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Mapping of QTLs conferring resistance to bacterial leaf streak in rice

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Abstract A large F_2 and a RI population were separately derived from a cross between two *indica* rice varieties, one of which was highly resistant to bacterial leaf streak (BLS) and the other highly susceptible. Following artificial inoculation of the RI population and over 2 years of testing, 11 QTLs were mapped by composite interval mapping (CIM) on six chromosomes. Six of the QTLs were detected in both seasons. Eight of the QTLs were significant following stepwise regression analysis, and of these, 5 with the largest effects were significant in both seasons. The detected QTLs explained 84.6% of the genetic variation in 1997. Bulked segregant analysis (BSA) of the extremes of the F_2 population identified 3 QTLs of large effect. The 3 QTLs were dentical to 3 of the 5 largest QTLs detected by CIM. The independent detection of the same QTLs using two methods of analysis in separate mapping populations verifies the existence of the QTLs for BLS and provides markers to ease their introduction into elite varieties.

Key words Rice · Bacterial leaf streak · Mapping · Bulked segregant analysis · QTL

Introduction

Bacterial leaf streak (BLS) is a major disease in cereals. It was first observed in rice in Guangdong, China and identified later as a new bacterial disease of rice caused by the pathogen *Xanthomonas oryzae* pv. *oryzicola*. It is now known that rice BLS occurs worldwide being especially serious in tropical and subtropical areas of Asia. In China, the large-scale release since the later 1970s of elite hybrid rice varieties that are susceptible to BLS has resulted in the disease spreading rapidly and becoming one of the major diseases in rice. Quarantine regulations are now in force for BLS in China.

Breeding resistant varieties is the most efficient way to control BLS in rice. Some highly resistant rice varieties such as Acc8558 and Acc8518 (Xia et al. 1992) have been identified that can be used as potential resistant parents for breeding programs. It has been shown that the level of BLS resistance in rice varies continuously, ranging from highly resistant to highly susceptible (Xia et al. 1991, 1992), implying that the trait is quantitatively inherited. This was confirmed in our previous study (Tang et al. 1998).

Genetic studies of quantitative traits are difficult because of their complicated genetic basis. The advent of molecular marker technologies such as restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) has opened a door for the genetic dissection of complex traits. Genetic studies on BLS resistance, including mapping relevant quantitative trait loci (QTLs), have been conducted in wheat (El Attari et al. 1996; Duveiller et al. 1993) and barley (El Attari et al. 1998; Alizadeh et al. 1994). With the aid of molecular markers, El Attari et al. (1998) identified 3 QTLs conferring BLS resistance in barley. This paper reports the first study of mapping QTLs controlling BLS resistance in rice.

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Materials and methods

Materials

An F_2 population consisting of 990 plants and a recombinant inbred (RI) population consisting of 131 ($F_{6:7}$) lines derived by single-seed descent were developed from a cross between two *indica* rice varieties, Acc8558 and H359, which had previously been identified to be respectively the most resistant and the most susceptible varieties to BLS amongst 969 rice varieties screened for

