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Development of a marker specific for the rice blast resistance gene Pi39 in the Chinese cultivar Q15 and its use in genetic improvement

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Blast, caused by the ascomycete fungus Magnaporthe oryzae, is one of the most devastating diseases of rice due to the high variation of the pathogen. Breeding high-yielding rice cultivar with durable resistance to rice blast is a priority in Southern China and in places where rice cultivation is an important branch in farming. Effectiveness and accuracy of resistant cultivar breeding largely depend on the development of markers specific to the target gene. In silico prediction of the resistance (R) Pi39 gene content of the interval was made and hence a candidate gene was identified to develop a perfect insertion–deletion (InDel) based marker for Pi39 gene selection. The Pi39 gene was successfully introgressed in two elite cultivars using both foreground (the InDel) and background (genome-wide microsatellites) genotypic and phenotypic selection. Five selected BC3F3 progeny lines were recovered and showed a high level of blast resistance. At least 97.5% of their genome was inherited from their recurrent parent. The agronomic performance of four lines (D94, D98, D112 and D113) was at least as good as that of their recurrent parent.

**Keywords:** rice; marker-assistant selection; resistance breeding

Introduction

Rice blast is caused by the fungus Magnaporthe oryzae and remains one of the most destructive rice crops diseases in China.[1,2] As deployment of the host plant’s resistance is considered to be the safest management option, the development of resistant cultivars is a continuing priority in rice breeding programmes, particularly in Southern China.[3,4] Genetic resistance, based on single genes, has a history of rapid breakdown, caused by pathogen adaptation.[5] One strategy for improving the durability of genetic resistance is to stack several genes into a single cultivar,[6–8] whereas another is to exploit genes which confer a broad resistance spectrum.[9–11] Nearly 100 distinct blast resistance genes have been identified, of which at least 14 (Pi1, Pi2, Pi9, Pi20(t), Pi33, Pi39, Pi40(t), Pi47, Pi48, Pi54rh, Pi56, Piz, Piz-t, and Pigm) have been described as conferring broad spectrum resistance.[4,12–22] The broad spectrum resistance gene Pi39, carried by the Yunnanese cultivar (cv.) Q15, is a homonym of the one carried by the cultivar Chubu 111 [23] and is also a particularly promising introgression target for rice that grows in the Guangdong Province, where the climate is favourable for the development of rice blast epidemics.[2,17]

DNA-based markers have an increasing impact on conventional breeding practices.[24] The most effective markers are those which lie within the sequence of the target gene itself, since otherwise there is always a risk of miss-selection when a recombination event has separated the marker from the target site.[25,26] Such ‘perfect’ markers have been developed for blast resistance genes, allowing them to be used as highly reliable selective aids. DNA markers specific for Pita and Pib genes have been used to follow their introgression into advanced breeding lines.[27,28] Allele-specific and insertion–deletion (InDel) marker sets are available for nine blast resistance genes, providing an efficient marker system for marker-assisted selection (MAS).[29,30] Recently, a functional marker for the blast resistance gene Pit has been developed and employed in the mining of this gene in diverse rice varieties or landraces.[16] In the case of Pik gene locus in rice, markers, which reflect allele-specific blast resistance, were identified. This allows the differentiation of Pik alleles from each other by applying MAS during
rice breeding processes.[4,31,32] The present study was focused on developing a ‘perfect’ genetic marker for Pi39 and using it to introgress the gene into two high yielding, but blast susceptible cultivars – ‘Yuexiangzhan’ (YXZ) and ‘Yueyinsimiao’ (YYSM).

