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BIOTECHNOLOGICAL APPROACHES TO DEVELOP RESISTANT RICE CULTIVARS AGAINST BACTERIAL PANICLE BLIGHT DISEASE

Asyraf Azmi [1], Mohamad Iqbal Hakim Mohd Azhan [1], Fatin 'Alyaa Zainuddin [2], and Muhammad Asyraf Md Hatta [1,*]

[1]*Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Malaysia*
[2]*Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Malaysia*

*Corresponding Author: Tel.: +6010-3958760; E-mail: m.asyraf@upm.edu.my

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Abstract

Bacterial Panicle Blight (BPB) caused by *Burkholderia glumae* is one of the severe seed-borne bacterial diseases of rice that has affected major rice producing countries worldwide. Over 70% of harvested rice exhibited grain rot or empty grain were reported from the infected fields. The development of disease-resistant rice cultivars remains to be the most sustainable approach to control this disease as opposed to chemical applications. However, rice cultivars that exhibit complete resistance to BPB is yet to be developed for deployment. Over the last century, conventional method has been used by breeders and cytogeneticists to introgress resistance (*R*) genes for crop improvement. The employment of this approach alone is often associated with a lengthy period and the simultaneous introduction of genetically linked undesired traits. The integration of genomics and molecular genetics into the conventional method has facilitated the breeding efforts in recent times. In this review, we discuss how recent advances in biotechnology can help to accelerate the process of developing resistant rice cultivars against bacterial panicle blight disease.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major dietary sources in the world, especially by the Asian population [1]. The countries located in regions that provide favorable conditions for the cultivation of paddy have a significant role in the production and consumption of rice as a primary crop. This is particularly evident in Asia, where approximately 90% of the world's rice is produced [2]. The global production of rice can be impacted by abiotic factors such as drought and heat stress, as well as biotic factors such as diseases and pests, which may result in food insecurity. The anticipated adverse effects of global climate change, including elevated temperatures, increased concentrations of carbon dioxide, rising sea levels, and an increased risk of severe weather events and irregular rainfall patterns, are projected to exert a substantial detrimental influence on crop yields in the coming century [3]. To reduce this impact, rice has undergone continuous genetic improvement through both conventional and modern approaches over the last few decades [4].

One of the major rice diseases is Bacterial Panicle Blight (BPB), caused by the gram-negative bacterium known as *Burkholderia glumae*, with yield losses ranging from 10 % to 75 % [5, 6]. It was initially dubbed Bacterial Grain Rot of rice when it was first discovered in Japan in 1956 [7]. BPB has since been detected across every rice-growing area of the world, including countries in Asia, Africa, North America, and Latin America [8-12].

B. glumae can infect rice plants at any growth stage, including seedling, vegetative growth, and reproductive stages. The bacterium causes seed rot, stunting, and chlorosis in seedlings, and sheath rot and grey lesions encircled by brown edges in leaves during the vegetative stages [13, 14]. The disease has the most damaging effects in reproductive tissues, where the symptoms are characterized by spikelet discoloration and impaired grain development [13] (Figure 1). In the case of severe infestation, the entire panicle appears straw-colored, and most of the panicles remain empty, aborted, and rotten [15]. Due to the lack of grain filling, panicles remain upright, as opposed to grain-filled panicles, which bend under the weight of the grain [13]. The detrimental impacts on the grain development resulting in total crop failure [5].

Figure 1. Bacterial panicle blight symptoms in rice. (a) Panicles with discoloration of spikelet and (b) sheath with lesions of infected rice plant.

In general, the bacterium favors high humidity and warmth (up to 40°C) during the flowering stages to multiply and spread as reported by disease outbreaks that had occurred amid record high temperatures, particularly at night [13]. With the increasing temperature every year due to climate change [16], it is expected that BPB will become more prevalent in the warm regions including tropical and semi-tropical countries.

Developing and deploying resistant rice cultivars remains an effective and sustainable approach for the control of this disease. However, there is little or no known resistance for BPB that occurs within the cultivated rice species [17-22]. Over the past decades, the genetic-based disease resistance via introgression breeding programs have been successful for other rice diseases such as bacterial leaf blight, sheath blight, and rice blast. However, this conventional breeding method is time consuming and often associated with linkage drag. Although marker-assisted breeding has significantly reduced the time required for early crop selection, the recent advances in genomics and molecular genetics can further accelerate the process. In addition, other new technologies such as gene editing, particularly CRISPR is also a valuable tool in engineering new resistance specificities and genome engineering against disease. Most recently, the advent of 'speed breeding' system provides a potential platform to reduce the generation time of rice for a rapid introgression and stacking of multiple resistance (*R*) genes [23].

In this review, we will discuss how recent advances in crop breeding can be used to accelerate the development of BPB-resistant rice. The potential strategies for employing and integrating various technologies in rice breeding program are presented.

THE VIRULENCE MECHANISM OF *BURKHOLDERIA GLUMAE*

The study of the mechanism by which pathogens interact with host defense responses is essential for understanding the strategies that enable them to overcome or suppress host defense, leading to enhanced virulence. Toxoflavin and lipase are known to be major virulence factors of *B. glumae*, and their production is dependent on the TofI/TofR quorum-sensing system. However, other factors such as the flagella and type III secretion system (T3SS) play a role for the full virulence effect of *B. glumae* [24].

Toxoflavin

Toxoflavin is a very efficient electron transporter and it produces reactive oxygen species (ROS) like hydrogen [25-27]. Hydrogen peroxide toxicity, which can interfere with photosynthesis, photorespiration, and other metabolic activities, may be the cause of toxoflavin's toxicity on rice [28, 29]. Toxoflavin production in *B. glumae* is governed by polycistronic genes which can be categorized into two groups, biosynthesis and transport. The biosynthesis process involved five genes, *ToxABCDE* [25, 30], while the transport of toxoflavin involved four genes, *ToxFGHI* [25]. *Tox*R controls both operons, while quorum sensing controls the expression of the transcriptional activator *tox*J. It is also suggested that the LysR transcriptional activator t*ox*R is

involved in the activation of the tox operon transcription since it can bind to the *tox*A promoter region [31]. Another crucial regulatory component for toxoflavin production is *tox*J, which encodes a transcriptional activator [25]. According to [32], the deficiency of the PidS/PidR two-component regulatory system (TCRS) in mutants has been found to result in reduced synthesis of the phytotoxin toxoflavin and decreased pathogenicity. However, it has been reported in some studies that the toxoflavin-deficient strains can still cause mild symptoms in rice cultivars that are vulnerable to the disease [24].

