Theor Appl Genet (2002) 104:547–552

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Mapping of the QTL (quantitative trait locus) conferring partial resistance to leaf blast in rice cultivar Chubu 32

Received: 15 March 2001 / Accepted: 13 August 2001

Abstract The rice cultivar Chubu 32 possesses a high level of partial resistance to leaf blast. The number and chromosomal location of genes conferring this resistance were detected by restriction fragment length polymorphism (RFLP) linkage mapping and quantitative trait locus (QTL) analysis. For the mapping, 149 F_3 lines derived from the cross between rice cultivar Norin 29, with a low level of partial resistance, and Chubu 32 were used, and their partial resistance to leaf blast was assessed in upland nurseries. A linkage map covering six chromosomes and consisting of 36 RFLP markers was constructed. In the map, only one significant QTL (LOD>2.0) for partial resistance was detected on chromosome 11. This QTL explained 45.6% of the phenotypic variation. The segregation ratio of the F3 lines was 3:1 for partial resistance to susceptibility. These results suggest that the partial resistance in Chubu 32 is controlled by a major gene.

Keywords Rice (*Oryza sativa*) · Leaf blast · Partial resistance · Restriction fragment length polymorphism (RFLP) · Quantitative trait locus (QTL)

Introduction

Blast, caused by *Pyricularia grisea* (Cooke) Sacc. (Rosseman et al. 1990), is one of the most severe diseases of rice (*Oryza sativa*) in Japan. The fungus colonizes leaves (leaf blast), panicles (panicle blast) and other parts of the rice plant, and causes crop loss in rice-growing areas worldwide (Ou 1985). To control the disease, the use of resistant cultivars is an effective measure; thus, rice breeders have been developing resistant cultivars.

Blast resistance in rice cultivars is generally classified into two types, qualitative (complete) and quantitative (partial) (Bonman and Mackill 1988; Lee et al. 1989). Complete resistance is characterized by prevention of blast fungus reproduction in incompatible combinations of the host and pathogenic strains, and each of the resistances is usually controlled by a single gene. On the other hand, partial resistance reduces the extent of pathogen reproduction in the compatible interaction (Jonson 1983; Parlevliet 1988). Most of the partial resistance is non-race specific, quantitative and polygenic (Maruyama et al. 1983; Higashi 1995). However, there are some exceptions such as *Pif* (Toriyama et al. 1968) and *Pb1*(t) (Fujii et al. 1999), which are single genes conferring partial resistance to blast.

Some genes conferring complete resistance to blast have been introduced into many rice cultivars to control the disease (Shinoda et al. 1971). However, their resistance broke down within a few years due to the appearance of new virulent blast races (Yunoki et al. 1969; Ou 1979; Watanabe 1980; Kiyosawa 1982). On the other hand, partial resistance is stable to different pathogenic races of the rice blast fungus; hence, the use of partial resistance is one of the most promising measures for blast control.

The rice cultivar Chubu 32 shows a stable and high level of partial resistance to leaf blast in Japan. It is regarded that the partial resistance in Chubu 32 is derived from a *japonica* upland rice cultivar (Koizumi and Fuji 1995) and that a major and some minor genes affect its level of partial resistance. Despite the usefulness of this partial resistance, its genetic analysis has not yet been performed.

Recent DNA marker-technology development, such as RFLP, RAPD, AFLP and other types of DNA markers, enables the construction of a high-resolution genetic map for isolating genes associated with important traits,

Communicated by H.C. Becker

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including disease resistance (McCouch et. al. 1988; Harushima et al. 1998). The first cloned disease resistance gene in rice was the bacterial leaf blight resistance gene, *Xa21*, which was isolated using a map-based cloning strategy (Song et al. 1995). For blast resistance genes, the complete resistance gene *Pib* was isolated by Wang et al. (1999). Some recent studies (Bao et al. 2000; Price et al. 2000) demonstrated that genetic linkage maps constructed with various DNA markers are very useful for the analysis and detection not only of qualitative trait loci but also of quantitative trait loci (QTLs). For example, Wang et al. (1994) identified ten QTLs affecting partial resistance to blast in the japonica rice cultivar Moroberekan; and Yamamoto et al. (2000) reported the characterization of epistatic interactions for two heading-date QTLs. However, successful cloning of these genes has not been reported, although some laboratories are attempting to isolate QTLs associated with agronomically important traits such as heading date (Yano et al. 1997; Yamamoto et al. 2000) and cold tolerance profiles (personal communication).