Materials and methods

Candidate gene annotation and sequence analysis

The gene annotation program RiceGAAS (http://ricegaas.dna.affrc.go.jp/rgadb/) was used to identify candidates for Pi39 within the Nipponbare genomic region defined by the closest flanking markers (39M11 and 39M22) (Figure 1(A) and 1(B)).[17] The full length of the chosen candidate, including its promoter and terminator, was amplified from the genomic DNA of the Pi39 donor Q15 by using long-range PCR (Takara, Dalian, China) with primer pair 39-LF/R, as described by Liu X et al.[17] The amplicon was inserted into the AscI restriction site of the vector pCAMBIA1300AscI and then it was sequenced (Figure 1). RACE (Rapid Amplification of cDNA Ends) PCR was conducted using a GeneRacer Kit (Invitrogen, Groningen, The Netherlands), following the manufacturer’s instructions. The Pi39 5′ RACE product was then amplified using nested PCR. The first reaction used reversed primer 39-5RACE1 and the GeneRacer 5′ primer, provided by the kit. The second round used nest reversed primer 39-5RACE2 in combination with the GeneRacer 5′ nest primer. The 3′ RACE employed forward 39-3RACE1 along with the GeneRacer 3′ primer. A mediate RT-PCR fragment was obtained by a pair of primers 39-RTF/R (Figure 1). Sequencing of amplicons derived from the cDNA of the blast susceptible cultivars Q1063, Kasalath and 93-11 was carried out using the primer pair 39-CDSF/R. All PCRs were based on a high-fidelity Taq polymerase (NEB, England). After an A-tailing procedure, the PCR amplicons were inserted into the pMD20 T-vector (TaKaRa, Dalian, China) and sequenced by Invitrogen (Guangzhou, China). All primers’ sequences are given in Table 1. Sequences alignments were performed using the DNAstar 7.10 (DNASTAR, Inc) software package.

Figure 1. The genomic region surrounding Pi39.
Note: Physical map of the Pi39 locus on chromosome 12 based on the ‘Nipponbare’ sequence (adapted from [17], Springer license number: 3551371059670 [Liu X, Yang Q, Lin F, Hua L, Wang C, Wang L, Pan Q. Identification and fine mapping of Pi39(t), a major gene conferring the broad-spectrum resistance to Magnaporthe oryzae. Mol Genet Genomics. 2007;278:403–410.] (A); according to RiceGAAS (http://ricegaas.dna.affrc.go.jp/), the Pi39 region harbours seven predicted genes, two of which have an NB-ARC domain (filled arrow) (B); the structure of OJ1115-G02 Autopredgene22 and OJ1115-G02 Autopredgene23, as predicted by RiceGAAS. The numbers shown at the top refer to cv. Nipponbare genomic sequence positions along chromosome 12 (C); the structure of Pi39, as determined by a comparison between its cDNA and gDNA sequence. Exons are shown as boxes and introns are shown as horizontal lines, connecting the exons. The positions of the Pi39-specific InDel and SNP markers are indicated by triangles (D).
Marker development

For target \( \text{Pi39} \), a primer pair (39SM) was chosen, which targeted an InDel differentiating the resistant from the non-resistant allele (Figure 1(D)). The amplification was performed in T100 machine (Bio-Rad Laboratories, Inc) and the programme was 94 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 1 min and 72 °C for 1 min, with a final extensional at 72 °C for 5 min. The PCR products were separated on 2.0% agarose gel, which contained ethidium bromide and was visualized by ultraviolet light.

| Primer name | Sequences | Use of primers |
|-------------|-----------|----------------|
| 39-L        | F: TGACTAGGCGGCTCCTCCACAAGACTCATCTCCACACAT | Gene structure determination |
|             | R: TCAGCGGATGGCGCGTGAGGACAGGACATTCTTGGTAGG | |
| 39-RT       | F: GTTCGTCTGCACAAGTCCCA | Gene structure determination |
|             | R: TCCTCGAATCGCACCATA | |
| 39-5RACE1   | F: CAAGACAGGTCGCGTGCTGCAG | 5’ RACE |
|             | R: GCAGCATTGTGACCTTGGGTCTGG | |
| 39-5RACE2   | F: CCAAGGTGACAATGCTGCCACTACAAG | 3’ RACE |
|             | R: ATGGAGCAATCGCCGCGCG | Allele sequencing |
| 39-3RACE1   | F: GCAGCATTGTGACCTTGGGTCTGG | 5’ RACE nest primer |
| 39-3RACE2   | R: CAAGACAGGTCGCGTGCTGCAG | |
| 39-CDS      | F: ATGGAGCAATCGCCGCGCG | Allele sequencing |
|             | R: CGTGTGTCGCGCGCGCG | |
| 39SM        | F: TGCAGGACTAGAGACCTTGTA | Pi39-specific marker |
|             | R: CACCCCATATAACTCTCTCC | |

Note: Forward primer (F); reverse primer (R); Rapid Amplification of cDNA Ends (RACE).

Table 1. Details of the PCR primers used in this study.

