Review

Strategy for Use of Rice Blast Resistance Genes in Rice Molecular Breeding

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Abstract: Rice blast is one of the most destructive diseases affecting rice production worldwide. The development and rational use of resistant varieties has been the most effective and economical measure to control blast. In this review, we summarized the cloning and utilization of rice blast resistance genes, such as *Pi1*, *Pi2*, *Pi9*, *Pi54*, *Pigm* and *Piz-t*. We concluded that three main problems in the current breeding of rice blast resistance are: availability of few *R* (resistance) genes that confer resistance to both seedling and panicle blast, the resistance effect of pyramided lines is not the result of a simple accumulation of resistance spectrum, and only a few *R* genes have been successfully used for molecular breeding. Therefore, novel utilization strategies for rice blast *R* genes in molecular breeding were proposed, such as accurately understanding the utilization of *R* genes in main modern rice varieties, creating a core resistant germplasm with excellent comprehensive traits, screening and utilizing broad-spectrum and durable resistance gene combinations. Lastly, the trends and possible development direction of blast resistance improvement were also discussed, including new genes regulating resistance identified via GWAS (genome-wide association study) and improving rice blast resistance using genetic editing.
Key words: rice blast; resistance gene; molecular breeding strategy

Rice blast, caused by *Magnaporthe oryzae* B.C. Couch, is one of the most destructive diseases encountered in rice production. Once rice is attacked by *M. oryzae*, pattern recognition receptors (PRRs) on the cell surface can specifically recognize pathogen-associated molecule patterns (PAMPs), and activate defense response by cell wall modification, callose deposition, and via expression of defense-related proteins in host cells, which is termed as PAMP-triggered immunity (PTI) (Jones and Dangl, 2006). However, PTI is a weak and non-specific resistance mechanism (Bernoux et al, 2011; Segonzac and Zipfel, 2011). In many cases, *M. oryzae* can secrete certain effectors to inhibit PAMP-induced PTI and can break resistance responses (Jones

and Dangl, 2006; Birch et al, 2009; Block and Alfano, 2011; Mentlak et al, 2012). At the same time, rice has acquired more specific resistance proteins that directly or indirectly recognize pathogen effector proteins. This recognition mechanism activates a second layer of the defense response in rice, known as effector-triggered immunity (ETI), which results in the production of ion (Ca^{2+}, K^+ and H^+) currents, superoxide, nitric oxide, and programmed cell death at the site of invasion (Dangl et al, 1996; Nurnberger et al, 2004). ETI is a highly specialized disease resistance mechanism in the host (Boller and He, 2009), which is activated in the gene-for-gene model upon recognition by an R (resistance) protein of the corresponding effector

protein of *M. oryzae*. Effector proteins are often encoded by avirulence genes in *M. oryzae*.

The *R* genes in rice correspond to the avirulence (*AVR*) genes in *M. oryzae* in a gene-for-gene manner (Flor, 1956), which ensures that the interaction between a specific R protein in rice and the corresponding AVR effector in the pathogen render resistant. To date, more than 40 *AVR* genes have been identified in *M. oryzae*, while 12 of them have been cloned. The cloned genes are *PWL1* (Kang et al, 1995), *PWL2* (Sweigard et al, 1995), *AvrPi-ta* (Jia et al, 2000), *ACE1* (Böhnert et al, 2004), *AvrPiz-t* (Li et al, 2009), *AvrPia* (Miki et al, 2009), *AvrPik/km/kp* (Yoshida et al, 2009), *Avr1-CO39* (Cesari et al, 2013), *AVRPii* (Fujisaki et al, 2015), *AVR-Pi9* (Wu J et al, 2015), *AVRPib* (Zhang et al, 2015) and *AVRPi54* (Ray et al, 2016). The R protein encoded by *R* genes interacts directly or indirectly with the effector protein, thus sensing pathogen invasion and inducing disease resistance. Among the cloned *AVR* and *R* gene pairs, *Pita/AvrPi-ta* (Jia et al, 2000; Orbach et al, 2000), *Pik/AvrPik* (Yoshida et al, 2009; Kanzaki et al, 2012), *Pia/AvrPia* (Miki et al, 2009; Ortiz et al, 2017), *Pi-CO39/Avr1-CO39* (Cesari et al, 2013) and *Pi54/AvrPi54* (Ray et al, 2016) can interact directly with each other. While *Pii/AVR-Pii* (Fujisaki et al, 2015; Singh et al, 2016) and *Piz-t/AvrPiz-t* (Park et al, 2012, 2016; Wang et al, 2016; Tang et al, 2017) require other proteins to complete the interaction. Despite the deployment of resistant varieties, blast epidemics can still occur, due to a lapse in host resistance and the emergence of new virulent pathotypes (Chuma et al, 2011). Several genetic events, including point mutations, insertion of transposable elements, deletion of partial or entire genes, etc, lead the function loss of *AVR* genes in *M. oryzae* (Li W T et al, 2019). Thus, the effectiveness of *R* genes highly dependents on the respective *AVR* gene. Deployment of blast resistant varieties requires regular monitoring of race dynamics, and current and future frequency of *AVR* genes across different regions (Selisana et al, 2017).

Currently, the uses of chemicals and resistant varieties are the main ways of rice blast management. In addition to increased costs, chemical control also causes serious environmental pollution and poses food safety risks. The use of *R* genes to breed resistant varieties remains the top-most economical and effective method to control rice blast (Wu Y Y et al, 2015). This review provided a summary of the identification, cloning and utilization of rice blast resistance genes, key problems in molecular breeding of rice to blast resistance and molecular breeding strategies based on cloned *R* genes. In addition, the trends and possible future development direction of blast resistance improvement were also discussed.

