Fine Mapping and Identification of Blast Resistance Gene *Pi-hk1* in a Broad-Spectrum Resistant *japonica* Rice Landrace

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ABSTRACT

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One *Japonica* rice landrace, Heikezijing, from the Taihu Lake region of China, exhibits broad-spectrum resistance to rice blast. As characterized in our previous research, a main-effect resistance (*R*) gene, *Pi-hk1*, in Heikezijing against five isolates (GD10-279a, JS2004-141-1, JS2004-185, JS90-78, and Hoku1) was roughly mapped on the long arm of chromosome 11. To fine map *Pi-hk1*, one recombinant inbred line (RIL), RIL72 ($F_{2.8}$), from the cross between Heikezijing and blast-susceptible variety Suyunuo, was further crossed and backcrossed with Suyunuo to produce a BC_1F_2 population of 477 individuals. Inoculation experiments

with the representative isolate Hoku 1 indicated that RIL72 carries a single dominant *R* gene for blast resistance. With the help of advanced BC_1F_3 (915 plants), BC_1F_4 (4,459 plants), and BC_1F_5 (2,000 plants) mapping populations, *Pi-hk1* was finally mapped to a 107-kb region between molecular markers P3586 and ILP3, and co-segregated with the markers P4098, RM7654, and P4099. By sequence analysis of Heikezijing bacterial artificial chromosome clones covering *Pi-hk1* region, 16 predicted genes were identified within this region, including three nucleotide-binding site leucine-rich repeat candidate genes. These results provide essential information for cloning of *Pi-hk1* and its application in rice breeding for broad-spectrum blast resistance by marker-assisted selection.

Additional keywords: gene prediction.

Rice is one of the most important food crops, feeding >50% of the world's population. Rice blast, caused by *Magnaporthe oryzae*, is one of the most devastating rice diseases worldwide. The annual loss of rice yield caused by blast was 10 to 30%, estimated to be enough for feeding ~60 million people (36). Breeding efforts have aimed to introduce blast resistance (*R*) genes into susceptible elite cultivars. However, resistant cultivars often remain effective for only a few years due to pathogenic variability of *M. oryzae* (10,28). Therefore, breeding for cultivars that display broad-spectrum and durable resistance has become a priority in rice improvement programs throughout the world (17).

Over the past several years, genetic characteristic of blast resistance in rice has been extensively studied, and a number of *R* genes have been documented (2,33,45). Most of the reported *R* genes are dominant and only *pi21* and *pi55* are recessive (13,14). In total, 20 *R* genes have been cloned (15,22,27,31,37,46,47), and some of them were organized into gene clusters, including *Pi2* loci (*Pi2/Piz-t/Pi9*) (30,48) and *Pik* loci (*Pik/Pik-m/Pik-p/Pi1*) (1,15,46,47). Of the cloned *R* genes, 18 genes encode nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins, except for *Pi-d2*, which encodes a B-lectin receptor kinase (9), and *pi21*, which encodes a proline-rich protein that includes a putative heavy metal-binding domain and putative protein-protein interaction motifs (13). It has been shown that at least six *R* genes—

Pi1 (24), *Pi2* (6), *Pi9* (21), *Pi5* (18), *Pi33* (4), and *Pigm* (11)—probably confer broad-spectrum resistance to a number of isolates or races from one or different countries. For instance, *Pi9*, located on the same region with *Pi2*, showed resistance to 43 isolates from 13 countries (21); *Pi5* was a locus associated with resistance to at least 6 blast races from the Philippines and 26 isolates from Korea (18); and *Pi33* showed resistance to >2,000 isolates originating from 55 countries (4).

In our previous research, it was found that Heikezijing, a *japonica* rice landrace from the Taihu region of China, exhibited broad-spectrum resistance to at least 300 isolates belonging to seven Chinese blast races (35,40). In total, 22 quantitative trait loci (QTLs) conferring resistance to these isolates were identified and mapped on rice chromosomes 1, 7, 9, 11, and 12, including the main-effect locus *qtl11-5-5*, named *Pi-hk1*, against five isolates, including GD10-279a(ZB5), JS2004-141-1(ZC5), JS2004-185(ZE3), JS90-78(ZF1), and Hoku1 on chromosome 11, which could explain 74.34% phenotypic variance against blast isolate Hoku 1 (35). In this article, we reported that fine mapping of *Pi-hk1* and prediction of candidate genes by four backcross (BC) populations (BC_1F_2 , BC_1F_3 , BC_1F_4 , and BC_1F_5) derived from the cross between one recombinant inbred line (RIL), RIL72 ($F_{2.8}$), from the cross Heikezijing × Suyunuo, and blast-susceptible variety Suyunuo.

