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K. P. Che · Q. C. Zhan · Q. H. Xing · Z. P. Wang
D. M. Jin · D. J. He · B. Wang

Tagging and mapping of rice sheath blight resistant gene

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Abstract Sheath blight (*Rhizoctonia solani* Kühn) is one of the severe rice diseases worldwide. In this study, an F_2 population from a cross between '4011' and 'Xiangzaoxian19' is used to identify molecular markers linked with the resistant trait. '4011' was a transgenic rice cultivar carrying a resistant gene to sheath blight, while 'Xiangzaoxian19' is a highly susceptible one. As a result, five molecular markers, including three RFLP markers converted from RAPD and AFLP markers, and two SSR markers were identified to link with the sheath blight resistant gene. This dominant resistant gene was named as R*sb 1* and mapped on rice chromosome 5. The linkage distance between the markers (E-AT:M-CAC_{120}, E-AT:M-CTA_{230}, OPN-16_{2000}, $RM164_{320}$ and $RM39_{300}$) and R*sb 1* was 1.6 cM, 9.9 cM, 1.6 cM, 15.2 cM and 1.6 cM, respectively.

Keywords Rice · Sheath blight resistance · Molecular markers · Gene mapping

Introduction

Rice sheath blight, caused by the pathogenic fungus *Rhizoctonia solani*, is one of the most prevalent rice diseases, causing great damage to rice yield and quality worldwide (Lee and Rush 1983; Rush and Lindberg 1996). Because of the semi-saprophytic pathogen with wide host ranges and inexplicit mechanisms responsible for resistance characters, no immunitive resistant rice germplasm has been found to-date. It was accepted that some partial resistant or tolerant varieties existed (Lee et al. 1999; Raina et al. 1999; Chen et al. 2000; Li et al. 2000; Meena et al. 2000).

Genetic studies have shown that there were multiple genes for resistance to sheath blight in some rice varieties (Sha and Zhu 1989; Li et al. 1995; Zou et al. 2000), whereas some researchers proposed that in some varieties, sheath blight resistance was controlled by major genes (Xie et al. 1992; Pan et al. 1999a, b). Xie et al. (1992) have reported that two resistant elite lines, LSBR-5 and LSBR-33, selected from a somaclonal mutant derived from the sheath blight susceptible cultivar Labelle, possessed major sheath blight resistant genes, respectively. In their research, genetic studies revealed that sheath blight resistance was controlled by one dominant and one recessive gene in LSBR-5, and by two independently inherited recessive genes in LSBR-33. Pan et al. (1999a) found that two resistant cultivars, Teqing and Jasmine85, each possessed a non-allelic dominant major resistant gene which segregated independently. They also suggested that major genes conferring high levels of partial resistance to sheath blight might be incorporated together into lines, to give near complete resistance. With a clonal F_2 population, Pan et al. (1999b) identified three major loci for sheath blight resistance in Jasmine85 on chromosomes 2, 3 and 7, respectively.

The objectives of this study were to find molecular markers tightly linked with the sheath blight resistance gene in resistant rice line '4011', in order to benefit marker-assisted selection (MAS) in rice breeding and to map the major dominant resistant gene on rice chromosomes to explore detailed knowledge about the resistant loci involved for further work.

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K.P. Che · Q.H. Xing · D.M. Jin · B. Wang (✉)
Plant Biotechnology Laboratory,
Institute of Genetics and Developmental Biology,
Chinese Academy of Sciences, Beijing 100101, China
e-mail: bwang@genetics.ac.cn
Tel.: +8610-64870491, Fax: +8610-64873428

Q.C. Zhan · Z.P. Wang · D.J. He
Rice Research Institute, Hunan Academy of Agricultural Sciences,
Changsha 410125, China

Materials and methods

Plant materials

The materials used in this study were an F_2 population developed from the cross between '4011' (resistant parent) and 'Xiangzaoxian19' (susceptible parent). '4011' is a stable transgenic rice line

with remarkable resistance to *R. solani* by introducing resistant DNA fragments of *Zizania caduciflora* (which is a water-growing wild kindred species of rice with high resistance to rice sheath blight) into *Indica* rice variety '90519' through the pollen-tube pathway transformation method (Zhan et al. 2001).