Identification and cloning of rice blast resistance gene

R genes are the foundation for disease resistance research and resistance breeding. The genetic analysis, gene mapping and cloning of rice blast resistance have been intensively studied. Since the first report of independently inherited three *R* genes *Pia*, *Pii* and *Pik* during 1960s (Yamasaki and Kiyosawa, 1966), more than 100 resistance genes or loci have been identified to date (Li W T et al, 2019; Li et al, 2020). *R* genes are distributed on 11 chromosomes of rice genome, except chromosome 3, and more than 64% are clustered in chromosomes 6, 11 and 12, representing 18%, 25% and 21%, respectively (Ashkani et al, 2016).

Since the cloning of first *R* gene, *Pib*, in 1999 (Wang et al, 1999), 31 *R* genes have been successfully cloned (Table 1). Except for *pi21*, which is a recessive *R* gene, the remaining 30 *R* genes are dominant. Among them, all genes except *pi21*, *Pi35*, *Pi63*, *Pb1* and *Pid3-I1* show complete resistance. *Pi-d2* encodes a B-lectin kinase domain protein (Chen et al, 2006), while *pi21* encodes a proline-rich protein with a heavy metal domain (Fukuoka et al, 2009), and *Ptr* encodes an atypical protein with an armadillo repeat (Zhao et al, 2018). Remaining 28 *R* genes encode nucleotide-binding site leucine-rich repeat (NBS-LRR) domain proteins. *Pik*, *Pikm*, *Pik-p*, *Pi1*, *Pike*, *Pi5*, *Pia* and *Pi-CO39* contain two NBS-LRR protein structural genes for blast resistance (Lee et al, 2009; Okuyama et al, 2011; Hua et al, 2012; Cesari et al, 2013). *Pi5-1*, *Pb1*, *pi21* and *Pi63* genes are induced by pathogen infection, while the remaining genes express constitutively. Majority of the cloned *R* genes induce resistance against leaf blast at the seedling stage, while only a few *R* genes, such as *Pb1*, *Pi25* and *Pi64*, confer resistance to panicle blast (Hayashi et al, 2010; Chen et al, 2011; Ma et al, 2015; Cao et al, 2019). Involvement of such a high numbers and types of *R* genes in rice blast resistance breeding applications indicates a complex genetics of this disease interaction.

Key problems in rice blast resistance breeding

Higher variability in *M. oryzae* population and a

Table 1. List of cloned blast resistance genes.

Gene name	Chromosome	Donor	Encoding protein	Reference
Pi37	1	St. No. 1	NBS-LRR	Lin et al, 2007
Pit	1	K59	CC-NBS-LRR	Hayashi and Yoshida, 2009
Pish	1	Shin-2	NBS-LRR	Takahashi et al, 2010
Pi35 [#]	1	Hokkai 188	NBS-LRR	Fukuoka et al, 2014
Pi64	1	Yangmaogu	NBS-LRR	Ma et al, 2015
Pib	2	Tohoku IL9	NBS-LRR	Wang et al, 1999
pi21 [#]	4	Owarihatamochi	Proline-rich metal binding protein	Fukuoka et al, 2009
Pi63/Pikahei-1(t) [#]	4	Kahei	NBS-LRR	Xu et al, 2014
Pi-d2	6	Digu	B-lectin receptor kinase	Chen et al, 2006
Pi9	6	75-1-127	NBS-LRR	Qu et al, 2006
Pi2	6	C101A51	NBS-LRR	Zhou et al, 2006
Piz-t	6	Toride 1	NBS-LRR	Zhou et al, 2006
Pi-d3	6	Digu	CC-NBS-LRR	Shang et al, 2009
Pi25	6	Gumei 2	CC-NBS-LRR	Chen et al, 2011
Pi50	6	Er-Ba-zhan (EBZ)	NBS-LRR	Su et al, 2015
Pigm	6	Gumei 4	NBS-LRR	Deng et al, 2017
Pid3-I1	6	MC276	CC-NBS-LRR	Inukai et al, 2019
Pi36	8	Q61	CC-NBS-LRR	Liu et al, 2007
Pi5	9	Moroberekan	CC-NBS-LRR	Lee et al, 2009
Pii	9	Hitomebore	NBS-LRR	Takagi et al, 2013
Pikm	11	Tsuyuake	NBS-LRR	Ashikawa et al, 2008
Pb1 [#]	11	Modan	CC-NBS-LRR	Hayashi et al, 2010
Pi54	11	Tetep	NBS-LRR	Sharma et al, 2010
Pia	11	Aichi Asahi	CC-NBS-LRR	Okuyama et al, 2011
Pik-p	11	K60	CC-NBS-LRR	Yuan et al, 2011
Pik	11	Kusabue	CC-NBS-LRR	Zhai et al, 2011
Pi1	11	C101LAC	NBS-LRR	Hua et al, 2012
Pi-CO39	11	CO39	CC-NBS-LRR	Cesari et al, 2013
Pike	11	Xiangzao 143	NBS-LRR	Chen et al, 2015
Pi-ta	12	Yashiro-mochi	NBS-LRR	Bryan et al, 2000
Ptr	12	Katy	Atypical protein with an armadillo repeat	Zhao et al, 2018

NBS-LRR, Nucleotide-binding site leucine-rich repeat; CC-NBS-LRR, Coiled-coil-nucleotide-binding site leucine-rich repeat. [#] *R* genes showing partial resistance.

frequent emergence of new virulent races cause a high selection pressure, resulting the resistant varieties often 'losing' resistance within 3–5 years of cultivation and becoming susceptible. Therefore, integration of broad-spectrum and durable resistance has become a key issue among rice breeders (Wu et al, 2007). However, because of a higher number of *R* genes in rice, their deployment and utilization become an important challenge in blast resistance breeding to achieve broad-spectrum and durable resistance. Generally, two main strategies are deployed, the use of broad-spectrum resistance genes and gene pyramiding. However, some problems with these strategies remain to be solved.

