Available online at www.sciencedirect.com

ScienceDirect

Rice Science, 2017, 24(1): 41−47

# Molecular Screening of Blast Resistance Genes in Rice Germplasms Resistant to *Magnaporthe oryzae*

LIANG Yan[1], YAN Bai-yuan[2], PENG Yun-liang[3], JI Zhi-juan[1], ZENG Yu-xiang[1], WU Han-lin[1], YANG Chang-deng[1]

([1]*State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 310006, China;* [2]*The Seed Management Station of Jiande, Jiande 311600, China;* [3]*Institute of Plant Protection, Sichuan Academy of Agricultural Sciences / Key Laboratory of Integrated Pest Management on Crops in Southwest China, Ministry of Agriculture, Chengdu 610066, China*)

**Abstract:** Molecular screening of major rice blast resistance genes was determined with molecular markers, which showed close-set linkage to 11 major rice blast resistance genes (*Pi-d2*, *Pi-z*, *Piz-t*, *Pi-9*, *Pi-36*, *Pi-37*, *Pi5*, *Pi-b*, *Pik-p*, *Pik-h* and *Pi-ta*$^2$), in a collection of 32 accessions resistant to *Magnaporthe oryzae*. Out of the 32 accessions, the *Pi-d2* and *Pi-z* appeared to be omnipresent and gave positive express. As the second dominant, *Pi-b* and *Piz-t* gene frequencies were 96.9% and 87.5%. And *Pik-h* and *Pik-p* gene frequencies were 43.8% and 28.1%, respectively. The molecular marker linkage to *Pi-ta*$^2$ produced positive bands in eleven accessions, while the molecular marker linkage to *Pi-36* and *Pi-37* in only three and four accessions, respectively. The natural field evaluation analysis showed that 30 of the 32 accessions were resistant, one was moderately resistant and one was susceptible. Infection types were negatively correlated with the genotype scores of *Pi-9*, *Pi5*, *Pi-b*, *Pi-ta*$^2$ and *Pik-p*, although the correlation coefficients were very little. These results are useful in identification and incorporation of functional resistance genes from these germplasms into elite cultivars through marker-assisted selection for improved blast resistance in China and worldwide.

**Key words:** rice; blast resistance gene; field evaluation; marker-assisted selection

Rice (*Oryza sativa* L.) is one of the world's most important crops, providing a staple food for nearly half of the global population. The demand for rice is expected to increase due to the steadily increasing population in Asia, Africa and Latin America (Wang and Li, 2005). In China, rice production will need to increase by approximately 20% by 2030 to meet the domestic demand if rice consumption per capita remains at its current level (Peng et al, 2009). However, rice production is continually threatened by disease, insects and other stress. Rice blast, one of the most damaging diseases affecting rice production worldwide, is caused by the non-obligate filamentous ascomycete *Magnaporthe oryzae* B. Couch (cyn. *Magnaporthe oryzae*). The control options for blast are adjusting planting time, spliting nitrogen fertilizer application in two or more treatments, flooding the field as often as possible, and planting resistant varieties (www.knowledgebank.irri.org). In rice production practices, rice blast is managed primarily by the use of chemical pesticides, which are both economically and environmentally costly. Moreover, the overuse of pesticides prompts the evolution of resistance in the disease, which in turn leads to disease resurgence. Therefore, the exploitation of host plant resistance has generally been considered as one of the most economical and environmentally friendly approaches to combat the disease (Khush and Tena, 2009).

Regarding the genetic basis of the resistance to *M. oryzae*, more than 86 dominant R genes and approximately 350 QTLs for resistance to rice blast have been identified, and 23 of them have been

**Received:** 4 May 2016; **Accepted:** 3 July 2016

**Corresponding author:** YANG Chang-deng (yangchangdeng@126.com; ycd311400@163.com)



Peer review under responsibility of China National Rice Research Institute

http://dx.doi.org/10.1016/j.rsci.2016.07.004

molecularly characterized: i.e. *pb1*, *Pi-a*, *Pi-b*, *Pi-d2*, *Pi-d3*, *Pi-k*, *Pik-h/Pi-54*, *Pik-m*, *Pik-p*, *Pi-sh*, *Pi-t*, *Pi-ta*, *Piz-t*, *Pi-1*, *Pi-2/Piz-5*, *Pi5*, *Pi-9*, *pi-21*, *Pi-25*, *Pi-36*, *Pi-37*, *Pi-35* and *Pi-64* upto date (RiceDate, 2012; Fukuoka et al, 2014; Ma et al, 2015). However, as each of these R genes usually act only against a subset of existing pathogen races, the identification of new R genes/alleles is still essential to the breeding of endurably resistant varieties by different strategies such as pyramiding of different resistance genes (Miah, 2013).

