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Characterization of quantitative trait loci (QTLs) in cultivated rice contributing to field resistance to sheath blight (*Rhizoctonia solani*)

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Abstract Sheath blight, caused by *Rhizoctonia solani*, is one of the most important diseases of rice. Despite extensive searches of the rice germ plasm, the major gene(s) which give complete resistance to the fungus have not been identified. However, there is much variation in quantitatively inherited resistance to *R. solani*, and this type of resistance can offer adequate protection against the pathogen under field conditions. Using 255 F_4 bulked populations from a cross between the susceptible variety 'Lemont' and the resistant variety 'Teqing', 2 years of field disease evaluation and 113 well-distributed RFLP markers, we identified six quantitative trait loci (QTLs) contributing to resistance to *R. solani*. These QTLs are located on 6 of the 12 rice chromosomes and collectively explain approximately 60% of the genotypic variation or 47% of the phenotypic variation in the 'Lemont' × 'Teqing' cross. One of these resistance QTLs *(QSbr4a)*, which accounted for 6% of the genotypic variation in resistance to *R. solani*, appeared to be independent of associated morphological traits. The remaining five putative resistance loci (*QSbr2a*, *QSbr3a*, *QSbr8a*, *QSbr9a* and *QSbr12a*) all mapped to chromosomal regions also associated with increased plant height, three of which were also associated with QTLs causing later heading. This was consistent with the observation that heading date and plant height accounted for 47% of the genotypic variation in resistance to *R. solani* in this population. There were also weak associations between resistance to *R. solani* and leaf width, which were likely due to linkage with a QTL for this trait rather than to a physiological relationship.

Key words RFLP markers · QTL mapping · Rice (*Oryza sativa* L.) · Disease resistance · Sheath blight (*Rhizoctonia solani*) · Morphological characters

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Introduction

Sheath blight, caused by *Rhizoctonia solani*, is one of the major diseases of rice, causing severe loss in yield and quality each year worldwide. The major gene(s) giving complete resistance to this fungus have not been found though extensive searches in both cultivated rice germplasm and related wild species have been made (GS Khush personal communication). However, considerable variation exists among different rice varieties for quantitatively inherited resistance to this fungus. The quantitative resistance in several of the most resistant varieties, such as 'Tetep' and 'Tadukan' can offer excellent protection against the pathogen under field conditions (Kikuo et al. 1983; Groth and Nowick 1992).

The mechanisms responsible for quantitative resistance to *R. solani* remain unclear. Resistance has been primarily attributed to differences in morphological and ecological characters because associations between resistance and several morphological characters, such as plant height and heading date, have been frequently observed (Groth and Nowick 1992; Hashiba et al. 1981). However, it has been noted that dark zones develop around the lesions in some of the most resistant varieties such as 'Tetep' and 'Tadukan' and that these varieties have fewer and smaller lesions, suggesting that other mechanisms of resistance may also exist (Groth and Nowick 1992).

Genetic studies on quantitative resistance to *R. solani* in rice have shown both polygenic and major gene inheritance (Groth and Nowick 1992; Sha 1987; Xie et al. 1990). However, breeding efforts to increase resistance to *R. solani* have been largely unsuccessful because of the com-

plexity of the resistance and a lack of detailed knowledge about the loci involved.

The objectives of the study presented here were to locate the genomic regions responsible for resistance to *R. solani* in rice in order to facilitate their manipulation in rice breeding and to examine the relationships between these loci and morphological characters associated with resistance to *R. solani*.

Materials and methods

Materials

The materials used in this study were progenies from the same cross between 'Lemont' (susceptible to *R. solani*) and 'Teqing' (resistant) as described previously (Li et al. 1995) except that bulk F_4 populations instead of individual F_4 lines were used for the evaluation of resistance to *R. solani*. Each of the bulk populations consisted of mixed seeds from 7 or more F_3 plants derived from single F_2 plants of the 'Lemont'×'Teqing' cross. These bulked F_4 populations were divided into groups for a 2-year replicated field study.