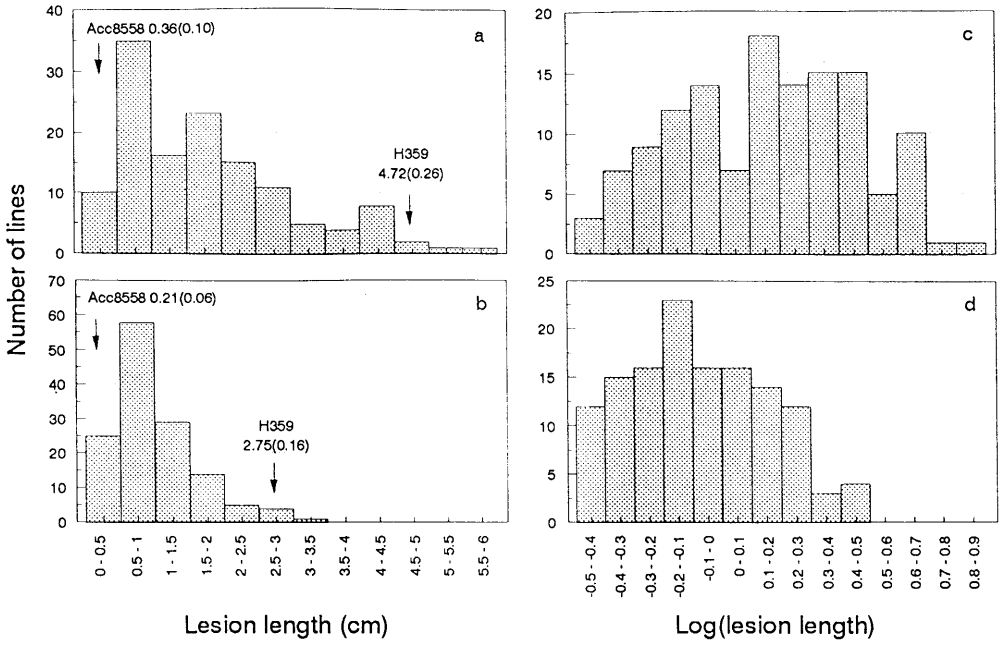

Fig. 1a–d Distributions of lesion length in the RI population. **a, c** in 1996; **b, d** in 1997. *Arrows* and the *numbers above arrows* show the mean values of lesion length of the two parents (*numbers* in *brackets* are standard errors)

resistance (Xia et al. 1992). A strong pathogen strain of *Xanthomonas campestris* pv *Oryzicola*, 89773–1-1, isolated in Fujian, China was kindly provided by Prof. Yihou Xia of the Department of Plant Protection, Fujian Agricultural University (FAU).

Field evaluation of BLS resistance

Field experiments were conducted at the FAU. The F_2 population was planted in 1996, and the RI population was planted in 1996 and 1997, with 5 and 7 seedlings per line, respectively. Five complete leaves of similar age from each plant were inoculated with the pathogen isolate at a concentration of 9×10^8 colony-forming units per milliliter using the pricking inoculation method at the active tillering stage. Lesion lengths on three leaves randomly selected from the five inoculated leaves of each plant were measured 20 days later. The resistance of each plant was indicated by the mean lesion length of three leaves, and the resistance of each RI line was indicated by the mean lesion length of 5 plants in 1996 or 7 plants in 1997.

Construction of molecular marker linkage map

A molecular map with 147 RFLP and 78 AFLP markers covering a length of 1480 cM was constructed based on the RI population. DNA of each RI line was extracted from young leaves of 5 plants using the CTAB method (Murray and Thompson 1980) with minor modifications. Six restriction endonucleases, *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV and *Hin*dIII, were used for RFLP analysis. Southern blotting were carried out according to standard procedures (Sambrook et al. 1989). The RFLP loci were revealed with 1 06 rice probes (prefixed C, G, L R, Y; Kurata et al. 1994) and 28 wheat probes (prefixed PSR; Gale et al. 1993). AFLP analysis was conducted following Zabeau and Vos (1993) and Vos et al. (1995) with minor modifications. Two restriction enzymes (*Pst*I and *Mse*I) were used to digest genomic DNA and five primer combinations (P19/M76, P22/M17, P75/M19, P76/M22 and P77/M25) were used for the polymerase chain reaction (PCR). The linkage map was constructed using MAPMAKER software version 3.0 (Lander et al. 1987). All heterozygous genotypes were treated as missing data in the map construction.

Composite interval mapping (CIM) of QTLs

The map generated shows some regions where markers are very closely linked (distance <1 cM). For QTL analysis, only 1 marker was selected from each of these regions. By removing redundant markers, a subset of 199 markers was selected for QTL analysis.

The log of the BLS lesion length obtained from the RI population was used, as the trait was asymmetrically distributed, skewing to the direction of smaller values (Fig. 1a, b). After the log conversion, symmetry of the trait distribution was improved (Fig. 1c, d). QTL analysis was performed with CIM (Zeng 1994) based on least squares estimation (Wu et al. 1996). Markers used as cofactors in the CIM model were selected by stepwise regression analysis (SRA) using a significance level of 5%. Because the RI population was not large and there were more markers than RI lines, cofactor selection was conducted on each chromosome separately. In addition, for each chromosome being analyzed in the CIM, only the markers selected from the same chromosome were used as cofactors. In this way we aimed to increase the degrees of freedom for model fitting. Moreover, three different window sizes (0 cM, 10 cM and 20 cM) were used in the CIM analyses to identify the most suitable window size for each QTL. For each CIM analysis, the significance threshold of the LOD statistic ($TLOD_\alpha$) for the overall (or genome-wise) significance level (α) required was estimated empirically by permutation tests (Churchill and Doerge 1994) with 1000 replicates. In this study, an overall significance level of 5% was used to declare the existence of a QTL (Zeng 1994).

