Dissection of broad-spectrum resistance of the Thai rice variety Jao Hom Nin conferred by two resistance genes against rice blast

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Abstract

Background: Rice (Oryza sativa) is one of the most important food crops in the world. Rice blast, caused by the fungal pathogen Magnaporthe oryzae, is one of the most destructive rice diseases worldwide. To effectively cope with this problem, the use of rice blast resistance varieties through innovative breeding programs is the best strategy to date. The Thai rice variety Jao Hom Nin (JHN) showed broad-spectrum resistance against Thai rice blast isolates. Two QTLs for blast resistance in JHN were reported on chromosome 1 (QTL1) and 11 (QTL11).

Results: Monogenic lines of QTL1 (QTL1-C) and QTL11 (QTL11-C) in the CO39 genetic background were generated. Cluster analysis based on the disease reaction pattern of QTL1-C and QTL11-C, together with IRBLs, showed that those two monogenic lines were clustered with IRBLsh-S (Pish) and IRBL7-M (Pi7), respectively. Moreover, sequence analysis revealed that Pish and Pi7 were embedded within the QTL1 and QTL11 delimited genomic intervals, respectively. This study thus concluded that QTL1 and QTL11 could encode alleles of Pish and Pi7, designated as Pish-J and Pi7-J, respectively. To validate this hypothesis, the genomic regions of Pish-J and Pi7-J were cloned and sequenced. Protein sequence comparison revealed that Pish-J and Pi7-J were identical to Pish and Pi7, respectively. The holistic disease spectrum of JHN was found to be exactly attributed to the additive ones of both QTL1-C and QTL11-C.

Conclusion: JHN showed broad spectrum resistance against Thai and Philippine rice blast isolates. As this study demonstrated, the combination of two resistance genes, Pish-J and Pi7-J, in JHN, with each controlling broad-spectrum resistance to rice blast disease, explains the high level of resistance. Thus, the combination of Pish and Pi7 can provide a practical scheme for breeding durable resistance in rice against rice blast disease.

Keywords: Magnaporthe oryzae, Quantitative trait loci (QTL), Durable resistance

Background

Rice is one of the most important staple food crops in the world. The increasing world population leads to increased demand for rice production. In order to feed the growing population, rice varieties that are resistant to known diseases and that can produce high yields should be used (Khush and Jena 2009). Rice blast, caused by an ascomycete Magnaporthe oryzae, is one of the most destructive diseases of rice worldwide (Ahn et al. 2000), which leads to economic losses of more than 70 billion dollars (Scheuermann et al. 2012). In many Asian countries, the outbreak of rice blast which led to complete losses in rice production has been reported (Sobrizal and Anggiani 2007; Variar 2007; Lei et al. 2016; Hairmansis et al. 2016; Khan et al. 2016; Nguyet et al. 2016).