Field evaluation of resistance to *R. solani* and morphological characters

Two hundred and fifty-five bulk F_4 populations were evaluated for field resistance to *R. solani* in both 1990 and 1991 in completely randomized field experiments at the Texas A&M University System Agricultural Research and Extension Center in Beaumont with three replications for each F_4 population. The test populations were drill-seeded in three-row plots 2.4 m long with 18 cm between rows and between plots. The plots were inoculated approximately 60 days after planting by broadcasting 100 ml per plot of a 2:1 (v:v) mixture of rice hulls and unhulled grains infested with the pathogen (Marchetti and Bollich 1991).

In 1990, the plots were rated only once, approximately 30 days after inoculation. To compensate for differences in maturity, in 1991 additional ratings were made at weekly intervals until the latest lines matured. The disease scores used for data analyses in 1991 were those acquired approximately 30 days after heading for each plot. The sheath blight response (SBR) rating system used was a 0–9 scale, with 0 indicating no evidence of infection and 9 indicating that the plants were killed and collapsing. Each unit of the scale approximated the proportion of the length of the plants showing symptoms, e.g. "5" indicated that about 50% of the height of the plants above the water line was diseased. Some bulked F_4 populations showing two distinct reactions were noted. Such mixed reactions were almost always associated with segregation for height or maturity.

To evaluate morphological traits putatively associated with resistance to *R. solani*, we also planted 2418 F_4 lines from the same 255 F_2 plants (7–11 lines from each of the F_2 plants) in the summer of 1990. The analysis and mapping of quantitative trait loci (QTL) for plant height and heading date in this material are described in the accompanying paper (Li et al. 1995). Leaf length and width were obtained by measuring the penultimate leaf on the main stems of 10 different plants in each F_4 row. Culm angle and leaf angle were also visually determined on the row bases according to standard procedures (IRRI 1980).

Statistical analyses and interval mapping of QTLs

Restriction fragment length polymorphism (RFLP) genotyping of the 255 original F_2 plants, construction of the linkage map and mapping of QTLs using the methods of interval mapping and ANOVA (including one-factor, two-factor and three-factor linear models) were performed as described previously (Li et al. 1995) using a LOD threshold of 2.4 for claiming the presence of a QTL. Correlation analyses and a multiple regression analysis were also performed to detect associations between resistance to *R. solani* and the morphological characters using SAS PROC CORR and GLM (SAS Institute 1987).

Results

Genetic variation for resistance to *R. solani*

The sheath blight response ratings (SBRs) of the bulked F_4 populations in 1990, 1991 and the 2-year averages were nearly normally distributed within the range spanned by the parents (Fig. 1). An ANOVA indicated that differences among the 255 F_4 bulk populations for SBR were highly significant and explained a majority of the total variation (R^2=0.696). Variation due to years and genotype year were also significant, but explained a small proportion of the total variation (R^2=0.025 and 0.088, respectively). The data from both years were highly correlated (r=0.78) and in only 2 F_4 populations were the disease ratings inconsistent between the two years, i.e. high in one year and low in the other. The discrepancy of these 2 populations was likely due to miscoring or poor inoculation in 1 year, and their data were eliminated from later mapping analyses.

Chromosomal locations and effects of QTLs for resistance to *R. solani*

Three QTLs (*QSbr2a*, *QSbr3a*, and *QSbr8a*) were identified on the basis of data from both 1990 and 1991, and they had the same chromosomal locations and relative magnitudes of additive gene effects (Table 1 and Fig. 2). However, there were also differences in the data from the 2 years.

Putative SBR QTLs on *chromosomes 4, 9* and *12* with LOD scores of 3.59, 4.08 and 2.83, respectively, were identified based on the 1991 data, but these had LOD scores of 1.65, 1.43 and 1.52 on the basis of 1990 data. These LOD scores, while below our threshold of LOD=2.4, correspond to significant levels of significance between 0.006 and 0.010, suggesting the possible presence of QTLs in the three regions. A region on *chromosome 11* was putatively associated with SBR and had a LOD score of 2.27 using 1990 data, but this region had a non-significant LOD of 0.64 (P>0.10) with the 1991 data. In addition, the magnitudes of the additive gene effects of identified SBR QTLs were all larger in 1991 than in 1990, except for *QSbr2a*, which remained unchanged.