Since the CIM was conducted on each chromosome separately, the effects of background QTLs located on other chromosomes were not statistically controlled and therefore might contribute to the position (putative QTL) being tested (Gimelfarb and Lande 1995). This could possibly generate false positives (Wu et al. 1999). For this reason, QTLs mapped by CIM were re-evaluated by SRA based on a multiple-QTL model. This would allow us to examine which of the putative QTLs were ghosts of other QTLs (Wu et al. 1999). To reduce the rate of second type error, we used a slightly less stringent significance level (10%) in the SRA.

The proportions of phenotypic variance explained by an individual QTL and overall were estimated by a^2_i and $[\sum_i a^2_i + \sum_{i \neq j} (1–2R_{ij})\ a_i a_j]/V_p$ (Zeng 1993), respectively, where a_i (or a_j) is the additive effect of QTL i (or j), $R_{ij} = 2r_{ij}/(1+2r_{ij})$, r_{ij} is the recombi-

nation frequency between the QTLs *i* and *j*, and V_p is the phenotypic variance of the population.

Bulked segregant analysis (BSA) for QTL detection

The 12 most resistant and 12 most susceptible plants in the F_2 population were selected. Leaves of the two groups were bulked to construct a pair of DNA pools. RFLP between the two DNA pools was examined with the same set of rice probes used for the map construction.

Results

$TLOD_{0.05}$ estimated in the case of window size = 0 cM was 2.928 for 1996 and 3.112 for 1997. These correspond to nominal (or comparison-wise) significance levels of 0.000241 and 0.000153, respectively, assuming that the likelihood ratio statistic in each test follows the chi-square distribution with 1 df.. Window size had little influence on $TLOD_{0.05}$.

With the $TLOD_{0.05}$, 9 and 8 QTLs were mapped by CIM in 1996 and 1997, respectively (Table 1) and, in total, 11 QTLs were identified, distributed on chromosomes 1, 2, 3, 4, 5 and 11. Six out of the eleven QTLs, i.e. *qBlsr*1, *qBlsr*2, *qBlsr*3b, *qBlsr*3d, *qBlsr*5a and *qBlsr*5b, were detected in both years, and their positions identified in separate seasons were very close except for *qBlsr*5a (Table 1).

With the results of CIM, QTLs were re-evaluated by SRA based on a multiple-QTL model (Table 1). Eight and five QTLs were significant in the SRA in 1996 and 1997, respectively. Interestingly, all the five QTLs significant in 1997 (*qBlsr*1, *qBlsr3*b, *qBlsr*3d, *qBlsr*5a and *qBlsr*5b) were also significant in 1996 and had the largest effects of the 8 QTLs significant in 1996. In addition, these 5 QTLs were amongst the 6 QTLs detected by CIM in both years. This provides strong support to the existence of these 5 QTLs and suggests that they are the most important QTLs in this population.

The proportions of phenotypic variation explained by individual QTLs varied from 2.64% to 12.84% in 1996 and from 7.27% to 15.93% in 1997, with *qBlsr*5a having the largest effect in both years (Table 1). In total, the QTLs explained 45.25% and 55.66% of phenotypic variation in 1996 and 1997, respectively. Analysis of variance (ANOVA) showed that the heritability for BLS resistance was 65.34% in 1996 and 65.80% in 1997. Thus, the proportion of genetic variation explained by the QTLs was 69.25% in 1996 and 84.59% in 1997. The 5 QTLs significant in both 1996 and 1997 in the SRA explained most of the genetic variation in the population in each of the years tested. This further confirms the importance of the 5 QTLs.

Most of the QTLs showed positive additive effects (Table 1). This means that the alleles from the susceptible parent H359 acted to increase the measured trait (i.e. lesion length) or to increase BLS susceptibility. However, there was 1 QTL (*qBlsr*3b) where the resistant allele was from H359, indicating that even a highly susceptible variety may possess resistant alleles.

In the BSA, among the 103 rice probes used, 4 probes (C49 and C409 on chromosome 1, C1419 on chromosome 2 and R1553 on chromosome 5) detected polymorphism between the two DNA pools, implying that these markers are linked to BLS-related QTLs. As C49 and C409 are only about 10 cM apart (Fig. 2), they might identify a single QTL. Thus, the BSA detected a total of 3 putative QTLs.

Positions of the QTLs detected in both populations are shown in Fig. 2. The markers identified by the BSA are either at or close to the positions of QTLs *qBlsr*1, *qBlsr*2 and *qBlsr*5b as estimated by the CIM, and these 3 QTLs are amongst the 5 most important QTLs detected by the CIM. Therefore, this result verifies the methodologies used here in that two different methods of analysis can detect identical QTLs for BLS resistance in two independently generated mapping populations. The fewer QTLs with large effects detected by BSA related to CIM also agrees with expectations.

