



Effectiveness and durability of Rmg8 and RmgGR119, wheat genes for resistance to the wheat blast fungus

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Doctoral Dissertation

**Effectiveness and durability of *Rmg8* and *RmgGR119*, wheat genes for
resistance to the wheat blast fungus**

**コムギのコムギいもち病菌に対する抵抗性遺伝子 *Rmg8* と
RmgGR119 の有効性と持続性**

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CHAPTER I: General Introduction

Plant disease is one of the most limiting factors to the human population, causing famine, economic crisis, and environmental degradation. It is a response of plant cells and tissues to a pathogenic organism or environmental factor that malfunctions and may lead to partial impairment or death of the plant (Agrios 2005). Based on their causal agents, plant diseases are divided into two categories, infectious and non-infectious diseases or biotic and abiotic diseases. Causal agents of biotic diseases include fungi, bacteria, viruses, nematodes, phytoplasma, and viroids. In contrast, abiotic factors include soil factors (physical properties/pH), nutrient toxicities/deficiencies, moisture extremes (deficiencies and excessive), temperature extreme (high/low), and chemical injuries (phytotoxicity) (Kennelly et al. 2012). Infectious plant diseases are transmittable from one plant to another in a short period and destroy massive plants in vast areas like coronavirus disease (COVID-19) in humans, as of this year. On the other hand, non-infectious diseases/disorders can predispose plants to diseases caused by infectious microbes.

In most cases, disease epidemics result in crop failures and starvation, or the presence of mycotoxins in foods results directly in death (Dickinson 2003). There are many unforgettable famines caused by plant diseases which led to death of millions and massive population displacement (Dickinson 2003). For instance, more than 1 million people died, and 1.5 million migrated due to Irish famine caused by potato late blight (*Phytophthora infestans*) in the 1840s. Similarly, 2.1-3 million people died, and many had displaced in Bengal due to Bengal famine caused by brown spots (*Cochliobolus*

miyabeanus) on rice in the 1940s. Pathogens with high virulence can cause up to 100% mortality in host plants leading to host extinction (Fisher et al. 2012). This situation results in environmental degradation by disturbing the biodiversity.

Globally, the demand for food is dramatically increasing due to fast population growth. Accordingly, food crop production is expected to rise to 3 billion tons to feed 9.1 billion world population in 2050 (FAO 2009). Annual cereal production has to increase by a billion tons (2.1 billion tons today). Wheat (*Triticum aestivum*) is one of the staple grain food sources for human consumption next to rice. According to FAOSTAT, the world wheat production in 2017 was 734 million tons from 214.3 million hectares, among which Asia takes the lion's share with 43.6% of production followed by Europe (32.6%) and America (17%). The global wheat demand has been continuously increasing due to the human population, changes in dietary habits, and the extended use of wheat as animal feed (Randhawa et al. 2019). In developing countries, for instance, in Bangladesh, the demand for wheat (5.35 million MT (metric ton)) was even 4.7-fold higher than the supply (1.15 million MT) for the last decade (Islam et al. 2019). Thus, about a 60% increase in wheat production is needed to meet the global demand for wheat by 2050.

On the other hand, though wheat is grown on more land than any other food crop, global wheat acreage of the past two decades decreased by 4% (Widmar 2017). The reduction in area planted is probably due to competition with other crops or due to farmland loss to the effect of degradation, desertification, urban sprawl, mining, recreation, toxic pollution, and rising sea level (Cribb 2010). Thus, to feed the growing world population, the potential to raise crop yield per unit area has seemed considerable

(FAO 2009; Widmar 2017). However, many factors hinder wheat production and productivity, among which wheat diseases and pests are the most limiting factor.

About 50 diseases and pests are considered to cause significant losses in major wheat-growing regions out of a documented list of 200 wheat diseases worldwide (Singh et al. 2016). Fungal diseases rank the highest in causing considerable damage to wheat, thus hindering wheat production. Pathogenic fungi are broadly classified as biotrophic and necrotrophic based on their lifecycles. Biotrophic fungi are highly specialized and require living plants for their survival. These include leaf rust (brown rust caused by *Puccinia triticina*), stripe rust (*Puccinia striiformis* f.sp. *tritici*), stem rust (black rust caused by *Puccinia graminis* f.sp. *tritici*) and powdery mildew (*Blumeria graminis* f.sp. *tritici*). On the other hand, necrotrophic fungi kill their hosts and live off dead tissue. Hemibiotrophs are defined as ‘fungi that initially form an association with living cells of the host, much like a biotroph, and then in the later stages of infection they become necrotrophic (De Silva et al. 2016). The most critical fungal wheat diseases categorized under hemibiotroph include fusarium head blight (FHB) and the leaf spotting diseases (LSDs), i.e., *Zymoseptoria tritici* blotch, tan spot, spot blotch, *Parastagonaspora nodurum* blotch, and wheat blast. Both biotrophs and hemibiotroph are threatening diseases to wheat production (Singh et al. 2016).

The overall actual loss of wheat yield due to diseases is estimated to be 13% of total wheat production (Randhawa et al. 2019). In a severe case, however, plant diseases can cause up to 100% yield losses. Unlike the visible epidemic and starvation incurs in a specific area at a time, the routine annual yield losses due to diseases are unseen and usually overlooked, but inadmissible. For instance, rice blast causes a yield loss, which

can feed 60 million people annually (Pennisi 2010). Likewise, the wheat blast has been causing significant yield losses in several regions of South America and recently in Asia (Bangladesh and India). The wheat blast had caused 40-100 % wheat yield loss in Paraguay in 1989 and infected 90,000 ha of wheat between 1997 and 2000 in Bolivia. It also caused up to 30% loss of wheat production in Brazil in 2009, and recently infected 15, 000 ha of a wheat field in Bangladesh in 2016 (CIMMYT 2010; Islam et al. 2016).

Wheat blast (*Pyricularia oryzae* Cavara) is a relatively new and fearsome wheat disease in South America. The outbreak of the disease in Bangladesh in 2016 was the first report of this disease other than South America since its discovery in 1985 in Brazil (Cruz and Valent 2017; Islam et al. 2016). Recently, because of its rising threat to world food security, scientists have given attention to combat the disease. Many international and national research organizations and Universities in U.S.A, UK, Bangladesh, and others have commenced working in a team for timely and efficient management of the disease. Furthermore, they have established an online information-sharing platform called OpenWheatBlast – making data instantly accessible (<http://www.wheatblast.net>) to provide free access to sequence data that highly enables swift investigation of the molecular aspect of the pathogen worldwide (Kamoun et al. 2019).

Plant disease control option includes cultural, chemical, host resistance, quarantine, and forecasting (O’Leary 2020). Culturally, wheat growers may control wheat blast through crop rotation, using disease-free seed, early sowing, removing grassy weeds, and not replanting in infected fields. Fungicides could be useful when advised by extension services. Unfortunately, the use of cultural methods and fungicides has proved ineffective in managing this disease due to the complex nature of the

pathogen. Profound knowledge of the pathogen epidemiology is needed to control this disease using cultural and chemical methods.

Generally, three elements – virulent pathogen, susceptible host, and conducive environment must be fulfilled for a plant disease to develop. These make up the disease triangle. Human intervention has a significant role in providing a suitable environment for a plant disease to occur. The expansion of conservation agriculture (CA), with stubble retention on the soil surface, attributes the occurrence of necrotrophs disease epidemics (Ugalde et al. 2007). Intensified wheat production, shorter crop rotations, monoculture, and susceptible cultivars also contributed significantly to the expansion of LSDs in epidemic proportions. According to Singh et al. (2016), the widespread cultivation of susceptible cultivars Kubsa and Galema, the presence of an aggressive Yr27-virulent race, and extremely favorable climate contributed to the epidemic of yellow rust in 2010, resulting in production losses on an estimated 0.6 million ha equivalent to 30% of the total area in Ethiopia. Increasing temperatures and drought due to climate change may further result in a higher degree of leaf senescence that will favor wheat blast diseases (Singh et al. 2016). Wheat blast is favored by continuous rainfall with average temperatures of 18-25°C during the flowering stage, followed by sunny, hot, and humid days (Islam et al. 2019; Kohli et al. 2011). This disease spreads through infected seeds, crop residues, and spores that can travel a long distance in the air.

Host resistance appears to be a more effective, affordable, and environmentally friendly method. It is believed to remain the best option for the majority of wheat farmers not only because it significantly reduces disease severity but also reduces the need for fungicide treatment (Singh et al. 2016). However, despite intensive searches in the past 30 years, no sources of durable resistance have been found (CIMMYT 2016).

Cultivars such as BH1146, BR18, IPR85, CD113, CNT8, and derivatives of CIMMYT line Milan displayed field resistance (Kohli et al. 2011; Maciel et al. 2014). Nevertheless, this has not been confirmed under artificial inoculation or multi-location studies (CIMMYT 2016). So far, nine resistance genes (R genes) (i.e., *Rmg1-8* and *RmgGR119*) and one chromosomal segment (2NS/2AS) have been identified (Anh et al. 2015; Cruz et al. 2016; Nga et al. 2009; Tagle et al. 2015; Takabayashi et al. 2002; Vy et al. 2014; Wang et al. 2018; Zhan et al. 2008). . However, the resistance conferred by 2NS has already been broken down by new races in Paraguay (Randhawa et al. 2019).

Besides, Castrogudin and his colleagues discovered a super race called virulence group A that is capable of causing blast on the entire set of tested wheat cultivars both at seedling and heading stage (Castroagudin et al. 2017). These virulent strains were widely distributed across regions of South America. As we experienced in the case of rice where R genes have frequently been broken down with new blast races, new races of wheat blast fungus with new degrees of virulence were presumed to evolve continuously, thus rendering R gene ineffective (Randhawa et al. 2019). Extensive gene flow between the populations of *P. oryzae* sampled from wheat, and other grasses and the presence of non-wheat hosts are supposed to play an essential role in the epidemiology of wheat blast and complicate control efforts (Ceresini et al. 2018).

Most race-specific R genes result in frequent “Boom and bust” cycles (Randhawa et al. 2019). Given that *P. oryzae* is highly diverse in genetic and virulence, breeding for resistance is expected to remain challenging. Therefore, intensive identification and combination of race non-specific R gene is considered an alternative to enhance the spectrum and durability of resistance (Castroagudin et al. 2016; Ceresini et al. 2018; Randhawa et al. 2019).

Disease resistance is divided into two categories, qualitative and quantitative, based on the type and number of genes involved. Qualitative resistance is controlled by a major gene and characterized as strong resistance and easy to be broken down by new races. In contrast, quantitative resistance is controlled by many minor genes and characterized as weak resistance but more stable.

Durable resistance is often conferred by quantitative trait loci (QTLs), which have small to intermediate but additive effects, and the accumulation of multiple QTLs can lead to a high level of APR (Adult Plant Resistance) approaching near immunity. There is a firm conviction that a combination of several minor genes of moderate-to-small effects leads to resistance durability. A practical and durable multiple undefeated genes should be identified and introgressed to develop a cultivar with a broad spectrum and durable resistance. This can be ensured via mobilizing genetic diversity for disease resistance in modern wheat cultivars through the use of advanced biotechnological interventions like markers-assisted selection, genomic selection, and genome editing.

A wide distribution of a given gene and functionality against several fungus lineages implies its effectiveness and durability. R genes confer resistance in interaction with its corresponding avirulence gene in the pathogen, gene-for-gene interaction (Flor 1971). Thus, the avirulence gene has to be concurrently durable and widely distributed across the majority of wheat blast fungus lineages. Up to date, no scientist is able to know the durability of an avirulent gene at the time of identification. As stated above, R genes are often broken down by pathogens a few years after release, which would cause a significant loss economically in the development of resistant cultivar as it appeared expensive. The host factors, the evolutionary potential of the pathogens, and the conduciveness of environments, influence durability (Nelson et al. 2018). So, it is

advised to predict the durability of the identified gene before embarking into further breeding programs (Anh 2016). To predict genes durability, one should consider from different aspects: the distribution, function, and molecular structure of the gene sequences. Avirulence genes corresponding to durable R genes must be widely distributed. All alleles of such avirulence genes in different fungal lineages should be functional, and the surrounding region of their coding region should be stable or not be surrounded with or have no insert of transposable elements (TE).

Resistance genes (R genes) are genes in plant genomes that produce R protein to convey plant disease resistance against pathogens. A nucleotide binding domain (NB) and a leucine rich repeat (LRR) domain(s) are the main class of R-genes. Similarly, pathogen avirulence genes (Avr genes) are the genes that help pathogen to colonize the host by manipulating host's cell physiology and development (Koeck et al 2011).

The mechanism of new race emergence is governed by the antagonistic interaction of plant defense and pathogen virulence (Kemen and Jones 2012). During co-evolution between plants and pathogens, plants have faced high selection pressure to detect, defend and survive pathogen pressure. Conversely, pathogenic fungi have also faced high selection pressure to avoid recognition or to suppress host defense, leading to an arms race between plant colonizing organisms and their hosts (Kemen and Jones 2012; Koeck et al. 2011).

Fungi are thought to have colonized terrestrial plants about 450 million year ago (Kemen and Jones 2012). Since then, different changes have undergone in genome size due to gene gain and loss in the plants and fungi during co-evolution. There are two types of plant resistance response; systematic acquired resistance which mediated by salicyclic acid and reactive oxygen species against biotrophs, and induces systematic

resistance which mediated by jasmonic acid and ethylene signaling against necrotrophs (De Silva et al. 2016). Salicylic acid dependent signaling pathway limits biotrophic colonization by activation of programmed cell death via a hypersensitive response. Plant chitinases are one of the main hydrolyzing enzymes that degrade fungal cell walls and limit colonization.

Plants also have primary and secondary defense responses. Initially, pathogen recognition receptors of the host plant recognize pathogen-associated molecular pattern (PAMPs) and thereby activate primary PAMP triggered defense response. This primary defense response is called PAMP Triggered Immunity (PTI). Most pathogens are, however, able to overcome this primary response by producing effector proteins that interact with host plant target and infect host plant tissue (De Silva et al. 2016; Koeck et al. 2011). Host plant produce resistance proteins that disrupt effector mediated interactions with a host target by activating secondary defense response (Koeck et al. 2011). This secondary defense response is called Effector Triggered Immunity (ETI).

Overall, the wheat blast is eventually spreading across the globe, posing a threat to world wheat production. Cultural control practices and fungicides treatment are ineffective as well as unreliable. Climate change is likely to exacerbate the disease pressure. To control the disease, the use of host resistance is the best option though sources of resistance are lacking. More effective and durable host resistance is required to combat the disease. R genes with corresponding avirulence genes should be continuously identified and introgressed into elite varieties. To develop multiline cultivars, knowledge on the genetic background for the host resistance and pathogen virulence is necessary (Koizumi et al. 2004).

Therefore, this study was initiated with the objectives to evaluate the effectiveness and durability of *Rmg8* and *RmgGR119* R genes against the wheat blast fungus.

CHAPTER 2: Effectiveness of the Resistance Gene *Rmg8* against Wheat Blast in Bangladesh

2.1 Introduction

Wheat blast caused by the *Triticum* pathotype (MoT) (Cruz and Valent 2017) of *Pyricularia oryzae* (Zhang et al. 2016) is one of the most destructive wheat diseases in South America and Asia. This disease was first observed in 1985 in Brazil (Urashima et al. 1993), then spread to adjacent countries such as Bolivia (in 1996), Paraguay (in 2002), and Argentina (in 2011), causing significant yield losses (Ceresini et al. 2018). In 2016, an outbreak of wheat blast occurred in Bangladesh (Callaway 2016; Islam et al. 2016; Malaker et al. 2016) and affected 15% of the country's total wheat area (Malaker et al. 2016). This was the first report of a wheat blast epidemic in a country other than in South America. The inoculum for this outbreak in Bangladesh was considered to originate from South America (Islam et al. 2016; Malaker et al. 2016).

Breeding for resistance is the most economical and labor-saving method for disease control. In the past 30 years, efforts have been made to breed wheat cultivars with improved blast resistance. Cruz et al. (2016) found that a 2NS chromosomal fragment derived from *Aegilops ventricosa* conferred resistance to head blast. Recently, BARI Gom33, a new wheat cultivar carrying the 2NS fragment, was released in Bangladesh (Islam et al. 2019). However, the resistance conferred by the 2NS fragment is not effective in some genetic backgrounds or against some fungal strains (Cruz et al. 2016). As such, it is crucial to identify new resistance genes against the wheat blast fungus.

Resistance genes against wheat blast must be valid at high temperatures and the heading stage of the plant, as the wheat blast is a spike disease which is favored by high temperature. Tosa and colleagues have identified five resistance genes against the wheat blast fungus, i.e., *Rmg2*, *Rmg3* (Zhan et al. 2008), *Rmg7* (Tagle et al. 2015), *Rmg8* (Anh et al. 2015), and *RmgGR119* (Wang et al. 2018). Among these, *Rmg2* and *Rmg3*, identified in common wheat, were temperature sensitive and not effective against spike infection. *Rmg7*, identified in emmer wheat, was effective against spike infection but not practical at high temperature. On the other hand, *Rmg8*, identified in common wheat cultivar S-615, was effective at both seedling and heading stages and relatively at high temperature (Anh et al. 2015; Wang et al. 2018). Recently, Wang et al. (2018) identified a new resistance gene, *RmgGR119*, in common wheat accession GR119 and proved that *Rmg8* combined with *RmgGR119* conferred strong resistance to *Triticum* isolates collected in Brazil.

The final goal for breeders is to develop a cultivar with broad-spectrum and durable resistance. In this context, the spectrum and durability of a resistance gene may be evaluated based on the distribution and stability of its corresponding avirulence gene (Tosa and Chuma 2014). Anh et al. (2018) cloned *AVR-Rmg8*, an avirulence gene corresponding to *Rmg8*, and found that it was recognized not only by *Rmg8* but also by *Rmg7* (Anh et al. 2018). Wang et al. (2018) analyzed nucleotide sequences of *AVR-Rmg8* homologs in *Triticum* isolates collected in Brazil and observed that it was divided into three types, eI, eII, and eII'.

In this study, we examined whether *Rmg8* and *RmgGR119* were effective against Bangladeshi isolates of *P. oryzae*. Infection assays coupled with analyses of the

avirulence gene suggest that *Rmg8*, and probably *RmgGR119*, are effective against wheat blast in Bangladesh.

2.2 Materials and Methods

2.2.1 Fungal Materials

Fungal materials used in the study comprised 11 *Triticum* isolates (isolates from *Triticum* spp. or isolates of the *Triticum* pathotype) of *P. oryzae*. Eight of these isolates (Table 1) were collected in Bangladesh in 2016/17 and provided by Md Tofazzal Islam, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Bangladesh, while another three isolates (Br48, Br5, and Br116.5) were collected in Brazil in 1990/92 (Wang et al. 2018). Br48ΔA8_d6, an *AVR-Rmg8* disruptant of Br48 produced by Wang et al. (2018), was used as a recipient of *AVR-Rmg8* homologs.

Table 1. Wheat blast isolates used in this study

Isolate	Original name	Location	Year of collection
Br5		Brazil Parana	1990
Br48		Brazil Mato Grosso do Sul	1990
Br116.5		Brazil Parana	1992
T-102	BTBa-B1	Bangladesh BSMRAU	2016
T-103	BTJP-4(1)	Bangladesh Jhenaidah	2016
T-104	BTGP-1(b)	Bangladesh Meherpur	2017
T-105	BTMP-2(b)	Bangladesh Meherpur	2017
T-106	BTGS-4(b)	Bangladesh Meherpur	2017
T-107	BTMB-5(a)	Bangladesh Meherpur	2017
T-108	BTMB-5(b)	Bangladesh Meherpur	2017
T-109	BTGP-6(e)	Bangladesh Meherpur	2017

2.2.2 Plant Materials

Common wheat (*Triticum aestivum*) tested were three susceptible lines (cv. Chinese spring (CS), cv. Hope, cv. Shin-Chunaga (Sch)), eighteen lines carrying *Rmg8* (cv. S-615, accession IL50, IL92, IL131, IL132, IL186, IL191, CP19, CP20, CP21, CP26, CP27, CP30, CP61, CP71, CP73, GR246, GR250), and one accession (GR119) carrying both *Rmg8* and *RmgGR119*. IL191 was selected as a representative of the 18 lines carrying *Rmg8* alone (Wang et al. 2018) as it displayed the highest resistance among them at the seedling stage.

