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Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (*Oryza sativa* L.)

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Abstract Rice sheath blight, caused by *Rhizoctonia solani* Kühn, is one of the three major diseases of rice. The present study was conducted with an F_2 clonal population of Jasmine 85/Lemont. The F_2 population, including 128 clonal families, was inoculated by short toothpicks incubated with a strain, RH-9 of the fungus. Based on field disease evaluations in 2 years and a genetic map with 118 evenly distributed molecular markers, we identified six quantitative trait loci (QTLs) contributing to sheath blight resistance. These QTLs, qSB-2, qSB-3, qSB-7, qSB-9-1, qSB-9-2 and qSB-11, were located on chromosomes 2, 3, 7, 9 and 11, respectively. The respective alleles of qSB-2, qSB-3, qSB-7, and qSB-9-2 from Jasmine 85 could explain 21.2%, 26.5%, 22.2% and 10.1% of the total phenotypic variation, respectively; while the alleles of qSB-9-1 and qSB-11 from Lemont could explain 9.8% and 31.2% of the total phenotypic variation. Of these qSB-2 and qSB-11 could be detected in both years, while remaining loci were detected only in a single year. Furthermore, four QTLs (qHD-2, qHD-3, qHD-5 and qHD-7) controlling heading date and three QTLs (qPH-3, qPH-4 and qPH-11) controlling plant height were also identified. Though rice sheath blight resistance may be influenced by morphological traits, such as heading date and plant height, in the present study most detected resistance loci were not linked to the loci for heading date or plant height.

Key words Rice · Sheath blight · F_2 clonal population · QTLs for resistance

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Introduction

Rice sheath blight, caused by *Rhizoctonia solani* Kühn, is one of the three major diseases of rice and severely impairs both rice yields and quality. The pathogen *R. solani* is a semi-saprophytic fungus with a wide range of hosts. So far no rice variety has been found completely resistant to this fungus; although considerable work has been carried out on the mechanisms responsible for some partial resistance of rice, no explicit and definitive result has been provided to date. It was generally believed that rice sheath blight is a typical quantitative trait controlled by polygenes (Sha and Zhu 1989), and that there are no major genes for resistance to this disease. However, it has been known for some time that there are significant differences in sheath blight resistance among rice varieties (Khush 1977; Guo et al.1985; Chang 1986; Groth and Nowick 1992;). On the other hand, some researchers (Hashioka 1951a, b; Masajo 1976; Xie et al. 1990) proposed that sheath blight resistance in some rice varieties is controlled by major genes. Pan et al. (1999a) reported that the resistant cultivars, Jasmine 85 and Teqing, each possessed a nonallelic dominant major resistance gene. Recently, we (Pan et al. 1999b) identified three major QTLs in an F_2 population of Jasmine 85/Lemont by bulked segregant analysis.

Most studies on QTL mapping in plants use F_2 or backcross populations, but it is difficult to replicate accurate phenotypic values in these populations for precise QTL mapping. Use of recombinant inbred lines (RIL) or doubled-haploid populations (DH) may resolve this problem in QTLs studies, but it takes a long time to develop RIL populations, and gamete selection may occur occassionally in developing DH populations by anther culture. Considering that F_2 populations are abundant in genetic segregation, we have developed an F_2 clonal population using the seeds of F_1 plants from a cross, Jasmine 85 × Lemont, in which the genotype of each F_2 plant can be multiplied to many homogeneous plants by clonal technique. This characteristic allows for the precise measurement of quantitative traits by repeated trials.

Based on previous studies (Pan et al. 1997, 1999b), we used this clonal F_2 population to search the rice genome for all possible loci controlling rice sheath blight resistance in 2 continuing years.

Material s and method

Plant materials

Jasmine 85, a sheath blight-resistant variety, and Lemont, a susceptible variety, were selected as parents. The two parents, their F_1 and clonal F_2 population were then grown in the experimental farm of the Agriculture colleage, Yangzhou University.

Development of F_2 clonal population

We developed the F_2 clonal population in 1996. The detailed method is as follows. Every seed from F_1 plants was placed on MS medium containing 20 mg/l of 6-BA (6-benzyladenine) to germinate. On this medium each seedling grew many adventitious buds, but its shoot and top bud were not able to grow due to the effect of 6-BA. After several subcultures, the adventitious buds were transferred into MS medium containing 1 mg/l of NAA to induce shoots. Using this method, the genotype of each F_2 plant could be multiplied to give a homogeneous clonal family. A total of 128 clonal families were included in the F_2 clonal population. We inoculated the same F_2 clonal population in 1997 and 1998. Each F_2 clonal family was divided into two replicates to plant at random in a field test, and each replicate contained eight plants. Meanwhile, seeds of the parents were also cultured for clonal plants, which were then planted as controls in the same field.

