DIFFERENT RICE BLAST RESISTANCE GENES CONTRIBUTING TO THE BROAD-SPECTRUM RESISTANCE IN ELITE MALE STERILE AND RESTORER LINES FOR HYBRID RICE BREEDING*

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SUMMARY

Rice blast is one of the most destructive diseases of rice worldwide. Three-line hybrid rice provides a high efficacy system to exploit heterosis that contributes to the yield increase in the past decades. A disease-resistant breeding program has released two elite male sterile lines, Gang Xiang 1A (GX1A) and D Xiang 4A (DX4A), and an elite restorer line, Shuhui 707 (SH707), which showed resistance to 102 isolates of Magnaporthe oryzae. To address the question whether the sterile lines and the restorer line contained different resistance genes, expression levels of the functionally cloned blast resistance genes were examined by real-time PCR (RT-PCR). Our data demonstrated that Pikm, Pia, Pid2, Pi-ta, and Pi5 were the candidate contributors of resistance in GX1A, DX4A and/or SH707, because of their high expression. Resistant spectrum assay and sequence polymorphism analysis narrowed down the potentially resistant contributors to Pikm and Pid2 in GX1A and DX4A, and Pi-ta, Pi5 and Pid2 in SH707, respectively. These data indicate that the male sterile lines and the restorer line possess different resistance genes and are highly valuable in disease-resistant breeding programs for developing new male sterile and restorer lines. Therefore, hybrid rice has the advantage to pyramid multiple disease resistance genes by introduction of different resistance genes into the parental male sterile lines and restorer lines.

Keywords: rice blast disease, Pikm, Pid2, Pi5, Pi-ta.

INTRODUCTION

Rice is one of the most important crops and the staple food for more than half the world’s population (Harlan, 1998). Rice blast disease, caused by the fungal pathogen Magnaporthe oryzae, is one of the most devastating diseases worldwide, which causes large loss in rice production annually (Babujee and Gnanamanickam, 2000; Bonman et al., 1992; Lee et al., 2009). Identification and employment of resistance genes to develop resistant cultivars are the most economical and effective approach for the disease control. In the past decades, about 100 rice blast resistance genes have been reported (Liu et al., 2014). These genes are distributed in all the rice chromosomes except chromosome 3. A total of 24 blast resistance genes have been functionally cloned and characterized. Except Pid2 that encodes a receptor-like kinase (RLK) protein and the wild type pi21 that encodes a proline-rich protein, all the resistance genes encode coiled-coil-nucleotide-binding Leucine-rich repeat (CC-NB-LRR) proteins. Mapping efforts have demonstrated that a number of these resistance genes are allelic or closely linked (Ashikawa et al., 2008; Chen et al., 2011; Hua et al., 2012; Qu et al., 2006; Shang et al., 2009; Wang et al., 1999; Yuan et al., 2011; Zhai et al., 2011). For instance, Pi2 and Pi-z are allelic and very close to Pi9 on chromosome 6; Pi1, Pi-k/b/Pi54, Pikm and Pik-p are at the same locus of Pik on chromosome 11; Pi3 and Pi25 are allelic genes on chromosome 6; Pia and PiCO39 are also allelic genes (Cesari, 2013). In addition, some of them act as paired genes and resistance phenotype requires two functional genes. For example, Pi5-1 and Pi5-2 are required for Pi5-mediated resistance, and Pik-1 and Pik-2 together confer Pik-mediated resistance (Ashikawa et al., 2008; Lee et al., 2009). In addition, Pia or Pi-CO39 locus consists of RGA4 and RGA5 (Cesari et al., 2013; Okuyama et al., 2011).