Type III secretion system

The T3SS is employed by diverse gram-negative bacterial pathogens to deliver proteinaceous virulence factors, or type III effectors, into eukaryotic host cells [33]. According to [34], the pathogenicity of *B. glumae* mutants, that lack T3SS is significantly less on rice panicles in comparison to the wild strain. In addition to the pathogenicity of bacteria, T3SS is necessary for inducing the hypersensitive reaction (HR) in non-host plants or resistant host plants [35, 36].

Lipase

Another virulence factor used by *B. glumae* is lipase, an enzyme that degrades the cell wall of plants during infection. Based on a study by [37], the expression of *lipA*, a gene responsible for encoding lipase has resulted in the reduced BPB symptoms in rice, suggesting that the *lipA* gene plays a crucial role in the biosynthesis of lipase. The enzyme is secreted by the pathogen via the type II secretion system [34]. Furthermore, it has been discovered that the TofI/TofR quorum sensing system facilitates the regulation of lipase activity [26, 37].

Host plants produce ROS as an initial defense mechanism, thus it is crucial for the pathogen to protect themselves against ROS [38]. Catalase which is encoded by the *katG* gene is one of the enzymes involved in this process in *B. glumae*. [27] reported the expression of *katG* is up-regulated by QsmR, a transcriptional activator for flagella-gene expression. In the same study, insertional mutation in the gene causes less severe disease symptoms on rice, indicating the key function of lipase as a virulence factor of *B. glumae* [27].

Exopolysaccharides

Another important virulence factor of *B. glumae* is exopolysaccharides (EPS). In general, EPSs produced by bacterial pathogens obstruct the host's vascular system, causing wilting to appear. The genes responsible for EPS biosynthesis are grouped in a chromosomal area, similar to the *hrp* genes for T3SS components. It has not been documented how EPSs contribute to the bacterial panicle blight of rice. However, the production of total and colloidal EPS was higher during dry seasons at the rice panicle initiation stage, according to the findings of [39]. The results of this study lend credence to the idea that high microbial activity and an increase in root biomass in the root rhizosphere region may be the causes of the EPS rise [40].

Polygalacturonases

In-depth research on the pectin-degrading enzyme, polygalacturonases in *B. glumae* has led to the discovery of its role in facilitating the pathogen's nutrient acquisition through the degradation of pectin layers in plant cell wall [41]. The genome of *B. glumae* has been found to contain endo-polygalacturonase PehA and PehB, which represent two isoforms of the enzyme. However, it has been reported that neither PehA nor PehB plays a direct role in the early phase of *B. glumae*'s pathogenicity [41].

Flagella

The flagella are crucial for bacterial movement within hosts, thereby contributing to the growth and development of bacterial pathogens [42, 43]. In *B. glumae*, the IcIR-type transcriptional regulator QsmR directly activates the expression of *flhDC*, a regulator responsible for the biosynthesis of flagella. The activation of FlhDC, in turn leads to the expression of genes associated with flagellum biosynthesis, motor functions, and chemotaxis [26]. In the same study, it was also observed that the mutants of *B. glumae* which were deficient in *qsmR*, *fliA*, and *flhDC* exhibited non-motile behavior and lost their pathogenicity in rice [26].

Quorum sensing

Quorum sensing (QS) is a cell-to-cell communication system in bacteria that is mediated by signaling molecules. This system enables bacteria to sense and respond to their environment, including regulation of virulence [44]. There are various signaling molecules employed by different bacteria, whereby the majority of signal molecules are N-acyl homoserine 8 lactones (AHLs), oligopeptides and autoinducer-2 (AI-2). The genes *tofI* and *tofR* in *B. glumae* exhibit homologies to the *luxI* and *luxR* genes found in *Vibrio fischeri*, which are known to function as a significant global regulatory system for the synthesis of virulence factors. This QS system mediated by AHL signal molecules regulates the production of virulence factors described above including toxoflavin, lipase, flagella, and catalase [25, 45].

Lipopolysaccharide

The outer membrane of gram-negative bacteria is primarily composed of lipopolysaccharide (LPS), which consists of three parts: lipid A, core oligosaccharide (OS), and O-antigen [46]. The early biosynthesis of core OS requires heptose addition to its inner core which facilitated by heptosyltransferase I through *waaC* gene expression [47]. The findings of [48] indicate that the absence of WaaC protein leads to the development of heptoseless LPS, which causes *B. glumae* more sensitive to acidic, osmotic, saline, and detergent stress condition, as well as to polymyxin B exposure. The disruption *waaC* gene also resulted in significant deficiency in the swimming and swarming motility, as well as a decrease in the virulence of *B. glumae* towards rice.

In short, an extensive study has been done to understand the virulence mechanism of *B. glumae* in causing BPB disease in rice. Similar to other bacterial pathogens, *B. glumae* possess or produces various virulence factors that facilitate in the colonization process including toxoflavin, lipase, catalase, exopolysaccharides, polygalacturonases, flagella, and lipopolysaccharide.

CURRENT MEASURES TO CONTROL BACTERIAL PANICLE BLIGHT DISEASE IN RICE

The current management strategies of BPB include cultural practices, chemical control, biological control, exclusion, and plant host resistance. To effectively and sustainably control this disease, integrating these strategies is crucial [49].

Cultural practices

Cultural practices aimed at minimizing the risk of disease incidence include the exclusion of the pathogen and the avoidance of conditions that favor the disease. A study on the evaluation of nitrogen application on BPB severity has demonstrated that the higher doses resulted in increased susceptibility of rice plants [50]. Thus, applying appropriate levels of nitrogen fertilizer can help reduce the damaging impacts of BPB. According to [51], another effective strategy to avoid disease progression is by practicing early planting or using early maturing rice cultivars, so that the flowering times do not occur during the hottest times of the growing season, which is favorable for BPB growth. The study also reveals that excessive seed planting can lead to an increase in BPB incidence and severity.

Chemical control

Chemical applications in accordance with the appropriate doses and timing can reduce the damage of BPB. It has been reported that the combined use of bactericide as seed treatment and foliar sprays at heading stage can effectively inhibit the multiplication of *B. glumae* on spikelets [52].