The most powerful tool to control rice blast is to use resistant varieties. These varieties protect rice plants against the pathogens, upon infection, via the induction of hypersensitive response (HR), which occurs via gene-for-gene recognition of a pathogen effector (Avr) and a host-encoded resistance (R) protein (Gururani et al. 2012). The majority of plant R genes encode proteins
that have putative central nucleotide-binding-site (NBS) and carboxy-terminal leucine-rich repeats (LRRs). These NBS-LRR proteins are divided into two major classes: the first class has an N-terminal domain which shares homology with the mammalian toll-interleukin-1-receptor (TIR) domain while the second class encodes an amino-terminal coiled-coil motif (CC-NBS-LRR) (DeYoung and Innes 2006; Meyers et al. 2003). To date, there are four types of coding proteins of R genes against rice blast: (1) NBS-LRR protein; (2) CC-NBS-LRR protein; (3) B-lectin binding and intracellular serine–threonine kinase domain protein; and (4) proline-rich protein (Chen et al. 2006; Zhou et al. 2006; Ashikawa et al. 2008; Fukuoka et al. 2009). The first rice blast resistance gene was cloned by Wang et al. 1999 and al. 2008; Fukuoka et al. 2009). The first rice blast protein (Chen et al. 2006; Zhou et al. 2006; Ashikawa et al. 2008; Fukuoka et al. 2009). The first rice blast resistance gene was cloned by Wang et al. 1999 and more than 20 R genes were cloned since then. These blast resistance genes are not randomly positioned on rice chromosomes (Sharma et al. 2012). Many R genes were cloned from chromosomes 6, 11, and 12. Some are clustered on a gene family (Sharma et al. 2012). Many allelic genes have been reported, for example Pish/PI35 on chromosome 1 (Takahashi et al. 2010; Fukuoka et al. 2014), Pi9/Pi2/Pizt/Pi50 on chromosome 6 (Qu et al. 2006; Zhou et al. 2006; Su et al. 2015), Pi5/Pi1 on chromosome 9 (Lee et al. 2009; Takagi et al. 2013), and Pikh/Pikm/Pik/Pikp/Pi1 on chromosome 11 (Ashikawa et al. 2008; Xu et al. 2008; Yuan et al. 2011; Zhai et al. 2011; Hua et al. 2012). To date, 11 avirulence (Avr) genes in rice blast fungus have been cloned. Nine out of 11 genes, namely; AvrPita (Orbach et al. 2000), ACE1 (Bohnert et al. 2004), AvrPia, AvrPi2, AvrPi (Yoshida et al. 2009), AvrPiz-t (Li et al. 2009), Avr1-CO39 (Zheng et al. 2011), AvrPib (Zhang et al. 2015), and AvrP19 (Wu et al. 2015), have their corresponding R genes in rice. However, there are no known R genes in rice for PWL1 (Kang et al. 1995) and PWL2 (Sweigard et al. 1995). There are many scenarios that describe the specificity of recognition between resistance proteins and avirulence proteins. For example, one R gene can recognize more than one Avr gene, an example of which is Pia that can recognize both AvrPia and Avr1-CO39 (Cesari et al. 2013), or it could also be that many R genes can recognize the same Avr gene, as in the case of AvrPik-D that can be recognized by Pik, Pikm, and Pikp (Yoshida et al. 2009). In another case, the presence of two R genes is required for the mediated resistance to rice blast: Pi-km1 and Pi-km2 from Tsuyuake (Ashikawa et al. 2008) and Pi5-1 and Pi5-2 from RIL260 (Lee et al. 2009).

Genes conferring broad-spectrum resistance are necessary in breeding programs. The presence of even only one R gene can induce resistance to many isolates of *M. oryzae* (Qu et al. 2006). Many broad-spectrum R genes have been validated such as Pi9 (Qu et al. 2006), Pi2, Piz-t (Zhou et al. 2006), Pi40 (Jeung et al. 2007), Pi20 (Li et al. 2008), Pi5 (Lee et al. 2009), Pi1 (Hua et al. 2012), Pi54rh (Das et al. 2012), Pi56 (Liu et al. 2013), and Pi50 (Su et al. 2015). Unfortunately, the resistance controlled by most race-specific R genes is prone to collapse often in a few years after introduction to the rice field due to the quick adaptation of rice blast pathogen (Kiyosawa 1982; Fukuoka et al. 2009).

Many strategies to keep the durability of resistance have been suggested, including multi-varietal planting, pyramiding of major R genes, and using partial resistance genes (Khush and Jena 2009). The use of partial resistance genes is one way to maintain the resistance of R gene(s) in the field. Partial resistance genes such as Pi34 (Zenbayashi-Sawata et al. 2005), pi21 (Fukuoka et al. 2009), and Pi35 (Fukuoka et al. 2014) have been reported. The pyramiding of partial resistance genes for enhancing the durability of resistance has been reportedly used in several breeding programs (Yasuda et al. 2015).