Inoculation and disease investigation

RH-9, a *R.solani* strain with strong pathogenicity, provided by the Plant Protection Institute of Jiangsu Academy of Agriculture, was used for infection. Inoculation was carried out with short woody toothpicks with a length of 0.8–1.0 cm. Autoclaved toothpicks were incubated with RH-9 strain on PDB medium for 3–5 days, then placed behind the leaf collar of the third sheath, counting from the top, at the first stem elongation stage of growth. After inoculation, the status of the sheath-holding stem should be nearly unchanged. Disease ratings were made about 30 days after heading using a 0–9 rating scale, as described by Rush et al. (1976), where 0 indicates no disease and 9 indicates plants dead or collapsed.

RFLP map construction and QTL detection

Rice total DNA was extracted, digested, and hybridized as described by McCouch et al. (1988). Seven restriction enzymes, *BglII, DraI, EcoRI, EcoRV, HindIII, ScaI* and *XbaI,* were used for RFLP analysis. A total of 118 polymorphic RFLP and microsatellite markers distributed on the 12 rice chromosomes were used to construct a rice linkage map using Mapmaker/EXP version 3.0 (Lander et al. 1987; Lincoln et al. 1993b). Interval QTL mapping was carried out using the software Mapmaker/QTL version 1.1 with a LOD threshold of 2.0 for declaring the presence of putative QTLs (Lander and Botstein 1989; Lincoln et al. 1993a). In addition, the additive effect and the percentage of variation explained by individual QTLs were also estimated.

Results

Inheritance of resistance to sheath blight in the F_2 clonal population

A total of 128 F_2 clonal families and their parents were used for genetic analysis of disease resistance. There was a significant difference in sheath blight resistance between Jasmine 85 and Lemont, and their resistant levels were steady, with disease ratings of 3–4 and 8–9, respectively. The results of variance analysis for disease resistance indicated that the variance between each F_2 clonal family far exceeded that within an F_2 clonal family (data not shown). The disease ratings in the F_2 clonal population were continuously distributed with wide ranges in both 1997 and 1998, but the distributions were not very normal (Fig. 1).

QTLs for resistance to sheath blight detection

Based on the F_2 clonal population a rice genetic map including 118 markers evenly distributed over all 12 chromosomes was constructed to identify QTLs for rice sheath blight resistance. The results are summarized in Table 1 and Fig. 2. A total of six quantitative trait loci, qSB-2, qSB-3, qSB-7, qSB-9-1, qSB-9-2 and qSB-11, for resistance were discovered. These QTLs were located on chromosome 2, 3, 7, 9 and 11, respectively. The respective alleles of qSB-2, qSB-3, qSB-7, and qSB-9-2 from Jasmine 85 could explain 21.2%, 26.5%, 22.2% and 10.1% of the total phenotypic variation, respectively; while qSB-9-1 and qSB-11 from Lemont could account for 9.8% and 31.2% of the phenotypic variation, respectively. Among these QTLs, only qSB-2 and qSB-11 were detected in both years, while other loci could be detected only in a single year.

Table 1 Map position of the QTLs for resistance to sheath blight

Year	Locus	Chr.	Marker interval	LOD score	% Variation	Additive[a]
97	qSB-2	2	G243–RM29	3.49	14.4	0.3915
	qSB-3	3	R250–C746	6.86	26.5	0.7332
	qSB-7	7	RG30–RG477	6.02	22.2	0.6722
	qSB-11	11	G44–RG118	5.40	20.5	–0.6676
98	qSB-2	2	RM29–RG171	5.07	21.2	0.7240
	qSB-9-1	9	C397–G103	2.27	9.8	–0.4471
	qSB-9-2	9	RG570–C356	2.54	10.1	0.2563
	qSB-11	11	G44–RG118	7.17	31.2	–1.0003

[a] The additive effect is the effect associated with substitution of a Jasmine 85 allele by the corresponding Lemont allele