2.2.3 Seedling infection assay

Wheat seeds were sown in vermiculite supplied with liquid fertilizer in a seedling case and grown in a controlled-environment room with a 12-h photoperiod of fluorescent lighting at 22°C. Primary leaves of 8-day-old seedlings were fixed onto a hard-plastic board using rubber bands immediately before inoculation. In the case of detached leaf inoculations, seedlings were grown in commercial soil (i.e., vermiculite) in a controlled-environment room as described above for eight days or in soil (Sakata Prime Mix, Sakata Seeds Corporation, Yokohama, Japan) in a greenhouse (18-26°C) for 17 days. The first leaves of the 8-day-old seedlings were used for initial evaluations of resistance/susceptibility, while the third leaves of the 17-day-old seedlings were used for precise evaluations of the degree of resistance. Leaves were cut at the base, inserted into Eppendorf tubes (2ml) containing distilled water, and fixed onto a hard-plastic board.

Fungal isolates/strains were grown on oatmeal agar media (sucrose 5g, oatmeal 40g, agar 20g/L) for seven days. Aerial mycelia from the 7-day-old cultures were removed by rubbing the mycelial surfaces with cotton balls. The cultures were incubated further under blue light to induce sporulation. Three-to-four days later, conidia were harvested, and a suspension (1×10^5 conidia/ml) containing 0.01% Tween 20 was evenly sprayed onto fixed leaves using an air compressor. Inoculated leaves were placed in a humid tray and incubated in the dark at 22 °C, 25°C, and 28°C. After 24 h, they were transferred to a dry condition with a 12-h photoperiod, and further incubated at 22 °C, 25°C, and 28°C for 3-4 days. Infection types were recorded based on the size and color of lesions. The size was rated on six progressive grades from 0 to 5: 0 = no visible infection; 1 = pinhead spots; 2 = small lesions (<1.5 mm); 3 = scattered lesions of intermediate size (<3mm); 4 = large lesion; and 5 = complete shriveling of leaf blades.

The color was designated as B for brown and G for green. A disease score (infection type) was composed of a number denoting the lesion size and a letter indicating the lesion color. For example, infection type 1B indicated brown pinhead spots. Infection types 0, 1B, and 2B were considered resistant (avirulent), while infection types 3G, 4G, and 5G were considered susceptible (virulent). Intermediate types, 3B and 3BG, were categorized as resistant because the browning of leaf tissues in these types had been shown to be caused by hypersensitive reactions (HR) (Hyon et al. 2012). In infection assays with primary leaves, infection types 4B and 5B were observed in some isolate/cultivar combinations. They were also categorized as resistant because the blighting or shriveling of whole leaves in these types had been shown to be caused by extensive HR (Murakami et al. 2000). Four leaves were employed for each

cultivar/isolate combination in one replication. The infection assays with primary leaves (intact/detached) and third leaves were replicated three times and twice, respectively.

2.2.4 Spike infection assay

Test plants were grown for 4-5 months in a field or greenhouse. At the stage of full head emergence (before flowering), a stem with a spike was cut at the base, trimmed to ~50 cm (including the spike), and put into a test tube (150 mm in length and 10 mm in diameter) with water containing antibiotics (MISAKI solution for cut flowers, O.A.T. Agrio, Tokyo, Japan). The spikes were inoculated with conidial suspension (1.5×10^5 conidia /ml), covered with a black plastic bag, and incubated in the dark at 25°C and 28°C. After 24 h, the plastic bag was removed, and the spikes were incubated further under a 12-h photoperiod at the same temperature as in the dark. Seven to eight days post-inoculation, infection was rated with six progressive grades from 0 to 5: 0 = no visible infection; 1 = brown pinhead spots; 2 = brown lesions of intermediate size (<3 mm); 3 = large lesions surrounded by brown tissues; 4 = extensive necrosis or chlorosis; and 5 = complete blighting of the spike. Infection types of 0 to 3 were considered resistant, and 4 to 5 were considered susceptible. Two spikes were employed for each cultivar/isolate combination in one replication. The infection assay with spikes was repeated three times.

2.2.5 Extraction of fungal genomic DNA

P. oryzae strains were cultured in liquid medium (3g yeast extract, 3g casamino acid, 5g sucrose/L) in a 200 mL Erlenmeyer flask for 4-5 days. Genomic DNA was extracted from harvested mycelia using a modified version of a protocol described by Nakayashiki et al. (1999). In short, 1g of a fungal sample (mycelial powder) in 1ml of

DNA extraction buffer (100mM LiCl, 100mM EDTA, 10mM Tris-HCl, pH7.5, 0.5%SDS) was incubated in a water bath at 65°C for 1h. After centrifuging at 15000 rpm, the supernatant was applied to PCI (phenol:chloroform:isoamyl alcohol =25:24:1) to remove protein, and then to 1ml of isopropanol to precipitate DNA after removing RNA with 1µl of RNase (10mg/mL), DNA was precipitated, washed with ethanol, and resuspended in 25 µl TE.

2.2.6 Sequencing of AVR-*Rmg8* homologs

A 550 bp fragment containing AVR-*Rmg8* was amplified from genomic DNA of Bangladeshi isolates of *P. oryzae* with primers, A8_ORF_Seq_F, and A8_ORF_Seq_R (Table 2). PCR products were checked by electrophoresis on a 0.7% agarose gel, purified by ethanol precipitation, and then sequenced using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and the ABI 3100 Genetic Analyzer following the manufacturer's instructions. Obtained nucleotide sequences were aligned with published sequences of eI (in Br48), eII (in Br5), and eII' (in Br116.5) types of AVR-*Rmg8* and those in databases (Table 6). Based on these nucleotide sequences, an ML (Maximum Likelihood) tree was constructed using the MEGA7 software (Kumar et al. 2016). Bootstrap values were generated from 1000 replications.

Table 2. Primers used in this study

Name	Sequence (5'--->3')
A8_ORF_Seq_F	TCTAGTTGCATTTTCTCACTCCA
A8_ORF_Seq_R	GGCCGTTTAACGTTTTTGG
AVR-Rmg8_multi_cloning_F	ATAAAAGCAATACGGTCGGA
AVR-Rmg8_multi_cloning_R	CACGTATAATTACGTTCTGTGTG
AVR-Rmg8_F2	ACCGCATCGGCTTTTTCTT
AVR-Rmg8_R2	TGCCTTCTAGTACCGGGAAGT

2.2.7 Preparation of protoplasts

Protoplasts were prepared from Br48ΔA8 (disruptant of *AVR-Rmg8*) by growing it in liquid CM medium (yeast extract 3g, casamino acid 3g, sucrose 5g/L) in Erlenmeyer flask for 3-4 days at 25°C and then, crushing, filtering with lysis enzyme (derived from *Trichoderma harzianum*, 500mg/1.5mL tube). The concentration of protoplast cells were adjusted to 10⁸ protoplast/mL under a microscope using a hemocytometer just before dispensing it in 1.5ml tubes and storing at -80°C.

2.2.8 Transformation of *P. oryzae* with *AVR-Rmg8* homologs

A 1.8kb fragment containing the *AVR-Rmg8* ORF, together with a 1000bp upstream region and a 500bp downstream region, was amplified from Br48 (carrying the eI type), Br5 (carrying the eII type) and Br116.5 (carrying the eII' type) using AVR-Rmg8_multi_cloning_F and AVR-Rmg8_multi_cloning_R primers (Table 2) and KOD-Plus-Neo polymerase (Toyobo, Osaka, Japan). The thermal cyclers setup comprised 94°C for 2 min, 35 cycles of 94°C for 30 sec, 60°C for 30 sec, and 68°C for 2 min. The amplicon was run on a 0.7% agarose gel and extracted from the gel using

QIAEX II[®] Gel Extraction Kit (QIAGEN, Hilden, Germany). The purified fragment was ligated with pBluescriptII S.K. (+) digested with *EcoRV*, and introduced into *Escherichia coli* DH5- α . The recombinant plasmids were introduced into Br48 Δ A8_d6 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. Positive colonies were selected on media containing G418 sulfate and confirmed by PCR using the primers mentioned above.

2.2.9 Southern blot analysis of transformants

Genomic DNAs of Br48, Br48 Δ A8_d6, and transformants were digested with *EcoRI* (Takara, Kusatsu, Japan), electrophoresed on a 0.7% agarose gel in the TBE buffer, and transferred to a nylon membrane (Hybond-N+, GE Healthcare, Buckinghamshire, England) through capillary blotting. The DNA fragments were fixed to a membrane by UV radiation. A 322bp *AVR-Rmg8* fragment amplified from the genomic DNA of Br48 with primers, AVR-Rmg8_F2, and AVR-Rmg8_R2 (Table 2), was labeled following the instruction of the ECL Direct Nucleic Acid Labeling and Detection System (GE Healthcare). The membrane was hybridized for 12h with the labeled probe in the Gold hybridization buffer (GE healthcare) at 42°C. After hybridization, the membrane was washed twice in the primary washing buffer (0.4% sodium dodecyl sulfate and 0.5 $\times$ SSC) for 10 min at 55°C, then once in 2 $\times$ SSC for 5 min at room temperature. Signals were detected following the manufacturer's instructions.

2.3 Results

2.3.1 Infection assay with intact primary leaves

Initially, intact primary leaves of 18 accessions carrying *Rmg8* alone, one accession carrying *Rmg8* and *RmgGR119*, and three accessions carrying no gene were inoculated with the eight Bangladeshi and two Brazilian isolates of *P. oryzae* and incubated at 22°C, 25°C, and 28°C. All accessions carrying the gene(s) were resistant to Bangladesh and Brazilian isolates at all levels of temperature (Tables 3-5). At high temperature, one gene carrying cultivars tended to show a lesion with a hypersensitive response recorded 1-2B. However, GR119 carrying two genes showed consistent resistance even at high temperature. On the other hand, the virulence of Br5, a Brazilian isolate, was relatively higher compared with the other test isolates. We chose Hope (-/-), IL191 (*Rmg8*/-), and GR119 (*Rmg8*/*RmgGR119*) as representative accessions and T-108 and T-109 as representatives of the Bangladeshi isolates for further analyses.

Table 3. Reactions of common wheat seedlings to Bangladeshi and Brazilian isolates at 22°C

No	Cultivar	Genotype	Infection types									
			T-102	T-103	T-104	T-105	T-106	T-107	T-108	T-109	Br48	Br5
1	CS	-	4G	4G	4G	4G	4G	4G	4G	4G	3GB	3GB
2	Hope	-	5G	4G	5G	4G	4G	5G	5G	5G	3GB	4GB
3	Sch	-	4G	5G	5G	5G	5G	4G	5G	4G	4GB	4GB
4	S-615	<i>Rmg8</i>	0	0	0	0	0	0	1B	0	0	2+B
5	IL50	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	1B
6	IL92	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	1B
7	IL131	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	1B
8	IL132	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	0
9	IL186	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	1B
10	IL191	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	0
11	CP19	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	1B
12	CP20	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	1B
13	CP21	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	1B
14	CP26	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	1B
15	CP27	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	0
16	CP30	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	1B
17	CP61	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	1B
18	CP71	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	1B
19	CP73	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	1B
20	GR246	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	1B
21	GR250	<i>Rmg8</i>	0	0	0	0	0	0	0	0	0	0
22	GR119	<i>Rmg8</i> + <i>RmgGR119</i>	0	0	0	0	0	0	0	0	0	0

Table 4. Reactions of common wheat seedlings to Bangladeshi and Brazilian isolates at 25°C

No	Cultivar	Genotype	Infection type									
			T-102	T-103	T-104	T-105	T-106	T-107	T-108	T-109	Br48	Br5
1	CS	-	5G	5G	5G	5G	5G	5G	5G	5G	5G	5G
2	Hope	-	5G	5G	5G	5G	5G	5G	5G	5G	5G	5G
3	Sch	-	5G	5G	5G	5G	5G	5G	5G	5G	5G	5G
4	S-615	<i>Rmg8</i>	1B	1B	1B	1B	1B	1B	1B	1-2B	1B	1B
5	IL50	<i>Rmg8</i>	1B	1B	1B	1B	1B	1B	1B	1B	0	1B
6	IL92	<i>Rmg8</i>	1B	1B	1B	1B	1B	1B	1B	1B	1B	2B
7	IL131	<i>Rmg8</i>	0	1B	0	0	0	0	1B	1B	0	1B
8	IL132	<i>Rmg8</i>	1B	1B	0	1B	1B	0	1B	1B	0	1B
9	IL186	<i>Rmg8</i>	0	1B	0	0	0	0	0	1B	0	1B
10	IL191	<i>Rmg8</i>	0	1B	0	0	0	0	0	1B	0	1B
11	CP19	<i>Rmg8</i>	0	1-2B	0	0	0	0	0	1B	0	2B
12	CP20	<i>Rmg8</i>	0	1B	0	0	0	0	0	1B	0	2B
13	CP21	<i>Rmg8</i>	0	1B	0	0	0	0	0	1B	0	2B
14	CP26	<i>Rmg8</i>	0	1B	0	0	0	1B	0	1B	0	2B
15	CP27	<i>Rmg8</i>	0	0	0	0	0	1B	0	0	0	1B
16	CP30	<i>Rmg8</i>	0	0	1B	0	0	1B	0	0	1B	2B
17	CP61	<i>Rmg8</i>	1B	2B	1B	2B	2B	1B	1-2B	2B	1B	2B
18	CP71	<i>Rmg8</i>	1B	1B	0	1B	1B	0	1-2B	1B	0	2B
19	CP73	<i>Rmg8</i>	1B	0	0	2B	1B	0	0	0	0	2B
20	GR246	<i>Rmg8</i>	1B	0	1B	1B	1B	0	0	0	0	1B
21	GR250	<i>Rmg8</i>	0	0	1B	0	0	1B	1-2B	0	0	2B
22	GR119	<i>Rmg8</i> + <i>RmgGR119</i>	0	0	0	0	0	0	0	0	0	1B

Table 5. Reactions of common wheat seedlings to Bangladeshi and Brazilian isolates at 28°C

No	Cultivar	Genotype	Infection type									
			T-102	T-103	T-104	T-105	T-106	T-107	T-108	T-109	Br48	Br5
1	CS	-	5G	5G	5G	5G	5G	5G	5G	5G	5G	5G
2	Hope	-	5G	5G	5G	5G	5G	5G	5G	5G	5G	5G
3	Sch	-	5G	5G	5G	5G	5G	5G	5G	5G	5G	5G
4	S-615	<i>Rmg8</i>	1-2B	1-2B	2B	1-2B	1-2B	2B	2B	1-2B	1-2B	1B
5	IL50	<i>Rmg8</i>	1B	0	1B	1B	1B	1B	2B	0	1B	1B
6	IL92	<i>Rmg8</i>	1B	1B	2B	1B	1B	2B	2+B	1B	2B	1B
7	IL131	<i>Rmg8</i>	0	1B	1B	0	0	1B	2B	1B	0	1B
8	IL132	<i>Rmg8</i>	0	1B	1B	0	0	1B	2B	1B	0	1B
9	IL186	<i>Rmg8</i>	0	1-2B	0	0	0	0	1B	1-2B	1-2B	2B
10	IL191	<i>Rmg8</i>	0	1B	0	0	0	0	1B	1B	0	1-2B
11	CP19	<i>Rmg8</i>	1-2B	2B	1-2B	1-2B	1-2B	1-2B	1-2B	2B	1-2B	2-3B
12	CP20	<i>Rmg8</i>	1B	1B	1-2B	1-2B	1-2B	1-2B	1-2B	1B	1B	3B
13	CP21	<i>Rmg8</i>	1-2B	1B	1B	1-2B	2B	1-2B	2B	1B	2B	3GB
14	CP26	<i>Rmg8</i>	2B	1B	1B	2B	2B	1-2B	2B	1B	2B	3B
15	CP27	<i>Rmg8</i>	1B	1B	0	1B	1B	0	1B	1B	1B	2B
16	CP30	<i>Rmg8</i>	1B	1B	0	1B	1B	0	1B	1B	1B	2-3B
17	CP61	<i>Rmg8</i>	2B	2B	2B	2-3B	2-3B	2B	2B	2B	2B	2-3B
18	CP71	<i>Rmg8</i>	2B	1B	2B	2B	2B	2B	2B	1B	0	2-3B
19	CP73	<i>Rmg8</i>	2B	1B	1B	2B	2B	1B	1B	2B	1B	2B
20	GR246	<i>Rmg8</i>	2B	1B	1B	2B	2B	1B	1B	1B	1B	2B
21	GR250	<i>Rmg8</i>	2B	1B	1B	2B	2B	1B	2B	1B	1B	2B
22	GR119	<i>Rmg8</i> + <i>RmgGR119</i>	1B	1B	1B	2B	2B	1B	1B	1B	1B	1B

2.3.2 Infection assays with detached leaves

When the two Bangladeshi isolates and three representative Brazilian isolates of *P. oryzae* (Br48, Br5, and Br116.5) were sprayed on detached primary leaves of Hope, IL191, and GR119, these isolates were divided into two groups, Group A (T-108, T-109, and Br48) and Group B (Br5 and Br116.5). At 25 °C, Group A was almost entirely avirulent on IL191 and GR119, while Group B produced some brown flecks (Fig. 1). At 28°C, Group A was still avirulent on IL191 and GR119, while Group B caused leaf shriveling (Fig. 1). It should be noted that these shriveled leaves were accompanied by tissue browning (infection types 5B and 4B – resistant reactions) in contrast to shriveled leaves of Hope (infection type 5G – susceptible reactions).

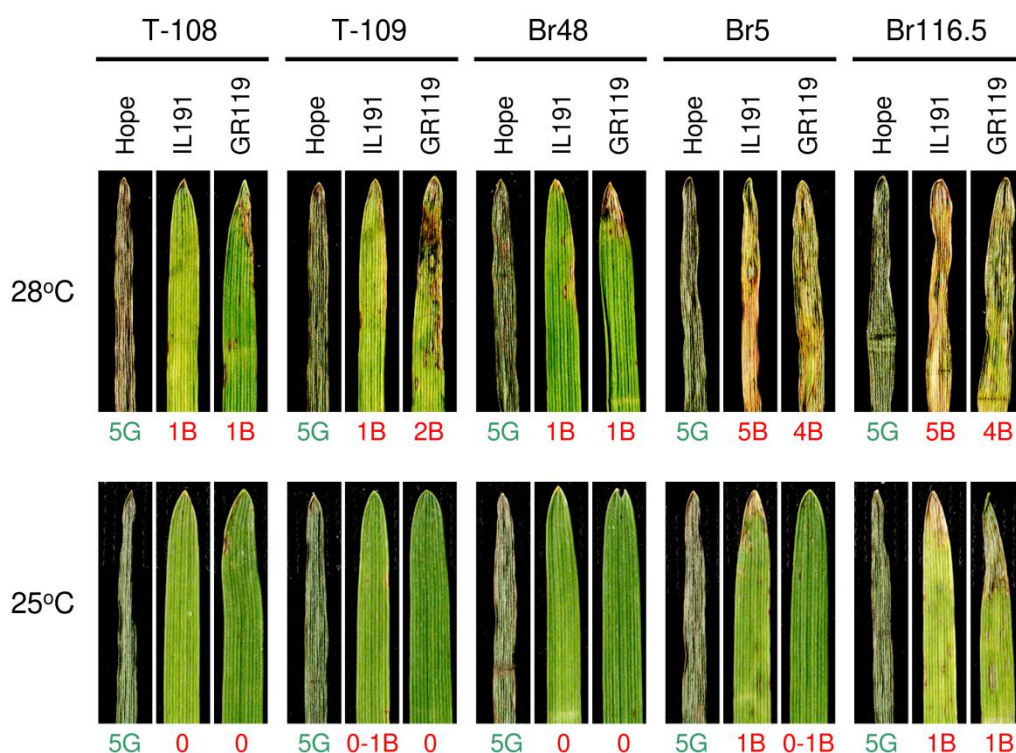


Fig. 1. Reactions of detached primary leaves of Hope (-/-), IL191 (*Rmg8*/-), and GR119 (*Rmg8/RmgGR119*) to *P. oryzae* isolates from Bangladesh (T-108, T-109) and Brazil (Br48, Br5, Br116.5) at 25°C and 28°C, 5 days after inoculation. Infection types are shown in red

(resistant) or green (susceptible) under each picture. The assay was repeated three times with similar results. The result of the third replication is shown.