Plant materials

The donor of \( \text{Pi39} \) was the native upland rice cv. Q15, while the recipient cvs. were YXZ and YYSM. Each of the \( F_1 \) hybrids Q15 × YXZ and Q15 × YYSM was backcrossed with its respective recurrent parent (Figure 2) and advanced lines were developed by following a backcrossing strategy using both genotypic and phenotypic selection. The presence of \( \text{Pi39} \) was ensured by the use of marker 39SM. \( BC_3F_1 \) generation was allowed to self-fertilize, and a combination of genotypic and phenotypic selections were applied to identify the individuals carrying \( \text{Pi39} \).
in a genetic background as close as possible to that of the recurrent parent. In field screening, where blast resistance was being monitored, the cv. Yueluzhan was included as a susceptible spreader. Four Pi39 susceptible cultivars, Tsuyuake, Q61, Kasalath and 93-11 were subjected to Pi39 candidate gene sequence. A panel of 121 accessions (16 landrace, 11 modern cultivars and 94 wild rice entries) (Table S1 in the Online Supplementary Appendix) were scanned to evaluate the specificity of the Pi39 marker.

**Background genotyping**

A set of 187 simple sequence repeat (SSR) marker assays, marking loci of known genomic location on each of the 12 chromosomes, was applied for background genotyping (Figure S1(A) and S1(B) in the Online Supplementary Appendix). Their location was based on the rice genetic map [33] as depicted in Gramene (http://www.gramene.org). PCR amplification and marker detection were done in the same way as previously described.[34]

**Blast resistance in the field**

Field screening for response to blast infection was carried out in a known disease hot spot in the Liutian county in the north Guangdong Province.[35] Around 20 grains of each BC3F2 and BC3F3 selections were sown in a line of 67 cm row, with an inter-row spacing of 23 cm, along with Q15 and the parental cultivar (XYZ and YYSM). The set of rows was surrounded by Yueluzhan plants. Disease reaction was scored on a 0—9 scale 10—15 days after the Yueluzhan plants showed severe signs of infection. The lines with a score of 0—3 were considered as resistant, 4—5 as moderately resistant, 6 as moderately susceptible and 7—9 as susceptible.[36]

**Agronomic performance**

A set of 20 plants of each selected advanced backcross lines were transplanted to a 20 cm × 17 cm space in a randomized complete block design with three replications during the late season of 2012 at the Guangdong Agriculture Academic Research Institution Dafeng research farm.

The following traits were recorded for 10 plants per line: number of panicles per plant, panicle length (cm), grains per panicle, filled grains (%), 1000-grain weight (g) and yield per plant (g). The least significant difference (LSD) statistic analysis was used to distinguish between mean values, and was obtained by using the software package SPSSv16.0 for Windows (SPSS Inc. Chicago, ILL, USA).

**Results and discussion**

**Identification of Pi39 candidate**

The fine-scale mapping of Pi39 succeeded in locating the gene within a 37 kb region, which harbours seven predicted genes according to RiceGAAS analysis (Figure 1(A) and 1(B)).[17] Two of these putative genes (OJ1115-G02 Autopredgene22 and OJ1115-G02 Autopredgene23) have an NB-ARC domain which is diagnostic for a major class of plant disease resistance genes (Figure 1(B) and 1(C)). Both the genomic DNA and the cDNA versions of OJ1115-G02 Autopredgene23 were sequenced and compared to identify the gene’s size and structure. Since there was an overlap between the 5’ and 3’ RACE products and the RT-PCR fragment, the complete transcribed region could be recognized (Figure 1(D)). The result of the gene structure identification indicated that OJ1115-G02 Autopredgene22 and OJ1115-G02 Autopredgene23 are essential in one and the same transcription (Figure 1(D)), which provided strong evidence that Autopredgene22 and Autopredgene23 had been split due to software miss annotation. Thus, Autopredgene22 and Autopredgene23 form a single gene, which was considered as a strong candidate gene for the Pi39 resistance. Based on the sequence analysis results, the Pi39 candidate gene contained a 3195-bp coding region, interrupted by one intron of 5949 bp and flanked by a 49-bp 5′-untranslated region (UTR) and a 344-bp 3′-UTR. A 1562-bp intron was present within the 3′-UTR (Figure 1(D)).