These differences may be due to the change in disease rating methods used during the 2 years. In 1990, all the plots were rated only once, approximately 30 days after infection. In contrast, multiple ratings were performed in 1991, and the data used for analysis were those taken approximately 30 days after heading. Since the disease index scored at 30 days after heading has proven to be highly correlated with the yield loss from the disease (Marchetti and Bollich 1991), the 1991 data should be more reliable in evaluating populations segregating for heading date.

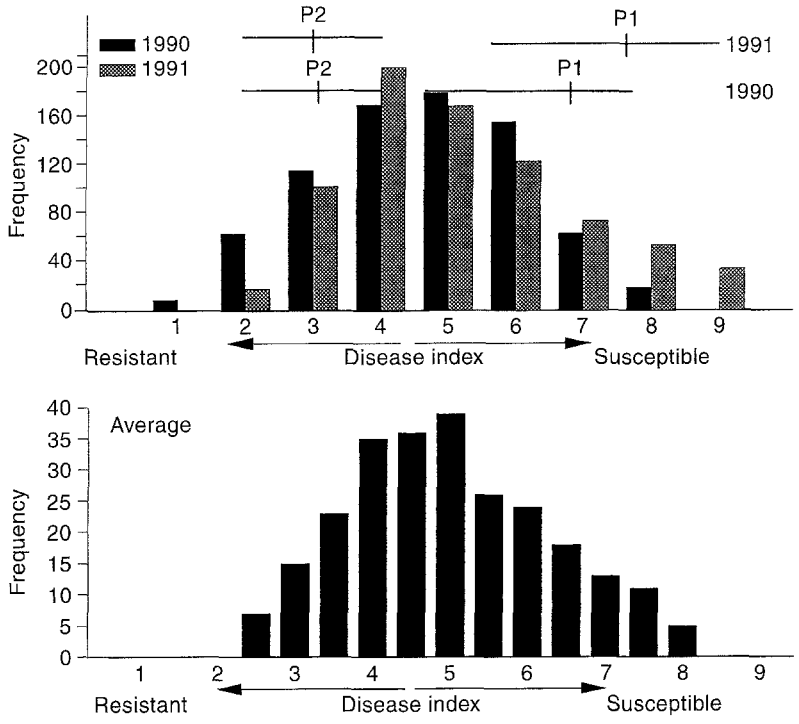


Fig. 1 The frequency distributions of sheath blight response ratings (SBR) of 255 bulked F_4 populations from the cross 'Lemont'×'Teqing' in a 2-year replicated field experiment

We anticipated that the replication afforded by the 2-year study might allow identification of additional QTLs with effect too small to be detected with single year data. When the 2-year mean was used, six QTLs contributing to resistance to *R. solani* were identified based on a threshold of 2.4 LOD. These were the same six QTLs identified using 1991 data alone. Analysis of a multi-QTL model indicated that these QTLs collectively accounted for 60% of the total genotypic variance or 47% of the phenotypic variance among the 255 F_4 bulk populations. QTLs mapped on the basis of the 2-year mean phenotype fit closely with the QTLs identified from the 1991 data. Analysis of 2-year phenotypic means detected an additional association between SBR and a region on *chromosome 9* (near the marker RZ276Q) that had a subthreshold LOD score of 2.11 (P=0.0018), suggesting a possible QTL for SBR at this region. The LOD scores at this position were 1.76 (P=0.0044) and 1.94 (P=0.0028) in 1990 and 1991, respectively.

ANOVA based on single-marker genotypes confirmed all SBR QTLs identified by interval mapping (including the region on *chromosome 9* that had subthreshold LODs), but genetic parameters obtained based on genotypes of single markers were smaller than those obtained by interval mapping (Table 1). General underestimation of genetic parameters using ANOVA was expected since all the SBR QTLs were located between flanking markers according to interval mapping.

The six putative resistance QTLs identified on the basis of the 2-year means and 1 or both individual year data were located on 6 different chromosomes. Five of the resistance alleles were from the resistant parent, 'Teqing', while one, *QSbr8a,* was identified in the susceptible parent, 'Lemont'. There was considerable variation among the QTLs for their additive gene effects, R^2 and LOD scores (Table 1). Overdominance towards resistance was observed for three of the SBR QTLs (*QSbr2a*, *QSbr9a* and *QSbr12a*), while *QSbr3a*, *QSbr8a* and the associated region on chromosome *9* showed partial or nearly complete dominance toward resistance. *QSbr4a* was the only QTL that exhibited small partial dominance towards susceptibility. However, since many of the bulked F_4 populations were segregating and a slight (average=21%) excess of heterozygotes in the F_2 genotypes was present (Li et al. 1995), the observed dominance effects at the SBR QTLs may have been slightly inflated.