Isolation, purification, cultivation and inoculation of *R. solani*, and disease investigation

The *R. solani* (Kühn) strain, which causes severe rice sheath blight disease, was isolated from sclerotia of sheath blight collected from heavily infected rice leaves. The sclerotia were scoured with distilled water and submerged in 70% ethanol for 2 mins. After flushing with sterile water, the strain was cultivated on potato dextrose agar (PDA) medium at 28 °C for 3–5 days until the hyphae developed. The hyphae were picked up and cultivated in autoclaved tubes on PDA medium untill the strain was purified completely. The autoclaved rice stalk with a length of 8 cm was incubated with the purified pathogenic strain grown on PDA medium for 4–6 days in darkness at 28 °C, then placed evenly at the bottom of the rice stem at the late tillering stage of growth both in the field trial and greenhouse tests. The disease scores were investigated about 30 days after heading using a 0–9 scale according to the method of Rush et al. (1976), in which 0 indicated no infection and 9 indicated plants dead or collapsed.

The cultivation and inoculation of the pathogen were carried out in the Plant Protection Institute, Hunan Academy of Agricultural Sciences, Changsha, China.

PCR analysis

Rice total genomic DNA was extracted and purified following the method of McCouch et al. (1988). Then, PCR analysis was carried out by the bulk segregant analysis (BSA) method with pooling of 15 resistant individuals as a resistant bulk, and 15 susceptible individuals as a susceptible bulk (Michelmore et al. 1991; Wang et al. 1995; Dong et al. 2000; Jia et al. 2000). In order to confirm the linkage relationship, the potential bands linked to the disease-resistant gene detected by BSA analysis were further assayed with a large number of F_2 plants.

RAPD analysis

The basic procedure of the RAPD reaction was as follows: 1 × reaction buffer, 0.1 mM of each dNTP, Mg^{2+} 2 mM, *Taq* polymerase 1.25 Units, template DNA 20 ng, primer 25 ng, then fixing to 25 µl with ultra-pure water, finally overlayed with one drop of mineral oil. The RAPD primers were purchased from Operon Technology Inc. The amplification reactions were carried out by the following profile: 94 °C 5 mins for one cycle, 94 °C 1 min, 37 °C 1 min and 72 °C 2 mins for 40 cycles, then 72 °C 5 mins. The amplification products were analyzed by electrophoresis in 1.0% agarose gels, followed by ethidium bromide staining and viewing under UV radiation.

SSR analysis

The basic procedure of the SSR reaction was as follows: 1 × reaction buffer, Mg^{2+} 2.0 mM, 0.1 mM of each dNTP, [α-^{32}P]dCTP 0.8 µCi (purchased from Amersham Technology Inc.), *Taq* polymerase 1.0 Unit, template DNA 20 ng, primer 30 ng, then fixing to 20 µl with ultra-pure water, finally overlayed with one drop of mineral oil. The amplification reactions were carried out by the following profile: 94 °C 1 min, 55 °C 1.5 mins, 72 °C 1.5 mins for 35 cycles, then 72 °C 5 mins. The amplification products were separated on 6% PAGE sequencing gel at 100 W for 2.0 h after pre-electrophoreses for 30 mins. The gel was then removed from the apparatus, dried with the BRL Model 583 gel dryer and exposed to X-ray film at –70 °C for 1–2 days until the film was adequately exposed.

AFLP analysis

AFLP analysis was performed following the Gibco-BRL AFLP Analysis System Kit (Life Technologies Inc., Gaithersburg, Mass.) with minor modifications. DNA (500 ng) was double-digested with *Eco*RI and *Mse*I, and the digested DNA fragments were ligated with *Eco*RI and *Mse*I adaptors, respectively. A pre-selective amplification was carried out with *Eco*RI+0 and *Mse*I+C primers, and the PCR product was diluted in a ratio of 1:15 with TE buffer and then used as a template for the selective amplification. Selective amplification using ^{33}P-labeled *Eco*RI primers was conducted and the products were separated on 6% PAGE sequencing gel at 100 W for 2.5 h after pre-electrophoreses for 30 mins. The gel was then removed, dried and exposed according to the procedure described above.

Mapping of the linkage markers and the sheath blight resistant gene

The target linkage AFLP and RAPD fragments were recovered, cloned and converted into RFLP markers according to the method of Li et al. (2000) and Jia et al. (2000). A double-haploid (DH) population from the cross between CT9993 and IR62266 (Zhang et al. 2001) was used for mapping the RFLP markers converted from AFLP and RAPD markers, with the MAPMAKER program (Lander et al. 1987). After the sheath blight resistant gene R*sb 1* was mapped on the chromosome, a number of SSR markers in the region surrounding R*sb 1* were selected for fine mapping of the sheath blight resistant gene. The phenotype, AFLP, SSR, and RAPD data were combined for linkage analysis using the MAPMAKER program and the linkage map of the specific chromosome region surrounding the resistant gene was constructed.