2.3.3 Nucleotide sequences of *AVR-Rmg8* in various isolates

Br48 in Group A carries the eI type *AVR-Rmg8*, while Br5 and Br116.5 in Group B carry its variants (the eII and eII' types) (Wang et al. 2018). To determine *AVR-Rmg8* types of Bangladeshi isolates, an *AVR-Rmg8* region (550bp) was amplified from the genomic DNA of the eight Bangladeshi isolates and sequenced. Nucleotide sequences of *AVR-Rmg8* in these isolates were aligned with those from databases and literatures (Table 6). A dendrogram showed that *AVR-Rmg8* in *Triticum* isolates worldwide was divided into two groups, eI and eII/eII' (Fig. 2). Isolates from South America harbored all the three types, whereas Bangladeshi isolates harbored the eI type alone. The nucleotide sequences of the eI type in Bangladeshi isolates were 100% identical to the eI type in Br48.

Table 6. *Pyricularia oryzae* isolates whose data in databases or literatures were used for construction of a dendrogram of *AVR-Rmg8*

Isolate	Host	Collection year	Locality	Sequence Source (Accession No.)	References
Br48	Common wheat	1992	Itapora, Mato Grosso do Sul, Brazil	LC223814.1	Wang et al. 2018
Br2	Common wheat	1990	Londrina, Parana, Brazil	LC383726.1	Wang et al. 2018
Br3	Common wheat	1990	Londrina, Parana, Brazil		Wang et al. 2018
Br5	Common wheat	1990	Jagupita, Parana, Brazil		Wang et al. 2018
Br8	Common wheat	1990	B.V. Praiso, Parana, Brazil		Wang et al. 2018
Br46	Common wheat	1990	Rio Brilhante, Mato Grosso do Sul, Brazil		Wang et al. 2018
Br49	Common wheat	1992	Dourados, Mato Grosso do Sul, Brazil	LC383727.1	Wang et al. 2018
Br50	Common wheat	1992	Rio Brilhante, Mato Grosso do Sul, Brazil		Wang et al. 2018
Br108.1	Common wheat	1992	Assai, Parana, Brazil		Wang et al. 2018
Br116.5	Common wheat	1992	Santa Mariana, Parana, Brazil		Wang et al. 2018
Br117.1	Common wheat	1992	Santa Mariana, Parana, Brazil		Wang et al. 2018
Br127.11	Common wheat	1992	Sertanopolis, Parana, Brazil	SAMEA4029889	Wang et al. 2018
Br128.1	Common wheat	1992	Sertanopolis, Parana, Brazil		Wang et al. 2018
Br130.8	Common wheat	1992	Rolandia, Parana, Brazil		Wang et al. 2018
PY5033	Common wheat	2005	Londrina, PR, Brazil		OPEN WHEAT BLAST ^a
PY6017	Common wheat	2006	Coromandel, Minas Gerais, MG, Brazil	SAMEA4029890	OPEN WHEAT BLAST ^a
PY0925	Common wheat	2009	Perdizes, MG, Brazil	SAMEA4029894	OPEN WHEAT BLAST ^a
PY36.1	Common wheat	2007	Brasília, DF, Brazil	SAMEA4029897	OPEN WHEAT BLAST ^a
BTBa-B2	Barley leaf	2016	BSMRAU(campus) Gazipur, Bangladesh	SAMEA104190807	OPEN WHEAT BLAST ^b
BTGP-6(f)	Wheat spike	2017	Gangni, Meherpur, Bangladesh	SAMEA104190810	OPEN WHEAT BLAST ^b
BTGP-6(g)	Wheat spike	2017	Gangni, Meherpur, Bangladesh	SAMEA104190811	OPEN WHEAT BLAST ^b
BTGP-6(h)	Wheat spike	2017	Gangni, Meherpur, Bangladesh	SAMEA104190812	OPEN WHEAT BLAST ^b
BTJP-4(3)	Wheat seed	2016	Jhenaidah, Bangladesh	SAMEA104190814	OPEN WHEAT BLAST ^b
BTJP-4(5)	Wheat seed	2016	Jhenaidah, Bangladesh	SAMEA104190815	OPEN WHEAT BLAST ^b
BTJP-4(6)	Wheat seed	2016	Jhenaidah, Bangladesh	SAMEA104190816	OPEN WHEAT BLAST ^b
BTJP-4(11)	Wheat seed	2016	Jhenaidah, Bangladesh	SAMEA104190817	OPEN WHEAT BLAST ^b
BTJP-4(12)	Wheat seed	2016	Jhenaidah, Bangladesh	SAMEA104190818	OPEN WHEAT BLAST ^b
BTJP-4(16)	Wheat seed	2016	Jhenaidah, Bangladesh	SAMEA104190819	OPEN WHEAT BLAST ^b
BTJP-4(18)	Wheat seed	2016	Jhenaidah, Bangladesh	SAMEA104190820	OPEN WHEAT BLAST ^b
BTMP-S-13(1)	Wheat seed	2016	Meherpur, Bangladesh	SAMEA104190821	OPEN WHEAT BLAST ^b
BTMP-S-13(2)	Wheat seed	2016	Meherpur, Bangladesh	SAMEA104190822	OPEN WHEAT BLAST ^b
BR32	Common wheat	1989	Brazil	SAMN08009575	The Magnaporthe GEMO database ^c
T25	Common wheat	1988	Parana, Brazil		Farman et al. 2017
Br7	Common wheat	1990	Parana, Brazil		Farman et al. 2017
Br130	Common wheat	1990	Mato Grosso do Sul, Brazil		Farman et al. 2017
Br80	Common wheat	1991	Brazil		Farman et al. 2017
P3	Durum wheat	2012	Canindeyu, Paraguay	SAMN08009568	Pieck et al. 2017 ^d
B2	Common wheat	2011	Bolivia	SAMN05580113	Pieck et al. 2017 ^d
B71	Common wheat	2012	Bolivia	SAMN04942725	Malaker et al. 2016

^a<https://doi.org/10.6084/m9.figshare.5231485.v1>

^b<https://doi.org/10.6084/m9.figshare.5236381.v1>

^c<http://genome.jouy.inra.fr/gemo>

^d<https://doi.org/10.1094/PDIS-04-16-0500-RE>

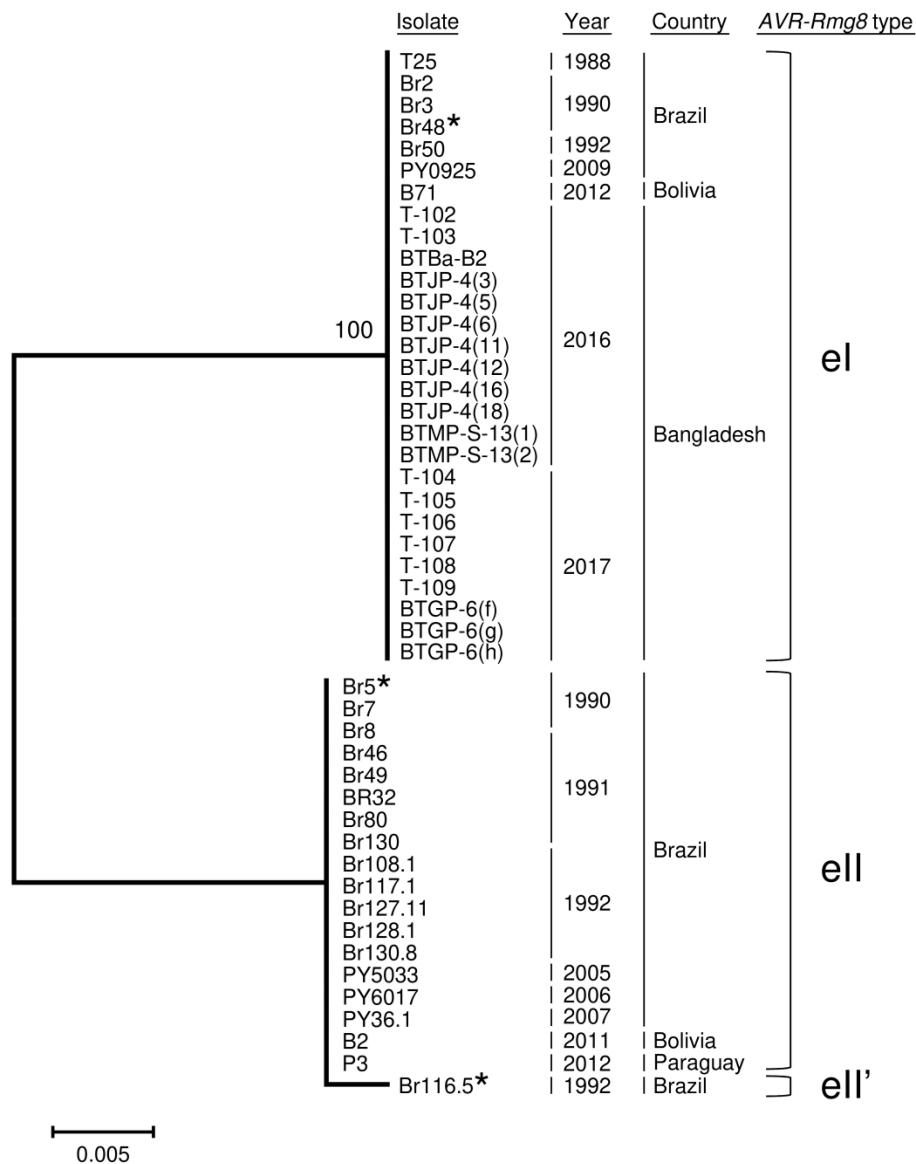


Fig. 2. Relationship of AVR-Rmg8 homologs in *Triticum* isolates used in this study and databases. The dendrogram was constructed based on their nucleotide sequences of AVR-Rmg8 ORF using the Maximum Likelihood method. The number at the node represents a percentage of 1000 bootstrap replicates. The scale bar represents 0.005 base substitutions per site. Asterisks indicate the three representative isolates.

2.3.4 Function of the three types of *AVR-Rmg8* as avirulence genes

To clarify the association of the *AVR-Rmg8* types with the phenotypes directly, 1.8kb fragments containing the eI, eII, and eII' types of *AVR-Rmg8* were isolated from Br48, Br5, and Br116.5, respectively, and introduced into Br48ΔA8_d6 (disruptant of *AVR-Rmg8*) which was virulent on both IL191 and Hope. Consequently, 11, 14, and 12 transformants were obtained from the transformation with the eI, eII, and eII' types, respectively. When sprayed on intact primary leaves of IL191 and Hope at room temperature (~22°C), six with the eI type (Br48ΔA8+eI), six with the eII type (Br48ΔA8+eII), and five with the eII' type (Br48ΔA8+eII') showed avirulence on IL191 (infection types 0 or 1B) without losing the high virulence on Hope (infection type 5G), indicating that all of these types are functional and recognized by *Rmg8*.

Three transformants were arbitrarily chosen as representatives from each of the three groups. The copy number of the transgenes in these transformants varied in each of Br48ΔA8+eI, Br48ΔA8+eII, and Br48ΔA8+eII' (Fig. 3). Nevertheless, the three transformants in each group showed similar tendency in virulence on IL191; when they were sprayed on detached third leaves of IL191 and incubated at 28°C, the eI transformants were completely avirulent while the eII and eII' transformants produced some brown spots or flecks (Fig. 4, 5). These results suggest that the eI type induces a higher level of resistance than the eII/eII' types in *Rmg8* carriers.

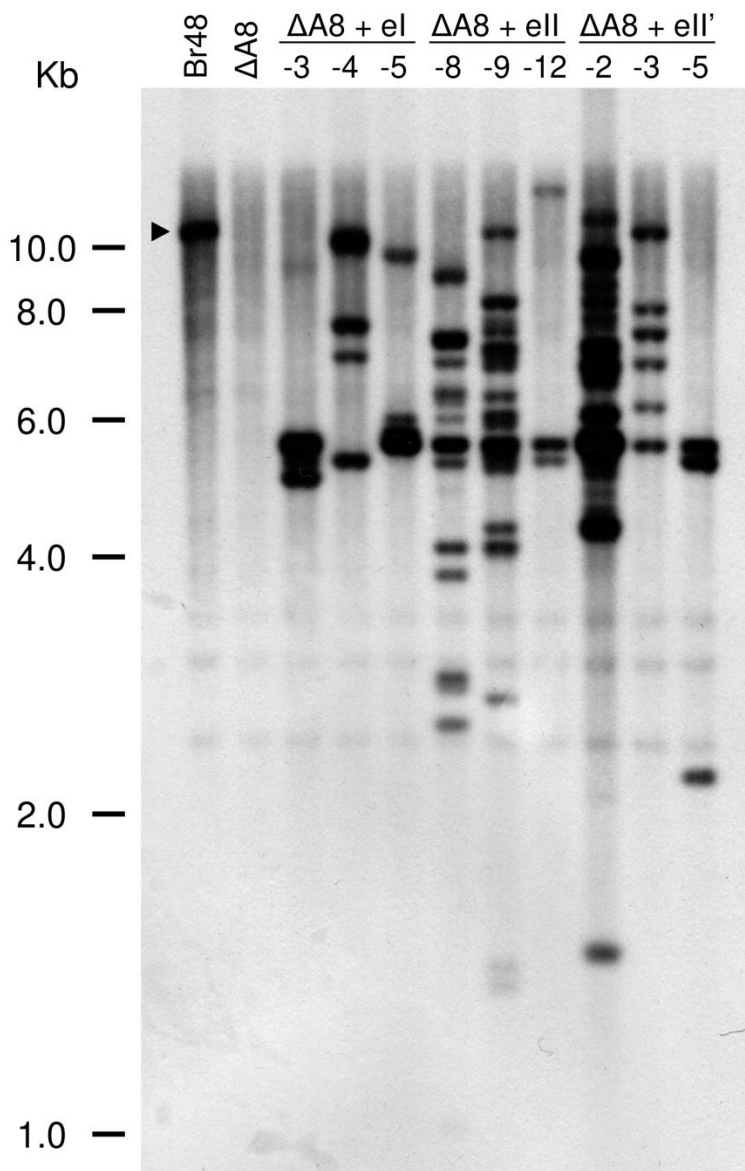


Fig. 3. Southern blot analysis of Br48, Br48 Δ A8 (Δ A8), and its transformants carrying the eI type (Δ A8+eI -3, 4, 5), eII type (Δ A8+eII -8, 9, 12), and eII' type (Δ A8+eII' -2, 3, 5) of *AVR-Rmg8* isolated from Br48, Br5, and Br116.5, respectively. A triangle indicates a fragment containing the endogenous *AVR-Rmg8* in Br48.

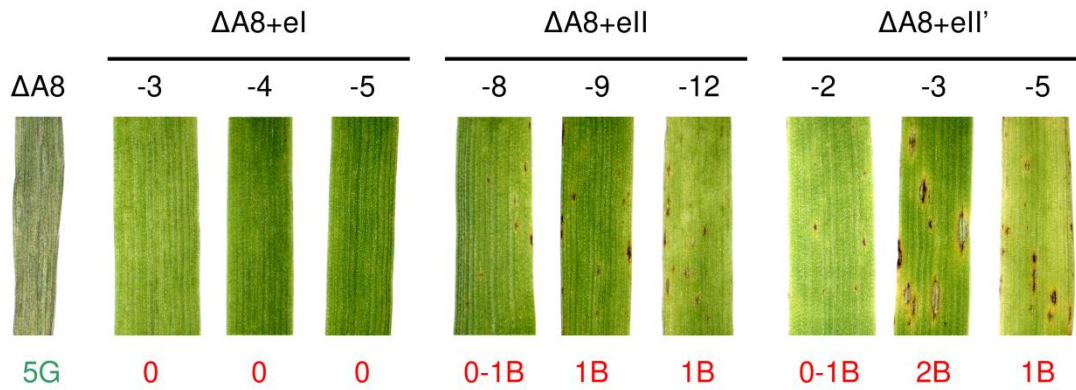


Fig. 4. Reactions of detached third leaves of IL191 (*Rmg8*/-) at 28°C to Br48ΔA8 (ΔA8) and its transformants carrying the eI type (ΔA8+eI -3, 4, 5), eII type (ΔA8+eII -8, 9, 12), and eII' type (ΔA8+eII'-2, 3, 5) of *AVR-Rmg8* isolated from Br48, Br5, and Br116.5, respectively, 4 days after inoculation. Infection types are shown in red (resistant) or green (susceptible) under each picture. The assay was repeated twice with similar results. The result of the second replication is shown.



Fig. 5. The whole images of the third leaves shown in Fig. 4. A portion of each leaf was magnified from the region enclosed with a red rectangle to produce Fig. 4.

2.3.5 Spike infection assay

To evaluate the effect of *Rmg8* and *RmgGR119* at the heading stage, spikes of Hope, IL191, and GR119 were inoculated with the representative Bangladeshi (T-108 and T-109) and Brazilian (Br48 and Br5) isolates of *P. oryzae*. IL191 was resistant to the eI carriers (T-108, T-109, Br48) but susceptible to the eII carrier (Br5) (Fig. 6). Infection assays with the transformants suggested that this contrastive reaction was attributed to the *AVR-Rmg8* types they harbored (Fig. 7). GR119 displayed higher levels of resistance than IL191 against T-109 and Br48 (Fig. 6). Furthermore, GR119 was resistant even against Br5 to which IL191 was susceptible.