MATERIALS AND METHODS

Plant materials and growth conditions. In our previous research, a population of RILs ($F_{2.8}$) was constructed from a cross between a resistant landrace, Heikezijing, and a susceptible variety, Suyunuo (35). One RIL, RIL72, carrying the target gene *Pi-hk1* from Heikezijing, was selected as *R* gene donor for developing fine-mapping populations. One F_2 population with 477

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plants, derived from a cross of RIL72 and the susceptible parent Suyunuo, were used for genetic analysis of resistance. Three advanced segregation populations—BC₁F₃ (915 plants), BC₁F₄ (4,459 plants), and BC₁F₅ (2,000 plants)—derived from self-cross of the heterozygous BC₁F₂ individuals were used for fine mapping of *Pi-hk1*. The mapping population plants grown in the fields and leaves were sampled two times at the five- to six-leaf stage and mature grain stage for DNA extraction and marker analysis. According to the genotype results, we selected the recombinants for inoculation.

Rice seed were sown in plastic trays of 60 by 30 by 5 cm with sieved garden soil, as described by Wang et al. (40). In each tray, Suyunuo was used as the susceptible control. Plants were grown in a greenhouse at 24 to 30°C with a light and dark cycle of 16 and 8 h, respectively, until they were at the four-leaf stage for disease evaluation. After disease evaluation, the leaves of susceptible plants were sampled for DNA extraction and marker analysis to conform the genotypes. For expression analysis of candidate genes, the leaves of Heikezijing seedlings inoculated by blast or water (control) were sampled as described (3).

Pathogen, inoculation, and disease evaluation. The *M. oryzae* isolate Hoku1 was used for all phenotypic analyses. Rice seedlings at the four-leaf stage were put into inoculation chambers and inoculated with a conidial suspension (5×10^4 conidia/ml) as described by Wang et al. (40). The inoculated plants were kept in the chambers at 26°C with 95% relative humidity and darkness for 24 h, and then transferred to a greenhouse (28 to 30°C, day and 20 to 22°C, night) for disease incubation with 100% relative humidity by intermittently spraying water for 1 to 2 min every

2 h. Each line was inoculated in two independent experiments and three replications for each experiment.

Lesion types on rice leaves were scored after 1 week of inoculation according to the referred standard (24). Lesion scores of 0 to 5 were based on lesion type with appropriate reference of the disease area of the highest score of each plant, as described by Shi et al. (35). Individuals with scores 0, 1, and 2 were grouped as resistant and those with scores 3, 4, and 5 as susceptible.

Marker development and identification. Total DNA was extracted from the 3-week-old rice seedlings using the sodium dodecyl sulfate method (29). Three types of polymerase chain reaction (PCR)-based markers were used in this study. For simple-sequence repeat (SSR) markers, the primer sets were adopted from the International Rice Microsatellite Initiative (<http://www.gramene.org>) (25). For further linkage analysis, intron length polymorphism (ILP) markers and insertion/deletion polymorphism (InDel) markers were developed in target region. ILP markers were designed around intronic regions which show higher polymorphism than exonic regions (42). For this type of marker, exon was first predicted by software tools GENSCAN (<http://genes.mit.edu/GENSCAN.html>) and softberry (<http://linux1.softberry.com/berry.phtml>). The predicted introns, ≈200 to 500 bp, were considered to be putative ILP markers candidate sequences, and the flanking exon sequences were used for primer designing. InDel markers were developed according to the sequence comparison between of 93-11 (<http://www.genomics.org.cn/>) and Nipponbare (<http://rgp.dna.affrc.go.jp/>) in the target region by Pairwise BLAST (<http://blast.ncbi.nlm.nih.gov/blast.cgi>). The sequence where a large deletion between two subspecies exists is

TABLE 1. Information on polymerase chain reaction (PCR)-based markers used for mapping of *Pi-hk1*