Interactions between QTLs for resistance to *R. solani*

The close linkage of most SBR QTLs to at least one flanking marker (generally<10 cM) and 2 years of data allowed us to examine if there were interactions between the SBR QTLs using linear models with two-way and three-way interactions (SAS PROC GLM, SAS Institute 1987). When the genotype replication variance was used as experimental error in F tests, 5 out of 15 possible digenic interactions (33%) and 3 out of 20 possible (15%) trigenic interactions among the SBR QTLs were significant at P<0.01 (i.e. where one would expect no more than 1% of the interactions to be significant due to chance alone). All but 1 of these interactions (between *QSbr2a* and *QSbr12a*, R^2=5.9%) had very small R^2, ranging from 0.010 to 0.028, with a mean of 0.020 and 0.019 for digenic and trigenic interactions, respectively. However, since the experimental error term in these analyses was very small due to the large number of families studied, these small but statistically significant interactions should not be interpreted as

Table 1 Genetic parameters of the QTL affecting field resistance to *R. solani* (SBR) identified in the cross of 'Lemont' × 'Teqing' as calculated by interval mapping for individual year and averaged data and by ANOVA for averaged data

Locus[a]	Flanking markers	Interval mapping										ANOVA using averaged data				
		1990				1991				Averaged data						
		a^b	d	R^2_G	LOD	a^b	d	R^2_G	LOD	a^b	d	R^2_G	LOD	a	d	R^2_G	pF	P
QSbr2a	RG654–RZ260	–0.54	–1.16	0.075	3.93	–0.52	–1.52	0.058	2.52	–0.52	–1.16	0.066	3.19	–0.46	1.11	0.054	21.15	<0.0001
QSbr3a	RG348–RG944	–0.99	0.28	0.229	11.73	–1.26	–1.88	0.323	17.61	–1.11	–0.59	0.277	15.26	–0.94	–0.46	0.210	98.37	<0.0001
QSbr4a	RG143–RG214	–0.13	0.48	0.040	1.65	–0.74	0.00	0.085	3.59	–0.58	0.20	0.067	2.83	–0.50	–0.18	0.050	19.76	<0.0001
QSbr8a	RG220–RG1034	0.79	–0.08	0.165	3.25	1.47	–4.68	0.559	3.45	0.86	–0.83	–0.189	3.38	–0.46	0.341	0.060	19.77	<0.0001
QSbr9a	RG910b–RZ777	–0.45	–1.36	0.044	1.43	–0.96	–1.60	0.130	4.08	–0.73	–1.32	0.094	3.20	–0.48	–0.44	0.050	17.53	<0.0001
QSbr12a	RG214a–RZ397	–0.16	–1.92	0.036	1.52	–0.09	–3.64	0.067	2.83	–0.14	–2.63	0.058	2.50	–0.10	–2.08	0.037	14.39	0.0024
									Total[c]	1.50	–3.27	0.596	28.67					

[a] Individual QTLs are designated with "Q" indicating QTLs with LOD≥2.4, abbreviation of the trait name and chromosome number followed by a letter is to accommodate situations when more than one QTL affecting a trait is identified on the same chromosome

[b] The additive effect (a) is the effect associated with substitution of a 'Lemont' allele by the corresponding 'Teqing' allele

[c] Estimates are obtained from a multi-QTL model fitting all seven SBR QTLs simultaneously

strong evidence for the presence of digenic and trigenic epistasis between or among the SBR QTLs.

Contribution of morphological characters to field resistance to *R. solani*

Simple correlation analyses indicated that culm angle and leaf length were not significantly correlated with SBR. However, heading date (HD) and plant height (PH) were both significantly (P<0.0001) correlated with SBR (r_G=0.65 and r_G=0.52, respectively). Significant genotypic correlations with SBR were also seen for leaf angle (r_G=0.33, P=0.0001) and leaf width (r_G=0.20; P=0.002). A multiple regression model constructed using heading date, plant height, leaf angle and leaf width against SBR indicated that these morphological characters collectively explained approximately 50% of the total genotypic variance in SBR (Table 2). The model also indicated that HD and PH individually explained 42% and 4.7% of the observed genotypic variation in SBR, respectively. Delayed heading date and increased plant height together resulted in a substantial decrease in the disease level (R^2=0.47).

