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Quantitative Trait Loci for Resistance to Stripe Disease in Rice (*Oryza sativa*)

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Abstract: In order to map the quantitative trait loci for rice stripe resistance, a molecular linkage map was constructed based on the F_2 population derived from a cross between Zhaiyeqing 8 and Wuyujing 3. Reactions of the two parents, F_1 individual and 129 $F_{2:3}$ lines to rice stripe were investigated by both artificial inoculation at laboratory and natural infection in the field, and the ratios of disease rating index were scored. The distribution of the ratios of disease rating index in Zhaiyeqing 8/Wuyujing 3 $F_{2:3}$ population ranged from 0 to 134.08 and from 6.25 to 133.6 under artificial inoculation at laboratory and natural infection in the field, respectively, and showed a marked bias towards resistant parent (Zhaiyeqing 8), indicating that the resistance to rice stripe was controlled by quantitative trait loci (QTL). QTL analysis showed that the QTLs detected by the two inoculation methods were completely different. Only one QTL, *qSTV7*, was detected under artificial inoculation, at which the Zhaiyeqing 8 allele increased the resistance to rice stripe, while two QTLs, *qSTV5* and *qSTV1*, were detected under natural infection, in which resistant alleles came from Zhaiyeqing 8 and Wuyujing 3, respectively. These results showed that resistant parent Zhaiyeqing 8 carried the alleles associated with the resistance to rice stripe virus and the small brown planthopper, and susceptible parent Wuyujing 3 also carried the resistant allele to rice stripe virus. In comparison with the results previously reported, QTLs detected in the study were new resistant genes to rice stripe disease. This will provide a new resistant resource for avoiding genetic vulnerability for single utilization of the resistant gene *Stvb-i*.

Key words: rice; resistance; rice stripe; quantitative trait loci; artificial inoculation; natural infection

Rice stripe, caused by rice stripe virus (RSV), is one of the most damaging diseases in temperate regions of East Asia, especially in China, Japan and Korea. RSV is transmitted by the small brown planthopper (SBPH, *Laodelphax striatellus* Fallen). In China, along with alteration of cropping system, the small brown planthopper population grew larger and larger, leading to the increasing of the severity of rice stripe [1]. In Jiangsu Province, approximately 0.6 Mha per year of rice were infected by RSV during the period from 2000 to 2003 and about 1 Mha in 2004. Rice yield was reduced by 30-40% in heavily infected fields, and in some of more infected fields, no harvest was possible (www.agri.gov.cn) [2]. Commonly, rice stripe is controlled by pesticide application, but this is both costly and environment-polluting, while utilization of the resistant varieties is the economic approach to control rice stripe, so there is a growing interest in the screening of disease-resistant varieties.

Previously, we had evaluated the resistance of 314 rice varieties to rice stripe by the field test, and found eight rice varieties including Zhaiyeqing 8 were resistant to the rice stripe, and the resistance of Zhaiyeqing 8 was characterized by the resistance to rice stripe virus and its vector, the small brown planthopper (SBPH) [3]. In order to detect QTLs associated with resistance to RSV and SBPH for utilizing Zhaiyeqing 8 in molecular breeding programs for the resistant rice varieties, a linkage map from Zhaiyeqing 8/Wuyujing 3 F_2 population was constructed and resistance to rice stripe of each $F_{2:3}$ line was evaluated using two evaluation methods, the artificial inoculation at laboratory and natural infection in the field.

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MATERIALS AND METHODS

Rice materials

The F_2 population involving 129 plants and F_1 plants from a cross between Zhaiyeqing 8 (resistant variety) and Wuyujing 3 (susceptible variety) were used in the experiment. Each F_2 plant was selfed to obtain $F_{2:3}$ lines. Nipponbare and Kasalash were used as susceptible and resistant controls, respectively.

Collection and raising of the small brown planthopper

The small brown planthoppers were collected in wheat fields at Hongze County, Jiangsu Province, China in April and May, 2004, and raised in a greenhouse of Nanjing Agricultural University.

Evaluation for resistance to rice stripe

Artificial inoculation

The artificial inoculation experiment was conducted in a greenhouse of Nanjing Agricultural University. Twenty to 25 seeds of each $F_{2:3}$ line, parents and check varieties were sown in a 5.8 cm × 6.0 cm soil-filled plastic cup with two replications,

which was placed in a 65 cm × 44 cm × 14 cm plastic case with water, then thinned at the one-leaf stage to 10 vigorous seedlings per genotype. The cup was covered with a cylinder capped with gauze. About sixty of the 2nd to 3rd instar SBPH nymphs were released into each cylinder at the 1.5-leaf stage of rice seedlings. The proportion of viruliferous SBPH was about 37.7% by the ELISA assay [4-5]. After two-day incubation, the SBPH were removed, and the seedlings were transplanted to a greenhouse and grew for about a month until disease symptoms were observed.

