RESEARCH ARTICLE

Fine mapping of Pi57(t) conferring broad spectrum resistance against Magnaporthe oryzae in introgression line IL-E1454 derived from Oryza longistaminata

Liying Dong¹, Shufang Liu¹, Peng Xu²,³, Wei Deng², Xundong Li¹, Didier Tharreau⁴, Jing Li², Jiawu Zhou², Qun Wang¹, Dayun Tao²*, Qinzhong Yang¹*

¹ Agricultural Environment and Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming, Yunnan, China, ² Food Crops Institute, Yunnan Academy of Agricultural Sciences, Kunming, Yunnan, China, ³ Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Kunming, Yunnan, China, ⁴ Centre de Coopération Internationale en Recherche Agronomique pour le Développement, UMR BGPI, Montpellier, France

* taody12@aliyun.com (DT); qzhyang@163.com (QY)

Abstract

Wild species of the genus Oryza are excellent gene pools for improvement of agronomic traits of Asian cultivated rice. The blast resistance gene Pi57(t) in the introgression line IL-E1454 derived from Oryza longistaminata was previously mapped on rice chromosome 12. Inoculation with 322 Magnaporthe oryzae isolates collected from 6 countries indicated that Pi57(t) conferred broad spectrum resistance against M. oryzae. Two mapping populations consisting of 29070 and 10375 F₂ plants derived from the crosses of resistant donor IL-E1454 with susceptible parents RD23 and Lijiangxintuanheigu respectively, were used for fine mapping of Pi57(t) locus. Based on genotyping and phenotyping results of recombinants screened from the two crosses, Pi57(t) was finally mapped to a 51.7-kb region flanked by two molecular markers (STS57-320 and STS57-372) on the short arm and close to the centromere of chromosome 12. Six candidate resistance genes were predicted in the target region according to the reference sequence of Nipponbare. These results could facilitate both marker-assisted selection for disease-resistant breeding and gene cloning.

Introduction

Rice blast, caused by the filamentous ascomycete Magnaporthe oryzae [1], is one of the most destructive diseases for rice (Oryza sativa L.), and is responsible for significant yield losses under favorable environmental conditions worldwide [2]. Rice-M. oryzae pathosystem follows the gene-for-gene relationship during the host-pathogen interaction [3–4]. The use of resistance (R) genes in rice breeding has been proved to be the most economic, effective and environment-friendly strategy for blast management. But, after the release of blast resistant varieties, the emergence of virulent races of the pathogen often cause the rapid loss of effectiveness of resistance conferred by monogenic resistance [5]. Few exceptions of monogenic
durable resistance exist [6]. Monogenic resistances may also contribute to durable resistance if appropriate management strategies are used: agronomic conditions, rotation and/or mixtures of varieties, etc. [7–10]. In addition, the pyramiding of multiple R genes with different resistance spectra to races of *M. oryzae* into a single variety through marker-assisted selection strategy is one of the most effective methods to breed durable varieties for durable control [11]. Pyramiding requires the characterization and identification of markers closely linked to the R genes of interest.

In the past decades, genetic studies on blast resistance in rice have been extensively conducted, and over 100 major blast R genes from *O. sativa* and its wild relatives have been identified and mapped on the 12 chromosomes of rice [12–16]. Clusters of functional genes were identified on chromosomes 6, 11 and 12. Most of the R genes are dominant, except 3 recessive genes, *pi21* [17], *pi55(t)* [18] and *pi66(t)* [16]. The availability of rice genome sequences of two subspecies of cultivated rice, *O. sativa ssp. japonica* cultivar Nipponbare [19] and *indica* cultivar 9311 [20], greatly facilitate the development of molecular markers for fine mapping of R genes, and comparison of R gene positions between mapping populations.

Blast R gene *Pi57(t)* is carried by a introgression line IL-E1454, and was introgressed from *O. longistaminata* into *indica* cultivar RD23. Previously, this gene was preliminary mapped on chromosome 12 of rice using a BC₂F₂ population derived from the cross between IL-E1454 and the recurrent parent RD23. *Pi57(t)* was mapped to a 6.07 Mb region between molecular marker RM27892 and RM28093 [21]. Although *Pi57(t)* can be differentiated from known R genes *Pita*, *Pita2*, *Pi12*, *Pi19* and *Pi20* also located on chromosome 12, through pathogen-testing with different *M. oryzae* isolates [21], its exact genomic position on chromosome 12 remains unclear. In this study, two mapping populations from ILE1454/RD23 and IL-E1454/Lijiangxintuanheigu (LTH) were used for further mapping of *Pi57(t)*.

