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Marker-Assisted Selection of *Xa21* Conferring Resistance to Bacterial Leaf Blight in *indica* Rice Cultivar LT2

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Abstract: Bacterial leaf blight of rice (BLB), caused by *Xanthomonas oryzae* pv. *oryzae*, is one of the most destructive diseases in Asian rice fields. A high-quality rice variety, LT2, was used as the recipient parent. IRBB21, which carries the *Xa21* gene, was used as the donor parent. The resistance gene *Xa21* was introduced into LT2 by marker-assisted backcrossing. Three *Xoo* races were used to inoculate the improved lines following the clipping method. Eleven BC_3F_3 lines carrying *Xa21* were obtained based on molecular markers and agronomic performance. The 11 lines were then inoculated with the three *Xoo* races. All the 11 improved lines showed better resistance to BLB than the recipient parent LT2. Based on the level of resistance to BLB and their agronomic performance, five lines (BC_3F_3 5.1.5.1, BC_3F_3 5.1.5.12, BC_3F_3 8.5.6.44, BC_3F_3 9.5.4.1 and BC_3F_3 9.5.4.23) were selected as the most promising for commercial release. These improved lines could contribute to rice production in terms of food security.

Key words: rice; backcrossing; bacterial leaf blight; marker-assisted selection

Rice bacterial leaf blight (BLB), caused by infection of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is a devastating disease resulting in 20%–30% annual reduction in rice production worldwide (Nino-Liu et al, 2006). The use of a resistant cultivar is the most effective and environmental friendly method to combat the disease. To date, more than 38 BLB- related genes have been identified in rice, and many of them have been utilized in rice breeding programs (Khan et al, 2014). Several near-isogenic lines (NILs) have been developed for BLB resistance genes with IR24 genetic background by International Rice Research Institute, such as IRBB1, IRBB2, IRBB3, IRBB4, IRBB5, IRBB7, IRBB10, IRBB11 and IRBB21. Some of these have been used to develop BLB-resistant varieties (Khush et al, 1989). Among these resistance genes, *Xa21* has been studied in depth (Song et al, 1997) and widely exploited in rice breeding programs (Williams et al, 1996; Singh et al, 2001; Perez et al, 2008). *Xa21*, derived from a wild African species *Oryza longistaminata* and physically mapped on the long arm of rice chromosome 11, was co-segregated with random amplied polymorphic DNA (RAPD) markers (RAPD248 and RAPD818) and restricted fragment length polymorphism (RFLP) marker (RG103) (Ronald et al, 1992). And then *Xa21* was cloned by Song et al (1995).

LT2 is a high-quality rice variety, preferentially grown in northern Vietnam with agronomically favorable features such as short growth duration, high yield, aroma and wide adaptability. However, LT2 is susceptible to BLB, leading to annual yield loss of about 15%–30% on average and up to 60% in the summer season. Of the reported BLB resistance genes, *Xa21* has stable and durable resistance and is effective in northern Vietnam (Furuya et al, 2012) as well as in India (Mishra et al, 2013). The objective of this study was to improve

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the BLB resistance level of LT2 by marker-assisted backcrossing.

MATERIALS AND METHODS

Rice materials

NIL IRBB21 carrying *Xa21* was used as the BLB-resistant donor. The F_1 plants derived from a cross between LT2 as the recipient parent and IRBB21 were backcrossed to LT2 to develop the BC_1F_1 generation. The same processes were conducted to generate the BC_3F_1 population. Self-pollination was conducted from selected BC_3F_1 individuals to generate the BC_3F_3 population.

Genomic DNA isolation and PCR amplification

Genomic DNA was isolated from the fresh leaves of the parents (LT2 and IRBB21), F_1, BC_1F_1, BC_2F_1, BC_3F_1 and BC_3F_2 following the mini preparation procedure, modified method of Zheng et al (1991) and TPS (100 mmol/L Tris-HCl buffer containing 10 mmol/L EDTA and 1 mol/L KCl) method (Monna et al, 2002). The DNA marker pTA248 (5′-AGACGCG GAAGGGTGGTTCCCGGA-3′; 5′-AGACGCGGTAATCGAA AGATGAAA-3′) was used to select plants that were heterozygous at the *Xa21* locus, which were then backcrossed to the recurrent parent. PCR amplification was performed as described by Huang et al (1997).