Few *R* genes are effective to both seedling and panicle blast

Among the identified and cloned resistance genes, *Pi1*, *Pi5*, *Pi33*, *Pi54*, *Piz*, *Piz-t*, *Pi2*, *Pi9*, *Pi40* and *Pigm* are broad-spectrum resistance genes to leaf blast. *Pi1* in the LAC23 cultivar from West Africa shows resistance to 98% of 792 *M. oryzae* isolates in China

(Chen et al, 2001). *Pi5* shows resistance against 6 races from the Philippines, and 26 of 29 isolates from Korea (Jeon et al, 2003). *Pi33*, which interacts with *AVR* gene *ACE1*, is located on the short arm of chromosome 8, and shows resistance to over 2000 isolates originating from 55 countries (Berruyer et al, 2003). *Pi54* identified in a highly resistant variety Tetep is also confirmed to have broad-spectrum resistance against predominant races found in India (Thakur et al, 2015). Six *R* genes, *Pi2*, *Pi9*, *Pi40*, *Pigm*, *Piz-t* and *Piz*, harbor alleles of the *Piz* locus located on the short arm near the centromere of rice chromosome 6 (Qu et al, 2006; Zhou et al, 2006; Jeung et al, 2007; Deng et al, 2017). The *Piz* gene cluster from the US cultivar Zenith shows resistant to five US races (IH-1, IG-1, IC-17, IE-1 and IE1k) (RoyChowdhury et al, 2012). *Pi9* confers a high level of resistance to 43 isolates collected from 13 countries (Qu et al, 2006). *Pi2* was cloned from a gene cluster composed of nine gene members (named *Nbs1-Pi2* to *Nbs9-Pi2*) encoding proteins with an NBS-LRR

structure (Zhou et al, 2006). Lines carrying *Pi2* confer resistance to 455 isolates collected from different regions of the Philippines and most of the 792 isolates from the 13 major rice regions of China (Chen et al, 1996). *Piz-t* strongly resembles to *Pi2* in sequence and structure having only eight amino-acid differences within the three leucine-rich repeats (Qu et al, 2006; Zhou et al, 2006). *Pi40* from *O. australiensis* shows broad-spectrum resistance to rice blast races from South Korea (Suh et al, 2009). *Pigm*, a resistance gene from Gumei 4, a local variety in China, is resistant to 50 isolates from all over the world (Deng et al, 2017; Zeng et al, 2018). *Bsr-d1*, cloned from a Chinese local variety Digu, is an atypical resistance gene that encodes a C_2H_2 transcription factor protein, which exhibits similar phenotypic incomplete resistance to several races of rice blast (Li et al, 2017). However, it should be noted that the broad-spectrum resistance of the above-mentioned *R* genes was mostly evaluated in different genetic backgrounds, thus the resistant phenotypes might have been masked by other *R* genes.

Among various disease symptoms caused by *M. oryzae*, leaf blast and panicle blast are the most common. However, resistance to leaf and panicle blast is often inconsistent, and many varieties with high resistance to leaf blast at the seedling stage show susceptibility to panicle blast at the heading stage (Puri et al, 2009; Ishihara et al, 2014). Transcriptomic analysis showed that distinct defense-related gene expression is induced by leaf blast and panicle blast, suggesting that the genetic mechanisms of leaf blast and panicle blast resistance might differ and are independently controlled by different *R* genes (Liu et al, 2016). Disease evaluation via artificial inoculation at the seedling stage is a high throughput method with clear resistance/susceptibility phenotyping, which ultimately leads to most research focusing on leaf blast screening. However, less research has been carried out on the genetics of panicle blast resistance because of the increased fieldwork, complex phenotype evaluation system and high influence of environmental conditions for artificial inoculation testing. Presently, the evaluation of panicle blast resistance is mainly performed in disease nurseries under natural conditions. The above-mentioned broad-spectrum *R* genes such as *Pi1*, *Pi2*, *Pi5*, *Pi9*, *Pi33*, *Pi40*, *Pi54*, *Pigm*, *Piz-t*, *Piz* and *bsr-d1* have not been evaluated using different isolates of *M. oryzae* by artificial inoculation at the heading stage. Therefore, it is not clear whether they exhibit broad-spectrum resistance to panicle blast.

Recently, Wu et al (2016, 2017) constructed a set of near-isogenic lines (NILs) for six resistance alleles of the *Piz* locus (*Pi2*, *Pi9*, *Pi40*, *Pigm*, *Piz-t* and *Piz*) in the genetic background of the *indica* rice Yangdao 6 and *japonica* rice 07GY31. Using an improved method of artificial inoculation, the panicle blast resistance evaluation of these NILs was carried out with representative isolates of *M. oryzae* collected from different ecological regions of China. Only *Pigm* show stable broad-spectrum resistance to leaf blast and panicle blast in the genetic background of *indica* and *japonica* rice (Wu et al, 2016, 2017). However, *Pi2*, *Pi9*, *Pi40*, *Piz-t* and *Piz* only show specific resistance to the blast population in some ecological areas, and the resistance frequency of panicle blast is significantly lower than that of leaf blast (Fig. 1) (Wu et al, 2016, 2017). Therefore, there are few of broad-spectrum *R* genes that can effectively protect against both seedling and panicle blast.