Molecular basis of plant disease resistance attributes to the direct or indirect interaction between a resistance gene product in the host and an avirulence gene product in the pathogenic microorganism that follows the classic...
gene-for-gene model (Narusaka et al., 2009). If the avirulence gene changes in the pathogen, the cognate resistance gene in the host will subsequently lose its resistant function. In rice production, many rice resistant cultivars released from disease-resistant breeding programs contain a single resistance gene that is risky in the epidemic occurrence of rice blast disease. In the three-line hybrid rice system, a maintainer line maintains the inheritance of a male sterile line. In turn, a male sterile line is used for hybrid rice seed production by cross-pollination with a restorer line. Thus, hybrid rice cultivar combines genes from both the male sterile and restorer lines. Therefore, pyramiding multiple resistance genes via hybrid rice breeding is an efficient and promising way for disease control.

Recently, two elite male sterile lines, Gang Xiang 1A (GX1A) and D Xiang 4A (DX4A), and an elite restorer line Shuhui 707 (SH707), were released in China, which showed high resistance to rice blast disease and several hybrid rice cultivars were developed based on these male sterile and restorer lines. To better exploit GX1A, DX4A and SH707 in rice blast disease-resistant breeding programs, it is necessary to know whether these lines contain different resistance genes. To this end, we analyzed the expression of the cloned blast resistance genes in these lines. Blast resistance spectrum of these lines and sequence polymorphism of expressed resistance genes were also examined to predict the potentially resistant contributors. Our data indicate that the candidate resistance genes in the male sterile lines were different from those in the restorer line. Therefore, the male sterile and restorer lines are valuable resistant germplasm and it is an advantage of hybrid rice in rice blast disease-resistant breeding programs to pyramid multiple resistance genes by introducing different resistance genes into the parental male sterile lines and restorer lines.

MATERIALS AND METHODS

Plant materials. Rice sterile lines Gang Xiang 1A (GX1A), D Xiang 4A (DX4A) and restorer line Shuhui 707 (SH707) (courtesy of Ming-Jing Zhou) were developed in a three-line hybrid rice breeding program. They showed high resistance to rice blast disease and several hybrid rice cultivars were developed based on these male sterile and restorer lines. To better exploit GX1A, DX4A and SH707 in rice blast disease-resistant breeding programs, it is necessary to know whether these lines contain different resistance genes. To this end, we analyzed the expression of the cloned blast resistance genes in these lines. Blast resistance spectrum of these lines and sequence polymorphism of expressed resistance genes were also examined to predict the potentially resistant contributors. Our data indicate that the candidate resistance genes in the male sterile lines were different from those in the restorer line. Therefore, the male sterile and restorer lines are valuable resistant germplasm and it is an advantage of hybrid rice in rice blast disease-resistant breeding programs to pyramid multiple resistance genes by introducing different resistance genes into the parental male sterile lines and restorer lines.

Fungal isolates and disease assay. Isolates Guy11, Zhong-10-8-14 (Li et al., 2014) and 100 M. oryzae strains from our disease nursery in Sichuan, China were used in resistance spectrum assay. The M. oryzae strains were cultured as described by Li et al. (2014). Briefly, M. oryzae strains were grown on complete medium with 15 g/l agar for 2 or 3 weeks at 26°C with a 12/12 h day/night regime for sporulation. Spores were suspended in 0.02% Tween 20 and adjusted to a concentration of 5 × 105 spores/ml as the inoculum. For spray inoculation, inocula were applied onto three-week-old seedlings of rice, which were then kept in dark chambers with high moisture for 24 h and transferred to a controlled growth room at 26°C. Disease symptoms were evaluated at 7-10 days post inoculation (dpi). Lesion type was scored with the Standard Evaluation System for Rice (Khoshkdaman et al., 2012). For punch inoculation, leaf fragments cut from 6 to 8-week-old plants were punch-wounded and inoculated as previously described (Park et al., 2012). Lesion size measurements and photographs were taken at 6 dpi.