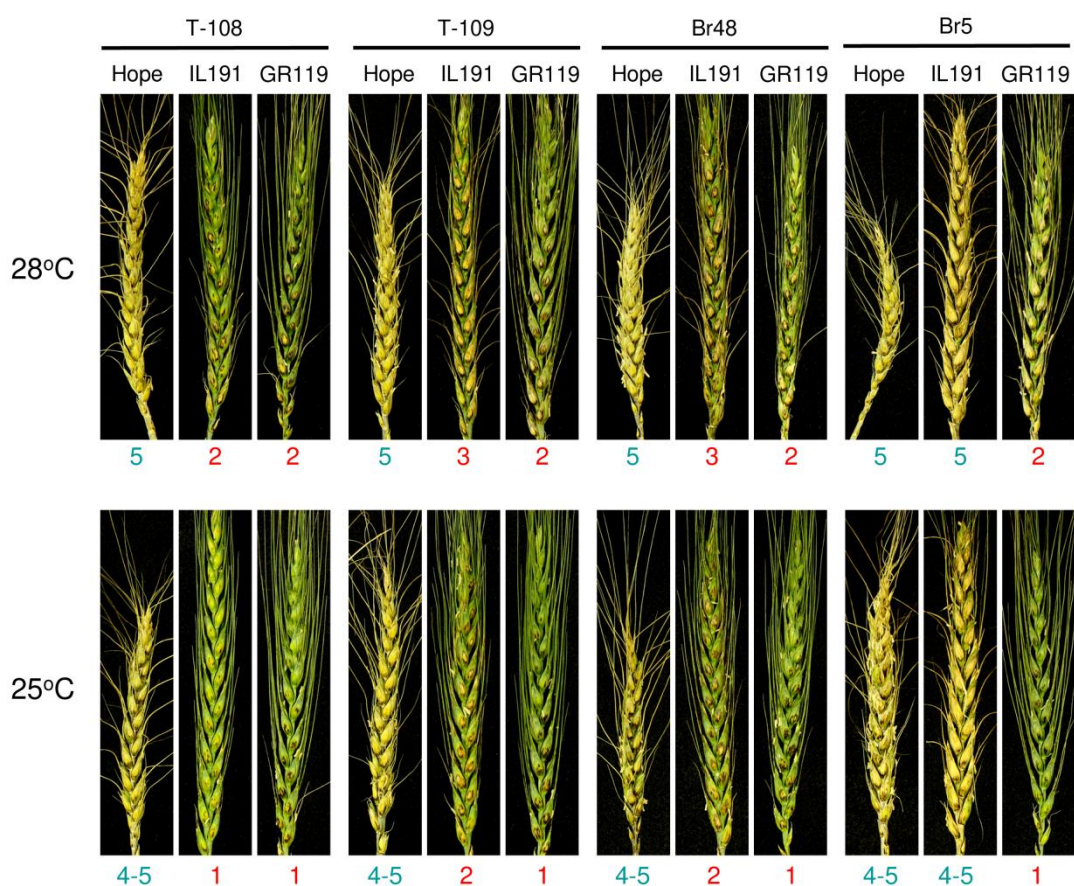


Fig. 6. Reactions of detached spikes of Hope (-/-), IL191 (*Rmg8*/-) and GR119 (*Rmg8/RmgGR119*) to *P. oryzae* isolates from Bangladesh (T-108, T-109) and Brazil (Br48, Br5) at 25°C and 28°C, 7 days

after inoculation. Infection types are shown in red (resistant) or green (susceptible) under each picture. The assay was repeated three times with similar results. The result of the third replication is shown.

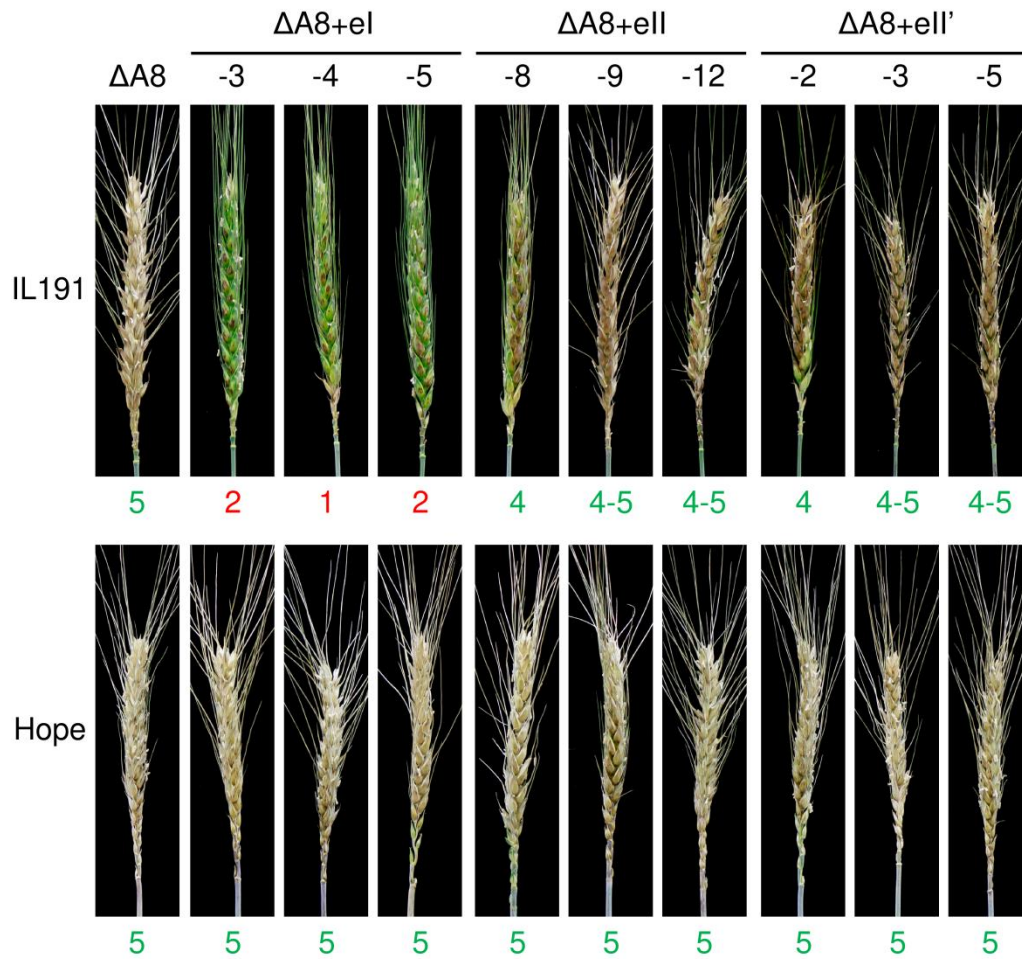


Fig. 7. Reactions of detached spikes of IL191 (*Rmg8*^{-/-}) and Hope (*-/-*) at 28 °C to Br48 Δ A8 (Δ A8) and its transformants carrying the eI type (Δ A8+eI-3, 4, 5), eII type (Δ A8+eII-8,9,12), and eII' type (Δ A8+-2,3,5) of *AVR-Rmg8* isolated from Br48, Br5, and Br116.5, respectively, 7 days after inoculation. Infection types are shown in red (resistant) or green (susceptible) under each picture.

2.4 Discussion

Wang et al. (2018) reported three types of *AVR-Rmg8*, i.e., eI, eII, and eII', in *Triticum* isolates collected in Brazil in 1990-1992. In their infection assay with intact leaves at 25°C, a

correlation was not recognized between the *AVR-Rmg8* types of these isolates and the degree of their avirulence on IL191 (carrying *Rmg8*). However, careful examination with detached leaves and spikes at 25-28°C revealed that Group A carrying the eI type was highly avirulent on IL191 while Group B carrying the eII/eII' types was more aggressive or virulent on this accession (Fig. 1, 6). Infection assays with the transformants revealed that the eI type induced higher levels of resistance in comparison with the eII/eII' types in this *Rmg8* carrier (Fig. 4, 7). In contrast to the Brazilian isolates, Bangladeshi isolates harbored the eI type with no exception (Fig. 2). The wheat blast population in Bangladesh is considered to be clonally derived from a single strain of one mating type (Cruz and Valent 2017), and therefore, is inferred to be composed of eI type carriers alone. These results suggest that *Rmg8* will be effective against wheat blast in farmer's fields in Bangladesh.

GR119 carrying two genes (*Rmg8/RmgGR119*) displayed higher levels of resistance than IL191 (*Rmg8*/-) against two of the three eI carriers tested at the heading stage (Fig. 6). In addition, GR119 was resistant even against the eII carrier to which IL191 was susceptible (Fig. 6). We suggest that the combination of *Rmg8* with *RmgGR119* will make the resistance long-lasting in Bangladesh, and also may be effective in South America where both of the eI and eII/eII' types are prevailing (Fig. 2).

Wang et al. (2018) observed brown flecks on detached leaves of S-615 inoculated with Br48 at 25°C. On detached leaves of IL191 inoculated with the same isolate at 25°C, however, no lesions were produced (Fig. 1). IL191 also showed similar immune responses against the other eI carriers at 25°C (Fig. 1). IL191 was selected as an accession displaying the highest level of resistance to Br48 at the seedling stage from the 18 lines carrying *Rmg8* alone, i.e., cv. S-615 and 17 accessions found by Wang et al. (2018). It is unlikely that IL191 carries another resistance gene because it is susceptible to Br48ΔA8_d6, an *AVR-Rmg8* disruptant of

Br48 (Fig. 4). IL191 and S-615 seem to carry different *Rmg8* alleles. For resistance breeding, it is advisable to choose the most effective allele of *Rmg8*.

Jensen et al. (2019) reported that S-615 carrying *Rmg8* was resistant to a Bangladesh isolate but susceptible to a Brazilian isolate. This Brazilian isolate may carry an *AVR-Rmg8* allele other than the eI type. The current global population of the wheat blast fungus is composed of strains that differ with respect to virulence on *Rmg8* (Fig. 2). Group A and Group B are regarded as physiological races differentiated on an *Rmg8* carrier, although their difference becomes obvious only at high temperature or at the heading stage. When a system of race identification is constructed (Cruppe et al. 2020), therefore, an *Rmg8* carrier should be included in the differential cultivars. It should be noted that this variation of *AVR-Rmg8* was already present in the population of wheat blast isolates collected in Brazil in 1990-1992, only 5 to 7 years after the first detection of this fungus. This variation does not seem to be caused by an adaptation to *Rmg8*. Further studies are needed to clarify the process of evolution of *AVR-Rmg8*.

CHAPTER 3: Polymorphisms of the Avirulence Gene *AVR-Rmg8* in *Pyricularia* isolates

3.1 Introduction

Knowing the spectrum and durability of a resistance gene at the initial stage of a breeding program is a breakthrough in the current disease management. Experiences tell that the resistance genes seldom withstand newly evolved virulent races of the pathogen because pathogens usually modify themselves to overcome the resistance. These biological modifications in the pathogen are supposed to have a relationship with nature/structure of the R gene. Up to date, there is no precise method to confirm the durability of a particular gene before releasing it into a field. However, some ideas have been proposed to predict the durability of R genes (Anh 2016; Chuma et al. 2011). For example, as an R gene confers resistance through a gene-to-gene interaction (Flor 1971), its corresponding avirulence gene must be present across a wide range of isolates of the specific pathogen. Avirulence gene homologs in other isolates have to be functional. Furthermore, the structure surrounding its coding region plays a vital role in keeping the gene stable. If the gene is surrounded by multiple transposable elements (TEs), there will be a high possibility of mutation, making the given avirulence gene unstable (Chuma et al. 2011; Yoshida et al. 2016).

TEs are DNA sequences known as jumping genes or mobile elements that move from one location of the genome to another (Kim et al. 2012; Pray 2008). They have been classified into two types; retrotransposons which transpose in a "copy-paste" fashion through reverse transcription and DNA transposons which transpose using a "cut and paste" mechanism without reverse transcription (Pray 2008). TE are found in almost all organisms and

constitutes more than 50% of DNA sequences of organisms (Goerner-Potvin and Bourque 2018; Pray 2008). For instance, the DNA sequence of *Oryza sativa* contains more than 80% of DNA transposons.

Although there is no definite answer to the question why organisms contain a lot of TE, several scholars have suggested its essential roles in driving the evolution of genes (Kumar et al. 2018; Seidl and Thomma 2017). Seidl and Thomma (2017) have reported that TEs direct the coevolution between the plant and microbes. Chuma et al. (2011) also reported that a critical event in the evolution of *AVR-Pita* flanks suggested by the topology of the phylogenetic tree was the insertion of the full length of Inago1. In the field of host resistance, their impact on the evolution of genes could be either positive or negative. Positively, TEs help the organism to modify itself and adjust to the new environment by facilitating the swift evolution of effector-rich regions in response to plant immunity by establishing genetic variability (Seidl and Thomma 2017).

In contrast, their insertion in a particular gene may cause loss of function. In rice blast disease management, most of the resistance genes identified were quickly broken down by new races of blast pathogens after releasing into the field (Yasuda et al. 2015). For wheat blast disease, even though about eight resistance genes have been identified, only *AVR-Rmg8* corresponding to *Rmg7* and *Rmg8* has been identified so far (Anh et al. 2018). *Rmg8* has not yet been introduced into farmer's field, and hence, it is crucial to predict its performance against new blast races in the field condition.

In this study, we analyzed the distribution of *AVR-Rmg8* in multiple *P. oryzae* isolates in the database.

3.2 Materials and Methods

3.2.1 Preparation of plasmid

Plasmid DNA (pBluescriptII SK(+)) was extracted from *E. coli* cultures using the FastGene kit (NipponGenetics, www.n-genetics.com), digested with *EcoRV* overnight to produce blunt ends, and purified by the FastGene kit. The extracted plasmid was dissolved in TE and stored at -20°C.

3.2.2 Southern blot analysis

Genomic DNA of 99 representative isolates from Kobe University collections were digested with *EcoRI* (New England Biolabs, www.neb.com/R3101) and hybridized as described in section 2.2.8.

3.2.3 Sequence analysis

To analyze the distribution of *AVR-Rmg8*, 140 genome sequence of *Pyricularia oryzae/gresea* originated from different hosts were collected from the database. The strain list included all the strains used in Gladieux et al. (2018). The eI type of *AVR-Rmg8* ORF extracted from Br48 were locally blasted using BASTN 2.10.0+ program (Altschul et al. 1997). Homologs of *AVR-Rmg8* extracted were aligned using muscle/align-by-codons option in the MEGA X tool. The phylogenetic tree was constructed using the Maximum Likelihood statistical method, and tested by the bootstrap method with 1000 replications. All sites of the data subset were used with nucleotide substitution type and Tamura-Nei model. Only isolates that carried full length of *AVR-Rmg8* ORF were included in the phylogenetic tree, except *Digitaria sanguinalis* (Dig41 and DSLIZ) used as an outgroup.

3.3 Results

3.3.1 Distribution of *AVR-Rmg8* across *Pyricularia oryzae* pathotypes

Southern blot analysis showed that the distribution of an avirulence gene, *AVR-Rmg8* in *Oryza*, *Setaria*, *Panicum*, *Eleusine*, *Avena*, *Triticum*, *Lolium*, *Brachiaria*, *Eragrostis* were 17/30(56.7%), 4/8(50%), 5/7(71%), 10/13(76.9%), 1/1(100%), 8/8(100%), 6/6(100%), 2/2(100%), and 1/1(100%), respectively. However, *AVR-Rmg8* was absent in *Pyricularia* spp. from *Leersia*, *Cenchrus*, *Digitaria*, and *Sasa* hosts (Table 7).

Also, the *AVR-Rmg8* sequence analysis of 140 *Pyricularia oryzae/gresea* isolates collected from databases (Supplementary Table S1) confirmed that 118 isolates out of 140 contained the full length of *AVR-Rmg8* homologs. Among these, 29 isolates of *Triticum* and *Avena* pathotypes contained the eI type which was 100% identical to *AVR-Rmg8* of Br48. Even though there was no signal in the southern blot analysis, the sequences of *Digitaria* isolates had a portion of *AVR-Rmg8*, 121bp with 69% identity (Supplementary Table S1).

A phylogenetic tree was constructed using *AVR-Rmg8* ORF to show evolutionary relationship and distribution of *AVR-Rmg8* in various pathotypes. Generally, the isolates were grouped into six groups; *Triticum* eI, *Triticum* eII/eII', *Setaria*, *Oryza*, *Lolium*, and *Eleusine* (Fig. 8). *Avena* pathotype was included in the group of *Triticum* eI. The *Eleusine* group was divided into some subgroups and two of them were clustered with the group of *Lolium* and *Triticum* eII/eII'. On the other hand, *Setaria* and *Oryza* groups were succinctly differentiated from other groups. The *Triticum* eI group mainly contained *Triticum* isolates and seemed to most recently evolved (Fig. 8).

Table 7. Distribution of *AVR-Rmg8* in *Pyricularia* isolates revealed by Southern blot analysis

Species	Isolate ^a	Host	Locality	Year	AVR- <i>Rmg8</i>	Percent distribution	Collector or reference ^b
<i>Pyricularia oryzae</i>	Guy11	<i>Oryza sativa</i>	Guiana (Combi)	1978	-		J.L. Notteghem
	Ken54-04 (MAFF235006)	<i>Oryza sativa</i>	Japan (Gifu)	1954	+		Kiyosawa (1974)
	Ken54-20 (MAFF235005)	<i>Oryza sativa</i>	Japan (Yamaguchi)	1954	+		Kiyosawa (1974)
	Hoku1	<i>Oryza sativa</i>	Japan (Hokkaido)	1948	+		Kiyosawa (1974)
	Ina72 (MAFF235003)	<i>Oryza sativa</i>	Japan (Nagano)	1957	+		Kiyosawa (1974)
	Ina168	<i>Oryza sativa</i>	Japan (Aichi)	1958	+		Kiyosawa (1974)
	Ken53-33	<i>Oryza sativa</i>	Japan (Aichi)	1953	+		Kiyosawa (1974)
	P-2b	<i>Oryza sativa</i>	Japan (Niigata)	1948	-		Kiyosawa (1974)
	0903-4	<i>Oryza sativa</i>	Japan (Tochigi)	1976	-		Yamada et al. (1976)
	2012-1	<i>Oryza sativa</i>	Japan (Ishikawa)	1976	+		Yamada et al. (1976)
	2403-1	<i>Oryza sativa</i>	Japan (Mie)	1976	+		Yamada et al. (1976)
	1836-3	<i>Oryza sativa</i>	Japan (Niigata)	1976	+		Yamada et al. (1976)
	88A	<i>Oryza sativa</i>	Japan (Ibaraki)	1976	-		Yamada et al. (1976)
	CHNOS 59-6-1	<i>Oryza sativa</i>	China (Yunnan)	1989	-	56.70%	N. Hayashi
	CHNOS 60-8-1	<i>Oryza sativa</i>	China (Yunnan)	1989	+		N. Hayashi
	Br10	<i>Oryza sativa</i>	Brazil (Parana)	1990	+		S. Igarashi
	Br13	<i>Oryza sativa</i>	Brazil (Parana)	1990	+		S. Igarashi
	Br15	<i>Oryza sativa</i>	Brazil (Parana)	1990	-		S. Igarashi
	Br18	<i>Oryza sativa</i>	Brazil (Parana)	1990	+		S. Igarashi
	PO-02-7306	<i>Oryza sativa</i>	Indonesia (Jawa Barat)	1973	+		A. Mukelar
	PO-02-7501	<i>Oryza sativa</i>	Indonesia (Jawa Barat)	1975	-		A. Mukelar
	PO-04-7501	<i>Oryza sativa</i>	Indonesia (Jawa Timur)	1975	-		A. Mukelar
	PO-12-7301-2	<i>Oryza sativa</i>	Indonesia (Lampung)	1973	-		A. Mukelar
	PO-12-7301	<i>Oryza sativa</i>	Indonesia (Lampung)	1973	-		A. Mukelar
	VHT6.1	<i>Oryza sativa</i>	Vietnam (Ha Tay)	1998	+		L. D. Don
	VTB6.1	<i>Oryza sativa</i>	Vietnam (Thai Binh)	1998	-		L. D. Don
	VHG4.5	<i>Oryza sativa</i>	Vietnam (Hau Giang)	1996	-		L. D. Don
	VHT3.3	<i>Oryza sativa</i>	Vietnam (Ha Tay)	1998	-		L. D. Don
	84R-62B	<i>Oryza sativa</i>	Japan (Aich)	1984	+		H. Naito
	Y93-245c-2	<i>Oryza sativa</i>	China (Yunnan)	1993	+		Y. Fujita
	GFSI1-7-2	<i>Setaria italica</i>	Japan (Gifu)	1977	+		H. Kato
	NRSI2-2-2	<i>Setaria italica</i>	Japan (Nara)	1977	+		H. Kato
	NRSI3-1-1	<i>Setaria italica</i>	Japan (Nara)	1977	+		H. Kato
	NNSI3-2-1	<i>Setaria italica</i>	Japan (Nagano)	1984	+	50%	H. Kato
	IN77-16-1-1	<i>Setaria italica</i>	India (Mysore)	1977	-		H. Kato
	IN77-20-1-1	<i>Setaria italica</i>	India (Mysore)	1977	-		H. Kato
	KANSV1-4-1	<i>Setaria viridis</i>	Japan (Kanagawa)	1975	-		H. Kato
	NI913	<i>Setaria viridis</i>	Japan (Chiba)	1974	-		N. Nishihara