**The development of a Pi39-specific marker**

The comparison between the Pi39 candidate cDNA sequences of Q15 and Tsuyuake (Table 2) revealed the

| Rice cultivars | Gene code | Position |
|---------------|-----------|----------|
| Q15           | Pi39ij    | 171 252 294 310 371 399 463 483 488 538 563 655 658 786 787 899 981 1019 1067 1084 90-bp |
| Tsuyuake      | Pi39ij    | +         |
| Q1063         | Pi39ij    | +         |
| Kasalath      | Pi39ij    | +         |
| 93-11         | Pi39ij    | +         |

Note: Resistance (R); Susceptible (S); indica (i); japonica (j); Ala (A); Asp (D); Ghu (E); Phe (F); Gly (G); His (H); Ile (I); Lys (K); Leu (L); Met (M); Asn (N); Pro (P); R: Arg (Rg); Ser (S); Thr (T); Val (V); Trp (W); Tyr (Y); and 90-bp indicate the 90 base pair of InDel at the 3′-UTR.
presence of a 90 bp InDel in the 3′-UTR, along with eight single nucleotide polymorphisms (SNPs) which affected the predicted peptide sequence. However, seven of the eight SNPs were simultaneously present in both Q15 and one (or more) of the susceptible Q1063, Kasalath and 93-11, so they cannot be diagnostic for the resistant allele. Thus either the leucine for arginine shift at position 171 and/or the 90 bp InDel are unlikely to be responsible for the *Pi39*-mediated resistance. The latter was convenient, because the target for marker development and its detection were very straightforward. The use of major resistance genes in breeding for blast resistant rice is complicated as many *Pi* genes confer resistance to overlapping spectra of blast phenotypes. Also they are often organized as clusters, which makes it difficult to monitor the presence of individual resistance genes and their introgression in breeding lines.[4,29,30] The elaboration of gene-specific DNA markers simplifies and accelerates the selection of lines carrying multiple resistance genes, since it avoids to carry out a complex set of progeny to test the presence of the resistance gene in each host.[29] The widely used *Pik* locus is multi-allelic and each known allele has been tagged with a specific marker. These assays have been effectively used for resistance breeding and searching for novel alleles in large germplasm sets. [4,29,30] *Pi39*, along with the genes *Pi20(t), Pita, Pita-2, Pi4, Pi6, Pi12, Pi19(t), Pi21, Pi24, Pi31, Pi32, Pi57, Pitq-6*, are all clustered to the pericentromeric region of chromosome 12, which is a part of the chromosome where recombination is suppressed.[17] Recombination suppression near the centromere region of the rice chromosome may inhibit the ability to separate these resistance (*R*) genes/alleles.[28,38] Thus the identification of gene-specific (as opposed to a *R* gene block-specific) DNA markers, distinguishing each of these *R* genes near the centromere region, is technically challenging. The *Pi39*-specific InDel marker, which is described in the present research, has the potential to greatly reduce the time and costs, required for disease screening, in order to distinguish the *Pi39* from other *Pi* genes in this cluster.

A screen of a 121 entry diversity panel showed that the InDel was not present in any of the landraces or modern cultivars, but was present in 11 wild rice accessions in heterozygosis state and in one wild rice accession (W067) in homozygous state (Table S1 in the Online Supplementary Appendix). An essential requirement for a molecular marker and its large-scale implementation in breeding programme is that it should be informative over a wide range of genetic backgrounds, so that the marker can be applied to a wide range of crosses.[39,40] A demonstration of the robustness of the *Pi39*-specific marker was given by the screen of the 121 accession diversity panel. Furthermore, the finding that the *Pi39*-specific marker was restricted to Q15 and a few wild rice accessions indicated that the gene has not yet been widely exploited in Chinese rice improvement programmes. Therefore, it is a valuable and potentially durable allele in contemporary breeding programmes.

### Introgression of *Pi39* Resistance

A selection of five F1 plants was made from the cross Q15 × YXZ and the heterozygosis of the *Pi39* loci confirmed their hybridity. After pollination by the recurrent parent YXZ, 150 BC1F1 progeny were generated, from which four *Pi39* heterozygotes plants were selected for the next round of backcrossing. A set of 150 BC2F1 plants was obtained from 15 BC2F1 selections, and eight of these were self-fertilized to generate 500 BC3F2 plants; 11 selected BC3F2 plants were self-fertilized once more to produce a population of 800 BC3F3 individuals. Finally, two BC3F3 blast resistant (*Pi39 homozygous*) selections were made (D94 and D98) (Table 3). Phenotypic selection at each backcross and selfing generation was conducted to eliminate partially sterile, tall and/or late flowering plants. [41] A similar strategy was applied for the introgression