Oxalinic acid is among the chemical agents employed for BPB disease prevention [53, 54]. The utilization of this bactericide was predominantly observed in Japan and Asia. However, its usage was prohibited in certain countries like USA owing to concerns regarding its safety [24]. According to [55], the mechanism of action of this chemical involves the specific targeting of DNA gyrase, which is composed of subunits encoded by *gyrA* and *gyrB* genes in *B. glumae* and other gram-negative bacterial species. However, excessive usage of oxolinic acid has caused the emergence of resistant strains in Japan since 1998 [52, 54, 56-58]. The resistance is conferred by the pathogen via an amino acid substitution in the GyrA protein [54, 58]. Therefore, ensuring the proper utilization of this bactericide is imperative in order to maintain its effectiveness.

It has also been reported that copper and copper-containing bactericides are effective at controlling BPB in rice [59-62]. Several copper products have been tested in the field trials in Lousiana and Texas, USA. Although the application of the chemicals at the boot and heading stages has significantly reduced the BPB severity, some degrees of phytoxicity on spayed leaves and panicles as well as yield reduction were observed in the fields [59-62]. As an alternative to foliar sprays, the copper-based bactericides can be used in seed treatment prior to planting. A study by [63] has showed that the application of copper hydroxide 77% at concentration of 5% has reduced bacterial populations on rice seeds.

Biological control

The deployment of biological agents has been proposed as a potentially safer method for managing the pathogen. Currently, there are present research efforts aimed at investigating the potential of utilizing non-pathogenic strains of *B. glumae* to induce infection. The application of biological control required the use of bacteria that exhibited antagonistic properties towards *B. glumae* bacteria [64]. According to [65], *B. glumae* strains isolated from the Southern United States did not demonstrate any pathogenicity towards rice, which led to no reduction in yield upon inoculation. In a later study, [32] also found that two non-pathogenic *B. glumae strains*, particularly 257sh-1 and396gt-2 obtained from rice fields were able to suppress BPB and prevent yield reduction.

Several species of *Bacillus*, including *B. methylotrophicus*, *B. amyloliquefaciens*, *B. subtilis*, and *B.*

oryzicola sp. nov. have been found to reduce the symptoms of *B. glumae*-induced disease through methods such as co-inoculation, soil drenches or foliar sprays [66, 67]. Other bacterial strain such as *Pseudomonas protegens* strain PBL3 and *Burkholderia cepacian* strain PBL18 inhibited the growth of *B. glumae in vitro* and reduced disease symptoms when co-inoculated with *B. glumae* [64].

In a more recent study, plant growth-promoting *Streptomyces* (PGPS) have been shown to be a potential biocontrol agent against *B. glumae*. Rice plants treated with three *Streptomyces* species exhibited reduced symptoms and enhanced growth as well as yield when challenged with the bacteria under greenhouse conditions [68].

Exclusion

Since BPB is seedborne, infected seeds are the main source of inoculum for this disease [13]. Therefore, the most effective measure relies on the exclusion of the pathogen from a disease-free geographic area through planting of seeds free of the *B. glumae*. To mitigate the risk of disease outbreaks, most countries implement phytosanitary regulations to inspect seeds upon arrival prior to distribution [17, 69]. The initial measure to prevent the spread of BPB is to quarantine seeds that have been imported from countries with a confirmed incidence of the disease [70]. In spite of the existence of a governmental regulatory body, the occurrence of activities such as smuggling and illegal seeds importation may potentially lead to a significant outbreak and subsequent epidemic within a rice field. Therefore, it is crucial to detect *B. glumae*-infected seed to ensure that the seeds being planted are pathogen-free.

During the first detection of BPB in Japan, the pathogen was identified primarily on the basis of the disease's morphology and symptoms [7]. Several years later, conventional biochemical and molecular techniques were employed to detect the bacterium *B. glumae*, as well as to characterize the disease into a broader range of knowledge through the use of culture method and real-time PCR. Currently, semi-selective media such as SP-G media and KB agar, as well as selective medium known as CCNT are utilized to distinguish *B. glumae* form other bacterial strains [71]. Following an incubation period of 2-4 days at 38°C, a yellowish colony will be grown on the media.

The SMART (Selective Medium-design Algorithm Restricted by two constraints) developed by [72] is an example of another selective medium that has been used for the detection of *B. glumae*. This medium not only identifies the target bacteria, but also provides insight into its ecology and epidemiology [70].

A faster approach in detecting the bacteria would be the use of PCR method. The identification of *B. glumae* in seed batches has been achieved with high sensitivity through the utilization of the 16S-23S recombinant DNA internal transcribed spacer (ITS) sequence and the *rhs* (rearrangement hotspots) gene of *B. glumae* in real-time PCR [73, 74]. [75] conducted a study wherein they utilized species-specific primers from the DNA gyrase gyrB sequence to develop a multiplex-PCR assay. The assay enabled the identification and specific detection of *B. glumae* and *B. gladioli*, both of which are known as causative agents of BPB.

Resistant rice cultivars

The development of BPB-resistant rice cultivars is the most sustainable measure to manage this disease. The process of incorporating BPB resistance into rice cultivars requires the identification of cultivars with higher resistance through screening against *B. glumae*. However, previous phenotyping studies revealed varying levels of disease responses, from high levels of susceptibility to moderate levels of resistance to BPB among existing rice cultivars. As a result, no individual genes or quantitative trait loci (QTLs) conferring complete resistance have been identified [17, 18, 20-22, 76]. These observations suggest that the resistance is quantitative and governed by multiple genes. In order to identify genes associated with quantitative resistance, several QTL mapping efforts have been conducted and will be further discussed in the next section.

In addition to conventional breeding strategies, researchers have generated transgenic rice lines that incorporate a transgene from other species, whose expression confers resistance against BPB in the rice plants [77, 78]. [77] demonstrated that rice plants overexpressing an oat thionin gene, *Asthi1* exhibited enhanced resistance to *B. glumae*. Seeds inoculated with *B. glumae* produced healthy seedlings, while non-transgenic wild-type seeds developed stunted seedlings characterised by stem discoloration. Furthermore, resistance to BPB was observed in transgenic lines overexpressing the *BSR1* (*Broad-Spectrum Resistance 1*), a gene encoding a BIK1-like receptor-like cytoplasmic kinase [78]. In addition to BPB, this gene was previously reported to confer resistance to rice blast and bacterial blight [79]. In addition to genes associated with BPB resistance, genes that facilitate the degradation of *B. glumae* virulence factors can also be introduced into rice plants. The introduction of the *TxDE* gene from *Paenibacillus polymyxa JH2* to rice, which eoncodes toxoflavin degrading enzyme has been found to confer resistance to BPB [80-82].