**Materials and methods**

**Mapping population construction, planting and resistance evaluation**

Resistant donor parent IL-E1454 was crossed with susceptible cultivars RD23 (*indica*) and LTH (*japonica*), respectively. The IL-E1454/RD23 and IL-E1454/LTH F₁ plants were grown in the greenhouse to generate F₂ populations for gene mapping. The germinated F₂ seeds of the IL-E1454/RD23 and IL-E1454/LTH cross combinations were sown in trays of 20×12×5 cm filled with compost, and each tray sowed with 95 seeds. Seedlings were inoculated with *M. oryzae* strain HN09-1C-7 by spraying at 4 leaf stages with 20 ml conidial suspension per tray. The inoculated rice plants were stored for one night in a controlled dark chamber at 25°C with 95% relative humidity, and then transferred back to the greenhouse. Lesion types on rice leaves were observed 6–7 days after inoculation and scored according to a standard reference scale [3]. Plants scored from 1 to 3 were considered to be resistant and scored from 4 to 6 were considered to be susceptible. Four hundred and seventy-five seedlings and 570 of the IL-E1454/RD23 and IL-E1454/LTH populations respectively were inoculated and evaluated for the expected 3:1 resistant: susceptible segregation ratio in F₂ populations [21]. To determine the resistance spectrum of *Pi57(t)* locus, IL-E1454 and 10 monogenic lines were inoculated with 322 isolates from 6 countries (S1 Table).

**M. oryzae isolate cultivation**

*M. oryzae* isolate HN09-1C-7, virulent to RD23 and LTH but avirulent to IL-E1454, and previously used to map *Pi57(t)* [21] was cultured on oatmeal medium (20 g of oatmeal, 15 g of agar, 10 g of sucrose and 1 L of distilled water) for 7 days in dark incubator at 25°C, and then aerial mycelia were washed off by gentle rubbing with distilled water and paintbrush. The colonies
were then successively exposed to fluorescent light for 3 days to induce sporulation at 25˚C. Conidia were harvested by softly scraping and flooding the medium surface with distilled water containing 0.01% Tween 20 detergent. The concentration of conidial suspension was adjusted to 50000 conidia/ml for inoculation.

Marker development and genetic map construction

Total DNA was extracted from fresh leaves of each plant following the method of Edwards et al. [22]. The SSR markers located in the genomic region carrying Pi57(t) and producing a polymorphic band between parents were used to genotype the mapping population. Sequence-tagged site (STS) markers were developed based on the alignment (using BLAST) within the critical region of the genomic sequences of 93–11 and Nipponbare.

PCR amplification conditions consisted of a denaturing step of 94˚C/3 min, followed by 35 cycles of 94˚C/30 s, annealing temperature (see Table 1)/30 s, and 72˚C/1 min, ending with an extension step of 72˚C/7 min. Amplicons were separated by 8% polyacrylamide gel electrophoresis and visualised by silver staining. Primer sequences and other relevant properties of the marker assays are summarized in Table 1. The polymorphism determined by all STS markers developed in this study among resistant donor IL-E1454, and susceptible parents RD23 and LTH were showed in S1 Fig. The genetic and linkage map of polymorphic markers was constructed using MAPMAKER/EXP 3.0 [23]. The Kosambi mapping function was used to transform recombination frequency to genetic distance (cM).

Physical map construction in silico and candidate gene prediction

To construct a physical map of Pi57(t) locus, all molecular markers used for gene mapping were landed on the IRGSP1.0 pseudomolecule of reference cv. Nipponbare released by IRGSP through BLASTN search (https://www.ncbi.nlm.nih.gov/Blast.cgi). Subsequently, the physical map spanning Pi57(t) locus was constructed based on the reference genomic sequence of Nipponbare. The candidate R genes in the target region were predicted based on the annotation information by Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/), GENSCAN (http://genes.mit.edu) and FGENSH (http://www.softberry.com/) software.

Amplification of candidate R genes from IL-E1454 by PCR

To amplify the candidate R genes from IL-E1454, PCR primers were designed according to the reference genomic sequence of Nipponbare. The long-range enzyme (PrimeSTAR GXL DNA polymerase, TAKARA BIO INC.) was used to amplify the target DNA fragments. The PCR amplification conditions and primers information were summarized in Table 1. After amplification, the PCR products were then sequenced and analyzed.

