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Two novel gene-specific markers at *Pik* locus facilitate the application of rice blast resistant alleles in breeding

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Abstract

Background: Rice blast disease, caused by *Magnaporthe oryzae*, is a major constraint for rice production in the world. Introgression of blast-durable resistance genes into high-yielding rice cultivars has been considered an agricultural priority in an effort to control the disease. The blast resistance *Pik* locus, located on chromosome 11, contains at least six important resistance genes, but these genes have not been widely employed in resistance breeding since existing markers hardly satisfy current breeding needs owing to their limited scope of application..

Results: In the present study, two PCR-based markers, *Pikp*-Del and *Pi1*-In, were developed to target the specific InDel (insertion/deletion) of the *Pik-p* and *Pi-l* genes, respectively. The two markers precisely distinguished *Pik-p*, *Pi-l*, and the K-type alleles at the *Pik* locus, which is a necessary element for functional genes from rice varieties.

Conclusions:Two gene-specific markers of *Pi-kp* and *Pi1* identified that only several old varieties contain the two genes, nearly half these varieties yet carry the K-type alleles. Therefore, these identified varieties can be new gene sources for developing blast resistant rice. The two newly developed markers should be highly useful for using *Pi-kp*, *Pi1* and other resistance genes at the *Pik* locus in marker-assisted selection (MAS) breeding programs.

Keywords: Rice, Blast disease, Molecular marker, *Pik-p*, *Pi-l*, K-type alleles

Background

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world that provides a staple food source for over half of the world's population (Khush 2005). One major obstacle encountered in rice production is the frequent widespread occurrence and damage of rice blast, caused by the ascomycete fungus *Magnaporthe oryzae* (Jeung et al. 2007). Cloning and utilizing resistance genes in rice breeding is an important and effective approach to enhance cultivar resistance in an economical and safe manner (Zeigler et al. 1994). To date, more than 100 resistance (*R*) genes have been found, and at least 28 of these genes have been successfully cloned (Deng et al. 2017; Li et al. 2019; Ma et al. 2015). Three of which are important clusters of blast *R* genes that have been reported to confer durable and broad-spectrum resistance (Chen et al. 2016; Deng et al. 2017; Wang et al. 2017). One such cluster is the *Pik* locus on chromosome 11, which has been commonly considered to be the major locus in control of rice blast disease since it contains at least 6 identified blast *R*-genes, including *Pi-1* (Hua et al. 2012), *Pik-h* (Zhai et al. 2014), *Pi-k* (Zhai et al. 2011), *Pik-m* (Ashikawa et al. 2008), *Pik-s* (Wang et al. 2009), and *Pik-p* (Yuan et al. 2011). Previous studies have shown that many *R*-genes from the *Pik* locus confer broad-spectrum resistance to a diverse array of *M. oryzae* isolates (Hua et al. 2012). Due to this fact, the *Pik* locus has the potential to be used in a marker-assisted selection (MAS) breeding program for the improvement of blast resistance in rice.

Gene-specific markers developed from DNA polymorphisms among the alleles are particularly useful for MAS breeding and screening large amounts of germplasm for allelic diversity (Brar and Khush 1997; Kumari et al. 2013; Nawaz et al. 2017; Shomura et al. 2008; Xu et al. 2005). Such markers have been developed for genes including *Pit*, *Pi-2*, *Pi-9*, and *Pig-m* (Hayashi et al. 2010; Tian et al. 2016; Wang et al. 2019). In the case of the *Pik* locus, which displays high similarity among *Pi-1*, *Pik-h*, *Pi-k*, *Pik-m*, *Pik-s*, *Pik-p*, and wild rice clusters from the same region (Hua et al. 2012; Zhai et al. 2011), it is difficult to differentiate these genes from

each other by simple sequence repeat (SSR) markers. Though several derived cleaved amplified polymorphic sequences (dCAPS) have been developed that allow for screening of *Pi-l*, *Pik-p*, *Pik-m*, and *Pi-k* genes in cultivars (Chen et al.2016; Hua et al. 2012; Zhai et al. 2011), it is inconvenient to apply these markers to large-scale breeding practices for dCAPS markers since they need to be digested with PCR products and restriction endonucleases, which is laborious and time consuming.