The objective of our study was to isolate the genes conferring partial resistance to leaf blast in Chubu 32. In this study, we located the partial resistance genes in Chubu 32 on the DNA-marker genetic map.

Materials and methods

Plant materials

Chubu 32, a japonica rice cultivar with a high level of partial resistance to leaf blast in Japan, was used as the donor parent of partial resistance genes in a cross with Norin 29, a japonica rice cultivar which has a low level of partial resistance. Chubu 32 is the unfixed cultivar involving the complete resistance gene. There are two kinds of Chubu 32 variety distributed in Japan, one carries the complete resistance gene *Pik- s,* and the other has *Pik-s* and the *Pia* resistance gene (Koizumi and Fuji 1995). We used Chubu 32, with *Pik-s* and *Pia,* and Norin 29, which has *Pik-s*. However, both of the genes are susceptible to the pathogenic races of *P. grisea* which are widely distributed in Japan and we have already checked that the races of *P. grisea* distributed in the test field were compatible to *Pik-s* and *Pia*. Due to this fact, 149 F_3 lines from the cross between Norin 29 and Chubu 32 were obtained from the Aichi Prefectural Agriculture Research Center in 1996 and these F_3 lines were used for the evaluation of partial resistance to leaf blast and for QTL analysis.

Evaluation of partial resistance to leaf blast in F_3 lines

To evaluate partial resistance to leaf blast in F_3 lines, an upland nursery trial was carried out in 1997 at the Tohoku National Agricultural Experiment Station (its present name is the National Agricultural Research Center for the Tohoku Region) in Omagari, Akita. In the trial, all 149 F_3 lines and the parental cultivars were used and a complete randomized block design was employed with three replications. Test entries were drilled with approximately 50 seeds per entry in rows 40 cm-long with 10-cm spacing, on June 9, 1997. Seeds from each F_3 line were sown in alternate rows and the parent cultivar seeds were drilled alternately between them. To increase the natural inoculum, the cultivar Sasanishiki, which is susceptible to field isolates of *P. grisea,* was used as a spreader. It was planted in ten rows at both sides of each block. Nitrogen was applied at 20 kg/1,000 m^2 in the test field as a basal fertilizer and top-dressed at 5 kg/1,000 m^2 on July 8 and July 18, 1997, to enhance disease development. Disease severity in each naturally infected row was visually estimated based on the percentage of diseased leaf area (%DLA) according to the scale of Asaga (1981), i.e. 0 to 10 in 0.5 increments. The disease measurements were performed on July 15, 18, 22, 25 and 28. The %DLA was calculated using the formula (Asaga 1981) as follows:

$\log y/1-y = 0.3687375x - 2.3644375$, where
$y \times 100 = \%DLA$ and
x = disease severity.

The partial resistance level of each line from July 22 to 28 was measured as the area under the disease progress curve (AUDPC) (Parlevliet 1988) for QTL analysis. The July 15 and 18 data were not used because the disease did not progress enough to be able to evaluate the resistance level.

RFLP and microsatellite analysis of parental cultivars and F_3 lines

DNA preparation, electrophoresis, blotting, probe labelling and detection were performed as previously reported (Kurata et al. 1994). To reveal the polymorphism between Chubu 32 and Norin 29, total DNA from fresh leaf tissue was extracted by the CTAB method (Murray and Thompson 1980) and digested with the restriction enzymes *Bam*HI, *Bgl*II, *Eco*RV, *Hin*dIII, *Dra*I, *Apa*I, *Eco*RI and *Kpn*I. The digested DNAs were subjected to electrophoresis on 0.6% agarose gels and separated products were transferred to a positively charged nylon membrane (Hybond N+, Amersham Pharmacia Biotech.) mediated by 0.4 N NaOH. The blotted filters were washed with 2×SSC, dried and baked at 120°C for 20 min. The 558 cDNA clones for Southern-blot hybridization were provided by the Rice Genome Research Program (RGP) (Harushima et al. 1998) via the Ministry of Agriculture, Forestry and Fisheries (MAFF) DNA bank, and eight clones were provided by Cornell University (McCouch et al. 1988). These insert DNAs were amplified by the polymerase chain reaction (PCR) using M13 primers. DNA probe labelling, hybridization and detection of chemiluminescence on X-ray films were carried out using an ECL direct-labelling and detection system (Amersham Pharmacia Biotech.).