Marker	Type ^a	Primer sequence (5'–3')	Product size (bp) expected	Anneal temperature (°C) ^b	Gel ^c
RM27088	SSR	F: tgcaaatgcaaaagcgtaaagc R: agcagcagcagtaggaacaagaagg	169 ...	56 ...	A ...
RM27154	SSR	F: tagtcgggcacatcctcttcc R: gttacctccgatgaaggcaagg	137 ...	55 ...	A ...
RM27189	SSR	F: ccgagcttaattgcactactgc R: tgcagattgtgttggaatgg	93 ...	58 ...	A ...
RM27230	SSR	F: tcctccagtgatccagtttg R: cggaggtagtatatcttctctcc	135 ...	58 ...	C ...
RM27248	SSR	F: cagcgactgctatgtttacc R: ctgaataaacggcgccataaagg	183 ...	55 ...	C ...
RM1233	SSR	F: atgggcacgtgtaattcattcg R: atctcgaagtaggagtaggaaagc	170 ...	55 ...	A ...
RM7221	SSR	F: ggatgccacatgctgattagg R: ctccctccgtttcacaataaagg	182 ...	55 ...	A ...
ID2	InDel	F: atctgcacggccaagaat R: gagctccgcatgaacacc	339 ...	58 ...	C ...
P3586	InDel	F: cccttaacacattccagcaa R: cccacccaatctagcaat	177 ...	52 ...	A ...
P4098	InDel	F: tggctctcttgaacccta R: agagacatcaagggaagcaaa	154 ...	55 ...	A ...
RM7654	SSR	F: cgcagctctcctaataaactgtgg R: ttacctaagcagctggcactgatgg	109 ...	55 ...	A ...
P4099	InDel	F: aaaaacattgaagtgtgcaaaga R: ggtgcaagggtttctgagg	169 ...	55 ...	A ...
P4261	InDel	F: tgaatttatgaacaaaactttca R: ataccccaactgagtgcaa	199 ...	52 ...	A ...
ILP3	ILP	F: ctcaagacaagagtgtaattgc R: gctacttgctgattatctgtct	432 ...	58 ...	B ...
RM27296	SSR	F: gggctctttgtacacattctgtgg R: ctggaaggatgagcagtatctcg	519 ...	53 ...	C ...
RM27318	SSR	F: tgaactggacatgcctttatcc R: atgcgccttactttgttagg	223 ...	60 ...	A ...
RM27342	SSR	F: tcattctcatcggtgtcttc R: ctgaaggaagttaagaggatgg	98 ...	55 ...	A ...
RM144	SSR	F: catgttgtgtgtctactgc R: agctagaggagatcagatggtagtc	245 ...	60 ...	A ...

^a SSR = simple-sequence repeat and InDel = insertion/deletion polymorphism.

^b All of the PCR reactions were conducted as below: after preheating for 5 min at 94°C, 35 PCR cycles (40 s at 94°C, 40 s at the corresponding temperature described above, and 40 s at 72°C) were followed by 10 min at 72°C.

^c A = 8% acrylamide, B = 6% acrylamide, and C = 2% agarose.

considered to be a candidate sequence for a putative InDel marker. All primers of these new markers were designed by Primer Premier 5.0 (PREMIER Bio-software International) (<http://www.premierbiosoft.com>). Information about primer sequences and PCR conditions is listed in Table 1.

Construction of genetic and physical maps. In total, 915 BC₁F₃ susceptible individuals were used to construct a genetic map of the *R* gene of RIL72 with SSR markers by recessive-class analysis methods (Fig. 1A) (49). In total, 6,459 plants of BC₁F₄ (4,459) and BC₁F₅ (2,000) were used for constructing a fine genetic linkage map on the basis of recombination frequencies between adjacent loci (Fig. 1B).

For constructing a physical map, a BAC library of Heikezijing was constructed in pIndigoBAC-5, as described (39), and screened with *Pi-hk1* linked markers as described (5). Three positive clones (numbers 66P14, 242, and 91C13) covering P3586 and ILP3 markers were obtained and sequenced by a shotgun sequencing method (12). Sequences of these three clones were analyzed and aligned by the bio-software tool Pairwise BLAST (<http://blast.ncbi.nlm.nih.gov/blast.cgi>) to construct the BAC contig spanning the *Pi-hk1* locus (Fig. 1C).