Resistance gene pyramiding effects is not simple accumulation of resistance spectrum of certain target *R* genes

Gene pyramiding is generally considered an effective way to develop varieties with broad-spectrum and durable resistance. The rice variety Jefferson, with the gene combination *Pik/Piz*, has remained resistant since its first application in 1997 (McClung et al, 1997; Fjellstrom et al, 2004). Chen et al (2001) showed that resistance frequencies of monogenic lines with *Pi1* and *Pi2* are 92.45% and 89.65%, respectively, and those of polygene pyramiding lines (PPLs) with *Pi1* and *Pi2* are as high as 98.04% against 715 isolates of *M. oryzae*. The additive effect of the monogenic lines broadens the resistance spectrum of the PPLs, resulting in an increase in the blast resistance. Similarly, pyramiding of *Pi46* and *Pi-ta* enhances resistance compared to *Pi46* and *Pi-ta* monogenic lines (Xiao et al, 2016). Yu et al (2013) confirmed pyramiding of two genes with different overlapping resistance spectra can improve the resistance of plants. However, the resistance effect of pyramiding lines does not comprise the simple accumulation of the resistance spectrum of target *R* genes. There is a significant interaction among the pyramided *R* genes, causing both positive and negative deviation (Tabien et al, 2000). Therefore, a random combination of two or more *R* genes can result a lower resistance effect of PPLs than the monogenic lines. International Rice Research Institute constructed a set of NILs carrying
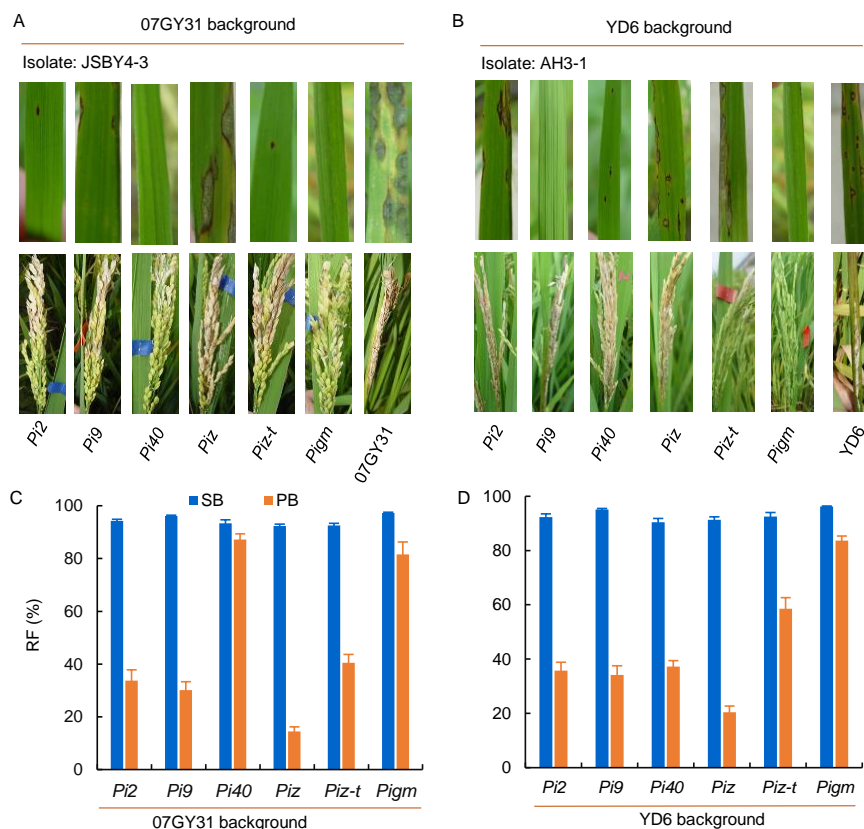
Fig. 1. Comparison of leaf blast and panicle blast resistance among different alleles of *Piz* locus.

A, Resistance reaction to leaf blast and panicle blast of different alleles from the *Piz* locus *japonica* rice 07GY31 against the *M. oryzae* isolate JSBY4-3. **B**, Resistance reaction to leaf blast and panicle blast of different alleles from the *Piz* locus in the background of *indica* rice Yangdao 6 (YD6) against the isolate of *M. oryzae* (AH3-1). **C**, Resistance frequency (RF) to leaf blast and panicle blast of different alleles of the *Piz* locus in the background of *japonica* rice 07GY31. **D**, RF to leaf blast and panicle blast of different alleles of the *Piz* locus in the background of *indica* rice Yangdao 6. SB, Seedling blast; PB, Panicle blast. Data are Mean ± SD (*n* = 3). Blast resistant phenotypes were reproduced from Wu et al (2016, 2017).

Pi1, *Pi2* and *Pi-ta*, as well as PPLs carrying 2–3 *R* genes in the background of CO39. The results showed that the resistance of PPL$^{Pi2/Pi-ta}$ is lower than that of the lines harboring only *Pi2* (Hittalmani et al, 2000). Similarly, He et al (2001) found that the resistance of PPL$^{Pi1/Pi-ta}$ is lower than that of the monogenic lines. In the background of *japonica* 07GY31, the resistance level of PPL$^{Piz-t/Pi54}$ is significantly higher than that of monogenic lines harboring a single *R* gene. However, the resistance level of PPL$^{Pi9/Pi54}$ is significantly lower than that of monogenic lines harboring *Pi9* (Xiao et al, 2017). In the *indica* background of Yangdao 6, the resistance frequency of monogenic lines with *Pi33* is slightly higher (70.8%) than that of the combination *Pi33* with *Pish* PPL$^{Pi33/Pish}$ (69.1%) (Xiao et al, 2018). Recently, Wu et al (2019) evaluated the resistance effects of different alleles of *Piz* locus (*Pigm*, *Pi40*, *Pi9*, *Pi2* and *Piz*) combined with other broad-spectrum *R* genes (such as *Pi1*, *Pi33* and *Pi54*) systematically. They found that different gene combinations produce different interaction effects, in which most PPLs show no resistance comprising the simple accumulation of the resistance spectra of the target *R* genes. Among them, *Pigm/Pi1*, *Pigm/Pi54* and *Pigm/Pi33* are the most effective gene combination patterns, displaying stable broad-spectrum resistance under various conditions. Therefore, the combination of *R* genes directly affects the resistance level of the PPLs. Thus, to achieve broad-spectrum resistance to both leaf blast and panicle blast, resistance gene combination patterns must be assessed first in gene pyramiding breeding.