Table 7. (Continued)

Species	Isolate ^a	Host	Locality	Year	AVR- Rmg8	Percent distribution	Collector or reference ^b
<i>Pyricularia oryzae</i>	NNPM1-2-1	<i>Panicum miliaceum</i>	Japan (Nagano)	1984	+		N. Hayashi
	NRPM1-1-1	<i>Panicum miliaceum</i>	Japan (Nara)	1990	+		H. Kato
	STPM1-3-2	<i>Panicum miliaceum</i>	Japan (Saitama)	1981	+		H. Kato
	STPM4-2-2	<i>Panicum miliaceum</i>	Japan (Saitama)	1981	+	71%	H. Kato
	SZPM1-1-1	<i>Panicum miliaceum</i>	Japan (Shizuoka)	1978	-		H. Kato
	YNPM4-1-1	<i>Panicum miliaceum</i>	Japan (Yamanashi)	1983	-		H. Kato
	NI922	<i>Panicum bisulcatum</i>	Japan (Tochigi)	1974	+		N. Nishihara
	G10-1	<i>Eleusine coracana</i>	Japan (Tochigi)	1977	+		S. Itoi
	Z2-1	<i>Eleusine coracana</i>	Japan (Kagawa)	1977	+		S. Itoi
	MZ5-1-6	<i>Eleusine coracana</i>	Japan (Miyazaki)	1976	+		H. Kato
	Ken15-15-1	<i>Eleusine coracana</i>	Japan (Tokyo)	1976	+		H. Kato
	SZEC1-1-1	<i>Eleusine coracana</i>	Japan (Shizuoka)	1978	+		H. Kato
	GFEC1-5-1	<i>Eleusine coracana</i>	Japan (Gifu)	1977	+		H. Kato
	IN77-31-1-1	<i>Eleusine coracana</i>	India (Bangalore)	1977	+	84.61%	H. Kato
	IN77-39-1-2	<i>Eleusine coracana</i>	India (Bangalore)	1977	+		H. Kato
	NP10-17-4-1-3	<i>Eleusine coracana</i>	Nepal (Syabru)	1975	+		S. Sakamoto
	NP10-28-1-1-1	<i>Eleusine coracana</i>	Nepal (Pokara)	1975	+		S. Sakamoto
	NI1006	<i>Eleusine africana</i>	Japan (Tochigi)	1975	-		N. Nishihara
	NI1011	<i>Eleusine boranensis</i>	Japan (Tochigi)	1975	-		N. Nishihara
	IN77-36-1-1	<i>Eleusine indica</i>	India (Mysore)	1977	+		H. Kato
	Br58	<i>Avena sativa</i>	Brazil (Parana)	1990	+	-	S. Igarashi
	Br3	<i>Triticum aestivum</i>	Brazil (Parana)	1990	+		S. Igarashi
	Br7	<i>Triticum aestivum</i>	Brazil (Parana)	1990	+		S. Igarashi
	Br8	<i>Triticum aestivum</i>	Brazil (Parana)	1990	+		S. Igarashi
	Br48	<i>Triticum aestivum</i>	Brazil (Mato Grosso do Sul)	1990	+	100%	S. Igarashi
	(IMI368172)		Brazil (Mato Grosso do Sul)	1990	+		S. Igarashi
	Br49	<i>Triticum aestivum</i>	Brazil (Mato Grosso do Sul)	1990	+		S. Igarashi
	(IMI368173)	<i>Triticum aestivum</i>	Brazil (Parana)	1992	+		A.S. Urashima
	Br115.7	<i>Triticum aestivum</i>	Brazil (Parana)	1992	+		A.S. Urashima
	Br116.5	<i>Triticum aestivum</i>	Brazil (Parana)	1992	+		A.S. Urashima
	Br118.2D	<i>Triticum aestivum</i>	Brazil (Parana)	1992	+		A.S. Urashima
	TP1	<i>Lolium perenne</i>	Japan (Tochigi)	1997	+		A. Tanaka, T. Tani
	TP2	<i>Lolium perenne</i>	Japan (Tochigi)	1997	+		A. Tanaka, T. Tani
	AK1	<i>Lolium perenne</i>	Japan (Akita)	1998	+	100%	A. Tanaka, T. Tani
	LW3	<i>Lolium perenne</i>	Japan (Yamanashi)	1999	+		A. Tanaka, T. Tani
	FI5	<i>Lolium perenne</i>	Japan (Chiba)	1998	+		A. Tanaka, T. Tani
	WK3-1	<i>Lolium perenne</i>	Japan (Yamaguchi)	1996	+		A. Tanaka, T. Tani
	Br35	<i>Brachiaria plantaginea</i>	Brazil (Parana)	1990	+	100%	S. Igarashi
	Bp3a	<i>Brachiaria plantaginea</i>	Brazil (Sao Paulo)	1996	+		A.S. Urashima
	NI986	<i>Eragrostis lehmanniana</i>	Japan (Kumamoto)	1975	+	-	N. Nishihara

Table 7. (Continued)

Species	Isolate ^a	Host	Locality	Year	AVR- Rmg8	Percent distribution	Collector or reference ^b
<i>Pyricularia</i> sp. (LS) ^c	NI919 (MAFF305509)	<i>Leersia oryzoides</i>	Japan (Chiba)	1974	-		N. Nishihara
	Br37	<i>Setaria geniculata</i>	Brazil (Parana)	1990	-		S. Igarashi
<i>Pyricularia</i> sp. (CE) ^c	NI981 (MAFF305501)	<i>Cenchrus ciliaris</i>	Japan (Kumamoto)	1975	-		N. Nishihara
	Br36	<i>Cenchrus echinatus</i>	Brazil (Parana)	1990	-		S. Igarashi
	Br38	<i>Echinochloa colonum</i>	Brazil (Parana)	1990	-		S. Igarashi
<i>Pyricularia grisea</i>	Dig41	<i>Digitaria sanguinalis</i>	Japan (Hyogo)	1990	-		Y. Iwamoto
	NI907	<i>Digitaria sanguinalis</i>	Japan (Tochigi)	1974	-		N. Nishihara
	IBDS4-1-1	<i>Digitaria sanguinalis</i>	Japan (Ibaraki)	1985	-		N. Hayashi
	NI980 Br29 (IMI368175)	<i>Digitaria smutsii</i>	Japan (Kumamoto)	1975	-		N. Nishihara
		<i>Digitaria horizontalis</i>	Brazil (Sao Paulo)	1990	-		S. Igarashi
	Br33	<i>Digitaria horizontalis</i>	Brazil (Parana)	1990	-		S. Igarashi
<i>Pyricularia zizaniaecola</i>	IBZL3-1-1	<i>Zizania latifolia</i>	Japan (Ibaraki)	1985	-	0%	N. Hayashi
	KYZL201-1-1	<i>Zizania latifolia</i>	Japan (Kyoto)	2003	-		K. Yoshida, K. Hirata
<i>Pyricularia zingiberi</i>	HYZiM101-1-1-1	<i>Zingiber mioga</i>	Japan (Hyogo)	1990	-		M. Ogawa
	HYZiM201-0-1	<i>Zingiber mioga</i>	Japan (Hyogo)	2002	-		H. Kato
	HYZiM202-1-2	<i>Zingiber mioga</i>	Japan (Hyogo)	2003	-		I. Chuma
	HYZiM201-1-1	<i>Zingiber mioga</i>	Japan (Hyogo)	2003	-		I. Chuma
<i>Pyricularia</i> sp. (SsPb) ^c	INA-B-92-45	<i>Sasa</i> sp. <i>Phyllostachys</i>	Japan (Aichi)	1992	-		S. Koizumi
	INA-B-93-19	<i>bambusoides</i>	Japan (Aichi)	1993	-		S. Koizumi
<i>Pyricularia</i> sp. (Kb) ^c	HYKB202-1-2	<i>Kyllinga brevifolia</i>	Japan (Hyogo)	2003	-		I. Chuma
	FKKB201-1-5	<i>Kyllinga brevifolia</i>	Japan (Fukuoka)	2003	-		I. Chuma
	FKKB201-3-2	<i>Kyllinga brevifolia</i>	Japan (Fukuoka)	2003	-		I. Chuma
<i>Pyricularia higginsii</i>	HYCI201-1-1	<i>Cyperus iria</i>	Japan (Hyogo)	2002	-		H. Kato

^a Accession numbers in public culture collections are shown in parentheses. (MAFF, Microorganisms Section of the NIAS Genebank, National Institute of Agrobiological Sciences, Tsukuba, Japan; IMI, CABI Bioscience, UK Centre, Egham, UK.)

^b Kiyosawa, S. (1974). Studies on genetics and breeding of blast resistance in rice. Misc. Publ. Bull. Natl. Inst. Agric. Sci. D No.1: 1-58 (In Japanese with English summary).

Yamada, M., Kiyosawa, S., Yamaguchi, T., Hirano, T., Kobayashi, T., Kushibuchi, K., and Watanabe, S. (1976). Proposal of a new method for differentiating races of *Pyricularia oryzae* Cavara in Japan. Ann. Phytopathol. Soc. Jpn. 42: 216-219.

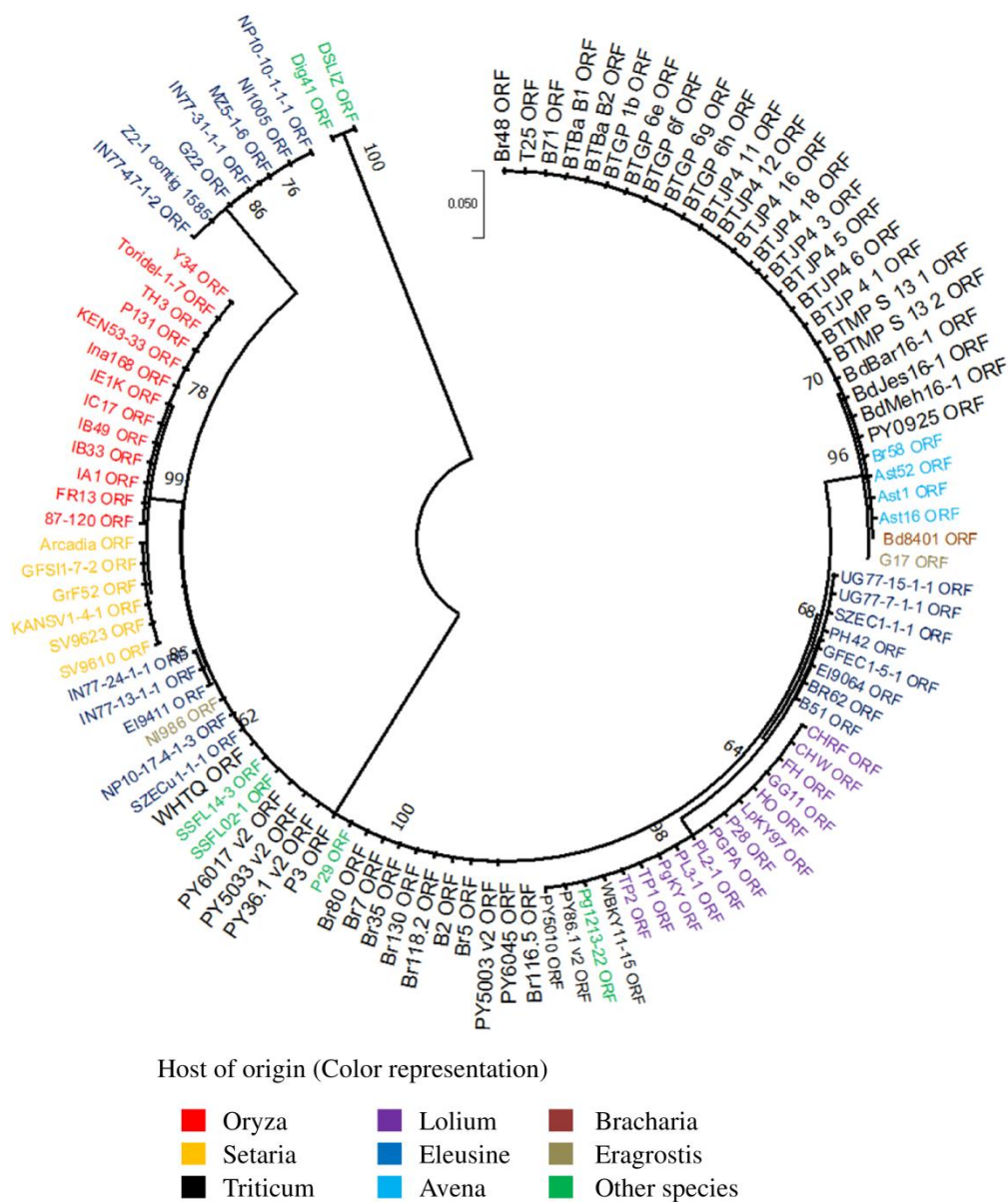


Fig. 8. The relationship of *AVR-Rmg8* in *P. oryzae* pathotypes. The phylogenetic tree was constructed using the Maximum Likelihood method and Tamura-Nei model, bootstrap with 1000 replicates. The tree is drawn to scale, with branch length measured in the number of substitutions per site. The evolutionary analysis was conducted in MEGA X (Kumar et al. 2018).

3.3.2 Analysis of amino acid sequences of *AVR-Rmg8* homologs

Lolium isolates have been reported to be most closely related to *Triticum* isolates (Asuke et al. 2020; Vy 2013). In this study, we observed that *AVR-Rmg8* in *Lolium* isolates

has amino acid substitution at 10th/110 (Isoleucine, I-to-Leucine, L) and at 15th/110 (Alanine, A-to-Valine, V) in reference to the eI type of *AVR-Rmg8* in Br48 (Supplementary Table S2). The amino acid substitution at 10th/110 was the same as that of in the eII/eII' type of *AVR-Rmg8* in the *Triticum* isolates.

Setaria and *Oryza* isolates are closely related pathotypes. Their *AVR-Rmg8* homologs varied from those of the *Triticum* pathotype at 3' region of *AVR-Rmg8* ORF. The homolog of *Setaria* isolates had an unique amino acid substitution at 75th/110, Glutamine (Q) to Lysine (K) while the homolog of *Oryza* isolates had a substitution at 95th, Tyrosine (Y) to Histidine (H) (Supplementary Table S2).

AVR-Rmg8 homologs of *Eleusine* isolates were classified into four subgroups. Group 1 and 2 included those with three stop codons (at 22nd, 31st, 75th) and one stop codon (at 32nd), respectively. On the other hand, Group 3 and 4 included those with no stop codons. Group 3 varied from the eII type by one amino acid substitution at 15th/110, Alanine (A) to Valine (V), but similar to those of *Lolium* isolates while Group 4 (homologs of SZECu1-1-1 and NP10-17-4-1-3) were 100% identical to the eII type of *AVR-Rmg8* (Supplementary Table S2). Group 1, 2, 3, and 4 were shared by 7, 3, 8 and 2 isolates, respectively.

3.3.3 Test of positive selection

Codon-based test of positive selection for averaging over all sequence pairs was conducted using the Nei-Gojobori method (Nei and Gojobori 1986) in MEGA X (Kumar et al., 2018). The ratio of nonsynonymous/synonymous substitution (dN/dS) was non-significant with p-value=1.0 and test statistic -2.29 (dN-dS). The variance of the difference was estimated by the bootstrap method (10000 replicates). There were a total of 105 positions in the final dataset.

3.4 Discussion

AVR-Rmg8 is widely distributed in the isolates from *Triticum*, *Lolium*, *Avena* and *Eleusine* but absent in *Pyricularia gresea* and other *Pyricularia* species. In the *Triticum* pathotype, two types (eI and eII/eII') were recognized but they were both functional. On the other hand, the *AVR-Rmg8* homologs in *Eleusine* isolates are supposed to be non-functional because they have stop codons (Group 1 and 2). However, homologs of Group 3 of *Eleusine* isolates do not have stop codon and are very similar to those of *Lolium* and *Triticum* pathotypes (the eII type). Therefore, the *AVR-Rmg8* homologs in the Group 3 *Eleusine* isolates are probably functional.

Although all Bangladeshi isolates collected from wheat carried full length of *AVR-Rmg8* homologs, the two *Pyricularia oryzae* isolates collected from Torpedo grass (*Panicum repens*) (BTTrp-5 and BTTrp-6) in Bangladesh did not carry the full length of *AVR-Rmg8* (Supplementary Table S1). There is a possibility that, in future, the full length gene in the wheat blast fungus may be replaced with the partial homolog. Castroagudin et al. (2017) reported unidirectional gene flow into the *P. oryzae* population infecting wheat from the one infecting other grasses. Gladieux et al. (2018) also clarified that gene flow was widespread across both historical times and lineages. At present, we have not yet tested whether this partial homolog maintain the function of *AVR-Rmg8*. Jiang et al. (2020) tested the function of *AVR-Rmg8* in *Lolium* pathotype. *AVR-Rmg8* in *Lolium* pathotype was divided into three types, eL1, eL2, and eL3. eL1 and eL2 were functional, while three eL3 carriers showed virulent reaction due to retrotransposon Pyret insertion into the ORF. Hence, we recommend further investigation in this regard to pinpoint the probable effect of the mutation.

CHAPTER 4: General Discussion

Blast is a dangerous foe that causes disease to several gramineous crops and grass species. Its damage to rice and wheat crops, which are the leading food crops in the globe, is posing a considerable threat to world food security. Currently, a wheat blast fungus caused by the MoT pathotype of *Pyricularia oryzae* has been introduced into Asia (Bangladesh and India). Due to the enormous contribution of the Eurasian continent to food supply, there is raising concern about the disease. It is also speculated that the pathogen may continue to spread in other parts of wheat-producing countries in the world. In addition, the Bangladeshi isolates showed higher virulence on *Lolium perenne* compared with Brazilian isolates (Fig. 9), indicating that the wheat blast fungus is evolving toward expanding its host range.

In the present study, we demonstrated that *Rmg8* is effective against wheat blast in Bangladesh. Bangladeshi isolates were reported to be derived from wheat-infecting South American single lineage of *P.oryzae* (Islam et al. 2016). We found that this lineage harbored the eI type of *AVR-Rmg8*. Interestingly, the eI type of *AVR-Rmg8* was strongly recognized by *Rmg8*. Therefore, introducing *Rmg8* into elite varieties contributes to the management of wheat blast disease in Bangladesh.

Recently, a resistant cultivar (BAR Gom 33), carrying a chromosomal segment of 2NS, has been released in Bangladesh. However, it is not dependable since the 2NS R gene has already been overcome by a virulent isolate of *P. oryzae* in Paraguay (Randhawa et al. 2019). Introgression of several R genes in a given line is needed to provide durable and robust resistance against a diversified population of wheat blast fungus. Stable and durable R genes will guarantee the strength and durability of the resulting cultivars. The characteristic of a corresponding avirulence gene will be used to predict the stability and durability of a

given R gene. *AVR-Rmg8* is widely distributed in the *Triticum*, *Lolium*, and *Avena* pathotypes. The homologs in *Triticum* and *Lolium* isolates are recognized by *Rmg8*. Even though the data we obtained so far suggest that *AVR-Rmg8* is durable compared with rice resistance genes such as *Pita*, *Pii*, *Pik*, *Pia* etc, further analyses are needed to predict its durability precisely.