The objectives of this study were:1) to analyze polymorphism in the *Pik* locus and develop *Pi-l*, *Pik-p*, and K-type allele marker systems; 2) using the newly developed marker systems to identify the presence of two blast *R*-genes in a large collection of 531 rice accessions; and 3) to verify the resistance of representative rice varieties by pathogenicity tests. Development of the gene-specific markers has great potential applicability in MAS breeding programs.

Results

Functional markers for *Pi-l*/*Pik-p*/K-type genotypes

To determine the genotypes of *Pi-l* and *Pik-p*, an extensive sequence alignment was conducted to analyze the InDel polymorphisms among the sequences of *Pi-l*, *Pi-k*, *Pik-m*, *Pik-p*, *Pik-s*, and *Pik-m*. A conserved 19-bp deletion at the -1015-bp location of *Pikp-l*, which is located at the promoter region of *Pikp-l*, and a 10-bp insertion positioned at +6816-bp of *Pi-l*-5-terminal were identified (Fig. 1A). To further verify the two identified InDel polymorphisms, two pairs of specific primers, *Pikp-Del* and *Pi-l-In*, were designed to develop a molecular marker that targeted the regions containing the identified InDel (Table 1). PCR fragments corresponding to the two regions of *Pi-l*, *Pik-p*, *Pi-k*, *Pik-m*, *Pik-h*, *Pik-s*, and non-functional N-type alleles were amplified from rice monogenic lines IRBL1-CL-Pi1, IRBLkp-K60-Pik-p, IRBLk-Ka-Pik, IRBLkm-Ts-Pik-m, IRBLkh-K3-Pik-h, IRBLks-S-Pik-s, and Nipponbare with specific primers

for the *Pikp*-Del and *Pi1*-In markers, respectively. Electrophoretic analysis showed that the band-type of *Pik-p* and *Pi-1* were different from the others, and the N-type from Nipponbare had invisible PCR product (Fig. 1D). Therefore, the two developed InDel markers could clearly distinguish the genotypes corresponding to the *Pi-1* and *Pik-p* loci from other functional and non-functional alleles.

Assessment of the *Pi-1*, *Pik-p*, and K-type alleles in 531 rice varieties

A total of 531 rice varieties were assessed for the presence of the *Pik-p*, *Pi-1*, and K-type alleles using the newly developed markers. The 531 rice accessions consisted of 98 *indica* restorer lines of hybrid rice, 49 maintainer/sterile lines of hybrid rice, 328 *indica* conventional cultivars/breeding materials, and 56 *japonica* conventional cultivars/breeding materials (supplementary Table S1). When using the *Pikp*-Del marker for screening, 5 out of 531 and 264 out of 531 accessions were separately identified displaying 368-bp and 377-bp PCR fragment, respectively (supplementary Table S2), suggesting that the 5 accessions might contain the *Pik-p* resistance genes and the 264 varieties had the functional K-type alleles. When using the *Pi1*-In marker for screening, 8 and 259 accessions produced specific patterns identical to that of the *Pi-1* and K-type alleles, respectively. Taken together, these results suggest that the 259 accessions may contain the other resistance genes except for the 8 that contain *Pi-1* and the 5 that have *Pik-p*.

The rice accessions containing *Pik-p*, *Pi1* and K-type identified by the *Pi1*-In and *Pikp*-Del markers were also confirmed with K - and N - type specific primer sets, two dCAPS markers of dCAPS-*Pi1* and dCAPS-795 (Zhai et al. 2011; Hua et al. 2012). Genotyping results of the those markers showed consistent with the results using the *Pikp*-Del and *Pi1*-In markers. The 5 accessions were identified to have a *Pik-p* genotype corresponding to the *Pik-p* allele (Fig. 2A, Table S2), and *Pi1*FPN analysis in the 8 accessions gave a restriction enzyme digestion pattern identical to the *Pi-1* allele (Fig. 2C, Table S2).