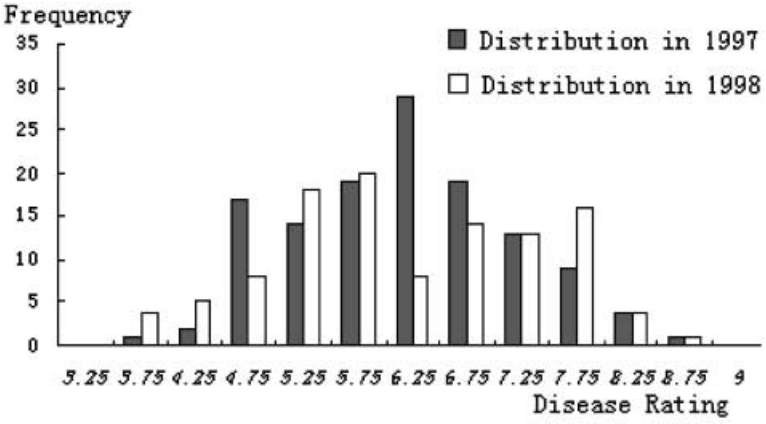
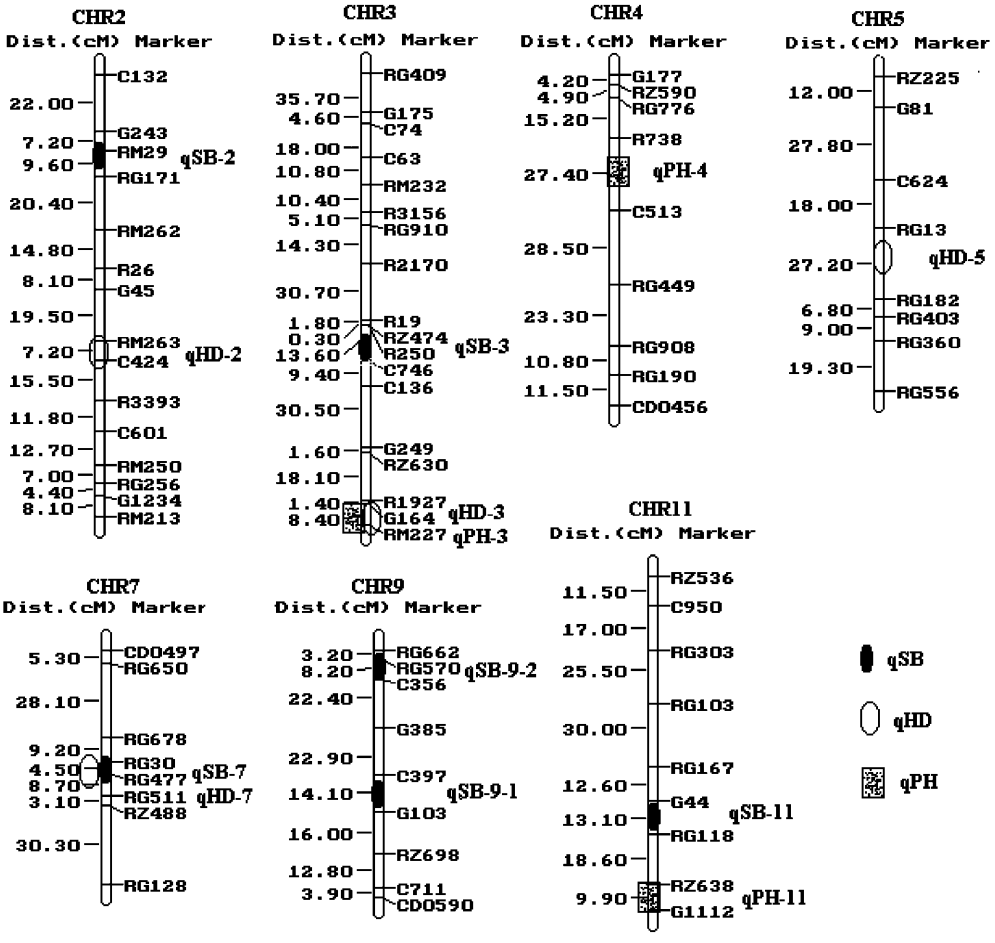


Fig 1 Frequency distributions of resistance to disease in the F_2 clonal population in 2 years



qSB QTLs for rice sheath blight resistance **qHD** QTLs for heading date **qPH** QTLs for plant height

Fig 2 Locations of QTLs on rice chromosomes

QTL s for plant height and heading date

The correlation between rice sheath blight resistance and several morphological characters of rice, such as plant height and heading date, have been frequently observed. However, inconsistent correlations between resistance and the two characters were found in the present study. As shown in Table 2, the correlations were not significant between resistance and heading date (r = – 0.054) in 1998

Table 2 The correlations between sheath blight resistance and two other characters of rice. The correlation in 1997 is indicated in front of, while the correlation in 1998 is at the back of the given values; ** P<0.01

Item	Resistance	Heading date	Plant height
Resistant	1	– 0.521**/– 0.054	– 0.197/– 0.503**
Heading date		1	– 0.285**/– 0.068
Plant height			1