Although complete resistance to BPB has not yet been achieved, the insights obtained through QTL mapping

and rice transgenic studies will facilitate the advancement of BPB-resistant lines in forthcoming research efforts.

MOLECULAR COMPONENTS OF PLANT DEFENSE: CURRENT INSIGHTS ON THE RICE-*B. GLUMAE* PATHOSYSTEM

Qualitative resistance of rice to diseases

To defend against pathogen attack, plants have evolved a sophisticated innate immune system. Pattern recognition receptors on the plant cell surface governs the first layer of defense through detection of highly conserved pathogen-associated molecular pattern (PAMP) secreted by the potential pathogen, resulting in the activation of PAMP-triggered immunity (PTI) [83].

The more adapted pathogens secrete molecules known as effectors that can suppress PTI [84-86]. In turn, plants have co-evolved resistance (*R*) genes that typically encode immune receptors that directly or indirectly recognize a subset of effectors known as avirulence molecules (Avrs) in plant cells and activate the second layer of defense, termed effector-triggered immunity (ETI) [83, 87]. This recognition triggers a set of defense responses that ultimately lead to resistance. The majority of plant *R* genes that have been cloned to date encode nucleotide-binding leucine-rich repeat (NLR) proteins that are often race-specific and function at all stages of plant development [88]. In rice, approximately 100 *R* genes against *Magnaporthe oryzae*, the causal agent of rice blast have been identified so far, 31 of which have been cloned. Among these genes, only three are non-NLR proteins while the others are all NLRs [89]. For bacterial blight caused by *Xanthamonas oryzae* (*Xoo),* at least 46 *R* genes have been identified in rice, of which 16 *R* genes have been cloned. Unlike rice blast, only five of these genes belong to NLR family [90, 91]. The non-NLRs are generally non-race specific and effective only at adult stages, conferring only partial resistance. Cloning of *R* genes offers better understanding of the interaction between pathogen and host which can facilitate the breeding process.

Genetic characterization of quantitative resistance of rice to BPB

In the case of BPB, no *R* gene has been identified so far, hence the molecular mechanisms underlying rice-BPB resistance remain poorly understood [90]. Evaluation of BPB resistance under greenhouse and field conditions reveal that majority of the commercial rice cultivars are highly susceptible, with only a few of them showed moderate resistance but no complete resistance response was found [6, 17-20].

The wide range of response suggests that the resistance to BPB in rice is quantitative. This type of resistance is usually controlled by multiple genes associated with QTL [64]. In the recent years, several studies have attempted to use QTL mapping to identify the genes that confer resistance to BPB [18, 76, 92-94]. Molecular markers were successfully employed in those studies to map the QTL in either recombinant inbred lines (RILs) [18, 94], backcross inbred lines (BILs) [76, 92], or chromosome segment substitution line (CSSLs) [93].

The first report of QTLs related to BPB resistance was presented by [18] using a set of RILs derived from crosses between Lemont (susceptible) and TeQing (resistant). Twelve QTLs were discovered in which one of them is a major QTL on chromosome 3 (Table 1). Among the identified QTLs, a total of eight (namely *qBPB-1-1*, *qBPB-1-2*, *qBPB-2-2*, *qBPB-3-1*, *qBPB-7*, *qBPB-8-1*, *qBPB-10*, and *qBPB-11*) were found to be derived from TeQing, while the remaining four (*qBPB-1-3*, *qBPB-2-1*, *qBPB-3-2*, and *qBPB-8-2*) were derived from Lemont. It is notable that QTLs *qBPB-2-1*, *qBPB-2-2*, *qBPB-3-1*, and *qBPB-8-2* have been found to be related to resistance to other diseases such as sheath blight, rice blast, and bacterial leaf blight, implying that these QTLs encode genes that provide broad-spectrum resistance to pathogens [18].

In the second QTL analysis, another locus was detected in chromosome 1 using 110 BILs developed from a cross between a BPB-resistant cultivar Kele and a BPB-susceptible cultivar Hitomebore [76]. The QTL was confirmed and named *RBG2* in the subsequent study [92] (Table 1).

Following that, the same research group revealed a QTL named *qRBS1/RBG1* in chromosome 10, utilizing a CSSL population derived from Nona Bokra (resistant) and Koshihikari (susceptible) [93] (Table 1).

More recently, 120 RILs developed from crosses between a variety with strong stress resistance Huazhan and a variety with widely compatible Nekken 2 were utilized to identify nine QTLs associated with BPB resistance [94] (Table 1). One and two of the QTLs detected on chromosomes 1 and 7, respectively, were consistent with a previous study by [18], confirming their resistance to BPB and may be employed in resistance breeding program.

The advancement of NGS technologies, combined with the use of association panels and multi-parental populations has accelerated the identification of a larger number of QTL associated with quantitative resistance in recent years. This approach can enable the discovery of additional markers that are closely linked with BPB resistance QTL, particularly those with minor effects and marker haplotypes representing allelic diversity at QTL. For example, in other rice diseases, genotyping-by-sequencing

Table 1. List of quantitative trait loci (QTLs) for resistance to bacterial panicle blight in rice.