Table 1 QTLs underlying BLS resistance in rice. A QTL is denoted by *qBlsr* followed first by a number indicating the chromosome on which it is located and then a lowercase letter when there is more than 1 QTL on the same chromosome. The positions were estimated by CIM and the additive effects by SRA (in 10-base logarithm scale)

QTL	Position (cM)		Additive effect		%Percentage of V_P explained	
	1996	1997	1996	1997	1996	1997
*qBlsr*1	75	76	0.0890	0.0767	8.45	10.47
*qBlsr*2	23	24	0.0564	–	3.39	–
*qBlsr*3a	–	28	–	–	–	–
*qBlsr*3b	113	113	–0.0786	–0.0639	6.59	7.27
*qBlsr*3c	131	–	0.0497	–	2.64	–
*qBlsr*3d	170	172	0.0774	0.0741	6.39	9.78
*qBlsr*4a	–	75	–	–	–	–
*qBlsr*4b	139	–	–	–	–	–
*qBlsr*5a	17	26	0.1097	0.0946	12.84	15.93
*qBlsr*5b	72	73	0.0630	0.0748	4.24	9.96
*qBlsr*11	0	–	0.0506	–	2.73	–
	9 [a]	8 [a]	8 [a]	5 [a]	45.25 [b]	55.66 [b]
					69.25 [c]	84.59 [c]

[a] Number of QTLs detected
[b] Total percentage of phenotypic variance explained
[c] Total percentage of genetic variance explained