Table 3 Map positions of the QTLs for heading date and plant height

Traits	Year	Locus	Chr.	Marker interval	LOD score	% Variation	Additive[a]
Heading date	97	qHD-2	2	RM263–C424	3.24	11.8	3.2023
		qHD-3	3	G164–RM227	3.42	11.6	–2.3972
		qHD-5	5	C624–RG13	2.11	9.1	2.8725
		qHD-7	7	RG30–RG477	15.46	46.1	–6.8686
	98	qHD-2	2	RM263–C424	3.47	13.5	2.3508
		qHD-5	5	C624–RG13	3.69	17.6	2.9762
		qHD-7	7	RG30–RG477	6.40	23.5	–3.5303
Plant height	97	qPH-3	3	G164–RM227	3.30	13.3	1.2189
	98	qPH-3	3	G164–RM227	4.12	18.7	2.7782
		qPH-4	4	R738–C513	2.47	27.2	–2.0551
		qPH-11	11	RZ638–G1112	2.81	14.6	4.1523

[a] The additive effect is the effect associated with substitution of a Jasmine 85 allele by its corresponding Lemont allele

and between resistance and plant height ($r = -0.197$) in 1997, while strong negative correlations were detected between resistance and heading date ($r = -0.521$) in 1997 and between resistance and plant height ($r = -0.503$) in 1998. Furthermore, the correlation ($r = -0.285$) between heading date and plant height in 1997 was also significant.

In order to explain the true relationship between sheath blight resistance and plant height or heading date, we also mapped the QTLs for these two characters in the same population. Four QTLs (qHD-2, qHD-3, qHD-5 and qHD-7) controlling heading date and three QTLs (qPH-3, qPH-4 and qPH-11) controlling plant height (Fig. 2 and Table 3) were detected in both years. Of these four QTLs (qHD-2, qHD-5, qHD-7 and qPH-3) were detectable in both years. By comparing the locations of different QTLs on chromosomes, it was found that qSB-7 and qHD-7 were located in the same interval on chromosome 7 in 1997. This coincidence may be consistent with the significant correlation observed between resistance and heading date in 1997. However, in 1998 not only was the qSB-7 not detectable, but also the correlation between resistance and heading date was insignificant in 1998. Furthermore, the two QTLs, qSB-11 and qPH-11, detected only in 1998, were both located on chromosome 11, and the distance between them was 18.6 cM, meaning that the two loci may have incomplete linkage. But, neither heading date loci nor plant height loci were identified near other disease resistance loci.

Discussion

Rice sheath blight resistance has been considered as a quantitative trait significantly affected by the environment. It is usually difficult to obtain accurate data on this type of quantitative trait. But getting accurate phenotypic data is always indispensable to precisely map QTLs. Paterson et al. (1991) and Lu et al. (1996) have studied the effects of environment on mapping QTLs. Both results suggested that the environmental effects on QTLs could be extremely significant. So, in our present study, in order to reduce environmental effects, we improved the inoculation and investigation method, and developed the clonal population as a permanent genetic-analysis population.

Although all these measures could increase the accuracy of the field test, the results between the 2 years were still not completely consistent. Among six QTLs for resistance, only two were significant in both years. There was a similar problem, though to a less extent, in identifying QTLs for heading date or plant height. From the present study, we may conclude that only the major genes explaining over 15% of the total phenotypic variation were robust in different environments, whereas the minor genes explaining under 10% of the total phenotypic variation could not be consistently detected. It should be noted that there were deficiencies in our field trial in 1997 when the field experienced drought at a later growing period. This shortage of water would result in low-moisture microclimates in the field, which could significantly affect disease development. Due to the same environmental factors, the growth of rice, including heading date and plant height, may be influenced to some extent. This influence was probably a reason why the simple correlation between traits in both years was inconsistent and why the qSB-7 and qHD-7 were discovered in the same location in 1997 while the qSB-7 was not found in 1998. Thus, this putative QTL was very likely to be a false locus due to the influence of heading date.

Li et al. (1995) identified six QTLs for sheath blight resistance in an F_4 population of Teqing/Lemont, but one allele on chromosome 8 for the resistance contributed by Lemont could not be identified in our clonal population, of which one parental variety was also Lemont. On the other hand, a major resistance QTL, qSB-11, on chromosome 11, which explained 31.2% of the total phenotypic variation, was identified in our study, and Li et al. also indicated that there might have been a putative resistant QTL in the same interval of qSB-11, though they did not give any further information about the effect of this locus possibly due to its low LOD score. In addition, they identified three QTLs for heading date and four QTLs for plant height in the resistance loci interval, and thus thought that the QTLs for sheath blight resistance were closely associated with the QTLs for heading date or plant height. Nevertheless, in the present study, most resistance loci and other trait loci were not located in same intervals of the rice chromosomes, and, therefore, sheath blight resistance should not be associated with the morphological traits. Though the genetic study on rice sheath

blight resistance has been a difficult task, we believe that it can be advanced by controlling environmental effects in an acceptable range.

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