Natural infection

The natural infection experiment was carried out in experiment farm at Louwang town, Yancheng City, Jiangsu Province, China in 2005, where rice stripe severely occurred. Fifty seeds for every $F_{2:3}$ line, parents and check varieties were sown per row (180 cm long) with an inter-row spacing of 10 cm. The rows were laid out in a randomized complete block design with two replications. At the one-leaf stage, thirty healthy seedlings per line were maintained after removing weak plants. The proportion of viruliferous SBPH was estimated at 45.6% by the ELISA assay [4-5]. The disease incidence of plants was symptomatically evaluated about 60 days after sowing.

Evaluation of rice stripe disease incidence

According to Washio et al, plant resistance to rice stripe was classified into six classes (A, B, Bt, Cr, C and D) based on symptoms [6]. The disease rating index was calculated as follows:

Disease rating index = (100A + 80B + 60Bt + 40Cr + 20C + 5D)/(Number of seedlings examined ×100)×100.

Where, A, B, Bt, Cr, C and D denote frequency within each respective class.

The ratio of the disease rating index (RDRI) compared to Nipponbare was calculated for each accession.

DNA preparation and PCR analysis

DNA samples were extracted from fresh leaves of each plant using the method described by Dellaporta et al [7] with minor modifications. The extracted DNA samples were dissolved in TE buffer (10 mmol/L Tris base, 0.1 mmol/L EDTA) and tested for quality and quantity with an MBA 2000 UV/Vis Spectrometer (Perkin-Elmer Co., Norwalk, CT, USA). The eligible samples were diluted into 20 ng/μL with double distilled water (ddH_2O) and stored at 4℃ for further analysis.

The original sources for simple sequence repeat (SSR) primers used in this study are given in the gramene database (http://www.gramene.org/) and McCouch et al [8]. SSR analysis was performed following the procedure of Chen et al [9] with minor modifications. Amplification reactions were performed in a 10 μL system containing 10 mmol/L Tris-HCl pH 8.3, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 50 μmol/L each of dNTPs, 0.2 μmol/L each of primers, 0.5 U *Taq* polymerase (TaKaRa, Dalian) and 20 ng of DNA template, using a PTC-200 thermal cycler (MJ Research Inc., Waltham, MA, USA) programmed as 94℃ for 5 min, followed by 35 cycles of 30 s at 94℃, 30 s at 55℃, 1 min at 72℃ with a final extension of 7 min at 72℃. Amplified DNA products were electrophoresed on 8% polyacrylamide non-denaturing gels in 0.5×TBE buffer and observed by the silver staining method based on Sanguinetti et al [10]. Amplified DNA fragments showing clear polymorphism were used for analysis of the F_2 population and linkage mapping.

Map construction and quantitative trait loci analysis

Linkage groups and the order of markers were determined using MAPMARKER/EXP3.0 [11]. The Kosambi mapping function was used to transform the recombination frequency to genetic distance (cM). Composite interval mapping was performed to identify QTLs by using the software package QTL Cartographer [12]. These permutations can account for non-normality in marker distribution and traits values. The LOD value for the level of significance for QTLs in this study was LOD=2.5 (P=0.05). Analysis of variance using marker genotypes as the groups was conducted using the general linear model (GLM) procedure of SAS [13]. QTL nomenclature followed the suggestion by McCouch et al [14].

RESULTS

Linkage map

Of a total of 667 pairs of primers surveyed, 153 pairs (34.5%) showed polymorphism. SSR linkage map was constructed using 105 pairs of selected primers evenly distributed on the total genome, and spanned a total of 1544 cM on all 12 chromosomes with an average interval of 14.7 cM between adjacent markers. The order of 105 markers agreed with Temnykh et al [15] and McCouch et al [8] (Fig. 1 only shows chromosomes 1, 5, and 7, the rest is omitted).

Rice stripe resistance evaluation of $F_{2:3}$ lines and QTL mapping under artificial inoculation

Under artificial inoculation, resistant control, Kasalath,

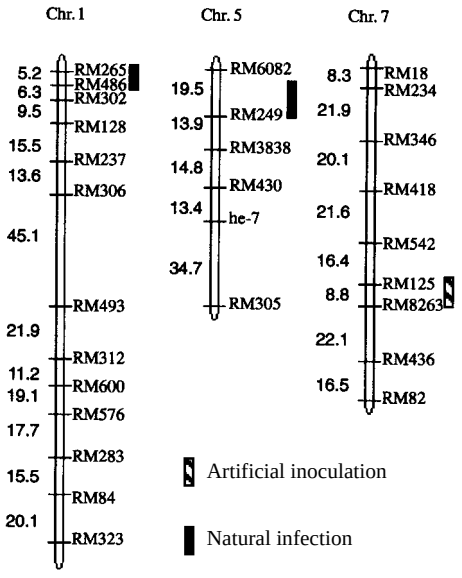

Fig. 1. Molecular linkage map constructed by SSR marker based on Zhaiyeqing 8/Wuyujing 3 F_2 population and distribution of QTLs for rice stripe resistance.