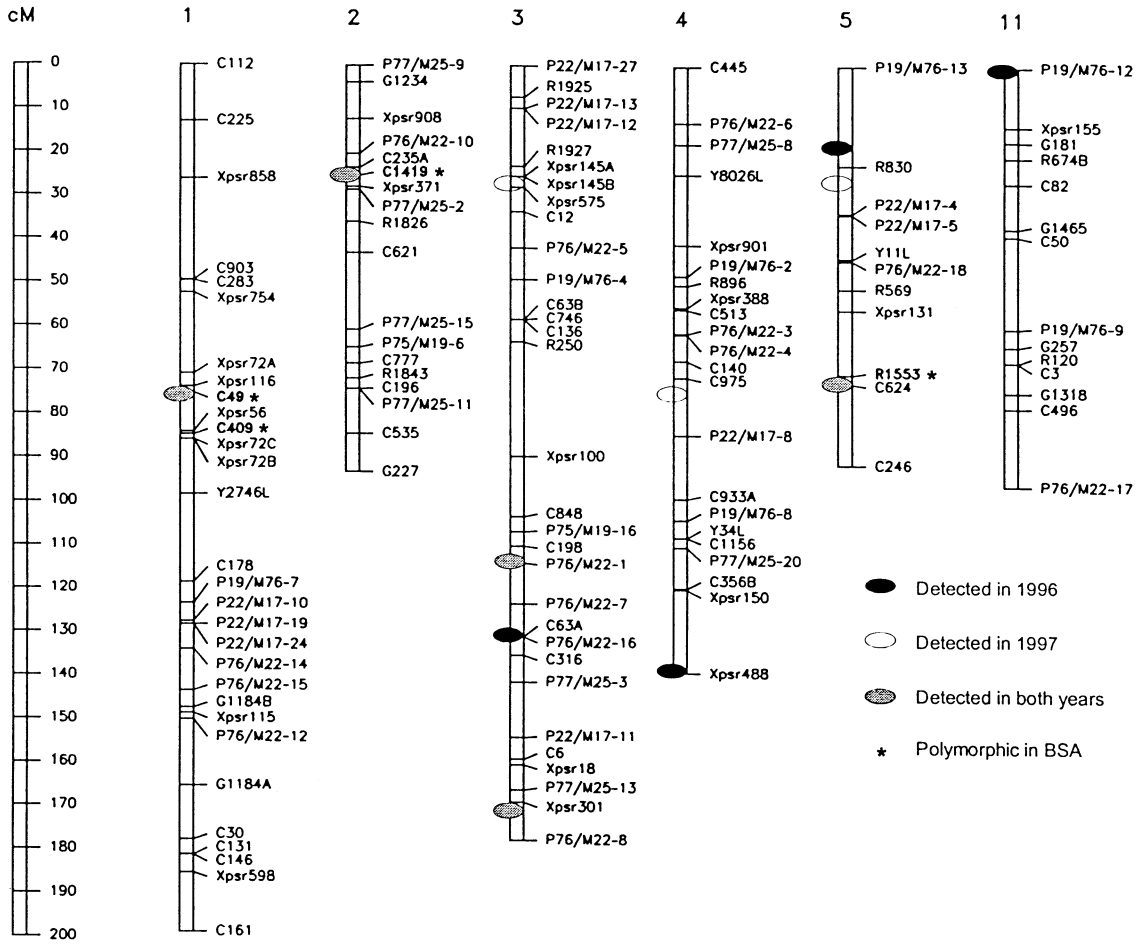

Fig. 2 Molecular marker linkage map showing positions of QTLs for BLS resistance in rice. The chromosome designation is given *above* each chromosome. To the *right* of each chromosome are the marker names, among which P_/M_ indicates AFLP markers; Xpsr_ indicates RFLP markers revealed by wheat probes and all others indicates RFLP markers revealed by rice probes. Positions of QTLs are indicated by *ovals*. The markers identified by BSA are printed in *bold* and indicated with *asterisks*

Discussion

Genetic basis of BLS resistance in rice

Several conventional genetic studies on BLS resistance in rice have been reported with differing conclusions (He et al. 1994; Xu et al. 1997; Tang et al. 1998). In the studies presented by He et al. (1994) and Xu et al. (1997), individuals were first classified into nine different resistance levels according to their lesion sizes and then further divided into resistant and susceptible groups. Based on such an approach, it was concluded that a few major genes control the BLS resistance in rice. As the study of He et al. (1994) showed the resistance level was clearly a unimodal distribution, the division of resistant and susceptible groups was artificial. Group dividing in the study of Xu et al. (1997) seemed reasonable as the resistance level showed bimodal distributions. However, the classification system of resistance level used by both He et al. (1994) and Xu et al. (1997) is based on an empirically designed nonlinear scaling system primarily devised for practical breeding. With such a scaling system, it is possible that a unimodal distribution of lesion size is converted into a bimodal distribution of resistance. A more reliable approach to study the inheritance of BLS resistance would be to analyze the distribution of realistically measured lesion sizes instead of the distribution of empirically defined resistance levels.

Our previous study (Tang et al. 1998) with two crosses between highly susceptible and highly resistant varieties (H359/Acc8558, H359/Acc8518) showed that BLS resistance in rice is a quantitatively inherited trait as the lesion length showed a continuous unimodal distribution in the various segregating populations (F_2, B_1 and B_2). In the present study, the RI population of H359/Acc8558 also showed a continuous unimodal distribution (Fig. 1), and no major gene was detected. Since the parents used in our studies belong to two extremes of resistance performance among 969 accessions (Xia et al. 1991, 1992), it seems that there are no major genes involved in the BLS resistance of rice.

Feasibility of using BSA for QTL detection

BSA was first described by Michelmore et al. (1991) for the rapid identification of molecular markers linked to any specific gene or genomic region. While the method was designed mainly for the mapping of major genes, it can be extended to the analysis of genetically complex traits if the trait is controlled by a few major genes (Michelmore et al. 1991). Wang and Paterson (1994) further evaluated the possibility of using this method for QTL mapping and concluded that phenotype-based DNA pools can be reliably used to tag QTLs of very large effect but may fail to tag QTLs of small effect. As the statistical power of BSA is relatively low, a large population size is usually required. In the present study, a large F_2 population consisting of 990 plants was used and a very small proportion of individuals of extreme phenotypes (about 1.2% for each extreme) was selected for the BSA. Three QTLs were identified and were consistent with the results of CIM. Our study thus verifies the feasibility of using BSA to tag QTLs of relatively large effect.

Potential for breeding elite rice varieties highly resistant to BLS

BLS resistance in rice appears to be controlled by a number of QTLs. Therefore, BLS resistance is a quantitative or horizontal resistance and should be stable and durable. This is a desirable characteristic for rice production. Moreover, BLS resistance in rice could reach a very high level. For example, the parent Acc8558 used in this study appears to be nearly immune to BLS. Under field conditions, it showed few BLS symptoms, while susceptible varieties suffered serious damage; under artificial pricking inoculation, it also only developed very short lesions, usually less than 4 mm in length, while susceptible varieties such as H359 could form long lesions of more than 10 cm. However, the present study has shown that Acc8558 still contains susceptible alleles at some loci. This implies that the resistance level of Acc8558 could be improved still further.

Therefore, the potential of breeding new rice varieties with high and durable resistance to BLS is good. Acc8558 could serve as a good donor parent for the introgression of resistant alleles to elite varieties by backcross breeding (Hospital and Charcosset 1997). The knowledge acquired in this study of linkage relationships between molecular markers and QTLs will facilitate the breeding program via marker-assisted selection.

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