Jao Hom Nin (JHN) is a Thai rice variety which shows broad-spectrum resistance against rice blast in Thailand. Only two out of 346 blast isolates collected from all the rice growing areas in Thailand can overcome the resistance of JHN (Sreewongchai 2008). Moreover, seven out of 124 blast isolates collected worldwide can overcome the resistance of JHN, two of the isolates are from Colombia while five are from China (Sreewongchai 2008). Two Quantitative Trait Loci (QTLs) which are associated with rice blast resistance were mapped on chromosomes 1 and 11 of JHN (Noenplab et al. 2006). The major QTL (QTL11) that controls complete resistance was mapped on chromosome 11 by the SSR markers RM144 and RM224. The minor QTL (QTL1) which confers partial resistance was mapped on chromosome 1 by the flanking markers RM319 and RM 212 (Noenplab et al. 2006). QTL11 is located on the Pik locus which is a gene family locus. Interestingly, QTL1 is located on the Pish locus which is composed of Pi37 (Lin et al. 2007), Pish (Takahashi et al. 2010), Pi35 (Fukuoka et al. 2014), and Pi64 (Ma et al. 2015). In Thailand, JHN is used as a rice blast resistant donor in breeding programs. Introductions of QTL1 and QTL11 from JHN via marker-assisted selection (MAS) into susceptible cultivars such as KDML105 (Noenplab et al. 2006) and RD6 (Wongsaprom et al. 2010) were successfully accomplished. In this study, the resistance gene(s) in both QTL1 and QTL11 of JHN, which confer broad-spectrum resistance against rice blast populations from Thailand and the Philippines, were cloned and characterized and their disease spectrum was validated. Revealing the JHN rice blast resistance genes discloses the powerful resistance gene combination for resistance and durability to rice blast fungus populations in Thailand, the Philippines, and worldwide.
Results

JHN controls broad-spectrum resistance through the combination of QTL1 and QTL11

To evaluate the resistance spectrum of JHN, 132 diverse blast isolates collected from the Philippines were used for disease assessment. It was found that JHN showed resistance to 120 out of the 132 isolates, representing 90.9% resistance frequency (Fig. 1 & Additional file 1: Table S1). The reactions of different IRRI-bred blast resistant lines (IRBLs) to these isolates were also assessed, revealing that each line had a varied resistance frequency (Fig. 1). Compared to the majority of IRBLs, JHN controlled a quite high resistance frequency to the Philippine isolates. The data indicated that JHN was a promising rice line controlling broad-spectrum resistance to the Philippine isolates as to the Thai isolates (Sreewongchai 2008). Given the fact that JHN contained at least two resistance loci, i.e., QTL1 on chromosome 1 and QTL11 on chromosome 11 (Noenplab et al. 2006), we generated two monogenic lines which were designated as QTL1-C and QTL11-C containing individual QTL1 and QTL11, respectively, in the CO39 genetic background via marker aided backcrossing (MABC) (Additional file 2: Figure S1). The derived homozygous BC3F3 monogenic lines were used for the spectrum analysis against 42 representative isolates selected from the set of 132 isolates as described above. Eight isolates showed virulence to QTL1-C, QTL11-C, and JHN, indicating that neither QTL1 nor QTL11 controlled resistance to these eight isolates (Table 1). Out of 34 avirulent isolates to JHN, three were virulent to QTL1-C but avirulent to QTL11-C, suggesting that QTL11 rather than QTL1 contributed to the resistance of JHN to these three isolates (Table 1). On the contrary, 12 isolates were avirulent to QTL1-C but virulent to QTL11-C, suggesting that QTL1 rather than QTL11 contributed to the resistance of JHN to these 12 isolates (Table 1). The remaining 19 isolates were avirulent to QTL1-C, QTL11-C, and JHN, suggesting that both QTL1 and QTL11 controlled the resistance to these 19 isolates (Table 1). We thus concluded that the resistance of JHN to these 34 isolates was additively controlled by QTL1 and QTL11.

