common wheat accessions in the world

Type of accessions and locality	No. of accessions	% accessions					
		<i>Rwt3/ Rwt4</i>	<i>Rwt3/ rwt4</i>	<i>rwt3/ Rwt4</i>	<i>rwt3/ rwt4</i>	<i>Rwt3</i> carrier	<i>Rwt4</i> carrier
Local landraces							
Americas							
South America	37	73.0	16.2	8.1	2.7	89.2	81.1
Northern/Central America	7	42.9	14.3	42.9	0.0	57.1	85.7
Africa							
Northern Africa	12	50.0	8.3	33.3	8.3	58.3	83.3
Eastern Africa	22	81.8	0.0	9.1	9.1	81.8	90.9
Asia							
Western Asia	43	58.1	7.0	14.0	20.9	65.1	72.1
Central Asia	10	60.0	20.0	10.0	10.0	80.0	70.0
Southern Asia	102	85.3	3.9	6.9	3.9	89.2	92.2
Eastern Asia	76	68.4	7.9	17.1	6.6	76.3	85.5
Europe							
Eastern Europe	59	71.2	5.1	15.3	8.5	76.3	86.4
Northern Europe	35	48.6	5.7	37.1	8.6	54.3	85.7
Southern Europe	39	66.7	5.1	28.2	0.0	71.8	94.9
Western Europe	57	70.2	7.0	19.3	3.5	77.2	89.5
Total	499	69.9	6.8	16.6	6.6	76.8	86.6
Improved cultivars							
Americas							
Brazil	36	69.4	2.8	25.0	2.8	72.2	94.4
USA	23	47.8	4.3	39.1	8.7	52.2	87.0

Table S4. Distribution of *Rwt3* and *Rwt4* in improved cultivars of common wheat.

Cultivars	Country	Infection type ^a w with			Presence/absence ^d	
		Br48	Br48+3 ^b	Br48+4 ^c	<i>Rwt3</i>	<i>Rwt4</i>
Hosto	Brazil	4-5G	3G	3G	-	-
Alegete	Brazil	4G	0	0	+	+
Carazinho	Brazil	4-5G	0-1B	1-2B	+	+
Super Precoz	Brazil	3-4G	1-2B	0	+	+
Superx	Brazil	4-5G	1B	0	+	+
Las 55	Brazil	3-5G	0-2B	3-4G	+	-
Marinqa	Brazil	3-5G	0	0	+	+
GD 83162	Brazil	5G	1B	1-2B	+	+
Toropi	Brazil	4-5G	3-4G	1B	-	+
K 6109.9	Brazil	4-5G	3G	1B	-	+
Jacui	Brazil	3-5G	1-2B	0-1B	+	+
BR 10	Brazil	5G	4-5G	0	-	+
Ticena 4	Brazil	3-4G	2B	0	+	+
CNT 9	Brazil	3-4G	1B	0	+	+
Nanbu	Brazil	5G	1-2B	0	+	+
C 33	Brazil	3-4G	0	0	+	+
Ca-iota	Brazil	3-5G	1B	1-3B	+	+
CEP 75203	Brazil	5G	4G	0	-	+
Cotipora	Brazil	3G	0-2B	0-1B	+	+
Encruzilhada	Brazil	5G	2-3B	0-2B	+	+
Iapar I-NI	Brazil	3G	1B	0	+	+
PEL 74142	Brazil	3G	0	0	+	+
PF 81910	Brazil	3-5G	1-3B	0	+	+
Tifton	Brazil	4-5G	1B	0	+	+
Clement	Brazil	3-5G	0-2B	0	+	+
IAC-5	Brazil	5G	0-1B	0	+	+
IAC 24	Brazil	3-4G	1B	0	+	+
Buck Buck	Brazil	3-4G	2B	0	+	+
Zaraguro	Brazil	5G	4G	0	-	+
Trigo	Brazil	4-5G	0	0	+	+
Anahuac	Brazil	5G	4-5G	0-1B	-	+
Ca-eias	Brazil	3-4G	1-2B	0	+	+
Butui	Brazil	3-5G	1-3B	0	-	+
Nobre	Brazil	4-5G	0	0	+	+
PAT 7219	Brazil	5G	3-5G	0	-	+
Alo-ra 4546	Brazil	5G	3-5G	0	-	+
Cadet	USA	5G	3-5G	4-5G	-	-
Nebred	USA	3G	0-1B	1B	+	+
Atlas 66	USA	4-5G	1-2B	0	+	+
Marquis	USA	4-5G	3-4G	5G	-	-
Turkey Red	USA	3-5G	1-3B	0-1B	+	+
Brevor	USA	5G	4-5G	0	-	+
Red fife	USA	3-5G	0	0-1B	+	+
Burt (CI 12696)	USA	4-5G	3-4G	0	-	+
Nugaines (CI 13968)	USA	4-5G	4-5G	1B	-	+
Scoutla- (CI 14075)	USA	3G	0	0	+	+
Yamhill (CI 14563)	USA	3G	1B	0	+	+
Neepaw a (CI 15073)	USA	4-5G	2B	3-4G	+	-
Centurk (CI 15075)	USA	4-5G	0-1B	0	+	+
Gaines	USA	4-5G	4-5G	0-1B	-	+
Marquillo (CI 6887)	USA	4-5G	1B	0-1B	+	+
Prelude	USA	5G	3-5G	0-1B	-	+
Hyslop (CI 14564)	USA	3-5G	3G	0-1B	+	+
Stephens	USA	3-4G	1B	0-1B	+	+
Tordo	USA	5G	4-5G	0	-	+
Anza	USA	4-5G	1-2B	0	+	+
Purple Straw (Alabama)	USA	4-5G	3G	0	-	+
Kanred	USA	4G	3G	0	-	+
Karl	USA	3G	3G	0	-	+

^a Refer to Materials and Methods^b M-16, a Br48 transformant carrying *PWT3*^c XB-6, a Br48 transformant carrying *PWT4*^d +, present; -, absent.