RNA extraction and RT-PCR analysis. Leaf samples from seedlings of rice were collected at 0, 12, 24 and 48 hpi. Total RNA was extracted by using the TRizol® Reagent (Invitrogen). Quantity and quality of RNA samples were determined using a NanoDrop 2000 Spectrophotometer (Thermo, USA) and 1% (w/v) denaturing agarose gel electrophoresis. gDNA was removed and cDNA was synthesized with ReverTra Ace qPCR RT Master Mix with gDNA Remover Kit (TOYOBO). RT-PCR was performed using EasyTaq DNA Polymerase (TransGen Biotech, China). RT-PCR was initiated with one cycle at 94°C for 3 min, followed by 28 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s, and was terminated with a final extension at 72°C for 5 min. The PCR products were examined by 1.5% (w/v) denaturing agarose gels. The OsActin1 of rice was used as the internal reference gene. Primers were listed in Supplementary Table S1.

Sequence analysis. Candidate rice blast resistance genes from the examined cultivars were amplified by KOD-Plus High Fidelity DNA Polymerase (TOYOBO) using cDNA as templates. The PCR products were either directly used for sequencing analysis or separated and purified by 1% (w/v) agarose gel electrophoresis and the Quick Gel Extraction Kit (CWBio, China), and cloned into the pEASY-Blunt Simple Cloning Vector (TransGen Biotech, China). Multiple positive clones were sequenced to ensure there were no PCR-generated mutations, and the resulting sequences were assembled. Sequence alignment was performed using VectorNTII Advance version 11.5.1. Primers were listed in Supplementary Table S1.

RESULTS

GX1A, DX4A and SH707 exhibit broad-spectrum resistance to rice blast disease. Hybrid rice sterile lines GX1A and DX4A, and restorer line SH707 showed high resistance to rice blast in field trials at different locations
across China. Artificial inoculation was performed to confirm the high resistance. Lijiangxin Tuan Heigu (LTH) was used as a susceptible reference. The results showed that GX1A, DX4A and SH707 all displayed complete resistance to *M. oryzae* mixed strains, including Guy11, a GFP-tagged strain Zhong-10-8-14 (Shang *et al.*, 2009) and seven other isolates purified from diseased rice plants in Sichuan Province, China. By contrast, leaves of LTH had severe disease symptoms (Fig. 1). Individual punch inoculation of the above nine isolates also confirmed the high resistance of GX1A, DX4A and SH707 (Fig. 2). To examine the resistance spectrum of GX1A, DX4A and SH707, spray inoculation was carried out in greenhouse using 102 *M. oryzae* isolates individually. We presented the data in Supplementary Table S2. The data demonstrated that the virulence of the 102 isolates were quite different. As expected, GX1A, DX4A and SH707 were resistant to all tested *M. oryzae* isolates, while LTH was susceptible (Supplementary Table S2). Therefore, GX1A, DX4A and SH707 possess broad spectrum of rice blast resistance and are valuable germplasms for rice blast resistant breeding.