To further examine the effects of PH and HD on SBR, the QTL likelihood maps of LOD scores for PH, HD and SBR were compared (Fig. 3). There was a notable correspondence between the LOD score profile of SBR and those for HD and PH on *chromosomes 2, 3, 8* and *9*. *QSbr3a, QSbr8a* and *QSbr9a* mapped to approximately the same locations as the major heading date QTLs *QHd3a*, *QHd8a*, and *QHd9a* and the corresponding plant height QTLs *QPh3a*, *QPh8a* and *QPh9a* (Li et al. 1995). While *QSbr2a* and *QSbr12a* did not coincide with HD QTLs, *QSbr2a* did correspond to a QTL (*QPh2a*) for PH and *QSbr12a* coincided with a region on *chromosome 12* that exhibited subthreshold association with PH (LOD=2.19, P=0.0016). In all cases, resistance alleles at these QTLs were associated with alleles that delayed heading and/or increased plant height.

We also mapped chromosomal regions that affected leaf width, leaf length, leaf angle and culm angle (data not presented). Of these, only one out of six QTLs for leaf width was mapped to approximately the same region as a SBR QTL, *QSbr4a*.

Table 2 Contributions of morphological characters to *R. s.* resistance

Character	Partial R^2	P
Heading date (HD)	0.4181	0.0001
Plant height (PH)	0.0468	0.0001
Leaf width (LW)	0.0229	0.0010
Leaf angle (LA)	0.0099	0.0283
Cumulative R^2	0.4977	
Regression model:		
SBR=8.09–0.098 HD – 0.041 PH + 1.663 LW + 0.302 LA		