Data analysis

The chi-square (χ^2) test was used in the analysis of the inheritance of sheath blight resistance. The linkage map of molecular markers on the rice chromosome was constructed using the MAPMAKER program (Lander et al. 1987). Genetic distance was expressed in centiMorgans.

Results

Pathological and genetic analysis of sheath blight resistance in the F_2 population

A total of 1,030 F_2 individuals was used to conduct genetic analysis of disease resistance. The resistant difference between '4011' and 'Xiangzaoxian19' was significant. '4011' was resistant to the *R. solani* pathogen with a disease score of 2–3, and 'Xiangzaoxian19' was susceptible with a disease score of 8–9. The disease-score distribution of the F_2 population (see Fig. 1) was bimodal, suggesting the involvement of a major resistant gene. All 765 individuals (with a disease score ≤5) of the F_2 population were considered as sheath blight resistance, and 265 individuals (with a disease score ≥6) were identified as susceptible ones. The segregation ratio of the F_2 population is indicated in Table 1.

The approximate resistant/susceptible segregation ratio of 3:1 ($P < 0.05$) in the F_2 population suggested that

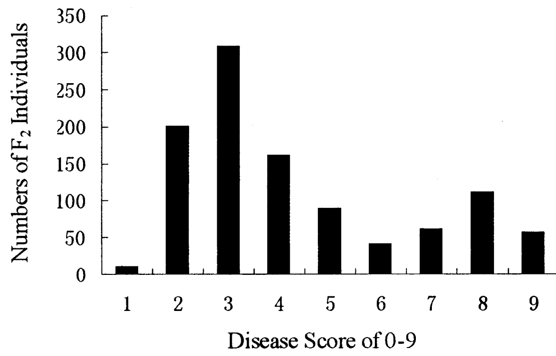

Fig. 1 Modal distribution of sheath blight disease scores in the F_2 population for resistance

the resistant trait of '4011' to *R. solani* was controlled by a major dominant gene, which was named R*sb 1*.

Identification of molecular markers linked to the R*sb 1* gene

Among all the fragments amplified by 230 RAPD primers and 128 AFLP primer combinations, three single-copy sequence molecular markers (OPN-16_{2000}, AT:M-CTA_{230} and E-AT:M-CAC_{120}) showed significant linkage with the resistant character. They were cloned and converted to RFLP markers according to the method of Li et al. (2000). The amplification segregation of these three

Fig. 2 The amplification segregation of molecular markers of OPN-16_{2000} 9(**A**), E-AT:M-CAC_{120}(**B**), E-AT:M-CTA_{230}(**C**), RM164_{320}(**D**) and RM39_{300}(**E**) in the F_2 population. The *arrow* indicates the relevant polymorphic amplification fragments between resistant individuals and susceptible ones. The *lane* with * represents the recombinant type. R_1 to R_{30} indicate the 30 resistant individuals of the F_2 population: S_1 to S_{30} indicated the 30 susceptible individuals of the F_2 population. In A, M_1 indicates the DNA molecular-weight marker III; M_2 indicates the DNA molecular weight marker 2000

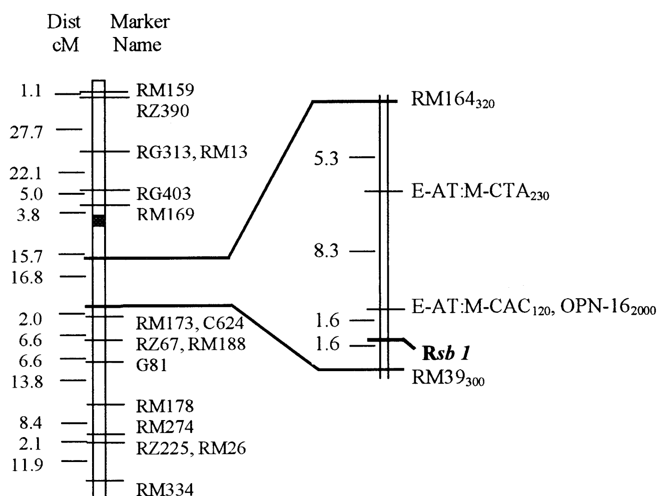

Fig. 3 Map showing the sheath blight resistant gene R*sb 1* and its linkage markers on chromosome 5

Table 1 The resistant/susceptible segregation ratio of the F_2 population

Total F_2 population numbers	Resistant individuals	Susceptible individuals	Segregation ratio	χ^2 test
1,030	765	265	3:1	0.98

molecular markers in the F_2 population is shown in Fig. 2 (A, B, and C).