Xanthomonas oryzae pv. *oryzae* (*Xoo*) inoculum preparation and inoculation

We used three *Xoo* isolates, 981.HUA10146, 996.HUA10147 and 1035.HUA10153, to evaluate the BLB resistance of plants. Culture and inoculation of the bacteria were conducted as described by Furuya et al (2012). The disease response was evaluated 18 d after inoculation by measuring the lesion lengths of three plants, and scored as follows: high resistance (R, lesion length < 8 cm), moderate resistance (M, lesion length 8–12 cm), and susceptiblity (S, lesion length > 12 cm).

Evaluation of agronomical traits

Six traits including growth duration, plant height, number of tillers per plant, number of grains per panicle, number of panicles per plant and 1000-grain weight were measured to evaluate agronomic performance. Grain quality was evaluated using polished ratio, gelatinization, amylose content, protein content and aroma. One kilogram of rice seeds was dehulled and polished, and then broken seeds were removed and measured. The polished rice rate was calculated by the polished unbroken seeds and polished seeds. Gelatinization was measured with 1.7% KOH at 30 ºC for 23 h. Amylose content was performed according to Juliano (1971). Add 1 mL of 95% ethanol and 9 mL NaOH into 100 mg grinded rice. The sample was heated for 10 min in water bath to gelatin the starch, and H_2O was added until 100 mL. The starch solution was mixed with 1 mL of 1 mol/L acid acetic and 2 mL iodine solution. Then, the solution was made up to 100 mL with distill water and shaken. After standing for 20 min, absorbance of the color was measured by spectrophotometer at 620 nm. Protein content was measured following the method of Lowry et al (1951). Aromatic testing was performed according to the method described by Kibria et al (2008). Briefly, 40 brown rice seeds were placed in a test tube, and 5 mL of 1.7% KOH was added. The tube was sealed and kept at room temperature for 15 min. Evaluation of aroma was performed by panelists and scored from grades of 1 to 9.

RESULTS

Marker-assisted selection of *Xa21* gene

The F_1 plants were backcrossed with LT2 to develop the BC_1F_1 generation (Fig. 1). Twenty-six heterozygous BC_1F_1 plants were selected based on the pTA248 marker, agronomic traits, and BLB resistance. Of these, eight plants were backcrossed to the recurrent parent LT2 to generate the BC_2F_1 population. In total, 36 of 63 plants were genotyped with the pTA248 marker, and 14 heterozygous plants were selected. Of these, 11 plants were then selected based on BLB resistance and backcrossed to the recurrent parent LT2 to generate the BC_3F_1 population. Based on the genotype of pTA248 and agronomic traits, 11 heterozygous plants were selected. The 11 plants were then narrowed down to 8 plants based on BLB resistance and these were then self-pollinated to generate the BC_3F_2 population. In total, 368 BC_3F_2 plants were planted and 138 were genotyped with the pTA248 marker (Fig. 2). Based on agronomic traits and BLB resistance, 11 lines were selected and self-pollinated to generate the BC_3F_3 population. These 11 BC_3F_3 lines were then planted, from which 8 plants were further selected based on agronomic traits. Finally, five of these eight plants, which

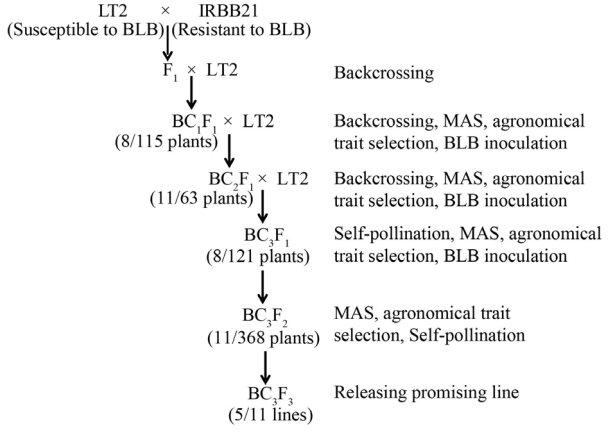

Fig. 1. Breeding scheme for MAS of *Xa21*.

The number of plants selected for self-pollination and total numbers of transplanted plants are indicated in parentheses.

MAS, Marker-assisted selection; BLB, Bacterial leaf blight.