Results

Genetic analysis of Pi57(t) locus

Altogether, 475 and 570 F2 plants derived from the crosses of IL-E1454/RD23 and IL-E1454/LTH, were inoculated with blast isolate HN-09-1C-7 for genetic analysis, respectively. As a result, phenotype of resistant (R) and susceptible (S) plants in both these populations fitted the expected segregation ratio of 3:1, i.e. 360 R: 115 S ($\chi^2 = 0.1579, P = 0.69$), and 437 R: 133 S ($\chi^2 = 0.8444, P = 0.35$), respectively. Hence, a single dominant gene in IL-E1454 confers resistance to M. oryzae isolate HN-09-1C-7, confirming previous results [21]. Therefore, both populations were used to finely map Pi57(t) locus.
Table 1. Summary of PCR markers used in this study.

| Marker   | Primer sequence (5’-3’)* | Genomic position (bp)b | Anneling temperature (˚C) | Expected size (bp) |
|----------|--------------------------|------------------------|---------------------------|--------------------|
| RM27892  | F: ATAAGAGATGGCGGATCTGAGACG R: GTGACACATGTCGACTCGAGACG | 9504613–9504635 | 55 | 153 |
| RM27921  | F: CTGCTTCCTCCTCTGCTCTTC R: GAGCTCTCCTGCTCGGTCCTCC | 10196011–10196033 | 55 | 199 |
| RM7102   | F: TAGGGATGTTTTAGGCTTCC R: TCGGTTTCCTATACATCATCAC | 13214191–13214172 | 55 | 168 |
| RM28093  | F: CTGTTTAGGAGCGTTTGAG R: ATTAAGTCAGGCCGTTCAC | 15572389–15572409 | 55 | 113 |
| STS57-1  | F: TGGATGAAAGAAATGTACCCAA R: GAAAGATGCCGAGTCAGACA | 10467998–10467977 | 55 | 105 |
| STS57-44 | F: TTAGAATGACAGGAAAAAC R: GACAGCTGCAAGCAGAGACACA | 10742010–10742030 | 55 | 81 |
| STS57-36 | F: CTAACCGGACCTATACCCAG R: GATGTGCTGCTGTAGATGTTTC | 108770604–108770624 | 55 | 96 |
| STS57-320| F: GAGGTGGAAGGTGGAGGTCGATAG R: ACATCCATCTCCTAGCTTTC | 10799295–10799318 | 60 | 85 |
| STS57-336| F: CTTCCAGGACAAAAACCT R: GAGCCGATCTGGGGCTGCTC | 108770604–108770624 | 55 | 106 |
| STS57-4  | F: CCCCACCGGTGTTGATCTCTCTTGAG R: GAAAGATGCCGAGTCAGACA | 108150853–108150872 | 55 | 95 |
| STS57-72 | F: TGGCAGACGATGCTGACTGGGA R: CAGCTATGACAAATATGTGGG | 11367028–11367052 | 55 | 231 |
| STS57-5  | F: CGAATTTCATGACCTCATGGAGAAATGTTACCCAA R: GAAAGATGCCGAGTCAGACA | 10848266–10848286 | 68 | 7960 |

* F forward, R reverse
b Genomic position of each marker along chromosome 12 as determined by BLASTN analysis against the Nipponbare genome sequence (IRGSP 1.0)

c The PCR were performed as following: after preheating for 1 min at 98˚C, 35 PCR cycles (10 s at 98˚C, 30 s at 65˚C, and 3 min at 68˚C), followed by 7 min at 72˚C, the PCR products were analyzed by 1% agarose gel
d The PCR were conducted as following: after preheating for 1 min at 98˚C, 35 PCR cycles (10 s at 98˚C, 30 s at 65˚C, and 10 min at 68˚C), followed by 10 min at 72˚C, the PCR products were analyzed by 0.8% agarose gel

https://doi.org/10.1371/journal.pone.0186201.t001
Fine mapping of the blast resistance gene Pi57(t) in Oryza longistaminata