Genetic validation of genomic location of QTL1 and QTL11 in JHN

To validate the genetic delimitation of QTL1 and QTL11 in JHN, two F3 populations were generated as illustrated in (Additional file 2: Figure S1), each derived from a single heterozygous F2 plant containing an individual QTLs determined by the flanking simple sequence repeat (SSR) markers of QTL1 (RM212 and RM11744) and of QTL11 (RM224 and RM144) as described previously (Noenplab et al. 2006). An F3 population consisting of 1177 plants for the genetic analysis of QTL1 was inoculated with a JHN-avirulent isolate BN111. A ratio of 907 resistant versus 270 susceptible progenies was observed, fitting an expected 3:1 ratio ($X^2$: 2.665 and $P$ value: 0.103) (Additional file 3: Table S2). On the other hand, an F3 population comprising of 1346 F3 progenies was inoculated with another JHN-avirulent isolate PO6-6 for the genetic analysis of QTL11. A ratio of 1024 resistant versus 322 susceptible plants was observed, which fitted an expected 3:1 ratio ($X^2$: 0.833, $P$: 0.361) (Additional file 3: Table S2). These data indicated that the resistance of the F3 plants of each population was controlled by a single $R$ gene to the isolate BN111 and PO6-6, respectively. Linkage analysis was further conducted to validate the association of QTL1 and QTL11 with respective linked markers described previously (Noenplab et al. 2006). Due to no polymorphism resolved between JHN and CO39 at RM319, another
polymorphic SSR marker, RM11744 was used instead together with RM212 as flanking markers for the genetic analysis of QTL1 (Fig. 2a & Additional file 4: Figure S2). All 270 susceptible F3 progenies showed CO39-pattern in PCR amplicon size at both flanking markers, suggesting no segregation of QTL1 from both markers (Fig. 2a).

For the linkage analysis of QTL11, RM224 and RM144 were used as flanking markers (Fig. 2b & Additional file 4: Figure S2). All 322 susceptible plants showed CO39-pattern in PCR amplicon size at both flanking markers, suggesting that QTL11 was co-segregated both markers (Fig. 2b). Our data verified that QTL1 and QTL11 were delimited into genomic intervals on chromosome 1 and chromosome 11, respectively, as characterized previously (Noenplab et al. 2006).

QTL1-C and QTL11-C controlled identical resistance spectra as IRBLsh-S and IRBL7-M, respectively

By aligning the positions of flanking SSR markers along respective chromosomes using the Nipponbare reference sequence (MSU Rice Genome Annotation Project, http://rice.plantbiology.msu.edu/), the genomic intervals of QTL1 and QTL11 were determined. The genomic interval of QTL1 corresponded to the region from 33,054,867 bp (RM212) to 33,678,804 bp (RM319) on chromosome 1 in Nipponbare, which is approximately 624 kb in size. Sequence analysis identified 5 predicted NBS-LRR genes in this region, 3 of which corresponded to cloned R genes or their alleles, namely Pi64, Pi37, and Pish/Pi35 (Table 2). On the other hand, the genomic interval of QTL11 was conformed to the region from 27,673,251 bp (RM224) to 28,281,693 bp (RM144) on chromosome 11 in Nipponbare, which is approximately 608 kb in size. Nine NBS-LRR genes were predicted in this region, two of which corresponded to the alleles of Pik-1 and Pik-2 (Table 2).

To validate whether QTL1 and QTL11 correspond to any of known R genes in respective delimited genomic intervals, we conducted cluster analysis of the resistance spectrum controlled by QTL1-C, QTL11-C, and other IRBLs. Intriguingly, QTL1-C and IRBLsh-S was clustered together while QTL11-C was clustered with IRBL7-M based on their reactions against 42 blast isolates (Fig. 3). This result promoted us to postulate that QTL1 and QTL11 in JHN are most likely alleles of Pish and Pi7, respectively. We thus named them as Pish-J and Pi7-J.

Pish-J and Pi7-J were alleles of known Pish and Pi7, respectively

To determine the sequence of Pish-J and Pi7-J in JHN, their genomic regions were cloned and completely sequenced. The sequence of Pish-J (GenBank accession number: KY225901) is 4356-bp in length. It contains a predicted 3873-bp coding sequence (CDS), which resulted
Fig. 2 An integrated genetic and physical map of QTL1 (a) and QTL2 (b). The chromosomal position of SSR markers delimiting the genomic interval of QTLs by referring to the one in the reference genome of Nipponbare was indicated below the line. The number of recombinants/toal F3 progeny at each SSR marker was indicated above the line. Four and 9 NBS-LRR type R genes predicted in the genomic intervals in Nipponbare for QTL1 and QTL11, respectively, were indicated in boxes with gene IDs. The figure was not draw in scale.

