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# Characterization and evaluation of rice blast resistance of Chinese *indica* hybrid rice parental lines☆

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ABSTRACT

The development of resistant varieties and hybrid combinations has been the most effective and economical strategy to control blast disease caused by *Magnaporthe oryzae*. However, the distribution of major R genes and blast resistance characterization in hybrid rice parents has not been well investigated, resulting in their limited use in hybrid rice blast-resistance breeding. In the present study, 88 elite *indica* hybrid rice parental lines were evaluated with 30 isolates of *M. oryzae* collected from the main planting area of *indica* hybrid rice in China and were characterized for the presence of 11 major resistance genes using molecular markers. The pathogenicity assays showed that four types of hybrid rice parent line showed some resistance to *M. oryzae*. However, the proportions of highly resistant lines and the mean resistance frequency (RF) varied among the four types, with resistance in decreasing order shown by three-line restorer lines, three-line maintainer lines, two-line sterile lines, and two-line restorer lines. All 88 hybrid rice parental lines carried more than one R gene, but none carried the R genes *Pi1* and *Pi2*. Although *Pid3* and *Pi9* were present only in three-line restorer lines and *Pigm* only in three-line maintainer lines, the remaining six R genes (*Pib*, *Pid2*, *Pi5*, *Pia*, *Pi54*, and *Pita*) were present in the four types of hybrid rice parent with significantly different distribution frequencies. The correlation between R genes and resistance reactions was investigated. The results are expected to provide useful information for rational utilization of major R genes in hybrid rice breeding programs.



## 1. Introduction

Rice (*Oryza sativa* L.) is the primary food crop of >50% of the world's population [1]. It accounts for 30%-50% of agricultural production and 35%-75% of the calories consumed by more than three billion Asians. Rice consumption is increasing and demand for rice is also increasing with population growth. To meet the increased demand for rice, production must be

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increased >40% by 2030 [1,2]. To meet the increasing food demand in the future, concerted efforts are required to increase rice productivity and minimize production losses to pests and disease. Among the biotic stresses afflicting rice, blast disease caused by *Magnaporthe oryzae* is one of the most devastating rice diseases, occurring in almost all rice production regions and commonly reducing yield by 10%-30%, an amount sufficient to feed >60 million people [3]. Thus, minimizing the occurrence of disease epidemics and reducing year-to-year losses are central to sustain rice productivity. Currently, use of pesticides and deployment of blast-resistant cultivars are the main methods of combating the disease. However, excessive use of pesticides not only increases the cost of rice production but also causes great environmental pollution. Development of resistant cultivars by introduction of major *R* genes into elite rice varieties has proven to be the most ecofriendly and sustainable approach for blast control [4].

Since the first rice blast resistance gene *Pia* was identified in the *japonica* variety Aichi Asahi by Yamasaki and Kiyosawa [5], *R* genes have been extensively studied in rice with the development of bioinformatics and molecular markers. To date, around 100 rice blast resistance genes have been identified: 45% in *japonica* cultivars, 51% in *indica* cultivars, and the remaining 4% in wild species of rice [6,7]. More than half of these genes were reported to be located in gene clusters on all rice chromosomes except chromosome 3. The three largest gene clusters are located on chromosomes 6, 11, and 12, comprising respectively 19, 27 and 22 *R* genes [6,8]. To date, 25 major *R* genes (*Pit*, *Pish*, *Pi37*, *Pi64*, *Pib*, *Pi63*, *Pid2*, *Pid3/Pi25*, *Pi2*, *Pi9*, *Pizt*, *Pi50*, *Pigm*, *Pi36*, *Pi5*, *Pia/Pi-CO39*, *Pi1*, *Pi54/Pi54rh*, *Pikm*, *Pikp*, *Pik*, and *Pita*) [8-10] and three partial-resistance genes (*pi21*, *Pi35*, and *Pb1*) [11-13] have been cloned and characterized. These cloned *R* genes could be classified into five clusters by the cause of the difference between resistance and susceptibility: 1) amino acid substitution or deletion, such as in *Pid2* [14], *Pi2/Pizt* [15], *Pi36* [16], *Pi37* [17], and *Pita* [18], 2) expression difference (*Pit*) [19]; 3) nonsense mutation resulting from a single nucleotide polymorphism (SNP) (*Pid3*) [20]; 4) expression of other genes via epigenetic regulation (*Pigm*) [10]; or 5) two adjacent NBS-LRR-type *R* genes acting together, such as in *Pi5* [21], *Pia/Pi-Co39* [22], *Pikm* [23], *Pik* [24] and *Pikp* [25]. Characterization of these *R* genes helps to elucidate the molecular mechanisms for rice resistance to *M. oryzae*. Information on molecular markers tightly linked to or derived from polymorphic sites within *R* genes is increasingly available and has been widely used in marker-assisted breeding programs to develop new cultivars with effective and broad-spectrum resistance to *M. oryzae* [26,27,28].