In a previous study, Pi57(t) gene were preliminary mapped in a region spanning the centromere of chromosome 12, and delimited between two SSR markers (RM27892 and RM28093). To further map its chromosomal position, 13395 F2 plants from IL-E1454/RD23 were genotyped by RM27892 and RM28093. As a result, 54 recombinants were found between markers RM27892 and RM28093. The recombinants were further genotyped with 2 known SSR markers RM27921, RM7102, and 3 new developed STS markers STS57-1, STS57-2 and STS57-4. The results showed that recombination events at RM27892, RM27921, STS57-1, STS57-4, STS57-2, RM7102 and RM28093 were 43, 16, 16, 0, 2 and 11, respectively (Fig 1a). Based on the genomic positions of the molecular markers, Pi57(t) locus was delimited between STS57-1 and STS57-2 (Fig 1a), and co-segregates with STS57-4. In order to finely narrow down the region carrying Pi57(t) locus, 15675 and 10375 additional F2 plants from the crosses of IL-E1454/RD23 and IL-E1454/LTH, respectively, were genotyped with STS57-1 and STS57-2. Altogether, 42 and 12 recombinants were identified at STS57-1 and STS57-2 (Fig 1a). Then, these recombinants were further genotyped with STS57-4 and 3 new developed polymorphic STS markers STS57-44, STS57-36 and STS57-72, which are located between STS57-1 and STS57-2. As showed in Fig 1a, 10 and 3 recombinants were found between STS57-44 or STS57-36 and STS57-1, respectively. Two recombinants were found between STS57-72 and...
STS57-2. Through phenotype assays of the recombinants, the \textit{Pi57(t)} locus was further mapped in the region of STS57-36 and STS57-72, and co-segregates with STS57-4 (Fig 1a).

Three additional STS markers (STS57-320, STS57-336 and STS57-372), were developed in the STS57-36/STS57-72 interval. Subsequently, the 54 recombinants between STS57-1 and STS57-2 were genotyped with these markers. As showed in Fig 1a and 1b, two recombinants were detected between STS57-320 and STS57-36, and only 1 recombinant was identified between STS57-372 and STS57-72. As a consequence of fine mapping, \textit{Pi57(t)} gene was finally narrowed down to the region between STS57-320 and STS57-372, and co-segregates with STS57-4 and STS57-336 (Fig 1a–1c).

**Construction of physical map of \textit{Pi57(t)} locus, candidate gene prediction and amplification**

All the molecular markers closely linked with \textit{Pi57(t)} gene were landed to the genome sequence of chromosome 12 of reference cultivar Nipponbare by BLAST analysis (Fig 1c), and the phenotypes of recombinants between STS57-320 and STS57-372 were showed in Fig 1b. Subsequently, \textit{Pi57(t)} locus defined by two flanking markers (STS57-320 and STS57-372), and co-segregated with two markers (STS57-336 and STS57-4). The resulting physical map is shown in Fig 1c with a physical distance of ca. 51.7 kb in the target region.

Based on the annotated Nipponbare genome sequence, 6 genes (\textit{LOC Os12g18690}, \textit{LOC Os12g18700}, \textit{LOC Os12g18710}, \textit{LOC Os12g18729}, \textit{LOC Os12g18750}, and \textit{LOC Os12g18760}) were predicted in the target region flanked by STS57-320 and STS57-372 (Chr12:10799294 to10850958). Among these candidate genes, all genes encode uncharacterized protein with the exception of \textit{LOC Os12g18760} that encode a putative peptidase family C78 domain containing protein. When amplifying these candidate genes in IL-E1454 using primers designed based on the genome sequence of Nipponbare, the candidate genes \textit{LOC Os12g18690}, \textit{LOC Os12g18710}, \textit{LOC Os12g18729}, and \textit{LOC Os12g18760} were successfully obtained, and showed high homology to the corresponding gene loci in Nipponbare at a level of 98.1%, 84.1%, 94.5% and 98.4%, respectively (Fig 1d). The candidate gene \textit{LOC Os12g18700} and \textit{LOC Os12g18750} could not be amplified. When new PCR primers were designed to amplify the remaining target region with IL-E1454 DNA as the template, two gaps located between \textit{LOC Os12g18690}/\textit{LOC Os12g18710}, and \textit{LOC Os12g18729}/\textit{LOC Os12g18760} could not be successfully filled out (data not shown), these suggested that \textit{LOC Os12g18700} and \textit{LOC Os12g18750} genes would be absent or inserted with large DNA fragments in the gaps region, respectively.