Disease reaction of varieties possessing *Pi-1* and *Pik-p* genotypes

To investigate whether the *Pi-kp*, *Pi-1*, and K-type varieties showed corresponding specific resistance, 25 representative rice varieties with *Pi-1*, *Pik-p*, and K-type alleles were selected and inoculated with seven blast pathogen isolates KJ201, CHNOS60-2-3, CHE86061, FJ86, FJ501-3, CHL768 (*AvrPi-1*), and CHL2110 (*AvrPik-p*) (Hua et al. 2012; Pan et al. 2003). The last two isolates were for resistance identification of the corresponding R genes, and the other 5 isolates were used for resistance comparison of the 25 representative varieties.

As shown in Table 2 and Figure 3, the 4 *Pik-p* genotype varieties showed high resistance to CHL2110, FJ86, FJ501-3, CHL768, and CHNOS60-2-3, but moderate resistance to CHE86061, which was similar to or even higher than the disease reactions in the *Pikp*-carrying line IRBLkp-K60 (Table 3). The 8 *Pi1*-type varieties also appeared equivalent resistance to or had stronger resistance than the *Pi1*-carrying near-isogenic line IRBL1-CL. The 6 K-type varieties showed differences in resistance spectrum compared to the *Pik-p*, *Pik-m*, *Pik-h*, or *Pi-1* donor lines, suggesting that these varieties likely possessed other blast R-genes from the *Pik* locus.

Discussion

The *Pik* locus has been considered to confer broad spectrum blast resistance. It contains at least six resistance genes (Ashikawa et al. 2008; Hua et al. 2012; Kiyosawa 1987; Wang et al. 2009, 2010; Zhai et al. 2011). *Pik-p* contributes to higher resistance responses in many regions in China including Guangdong, Jiangsu, and Sichuan provinces, indicating that this gene has greater applicability in these regions (Wang et al. 2009, 2017). *Pi-1* is regarded as having great potential for achieving broad spectrum and durable-resistance against blast (Hu et al. 2010; Jiang et al. 2012; Li et al. 2005; Wang et al. 2017; Yang et al. 2008; Zhang et al. 2010). The other resistance genes at the locus also have different resistance spectra to the blast fungal pathogen (Hua et al.

2012).

Utilization of molecular markers has made it feasible to test for the presence of different R genes, which was previously unattainable whenever several R genes conferred resistance to overlapping spectra of race-resistance in an inoculation test. With the availability of sequence data for functional *R*-genes, gene-specific DNA markers have been developed for several blast *R*-genes, such as *Pit* (Hayashi et al. 2010), *Pi54* (Ramkumar et al. 2011), *Pi-2/Piz-t/Pi-9* (Hua et al. 2015; Tian et al. 2016), and *Pita* (He et al. 2014) by using bioinformatics strategies. Not only can these gene-specific markers be used for screening for novel alleles in germplasm sets, but they can also be an effective selection tools for multiple allelic pyramid breeding.

Since the *Pik* locus contains *Pik-p*, *Pik-m*, *Pik-h*, *Pi-l*, *Pi-k*, and *Pik-s* alleles, which confers broad-spectrum and durable-resistance to rice blast disease (Hua et al. 2012), it is of great significance to accelerate its application in breeding practices. The current study developed *Pikp*-Del and *Pi1*-In markers based on the gene-specific states of *Pik-p* and *Pi-l* genes, respectively. The *Pikp*-Del marker lies in between *Pik-p1*(K1) and *Pik-p2*(K2), only 830-bp and 2,576-bp away from the K1 and K2 marker, respectively. And the *Pi1*-In marker located at the +6816 bp away from the start position of *Pi1*-5(Fig.1). All in all, the present study provides a unique opportunity to swiftly screen large scale germplasm resources for the target gene with high accuracy, which is very different from former dCAPS marker method which needed to digest PCR products with restriction endonuclease. So the newly developed markers have the ability to fix alleles in a population with more efficiency, which will be particularly useful in MAS breeding methods.