Candidate gene prediction and analysis. Open reading frames (ORFs) were predicted by GENSCAN (<http://genes.mit.edu>), FGENSH (<http://linux1.softberry.com/>), and Rice Genome Automated Annotation System (RiceGAAS) (<http://rgp.dna.affrc.go.jp>) software (32). The DNA and full-length cDNA of 16 predicted genes from two parents were amplified by PCR methods and sequenced (Invitrogen, Shanghai). Then, DNA and cDNA sequences were used to search the nucleotide collection (nr/nt) and expressed sequence tag (EST) database by BLAST tools (<http://www.ncbi.nlm.nih.gov/blast>).

The expression patterns of 16 candidate genes were detected by quantitative real-time reverse-transcription (RT)-PCR methods as described by Huang et al. (16). The fold change in cDNA (target candidate gene) relative to the reference gene (18s-rRNA) was determined by the comparative cycle threshold method (23). Relative quantification relates the PCR signal of the target transcript in inoculated rice seedlings to that of uninoculated rice plants. The primers for 16 candidate genes and 18s-rRNA in quantitative real-time RT-PCR assay are shown in Supplemental Table 1.

RESULTS

Genetic analyses. One BC₁F₂ population consisting of 477 individuals, derived from a backcross of one blast-resistant line, RIL72, carrying *Pi-hk1* locus and susceptible parent Suyunuo were inoculated with the isolate Hoku1. The segregation ratio of the resistant individuals to susceptible individuals (R/S) showed 3:1 (350R:127S) ($\chi^2 = 0.67$, $P = 0.41$), indicating that RIL72 carries a single dominant *R* gene, *Pi-hk1*, that conferred resistance to isolate Hoku1.

Mapping of *Pi-hk1*. In all, 46 SSR markers located in the region of target gene were selected and identified for their genetic polymorphisms between two parents, Heikezijing and Suyunuo. Seven markers (RM144, RM27342, RM27318, RM7654, RM27248, RM27230, and RM27189) showed polymorphisms between two parents. In total, 915 susceptible individuals selected from a BC₁F₃ population were subjected to linkage analysis with these SSR markers. There were 15, 15, and 2 recombinants that could be identified by RM144, RM27342, and RM27318, respectively, toward the telomeric side and 11, 9, and 3 recombinants identified by RM27189, RM27230, and RM27248, respectively,

Fig. 1. Genetic and physical maps of the region of the *Pi-hk1* locus. **A**, Genetic map of the resistance gene, *Pi-hk1*. Numbers in parenthesis are the recombination events in the mapping populations; digits below this map are relative genetic distances in centimorgans. **B**, Physical map of *Pi-hk1*, constructed from the 'Nipponbare' genomic sequence and sequenced BAC clones of 'Heikezijing'. Numbers in parenthesis are the recombination events in the mapping populations. Numbers below the map are physical distance. **C**, Contig map and candidate genes of the *Pi-hk1* locus. Short horizontal lines represent BAC clones of Heikezijing. These clones are anchored by the corresponding markers linked to the *Pi-hk1* locus. Arrows represent open reading frames (ORFs) predicted in the *Pi-hk1* region by the Rice Genome Automated Annotation System.

on the centromeric side. No recombinants were detected by RM7654, indicating that RM7654 co-segregated with the *R* gene. Based on these results, *Pi-hk1* might be flanked by markers RM27248 and RM27318. The genetic distances of *Pi-hk1* to RM144, RM27342, RM27318, RM27248, RM27230, and RM27189 were 0.82, 0.82, 0.11, 0.16, 0.65, and 0.76 centimorgans (cM), respectively (Fig. 1A).

RM27248 and RM27318 were tested in the 4,459 individuals of BC₁F₄ and 2,000 of BC₁F₅ populations and 45 recombinants were selected in this region. Within the interval flanked by RM27248 and RM27318, eight polymorphic markers, including three SSR makers (RM7221, RM1233, and RM27296) and five ILP or InDel markers (ILP3, ID2, P3586, P4098, and P4099) developed in this research were tested in 45 recombinants. There were 32, 29, 2, and 2 recombinants that could be detected by RM1233, RM7221, ID2, and P3586, respectively, on the centromeric side, and 3 and 2 recombinants detected by RM27296 and ILP3, respectively, on the telomeric side. There were no recombinants detected by P4098 and P4099. In all, 45 recombinants were inoculated for fine-mapping *Pi-hk1*. Therefore, *Pi-hk1* was finally delimited into an interval flanked by P3586 and ILP3 and co-segregated with P4098, RM7654, and P4099. The genetic region spanning the *Pi-hk1* locus was estimated to be 0.031 cM in length (Fig. 1B).