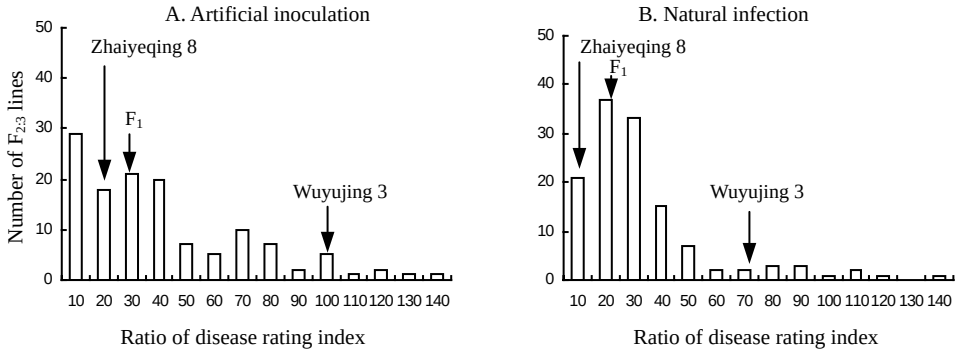

Fig. 2. Frequency distribution of ratio of disease rating index in Zhaiyeqing 8/Wuyujing 3 $F_{2:3}$ population.

had no typical symptom with a disease rating index 8.3, while susceptible control, Nipponbare showed the disease symptoms with a disease rating index 76.8, suggesting that the artificial inoculation was practicable. RDRIs differed significantly between Zhaiyeqing 8 and Wuyujing 3, which were 18.17 and 97.75, respectively. The frequency distributions of RDRIs in $F_{2:3}$ lines were shown in Fig. 2-A, which displayed a continuous distribution, ranging from 0 to 134.08, with transgressive segregation and bias towards to resistant parent Zhaiyeqing 8, suggesting that the resistance was quantitative trait and controlled by polygenes.

In the entire genome map, only one QTL for rice stripe resistance was detected on chromosome 7 designated as *qSTV7*, which was linked with the SSR markers, RM8236 and RM125, with a LOD score of 2.83 and explained 12.98% of the phenotypic variance. The positive resistant effect at the locus was contributed by Zhaiyeqing 8 (Table 1, Fig. 1).

Rice stripe resistance evaluation of $F_{2:3}$ lines and QTL mapping under natural infection

Under natural infection, no typical symptom was found in the resistant control, Kasalath, with a disease rating index of 6.4, while the typical disease symptoms were noted in the susceptible control, Nipponbare, with a disease rating index of 60.25. RDRI differed significantly between Zhaiyeqing 8 and Wuyujing 3, being 8.97 and 69.24, respectively. The frequency distributions of RDRIs in $F_{2:3}$ lines displayed a continuous distribution, ranging from 6.25 to 133.6, with transgressive segregation and bias towards to the resistant parent (Fig. 2-B), suggesting that the resistance was quantitative trait and governed by multi-genes also under natural conditions.

In the entire genome map, two QTLs for rice stripe resistance were found on chromosomes 1 and 5 (Fig. 1), respectively, designated as *qSTV1* and *qSTV5*. Among them, *qSTV1* was linked with the SSR markers, RM265 and RM486, with a 2.78 LOD score and explained 8.27% of the phenotypic variance, and the positive resistant effect at the locus was contributed by Wuyujing 3. *qSTV5* was linked with the SSR markers, RM6082 and RM249, with a 3.06 LOD score and explained 9.93% of the phenotypic variance, and the positive resistant effect at the locus was contributed by Zhaiyeqing 8 (Table 1, Fig. 1).

DISCUSSION

In this study, Zhaiyeqing 8 and Wuyujing 3 were all highly resistant and susceptible under the two evaluation methods, showing that the two evaluation methods are practically feasible, and resistance of each accession was evaluated according to RDRI so that environmental effect was eliminated. The artificial inoculation got rid of the small planthopper feeding hobbies. During the artificial inoculation, SBPH in every cylinder were disturbed so that they sucked more plants as possible as they could, ensuring that each plant with no typical symptom was due to resistance, not to be left out by insects. Therefore, it was inferred that the QTL, *qSTV7* was associated with the tolerance to virus. Under natural infection, SBPH sucked plants based on their hobbies, each plant was randomly sucked. We inferred the two QTLs detected under the natural infection were more possibly associated with resistance to virus infection or insect. However, it had been reported that Zhaiyeqing 8 was not resistant to virus infection [3], suggesting that *qSTV5* was related to resistance to insect. At *qSTV1*, the positive resistant effect was contributed by Wuyujing 3. Similar phenomena had been reported previously by other researches [16-17]. Using the two evaluation methods, the frequency distributions of RDRIs in $F_{2:3}$ lines were all continuous, indicating polygenic control of the resistance.

