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RESEARCH ARTICLE

Pyramiding blast, bacterial blight and brown planthopper resistance genes in rice restorer lines

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Abstract

Rice blast, bacterial blight (BB) and brown planthopper (BPH) are the three main pests of rice. This study investigated pyramiding genes resistant to blast, BB and BPH to develop restorer lines. Ten new lines with blast, BB and/or BPH resistance genes were developed using marker-assisted selection (MAS) technique and agronomic trait selection (ATS) method. Only HR13 with resistance genes to blast, BB and BPH was obtained. In addition to blast and BB resistance, four lines (HR39, HR41, HR42, HR43) demonstrated moderate resistance to BPH, but MAS for BPH resistance genes were not conducted in developing these four lines. These data suggested that there were unknown elite BPH resistance genes in the Zhongzu 14 donor parent. A more effective defense was demonstrated in the lines with *Pi1* and *Pi2* genes although the weather in 2012 was favorable to disease incidence. Blast resistance of the lines with a single resistance gene, *Pita*, was easily influenced by the weather. Overall, the information obtained through pyramiding multiple resistance genes on developing the restorer lines is helpful for rice resistance breeding.

Keywords: rice, blast, bacterial blight, brown planthopper, resistance, pyramid

1. Introduction

Rice (*Oryza sativa* L.) is a staple food crop in China that feeds more than 60% of the population, and it contributes nearly 40% of the total calorie intake (Cheng *et al.* 2007). Compared with conventional varieties, hybrid rice can significantly increase rice yields and has made a large contribution to the self-sufficiency of the food supply in China. However, most of the hybrid rice varieties do not have resistance to specific biotic stresses (Khush and Jena 2009).

Rice blast, bacterial blight (BB) and brown planthopper (BPH) caused by *Magnaporthe grisea*, *Xanthomonas oryzae* pv. *oryzae* (Xoo) and *Nilaparvata lugens* Stål, respectively, are the most destructive diseases and insects causing significant reduction in rice production throughout China and in other Asian rice-growing countries. Rice blast alone can cause annual yield losses of between 10 and 30% of the total harvest, and its occurrence was reported by the Ministry of Agriculture of China to be as high as 20% of the hybrid rice fields cultivated in 2006 (Jiang *et al.* 2012). BB disease, in its severe form, is known to cause yield losses ranging from 74 to 81% (Srinivasan and Gnanamanickam

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2005). The damage caused by BPH feeding has the greatest effect on the growth and crop yield of the susceptible rice plant through the removal of assimilates and the reduction in photosynthetic rate of leaves, ultimately causing plant death in its severe form (Jirapong *et al.* 2007). Deployment of host plant resistance is considered to be the best option for managing the diseases and insects. Breeding rice varieties with multiple disease and insect resistance genes will broaden the resistance spectrum and increase the resistance durability for the varieties.

With the development of gene identification technologies, the marker-assisted selection (MAS) technique is typically used to improve disease and insect resistance. The scope of MAS breeding for targeted introgression of BB resistance genes (Huang *et al.* 1997; Chen *et al.* 2000; Chen *et al.* 2001; Sundaram *et al.* 2008, 2009), blast resistance genes (Amante-bordeos *et al.* 1992; Hittalmani *et al.* 2000) and BPH resistance genes (Sharma *et al.* 2004; Jena *et al.* 2006) has been successfully demonstrated. In addition, the introgression of two different diseases or insect resistances has been conducted (Jiang *et al.* 2004). However, to the best of our knowledge, there is no report on the simultaneous introgression of BB, blast and BPH resistance into the lines of hybrid rice.

Currently, the production of hybrid rice is primarily based on the three-line hybrid system, which involves a cytoplasm male sterile (CMS) line, a corresponding isonuclear maintainer line and a genetically diverse restorer line. In addition, the sterile line is maintained by being crossed with its maintainer line, and hybrid seed is produced by crossing the sterile line with the restorer line (Cheng *et al.* 2012). Generally, restorer lines are much easier to be improved through breeding techniques than sterile lines because no sterility is considered. Shuhui 162 and Zhongzu 14 are two restorer lines in hybrid rice. Shuhui 162 is resistant to only blast. Zhongzu 14 is resistant to BB, blast and BPH, but its resistance gene to BB is recessive, which cannot demonstrate its resistance in heterozygous-genotype hybrid rice. Hence, in this study, new restorers with multiple resistances to diseases and BPH were developed using the MAS technique and further evaluated by artificial inoculation in two years. These results impart valuable information for breeding resistance in rice.

2. Materials and methods

2.1. Plant materials and breeding strategy

Five parents were used to pyramid disease and BPH resistance into the new lines (Table 1). The Shuhui 162 restorer line contains the *Pita* gene. The Zhongzu 14 restorer line contains *Pi1*, *Pi2* and *xa5* genes, and it is resistant to BB, blast and BPH. The BPH-resistance gene donor RH contains the *Bph3* gene. CBB23 and HN88 contain the *Xa23* gene. HN88 originated from CBB23 and is a new restorer line with high productive-tiller-rate and thousand-grain weight.

Two crosses, namely Shuhui 162/CBB23//HN88///RH (cross 1) and Zhongzu 14/CBB23 (cross 2), were conducted. After obtaining compound F_1 or F_1, self-pollination was continuously performed for several generations to make the resistance genes homozygous using the MAS technique and to stop other agronomic traits segregation through the agronomic trait selection (ATS) method and pedigree selection. Herein, the ATS method involves selecting agronomic traits of the progenies similar to the restorer parents by artificially judging for the background selection. Crosses between Xieqingzao A and the new lines were further conducted to evaluate their restoring fertility for CMS lines.

2.2. MAS technique

Six markers were used to select corresponding genes in the breeding of each generation (Table 2). DNA samples were extracted from fresh leaves using a simple one-step method (Ji *et al.* 2014). Leaves with a length of approximately 3 mm were immersed in buffer A containing 100 mmol L^{-1} Tris-HCl (pH 9.5), 1 mol L^{-1} KCl and 10 mmol L^{-1} EDTA ($EDTANa_2 \cdot 2H_2O$). The samples were crushed using a multi-sample tissue lyser (Jingxin Technology Co. Ltd., Shanghai, China), and the supernatants were collected by centrifugation at 4 000 r min^{-1} for 5 min for DNA amplification.

Polymerase chain reaction (PCR) was performed in a 15-μL reaction volume containing 0.8 μL of supernatant, 2× PCR buffer (including Tris-HCl, KCl and $MgCl_2$), 2 mmol L^{-1} dNTPs, 0.9 μmol L^{-1} primer pairs, and 0.3 U KOD FX polymerase (Toyobo Co. Ltd., Shanghai, China). The reaction mixture was initially denatured at 94°C for 2 min followed by 30 cycles of PCR amplification with the following parameters: 10 s of denaturation at 98°C, 30 s of primer annealing at 50°C (53°C for marker C189), and 1 min of primer extension at 68°C. Finally, the reaction mixture was maintained at 68°C for 7 min before completion. The amplified product was electrophoretically resolved on a 2% agarose gel using Gelrad staining for C189 and YL155/YL187, and it was also resolved on an 8% denaturing polyacrylamide gel using silver staining for RM122, RM224, (Indel) PI2-4 and RM589.

2.3. Disease and BPH resistance evaluation

After several successive segregating generations, new lines pyramiding multiple resistance genes were sown on June