**Resistance spectrum determination of \textit{Pi57(t)} gene**

To determine the resistance spectrum, identity and potential usefulness of \textit{Pi57(t)} in rice breeding for disease resistance, IL-E1454 and 10 monogenic lines were tested with 322 \textit{M. oryzae} isolates from Cambodia, Laos, Myanmar, Thailand, Vietnam and China (Table 2). The inoculation results showed that IL-E1454 was resistant to 300 isolates (93.17%) of the total tested isolates. Compared with monogenic lines carrying \textit{R} genes located on chromosome 12, IL-E1454 was resistant to all isolates from Laos and Myanmar, indicated that \textit{Pi57(t)} could be differentiated from \textit{Pi12, Pi19, Pi20, Pita} and \textit{Pita-2}, due to their susceptibility to part of the isolates from Laos and/or Myanmar. Meanwhile, \textit{Pi57(t)} showed a high resistant frequency to the isolates used in this study with the comparison of the known broad-spectrum \textit{R} genes (\textit{Pi5, Piz, Piz-5, Piz-t and Pi9}), suggesting that \textit{Pi57(t)} gene conferred a broad spectrum resistance against \textit{M. oryzae}. 
Table 2. Comparison of resistant percentage of IL-E1454 and 10 monogenic lines to 322 *Magnaporthe oryzae* from 6 countries.

| Number of *M. oryzae* isolates | Country of origin | Lines |  |
|-------------------------------|-------------------|-------|---|
|                               |                   | IL-E1454 (Pi57(t)) | IRBL12-M (Pi12) | IRBL19-A (Pi19) | IRBL20-IR24 (Pi20) | IRBLTA-K1 (Pita) | IRBLTA2-PI (Pita-2) | IRBL5-M (Pi5) | IRBLZ FU (Piz) | IRBLZ5-CA (Piz-5) | IRBLZT-T (Piz-I) | IRBL9-W (Pi9) | LTH |
|                               |                   | 44    | 95.45 | 50.00 | 9.09 | 50.00 | 79.55 | 100.00 | 97.73 | 54.55 | 47.73 | 18.18 | 100.00 | 0 |
|                               |                   | 30    | 100.00 | 43.33 | 80.00 | 86.67 | 56.67 | 6.67 | 70.00 | 66.67 | 70.00 | 100.00 | 100.00 | 0 |
|                               |                   | 25    | 100.00 | 40.00 | 20.00 | 76.00 | 64.00 | 100.00 | 100.00 | 96.00 | 96.00 | 12.00 | 96.00 | 0 |
|                               |                   | 19    | 83.33 | 78.95 | 26.32 | 21.05 | 100.00 | 100.00 | 100.00 | 94.74 | 89.47 | 36.84 | 100.00 | 0 |
|                               |                   | 28    | 78.57 | 89.29 | 21.43 | 21.43 | 85.71 | 75.00 | 89.29 | 64.29 | 57.14 | 82.14 | 100.00 | 0 |
|                               |                   | 176   | 90.91 | 81.25 | 20.45 | 69.32 | 58.52 | 68.18 | 96.02 | 55.68 | 51.14 | 68.18 | 97.16 | 0 |

*a* Resistant gene carrying in monogenic line;

*b* Resistance percentage (%), Number of isolates avirulent to a line or *R* gene/total number of isolates tested × 100

https://doi.org/10.1371/journal.pone.0186201.t002
Discussion

In a previous study, Pi57(t) was identified and preliminary mapped in 6.07 Mb region on chromosome 12 of rice [21]. In this study, this *O. longistaminata*-derived gene was finely mapped to a region of 51.7 kb on the short arm proximal to centromeric position of chromosome 12 of rice, by using two mapping population from IL-E1454/RD23 and IL-E1454/LTH. It has been well documented that the recombination frequency along a chromosome is quite different in plant, and that the chromosomal recombination was significantly suppressed in the region with more repetitive DNA sequences and/or close to the centromeric regions than other regions [26–28]. Pi57(t) was located in the region close to centromere of chromosome 12 and we observed low recombination frequency: after mapping with 13395 F2 individuals, Pi57(t) locus was still mapped in a large chromosomal region flanked by molecular markers STS57-1 and STS57-2. Although the population size used in this study are relatively larger than those used in other genes mapping [13, 25], increasing mapping population consisting of 39445 F2 individuals could finally delimit this locus to an estimated 51.7 kb, based on the physical distance determined by in silico mapping on *O. sativa* reference genome.