### Table 3. Reaction of the five selected BC3F3 lines to rice blast in field.

| Cross combination | Line selected (BC3F3) | Number of plants for each disease reaction scale | Phenotypes |
|-------------------|-----------------------|------------------------------------------------|-------------|
|                   |                       | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Total | R+MR : MS+S |
| Q15 × YXZ         | D94                   | 1 | 2 | 3 | 12| 1 | 1 | 0 | 0 | 0 | 0 | 20 | 20 : 0 |
|                   | D98                   | 0 | 3 | 5 | 10| 2 | 1 | 0 | 0 | 0 | 0 | 21 | 21 : 0 |
| Q15 × YYSM        | D112                  | 1 | 1 | 4 | 9 | 1 | 2 | 0 | 0 | 0 | 0 | 18 | 18 : 0 |
|                   | D113                  | 1 | 4 | 5 | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 20 | 20 : 0 |
|                   | D114                  | 0 | 1 | 4 | 12| 1 | 1 | 0 | 0 | 0 | 0 | 19 | 19 : 0 |
| Q15               |                       | 7 | 5 | 4 | 4 | 0 | 0 | 0 | 0 | 4 | 8 | 4 | 16 | 20 : 0 |
| YXZ               | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 12| 3 | 18 | 0 : 18 |
| YYSM              | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 12| 3 | 18 | 0 : 18 |

Note: For 0–9 scale: score 0 – resistant (R), 4–5 – moderately resistant (MR), 6 – moderately susceptible (MS) and 7–9 – susceptible (S).
of *Pi39* into YYSM, producing the three BC3F3 selections (D112, D113 and D114). Field blast resistance analysis of all five selected BC3F3 lines suggested that they show resistance to the rice blast in field and the resistance phenotypes are perfectly associated with the *Pi39* genotypes in selection (Table 3).

### SSR-based genetic background profiling of BC3F3 lines

Out of 187 SSRs, 96 were informative between Q15 and YXZ and 95 between Q15 and YYSM (Figure S1(A) and S1(B) in the Online Supplementary Appendix). Background selection was applied to the BC3F3 generation, in order to achieve an average recovery of 99.3% of the YXZ and 98.3% of the YYSM genome (Table 4). Donor DNA was restricted to small regions in chromosome 3, 4, 6, 9 and 10, as well as in the vicinity of *Pi39*, which lies in chromosome 12.[17] The D94 background was 99.5% recipient, that of D98 was 99.0%, that of both D113 and D114 was 98.7% and that of D112 was 97.6% (Table 4). Without any background selection, simulations assumed a 50% reduction in donor genome with each backcross in a standard backcross strategy.[41] On this basis, six backcrosses are needed to cover an average of 99.2% of the recurrent parent genome.[41,42] The advantage of deploying background selection is that the number of the required backcross generations can be decreased, which represents a substantial reduction of the time to complete the breeding task.[43] A combined foreground and background selection strategy enabled the selection of a three gene introgression line (*xa5, xa13* and *Xa21*) having a 97% level of the recurrent parent background by the fourth backcross generation.[44] In the present study, the recovery had already reached 99% by the third backcross, thanks to the effective deployment of background phenotypic and SSR-based selection.

### Agronomic performance of improved selections

The field performance of the five selected backcross lines D94, D98, D112, D113 and D114 was evaluated during the late season of 2012 (Table 5). The YXZ-derived selection D94 produced more panicles per plant, a greater percentage of filled grains and a higher yield per plant than YXZ, whereas plants of the other selection, D98, formed longer panicles, set more grains per panicle and also produced a greater yield per plant than YXZ. All of the three YYSM-derived lines were characterized by longer panicles than those of recurrent parent YYSM. Plants D112 formed more panicles per plant and had a better yield than YYSM, but there was no significant variation with respect to 1000 grain weight among the improved lines and their recurrent parents. The presence of *Pi39* in these improved lines can be expected to provide a level of resistance against most of the rice blast strains present in the Guangdong region, so this should have a positive impact on yield, stability and sustainability of the local rice crop. Grain yield and quality of the four *Pi39* breeding lines (D94, D98, D112 and D113) are at least as good as, if not better than, those of the respective recurrent parent, which confirms the absence of any unfavourable linkage drag associated with the *Pi39* gene. Therefore, these lines can be used directly as improved cultivars or as donors of *Pi39* in ongoing breeding programmes.