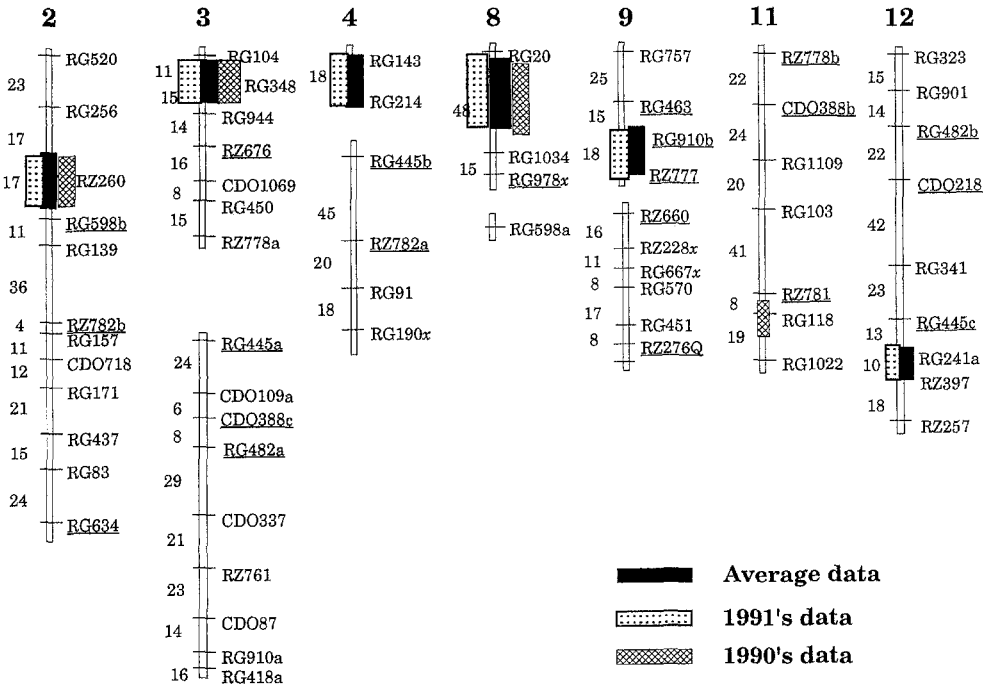
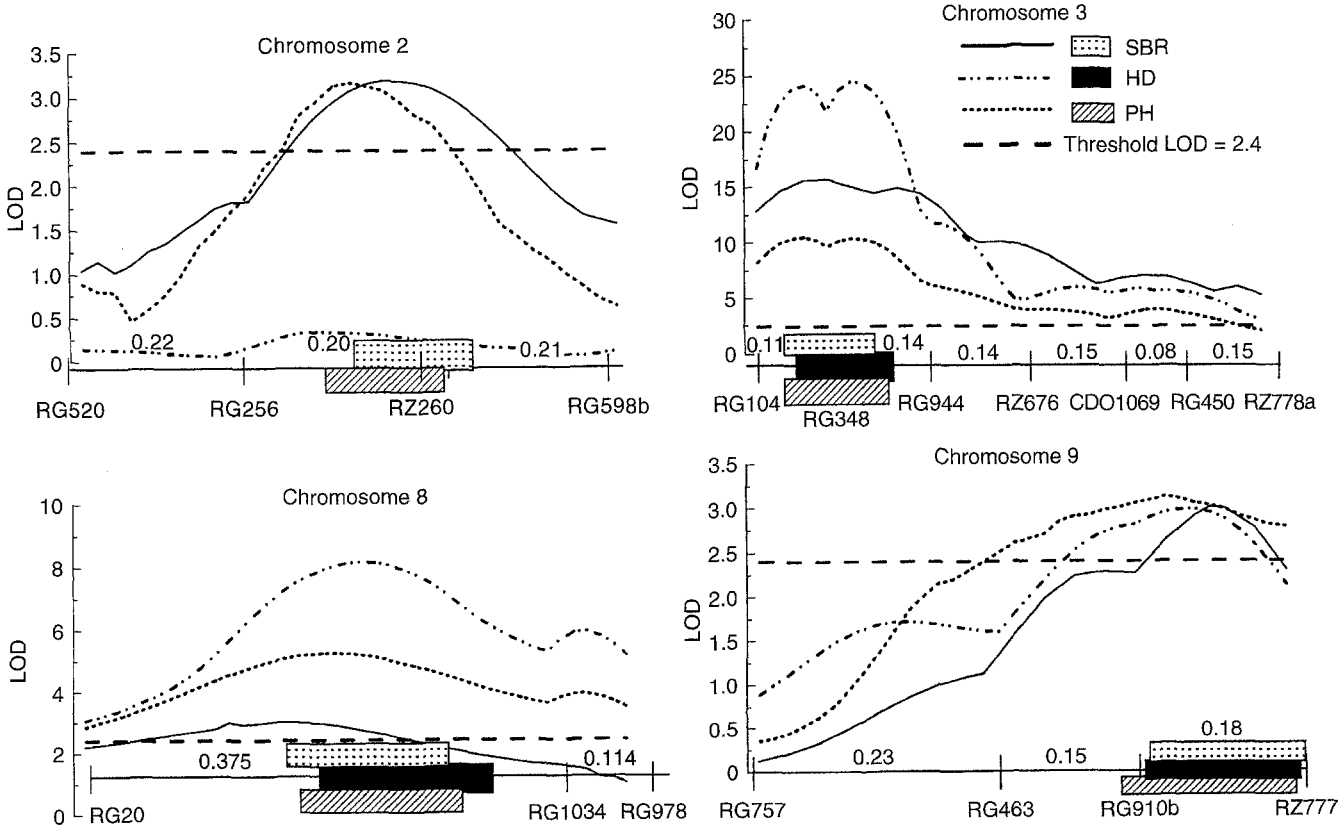


Fig. 2 Chromosomal locations of putative QTLs contributing to resistance to *R. solani* identified in the cross 'Lemont'× 'Teqing'. The *boxed* regions cover the chromosomal regions where the likelihood of the presence of a QTL was within tenfold (1 LOD) of its maximal value

Fig. 3 QTL likelihood maps of LOD scores for heading date (HD), plant height (PH), and sheath blight response (SBR) on chromosomes *2*, *3*, *8* and *9*. The associated RFLP markers and their approximate recombination fractions are indicated *below* the LOD score graphs



Discussion

Many important rice cultivars, such as 'Lemont' are very susceptible to *R. solani*, showing typical yield losses of 35–40% in inoculated plots (Marchetti and Bollich 1991). No major genes conferring immunity to *R. solani* have been identified, but some resistant varieties such as 'Teqing' and 'Tetep' offer sufficient quantitative resistance to *R. solani* under field conditions to be agriculturally useful.