Only a few *R* genes have been successfully used in molecular breeding practice

The development of molecular biology has brought rice breeding to the stage of combining biotechnology with conventional technology. In the last decade, molecular breeding technology, represented by molecular marker-assisted selection, has played an important role in the improvement of rice blast

resistance in elite recipients. Many studies have been reported in this area, and some successful and representative examples are listed in Table 2. The donor *R* genes used in these molecular breeding studies are mainly alleles or tightly linked *R* genes from three loci, *Piz*, *Pik* and *Pi-ta*, including *Pi2*, *Pi9*, *Pi40*, *Piz-t* and *Pigm* of the *Piz* locus; *Pi1* and *Pi54* in the *Pik* locus, and *Pi-ta* from *Pi-ta* locus. Since there are so many identified *R* genes, why is molecular breeding practice only limited to few *R* genes? The reasons might be as follows: Firstly, the alleles or tightly linked *R* genes from these loci often exhibit relatively broad-spectrum resistance, especially in leaf blast resistance at the seedling stage. However, many *R* genes in other loci have a narrower resistance spectrum or less resistance effect, resulting in no obvious effect using other *R* genes in resistant improvement. Secondly, the cumbersome chain of linkage drag of some *R* genes produces negative effects on agronomic traits, which limits their utilization in breeding practice.

Utilization strategies of rice blast resistance genes in molecular breeding

Understanding utilization of rice blast *R* genes in main modern varieties

An important prerequisite for molecular breeding improvement of rice blast resistance is to understand which *R* genes have been utilized in modern varieties and whether the resistance performance of these *R* genes is effective. Currently, the analysis of resistance genotypes in modern improved varieties uses linked markers or functional markers for cloned *R* genes. For example, Xiang et al (2018) analyzed distribution and use of rice blast resistant genes in the main cultivated rice varieties from Heilongjiang Province, China. The distribution frequencies (DFs) of *Pi-ta*, *Pi5* and *Pib* are higher than those of other genes, reaching 31.37%, 29.41% and 18.62%, followed by *Pi2*, *Pi-d2* and *Pi-d3* with DFs of 9.80%, 1.96% and 1.96%, respectively. However, no *Pi9* is detected. *Pi-ta*, *Pib*, *Pikm*, *Pi54* and *Pi5* are detected in the core rice germplasms in Ningxia Province, China (Li Y D et al, 2019). Ma et al (2018) identified relatively high DFs of *Pi5* and *Pi54* in local varieties in Guizhou Province, China, at 32.35% and 30.86%, respectively. While the DFs of *Pi9* and *Pi2* are relatively lower, at 2.56% and 2.47%, respectively. Wu Y Y et al (2015) analyzed the distribution of cloned *R* genes in 277 main *indica* and *japonica* parental lines and showed that *Pi5*, *Pi-d2*, *Pi-ta*, *Pia*, *Pib*, *Pb1*, *Pi54*, *Pish* and *Piz* have relatively higher DFs (> 15%), whereas *Pi2*, *Piz-t*, *Pi-d3*, *Pik* and *Pit* have relatively lower DFs (< 10%), and *Pi1*, *Pi9*, *Pi40* and *Pigm* have DFs of less than 2% and are found in local varieties, related wild species,

Table 2. Successful examples of application of broad-spectrum resistance genes in rice breeding practice.

Target gene	Recipient parent	Chromosome	DNA marker used	Reference
Pi2	C815S	6	RM527	Jiang et al, 2015
Pigm	Kongyu 131, Longjing 26, Kenjiandao 6	6	M80362	Tian et al, 2016
Pigm	KT27S	6	G8900	Dai et al, 2018
Pi9	E32	6	Ins2-3	Zou et al, 2017
Pi9	R288	6	Clon2-1	Xing et al, 2016
Pi9	Q211S	6	Nbs21	Huang et al, 2018
Pi40	Osmancik-97, Halilbey	6	9871.T7E2b	Beser et al, 2016
Pi1	BPT5204	11	RM224	Kumar et al, 2019
Pi54	BPT5204	11	Pi-54MAS	Kumar et al, 2018
Pi54	R1, R2	11	RM224	Khan et al, 2018
Pi1/Pi2	GD-7S	6, 11	RM144, AP22	Liu et al, 2008
Pi1/Pi2	Pusa RH-10	6, 11	RM5926, AP5659-5	Gouda et al, 2013
Pi1/Pi2, Pi1/Pigm	GZ63S, 97S, R084, R609	6, 11	RM224, ZJ58.7, AP22	Yu et al, 2013
Pi9/Pi-ta	Pusa Basmati 1	6, 12	AP5659-5, YL155/YL87//YL155/87	Khanna et al, 2015
Pi46/Pi-ta	Hanghui 179	11, 12	RM224, YL155/YL87//YL155/87	Xiao et al, 2016
Pib/Pik	K6415	2, 11	NSb, K6415	Hayashi et al, 2006
Pib/Pi54	MR219	2, 11	RM208, RM206	Tanweer et al, 2015
Pi2/Pi54	PB1121	6, 11	AP5659-5, RM206	Ellur et al, 2016
Pi2/Pi54	PRR78	6, 11	AP5930, RM206	Singh et al, 2012
Piz-t/Pi54, Pi9/Pi54	07GY31	6, 11	Z4794, Pikh-1	Xiao et al, 2017
Pi1/Pi2/Pi33	Kuboyar	6, 8, 11	RM224, RM527, RM310	Usatov et al, 2016
Pi1/Pi2/Pi33	ADT43	6, 8, 11	RM224, RM527, RM25	Divya et al, 2014
Pi1/Pi2/D12	Jin 23B	6, 11, 12	RM144/RM224, PI2-4/HC28, RM277/RM309	Jiang et al, 2012
Pi1/Pi54/Pi-ta	Mushk Budji	11, 12, 12	Pi54MAS, RM224, YL155/YL87//YL155/87	Khan et al, 2018