| QTL | Chromosome Chromosomal location of genomic interval (bp) | Annotation of NBS-LRR genes | Allelic genes | References |
|-----|--------------------------------------------------------|------------------------------|---------------|------------|
| QTL1 | 1 33,054,867-33,678,804 | LOC_Os01g57270 33,091,703 – 33,096,363 | NA | |
|     | | LOC_Os01g57280 33,099,464 – 33,103,906 | Pi64 | Ma et al. 2015 |
|     | | LOC_Os01g57310 33,116,117 – 33,124,371 | Pi37 | Lin et al. 2007 |
|     | | LOC_Os01g57340 33,141,127 – 33,145,609 | Psh/P35 | Takahashi et al. 2010 and Fukuoka et al. 2014 |
|     | | LOC_Os01g57870 33,463,913 – 33,460,254 | NA | |
| QTL11 | 11 27,673,251-28,281,693 | LOC_Os11g45750.1 27,695,070 – 27,683,753 | NA | |
|     | | XM_015761791.1 27,692,238 – 27,694,418 | NA | |
|     | | LOC_Os11g45790 27,707,310 – 27,703,761 | NA | |
|     | | LOC_Os11g45930 27,793,777 – 27,797,821 | NA | |
|     | | LOC_Os11g45970 27,812,251 – 27,818,431 | NA | |
|     | | NP_001068480.1 27,821,921 – 27,824,507 | NA | |
|     | | XM_015760098.1 27,885,376 – 27,886,175 | NA | |
|     | | LOC_Os11g46200.1 27,978,368 – 27,983,597 | Pik-1 alleles | Ashikawa et al. 2012 |
|     | | LOC_Os11g46210 27,984,697 – 27,989,134 | Pik-2 alleles | Ashikawa et al. 2012 |
in a polypeptide product composed of 1290-amino-acids. Sequence comparison revealed that Pish-J is identical in nucleotide sequence to Pish in Nipponbare (GenBank accession number: AP014957.1). We conclude that Pish-J in JHN is an identical allele of Pish. Given that Pik’s functionality is fulfilled by two adjacent NBS-LRR genes, Pik-1 and Pik-2 (Ashikawa et al. 2012), we determined the sequences of their alleles in JHN, designated as Pi7-J-1 and Pi7-J-2, respectively. Pi7-J-1 is 6489-bp in length (GenBank accession number: KY225902). By referring to sequence annotation of Pik-1 (GenBank accession number: AB616658), Pi7-J-1 was predicted to contain three exons interrupted by two introns in the coding region (Additional file 5: Figure S3). It encoded a 1142-amino-acid protein product. Pi7-J-2 is 3263-bp in length (GenBank accession number: KY225903). It contains two exons and encodes a 1021-amino-acid protein product (Additional file 5: Figure S3).

Sequence comparison revealed that Pi7-J-1 showed 99% identity in nucleotide sequence with Pi7-1 (GenBank accession number: HQ660231). It contains two sequence differences along the whole genomic region. One is composed of a single nucleotide “T” deletion at position 1369-bp in the intron and another is a nucleotide transition of G to A at position 5470-bp in the exon 2 of Pi7-J-1 from Pi7-1. The sequence change in the exon belongs to a synonymous substitution and thus does not alter the protein sequence of Pi7-J-1 from Pi7-1. No difference in nucleotide sequences was observed between Pi7-J-2 and Pi7-2 (GenBank accession number: HQ660231). Taken together, we concluded that Pi7-J represented a new allele of Pi7 containing two silent mutations in genomic sequence.

The sequence similarity of Pi7-J-1 and Pi7-J-2 with other known Pi7-1 and Pi7-2 alleles was further investigated. It was found that Pi7-J-1 shared 99% identity in amino acid to Pikp-1 and Pikh-1 while it shared 95% identity to Pik-1, Pikm-1, and Pi1-5 (Additional file 6: Table S3). Pi7-J-2 shared 100% identity in amino acid sequence to Pikp-2 and Pikh-2 while it shared 99% sequence identity to Pik-2, Pikm-2, and Pi1-6 (Additional file 6: Table S3).