Some cloned blast resistance genes are differentially expressed in GX1A, DX4A and SH707. To better employ GX1A, DX4A and SH707 in rice blast resistance breeding, identifying the candidate resistance genes in them is important. To this end, we employed a gene-expression based method: if there was a transcript of a gene, the gene existed. Then sequencing analysis of the gene could tell whether the transcript was from the functional allele or from the non-functional allele. First, RT-PCR was conducted to examine the expression levels of the functionally cloned blast resistance genes in these lines before and 48 h after inoculation (hpi) of *Magnaporthe oryzae*. The results indicated that *Pib*, *Pi-ta*, *Pid2*, *Pia* (RGA4 and RGA5), and *Pikm* (*Pikm1-TS* and *Pikm2-TS*) were obviously expressed in GX1A and DX4A at 0 and 48 hpi of *M. oryzae*, while expression of the other resistance genes were not or barely detected even at 48 hpi (Fig. 3). In SH707, *Pib*, *Pid2*, *Pia* (RGA4 and RGA5), and *Pi5* (*Pi5-1* and *Pi5-2*) were highly expressed, but other genes were not/lowly expressed after inoculation of *M. oryzae* (Fig. 3). Out of our expectation, transcripts of *Pid2*, *Pia-RGA4*, *Pia-RGA5*, *Pikm1-TS* and *Pikm2-TS* were also detected in LTH (Fig. 3), implying that the non-functional allele of these genes exists in LTH. Interestingly, expression of *Pi5* (*Pi5-1* and *Pi5-2*) was only detected in SH707. Moreover, time-course expression analysis confirmed that *Pib*, *Pi-ta*, *Pid2*, *Pia-RGA4* and *Pia-RGA5* were expressed at different time points after inoculation of *M. oryzae* and appeared to be induced by infection, although at different time points for GX1A, DX4A and SH707 (Fig. 4), confirming the existence of these genes. *Pikm1-TS* was preferentially induced at 48 hpi in GX1A and DX4A, while *Pikm2-TS* seemed to be constitutively expressed (Fig. 4). Expression of *Pi5-1* and *Pi5-2* particularly increased at 24 hpi in SH707 (Fig. 4). The expression patterns of *Pikm* and *Pi5* were consistent with previous reports (Ashikawa *et al.*, 2008; Lee *et al.*, 2009). These data suggest that high expression of the resistance genes presumably contribute to the resistance in GX1A, DX4A and SH707.

Monogenic lines harboring different single resistance gene exhibit different resistance spectrum. To narrow down the resistance gene candidates in GX1A,
DX4A and SH707, we compared the resistance spectrum among them together with the monogenic lines containing the corresponding single blast resistance genes, i.e. *Pib, Pi-ta, Pia, Pikm* or *Pi5*; whilst monogenic line of *Pid2* was not included because such a line was not available. Zhong-10-8-14, Guy11 and other 100 *M. oryzae* field isolates were used for artificial inoculation. While the monogenic line containing *Pib, Pi-ta*, and *Pia* exhibited narrow spectrum of resistance to 5.88%, 13.86%, and 2.91% of the tested isolates, *Pikm* and *Pi5* were resistant to 90.21% and 26.04% of the tested isolates, respectively (Supplementary Table S2), implying that *Pikm* and *Pi5* might be among the main resistance contributors in these lines. Nevertheless, because GX1A, DX4A and SH707 showed resistance to all tested isolates (100%), there might be novel resistance genes or *Pid2* contributing to the resistance in these lines. However, further experiments are necessary to identify the all contributors for the broad-spectrum resistance in these lines.

Polymorphism analysis reveals potentially functional resistance genes in GX1A, DX4A and SH707. The detection of *Pikm1-TS, Pikm2-TS, Pia-RGA4, Pia-RGA5, Pia-RGA6, Pia-RGA7, Pb1, Pit, Pi36, Pi37, Pi5-1, Pi5-2, Pi-k*, *Pi25* and *Pi56* were examined. Rice OsActin1 was set as the control. The experiment was repeated at least three times and typical images were presented.
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and Pt-ta in GX1A, DX4A and/or SH707 indicated that these genes might contribute to the resistant phenotypes in these lines. However, the transcripts of these genes were also detected in the susceptible line LTH. We speculated that the detected transcripts of these genes must be from either the susceptible or resistant alleles. To this end, we analyzed the full-length cDNA sequences of these genes from the corresponding lines.

The Pikm locus is composed of two closely linked NBS-LRR genes (i.e. Pikm1-TS and Pikm2-TS) and both are required for the resistance function. Pikm1-TS and Pikm2-TS are initially cloned from a blast-resistant cultivar Tsuyuake (Ashikawa et al., 2008), and several allelic genes, such as Pik, Piks, Pik-p and Pi1, were identified at this locus. Sequence analysis revealed that polymorphism mainly located at the CC domain (400-1000 bp after the start codon) among the resistant alleles, such as Pik-1, Pikm1-TS, Pikp-1, Piks-1, and Pi1-5C, and susceptible alleles (Fig. 5a).