Parents	Type of crossing	Population size	QTL			References
			Locus	Chr.	Marker interval	
Lemont X TeQing	RIL	300	*qBPB-1-1*	1	RG472–C131	[18]
			qBPB-1-2	1	CDO455–CDO118	
			qBPB-1-3	1	RG236–C112x, RZ14–RZ801	
			qBPB-2-1	2	C624x–RG139	
			qBPB-2-2	2	on end at RG520	
			qBPB-3-1	3	C515–RG348x	
			qBPB-3-2	3	G249–RG418	
			qBPB-7	7	BCD855–CDO497	
			qBPB-8-1	8	on end at C424x	
			qBPB-8-2	8	C825x–G104	
			qBPB-10	10	RG214x–CDO98, CDO98–Y1065La	
			qBPB-11	11	RZ900–G44	
Kele X Hitomebore	BIL	110	*RBG2*	1	RM1216-RM11727	[76, 92]
Nona Bokra X Koshihikari	CSSL	44	*qRBS1/RBG1*	10	RM24930-RM24944	[93]
Jupiter X Trenasse	RIL	286-300	*qBPB1.1*	1	SNP76-SNP280	[97]
			qBPB2.1	2	SNP1270-SNP43	
			qBPB3.1	3	SNP225-SNP91	
			qBPB3.2	3	SNP91-SNP103	
			qBPB4.1	4	SNP431-SNP337	
			qBPB5.1	5	SNP329-SNP341	
			qBPB11.1	11	SNP462-SNP474	
Huazhan X Nekken 2	RIL	120	*qBPB-1*	1	NR	[94]
			qBPB-3	3	NR	
			qBPB-4	4	NR	
			qBPB-5	5	NR	
			qBPB-7.1	7	NR	
			qBPB-7.2	7	NR	
			qBPB-8	8	NR	
			qBPB-9	9	NR	
			qBPB-10	10	NR	

RIL: Recombinant inbred lines
BIL: Backcross inbred lines
CSSL: Chromosome segment substitution lines
NR: Not reported

(GBS)-based genetic analysis and association mapping of African rice cultivars has allowed identification of rice blast *R* genes/QTLs against *M. oryzae* [95]. A similar approach was employed to identify novel QTLs that contribute to resistance against bacterial blight in rice diverse germplasm [96].

Recently, a major QTL associated with BPB was detected on the upper arm of chromosome 3 using a RIL population developed from a moderately resistant U.S. cultivar Jupiter and a susceptible U.S. cultivar Trenasse [97]. This QTL, namely *qBPB3.1* overlaps with a QTL discovered in a previous study by [18]. To complement the QTL linkage analysis, a set of WGS data (QTL-seq) generated from 15 selected RILs was utilized, which resulted in the identification of another major QTL on chromosome 1 and several minor QTLs. At least nine candidate genes with putative roles in defense or flowering were found within the identified QTLs [97].

The genetic information gained from the aforementioned QTL studies provides a valuable resource for breeding program aimed at developing BPB-resistant cultivars. It will also facilitate the cloning of *R* gene to elucidate the underlying genetic mechanism of rice resistance to this important disease.

EMPLOYING VARIOUS APPROACHES TO IDENTIFY QUALITATIVE RESISTANCE

Conventionally, isolating *R* genes has been a laborious and resource-intensive process. Crossing parents with contrasting phenotypes to generate a biparental mapping, and selfing the offspring to establish a segregating population is the first step in this long and arduous process. The population is subsequently genotyped and phenotyped to enable rough mapping of the *R* gene locus, followed by positional fine mapping.

Challenges of map-based cloning

This method of cloning *R* gene is hampered by two factors: the lack of available markers and the recombination suppression between the mapping parents. Although the problem of developing markers can now be addressed by NGS technologies, such map-based approximation approaches can be challenging to implement due to a limited recombination rate, especially when the target *R* gene resides in chromatin from the wild relatives or at the centromeric and pericentromeric regions [98]. An extreme example is demonstrated by [99] in which all crossover events on wheat chromosome 3B occurred in only 13% of the chromosome. In the case of rice, a significant recombination suppression was found near the centromere on chromosome 12 in the crosses of indica and japonica rice [100].

Mutational genomics

An *R* gene cloning approach that can overcome the limitation of recombination is mutational genomics, which is based on mutagenesis e.g., with ethyl methanesulfonate, followed by phenotypic screening for mutants and sequencing of multiple independently derived susceptible mutants to identify candidate genes. A blast *R* gene, *Pii* was cloned from the rice cv. Hitomebore using this strategy [101]. For crops with large genomes such as wheat, this method can be combined with capture-based techniques e.g., RenSeq [102] or chromosome flow sorting to reduce genome complexity [103]. However, it is important to note that unlike hexaploid wheat, diploid plants such as rice have a limited degree of genetic redundancy, necessitating the generation and phenotypic screening of large mutant populations to ensure the recovery of enough independent mutants.

Association genetics

An alternative approach that offers wider application in plants is association genetics. This method exploits historical recombination (linkage disequilibrium) and natural mutations in a diversity panel, which typically includes several hundreds of genetically diverse individuals. Association analyses between marker polymorphisms and phenotypic variance across the genomes of the diversity panel enable identification of QTL or genes associated with agronomic traits. In recent years, genome-wide association study (GWAS) has been applied to mine *R* genes in rice. [104] and [105] discovered 12 bacterial blight resistance-related loci and 27 sheath blight resistance-related loci, respectively, using GWAS on rice diversity panels. More recently, 27 rice blast resistance loci have been detected by performing GWAS of 584 rice germplasms [106]. Although this method allows identification of candidate genes, very few studies have led to direct *R* gene cloning due to a paucity of genetically mapped markers and linkage disequilibrium. These limitations can be overcome by detecting and analyzing the expression of candidate genes of different genotypes. A recent study has combined GWAS and transcriptome analysis to identify a set of candidate genes associated with bacterial blight resistance in rice [107].

Exome capture sequencing

So far, most association studies have used arrays or reduced-representation sequencing which typically discover single-nucleotide polymorphisms (SNPs). These approaches capture only a fraction of a population's genetic variation and overlook structural variants such as copy number variations and insertion-deletions (indels). An alternative way is to perform reduced representation sequencing that focuses on a particular fraction of the genome such as exome capture or gene-family capture sequencing, or whole genome sequencing. GWAS in combination with NLR gene-targeted sequencing (RenSeq) has allowed the rapid identification of four stem rust *R* genes from the *Aegilops tauschii* diversity panel [108]. The declining sequencing cost will allow routine whole genome sequencing and the development of pan-genome reference sequences for rice and its wild relatives. This, along with access to sequence-configured diversity panels, will accelerate the cloning of the first BPB *R* gene in the years to come (Figure 2).

Transcriptome sequencing

In addition, transcriptome analysis using RNA sequencing may also accelerate the identification of candidate *R* genes.