The resistance of QTL1-C was controlled by Pish-J rather than Pi64-J and Pi37-J
To exclude the possibility of the contribution of Pi64 and Pi37 to the resistance spectrum conferred by QTL11, their homologues in JHN, named Pi64-J and Pi37-J, respectively, were cloned and sequenced. Pi64-J was 4406-bp in length covering a predicted 3867-bp CDS while Pi37-J was 4474-bp in length covering a predicted 3873-bp CDS. Sequence analysis revealed that Pi64-J
and Pi37-J shared 90 and 99% identity in amino acid sequence to Pi64 (Ma et al. 2015) and Pi37 (GenBank accession number: DQ923494.1), respectively, indicating that they were different from known genes. Instead, they were identical in sequence to LOC_Os01g57280 and LOC_Os01g57310, respectively in Nipponbare. Since Nipponbare was used as a reference susceptible variety for both Pi64 and Pi37 (Lin et al. 2007; Ma et al. 2015), we thus concluded that Pi5h-J rather than Pi64-J and Pi37-J was responsible for the resistance of QTL1-C.

Discussion

The most efficient way to control rice blast disease is the utilization of resistant varieties. It is also low-cost and environment-friendly (Manandhar et al. 1998). The JHN rice variety showed broad-spectrum resistance against Thai and worldwide blast isolates (Sreewongchai 2008). The resistance of JHN was documented in this study by testing it against 132 Philippine blast isolates and found to be effective to most isolates, indicating that it is also quite promising in resistance against rice blast in the Philippines. The dissection of mechanisms underlying the broad-spectrum resistance of JHN against diverse sets of rice blast isolates worldwide is vital for the utilization of JHN-embedded R genes in varietal improvement against rice blast via marker aided selection approach. Noenplab et al. (2006) documented that two QTLs, namely QTL1 on chromosome 1 and QTL11 on chromosome 11, were responsible for the broad-spectrum resistance of JHN. In this study, we employed a multifaceted approach to further dissect the molecular mechanisms of both QTL1 and QTL11. The genomic interval spanning QTL1 and QTL11 were first delimited within respective regions by flanking markers. Sequence analyses revealed that the Pi5h and Pi5k loci were present in the delimited genomic intervals of QTL1 and QTL11, respectively. Cluster analysis of the resistance reactions of derived QTL1 and QTL11 monogenic lines and IRBLs led to the finding of their association with Pi5h and Pi5k, respectively. Finally, gene cloning and sequence analysis revealed that QTL1 and QTL11 encoded alleles of Pi5h and Pi5k, respectively.

The Pi5h gene was mapped on chromosome 1 by an analysis of QTL due to its moderate resistance and isolated by extensive characterization of retrotransposon-tagged suppressive mutants (Araki et al. 2003; Takahashi et al. 2010). It is located in a gene cluster with a tandem array of four NBS-LRR genes (RGA1 through RGA4) in Nipponbare. It was found that RGA4 rather than RGA1 through RGA3 was responsible for Pi5h (Takahashi et al. 2010). Significant sequence similarity among different homologues suggests that they were likely derived from successive rounds of duplication from a common progenitor (Takahashi et al. 2010). In addition to Pi5h, three other functional R genes have been molecularly characterized, namely Pi64, Pi37, and Pi35 corresponding to alleles of RGA2, RGA3, and RGA4 in respective resistant rice varieties (Lin et al. 2007; Ma et al. 2015; Fukuoka et al. 2014). Sequence comparison further revealed that multiple functional polymorphisms cumulatively resulted in the conversion from Pi5h-mediated race specific resistance to Pi35-mediated non-race specific resistance (Fukuoka et al. 2014). The functionality of these Pik alleles is required by the co-existence of two adjacent NBS-LRR genes at the locus. Different Pik alleles activate immunity to rice blast isolates by recognizing different haplotypes of AvrPik allele, demonstrating a classical arms race coevolution model (Kanzaki et al. 2012). Biochemical and structural biology assays revealed that the delicate binding between a heavy-metal associated domain (HMA) in Pik-1 and AvrPik-D initiated the host immune response to rice blast pathogen (Maqbool et al. 2015). In this study, we demonstrated that JHN contained an allele of Pi7 in IRBL7-M sharing limited sequence differences in nucleotide but not in protein sequence. Resistance spectrum analysis of IRBLs containing different Pik alleles against isolates from Bohol clearly indicated that Pi7, Pikp, and Pik controlled resistance to the isolates containing AvrPik-D but not other AvrPik haplotypes (Selisana et al. 2017). Interestingly, a pathotype test of 2476 rice blast isolates collected from the north, northeast, and south of Thailand in 2007 against IRBLs revealed that IRBLk-ka (Pik) and other Pik-allele containing IRBLs were resistant to most isolates (Mekwatanakarn et al. 2007). A similar result was obtained in other pathotype tests using 2293 and 1375 Thai rice blast isolates collected in 2011 and 2013, respectively (Mekwatanakarn et al. 2011; Mekwatanakarn et al. 2013). Moreover, Pi7 was reported to show high frequency of resistance against rice blast isolates in Cambodia (Fukuta et al. 2014) and Vietnam (Nguyen et al. 2015).