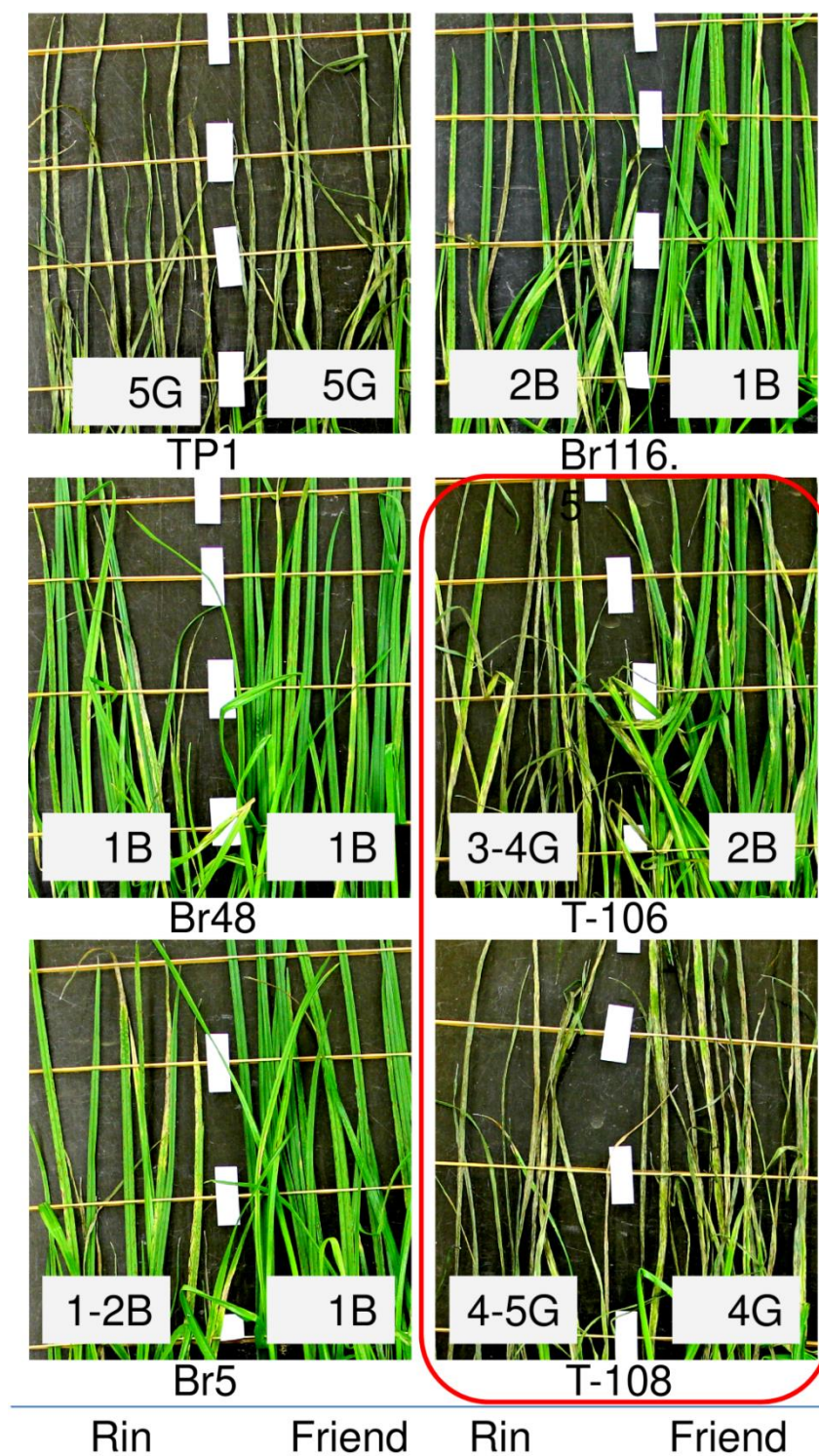


Fig. 9. Reactions of perennial ryegrass leaves to *P. oryzae* isolates from Bangladesh (enclosed in a red rectangle) and Brazil, 5 days after inoculation. The experiment was done at room temperature.

SUMMARY

In this study, I evaluated the effectiveness and durability of *Rmg8* and *RmgGR119*, recently identified R genes in wheat against wheat blast in Bangladesh. Eighteen wheat lines carrying *Rmg8* and an accession (GR119) carrying *Rmg8* and *RmgGR119* were inoculated with eight Bangladeshi isolates (T-102 through T-109) and two Brazilian isolates (Br48 and Br5). At 22°C and 25°C, they were indiscriminately resistant to all Bangladeshi and Brazilian isolates. However, further investigation at higher temperatures with intact and detached primary or third leaves revealed that these isolates were divided into two groups. Group A consisting of a Brazilian isolate (Br48) and all Bangladeshi isolates showed an entirely avirulent reaction, while Group B consisting Br5 and Br116.5 produced some brown flecks accompanied by tissue browning. Sequence analysis revealed that Group A harbored the eI type of *AVR-Rmg8*, which was 100% identical to that of Br48, while Group B harbored the eII and eII' types of *AVR-Rmg8*. All Bangladeshi isolates exclusively harbored the eI type.

The three types of *AVR-Rmg8* were cloned and transformed into Br48ΔA8 (disruptant of *AVR-Rmg8*). The resulting transformants were sprayed to seedlings and spikes of common wheat cv. Hope (-/-), accession IL191 (*Rmg8*/(-)), and GR119 (*Rmg8*/*RmgGR119*). IL191 was resistant to transformants with the eI type but susceptible to those with the eII and eII' type, suggesting that the different reaction is attributed to the difference of *AVR-Rmg8* alleles. GR119 was resistant to transformants with all types of *AVR-Rmg8*, even at high temperatures.

Southern blot and sequence analyses of multiple isolates from different pathotypes revealed that *AVR-Rmg8* was widely distributed in the *Triticum*, *Lolium*, and *Avena* isolates, but lacking in the isolates collected from *Leersia* sp., *Cenchrus* sp., *Digitaria* sp. and *Sasa* sp. hosts.

Taken together, I conclude that *Rmg8* and probably, *RmgGR119* are effective against *P. oryzae* in Bangladesh and other countries that have or will have similar wheat blast disease conditions.

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APPENDIX

Supplementary Table S1. Sequence analysis of AVR-Rmg8 in *P. oryzae/gresea* isolates

Isolate	Host	country	year	Identity	Hits	Collector/References
4-1	<i>Avena sativa</i>			96	312/330	
Ast1	<i>A. sativa</i>			100	330/330	
Ast16	<i>A. sativa</i>			100	330/330	
Ast52	<i>A. sativa</i>			100	330/330	
Br58	<i>A. sativa</i>	Brazil	1990	100	330/330	Yoshida et al. 2016
Bd8401	<i>Brachiaria distachya</i>	Philippines	1984	99	329/330	Gladieux et al. 2018
Bm88324	<i>Brachiaria mutica</i>	Philippines	1988	96	318/330	Gladieux et al. 2018
Br35	<i>Brachiaria plantaginea</i>	Brazil	1990	97	319/330	Urashima et al. 1999
P28	<i>Bromus tectorum</i>	Paraguay	2014	96	316/330	Pieck et al. 2017
P29	<i>B. tectorum</i>	Paraguay	2014	97	319/330	Pieck et al. 2017
Br36	<i>Cenchrus echinatus</i>	Brazil	1990	0		Urashima et al. 1999
HYZiM101-1-1-1	<i>Cyperus iria</i>	Japan	2002	0		M. Ogawa
HYCI201-1-1	<i>Cyperus iria</i>	Japan	2002	0		H. Kato
BR29	<i>Digitaria sanguinalis</i>	Brazil	1989	69	75/108	Chiapello et al. 2015
Dig41	<i>D. sanguinalis</i>	Japan	ND	69	84/121	Yoshida et al. 2016
DSLIZ	<i>D. sanguinalis</i>	KY, USA	2000	69	84/121	Pieck et al. 2017
VO107	<i>D. sanguinalis</i>	USA	1981	69	84/121	Gladieux et al. 2018
NI907	<i>D. sanguinalis</i>	Japan	1974	69	84/121	N. Nishihara
UG77-15-1-1	<i>Eleusine indica</i>	Uganda	ND	97	319/330	J. Mukiibi
NI1005	<i>Eleusine africana</i>	Japan	ND	89	294/330	N. Nishihara
G22	<i>Eleusine coracana</i>	Japan	1976	89	294/330	Gladieux et al. 2018
Z2-1	<i>E. coracana</i>	Japan	1977	89	294/330	Urashima et al. 1999
PH42	<i>E. coracana</i>	Philippines	1983	97	319/330	Farman et al. 2017
IN77-13-1-1	<i>E. coracana</i>	India	1977	96	318/330	H. Kato
IN77-24-1-1	<i>E. coracana</i>	India	1977	96	317/330	H. Kato
IN77-31-1-1	<i>E. coracana</i>	India	1977	89	294/330	H. Kato
IN77-47-1-2	<i>E. coracana</i>	India	1977	89	281/315	H. Kato
GFEC1-5-1	<i>E. coracana</i>	Japan	1977	97	319/330	H. Kato
MZ5-1-6	<i>E. coracana</i>	Japan	1976	89	294/330	
NP10-10-1-1-1	<i>E. coracana</i>	Nepal	1975	89	294/330	S. Sakamoto
NP10-17-4-1-3	<i>E. coracana</i>	Nepal	1975	97	319/330	Urashima et al. 1999
SZEC1-1-1	<i>E. coracana</i>	Japan	1978	97	319/330	H. Kato
SZECu1-1-1	<i>E. coracana</i>	Japan	1978	97	319/330	H. Kato
CH90-1-1	<i>E. coracana</i>	China	ND	89	293/330	M. Iwano
UG77-7-1-1	<i>E. indica</i>	Uganda	ND	97	319/330	J. Mukiibi
EI9064	<i>E. indica</i>	China	1996	97	319/330	Zhong et al. 2016
EI9411	<i>E. indica</i>	China	1990	96	318/330	Zhong et al. 2016
CD156	<i>E. indica</i>	Ivory Coast	1989	96	317/330	Chiapello et al. 2015
B51	<i>E. indica</i>	Bolivia	2012	97	319/330	Pieck et al. 2017
BR62	<i>E. indica</i>	Brazil	1991	97	319/330	Islam et al. 2016
NI915	<i>E. indica</i>	Japan	ND	0		N. Nishihara
NI986	<i>Eragrostis lehmanniana</i>	Japan	1975	97	319/330	N. Nishihara
G17	<i>Eragrostis curvula</i>	Japan	1976	99	328/330	Open Wheat Blast
FSECu1-1-1	<i>E. curvula</i>	Japan	ND	97	319/330	N. Hayashi
Pg1213-22	<i>Festuca arundinaceum</i>	USA	1999	96	316/330	Pieck et al. 2017
BTBa_B2	<i>Hordeum vulgare</i>	Bangladesh	2016	100	330/330	Open Wheat Blast
BTBa_B1	<i>Hordeum vulgare</i>	Bangladesh	2016	100	330/330	Open Wheat Blast
HYKB202-1-2	<i>Kyllinga brevifolia</i>	Japan	2003	0		I. Chuma

Supplementary Table S1. (Continued)

Isolate	Host	country	year	Identity	Hits	Collector/References
NI919	<i>Leersia oryzoides</i>	Japan	1974	0		N. Nishihara
GG11	<i>Lolium perenne</i>	USA	1997	96	316/330	Farman et al. 2017
LpKY97	<i>L. perenne</i>	USA	1997	96	316/330	Pieck et al. 2017
PgKY	<i>L. perenne</i>	USA	2000	96	316/330	Islam et al. 2016
PL2-1	<i>L. perenne</i>	USA	2002	96	316/330	Farman et al. 2017
PL3-1	<i>L. perenne</i>	USA	2002	96	316/330	Farman et al. 2017
CHRF	<i>L. perenne</i>	MD	1996	96	316/330	Pieck et al. 2017
CHW	<i>L. perenne</i>	MD	1996	96	316/330	Pieck et al. 2017
FH	<i>L. perenne</i>	MD	1997	96	316/330	Pieck et al. 2017
HO	<i>L. perenne</i>	USA	1996	96	316/330	Farman et al. 2017
PGPA	<i>L. perenne</i>	USA	1998	96	316/330	Pieck et al. 2017
TP1	<i>L. perenne</i>	Japan	1997	96	316/330	A. Tanaka, T. Tani
TP2	<i>L. perenne</i>	Japan	1997	96	312/330	A. Tanaka, T. Tani
Y34	<i>Oryza sativa</i>	China	1982	95	312/330	Farman et al. 2017
70-15_8	<i>O. sativa</i>	Lab. Cross	-	0		Pieck et al. 2017
FR13	<i>O. sativa</i>	France	1988	95	312/330	Chiapello et al. 2015
Guy11	<i>O. sativa</i>	Guiana	1988	0		J.L. Notteghem
Ina168	<i>O. sativa</i>	Japan	1958	95	312/330	Yoshida et al. 2016
KEN53-33	<i>O. sativa</i>	Japan	1953	95	312/330	Kiyosawa 1974
P131	<i>O. sativa</i>	Japan	ND	95	312/330	Xue et al. 2012
P-2	<i>O. sativa</i>	Japan	1948	88	291/330	Yoshida et al. 2016
ML33	<i>O. sativa</i>	Mali	1995	0		Gladieux et al. 2018
TH3	<i>O. sativa</i>	Thailand	ND	95	312/330	Gladieux et al. 2018
IA1	<i>O. sativa</i>	USA	2009	95	312/330	Pieck et al. 2017
IB33	<i>O. sativa</i>	USA	ND	95	312/330	Gladieux et al. 2018
IB49	<i>O. sativa</i>	USA	1992	95	312/330	Pieck et al. 2017
IC17	<i>O. sativa</i>	USA	1992	95	312/330	Pieck et al. 2017
IE1K	<i>O. sativa</i>	USA	2003	95	312/330	Pieck et al. 2017
87-120	<i>O. sativa</i>	-	ND	95	312/330	Gladieux et al. 2018
FR13	<i>O. sativa</i>	France	1988	94	310/330	Chiapello et al. 2015
PO12-7301-2	<i>O. sativa</i>	Indonesia	1973	0		A. Mukelar
TorideI-1-7	<i>O. sativa</i>	Japan	ND	95	312/330	
BTT _{rp} _5	<i>Panicum repens</i>	Bangladesh	2016	99	295/298	Open Wheat Blast
BTT _{rp} _6	<i>Panicum repens</i>	Bangladesh	2016	99	295/298	Open Wheat Blast
PM1	<i>Pennisetum americanum</i>	USA	1990	0		Gladieux et al. 2018
SSFL02-1	<i>Stenotaphrum secundatum</i>	Disney World, FL	2002	97	319/330	Pieck et al. 2017
SSFL14-3	<i>S. secundatum</i>	New Smyrna, FL	2014	97	319/330	Gladieux et al. 2018
GFSI1-7-2	<i>Setaria italica</i>	Japan	1977	95	312/330	Yoshida et al. 2016
US71	<i>Setaria viridis</i>	USA	ND	94	309/330	Chiapello et al. 2015
SV9610	<i>Setaria viridis</i>	China	1996	95	312/330	Zhong et al. 2016
SV9623	<i>S. viridis</i>	China	1996	95	312/330	Zhong et al. 2016
KANSV1-4-1	<i>S. viridis</i>	Japan	1975	95	312/330	Yoshida et al. 2016
SA05-43	<i>S. viridis</i>	Japan	2005	94	309/330	Yoshida et al. 2016
Arcadia	<i>S. viridis</i>	USA	1998	95	312/330	Farman 2002
GrF52	<i>S. viridis</i>	USA	2001	95	312/330	Gladieux et al. 2018
INA-B-93-19	<i>Sasa sp.</i>	Japan	1992	0		S. Koizumi

Supplementary Table S1. (Continued)

Isolate	Host	country	year	Identity	Hits	Collector/References
P3	<i>Triticum durum</i>	Paraguay	2012	97	319/330	Pieck et al. 2017
BdBar16-1	<i>T. eastivum</i>	Bangladesh	2016	100	330/330	Makaker et al. 2016
BdJes16-1	<i>T. eastivum</i>	Bangladesh	2016	100	330/330	Makaker et al. 2016
BdMeh16-1	<i>T. eastivum</i>	Bangladesh	2016	100	330/330	Makaker et al. 2016
BTGP_6f	<i>T. eastivum</i>	Bangladesh	2017	100	330/330	Open Wheat Blast
BTGP_6g	<i>T. eastivum</i>	Bangladesh	2017	100	330/330	Open Wheat Blast
BTGP_6h	<i>T. eastivum</i>	Bangladesh	2017	100	330/330	Open Wheat Blast
BTJP4_11	<i>T. eastivum</i>	Bangladesh	2016	100	330/330	Open Wheat Blast
BTJP4_12	<i>T. eastivum</i>	Bangladesh	2016	100	330/330	Open Wheat Blast
BTJP4_16	<i>T. eastivum</i>	Bangladesh	2016	100	330/330	Open Wheat Blast
BTJP4_18	<i>T. eastivum</i>	Bangladesh	2016	100	330/330	Open Wheat Blast
BTJP4_3	<i>T. eastivum</i>	Bangladesh	2016	100	330/330	Open Wheat Blast
BTJP4_5	<i>T. eastivum</i>	Bangladesh	2016	100	330/330	Open Wheat Blast
BTJP4_6	<i>T. eastivum</i>	Bangladesh	2016	100	330/330	Open Wheat Blast
B2	<i>T. eastivum</i>	Bolivia	2011	97	319/330	Pieck et al. 2017
B71	<i>T. eastivum</i>	Bolivia	2012	100	330/330	Makaker et al. 2016
Br130	<i>T. eastivum</i>	Brazil	1990	97	319/330	Farman et al. 2017
BR32	<i>T. eastivum</i>	Brazil	1991	96	319/330	Chiapello et al. 2015
Br48	<i>T. eastivum</i>	Brazil	1990	100	330/330	Urashima et al. 1999
Br7	<i>T. eastivum</i>	Brazil	1990	97	319/330	Pieck et al. 2017
Br80	<i>T. eastivum</i>	Brazil	1991	97	319/330	Pieck et al. 2017
PY0925	<i>T. eastivum</i>	Brazil	2009	100	330/330	Open Wheat Blast
PY36.1_v2	<i>T. eastivum</i>	Brazil	2007	97	319/330	Open Wheat Blast
PY5003_v2	<i>T. eastivum</i>	Brazil	2005	96	318/330	Open Wheat Blast
PY5010	<i>T. eastivum</i>	Brazil	2005	96	316/330	Open Wheat Blast
PY5033_v2	<i>T. eastivum</i>	Brazil	2005	97	319/330	Open Wheat Blast
PY6017_v2	<i>T. eastivum</i>	Brazil	2006	97	319/330	Open Wheat Blast
PY6045	<i>T. eastivum</i>	Brazil	2006	96	318/330	Open Wheat Blast
PY86.1_v2	<i>T. eastivum</i>	Brazil	2008	96	316/330	Open Wheat Blast
T25	<i>T. eastivum</i>	Brazil	1988	100	330/330	Pieck et al. 2017
WHTQ	<i>T. eastivum</i>	Brazil	ND	97	319/330	Farman et al. 2017
WBKY11-15	<i>T. eastivum</i>	USA	2011	96	316/330	Farman et al. 2017
Br116.5	<i>T. eastivum</i>	Brazil	1992	96	318/330	Urashima et al. 1999
Br118.2	<i>T. eastivum</i>	Brazil	1992	97	319/330	Urashima et al. 1999
BTGP_1b	<i>T. eastivum</i>	Bangladesh	2016	100	330/330	Open Wheat Blast
BTGP_6e	<i>T. eastivum</i>	Bangladesh	2017	100	330/330	Open Wheat Blast
BTGP1b	<i>T. eastivum</i>	Bangladesh	2017	99	327/330	Open Wheat Blast
BTGP6F	<i>T. eastivum</i>	Bangladesh	2017	99	326/330	Open Wheat Blast
BTJP_4_1	<i>T. eastivum</i>	Bangladesh	2016	100	330/330	Open Wheat Blast
BTJP4_1	<i>T. eastivum</i>	Bangladesh	2016	99	327/330	Open Wheat Blast
BTMP_S_13_1	<i>T. eastivum</i>	Bangladesh	2016	100	330/330	Open Wheat Blast
BTMP_S_13_2	<i>T. eastivum</i>	Bangladesh	2016	100	330/330	Open Wheat Blast
BTMP13_1	<i>T. eastivum</i>	Bangladesh	2016	98	325/330	Open Wheat Blast
PY6025	<i>T. eastivum</i>	Brazil	2006	99	213/215	Open Wheat Blast
IBZL3-1-1	<i>Zizania latifolia</i>	Japan	1985	0		N. Hayashi
HCP2				100	330/330	