Full-length cDNA sequence analysis revealed that several point mutations led to amino acid substitutes in LTH that might distinguish the susceptible allele of Pikm1-TS in LTH from the resistant alleles; these sites included K190T, A212K, A221S and K285R substitutions (Fig. 5a). While the site A212K was also found in GX1A, the site P673 was...
converted into S in GX1A; A221 site was converted into T and E49 site was converted into K in DX4A; all the other substitutions in GX1A and DX4A were identical to one of the resistant alleles (Fig. 5a). These data imply that the Pikm1-TS allele might be functional in both GX1A and DX4A. Nevertheless, functional analysis through transgenic approach is required to examine whether the specific A212K and P673S substitutions in GX1A, and E49K and A221T substitutions in DX4A led to any changed functions. On the contrary, Pikm2-TS locus showed high conservation in both cDNA and protein sequences. Five SNPs were detected, resulting in four amino acid substitutions, but none of them could tell the susceptible allele in LTH from the resistant alleles (Fig. 5b), implying that all the alleles of Pikm2-TS might be functional and the resistant function of Pikm might merely rely on the variation of Pikm1-TS.

The Pia locus is also composed of two adjacent NBS-LRR genes, namely RGA4 and RGA5, both of which are required for resistance to M. oryzae strains containing Avr-Pia (Okuyama et al., 2011). Sequence alignment revealed multiple SNPs and InDels in both Pia-RGA4 and Pia-RGA5 among GX1A, DX4A, SH707 and Sasanishiki (a rice cultivar from which the resistant alleles of Pia-RGA4 and Pia-RGA5 were first isolated). Large fragment deletions of Pia-RGA4 in GX1A and SH707 suggested that it was not functional in these two lines (Fig. 5c); compared with the resistant allele in Sasanishiki, a fragment from nucleotide position 208 to 583 was deleted resulting in a deletion of 125 amino acid residues of Pia-RGA4 in GX1A, and from nucleotide position 32 to 554 was deleted leading to deletion of 171 amino acid residues located in the CC domain of Pia-RGA4 in SH707. On the contrary, no polymorphism was detected for Pia-RGA5 between in Digu and LTH was amplified and sequenced. As shown in Table 1, analysis on amino acid sequences revealed that Pid2 in all the male sterile and restorer lines are functional and thus likely contribute to the blast resistance in these lines.

We also compared the sequences of Pi-ta amplified from GX1A, DX4A and SH707 with those from previously reported cultivars including Yashiro-mochi, C101A51 and Tsuyuaka (Bryan et al., 2000). Polymorphisms were found at five amino acid residues of Pi-ta in GX1A, DX4A and SH707. GX1A and DX4A shared the same type of polymorphism in Pi-ta with C101A51 (Table 2), from which the reported resistant allele in Digu, from which Pid2 was cloned (Chen et al., 2006). By contrast, an A to G point mutation at the nucleotide position 1383 leading to aa site I461 substituted with M of Pid2 in LTH (Table 1). These data suggest that Pid2 in all the male sterile and restorer lines are functional and thus likely contribute to the blast resistance in these lines.

**DISCUSSION**

In the past decades, rice yield-increasing and blast disease control have been attributed greatly to the success of three-line hybrid rice system in China. Hybrid rice is generated by cross-pollination of a male sterile line with a restorer line. Thus, genes controlling agronomically important traits in either the male sterile line or the restorer line can be expressed in hybrid rice. To better exploit the released male sterile and restorer lines, it is important to know the agronomically important trait-related genes, particularly the disease resistance genes in individual lines. The two male sterile lines GX1A and DX4A, and the

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<th>Rice variety</th>
<th>Response to blast</th>
<th>Polymorphism site&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Digu&lt;sup&gt;b&lt;/sup&gt;</td>
<td>R</td>
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<tr>
<td>GX1A</td>
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<td>DX4A</td>
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<td>SH707</td>
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<td>LTH</td>
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<sup>a</sup>Sequence polymorphism was detected at amino acid position 461. I and M indicate amino acid Isoleucine and Methionine, respectively.