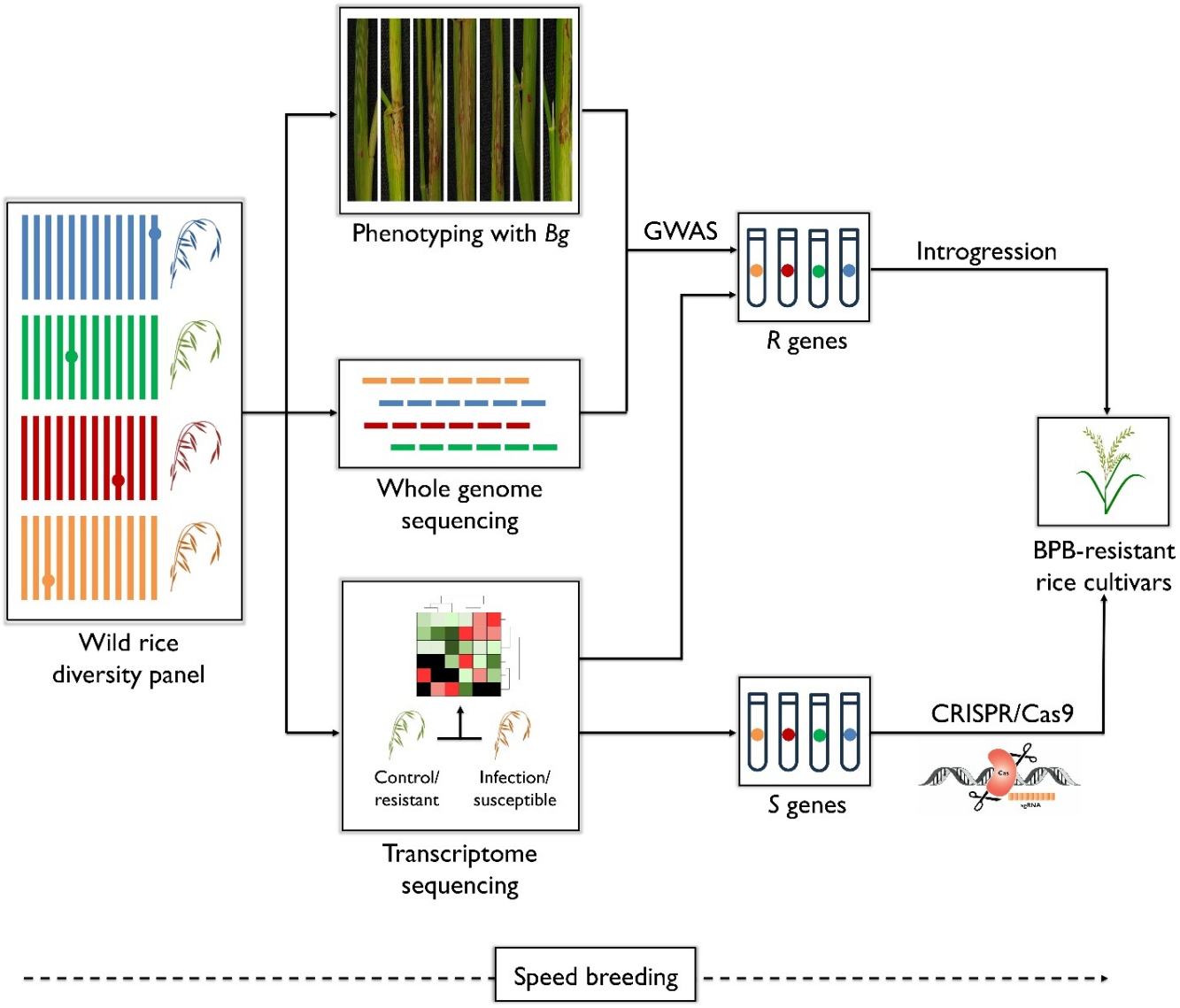

Figure 2. Employing various biotechnological approaches to harness genetic diversity in wild rice relatives for the development of Bacterial Panicle Blight (BPB)-resistant rice cultivars. A genetically diverse panel of wild rice relatives contains high genetic diversity in important traits including disease resistance. Phenotypic characterization with *Burkholderia glumae* (*Bg*) and whole genome sequencing of the diversity panel will allow genome-wide association study (GWAS) for BPB resistance (*R*) genes cloning. Transcriptomic analysis may further facilitate the selection of candidate *R* genes by identifying upregulated genes in the resistant line in comparison with the susceptible line. The introgression of *R* genes into the cultivated rice species background can be performed using marker-assisted breeding. A detailed analysis of the differentially upregulated genes between the infected and non-infected susceptible cultivars against BPB may reveal candidate susceptibility (*S*) genes. CRISPR/Cas9 technology can be employed for targeted gene knockout of *S* genes to enhance BPB resistance in rice. Speed breeding (SB) will accelerate the development of diversity panel, the BPB disease screening of diversity panel, and the introgression of BPB *R* genes into desired cultivars.

A transcriptomic study of the rice-*B. glumae* interaction discovered differentially expressed transcripts between the moderately resistant cultivar Clearfield 161 (CL161) and the susceptible cultivar Clearfield 151 (CL151) upon inoculation with *B. glumae* [109]. A set of upregulated genes belonging to the families of NLR and NB-ARC (nucleotide-binding adaptor shared by APAF-1, R proteins, and CED-4) has been revelead in the CL161 cultivar in comparison with the CL151 cultivar. Further validation of these genes using quantitative reverse transcription PCR has confirmed that

three of them, namely LOC_Os11g12330, LOC_Os11g12040, LOC_Os11g12300, and LOC_Os11g12000 exhibited significant upregulation following inoculation with *B. glumae* in the CL161 [109]. By integrating the transcriptomic data with the GWAS data, it will facilitate the selection of the candidate *R* genes prior to functional characterization studies (Figure 2).

EXPLORING WILD RELATIVES AS NOVEL SOURCES OF RESISTANCE AGAINST BPB

Since most of the cultivated rice cultivars are susceptible to BPB, the search for novel *R* genes is challenging. This lack of resistance is primarily due to the domestication process which has narrowed down the genetic diversity of commercial cultivars, particularly in terms of diverse allelic forms of *R* genes. To broaden the genetic base, wild rice relatives could be a rich source of resistance due to the untapped reservoir of *R* gene.