In this study, we demonstrated that the resistance spectrum of JHN is attributable to the additive contribution from both Pi7-J and Pi5h-J, which underpinned the mechanism of broad-spectrum of resistance to rice blast in JHN. It is indeed that JHN has been widely used as an elite resistant donor in conventional and molecular
breeding programs against rice blast disease in Thailand. These breeding programs aim to introgress \textit{QTL1} and \textit{QTL11} of JHN into commercial Thai rice varieties which are susceptible to rice blast. Examples of these varieties are RD6 (Wongsaprom et al. 2010), Khoa Dok Mali 105 (Nalampangnoenplab 2011), IR77955-24-75-284 (Kotchasantit 2013), Ban Tang (Kaewcheenchai et al. 2014), Jao Hawm Phitsanulok51 (Noenplab et al. 2015), and San Par Tong1 (Yajai and Ketsuwan 2015).

**Conclusions**

In this study, the \textit{R} genes which control the resistance of \textit{QTL1} and \textit{QTL11} in the JHN rice variety were identified. The \textit{Pish-J} in \textit{QTL1} showed partial resistance while the \textit{Pi7-J} gene in \textit{QTL11} showed complete resistance against Thai and Philippine rice blast isolates. The combination of the two broad spectrum blast resistance genes explains why JHN can still show a high level of resistance for a long period of time in Thailand. The results indicate that \textit{Pish-J} and \textit{Pi7-J} in JHN are broad-spectrum resistance genes which are excellent candidate genes to be included in Thailand and the Philippines’s rice breeding pipeline to maintain rice blast resistance.

**Methods**

**Plant materials**

Jao Hom Nin (JHN), a Thai glutinous rice variety, was used as source of resistance genes for cloning and characterization. CO39, a susceptible rice variety, was used as a recipient variety for genetic linkage analysis. LTH and IRBLs from the Gene Bank of the International Rice Research Institute (IRRI) were used for disease spectrum analysis.

**Generation of monogenic lines of \textit{QTL1} and \textit{QTL11} of JHN in the CO39 genomic background using marker assisted backcrossing (MABC)**

The monogenic lines of \textit{QTL1} and \textit{QTL11} of JHN in the CO39 genomic background were generated as illustrated in Additional file 2: Figure S1. In brief, JHN was first crossed with the susceptible rice variety, CO39. The SSR marker RM144 was used to check the real cross of \textit{F1} plants (Additional file 7: Table S4). Genomic DNA was extracted from frozen rice leaves by CTAB method as described by Doyle and Doyle (1987). To separate \textit{QTL1} and \textit{QTL11}, the resistant \textit{F2} plants selected by the inoculation of the isolate PO6-6 were screened using SSR markers, RM212 and RM11744 for \textit{QTL1} and RM224 and RM144 for \textit{QTL11} (Additional file 7: Table S4). The homozygous \textit{F2} plants for individual \textit{QTL1} and \textit{QTL11} were further used for the generation of monogenic lines at the generation of BC3F1 by MABC. The derived homozygous BC3F3 plants of \textit{QTL1}, designated as QTL1-C, and of \textit{QTL11}, designated as QTL11-C were used for spectrum analysis of \textit{QTL1} and \textit{QTL11}, respectively.