or improved intermediate materials. Further analysis showed that some *R* genes are specifically distributed in the genomes of rice sub-species, for example, *Pi5*, *Pit*, *Pi-d2* and *Pi-d3* are mainly distributed in *indica*-type accessions, and *Piz*, *Piz-t*, *Pb1*, *Pik* and *Pish* are mainly harbored in *japonica*-type accessions, while *Pi-ta*, *Pib*, *Pia* and *Pi54* are evenly distributed in both accessions. The above results provide us with a general understanding of the *R* genes used in the main rice parental lines in China. However, it should be noted that the judgment of the existence of the target *R* genes in the above studies are based on closely linked molecular markers or functional markers. Therefore, detection using molecular markers has a certain degree of correlation with *R* genes, but is more of an inference than a certainty, and cannot accurately reflect the presence of the target *R* gene. With the advances in third generation genome sequencing technology and the reduction of sequencing costs, it has become easier to quickly obtain high quality whole genome sequences.

Creation of core resistant germplasm with excellent comprehensive traits

The existence of a defined resistant germplasm with an *R* target gene is an important prerequisite for breeding applications. Apart from the above-mentioned cloned *R* genes and some *R* genes used in modern improved varieties, many broad-spectrum genes, including *Pigm*, *Pi9*, *Pi40*, *Pi1*, *Pi33* and *Pi56*, have not been utilized in modern varieties. These *R* genes are mainly distributed in local varieties or germplasms and might exhibit drag, causing poor agronomic traits and low yield. To promote the utilization of these genes in molecular breeding, it is necessary to overcome the linkage drag and create core resistant germplasms with excellent and comprehensive agronomic traits. Some explorations in this field have been made. For example, *Pigm* is a broad-spectrum *R* gene against both leaf and panicle blast (Wu et al, 2016, 2017). However, *Pigm* also has the effect of reducing grain weight but increasing grain number (Deng et al, 2017). Although it can achieve yield balance, decrease in grain weight will lead to a reduction in rice yield and its marketability. Grain weight is a quantitative trait controlled by multiple genes, and therefore, it is affected by many other genes besides *Pigm*. In theory, individuals with no significant decrease in grain weight can be obtained by large-scale selection. Therefore, Wu et al (2016, 2017) used the elite restorer line Yangdao 6

(9311) as the recurrent parent, crossed it with the *Pigm* donor Gumei 4, and backcrossed the progeny continuously. A series of NILs have been obtained through foreground selection of target genes and large-scale screening of agronomic traits. The results of agronomic trait investigation showed that although the grain weight of some NILs is decreased, there are also some lines with similar grain weight and other elite agronomic traits to the recurrent parents (Fig. 2-A and B). Finally, core resistance germplasm carrying *Pigm* was selected and named as R9311. Using R9311 as the restorer line, two-line hybrid rice Yangliangyou 309 is bred. Moreover, using R9311 as the *R* gene donor parent, two-line cytoplasmic male sterility (CMS) line Yangxian 6S and three-line CMS line Yangxian 9A are bred, followed two-line hybrid rice Yangliangyou 612 and three-line hybrid rice Yangxianyou 919 (Fig. 2-C). These hybrid rice combinations performed well in regional trials and production tests (Fig. 2-D), and have also been approved by national certification. This serves as a successful example of the creation of core germplasm and molecular breeding for rice blast resistance.

Screening and utilization of broad-spectrum and durable resistance gene combinations