Generally, R genes for *M. oryzae* are identified in landraces, cultivars or wild rice using differential physiological races of *M. oryzae* (Tanksley and McCouch, 1997). The development of improved rice cultivars has led to the replacement of landraces and traditional varieties by modern cultivars, which has resulted in a decline in the diversity of agriculturally used rice. However, the diversity lost in the elite materials is somewhat preserved in crop gene banks, wild rice collections and breeding resources, thus, these rice materials provide the basis for genetic improvement of crops for specific traits and represent rich sources of novel allelic variation. In this research, a collection of 32 rice accessions have been constructed by inoculation tests, which were taken in lab and in field evaluations in multiple locations over years, and showed complete and moderate resistance to *M. oryzae*. These accessions were carefully assessed with the response to *M. oryzae* to ensure the accuracy of phenotyping.

Molecular genetic markers are now widely used to characterize gene bank collections that contain untapped resources of distinct alleles, which will remain hidden unless efforts are initiated to screen them for their potential use and function (Cho et al, 2007; RoyChowdhury et al, 2012a, b; Imam et al, 2013; Vasudevan et al, 2014; Singh et al, 2015). Little is currently known about the genetic basis of blast resistance among the collection of 32 rice germplasm. In this study, molecular screening and field evaluation were both carried out to acquire the information for 11 functional blast R genes in the selected collection, and the efforts can be utilized to develop high-yielding rice varieties with resistance to blast through marker-assisted selection.

## MATERIALS AND METHODS

### Rice materials

Thirty-two rice accessions resistant to *M. oryzae* with different agronomic characters were selected from 347 landraces and cultivars based on the field evaluation in multiple locations in Xuyong, Pujiang and Ya'an in Sichuan Province, China. The detail information of 32 accessions and the geographical origins are shown in Supplemental Table 1. These materials were continued investigated in 2011 in Puling in Chongqing, Enshi in Hubei Province, Pingxiang and Jinggangshan in Jiangxi Province, Jing County and Xiuning in Anhui Province, Jintan in Jiangsu Province and Lin'an in Zhejiang Province, China, and showed complete resistance except IR72903-99-2-3-2, A10 and 237, which showed moderate resistance in the Enshi nursery in Hubei Province (data not shown). Lijiangxintuanheigu (LTH), Yuanfengzao and CO39 were used as negative controls in the field evaluation, and CO39 was used as negative control in molecular screening. The positive controls in molecular screening were Digu for *Pi-d2*, and Chunjiangnuo for *Pi-36* and *Pi-37*, which were conserved in the State Key Laboratory of Rice Biology, China National Rice Research Institute. The monogenetic lines, IRBLz-Fu for *Pi-z*, IRBLzt-T for *Piz-t*, IRBL9-W for *Pi-9*, IRBL5-M for *Pi5*, IRBLb-B for *Pi-b*, IRBLkp-K60 for *Pik-p*, IRBLkh-K3 for *Pik-h* and IRBLta2-Pi for *Pi-ta*$^2$, were donated by Prof. WANG Guo-liang at Ohio State University, USA.

### Methods

*DNA isolation and DNA marker analysis*

Genomic DNA was isolated from 100 mg of young leaf tissue from each accession using modified cetyl-trimethyl ammonium bromide method (Warude et al, 2003). The quality and quantity of extracted genomic DNA was measured according to Imma et al (2014).

The PCR markers for blast resistance genes *Pi-d2*, *Pi-z*, *Piz-t*, *Pi-9*, *Pi-36*, *Pi-37*, *Pi5*, *Pi-b*, *Pik-p*, *Pik-h* and *Pi-ta*$^2$ were listed in Table 1. All of them were synthesized by Dingguo Biotech. Co. Ltd, Beijing, China. The PCR analyses were conducted and templates for PCR reaction set up with modifications according to the instructions: 2.5 μL of 10× LA PCR Buffer ($Mg^{2+}$ Plus), 4 μL of dNTP mixture (2.5 mmol/L), 1 μL of each primer (10 μmol/L), 50 ng of genomic DNA template, 0.25 μL of TaKaRa LA *Taq* DNA polymerase (5 U/μL) (Takara Bio Inc., Shanghai, China), and dd$H_2O$ water to final volume of 25 μL. PCR amplification was performed with the following profile: 94 ºC for 5 min, 35 cycles of 94 ºC for 30 s, primer annealing at different temperature for 45 s (Table 1), and 72 ºC for 2 min, and 72 ºC for 5 min. All PCR reactions for each sample were repeated three times to confirm the results. To detect polymorphisms,