Additionally, we found that *Pi-l* or *Pik-p* only exists in several older local or bred varieties, such as RCPL3-2, Ruanmigu and Zaohui62, and few newly-bred varieties contained them, So we can infer that the two genes have not been widely applied in rice breeding. In addition, the 6 representative K-type allelic varieties also showed different resistance responses from the four

isogenic-lines, which implies that there may be some new R alleles in these particular varieties.

Accurate identification of R genes in diverse elite germplasm panels through the use of suitable genetic markers and diverse blast races is the key to correctly utilize the R gene in MAS for breeding programs (RoyChowdhury et al. 2012). Thus these results will be beneficial to rice breeders who seek appropriate and even new R alleles for the improvement of blast resistance from a wider range of germplasm, and contribute towards the development of novel molecular breeding practices.

Conclusions

In this study, we developed two gene-specific markers for *Pik-p* and *Pil*, and identified the presence of the two blast *R*-genes in 531 rice varieties based on the two new developed marker. The results revealed that *Pi-l* or *Pik-p* only exists in several older local or bred varieties and nearly half the identified varieties carry the K-type alleles, supporting that the two blast-*R* genes and the K-type alleles can be important gene sources for breeding of blast resistant in future. Furthermore, the *Pikp-Del* and *Pi1-In* markers will be highly useful in MAS breeding for blast resistance.

Methods

Materials and Methods

Plant material and pathogen isolates

The 531 rice varieties were maintained at Fujian Provincial Key Laboratory of Genetic Engineering for Agriculture, Fujian Academy of Agricultural Sciences, Fuzhou, China. Six

monogenic lines, IRBLkh-K3 (*Pik-h*), IRBLkm-Ts (*Pik-m*), IRBLkp-K60 (*Pik-p*), IRBLk-Ka (*Pi-k*), IRBLks-F5 (*Pik-s*) and IRBL1-CL (*Pi-l*), were developed by International Rice Research Institute (IRRI) (Tsunematsu et al. 2000) and provided by Dr. Di Liu at Chinese Academy of Tropical Agricultural Sciences. Seven *Magnaporthe oryzae* isolates, KJ201, CHE86061, CHNOS60-2-3, FJ86, PO6-6, CHL2110 and CHL768, were kindly provided by Dr. Qinghua Pan (Laboratory of Plant Resistance and Genetics, South China Agricultural University) and Dr. Guo-Liang Wang (Department of Plant Pathology, Ohio State University). *M. oryzae* isolates were cultured on oatmeal agar plates in the dark for 7 days at 28°C and then grown under light and dark cycles of 12/12 h for 5 days.

Rice blast inoculation assay

Rice blast inoculation assays were conducted in a greenhouse with an automation system to maintain a suitable temperature around 28°C and relative humidity of >95%. About 2 weeks after sowing, 20 rice seedlings of each of 25 rice varieties were spray-inoculated with spore suspensions of the 7 isolates separately at a concentration of 5×10^5 spores/ml until run-off. After inoculation, the cement tank was completely covered with a tent, and the inoculated seedlings were maintained under dark conditions for 24 h. The tent was then removed from the tank, and the seedlings were grown in the greenhouse under high humidity (>95%) for an additional week for symptom evaluation. The 0-5 standard scale was used to score disease severity following Campbell et al. (2004). Plants with lesion scores 0, 1 and 2 were considered to be resistant, 3, 4 and 5 were considered as moderately resistant, susceptible and highly susceptible, respectively.

DNA extraction

Leaf tissues of rice plants were collected for DNA extraction. About 50 mg of leaf samples collected in Eppendorf tubes were frozen in liquid nitrogen and were processed into fine powder with a TissueLyser (Qiagen, Haan, Germany). Rice genomic DNA was extracted from ground

leaf tissue by the Cetyltrimethyl Ammonium Bromide (CTAB) method as described by Murray and Thompson (1980).