Supplementary Table S2. Alignment of amino acid sequences of AVR-Rmg8 in different pathotypes of *P. oryzae*

Isolate	Host	Protein Alignment																													
Br48 ORF	<i>T. aestivum</i>	M	H	R	I	G	F	F	P	I	L	I	A	G	A	M	A	L	P	A	P	Q	P	M	P	P	S	R	P	G	[30]
Br116.5 ORF	<i>T. aestivum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
Br5 ORF	<i>T. aestivum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
B71 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTGP 1b ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTGP 6e ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTGP 6f ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTGP 6g ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTGP 6h ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTJP4 11 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTJP4 12 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTJP4 16 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTJP4 18 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTJP4 3 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTJP4 6 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTJP 4 1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTMP S 13 1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTMP S 13 2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTJP4 5 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BdBar16-1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BdJes16-1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BdMeh16-1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
PY0925 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
T25 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
PY5010 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	L	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
PY86.1 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	L	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
WBKY11-15 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	L	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
B2 ORF	<i>T. aestivum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
PY36.1 v2 ORF	<i>T. aestivum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
PY5003 v2 ORF	<i>T. aestivum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
PY5033 v2 ORF	<i>T. aestivum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
PY6017 v2 ORF	<i>T. aestivum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
PY6045 ORF	<i>T. aestivum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
Br118.2 ORF	<i>T. aestivum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
Br130 ORF	<i>T. aestivum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
Br7 ORF	<i>T. aestivum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
Br80 ORF	<i>T. aestivum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
WHTQ ORF	<i>T. aestivum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
P3 ORF	<i>T. durum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
BTBa B1 ORF	<i>H. vulgare</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
BTBa B2 ORF	<i>H. vulgare</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
Ast16 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
Ast1 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
Ast52 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
Br58 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
Bd8401 ORF	<i>B. distachya</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
G17 ORF	<i>E. curvula</i>	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[30]
Br35 ORF	<i>B. plantaginea</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
SSFL02-1 ORF	<i>S. secundatum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]
SSFL14-3 ORF	<i>S. secundatum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	S	.	[30]

Supplementary Table S2. (Continued)

Isolate	Host	Protein Alignment																				
CHRF ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
CHW ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
FH ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
GG11 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
HO ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
LpKY97 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
PGPA ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
PL2-1 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
PL3-1 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
PgKY ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
TP1 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
TP2 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
87-120 ORF	<i>O. sativa</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
FR13 ORF	<i>O. sativa</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
IA1 ORF	<i>O. sativa</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
IB33 ORF	<i>O. sativa</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
IB49 ORF	<i>O. sativa</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
IC17 ORF	<i>O. sativa</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
IE1K ORF	<i>O. sativa</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
Ina168 ORF	<i>O. sativa</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
KEN53-33 ORF	<i>O. sativa</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
P131 ORF	<i>O. sativa</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
TH3 ORF	<i>O. sativa</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
TorideI-1-7 ORF	<i>O. sativa</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
Y34 ORF	<i>O. sativa</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
Arcadia ORF	<i>S. viridis</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
GFS11-7-2 ORF	<i>S. italica</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
GrF52 ORF	<i>S. viridis</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
KANSV1-4-1 ORF	<i>S. viridis</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
SV9610 ORF	<i>S. italica</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
SV9623 ORF	<i>S. viridis</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
Z2-1 contig 1585	<i>E. coracana</i>	.	.	C	.	.	.	.	.	F	F	.	.	.	.	.	.	.	.	I	.	[30]
IN77-31-1-1 ORF	<i>E. coracana</i>	.	.	C	.	.	.	.	.	F	F	.	.	.	.	.	.	.	.	I	.	[30]
IN77-47-1-2 ORF	<i>E. coracana</i>	-	-	-	-	.	.	.	.	F	F	.	.	.	.	.	.	.	.	I	.	[30]
MZ5-1-6 ORF	<i>E. coracana</i>	.	.	C	.	.	.	.	.	F	F	.	.	.	.	.	.	.	.	I	.	[30]
NI1005 ORF	<i>E. africana</i>	.	.	C	.	.	.	.	.	F	F	.	.	.	.	.	.	.	.	I	.	[30]
NP10-10-1-1-1 ORF	<i>E. coracana</i>	.	.	C	.	.	.	.	.	F	F	.	.	.	.	.	.	.	.	I	.	[30]
G22 ORF	<i>E. coracana</i>	.	.	C	.	.	.	.	.	F	F	.	.	.	.	.	.	.	.	I	.	[30]
EI9411 ORF	<i>E. indica</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
IN77-13-1-1 ORF	<i>E. coracana</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
IN77-24-1-1 ORF	<i>E. coracana</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
NP10-17-4-1-3 ORF	<i>E. coracana</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
NI986 ORF	<i>E. lehmannia</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
B51 ORF	<i>E. indica</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
BR62 ORF	<i>E. indica</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
EI9064 ORF	<i>E. indica</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
GFEC1-5-1 ORF	<i>E. coracana</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
PH42 ORF	<i>E. coracana</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
SZEC1-1-1 ORF	<i>E. coracana</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
SZECu1-1-1 ORF	<i>E. coracana</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
UG77-15-1-1 ORF	<i>E. indica</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
UG77-7-1-1 ORF	<i>E. indica</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
P28 ORF	<i>B. tectorum</i>	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]
P29 ORF	<i>B. tectorum</i>	.	.	C	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	[30]
Pg1213-22 ORF	<i>F. arundinaceum</i>	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	V	[30]

Supplementary Table S2. (Continued)

Isolate	Host	Protein Alignment																														
Br48 ORF	<i>T. aestivum</i>	Q	G	G	R	G	G	N	G	R	G	P	G	G	P	P	P	Q	Q	Y	E	E	P	V	P	Y	H	Q	T	A	[60]	
Br116.5 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
Br5 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
B71 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTGP 1b ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTGP 6e ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTGP 6f ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTGP 6g ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTGP 6h ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTJP4 11 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTJP4 12 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTJP4 16 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTJP4 18 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTJP4 3 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTJP4 6 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTJP 4 1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTMP S 13 1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTMP S 13 2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTJP4 5 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BdBar16-1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BdJes16-1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BdMeh16-1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
PY0925 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
T25 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
PY5010 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
PY86.1 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
WBKY11-15 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
B2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
PY36.1 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
PY5003 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
PY5033 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
PY6017 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
PY6045 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
Br118.2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
Br130 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
Br7 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
Br80 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
WHTQ ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
P3 ORF	<i>T. durum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTBa B1 ORF	<i>H. vulgare</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
BTBa B2 ORF	<i>H. vulgare</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
Ast16 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
Ast1 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
Ast52 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
Br58 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
Bd8401 ORF	<i>B. distachya</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
G17 ORF	<i>E. curvula</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
Br35 ORF	<i>B. plantaginea</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
SSFL02-1 ORF	<i>S. secundatum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]
SSFL14-3 ORF	<i>S. secundatum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[60]

Supplementary Table S2. (Continued)

Isolate	Host	Alignment										
CHRF ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	[60]
CHW ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	[60]
FH ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	[60]
GG11 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	[60]
HO ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	[60]
LpKY97 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	[60]
PGPA ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	[60]
PL2-1 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	[60]
PL3-1 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	[60]
PgKY ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	[60]
TP1 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	[60]
TP2 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	[60]
87-120 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	.	.	.	[60]
FR13 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	.	.	.	[60]
IA1 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	.	.	.	[60]
IB33 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	.	.	.	[60]
IB49 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	.	.	.	[60]
IC17 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	.	.	.	[60]
IE1K ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	.	.	.	[60]
Ina168 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	.	.	.	[60]
KEN53-33 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	.	.	.	[60]
P131 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	.	.	.	[60]
TH3 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	.	.	.	[60]
TorideI-1-7 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	.	.	.	[60]
Y34 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	.	.	.	[60]
Arcadia ORF	<i>S. viridis</i>	.	.	.	.	.	.	.	.	.	.	[60]
GFS11-7-2 ORF	<i>S. italica</i>	.	.	.	.	.	.	.	.	.	.	[60]
GrF52 ORF	<i>S. viridis</i>	.	.	.	.	.	.	.	.	.	.	[60]
KANSVI-4-1 ORF	<i>S. viridis</i>	.	.	.	.	.	.	.	.	.	.	[60]
SV9610 ORF	<i>S. italica</i>	.	.	.	.	.	.	.	.	.	.	[60]
SV9623 ORF	<i>S. viridis</i>	.	.	.	.	.	.	.	.	.	.	[60]
ZZ-1 contig 1585	<i>E.corocana</i>	*	.	.	.	.	.	K	.	.	.	[60]
IN77-31-1-1 ORF	<i>E. coracana</i>	*	.	.	.	.	.	K	.	.	.	[60]
IN77-47-1-2 ORF	<i>E. coracana</i>	*	.	.	.	.	.	K	.	.	.	[60]
MZ5-1-6 ORF	<i>E. coracana</i>	*	.	.	.	.	.	K	.	.	.	[60]
NI1005 ORF	<i>E. africana</i>	*	.	.	.	.	.	K	.	.	.	[60]
NP10-10-1-1-1 ORF	<i>E. coracana</i>	*	.	.	.	.	.	K	.	.	.	[60]
G22 ORF	<i>E. coracana</i>	*	.	.	.	.	.	K	.	.	.	[60]
EI9411 ORF	<i>E. indica</i>	.	*	.	.	.	.	.	.	.	.	[60]
IN77-13-1-1 ORF	<i>E. coracana</i>	.	*	.	.	.	.	.	.	.	.	[60]
IN77-24-1-1 ORF	<i>E. coracana</i>	.	*	.	.	.	.	.	.	.	.	[60]
NP10-17-4-1-3 ORF	<i>E. coracana</i>	.	.	.	.	.	.	.	.	.	.	[60]
NI986 ORF	<i>E. lehmannia</i>	.	.	.	.	.	.	.	.	.	.	[60]
B51 ORF	<i>E. indica</i>	.	.	.	.	.	.	.	.	.	.	[60]
BR62 ORF	<i>E. indica</i>	.	.	.	.	.	.	.	.	.	.	[60]
EI9064 ORF	<i>E. indica</i>	.	.	.	.	.	.	.	.	.	.	[60]
GFECl-5-1 ORF	<i>E. coracana</i>	.	.	.	.	.	.	.	.	.	.	[60]
PH42 ORF	<i>E. coracana</i>	.	.	.	.	.	.	.	.	.	.	[60]
SZEC1-1-1 ORF	<i>E. coracana</i>	.	.	.	.	.	.	.	.	.	.	[60]
SZECu1-1-1 ORF	<i>E. coracana</i>	.	.	.	.	.	.	.	.	.	.	[60]
UG77-15-1-1 ORF	<i>E. indica</i>	.	.	.	.	.	.	.	.	.	.	[60]
UG77-7-1-1 ORF	<i>E. indica</i>	.	.	.	.	.	.	.	.	.	.	[60]
P28 ORF	<i>B. tectorum</i>	.	.	.	.	.	.	.	.	.	.	[60]
P29 ORF	<i>B. tectorum</i>	.	.	.	.	.	.	.	.	.	.	[60]
Pg1213-22 ORF	<i>F. arundinaceum</i>	.	.	.	.	.	.	.	.	.	.	[60]

Supplementary Table S2. (Continued)

Isolate	Host	Protein Alignment																														
Br48 ORF	<i>T. aestivum</i>	A	A	A	W	Q	P	Y	P	G	H	V	P	G	G	Q	R	P	T	E	H	S	E	L	I	P	D	D	Y	P	Q	[90]
Br116.5 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
Br5 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
B71 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTGP 1b ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTGP 6e ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTGP 6f ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTGP 6g ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTGP 6h ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTJP4 11 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTJP4 12 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTJP4 16 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTJP4 18 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTJP4 3 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTJP4 6 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTJP 4 1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTMP S 13 1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTMP S 13 2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTJP4 5 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BdBar16-1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BdJes16-1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BdMeh16-1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
PY0925 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
T25 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
PY5010 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
PY86.1 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
WBKY11-15 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
B2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
PY36.1 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
PY5003 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
PY5033 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
PY6017 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
PY6045 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
Br118.2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
Br130 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
Br7 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
Br80 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
WHTQ ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
P3 ORF	<i>T. durum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTBa B1 ORF	<i>H. vulgare</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
BTBa B2 ORF	<i>H. vulgare</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
Ast16 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
Ast1 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
Ast52 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
Br58 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
Bd8401 ORF	<i>B. distachya</i>	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
G17 ORF	<i>E. curvula</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
Br35 ORF	<i>B. plantaginea</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
SSFL02-1 ORF	<i>S. secundatum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]
SSFL14-3 ORF	<i>S. secundatum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]

Supplementary Table S2. (Continued)

Isolate	Host	Alignment																				
CHRF ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
CHW ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
FH ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
GG11 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
HO ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
LpKY97 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
PGPA ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
PL2-1 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
PL3-1 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
PgKY ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
TP1 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
TP2 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
87-120 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	Q	.	.	[90]	
FR13 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	Q	.	.	[90]	
IA1 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	Q	.	.	[90]	
IB33 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	Q	.	.	[90]	
IB49 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	Q	.	.	[90]	
IC17 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	Q	.	.	[90]	
IE1K ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	Q	.	.	[90]	
Ina168 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	Q	.	.	[90]	
KEN53-33 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	Q	.	.	[90]	
P131 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	Q	.	.	[90]	
TH3 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	Q	.	.	[90]	
TorideI-1-7 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	Q	.	.	[90]	
Y34 ORF	<i>O. sativa</i>	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	Q	.	.	[90]	
Arcadia ORF	<i>S. viridis</i>	.	.	.	.	.	.	.	Y	.	.	K	.	.	.	.	.	Q	.	.	[90]	
GFS11-7-2 ORF	<i>S. italica</i>	.	.	.	.	.	.	.	Y	.	.	K	.	.	.	.	.	Q	.	.	[90]	
GrF52 ORF	<i>S. viridis</i>	.	.	.	.	.	.	.	Y	.	.	K	.	.	.	.	.	Q	.	.	[90]	
KANSV1-4-1 ORF	<i>S. viridis</i>	.	.	.	.	.	.	.	Y	.	.	K	.	.	.	.	.	Q	.	.	[90]	
SV9610 ORF	<i>S. italica</i>	.	.	.	.	.	.	.	Y	.	.	K	.	.	.	.	.	Q	.	.	[90]	
SV9623 ORF	<i>S. viridis</i>	.	.	.	.	.	.	.	Y	.	.	K	.	.	.	.	.	Q	.	.	[90]	
ZZ-1 contig 1585	<i>E. coracana</i>	.	.	V	.	.	.	.	.	.	*	.	K	.	.	F	.	N	N	.	[90]	
IN77-31-1-1 ORF	<i>E. coracana</i>	.	.	V	.	.	.	.	.	.	*	.	K	.	.	F	.	N	N	.	[90]	
IN77-47-1-2 ORF	<i>E. coracana</i>	.	.	V	.	.	.	.	.	.	*	.	N	K	.	F	.	N	N	.	[90]	
MZ5-1-6 ORF	<i>E. coracana</i>	.	.	V	.	.	.	.	.	.	*	.	K	.	.	F	.	N	N	.	[90]	
NI1005 ORF	<i>E. africana</i>	.	.	V	.	.	.	.	.	.	*	.	K	.	.	F	.	N	N	.	[90]	
NP10-10-1-1-1 ORF	<i>E. coracana</i>	.	.	V	.	.	.	.	.	.	*	.	K	.	.	F	.	N	N	.	[90]	
G22 ORF	<i>E. coracana</i>	.	.	V	.	.	.	.	.	.	*	.	K	.	.	F	.	N	N	.	[90]	
EI9411 ORF	<i>E. indica</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
IN77-13-1-1 ORF	<i>E. coracana</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
IN77-24-1-1 ORF	<i>E. coracana</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
NP10-17-4-1-3 ORF	<i>E. coracana</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
NI986 ORF	<i>E. lehmannia</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
B51 ORF	<i>E. indica</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
BR62 ORF	<i>E. indica</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
EI9064 ORF	<i>E. indica</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
GFEC1-5-1 ORF	<i>E. coracana</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
PH42 ORF	<i>E. coracana</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
SZEC1-1-1 ORF	<i>E. coracana</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
SZECu1-1-1 ORF	<i>E. coracana</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
UG77-15-1-1 ORF	<i>E. indica</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
UG77-7-1-1 ORF	<i>E. indica</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
P28 ORF	<i>B. tectorum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
P29 ORF	<i>B. tectorum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	
Pg1213-22 ORF	<i>F. arundinaceum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[90]	

Supplementary Table S2. (Continued)

Isolate	Host	Protein Alignment																				
Br48 ORF	<i>T. aestivum</i>	F	V	K	D	Y	D	T	Y	F	F	G	G	L	P	G	T	R	R	Q	*	[110]
Br116.5 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	.	.	.	[110]
Br5 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
B71 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTGP 1b ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTGP 6e ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTGP 6f ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTGP 6g ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTGP 6h ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTJP4 11 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTJP4 12 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTJP4 16 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTJP4 18 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTJP4 3 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTJP4 6 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTJP 4 1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTMP S 13 1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTMP S 13 2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTJP4 5 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BdBar16-1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BdJes16-1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BdMeh16-1 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
PY0925 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
T25 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
PY5010 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
PY86.1 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
WBKY11-15 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
B2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
PY36.1 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
PY5003 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	.	.	.	.	.	[110]
PY5033 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
PY6017 v2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
PY6045 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	K	.	.	.	[110]
Br118.2 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
Br130 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
Br7 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
Br80 ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
WHTQ ORF	<i>T. aestivum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
P3 ORF	<i>T. durum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTBa B1 ORF	<i>H. vulgare</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
BTBa B2 ORF	<i>H. vulgare</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
Ast16 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
Ast1 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
Ast52 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
Br58 ORF	<i>A. sativa</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
Bd8401 ORF	<i>B. distachya</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
G17 ORF	<i>E. curvula</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
Br35 ORF	<i>B. plantaginea</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
SSFL02-1 ORF	<i>S. secundatum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]
SSFL14-3 ORF	<i>S. secundatum</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]

Isolate	Host	Alignment																		
CHRF ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]	
CHW ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]	
FH ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]	
GG11 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]	
HO ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]	
LpKY97 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]	
PGPA ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]	
PL2-1 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]	
PL3-1 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]	
PgKY ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]	
TP1 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]	
TP2 ORF	<i>L. perenne</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	[110]	
87-120 ORF	<i>O. sativa</i>	.	.	.	H.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
FR13 ORF	<i>O. sativa</i>	.	.	.	H.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
IA1 ORF	<i>O. sativa</i>	.	.	.	H.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
IB33 ORF	<i>O. sativa</i>	.	.	.	H.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
IB49 ORF	<i>O. sativa</i>	.	.	.	H.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
IC17 ORF	<i>O. sativa</i>	.	.	.	H.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
IE1K ORF	<i>O. sativa</i>	.	.	.	H.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
Ina168 ORF	<i>O. sativa</i>	.	.	.	H.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
KEN53-33 ORF	<i>O. sativa</i>	.	.	.	H.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
P131 ORF	<i>O. sativa</i>	.	.	.	H.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
TH3 ORF	<i>O. sativa</i>	.	.	.	H.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
Toride1-7 ORF	<i>O. sativa</i>	.	.	.	H.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
Y34 ORF	<i>O. sativa</i>	.	.	.	H.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
Arcadia ORF	<i>S. viridis</i>	.	.	.	.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
GFSI1-7-2 ORF	<i>S. italica</i>	.	.	.	.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
Gf52 ORF	<i>S. viridis</i>	.	.	.	.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
KANSV1-4-1 ORF	<i>S. viridis</i>	.	.	.	.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
SV9610 ORF	<i>S. italica</i>	.	.	.	.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
SV9623 ORF	<i>S. viridis</i>	.	.	.	.	A	T	I.	.	.	.	.	.	.	.	.	.	.	[110]	
Z2-1 contig 1585	<i>E.coracana</i>	.	.	.	N.	.	.	.	.	.	.	.	.	.	.	K.	.	.	[110]	
IN77-31-1-1 ORF	<i>E. coracana</i>	.	.	.	N.	.	.	.	.	.	.	.	.	.	.	K.	.	.	[110]	
IN77-47-1-2 ORF	<i>E. coracana</i>	.	.	.	N.	.	.	.	.	.	.	.	.	.	.	K.	.	.	[110]	
MZ5-1-6 ORF	<i>E. coracana</i>	.	.	.	N.	.	.	.	.	.	.	.	.	.	.	K.	.	.	[110]	
NI1005 ORF	<i>E. africana</i>	.	.	.	N.	.	.	.	.	.	.	.	.	.	.	K.	.	.	[110]	
NP10-10-1-1-1 ORF	<i>E. coracana</i>	.	.	.	N.	.	.	.	.	.	.	.	.	.	.	K.	.	.	[110]	
G22 ORF	<i>E. coracana</i>	.	.	.	N.															