Classical genetics and molecular data have demonstrated that many resistance genes in plant are often clustered in a certain chromosomal region as a complex locus [29]. To date, 19 R genes *Pita*, *Pita*2, *Pita*6, *PiGD*-3, *Pi6* (t), *Pi12* (t), *Pi19* (t), *Pi20* (t), *Pi21* (t), *Pi24* (t), *Pi31* (t), *Pi32* (t), *Pi39* (t), *Pi41*, *Pi42* (t), *Pi57* (t), *Pi58* (t), *Pi61* (t) and *Pi157* (t) have been mapped on chromosome 12, and most of them are concentrated around the centromere region [12, 13, 21, 30, 31–34]. Most of them were mapped to a relative large chromosomal region spanning over several Mb on the short arm of chromosome 12 [12, 31, 33–34]. By using a large number of *M. oryzae* strains, Pi57(t) could be differentiated from genes located in the same genomic regions and introgressed in monogenic lines (*Pita*, *Pita*-2, *Pi12*, *Pi19* and/or *Pi20*). However, the positional or allelism relationship among these genes could not be compared with each other in detail, due to the limited information about their rough mapping position and different *M. oryzae* strains used in gene mapping research [13, 21, 31, 33–34]. Fine mapping of R genes provide direct information about the relationship among the genes in a cluster. For example, by comparing with the chromosomal position of cloned *Pita* gene, *Pi61* (t) gene was mapped at ca. 200 kb region on the telomere side, and *Pi39* (t) was localized at 37 kb region on the centromeric side in the short arm of chromosome 12 [13, 24–25]. Pi57(t) was mapped to the proximal side to the centromere compared with *Pi39* (t) location, indicating that this is a new locus conferring resistance to rice blast. With the characterization of broad spectrum resistance against *M. oryzae*, this gene would be a very useful gene resource for improvement of resistance to rice blast in rice breeding program.

Most of the R genes cloned from plants so far encode protein with nucleotide-binding site and leucine repeat repeat (NBS-LRR), LRR-kinase or kinase structure [35]. To date, all cloned rice blast R genes encode NBS-LRR proteins, except for *Pid*-2 and the recessive *pi21*, which encode a receptor-like kinase protein and a proline-rich protein, respectively[36, 37]. In this study, Pi57(t) gene was mapped in a region containing 6 predicted genes without any similarity to known R genes, based on the gene annotation results of reference genomic sequence of *O. sativa* ssp. *japonica* cultivar Nipponbare. In the present study, because two gaps exit in target region in IL-E1454 with the comparison of Nipponbare genomic sequence, whether Pi57(t) encodes protein with either novel structure similar to the annotated candidate gene, or a known R gene structure but located in gap region in IL-E1454 remains to be clarified. Currently, the gap-filling with genome walking strategy and genetic transformation for candidate genes are undergoing.
Supporting information

S1 Fig. Polymorphic analysis of resistance donor IL-E1454, and susceptible parents RD23 and LTH with STS markers developed in this study.
(PDF)

S1 Table. Resistance reaction of IL-E1454 and 10 monogenic lines to 322 Magnaporthe oryzae strains.
(DOC)

Acknowledgments

We are grateful to Dr. Khay S, Dr. Douangboupha B, Dr. Somrith A and Dr. Chung NH for the provision of blast isolates. This research was supported by grants from National Natural Science Foundation of China (31360462, 31560493), Yunnan Provincial Science and Technology Department (2014GA009), the Special Fund for Agro-Scientific Research in the Public Interest (201303129), Scientific Observing and Experimental Station of Crops Pests in Kunming, Ministry of Agriculture, China.

Author Contributions

Conceptualization: Dayun Tao, Qinzhong Yang.

Data curation: Liying Dong.

Formal analysis: Liying Dong, Peng Xu.

Funding acquisition: Dayun Tao, Qinzhong Yang.

Investigation: Liying Dong, Shufang Liu, Peng Xu, Wei Deng, Xundong Li, Qun Wang.

Methodology: Liying Dong, Shufang Liu, Peng Xu.

Project administration: Liying Dong, Dayun Tao, Qinzhong Yang.

Resources: Liying Dong, Wei Deng, Jing Li, Jiawu Zhou.

Software: Shufang Liu, Xundong Li, Didier Tharreau.

Validation: Liying Dong, Didier Tharreau.

Visualization: Liying Dong, Dayun Tao, Qinzhang Yang.

Writing – original draft: Liying Dong, Dayun Tao, Qinzhong Yang.

Writing – review & editing: Liying Dong, Didier Tharreau, Dayun Tao, Qinzhang Yang.