Cloning of rice R genes from wild relatives

The first bacterial blight *R* gene, *Xa21* was cloned from a wild African species *O. longistaminata* which encodes a receptor kinase-like protein containing LRRs in the putative extracellular domain, a single pass transmembrane domain, and a serine/threonine kinase intracellular domain [110]. Another important bacterial blight *R* gene, *Xa23* gene, was identified from a common wild rice, *O. rufipogon*, also known as the putative progenitor of Asian cultivated rice. This gene encodes an executor R protein and appears to confer completely dominant and broad-spectrum resistance against the disease [111, 112]. The tetraploid wild rice *O. minuta* is also one of the bacterial blight resistance sources from which the *Xa27* gene was discovered and transferred into the cultivated rice prior to gene cloning. The encoded executor R protein confers resistance to diverse strains of *Xoo* and has been widely used in rice breeding program [113, 114]. In addition, there are other bacterial blight *R* genes that have been identified in wild rice species and yet to be cloned including *Xa29(t)* from *O. officialis* [115], *Xa30(t)* from *O. rufipogon* [116], *Xa32(t)* from *O. australiensis* [117], *Xa33(t)* and *Xa38* from *O. nivara* [118, 119], and *Xa35(t)* from *O. minuta* [120].

In case of rice blast, many genes from wild species have been reported to be effective against this disease such as *Pi9* from *O. minuta*, *Pi-40(t)* from *O. australiensis*, *Pirf2-1(t)* and *Pid3-A4* from *O. rufipogon*, *Pi54rh* from *O. rhizomatis*, *Pi54of* from *O. officinalis* and *Pi57(t)* from *O. longistaminata* [121-123]. Out of these, *Pi9* [124] and *Pi54of* [125] have been cloned which encode for NLR.

Challenges of wild relatives in breeding

Based on these findings, wild relatives of rice are predicted to play a crucial role in generating durable BPB resistant rice varieties. Whole genome sequencing and pan-genome analysis of wild rice will speed up the identification of *R* genes from wild relatives of rice and may provide insights into the pathways associated with the evolution of different *R* genes. A transcriptomic analysis of rice infected with *B. glumae* also revealed that resistance may have existed immediately prior to rice domestication indicating that the sources of resistance may be present in wild species [109].

Although wild rice species offers a tremendous source of genetic variation for BPB resistance, its utilization in breeding is hampered by several challenges. In the quest to identify and clone *R* genes, rice breeders and researchers must structure their germplasm which typically involves the generation of a biparental mapping population, followed by phenotyping and genotyping to allow the approximate localization of a major effect locus. This can pose a significant challenge in undomesticated grasses owing to their poor agronomy such as a lengthy generation period, seed shattering, difficulty in seed threshing, poor germination or dormancy, and unruly growth pattern. The introgression of *R* genes from wild relatives into the cultivated rice species background through conventional breeding or marker-assisted breeding overcomes the issue of poor agronomy. However, many introgression lines cannot be fully utilized in agriculture due to the persistent failure to eliminate the undesirable traits that are closely linked to the target trait, often known as ‘linkage drag’ and also other challenges including hybrid sterility and sexual incompatibility barriers [126].

With the advent of molecular tools and omics technologies such as trait-associated DNA markers, next-generation sequencing (NGS)-based genotyping and high-throughput phenotyping approaches, the rich source of genetic variation found in the wild relatives can be fully exploited by rapid cloning of BPB *R* genes and accelerated introgression into elite rice cultivars (Figure 2).

ACCELERATING THE DEVELOPMENT OF BPB-RESISTANT RICE CULTIVARS VIA SPEED BREEDING

The development of disease-resistant rice cultivars (i.e., BPB) via crossbreeding is time-consuming, often requiring more than 5 years, involving the crossing of selected parents, the selection and evaluation of genetically stable lines, and the establishment of pure lines [127]. Hence, it is crucial to shorten the breeding cycle by increasing the number of generations per year in rice breeding.

Principles of speed breeding

Speed breeding (SB) is an exciting new approach for accelerating genetic gain by manipulating plant growth environments [128]. This method entails growing plants under a better quality of light (i.e., light-emitting diodes, LED) at a higher intensity for an extended period of time (20-22 h), allowing them to photosynthesize longer which results in faster growth. Depending on available resources and crop type, plant growth conditions can be manipulated using a DIY benchtop cabinet, growth chambers, or glasshouses [129]. Speed breeding protocol has been adopted by many labs around the globe for a wide variety of crop species, mainly long-day crops including wheat [130, 131], barley [132], oats [133], and canola [134].

Current research on speed breeding in rice

Application of this approach in rice breeding would significantly reduce its long generation time, which typically takes about 3-6 months [135]. However, unlike abovementioned examples of crop, rice is a short-day crop, which means it requires extended, uninterrupted periods of darkness to induce flowering [136]. Therefore, the optimization of the current SB protocols is needed for rice as the prolonged photoperiod may prevent its flowering. Recently, a speed breeding system has been developed using LED for three short-day crops: rice, soybean, and amaranth [136]. By optimizing photoperiod to 10 h and employing a blue-light enriched, far-red-deprived light spectrum, accelerated growth and development, early flowering, and maturity was achieved for soybean, allowing for five generations per year. In a similar study, the use of far-red light in rice led to earlier flowering by 20 days for two genotypes, 'Primavera' and 'Nerica L-19'. However, unlike soybean, photoperiod manipulation was not performed for rice. It would be interesting to grow the two rice genotypes under the same photoperiod duration as soybean and examine its effects on the growth rate.

Apart from manipulating supplementary lighting, exerting physiological stress to trigger flowering and earlier seed setting has also been used as a basis for rapid generation advance in crops. Increasing the concentration of CO_2 can also promote growth, especially for C3 plants like rice in which higher level of CO_2 increases photosynthetic efficiency, leading to early flowering and higher biomass. Earlier studies by Japanese researchers reported this approach by combining restricted root growth and canopy thinning with high CO_2 concentration, followed by early harvest and embryo rescue to cut down generation times of 'Nipponbare' and several other rice varieties, known as rapid generation advance (RGA) [137, 138]. RGA method has several advantages compared to other conventional breeding methods, as it requires less space and resources and many germplasms or lines can be screened in shorter time.