Development of allele-specific markers for *Pi-1* and *Pik-p*

Based on the multiple sequence alignment of *Pi-1* (HQ606329), *Pik-h* (HQ662330), *Pi-k* (HM048900), *Pik-m* (GU811852), *Pik-s* (HQ662329), and *Pik-p* (HM035360), *Pikp-Del* and *Pi1-In* markers were designed specifically targeting the InDel polymorphisms of *Pik-p* and *Pi-1* genes among *Pi-1*, *Pik-h*, *Pi-k*, *Pik-m*, *Pik-s*, and *Pik-p*. PCR was performed in a 25 µl reaction that contained 12.5 µl of 2x mix buffer (Mg2+Plus), 1 µl of each primer (10 mM), and 0.2 µl Taqase (5U/ml). For each reaction, 20~200 ng of genomic DNA was used. PCR was performed with the following thermal profile: initial denaturation at 94°C for 5 min, followed by 34 cycles of denaturation at 94°C for 30 s, 55°C for 30 s, and extension at 72 °C for 30 s, and a final extension at 72°C for 10 min. The PCR products were separated in 3% agarose gels and then visualized by ethidium bromide staining.

Genotyping diverse rice varieties with allele-specific markers

Genomic DNA of the 531 varieties was subjected to genotyping the presence of *Pi-1*, *Pik-p*, and K-type alleles using the *Pi1-In* and *Pikp-Del* markers. In addition, two previously reported dCAPS markers *Pi1FNP* and dCAPS-795 (Hua et al. 2012; Zhai et al. 2011) which targeted the functional regions of *Pi-1* and *Pik-p*, respectively, were used for verification. For genotyping using the *Pi1FNP* marker, PCR amplicons were digested with *RsaI*. For genotyping using the dCAPS-1879 marker, PCR amplicons were digested with *Nde I*. All the above products were separated electrophoretically in 3% agarose gels and then visualized by ethidium bromide staining.

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Abbreviations

InDel insertion/deletion

MAS marker-assisted selection

PCR polymerase chain reaction

SSR simple sequence repeat

dCAPS derived cleaved amplified polymorphic sequences

CTAB Cetyltrimethyl Ammonium Bromide

Authors' contributions

DT and ZW conceived and designed the experiments. DT, ZQ C, ZJ C, JL and YL conducted experiment and analyzed the data. DT wrote and PJ, MW, S Chen, FW and ZW revised the manuscript. All authors read and approved the final manuscript.

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258 **Availability of data and materials**

259 The datasets supporting the conclusions of this article are included within the article and its
260 additional files.

261 **Ethics approval and consent to participate**

262 Not applicable.

263 **Consent for publication**

264 Not applicable.

265 **Competing interests**

266 The authors declare no conflicts of interest.

267 **Additional files**

268 **Additional file 1: Table S1.** List of rice varieties assessed in this study

269 **Additional file 2:Table S2.** Summary of identification results with Pi1-In and Pkp-Del markers
270 in 531 rice varieties

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396 **Table 1.** List of primers in the present study

Primers	Sequence: 5'-3'	Expected size (bp)	Comments
PikpDel-F	TGGTTAAATAGGACTCCCTC	368 for <i>Pik-p</i> allele, 377 for other functional alleles of <i>Pik</i> locus	Forward primer to amplify the fragments corresponding to the <i>Pik</i> locus region for allele mining
PikpDel-R	CATTCGCAGACTCGTTGA	368 for <i>Pik-p</i> allele, 377 for other functional alleles of <i>Pik</i> locus	Reverse primer to amplify the fragments corresponding to the <i>Pik</i> locus region for allele mining
Pi1In-F	TGAGGTAGAAGCGGGAGC	218 for <i>Pi-I</i> allele, 207 for other functional alleles of <i>Pik</i> locus	Forward primer to amplify the fragments corresponding to the <i>Pik</i> locus region for allele mining
Pi1In-R	GGTTGGTCGAAACCAGAA	218 for <i>Pi-I</i> allele, 207 for other functional alleles of <i>Pik</i> locus	Reverse primer to amplify the fragments corresponding to the <i>Pik</i> locus region for allele mining
Pi1FNP-F	CAATAGTCCAGCTAAAACGG	159 for <i>Pi-I</i> allele, 178 for non- <i>Pi-I</i> alleles	Forward primer for allele mining between <i>PiI</i> and non- <i>PiI</i> alleles, and for dCAPS marker Pi1FNP
Pi1FNP-R	CATTGCGCCTTTACCTTGT	159 for <i>Pi-I</i> allele, 178 for non- <i>Pi-I</i> alleles	Forward primer for allele mining between <i>PiI</i> and non- <i>PiI</i> alleles, and for dCAPS marker Pi1FNP
dCAPS-1879-F	CGTGGAAGTTCAACAAAAGG	73 for <i>Pik-p</i> allele, 115 for non- <i>Pik-p</i> alleles	Forward primer for allele mining between <i>Pik-p</i> and non- <i>Pikp</i> alleles, and for dCAPS marker dCAPS-1879
dCAPS-1879-R	CAGCACCTGTATTATCCCAT	73 for <i>Pik-p</i> allele, 115 for non- <i>Pik-p</i> alleles	Forward primer for allele mining between <i>Pik-p</i> and non- <i>Pikp</i> alleles, and for dCAPS marker dCAPS-1879