References

1. Couch BC, Kohn LM (2002) A multilocus gene genealogy concordant with host preference indicates segregation of a new species, Magnaporthe oryzae, from M. grisea. Mycologia 94: 683–693 PMID: 21156541

2. Ou SH (1985) Rice diseases, 2nd edn. Commonwealth Mycological Institute, Kew, pp 109–201

3. Silué D, Notteghem JL, Tharreau D (1992) Evidence of a gene for gene relationship in the Oryza sativa-Magnaporthe grisea pathosystem. Phytopathology 82:577–580

4. Jia Y, McAdams SA, Bryan GT, Hershey HP, Valent B (2000) Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. The EMBO Journal 19: 4004–4014 https://doi.org/10.1093/emboj/19.15.4004 PMID: 10921861
5. Zeigler RS, Tohme J, Nelson J, Levy M, Correa F. Linking blast population analysis to resistance breeding (1994) A proposed strategy for durable resistance. In: Zeigler RS, Leong SA, Teng PS (eds) Rice Blast Disease. CAB International and IRRI, Wallingford, United Kindom, p16-26

6. Deng Y, Zhai K, Xie Z, Yang D, Zhu X, Liu J, et al. (2017) Epigenetic regulation of antagonistic receptors confers rice blast resistance with yield balance. Science 355:962–965. https://doi.org/10.1126/science.aai8898 PMID: 28154240

7. Zhu Y, Chen H, Fan J, Wang Y, Li Y, Chen J, et al. (2000) Genetic diversity and disease control in rice. Nature 406:718–722. https://doi.org/10.1038/35021046 PMID: 10963595

8. Raboin LM, Ramantantsoaninirina A, Dusserre J, Razasolofanahary F, Tharreau D, Lannou C, et al. (2012) Two-component cultivar mixtures reduced rice blast epidemics in an upland agrosystem. Plant Pathology 61: 1103–1111.

9. Sester M, Raveloson H, Tharreau D, Dusserre J (2014) Conservation agriculture cropping system to limit blast disease in upland rainfed rice. Plant Pathology 63: 373–381. https://doi.org/10.1111/ppa.12099

10. Liao J, Huang HC, Meusnier I, Adreit H, Ducasse A, Bonnot F, et al. (2016) Pathogen effectors and plant immunity determine specialization of the blast fungus to rice subspecies. e-Life 5:e19377. https://doi.org/10.7554/eLife.19377 PMID: 28008850

11. Hittalmani S, Parco A, Mew TV, Zeigler RS, Huang N (2000) Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor Appl Genet 100:1121–1128

12. Ballini E, Morel JB, Droc G, Price A, Courtouis B, Notteghem JL, et al. (2008) A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. Mol Plant Microbe Interact 21: 859–868 https://doi.org/10.1094/MPMI-21-7-0859 PMID: 18538277

13. Lei C, Hao K, Yang Y, Ma J, Wang S, Wang J, et al. (2013) Identification and fine mapping of two blast resistance genes in rice cultivar 93–11. The Crop Journal 1: 2–14

14. Su J, Wang W, Han J, Chen S, Wang C, Zeng L, et al. (2015) Functional divergence of duplicated genes results in a novel blast resistance gene Pi50 at the Pi2/9 locus. Theor Appl Genet 128: 2213–2225. https://doi.org/10.1007/s00122-015-2579-9 PMID: 26183036

15. Wang R, Fang N, Guan C, He W, Bao Y, Zhang H (2016) Characterization and fine mapping of a blast resistant gene Pj-nw1 from the japonica rice landrace Jiangnanw. PLOS one 11(12): e0169417 https://doi.org/10.1371/journal.pone.0169417 PMID: 28036378

16. Liang Z, Wang L, Pan Q (2016) A new recessive gene conferring resistance against rice blast. Rice 9:47 https://doi.org/10.1186/s12284-016-0120-7 PMID: 27637926

17. Fukuoka S, Okuno K (2001) QTL analysis and mapping of Pi-jnw1, a recessive gene for field resistance to rice blast in Japanese upland rice. Theor Appl Genet 103: 185–190

18. He X, Liu X, Wang L, Wang L, Lin F, Cheng YQ, et al. (2012) Identification of the novel recessive gene pi55(t) conferring resistance to Magnaporthe oryzae. Sci China Life Sci 55(2):141–9 https://doi.org/10.1007/s11427-012-4282-2 PMID: 22415685

19. International Rice Genome Sequencing Project. The map-based sequence of the rice genome. Nature, 2005, 436: 793–800 https://doi.org/10.1038/nature03895 PMID: 16100779