<sup>b</sup>Digu is the rice variety from which the Pid2 gene is cloned (Chen et al., 2006).

The two male sterile lines GX1A and DX4A, and the restorer line SH707 may not functional and thus not contribute to their blast resistance.

Full length of Pid2 gene from GX1A, DX4A, SH707 and LTH was amplified and sequenced. As shown in Table 1, analysis on amino acid sequences revealed that Pid2 from GX1A, DX4A and SH707 was identical to the reported resistant allele in Digu, from which Pid2 was cloned (Chen et al., 2006). By contrast, an A to G point mutation at the nucleotide position 1383 leading to aa site I461 substituted with M of Pid2 in LTH (Table 1). These data suggest that Pid2 in all the male sterile and restorer lines are functional and thus likely contribute to the blast resistance in these lines.

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**Table 1.** Sequence polymorphism of Pid2 (ACR15163.1).

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</tr>
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<tbody>
<tr>
<td>Yashiro-mochi</td>
<td>R</td>
<td>I R H D A</td>
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<tr>
<td>C101A51</td>
<td>S</td>
<td>I R H D S</td>
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<tr>
<td>Tsuyuaka</td>
<td>S</td>
<td>S Q V S</td>
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<tr>
<td>GX1A</td>
<td>R</td>
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<td>DX4A</td>
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<td>I R H D S</td>
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<tr>
<td>SH707</td>
<td>R</td>
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<sup>a</sup>Yashiro-mochi is resistant to blast pathogens possessing AVR-Pi-ta gene, while C101A51 and Tsuyuaka were susceptible (Bryan et al., 2000).

Sequence polymorphisms were detected at five amino acid residues of Pi-ta from resistant and susceptible alleles.
restorer line SH707 were released in China recently and a few hybrid rice cultivars were developed based on these lines. In field trials, GX1A, DX4A and SH707 exhibit high resistance to both leaf and panicle-neck blast diseases. In the present study, the broad-spectrum resistance feature of these lines was confirmed by inoculation of 102 M. oryzae isolates in controlled conditions (Supplementary Table S2; Fig. 1; Fig. 2). Thus our present focus was on addressing the question whether the candidate genes contributing to the broad-spectrum resistance in the male sterile lines were different from those in the restorer line.

Because there are 24 blast resistance genes being functionally cloned, the first step and probably a “short-cut” strategy to identify the potential contributor of resistance in these lines is to examine the expression of the cloned resistance genes in these lines upon M. oryzae infection. If there were not any expression of tested genes in a line, then these genes must not be functional or non-existent in the line. Following this scenario, we examined the transcripts of all the cloned blast resistance genes in GX1A, DX4A and SH707, except p21, which is a recessive mutant and does not exist in indica varieties (Fukuoka and Okuno, 2001). As expected, we detected the transcripts of Pi9, Pi-5a, Pia (i.e. RGA4 and RGA5), Pikm (i.e. Pikm1-TS and Pikm2-TS) in GX1A and DX4A; and Pib, Pi-5a, Pia (RGA4 and RGA5), and Pi5 (P5-1 and P5-2) in SH707 (Fig. 3; Fig. 4). Therefore, these genes were candidate contributors for the broad-spectrum resistance in GX1A, DX4A and SH707. However, the transcripts of Pi-5a, Pia (RGA4 and RGA5) and Pikm (Pikm1-TS and Pikm2-TS) were also detected in the susceptible line LTH, indicating that the detected transcripts could be from either the resistant or the susceptible alleles (Fig. 3).