Three-line hybrid rice has been successfully commercialized in China since 1973 because of its yield advantages over conventional rice [29]. Breeding of the two-line super hybrid rices Liangyoupeijiu and Yangliangyou 6 and others has marked China entry into the new era of combined use of three-line and two-line hybrid rice. Currently, hybrid rice accounts for about 60% of total rice acreage and contributes 65% of total rice production in China [30]. Although the adoption of hybrid rice varieties has been rapid, only a small number of such varieties have been extensively cultivated over a large area for many years. This uniformity reduces genetic diversity and the limited number of major *R* genes present in hybrid rice parents has severely restricted the development of hybrid rice. Blast disease has occurred more frequently and severely with the speedy emergence of virulent races in recent years. The appearance of new races of *M. oryzae* in China has resulted in a breakdown of resistance, leaving 20% of hybrid rice fields infected in 2006, as reported by the Ministry of Agriculture of China [31]. In 2008, Leizhou city in Guangdong province witnessed >1500 ha of hybrid rice damaged by blast disease, of which over 250 ha gave no yield [29]. The resistance of hybrid combinations is strongly associated with the resistances in the hybrid rice parents; at least one of the parental lines used in hybrid rice blast resistance breeding must be resistant to *M. oryzae*. Accordingly, identification of major *R* genes and characterization of blast resistance in hybrid rice parental lines play vital roles in rational utilization of *R* genes and disease-resistance improvement in hybrid rice.

The objectives of this study were to evaluate the disease reactions of 88 hybrid rice parental lines to 30 isolates collected from the main planting area of *indica* hybrid rice in China and to identify 11 major *R* genes in these lines using molecular markers. The results of this study were expected to provide useful information for rational utilization of major *R* in hybrid rice breeding programs, and germplasm characterized in the study could be used in marker-assisted selection (MAS) for improving blast resistance in hybrid rice.

## 2. Materials and methods

### 2.1. Plant materials

Eighty-eight elite *indica* hybrid rice parent lines were analyzed, including 39 three-line restorer lines, 22 three-line maintainer lines, 18 two-line restorer lines, and nine two-line sterile lines. For investigating the resistance of the *R* genes, 13 accessions carrying the major *R* genes were used as standard check cultivars (Table S1).

### 2.2. Pathogens

A total of 30 isolates collected from Sichuan (SC), Guangdong (GD), Anhui (AH), Jiangxi (JX), Hainan (HN), Hubei (HB), and Zhejiang (ZJ) provinces in 2010-2016 were employed (Table S1).

### 2.3. Blast inoculation and disease evaluation

The plants used for inoculation were grown in 60 cm × 30 cm × 4 cm plastic trays with sieved garden soil. In each tray, 42 experimental lines and two highly susceptible cultivars Co39 and LTH (susceptible control) were sown, with 10 seeds per lines for each inoculation. Three-week-old rice seedlings were placed into inoculation chambers and inoculated by spraying with 40 mL conidial suspensions ($5 \times 10^{-4}$ conidia $mL^{-1}$) with 0.02% Tween 20 using a hand atomizer (100 kPa) connected to an air compressor [32]. The inoculated seedlings were kept in dark chambers with moisture-saturated atmosphere at 26 °C for 24 h and then transferred to the

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