20. Yu J, Hu S, Wang J, Wong GK, Li S, Liu B, et al. (2002) A draft sequence of the rice genome (Oryza sativa L. ssp. indica). Science 296: 79–92 https://doi.org/10.1126/science.1068037 PMID: 11935017

21. Xu P, Dong L, Zhou J, Li J, Zhang Y, Hu F, et al. (2015) Identification and mapping of a novel blast resistance gene Pi57(t) in Oryza longistaminata. Euphytica 205: 95–102

22. Edwards K, Johnstone C, Thompson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res 19: 1349 PMID: 2030957

23. Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, et al. (1987) MAPMAKER: an interactive computing package for constructing primary genetic linkages of experimental and natural populations. Genomics 1: 174–181. PMID: 3692487

24. Bryan GT, Wu KS, Farrall L, Jia Y, Hershey HP, McAdams SA, et al. (2000) A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene Pi-ta. Plant Cell 12: 2033–2045 PMID: 11090207

25. Liu X, Yang Q, Lin F, Hua L, Wang C, Wang L, et al. (2007) Identification and fine mapping of Pi39(t), a major gene conferring the broad-spectrum resistance to Magnaporthe oryzae. Mol Genet Genomics 278: 403–410 https://doi.org/10.1007/s00438-007-0258-5 PMID: 17576597

26. Wu JZ, Mizuno H, Hayashi-Tsgane M, Ito Y, Chiden Y, Fujisawa M, et al. (2003) Physical maps and recombination frequency of six rice chromosomes. Plant J 36:720–730 PMID: 14617072
27. Farkhari M, Lu Y, Shah T, Zhang S, Naghavi MR, Rong T, et al. (2011) Recombination frequency variation in maize as revealed by genome wide single-nucleotide polymorphisms. Plant Breeding 130: 533–539

28. Jia Y, Jia MH, Wang X, Liu G (2012) Indica and Japonica crosses resulting in linkage block and recombination suppression on rice chromosome 12. PLoS ONE 7(8): e43066. https://doi.org/10.1371/journal.pone.0043066 PMID: 22912788

29. Michelmore RW, Meyers BC (1998) Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res 8:1113–1130 PMID: 9847076

30. Sallaud C, Lorieux M, Roumen E, Tharreau D, Berruyer R, Svestasrani P, et al. (2003) Identification of five new blast resistance genes in the highly blast-resistant rice variety IR64 using a QTL mapping Strategy. Theor Appl Genet 106: 794–803 https://doi.org/10.1007/s00122-002-1088-9 PMID: 12647052

31. Li W, Lei C, Cheng Z, Jia Y, Huang D, Wang J, et al. (2008) Identification of SSR markers for a broad-spectrum blast resistance gene Pi20(t) for marker-assisted breeding. Mol Breeding 22: 141–149

32. Yang Q, Lin F, Wang L, Pan Q (2009) Identification and mapping of Pi41, a major gene conferring resistance to rice blast in the Oryza sativa subsp. indica reference, 93–11. Theor Appl Genet 118: 1027–1034 https://doi.org/10.1007/s00122-008-0959-0 PMID: 19153709

33. Kumar P, Pathania S, Katoch P, Sharma TR, Plaha P, Rathour R (2010) Genetic and physical mapping of blast resistance gene Pl-42(t) on the short arm of rice chromosome 12. Mol Breeding 25: 217–228

34. Koide Y, Telebanco-Yanoria JT, Fukuta Y, Kobayashi N (2013) Detection of novel blast resistance genes, Pi58(t) and Pi59(t) in a Myanmar rice landrace based on a standard differential system. Mol Breeding 32: 241–252

35. Dangl JL, Jones JDG (2001) Plant pathogens and integrated defence responses to infection. Nature 411: 826–833 https://doi.org/10.1038/35081161 PMID: 11459065

36. Chen X, Shang J, Chen D, Lei C, Zou Y, Zhai W, et al (2006) A B-lectin receptor kinase gene conferring rice blast resistance. Plant J 46: 794–804 https://doi.org/10.1111/j.1365-313X.2006.02739.x PMID: 16709195

37. Fukuoka S, Saka N, Koga H, Ono K, Shimizu T, Ebana K, et al. (2009) Loss of function of a proline-containing protein confers durable disease resistance in rice. Science 325: 998–1001 https://doi.org/10.1126/science.1175550 PMID: 19696351