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400 **Table 2.** Disease reactions of representative varieties *Pi-l*, *Pik-p*, K-type-contained using diverse
401 *Magnaporthe grisea* strains

Genes	Cultivars	KJ201	CHNOS60-2-3	CHE86061	FJ501-3	FJ86	CHL2110	CHL768
<i>Pi-l</i>	Enhui 99-64	0	0	0	0	0	0	0
	IR0437	0	0	2	0	0	0	0
	IR56455-206-2-1-2R	0	0	1	0	1	2	0
	IR0436	0	0	0	1	0	0	0
	Chenghui 448	0	0	0	0	0	0	0
	RGD-7S	0	0	3	0	0	0	0
	Jinkang1B	0	0	1	0	0	0	0
	BL123	0	0	0	0	0	0	0
<i>Pik-p</i>	RCPL3-2	0	0	2	0	0	0	0
	Ruanmigu	0	0	0	0	0	0	0
	Zaohui 62	0	0	0	0	0	0	0
	Huifeng A	0	0	0	0	0	0	0
K-type	Gufeng A	0	0	0	0	0	4	0
	Minhui3301	0	0	0	5	0	0	0
	Shuiyuan 290	0	0	0	5	0	0	0
	IR04557	0	0	0	5	0	0	0
	IR73712-68-3-1-2	0	0	0	5	0	0	0
	GIZA176	0	0	0	5	5	0	0
Monogenic lines	IRBLkp-K60(<i>Pik-p</i>)	1	0	5	0	0	0	0
	F-98-7 (<i>Pik-m</i>)	0	0	5	0	0	0	0
	IRBL1-CL(<i>Pi-l</i>)	0	0	4	0	1	4	0
	IRBLkh-K3(<i>Pik-h</i>)	1	0	5	1	0	0	0
Negative (CK)	Nipponbare	3	5	2	5	5	3	5
	Co39	5	5	2	5	5	4	5

Figure Legends

Fig.1 Sequence alignment showing the 19-bp and 11-bp InDel in the Pik-p-1-1015-bp and Pi1-5+6816-bp alleles, respectively. (A) Physical location of the identified InDel region in the Pik locus. (B) and (C) Sequence alignment showing the 19-bp InDel between the Pik-p and non-Pik-p alleles and the 11-bp InDel between the Pi-1 and non-Pi-1 alleles in IRBL1-CL-Pi1(Pi-1), IRBLkp-K60-Pik-p (Pik-p), IRBLk-Ka-Pik (Pi-k), IRBLkm-Ts-Pik-m (Pik-m), IRBLkh-K3-Pik-h (Pik-h), and IRBLks-S-Pik-s (Pik-s). (D) PCR amplified with markers Pi1-In and Pikp-Del, respectively, derived from IRBL1-CL-Pi1(Pi-1), IRBLkp-K60-Pik-p (Pik-p), IRBLk-Ka-Pik (Pi-k), IRBLkm-Ts-Pik-m (Pik-m), IRBLkh-K3-Pik-h (Pik-h), IRBLks-S-Pik-s (Pik-s), and NPB were used for electrophoresis analysis.