Sixty two microsatellite markers located on chromosomes 1,2,3,4,9,10,11 and 12 (Temnykh et al. 2000) were also tested between parental cultivars. Primers were synthesized by Amersham Pharmacia Biotech, Japan Ltd, and Invitrogen Ltd, Tokyo, Japan. PCR was performed in a PTC-100 Programmable Thermal Controller (MJ Research Inc.) as described by Chen et al. (1997) with the exception that 25 µl of the reaction mixture was used. The PCR program was 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 55°C, 2 min at 72°C, and 5 min at 72°C for final extension. PCR products were separated on 2.5% NuSieve GTG agarose gels in 1×TBE buffer and marker bands were revealed using ethydium-bromide staining.

Linkage map construction and mapping of putative QTLs

The program MAPMAKER/EXP ver.3.0 (Lander et al. 1987) was used to establish a RFLP map, while MAPMAKER/QTL ver.1.1 (Lincoln et al. 1993) was used to identify putative loci affecting partial resistance. Correspondence of linkage groups to chromosomes was inferred based on the existing RFLP linkage map of rice (Harushima et al. 1998). The marker order and map distances were computed using the F_2 algorithm in MAPMAKER/EXP, based on the segregation data on the bulked F_3 lines. All map distances were in Kosambi centimorgans (cM). The interval analysis programmed in MAPMAKER/QTL was carried out to identify putative QTLs. The average data of three replication plots of disease severity and that of AUDPC were used as trait data in MAPMAKER/QTL. Putative QTLs were identified in regions exceeding 2.0 LOD (log-likelihood value). To identify the mode of inheritance, putative QTL regions were examined by three con-

Fig. 1 Leaf blast severity in F_3 lines derived from the cross between Norin 29 and Chubu 32, and their parents, in an upland nursery trial: Norin 29 (*N*), Chubu 32 (*C*), partially resistant F_3 lines (*R*) and susceptible F_3 lines (*S*)

strained genetic tests such as "dominance", "recessive" and "additive" using MAPMAKER/QTL. A 1-LOD reduction in likelihood (a 1:10 likelihood ratio) has been used as the criterion for ruling out the mode of inheritance. The phenotypic effects, such as the additive effects, the dominance effects and the percent of total phenotypic variation explained by putative QTLs, were also examined by MAPMAKER/QTL.

Results

Phenotypic segregation

Genes conferring partial resistance to blast can only be detected in the absence of effective complete resistance genes. We confirmed the pathogenicity of the blast isolates distributed in the trial field. All blast isolates from the field could overcome the complete resistance genes *Pik-s* and *Pia* of the tested F_3 lines. The natural infection of leaf blast occurred 1 month after sowing and the disease developed rapidly in the middle of July (5–6 weeks after sowing). The average score of leaf blast severity in Chubu 32 at the final assessment was 6.29, which is approximately equal to 45% DLA, and that in Norin 29 was 9.19 (92% DLA). The parental cultivars showed a significant ($P<0.01$) difference in leaf blast resistance (Figs. 1 and 2). The frequency distribution of AUDPC from July 22 to 28 in F_3 lines was bimodal, between 2.0 and 5.5 with 4.2 being the boundary score (Fig. 2). It was indicated that disease severity was strongly correlated with AUDPC in the F_3 lines ($r^2=0.904$, $P<0.001$) by simple linear regression. The F_3 lines were divided into two groups R (partially resistant) and S (susceptible) with a disease severity boundary score of 8.5, and the segregation ratio of the number of R and S lines corresponded to the expected ratio of 3:1 (R:S) using the chi-square test ($0.2<P<0.3$). The same test was performed for AUDPC, the segregation ratio also corresponded to the expected 3:1 ratio ($0.3<P<0.5$). The result suggests that the partial resistance is controlled by a single dominant gene.