Tagging and mapping of the sheath blight resistant gene R*sb 1*

Two of the three selected single-copy sequence markers (OPN-16_{2000} and E-AT:M-CTA_{230}) were used for mapping the R*sb 1* gene with the DH population from the cross between CT9993 and IR62266 (Zhang et al. 2001). After analysis with MAPMAKER software, OPN-16_{2000} and E-AT:M-CTA_{230} were mapped on the long arm of chromosome 5 near the centromere region (see Fig. 3).

After R*sb 1* was mapped on chromosome 5, in order to further confirm the map result and to find more markers closely linked with R*sb 1*, ten SSR markers were selected from the region surrounding E-AT:M-CTA_{230} and OPN-16_{2000} on chromosome 5, and were analyzed by the linkage relationship with R*sb 1*. The results indicated that two SSR markers, $RM164_{320}$ and $RM39_{300}$, showed linkage with R*sb 1* (see Fig. 2D and E). The linkage distance between R*sb 1* and the linkage markers OPN-16_{2000}, E-AT:M-CTA_{230}, E-AT:M-CAC_{120}, $RM164_{320}$ and $RM39_{300}$, was 1.6 cM, 9.9 cM, 1.6 cM, 15.2 cM and 1.6 cM, respectively (see Fig. 3).

Discussion

It was generally considered that rice sheath blight resistance was a quantitative trait controlled by multiple genes (Sha et al. 1989; Li et al. 1995; Zou et al. 2000). However, with the progress of recent research it was confirmed that in some rice varieties, sheath blight resistance was controlled by major genes. These results suggest that are important loci in the rice chromosome responsible for the resistant character (Xie et al. 1992; Pan et al. 1999a, b). Zhan et al. (2001) developed a transgenic line ‘4011’ resistant to sheath blight by injecting sheared *Z. caduciflora* (which is the kindred species of rice and with high resistance to sheath blight) DNA into the pollen tube of *Indica* rice variety ‘90519’, which is susceptible to sheath blight disease. After cultivation and selection for several generations, ‘4011’ became stable in the sheath blight resistant character. ‘4011’ has passed the rice variety comparison test of Hunan Province in 1999 and was confirmed to be a good resistant cultivar to sheath blight disease with a disease score rating of 2–3, and has spread rapidly in Hunan Province. Through genetic analysis, it is considered that there is a dominant major locus in ‘4011’ controlling the resistance to this disease.

In theory, it is possible to develop resistant germplasm controlled by a single dominant locus by introducing a foreign resistant gene. In the case of ‘4011’, when the sheared *Zizania* DNA fragments involving resistant loci were transformed into the rice genome through genetic manipulation, one or more fragments might be integrated into the rice genome. In our case, the sheath blight resistance of ‘4011’ was identified to be controlled only by a single locus. Therefore, the resistance level of ‘4011’ was comparatively lower than that of *Z. caduciflora*.

In the five identified molecular markers linked with rice sheath blight resistance, the co-dominant linkage marker E-AT:M-CAC_{120} was used to screen the F_2 individuals to test the verification of the resistant/susceptible phenotypic segregation ratio. The amplification result (data not shown) segregated in an approximate ratio of 1:2:1, and supported the genetic analysis results at the molecular level. The resistant gene was mapped on rice chromosome 5 according to the linkage markers. The tightly linked markers will be useful for further research to clone the resistant gene through the map-based cloning method.

The identification of the molecular markers linked with R*sb 1* provides a superior selective screen to assist in transferring resistant loci into cultivars. The SSR markers $RM164_{320}$ and $RM39_{300}$ can be used efficiently in MAS. As for the AFLP markers E-AC:M-CAC_{120} and E-AT:M-CTA_{230}, as well as the RAPD marker OPN-16_{2000} and the RFLP markers converted from PCR markers, they may not be ideal for selection purposes directly. After converting these three PCR-based markers into SCAR (sequence characterized amplified region) or STS (sequence tagged site) markers in further work, we can exploit the specific markers as strong aids to promote the breeding process of rice sheath blight resistance.

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