To tell whether the transcripts detected by RT-PCR was from the resistant or the susceptible allele, the simplest method was to perform sequence analysis. Therefore, we performed sequence analysis on Pikm, Pia, Pi-5a and Pi5. We excluded Pib and Pi5 in the sequence analysis because Pib was only detected in the resistant lines and Pi5 was only detected in SH707, and they were most likely among the resistant contributors, although future sequence and functional analyses might be inevitable. Resistance genes are among the most diverse class of genes in Arabidopsis thaliana (Clark et al., 2007). In rice, polymorphisms are also widely distributed in resistance genes, which lead to generation of different resistant alleles. For example, high nucleotide polymorphism level has been reported in Pikm locus, particularly in the region between 400 bp and 1000 bp from the start codon of Pikm1-TS (Costanzo and Jia, 2010), resulting in the resistant alleles of Pik, Piks, Pik-p and Pik-t. The amino acid sequence identities of Pikm1-TS from 15 tested rice cultivars range from 57.9% to 100%. Polymorphism also exists in Pi5. Although only two base changes leading to two amino acid substitutions are found among different indica and japonica rice cultivars, the I461M substitution is enough to distinguish resistant and susceptible Pi5 alleles (Chen et al., 2006). LTH is highly susceptible to blast fungus and has no functional blast resistance genes (Tsunematsu et al., 2000). However, the transcripts of Pi-5a, Pi2, Pia-RGA4, Pia-RGA5, Pikm1-TS and Pikm2-TS were detected in LTH (Fig. 3). Sequence analysis on these genes indicated that LTH contained susceptible alleles of these genes, while GX1A and DX4A contained resistant alleles of Pi2 and Pikm, SH707 contained resistant alleles of Pi-5a and Pi2 (Table 1; Table 2; Fig. 5). Nevertheless, whether the mutation site in Pikm-TS from GX1A and DX4A identical to the one from LTH affects the resistant function of Pikm is a future research focus.

In addition, the contribution of a resistance gene with narrow resistant spectrum may be very limited, and thus can be excluded among the resistant contributors. To this end, we examined the resistance spectrum of monogenic lines harboring Pib, Pi-5a, Pia and Pikm in LTH background (Tsunematsu et al., 2000), respectively, with 102 isolates. Resistance spectrum assay revealed that all the five monogenic lines exhibited resistance to certain of the tested isolates, with the lowest resistance frequency of 2.91% for Pia and the highest frequency of 90.21% for Pikm (Supplementary Table S2). The resistance spectrum for Pi2 could not be determined because its monogenic line was not available. Pi2 encodes a receptor-like kinase protein, representing a new class of plant resistance genes due to its predicted extracellular domain of a bulb-type manno specific binding lectin (Chen et al., 2004). Transgenic rice plants expressing Pi2 display resistant frequency as high as 91.7% to 39 rice blast fungal strains, suggesting a broad resistance spectrum (Chen et al., 2010). Thus, Pi2 must be among the resistant contributors to the broad-spectrum resistance in GX1A, DX4A and SH707.

Nevertheless, we cannot exclude that there might be novel resistance genes contributing the broad-spectrum resistance in GX1A, DX4A and SH707. Because identification and functional characterization of novel genes are both a labor- and time-consuming project, it is beyond the scope of current study and will be the first priority in the future research focus.

In conclusion, this study described that two rice male sterile lines GX1A and DX4A, and a restorer line SH707 had a broad blast resistance spectrum. The potentially resistant contributors in the male sterile lines were different from that in the restorer line. While Pikm and Pi2 may be the major resistant contributors in GX1A and DX4A, Pi2, Pi-5a and Pi5 are most likely among the resistant contributors in SH707. In a previous study, we detected that Pi2, a broad-spectrum resistance gene on chromosome 6, contributes to the broad-spectrum resistance in the elite restorer line YH2115 (Shi et al., 2015). Therefore, it is tempting to combine Pi5 on chromosome 9 with Pi2 by crossing SH707 with YH2155 in developing new restorer line. All the male sterile and restorer lines reported here
are highly valuable in three-line hybrid rice disease-resistant breeding programs.

ACKNOWLEDGEMENTS

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