Prediction of candidate genes. To physically cover the *Pi-hk1* locus, a BAC library of Heikezijing was constructed, which included ≈39,936 clones with an average DNA insert size of 70 kb, corresponding to 6.5 genome equivalents (*unpublished data*). By screening with co-segregating markers P3586, RM7654, and ILP3, three positive clones (numbers 66P14, 242, and 91C13) spanning the *Pi-hk1* region were obtained and sequenced. The results showed that the physical distance between P3586 and ILP3 in Heikezijing was ≈107 kb (Fig. 1C).

Sequence analyzed by GENSCAN (<http://genes.mit.edu>), FGENSH (<http://linux1.softberry.com/>), and RiceGAAS (<http://rgp.dna.affrc.go.jp>) software suggests that the region flanked by P3586 and ILP3 contains 16 predicted genes, *P1* to *P16*. They were used to search the full-length cDNA and EST database; *P3* and *P8* had no hits to ESTs and unknown functions and the other 14 genes had hits to full-length cDNA or EST. Eight genes (*P2*, *P4*, *P7*, *P9*, *P13*, *P14*, *P15*, and *P16*) showed sequence differences between two parents (Table 2). In order to narrow down the

range of candidate genes, real-time RT-PCR was performed to examine the expression pattern of candidate genes in Heikezijing seedlings induced by Hoku1. The results showed that *P1*, *P2*, *P11*, and *P14* were not expressed before or after inoculation; *P3*, *P4*, and *P8* were not induced by inoculation; *P5*, *P6*, *P7*, *P9*, and *P10* were slightly induced (2.1-, 2.2-, 3.5-, 2.1-, and 1.6-fold, respectively) by inoculation; and *P12*, *P13*, *P15*, and *P16* were strongly induced (6.1-, 80.5-, 8.2-, and 31.5-fold, respectively) by inoculation (Fig. 2). According to these results, five genes (*P7*, *P9*, *P13*, *P15*, and *P16*), including three encoded NBS-LRR disease resistance proteins, might be considered to be the candidates of the *Pi-hk1* gene.

DISCUSSION

As characterized in the previous study, *Pi-hk1*, conferring resistance to blast in landrace Heikezijing, was roughly mapped between RM27187 and RM27381 on the long arm of rice chromosome 11 (35). In this study, *Pi-hk1* was mapped in the 107-kb region between P3586 and ILP3 co-segregated with markers P4098, RM7654, and P4099 on rice chromosome 11. Five other blast *R* genes—*Pikh*, *Pik-m*, *Pik*, *Pik-p*, and *Pil*—were reported to locate near this region (15,20,34,41). In order to distinguish *Pi-hk1* from them, the six genes were integrated into the same chromosome physical map according to their linked markers located on the Nipponbare sequence (Fig. 3). The results showed that the distance between the region flanked by P3586 and ILP3 and the region of *Pi-kh* was 2,334 kb and to the *Pik* cluster was 114 kb. In our previous work, these five *R* genes showed significantly different resistance-spectra with *Pi-hk1* (40). All of these results suggested that *Pi-hk1* is a new blast *R* gene.

With help of four populations—BC₁F₂ (477 plants), BC₁F₃ (915 plants), BC₁F₄ (4,459 plants), and BC₁F₅ (2,000 plants)—*Pi-hk1* was mapped in a 107-kb region. Recombinant events were rarely detected in the *Pi-hk1* region. In the interval between RM7654 and RM27318, only three recombinants were found in 6,459 plants. According to Wu and Tanksley's method, the physical/genetic (P/G) ratio between RM7654 and RM27318 was estimated as 1,759,431.6 kb/cM, which was much higher than the average P/G ratio of 260 to 280 kb/cM estimated in the rice genome (44). There was even no recombination break point observed in the interval spanned by RM27342 and RM144. Re-