**Genetic analysis of \textit{QTL1} and \textit{QTL11} of JHN**

During the generation of monogenic lines of \textit{QTL1} and \textit{QTL11}, the heterozygous \textit{F2} plants which harbored either \textit{QTL1} or \textit{QTL11} were self-pollinated to produce \textit{F3} lines for the genetic linkage analysis (Additional file 2: Figure S1). They were inoculated with the differential blast isolates for \textit{Pish} and \textit{Pik}, BN111 and PO6-6, respectively (Telebanco-Yanoria et al. 2008). The DNA of susceptible plants was extracted and used for segregation analysis for \textit{QTL1} with SSR markers of RM212 and RM11744 and for \textit{QTL11} with RM144 and RM224.

**Pathotype analysis**

JHN was inoculated with 132 Philippine blast isolates (Additional file 8: Table S5) using \(1 \times 10^5\) conidia/ml suspension. Pathogen inoculation and disease evaluation were performed as previously described by Zhu et al. (2012). The BC3F3 homozygous lines of \textit{QTL1-C} and \textit{QTL11-C} were inoculated with 42 representative Philippine blast isolates. These 42 representative isolates can be classified in four categories based on the reactions to \textit{Pish} and \textit{Pik} including (I) avirulent to both \textit{Pish} and \textit{Pik}, (II) virulent to both \textit{Pish} and \textit{Pik}, (III) avirulent to \textit{Pish} but virulent to \textit{Pik}, (IV) virulent to \textit{Pish} but avirulent to \textit{Pik}. The disease reactions of \textit{QTL1-C} and \textit{QTL11-C} were clustered with those of different IRBLs using NTSYS program version 2.21q.

**Cloning and comparative sequence analysis of known \textit{R} genes in the regions of \textit{QTL1} and \textit{QTL11} in JHN**

The genomic DNA of JHN was used as the DNA template for cloning the homologues of \textit{Pik64}/\textit{Pik37}/\textit{Pish} and \textit{Pik} in the regions of \textit{QTL1} and \textit{QTL11} in JHN with the primer pairs listed in Additional file 7: Table S4. PCR amplicons were sequenced (Macrogen Inc, South-Korea) and the online software MAFFT version7 (http://mafft.cbrc.jp/alignment/server/) was used sequence analysis and comparison. Gene and protein sequence similarity was determined via BLASTN and BLASTP, respectively (https://www.ncbi.nlm.nih.gov/). The encoded protein sequences were deduced using the translation program at ExPASy (http://web.expasy.org/translate/).

**Additional files**

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**Additional file 1: Table S1.** The resistance reactions of JHN and different IRBLs to 132 Philippines isolates. The 42 representative isolates used for the cluster analysis of \textit{QTL1} and \textit{QTL11} with different target \textit{R} genes in IRBLs were indicated in superscript letter a: IRBL: International Rice Research Institute bred lines; R: resistant; S: susceptible. (DOC 385 kb)
Acknowledgements
We appreciated Dr. Cailin Lei at Chinese Academy of Agricultural Sciences for providing the genomic sequence of Pi64. We would also like to thank Mr. Robert Harman II for grammatically reviewing the manuscript. This work was supported by Funding from LEE Foundation, International Rice Research Institute (IRRI), the National Center for Genetic Engineering and Biotechnology (BIOTEC), and National Science and Technology Development Agency (NSTDA). Thailand NSTDA Research Chair Grant (No. P12-01898) and Kasetsart University Research and Development Institute (KURDI).

Authors' contributions
Project conception (CI. BZ). Mapping population construction (CC, TT, AV). Pathotype screening (CC, MJ, BQ, AL, SK, SK). Gene cloning and sequence analysis (CC, BZ). Manuscript preparation (CC, CJ, BZ). All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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Received: 29 December 2016 Accepted: 4 May 2017
Published online: 11 May 2017

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