| Chromosome | Q15 × YXZ |  | Q15 × YYSM |  |
|------------|-----------|--|------------|--|
|            | PM (%) between | RP segment in selected line | PM (%) between | RP segment in selected line |
|            | DP/RP | D94 | D98 | DP/RP | D112 | D113 | D114 |
| 1          | 39.1 | 100.0 | 100.0 | 43.5 | 100.0 | 100.0 | 100.0 |
| 2          | 57.1 | 100.0 | 100.0 | 57.1 | 100.0 | 100.0 | 100.0 |
| 3          | 50.0 | 97.2 | 97.2 | 27.8 | 100.0 | 94.4 | 94.4 |
| 4          | 57.1 | 100.0 | 100.0 | 50.0 | 100.0 | 92.9 | 92.9 |
| 5          | 41.7 | 100.0 | 100.0 | 50.0 | 100.0 | 100.0 | 100.0 |
| 6          | 68.8 | 100.0 | 90.6 | 68.8 | 100.0 | 96.9 | 96.9 |
| 7          | 26.7 | 100.0 | 100.0 | 20.0 | 100.0 | 100.0 | 100.0 |
| 8          | 30.8 | 100.0 | 100.0 | 46.2 | 88.5 | 100.0 | 100.0 |
| 9          | 66.7 | 100.0 | 100.0 | 44.4 | 83.3 | 100.0 | 100.0 |
| 10         | 42.9 | 96.4 | 100.0 | 42.9 | 100.0 | 100.0 | 100.0 |
| 11         | 70.0 | 100.0 | 100.0 | 60.0 | 100.0 | 100.0 | 100.0 |
| 12         | 27.8 | 100.0 | 100.0 | 38.9 | 100.0 | 100.0 | 100.0 |
| Average (Total) | 48.2 | 99.5 | 99.0 | 46.4 | 97.6 | 98.7 | 98.7 |

Note: Polymorphism marker (PM); recurrent parent (RP); donor parent (DP).
Table 5. Comparison of principal agronomic traits between introgression lines and their recurrent parents.

| Cross recombination | Variety and line selected | Number of panicles per plant | Panicle length (cm) | Number of grains per panicle | Filled grains (%) | 1000 grain weight (g) | Yield per plant (g) |
|---------------------|---------------------------|------------------------------|---------------------|-----------------------------|------------------|----------------------|---------------------|
| 39MAS-1             | D94                       | 16.0 ± 3.5**                 | 21.1 ± 0.3          | 165.6 ± 14.0                | 93.7 ± 2.0**     | 20.1 ± 1.1           | 39.4 ± 3.0**        |
|                     | D98                       | 11.0 ± 1.2                   | 22.38 ± 1.46*      | 221.7 ± 13.0**              | 89.5 ± 1.9*      | 19.2 ± 1.0           | 39.5 ± 1.1**        |
|                     | YXZ                       | 10.8 ± 2.3                   | 20.7 ± 0.5         | 158.1 ± 18.1                | 93.6 ± 2.4       | 18.5 ± 0.3           | 29.2 ± 8.5          |
| 39MAS-2             | D112                      | 12.0 ± 1.3*                  | 23.75 ± 1.03**     | 187.8 ± 20.0                | 83.3 ± 1.2       | 21.4 ± 0.4           | 38.5 ± 1.3**        |
|                     | D113                      | 11.25 ± 2.72                 | 24.25 ± 0.79**     | 191.5 ± 16.2                | 64.9 ± 15.2      | 22.4 ± 0.2           | 27.6 ± 6.6          |
|                     | D114                      | 9.25 ± 1.52                  | 25.50 ± 0.65**     | 36.6 ± 7.2**                | 49.2 ± 5.0**     | 22.2 ± 0.1           | 21.4 ± 5.0**        |
|                     | YYSM                      | 9.5 ± 1.6                    | 22.6 ± 1.5         | 189.9 ± 23.0                | 92.7 ± 1.0       | 21.4 ± 0.3           | 29.3 ± 6.2          |

Note: Lines derived from the cross of Q15 and YXZ are named 39MAS-1, and those from cross Q15 and YYSM are named 39MAS-2; means with * and ** indicate significant difference at 0.05 and 0.01 level, respectively, between pyramid line and their recurrent parents; Yuxiangzhan (YXZ); Yueyinsimiao (YYSM);

Conclusions
In this study, a ‘perfect’ InDel-based marker for Pi39 gene selection was developed. Robustness of the Pi39-specific marker was verified by the screen of the 121 accession genotypic and phenotypic selection. Five selected BC3F3 progeny lines were recovered and showed a high level of blast resistance. At least 97.5% of their genome was inherited from their recurrent parent. The agronomic performance of four lines (D94, D98, D112 and D113) was at least as good as that of their recurrent parent.

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No potential conflict of interest was reported by the authors.

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Supplemental data
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