Gene pyramiding helps to develop varieties with broad-spectrum and durable resistance to *M. oryzae*. Many studies have shown that resistance is significantly associated with the number of *R* genes, which means that the greater the number of *R* genes found in the accessions, the higher their resistance against *M. oryzae* (Wu Y Y et al, 2015; Li et al, 2019). However, the number of *R* genes is not the only factor affecting resistance. On one hand, as the number of *R* genes increases, the improvement in the resistance level would gradually slow down, which term as law of 'diminishing returns' between the number of pyramided *R* genes and resistance (Xiao et al, 2018; Wu et al, 2019). On the other hand, as the number of pyramided *R* genes increases, in addition to increasing the workload, the linkage drag with unacceptable traits might also increase. Therefore, how to employ a few of these *R* genes to achieve broad-spectrum and durable resistance must be considered during rice blast resistance breeding (Yang et al, 2008). From the perspective of pyramided *R* genes and their corresponding resistance, the combination pattern of *R* genes is a key factor in determining the resistance level of different varieties against *M. oryzae* (Wu Y Y et al, 2015). Once
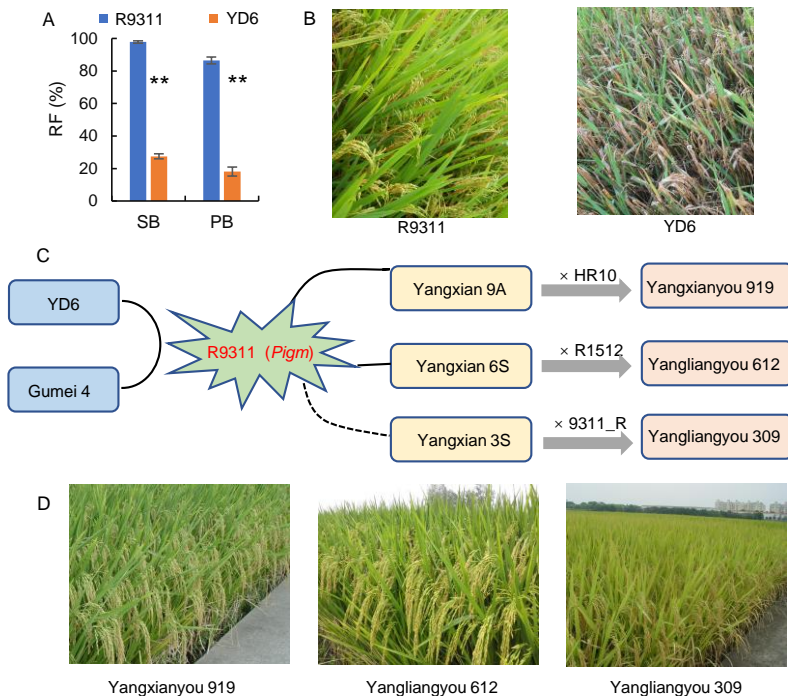
Pigm, should be used as the backbone and core for gradual pyramiding of other *R* genes at three levels (Fig. 3). Firstly, pyramiding with *Pi54* from the *Pik* locus, *Pi-ta/Ptr* from the *Pi-ta* locus, and the independently distributed *R* genes *Pi5* and *Pish*. These *R* genes have higher DFs, less linkage drag of poor agronomic traits, and are relatively easy to integrate. Secondly, pyramiding with *Pi1* from the *Pik* locus, *Pi48* from the *Pi-ta* locus, and the independently distributed *R* genes *Pi33* and *Pi56*. These *R* genes are generally distributed in local varieties or original germplasms and can be used in pyramiding breeding by creating core germplasms carrying target *R* genes and showing elite agronomic traits. Thirdly, on the basis of further clarifying the resistance effect of gene combinations, pyramiding could be performed using certain partial resistance genes, such as *Pb1*, *Pi35*, *Pi63* and *bsr-d1*.

Conclusions and future perspectives

The development of genome sequencing technology has promoted the rapid identification and cloning of rice blast *R* genes and *AVR* genes, which has deepened understanding of the molecular mechanisms of rice blast fungus interaction and their co-evolution. It provides not only new genetic resources for rice blast resistance improvement, but also new technical ideas to regulate disease-resistance signaling pathways via genetic editing to achieve resistance improvement.

High-throughput whole genome sequence or target gene sequencing in the elite rice cultivars or core resistant germplasm will provide useful technological means for breeding selection. Since the cloning of *Pi2*, *Pi9* and *Pigm* (Deng et al, 2017; Yang et al, 2019), functional single nucleotide polymorphism related to resistant genotypes can be used to design Kompetitive Allele-Specific PCR (KASP) markers and used in

different *R* genes are pyramided, some gene combinations will show positive interactions, while other gene combinations might exhibit negative interactions (Hittalmani et al, 2000; Xiao et al, 2017; Wu et al, 2019). Therefore, it is essential to screen and determine the combination pattern of *R* genes that exhibit broad-spectrum and durable resistance to promote the practical use of molecular breeding for blast resistance.

Several successful molecular breeding studies (Table 2) have indicated that a single *R* gene, such as *Pi2*, *Pi9*, *Piz-t* or *Pi40* from the *Piz* locus shows broad-spectrum resistance to leaf and specifically exhibits resistance to panicle blast in some ecological regions. However, only *Pigm* shows broad-spectrum resistance to both leaf and panicle blast, and has an important practical value in breeding. Combining *Pi2* with *Pi1* from the *Pik* locus is the most successful example of the application of gene pyramiding, and there have also been reports about pyramiding with *Pi5* and *Pi-ta*. Wu et al (2019) reported that *Pigm* exhibits high resistance to leaf and panicle blast after pyramiding with *Pi1*, *Pi33* and *Pi54*, respectively. Moreover, they also found that the *R* genes of the alleles from the *Piz* locus exhibit excellent resistance after combining them with certain independently distributed *R* genes, such as *Pi56* (unpublished data) and *Pish* (Xiao et al, 2018). Based on the distribution and utilization of cloned genes, we proposed that the broad-spectrum *R* genes of the *Piz* locus, especially
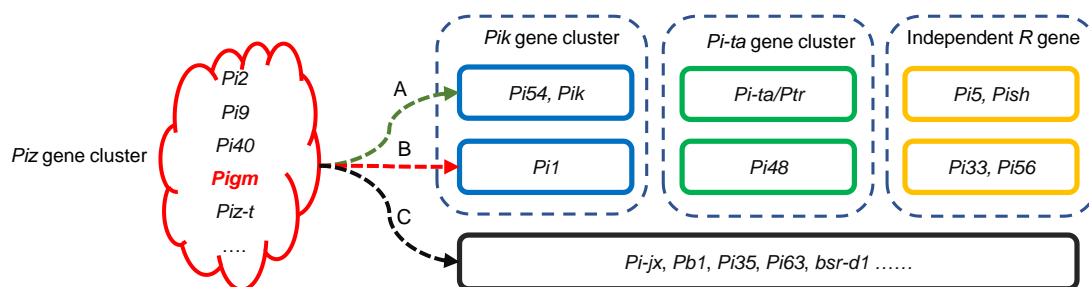
Fig. 3. Molecular breeding strategies using rice blast resistance genes.
  A, Breeding strategies for improving blast resistance in the previous studies. **B**, Breeding strategies that have been used. **C**, Novel breeding strategies that can be utilized in further research.

marker-assisted selection. Wang et al (2019) sequenced an important broad-spectrum blast resistant germplasm (Tetep) that is the donor of *Pi5*, and obtained a high-quality assembly, in which 455 nucleotide-binding site leucine-rich repeat (NLR) genes are annotated. Based on this, a few molecular markers have been designed to rapidly introduce clustered and paired NLRs in the Tetep genome to breed new resistant cultivars. With more donors of *R* genes being sequenced, the *R* genes confering a resistant genotype on elite rice cultivars or core resistant germplasms could be identified.