By using a detailed RFLP linkage map with 115 well-distributed marker loci, we were able to identify six SBR QTLs in a cross between 'Teqing' and 'Lemont'. These QTLs collectively explained 60% of the total genotypic variance and 47% of the phenotypic variance among the 255 F_4 bulk populations.

Our results confirmed previously reported associations of PH (Marchetti 1983) and HD (Marchetti and McClung 1994) with SBR and provided more detailed genetic information on the QTLs involved. The association between decreased SBR and increased PH is not surprising since infection begins at the water line and progresses upward, and our scoring system for SBR was based on the infected proportion of vegetative parts. A smaller proportion of tall plants would be infected even if the disease spreads at the same rate on short and tall plants. Since we were working with segregating populations, the micro-environmental conditions for disease development at the upper portions of tall plants may have been less favorable. Furthermore, because shorter plants could be hidden by the taller plants whose upper portion generally was not infected, disease scores for these latter segregating populations might have been biased toward lower ratings, resulting in overestimation of the dominance effects of the identified SBR QTLs. While we observed consistent correspondence between increased PH and decreased SBR for the additive gene effects, such a relationship was not consistent for the dominance effects (Table 1). Sampling errors and separate experiments for the measurement of SBR and PH may have contributed to this inconsistency. The relationship between the SBR QTLs and the PH QTLs we identified in this cross is consistent with the empirical observation that semidwarf rice varieties are more susceptible to *R. solani* than traditional tall varieties (Marchetti 1983). However, although 'Lemont' and 'Teqing' both contain the *sd-1* semidwarf gene and differ in height by only 10 cm (Li et al. 1995), they have very different susceptibilities to *R. solani*. Thus, the utilization of *sd-1* is not the direct cause of the increased severity of sheath blight in semidwarf varieties, and it should be possible to develop *R. solani*-resistant semidwarf rice varieties .

It might be expected that increased resistance would be associated with earlier heading because of a decreased time for disease development. Instead, alleles at three of the SBR QTLs were found to map to the same location as loci associated with delayed heading date, possibly due to a pleiotropic effect of HD and PH genes (Li et al. 1995).

Unfortunately, the resolution of 10–20 cM between the markers in the present study does not allow tight linkage of several QTLs for different morphological characters to be distinguished from pleiotropic effects of a single gene, or from close linkage between resistance QTLs and QTLs influencing morphological traits. To address these questions, further investigations will be needed using more closely linked markers, recombinant inbred lines or specifically constructed isogenic lines that allow detailed examination within these genomic regions. Examination of the effects of the SBR QTLs on resistance components such as the rate of fungal growth, number of lesions per plant and lesion expansion rate might clarify whether the observed association is due to linkage or pleiotropy.

The fact that *QSbr4a* maps to approximately the same chromosomal location as a QTL with a large effect on leaf width provides a reasonable explanation for the observed weak correlation between SBR and leaf width. Whether the relationship between *QSbr4a* and the leaf width QTL is due to pleiotropy is unknown, but it seems unlikely since other leaf width QTLs with comparable effects did not correspond to any of the SBR QTLs identified in this cross. The weak association between SBR and leaf angle is unlikely to be due to linkage or pleiotropy of QTLs since no QTLs for the two traits were detected at approximately the same chromosomal regions in this cross (data not presented).

While morphological traits like plant height and heading date have a major impact on field resistance to *R. solani*, *QSbr4a* appeared to be independent of associated morphological traits. *QSbr4a* explained 6.7% of the total observed genotypic variation, which correlates with a fairly large phenotypic effect of 0.6 disease index units. QTLs with such magnitudes can be agriculturally significant according to the relationship 'percent yield loss=–1.8+5.1 (sheath blight rating)', described by Marchetti and Bollich (1991). Thus, transfer of the resistant allele from 'Teqing' at *QSbr4a* into 'Lemont' would be expected to reduce yield loss from sheath blight by 4.9% assuming that this QTL has an equivalent effect in the 'Lemont' genetic background.

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