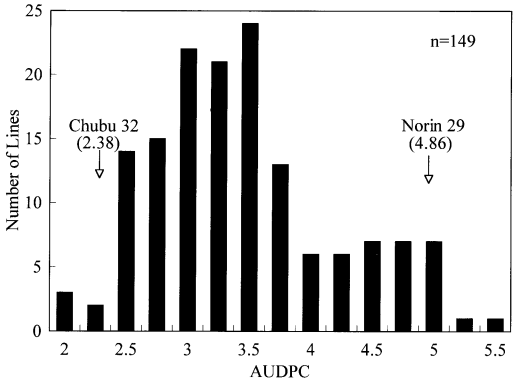

Fig. 2 Frequency distribution of AUDPC in F_3 lines derived from the cross between Norin 29 and Chubu 32. The *two arrows* indicate the average scores of disease severity and AUDPC in the parental cultivars

Analysis of parental polymorphism and segregation of RFLPs in F_3 lines

We tested 566 RFLPs and 62 microsatellite markers previously mapped on the rice genetic maps, and 89 of these markers were polymorphic between Chubu 32 and Norin 29 for one or more of the eight tested enzymes. The percentage of polymorphism detected between the two parents was 12.5%. This is much less than that of the cross between the indica and japonica rice cultivars.

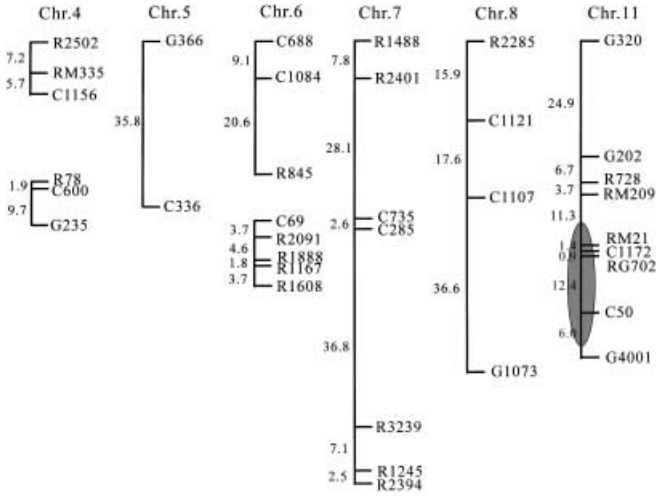

Fig. 3 RFLP linkage map in rice derived from segregation data of the Norin 29/Chubu 32 bulked F_3 lines using MAPMAKER/EXP 3.0 and quantitative trait locus (QTL) related to partial resistance to leaf blast. Markers are indicated by clone names. Markers denoted with *C, R or G numbers* are obtained from RGP. The *first letter* of the marker's name indicates the category of mapped clones as follows: *C* cDNA clones derived from the callus library; *R* cDNAs from the root library; and *G* random genomic clones. Marker RG702 mapped on chromosome 11 was obtained from Cornell University. Three microsatellite markers were designated *RM*. The location of the QTL enclosed in an *oval* was determined by interval mapping using MAPMAKER/QTL 1.1

Fifty markers that clearly showed polymorphism between the parents were used for the segregation analysis of the bulked F_3 lines. There were 36 (32 RFLPs, three microsatellites, one CAPS) out of the 50 markers which were codominant, distinguishing the heterozygotes in the bulked F_3 lines.

Genetic map construction and linkage mapping of putative QTLs

A linkage map, which covers 327 cM on six chromosomes and consists of eight linkage groups with a total of 36 markers, was constructed using MAPMAKER/EXP (Fig. 3). The order of markers in each linkage group mostly corresponded to that of the RGP map.