Another approach to shorten the generation time is the doubled haploid (DH) method. However, a well-established protocol in tissue culture is required to produce DH lines. In rice, it has been shown that japonica germplasm is more amenable to tissue culture than indica germplasm, although there have been successful examples [139]. Mapping of resistant QTLs for rice sheath blight disease has been reported using a DH population generated from a cross between a japonica and an indica variety [140].

Prospects for speed breeding applications

In the context of breeding for BPB resistance, since it is an adult plant trait, shortening the rice generation time will accelerate the screening of germplasm (Figure 2) although some studies showed that BPB symptoms can also be observed at seedling stage. In wheat, the SB technique has been previously demonstrated suitable for phenotyping adult plant resistance to wheat stripe rust (caused by *Puccinia striformis* f. sp. *tritici*) and wheat leaf rust (caused by *Puccinia triticina* f. sp. *tritici*) as well as adult plant phenotype such as fusarium head blight (FHB) caused by *Fusarium graminearum* [141]. Thus, by growing rice under SB conditions, the BPB progression on the panicle of inoculated rice plants could be scored faster than under the normal conditions.

Apart from rapid screening of rice germplasm for resistance to BPB, SB can also accelerate genetic structuring and molecular studies such as gene transformation. The SB can be combined with transformation of rice to speed up the process of generating transgenic rice plants. It has been previously shown that the integration of SB in barley transformation pipelines resulted in transformation efficiency similar to standard pipelines and the viable transformed seeds can be obtained at least 6 weeks earlier than the standard control conditions [141].

The crossing of rice plants for transferring single or multiple *R* genes or QTLs associated with BPB into desired cultivars can also be accelerated using SB technique (Figure 2). A previous study in barley reported that the development of resistant lines against different diseases such as leaf rust, net and spot forms of net blotch and spot blotch have been achieved within two years when backcrossing scheme was combined with SB [142].

DEVELOPING BPB-RESISTANT RICE CULTIVARS VIA GENOME EDITING

The application of genome editing tools has significantly transformed and accelerated plant research within the past

two decades. Following the discovery of meganucleases, zinc finger nucleases (ZFNs), and transcriptional activator-like effector nucleases (TALENs), the clustered regularly interspaced short palindromic repeat/CRISPR-associated system has emerged as a more powerful genome editing tool. While the generation of DNA double-strand breaks (DSBs) at a targeted genomic location remains a common principle for all methods, the CRISPR editing system offers a more straightforward design, greater efficiency, and lower cost. The plants's endogeneous repair mechanism then repaired the DSBs either via non-homologous end joining (NHEJ) or homology-directed repair (HDR) [143], which often imperfect, leading to DNA modifications [144]. The introduction of specific DNA changes, including substitutions and insertion/deletion events (indels), can potentially impact gene function and subsequently modify external characteristics (traits).

Targeted gene knockout of susceptibility genes

Since it was first emerged, CRISPR/Cas9-mediated targeted gene knockout has been rountinely employed to functionally characterize candidate genes that may play roles in the molecular mechanisms underlying different agriculturally important traits in crops. To engineer BPB-resistant rice cultivars, CRISPR/Cas9 technology can be used to target disease susceptibility (*S*) genes, a set of host genes that contribute to pathogen colonization. By disabling the *S* genes, it may potentially reduce the ability of the *B. glumae* to colonize rice and lead to pathogen-specific resistance. In a recent work done by [145], the knockout of two *S* genes, *OsDjA2* and *OsERF104* using CRISPR/Cas9 has resulted in enhanced resistance to blast disease. For improvement of rice towards bacterial leaf blight disease, three *S* genes have been targeted, *SWEET11*, *SWEET13*, and *SWEET13* which led to broad-spectrum resistance to the pathogen *Xoo* [146]. Rice cultivars that exhibit enhanced resistance to both blast and bacterial leaf blight have also been engineered using this approach in which three genes were simultaneously inactivated [147].

In the case of BPB, so far no *S* genes have been identified. Since it is known that upon infection, pathogens typically employ overexpression of *S* genes as one of the strategies to invade the hosts, transcriptomics data on the BPB-infected rice plant could provide insights on the potential target genes by the *B. glumae*. Although a transcriptomic study has been performed on the resistant and susceptible rice cultivars as reported by [109], the authors focussed more on the molecular interaction between the rice and *B. glumae* rather than on the host susceptible mechanism. Thus, a detailed analysis of the differentially upregulated genes between the infected and non-infected susceptible cultivar used in the study may reveal some candidate *S* genes (Figure 2).

Typically, *S* genes exhibit a high degree of conservation among various species. The decreasing costs of sequencing may facilitate the identification of orthologous *S* genes, owing to the accessibility of pan-genomic, whole-genomic, and transcriptomic data [148]. One of the promising target is the *OsERF922* gene, which has been shown to be a negative regulator of resistance to multiple pathogens including *M. oryzae* and *X. oryzae* pv. *oryzae* [147, 149]. It would be interesting to challenge the rice plants with edited copy of the gene with *B. glumae*.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

In contrast to chemical treatments, breeding for BPB-resistant rice cultivars remains the most sustainable approach to controlling this disease. Thus, considerable efforts should be made to clone the first *R* gene. Since cultivated rice's genetic variation for BPB resistance is very narrow, novel sources of resistance need to be sought from wild relatives or other closely related species. The integration of current biotechnological tools such as pan-genomics, genomics, transcriptomics, genome editing, and speed breeding into conventional methods can accelerate the breeding process. With increased knowledge at the genomic and transcriptomic levels, the genetic dissection of the resistance mechanism of rice towards BPB disease can be further elucidated. The development of an established speed breeding protocol for rice will speed up the phenotyping process at the adult stage, in addition to shortening timelines for generating resistant inbred lines for cultivation.

In contrast to conventional strategies for cloning *R* genes, which are often associated with suppressed recombination and a lengthy process, we have proposed the application of association genetics coupled with whole genome sequencing as an alternative approach. The historical recombination (linkage disequilibrium) and natural mutations present in a diversity panel of wild relatives of rice can be exploited in the search for novel *R* genes. However, phenotypic data collection from the diversity panel might be seen as a major constraint due to the poor agronomy of wild species. Therefore, a collective effort must be made by the rice research community to accelerate the process.

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CONFLICTS OF INTEREST

The authors declare no conflict of interests regarding the publication of this manuscript.

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