TABLE 2. Candidate genes at the *Pi-hk1* region in one parent Heikezijing

Genes ^a	TIGR/IRGSP gene identity	Gene products ^b	Conserved domain	cDNA or EST ^c	aa ^d	Diff. ^e
<i>P1</i>	Os11g0681600	Cysteine-rich receptor-like protein kinase	Catalytic domain	AK243250	625	No
<i>P2</i>	Os11g0622600	BTB/POZ domain like protein	BTB/POZ domain	AU094686(EST); AU094687(EST)	344	Yes
<i>P3</i>	None	Unknown Protein	None	No EST	143	No
<i>P4</i>	Os11g0682000	Cadmium tolerance factor	None	AK241956	688	Yes
<i>P5</i>	Os02g0764100	AP2 domain-containing transcription factor	AP2 domain	AK066197	182	No
<i>P6</i>	Os11g0682300	Nucleic acid binding protein	Nucleotide-binding	AK066311	613	No
<i>P7</i>	Os11g0682600	NBS-LRR disease resistance protein	NBS-LRR domain	AK242652	1,180	Yes
<i>P8</i>	None	Unknown Protein	None	No EST	102	No
<i>P9</i>	Os11g0683600	Unknown Protein	None	AK119461	167	Yes
<i>P10</i>	Os11g0683500	Putative β-glycosidase	None	AK067619	647	No
<i>P11</i>	Os11g0683700	Pectin lyase fold domain containing protein	Pectin lyase fold domain	AK072863	399	No
<i>P12</i>	Os11g0683800	Pectin lyase fold domain containing protein	Pectin lyase fold domain	AK070275	426	No
<i>P13</i>	Os11t0684000	Myb transcription factor	Myb-type HTH DNA-binding domain	AK069082	285	Yes
<i>P14</i>	Os01g0265300	Transposon	Transposon	AK288785	1,102	Yes
<i>P15</i>	Os11t0684100	NBS-LRR disease resistance protein	NBS-LRR domain	AK066053	1,361	Yes
<i>P16</i>	Os11t0684200	NBS-LRR disease resistance protein	NBS-LRR domain	AK287819	963	Yes

^a Predicted genes.

^b NBS-LRR = nucleotide-binding site leucine-rich repeat.

^c Full-length cDNA or expressed sequence tag (EST).

^d Number of amino acids.

^e No and Yes indicate that there is no sequence difference or is a sequence difference, respectively, between two parents detected by polymerase chain reaction amplification and sequencing.

cently, distorted segregation has frequently been identified in some regions of the rice chromosome (7,43). Four *R* genes were reported in regions with low recombination, such as *Pi5* (18), *Pi37* (8), *Pi-km* (20), and *Pita2* (26). The crossover suppression might be explained by two reasons. The first is the lack of pairing and subsequent strand exchange between homologous regions in two parents, which can be supported by the fact that some of the markers we developed only amplified one of the parents. The second is a segregation distortion locus (19), such as when the

gamete gene and sterile gene may be located in the region RM27342 and RM144, because there was no recombination event observed in this region. The impact of the distortion will depend on the linkage distance between the distorted loci and target gene; the closer distance showed more serious distorted segregation (19).

Sequence analysis of the BAC contig of Heikezijing covering the *Pi-hk1* region between P3586 and ILP3 identified 16 genes. The sequences comparing the promoter and gene region, EST

Fig. 2. Real-time polymerase chain reaction analysis for the expression patterns of candidate genes in Heikezijing induced by inoculation Hoku1. 18S-rRNA was used as an internal control. Data represent means and standard errors of three replicates.

Fig. 3. Integrated physical mapping of six resistance genes (*Pikh*, *Pi1*, *Pik*, *Pik-p*, *Pik-m*, and *Pi-hk1*), which were mapped on telemetric region of chromosome 11. Positions of the resistance genes and markers were integrated on the basis of data from the following references: *Pi-hk1* (this article), *Pikh* (34), *Pi1* (15), *Pik* and *Pik-p* (41), and *Pikm* (20). Markers used in this study and their physical distances were mapped below the chromosome

database searching, and expression patterns analysis of these genes showed that only five genes could be the potential candidate genes. They encode an unknown protein (*P9*), an Myb transcription factor (*P13*), and three NBS-LRR disease resistance proteins (*P7*, *P15*, and *P16*), respectively. Especially three NBS-LRR domain proteins (*P7*, *P15*, and *P16*) are most likely to have plant resistance function because an NBS-LRR disease resistance protein had been demonstrated to be involved in plant disease resistance or associated with cell death in animals (38). In this report, we only considered the expressed genes because our future work will focus on identifying which candidate genes represent the broad-spectrum resistance of *Pi-hk1* to blast using *Agrobacterium*-mediated transformation technology.

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