LITERATURE CITED

- Agrios, G. N. 2005. Plant pathology: Fifth edition. Elsevier Inc., 922pp
- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J. Z., Miller, W. and Lipman, D. J. 1997. Gapped blast and psi-blast: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402.
- Anh, V. L. 2016. Identification of a resistance gene against the wheat blast fungus in common wheat and cloning of its corresponding avirulence gene. Ph.D thesis, Kobe University.
- Anh, V. L., Anh, N. T., Tagle, A. G., Vy, T. T. P., Inoue, Y., Takumi, S., et al. 2015. *Rmg8*, a new gene for resistance to *Triticum* isolates of *Pyricularia oryzae* in hexaploid wheat. Phytopathology 105: 1568-1572.
- Anh, V. L., Inoue, Y., Asuke, S., Vy, T. T. P., Anh, N. T., Wang, S., et al. 2018. *Rmg8* and *Rmg7*, wheat genes for resistance to the wheat blast fungus, recognize the same avirulence gene AVR-*Rmg8*. Molecular Plant Pathology 19: 1252–1256.
- Asuke, S., Tanaka, M., Hyon, G., Inoue, Y., Thi, T., Vy, P., et al. 2020. Evolution of an *Eleusine* -specific subgroup of *Pyricularia oryzae* through a gain of an avirulence gene. Molecular Plant-Microbe Interactions 33: 153-165.
- Callaway E. 2016. Devastating wheat fungus appears in Asia for first time. Nature 532: 421–422.
- Castroagudín, V. L., Moreira, S. I. S., Pereira, D. A. S., Moreira, S. I. S., Brunner, P. C., Maciel, J. L. N., et al. 2016. *Pyricularia graminis-tritici*, a new *Pyricularia* species causing wheat blast. Persoonia: Molecular Phylogeny and Evolution of Fungi 37: 199–216.

- Castroagudín, V. L., Danelli, A. L. D., Moreira, S. I., Reges, J. T. A., McDonald, G. C., Maciel, J. L. N., et al. 2017. The wheat blast pathogen *Pyricularia graminis-tritici* has complex origins and a disease cycle spanning multiple grass hosts. *BioRxiv* preprint.
- Ceresini, P. C., Castroagudín, V. L., Rodrigues, F. Á., Rios, J. A., Eduardo A. C., Moreira, S. I., et al. 2018. Wheat blast: past, present, and future. *Annu. Rev. Phytopathol.* 56: 427–456.
- Chiapello, H., Mallet, L., Guérin, C., Aguilera, G., Amselem, J., Kroj, T. 2015. Deciphering genome content and evolutionary relationships of isolates from the fungus *Magnaporthe oryzae* attacking different host plants. *Genome Biology and Evolution* 7: 2896–2912.
- Chuma, I., Isobe, C., Hotta, Y., Ibaragi, K., Futamata, N., Kusaba, M., et al. 2011. Multiple translocation of the *AVR-Pita* effector gene among chromosomes of the rice blast fungus *Magnaporthe oryzae* and related species. *PLoS Pathogens* 7: e1002147.
- CIMMYT 2010. First international wheat blast meeting held in Brazil, 2pp. Retrieved from <https://www.cimmyt.org/news/first-international-wheat-blast-meeting-held-in-brazil>, (Accessed 21 April 2020).
- CIMMYT 2016. Understanding and managing the threat of wheat blast in South Asia, South America, and beyond, 4. Retrieved from <https://repository.cimmyt.org/bitstream/handle/10883/16947/57941.pdf?sequence=1&isAllowed=y>, (Accessed 11 May 2020).
- Cribb J. 2010. The coming famine: The global food crisis and what we can do to avoid it. Berkeley: University of California Press 248pp.
- Cruppe, G., Cruz, C. D., Peterson, G., Pedley, K., Asif, M., Fritz, A., et al. 2020. Novel sources of wheat head blast resistance in modern breeding lines and wheat wild relatives. *Plant Disease* 104: 35-43.

- Cruz, C. D., Peterson, G. L., Bockus, W. W., Kankanala, P., Dubcovsky, J., Jordan, K. W., et al. 2016. The 2NS translocation from *Aegilops ventricosa* confers resistance to the *Triticum* pathotype of *Magnaporthe oryzae*. *Crop Science* 56: 990-1000.
- Cruz, C. D., and Valent, B. 2017. Wheat blast disease: danger on the move. *Tropical Plant Pathology* 42: 210–222.
- De Silva, N. I., Lumyong, S., Hyde, K. D., Bulgakov, T., Phillips, A. J. L., Yan J. Y. 2016. Mycosphere Essay 9: Defining biotrophs and hemibiotrophs. *Mycosphere* 7: 545-559.
- Dickinson, M. 2003. Molecular plant pathology. BIOS Scientific Publisher, Taylor & Francis Group, London and New York, 273pp.
- Duba, A., Goriewa-Duba, K., and Wachowska, U. 2018. A review of the interactions between wheat and wheat pathogens: *Zymoseptoria tritici*, *Fusarium* spp., and *Parastagonospora nodorum*. *International Journal of Molecular Sciences* 19: 1138.
- Dufresne, M., and Daboussi, M. J. 2010. Development of impala-based transposon systems for gene tagging in filamentous fungi. In A. Sharon (Ed.), Springer, Dordrecht, New York: Humana Press 41–54 pp.
- FAO 2009. 2050: A third more mouths to feed. 5pp. Retrieved from <http://www.fao.org/news/story/en/item/35571/icode/>, (Accessed 14 January 2020).
- Farman, M., Peterson, G., Chen, L., Starnes, J., Valent, B., Bachi, P., et al. 2017. The *Lolium* pathotype of *Magnaporthe oryzae* recovered from a single blasted wheat plant in the United States. *Plant Disease* 101: 684–692.
- Fisher, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L., et al. 2012. Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484:186–194.
- Flor, H. H. 1971. Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* 275–296.

- Gladieux, P., Condon, B., Ravel, S., Soanes, D., Maciel, J. L. N., Nhani, A., et al. 2018. Gene flow between divergent cereal and grass-specific lineages of the rice blast fungus *Magnaporthe oryzae*. *MBio* 9:1–19.
- Goerner-Potvin, P., and Bourque, G. 2018. Computational tools to unmask transposable elements. *Nature Reviews Genetics* 19: 688–704.
- Inoue, Y., Vy, T. T. P., Yoshida, K., Asano, H., Mitsuoka, C., Asuke, S., et al. 2017. Evolution of the wheat blast fungus through functional losses in a host specificity determinant. *Science* 357: 80–83.
- Islam, M. T., Croll, D., Gladieux, P., Soanes, D. M., Persoons, A., Bhattacharjee, P., et al. 2016. Emergence of wheat blast in Bangladesh was caused by a South American lineage of *Magnaporthe oryzae*. *BMC Biology* 14: 1–11.
- Islam, M. T., Kim, K. H., and Choi, J. 2019. Wheat blast in Bangladesh: The current situation and future impacts. *Plant Pathology Journal* 35: 1–10.
- Jensen, C., Tosa, Y., Islam, T., Talbot, N. J., Kamoun, S., and Saunders, D. G. O. 2019. *Rmg8* confers resistance to the Bangladeshi lineage of the wheat blast fungus. *Zenodo*, 4–5
- Jiang, Y., · Asuke, S., · Vy, T. T. P., Inoue, Y., and · Tosa, Y. 2020. Evaluation of durability of blast resistance gene *Rmg8* in common wheat based on analyses of its corresponding avirulence gene. *Journal of General Plant Pathology* (In press).
- Hyon, G.-S., Nga, N. T. T., Chuma, I., Inoue, Y., Asano, H., Murata, N., et al. 2012. Characterization of interactions between barley and various host-specific subgroups of *Magnaporthe oryzae* and *M. grisea*. *Journal of General Plant Pathology* 78: 237–246.
- Kamoun, S., Talbot, N. J., and Tofazzal Islam, M. 2019. Plant health emergencies demand open science: Tackling a cereal killer on the run. *PLoS Biology* 17: 1–6.
- Kato, H., Yamamoto, M., Yamaguchi-Ozaki, T., Kadouchi, H., Iwamoto, Y., Nakayashiki, H., et al. 2000. Pathogenicity, Mating ability and DNA restriction fragment length

- polymorphisms of *Pyricularia* populations isolated from Gramineae, Bambusideae and Zingiberaceae Plants. J. Gen. Plant Pathol. 66: 30–47.
- Kemen, E. and Jones, J. D. G. 2012. Obligate biotroph parasitism: can we link genomes to lifestyles? Trends in Plant Science 17: 448-457.
- Kennelly, M., O'Mara, J., Rivard, C., Miller, G. L., and Smith, D. 2012. Introduction to abiotic disorders in plants. The Plant Health Instructor 42: 142–145.
- Kim, Y.-J., Lee, J., and Han, K. 2012. Transposable Elements: No More 'Junk DNA.' Genomics and Informatics 10: 226 – 233.
- Koeck, M., Hardham, A.R., and Dodds, P.N. 2011. The role of effectors of biotrophic and hemibiotrophic fungi in infection. Cell Microbiology 13: 1849-1857.
- Kohli, M. M., Mehta, Y. R., Guzman, E., de Viedma, L., Cubilla, L. E., Islam, M. T., et al. 2011. *Pyricularia* blast – a threat to wheat cultivation. Czech Journal of Genetics and Plant Breeding 47: 130–134.
- Koizumi S., Ashizawa T., Zenbayashi K.S. 2004. Durable control of rice blast disease with multilines. In: Kawasaki S. (eds) Rice Blast: Interaction with rice and control. Springer, Dordrecht 191 – 199.
- Kumar, I. S., Amran, N. A., and Nadarajah, K. 2018. In silico analysis of qBFR4 and qLBL5 in conferring quantitative resistance against rice blast. Journal of Pure and Applied Microbiology 12: 1703–1718.
- Kumar, S., Stecher, G., and Tamura, K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35: 1547–1549.

- Maciel, J. L. N., Ceresini, P. C., Castroagudin, V. L., Zala, M., Kema, G. H. J., and McDonald, B. A. 2014. Population structure and pathotype diversity of the wheat blast pathogen *Magnaporthe oryzae* 25 years after its emergence in Brazil. *Phytopathology* 104: 95–107.
- Malaker P. K., Barma N. C. D., Tiwari T. P., Collis W. J., Duveiller E., Singh P. K., et al. 2016. First report of wheat blast caused by *Magnaporthe oryzae* pathotype *Triticum* in Bangladesh. *Plant Dis* 100:2330.
- Murakami, J., Tosa, Y., Kataoka, T., Tomita, R., Kawasaki, J., Chuma, I., et al. 2000. Analysis of host species specificity of *Magnaporthe grisea* toward wheat using a genetic cross between isolates from wheat and foxtail millet. *Phytopathology* 90: 1060-1067.
- Nakayashiki, H., Kiyotomi, K., Tosa, Y., and Mayama, S. 1999. Transposition of the retrotransposon MAGGY in heterologous species of filamentous fungi. *Genetics* 153: 693–703.
- Namiki, F., Matsunaga, M., Okuda, M., Inoue, I., Nishi, K., Fujita, Y., et al. 2001. Mutation of an arginine biosynthesis gene causes reduced pathogenicity in *Fusarium oxysporum* f. sp. *melonis*. *Mol. Plant-Microbe Interact.* 14:580-584.
- Nei, M., and Gojoborit, T. 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Molecular Biology and Evolution* 3: 418–426.
- Nelson, R., Wiesner-Hanks, T., Wisser, R., and Balint-Kurti, P. 2018. Navigating complexity to breed disease-resistant crops. *Nature Reviews Genetics* 19: 21–33.
- Nga, N. T., Hau, V. T., and Tosa, Y. 2009. Identification of genes for resistance to a *Digitaria* isolate of *Magnaporthe grisea* in common wheat cultivars. *Genome* 52: 801-809.

- O’Leary, M. 2020. Blast and rust forecast: New project to deliver wheat disease warnings directly to farmers’ phones in Bangladesh and Nepal . CIMMYT. Retrieved from <https://www.cimmyt.org/news/blast-and-rust-forecast/>, (Accessed 7 June 2020).
- Pennisi, E. 2010. Armed and dangerous. *Science* 327: 804–805.
- Pieck, M. L., Ruck, A., Farman, M. L., Peterson, G. L., Stack, J. P., Valent, B., et al. 2017. Genomics-based marker discovery and diagnostic assay development for wheat blast. *Plant Disease* 101: 103–109.
- Pray, L. A. 2008. Transposons: The jumping genes. *Nature Education*, 1: 204. Retrieved from <http://www.nature.com/scitable/topicpage/transposons-the-jumping-genes-518>, (Accessed 13 May 2020).
- Randhawa, M. S., Bhavani, S., Singh, P. K., Huerta-Espino, J., and Singh, R. P. 2019. Disease resistance in wheat: present status and future prospects. In *Disease Resistance in Crop Plants*. Cham: Springer International Publishing 61–81.
- Seidl, M. F., and Thomma, B. P. H. J. 2017. Transposable elements direct the coevolution between plants and microbes. *Trends in Genetics* 33: 842–851.
- Singh, R. P., Singh, P. K., Rutkoski, J., Hodson, D. P., He, X., Jørgensen, L. N., et al. 2016. Disease impact on wheat yield potential and prospects of genetic control. *Annual Review of Phytopathology* 54: 303–322.
- Tagle, A. G., Chuma, I., and Tosa, Y. 2015. *Rmg7*, a new gene for resistance to *Triticum* isolates of *Pyricularia oryzae* identified in tetraploid wheat. *Phytopathology* 105: 495-499.
- Takabayashi, N., Tosa, Y., Oh, H. S., and Mayama, S. 2002. A gene-for-gene relationship underlying the species-specific parasitism of *Avena/Triticum* isolates of *Magnaporthe grisea* on wheat cultivars. *Phytopathology* 92: 1182-1188.

- Tosa, Y., and Chuma, I. 2014. Classification and parasitic specialization of blast fungi. *J. Gen. Plant Pathol.* 80: 202-209.
- Tosa, Y., Osue, J., Eto, Y., Oh, H. S., Nakayashiki, H., Mayama, S., et al. 2005. Evolution of an avirulence gene, *AVR1-CO39*, concomitant with the evolution and differentiation of *Magnaporthe oryzae*. *Mol. Plant-Microbe Interact* 18:1148-1160.
- Ugalde, D., Brungs, A., Kaebernick, M., McGregor, A., and Slattery, B. 2007. Implications of climate change for tillage practice in Australia 97: 318–330.
- Urashima, A. S., Hashimoto, Y., Don, L. D., Kusaba, M., Tosa, Y., Nakayashiki, et al. 1999. Molecular Analysis of the Wheat Blast Population in Brazil with a Homolog of Retrotransposon MGR583. *Japanese Journal of Phytopathology* 65: 429–436.
- Urashima, A. S., Lavorent, N. A., Goulart, A. C. P., and Mehta, Y. R. 2004. Resistance spectra of wheat cultivars and virulence diversity of *Magnaporthe grisea* isolates in Brazil. *Fitopatologia Brasileira*, 29: 511–518.
- Vy, T. T. P. 2013. Genetic and molecular mechanisms of the evolution of wheat blast fungus from its relatives, Ph.D thesis, Kobe University.
- Wang, S., Asuke, S., Vy, T. T. P., Inoue, Y., Chuma, I., Win, J., et al. 2018. A new resistance gene in combination with *Rmg8* confers strong resistance against *Triticum* isolates of *Pyricularia oryzae* in a common wheat landrace. *Phytopathology* 108: 1299–1306.
- Widmar, D. 2017. Trends in global wheat production change in acres. *Agricultural Economics Insight*, Aei, 1–6. Retrieved from <https://aei.ag/2017/10/23/trends-global-wheat-production>, (Accessed 2 June 2020).
- Yasuda, N., Mitsunaga, T., Hayashi, K., Koizumi, S., & Fujita, Y. (2015). Effects of pyramiding quantitative resistance genes *pi21*, *Pi34*, and *Pi35* on rice leaf blast disease. *Plant Disease*, 99: 904–909.

- Yoshida, K., Saunders, D. G. O., Mitsuoka, C., Natsume, S., Kosugi, S., Saitoh, H., et al. 2016. Host specialization of the blast fungus *Magnaporthe oryzae* is associated with dynamic gain and loss of genes linked to transposable elements. BMC Genomics 17: 1–18.
- Zhan, S. W., Mayama, S., and Tosa, Y. 2008. Identification of two genes for resistance to *Triticum* isolates of *Magnaporthe oryzae* in wheat Genome 51: 216–221.
- Zhang, N., Luo, J., Rossman, A. Y., Aoki, T., Chuma, I., Crous, P. W., et al. (2016). Generic names in Magnaporthales. IMA Fungus 7: 155–159.
- Zhong, Z., Norvinyeku, J., Chen, M., Bao, J., Lin, L., Chen, L., et al. 2016. Directional selection from host plants is a major force driving host specificity in *Magnaporthe* species. Scientific Reports 6: 1–13.