  With the increase in genetic research into blast resistance, understanding of the partial resistance of non-race specificity, controlled by multiple quantitative trait loci (QTLs), has became a hot topic. Partial resistance is generally regarded as a quantitative trait. It does not prevent infection by *M. oryzae* but can reduce the proliferation of pathogens in the host and maintain a relatively low selection pressure on the population of *M. oryzae*, thus maintaining broad-spectrum and durable resistance (Niks et al, 2015). Since Wang et al (1994) first used restriction fragment length polymorphism markers to identify 19 QTLs controlling partial resistance in the durable resistance African upland rice cultivar Moroberekan, at least 500 QTLs for resistance to rice blast have been identified on the 12 chromosomes of rice to date (Li et al, 2019). In recent years, with the rapid development of molecular biology, a large number of partial resistance genes such as *pi21* (Fukuoka et al, 2009), *Pb1* (Hayashi et al, 2010), *Pi35* (Fukuoka et al, 2014), *Pi63* (Xu et al, 2014), *bsr-d1* (Li et al, 2017), *Bsr-k1* (Zhou et al, 2018) and *Pid3-I1* (Inukai et al, 2019) have been cloned, suggesting that the rapid introduction of these *R* genes into rice varieties through molecular breeding is feasible (Pilet-Nayel et al, 2017). Fukuoka et al (2015) first reported that the four partial resistance genes, *pi21*, *Pi34*, *qBR4-2* and *qBR12-1*, can be combined to improve durable resistance of rice.

However, because of the small resistant effect of a single partial *R* gene, it must be combined with multiple partial resistance genes to obtain effective resistance. With the establishment of high-throughput molecular breeding methods, the creation of core germplasms harboring target partial resistance without linkage drag will become an important step in rice blast resistance improvement in the future.

  In recent years, a series of important advances have been made in understanding the molecular mechanism of rice blast resistance. Significant progress has been achieved in cloning and identifying a number of key PTI and ETI signal regulation genes (Liu et al, 2014; Nasir et al, 2018), downstream signaling pathway related genes (Choi et al, 2015), and R proteins, especially downstream signaling molecules directly regulated by NBS-LRR proteins. However, compared with those 30 *R* genes that have been cloned, there has been less research on the downstream signaling molecules of *R* genes. Currently, three kinds of downstream signaling molecules that interact directly with *R* genes have been identified: *OsRac1* signaling pathways downstream of *Pit*, *Pia* and *Pi-d3* (Chen et al, 2010; Kawano et al, 2010; Wang et al, 2018; Zhou et al, 2019); the ARM repeats of *OsPUB15* and *Ptr*(t) downstream of *Pi-d2* and *Pi-ta*, respectively (Jia and Martin, 2008; Wang et al, 2015; Zhao et al, 2018); and transcription factor signaling pathways of *WRKY45* and *OsBIHD1*, which act downstream of *Pb1* and *Pik-H4*, respectively (Inoue et al, 2013; Liu et al, 2017). *OsRac1* encoding a guanylate triphosphatase (GTPase), a member of the RhoGTPase family, is a key regulator of rice resistance to pathogens and it can participate in both PTI (Akamatsu et al, 2013) and ETI (Kawano et al, 2010). Zhou et al (2019) suggested that *OsRac1* might be a common regulatory factor downstream of rice NBS-LRR proteins. Therefore, strengthening research into the interaction between *OsRac1* and other *R* genes, especially the study of

different broad-spectrum alleles from the *Piz* and *Pik* loci, should be promoted. At the same time, further identification of new signaling genes and analysis of the molecular regulatory mechanism of broad-spectrum resistance against *M. oryzae* are needed. *OsERF922*, an APETELA2/ethylene response factor (AP2/ERF) type transcription factor in rice, is rapidly and strongly induced by avirulent pathovars of *M. oryzae*. When the expression of *OsERF922* was inhibited in RNAi transgenic lines, their blast resistance was enhanced (Liu et al, 2012). Similar research showed that mutant lines of *OsERF922* edited by CRISPR/Cas9 show significantly higher resistance to *M. oryzae* compared with the wild type (Xu et al, 2019). The expression levels of defense-related genes involved in signaling pathways of salicylic acid, jasmonic acid and ethylene metabolisms are upregulated in the mutant lines after inoculation of the physiological races of *M. oryzae*. Otherwise, some QTLs identified as necessary loci are required for resistance to rice panicle blast. Inoue et al (2017) mapped four QTLs that contribute to *Pb1*-mediated panicle blast resistance. In addition, a genome-wide association study of blast resistant loci or genes was used to identify novel *R* genes, and new *Pik* alleles, *Pikx* and *Pif*, respectively (Wang et al, 2014; Li C G et al, 2019). These identified genes not only provide new genetic resources for breeding broad-spectrum and durable rice cultivars, but also provide new strategies to improve resistance to rice blast.

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