Fig. 2 Electrophoresis profiles of the Pikp-Del and Pi1-In marker that differentiates the Pik-p and Pi-1 alleles. (A) Pikp-Del and dCAPS -1879 marker that differentiates the Pik-p and non-Pik-p alleles. Upper panel showing the PCR products with Pikp-Del and lower panel showing the dCAPS -1879 PCR products digested with NdeI. (B) Pikp-Del, Ku1-GT, and Ku2-GT marker that differentiates the K genotype and non-K genotype alleles. Upper panel showing the PCR products with Pikp-Del, middle panel showing PCR products with Ku1-GT, and lower panel showing PCR products with Ku2-GT. (C) Pi1-In and Pi1FNP marker that differentiates the Pi-1 and non-Pi-1 alleles. Upper panel showing the PCR products with Pi1-In and lower panel showing the PCR products of Pi1FNP digested with Rsa I.

Fig. 3 Co-segregation analysis on Pikp-Del and Pi1-In with blast resistance using rice varieties containing Pik-p and Pi-1. A total of 8 varieties containing Pi-1, 4 containing Pik-p and 3

426 isogenic lines as check varieties were used for inoculation identification by *Magnaporthe oryzae*
427 isolates CHL768 (contain-AvrPi-1) and CHL2110 (contian-AvrPik-p), respectively.

428

Figures

Figure 1

Sequence alignment showing the 19-bp and 11-bp InDel in the Pik-p-1-1015-bp and Pi1-5+6816-bp alleles, respectively. (A) Physical location of the identified InDel region in the Pik locus. (B) and (C) Sequence alignment showing the 19-bp InDel between the Pik-p and non-Pik-p alleles and the 11-bp InDel between the Pi-1 and non-Pi-1 alleles in IRBL1-CL-Pi1(Pi-1), IRBLkp-K60-Pik-p (Pik-p), IRBLk-Ka-Pik (Pi-k), IRBLkm-Ts-Pik-m (Pik-m), IRBLkh-K3-Pik-h (Pik-h), and IRBLks-S-Pik-s (Pik-s). (D) PCR amplified with markers Pi1-In and Pikp-Del, respectively, derived from IRBL1-CL-Pi1(Pi-1), IRBLkp-K60-Pik-p (Pik-p), IRBLk-Ka-Pik (Pi-k), IRBLkm-Ts-Pik-m (Pik-m), IRBLkh-K3-Pik-h (Pik-h), IRBLks-S-Pik-s (Pik-s), and NPB were used for electrophoresis analysis.

Figure 2

Electrophoresis profiles of the Pikp-Del and Pi1-In marker that differentiates the Pik-p and Pi-1 alleles. (A) Pikp-Del and dCAPS -1879 marker that differentiates the Pik-p and non-Pik-p alleles. Upper panel showing the PCR products with Pikp-Del and lower panel showing the dCAPS -1879 PCR products digested with NdeI. (B) Pikp-Del, Ku1-GT, and Ku2-GT marker that differentiates the K genotype and non-K genotype alleles. Upper panel showing the PCR products with Pikp-Del, middle panel showing PCR products with Ku1-GT, and lower panel showing PCR products with Ku2-GT. (C) Pi1-In and Pi1FNP marker that differentiates the Pi-1 and non-Pi-1 alleles. Upper panel showing the PCR products with Pi1-In and lower panel showing the PCR products of Pi1FNP digested with Rsa I.

Figure 3

Co-segregation analysis on Pikp-Del and Pi1-In with blast resistance using rice varieties containing Pik-p and Pi-1. A total of 8 varieties containing Pi-1, 4 containing Pik-p and 3 isogenic lines as check varieties were used for inoculation identification by *Magnaporthe oryzae* isolates CHL768 (contain-AvrPi-1) and CHL2110 (contian-AvrPik-p), respectively.

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