Interval mapping was performed using MAPMAKER/QTL to identify QTLs associated with partial resistance to leaf blast. In the first step of analysis, two putative QTLs were detected on chromosome 11 based on a single-QTL model. One QTL peak for *QTL1* (LOD=19.7) was found to be located in the C1172-RG702 interval (0.8 cM) and another QTL, *QTL2* (LOD=10.7) was mapped in the G320–G202 marker interval (30.9 cM). To verify the substance of these QTLs, the effect of one of them was removed using the "sequence" command of MAPMAKER/QTL and the effect of another QTL was checked based on the multiple-QTL model. When *QTL2* was "fixed", the log-likelihood of the two QTL map (20.7) was much lower than the sum of the QTL's independent likelihoods (19.7+10.7=29.7). This result indicates epistasis or a spurious result, or else some other condition. On the other hand, when we "fix" *QTL1* and re-scan the genome, no peaks with a LOD score above the threshold were found. Furthermore, the interval of G320–G202 was very long (30.9 cM) and a putative LOD peak was indicated at the middle of the interval. From these results, it was suggested that *QTL2* was the spurious QTL. *QTL1* was located between RM209 and G4001 by "fix" analysis (Fig. 3). The mode of inheritance of *QTL1* was tested based on a LOD reduction criterion; a "dominant" was most likely, though "recessive" and "additive" could be ruled out. This QTL explained about 45.6% of the total phenotypic variation in the F_3 lines.

Discussion

Partial resistance to plant diseases is generally a polygenic characteristic. However, the result of this study indicates that the QTL associated with partial resistance to leaf blast in Chubu 32 was a single dominant gene. This is consistent with the supposition based on the segregation data on leaf blast severity in F_3 lines in the upland nursery trial. Fukuoka and Okuno (1999) and Miyamoto et al. (1999) have reported on QTLs associated with partial resistance to blast. They used two different Japanese domestic upland rice cultivars for analysis of partial resistance and showed that t he QTL with the largest effect in each cultivar was located on chromosome 4. Interestingly, in both cultivars used, they detected QTLs with a relatively small effect on partial resistance to blast at approximately the same locus where the most effective QTL in Chubu 32 was found (the nearest marker to this QTL was C1172). Moreover, Fujii et al. (2000) reported that Pb1, the resistance gene to panicle blast with a quantitative nature is located on Chromosome 11, and such a locus is located near the QTL we have found.

Unfortunately, although we used more than 600 clones to analyze DNA polymorphisms, only 36 markers were mapped on the genetic linkage map. Generally, the higher the genetic similarity between the tested rice cultivars, the less-frequent polymorphism occurs between them. Both parental cultivars used in this study are japonica lowland rice cultivars and their genetic similarity is supposed to be relatively high. Therefore, the genetic linkage map constructed in this study did not include all of the genome and had very low-density markers including the QTL region. For this reason, it seems to be very difficult to construct a physical map accurate enough to be able to isolate a gene conferring partial resistance to leaf blast using the cross between Norin 29 and Chubu 32.

To solve this problem, we used three new materials distributed from RGP; that is, chromosomal-fragment substitution lines. The lines basically consist of japonica cultivar Koshihikari alleles. However, a part of their

chromosome 11 is substituted with the indica cultivar Kasalath alleles. We have already confirmed that these lines have a low level of partial resistance to leaf blast, as low as that of Koshihikari, with no complete resistance genes to most of the blast fungus strains found in Japan. We expect that use of these lines enables the construction of a high-density linkage map and the determination of useful markers necessary for the physical map construction of a target gene.

Isolation of some of the genes conferring partial resistance to blast described above has been attempted using upland rice cultivars (Fukuoka, personal communication). In this study, we obtained basic information for the isolation of the resistance gene. Moreover, the identified QTL in this study will be very useful for further analysis of the genetic basis of par tial resistance to blast because it is considered that this QTL is controlled by a major gene.

Acknowledgements We gratefully thank Drs. M. Yano and T. Sasaki, National Institute of Agricultural Resources, Japan, for helpful suggestions during the planning stages of this work, members of the Rice Genome Research Program (RGP) for technical cooperation and providing rice clones, and Dr. S. McCouch for also providing rice clones. We also thank A. Konno for technical support. This work was supported by funds from the Rice Genome Project sponsored by the Ministry of Agriculture, Forestry and Fisheries of Japan.

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