The QTLs detected by the artificial inoculation and natural infection were not repeatable in this study. Similar reports were found in other research, e.g., using Kanto 72/Nippobare F_2 population, *Stvb* gene was detected under mass inoculation and individual inoculation, while another QTL on chromosome 2, which might be related to the tolerance to virus, was only

Table 1. Putative QTL for rice stripe resistance detected by two evaluation methods.

Evaluation method	Locus	Chromosome	Marker interval	LOD	Variance explained (%)	Additive effect
Artificial inoculation	*qSTV7*	7	RM125-RM8263	2.83	12.98	-5.27
Natural infection	*qSTV1*	1	RM265-RM486	2.78	8.27	6.26
	qSTV5	5	RM6082-RM249	3.06	9.93	-3.07

detected under individual inoculation [18]. Using Nipponbare/ Kasalash//Nipponbare BIL population, *qSTV11* could be mapped under the mass and individual inoculations, while another QTL on chromosome 7 was detected under the individual inoculation [19-20]. Thus, because of the complexity of resistant mechanism to rice stripe, different QTLs could be detected using different evaluation methods.

A number of studies has been undertaken to identify QTL underlying RSV resistance. Hayano-Saito et al [21] mapped *Stvb-i* from 'Modan' on a 1.8 cM interval between XNpb220 and XNpb257 on chromosome 11, and both Maeda et al [18, 22] and Ding et al [17, 19] detected a QTL in this same region from Milyang, Kanto 72 and DV85, Kasalath, respectively. It indicated that the expression of this QTL or *Stvb-i* is stable under different environmental and genetic backgrounds. In addition, Japanese breeders had bred a number of japonica varieties with *Stvb-i*. In our study, no QTL in the vicinity of XNpb257 was detected, showing the QTLs detected in the study were new.

Zhaiyeqing 8 was bred by Guangdong Academy of Agricultural Sciences, which is resistant to blast [23]. In this study, QTLs for SBPH resistance and rice stripe virus tolerance were mapped, showing that Zhaiyeqing 8 is a multi-resistant variety, and these loci are different from major gene, *Stvb-i*, and suggesting that it will provide a new resistant resource for avoiding genetic vulnerability by utilization of single resistant gene, *Stvb-i*, and will be very useful for marker assisted selection in rice stripe resistance breeding programs.

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REFERENCES

1 Cooperation Research Group on Rice Stripe in Jiangsu Province. Cooperation research progress on rice stripe in Jiangsu province. *Jiangsu Agric Sci*, 2003(Suppl): 1-2. (in Chinese)

2 Wei T Y, Wang H, Lin H X, Wu Z J, Lin Q Y, Xie L H. Primary analysis of population genetic structure of rice stripe virus in China. *Acta Phytopath Sin*, 2003, **33**(3): 284-285. (in Chinese with English Abstract)

3 Sun D Z, Jiang L, Zhang Y X, Cheng X Y, Wang C M, Zhai H Q, Wan J M. Resistance to rice stripe in eight rice varieties. *Chinese J Rice Sci*, 2006, **20**(2): 219-222. (in Chinese with English abstract).

4 Zhou Y J, Liu H J, Wang G Z, Huang X, Cheng Z B, Chen Z X, Zhou X P. Immune test of rice stripe virus taken with small brown planthopper (*Laodelphax striatellus* Fallen). *Jiangsu Agric Sci*, 2004(1): 50-51. (in Chinese)

5 Wang G Z, Zhou Y J, Chen Z X, Zhou X P. Production of monoclonal antibodies to rice stripe virus and application in virus detection. *Acta Phytopath Sin*, 2004, **34**(4): 302-306. (in Chinese with English abstract)

6 Washio O, Ezuka A, Toriyama K, Sakurai Y. Testing method for genetic of and breeding for resistance to rice stripe disease. *Bull Chugoku Agric Ext Sta Ser*, 1968, **16**: 39-197.

7 Dellapporta S L, Wood J, Hicks J B. A plant DNA mini preparation: Version Ⅱ. *Plant Mol Biol Rep*, 1983, **1**: 19-21

8 McCouch S R, Teytelman L, Xu Y. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Res*, 2002, **9**: 199-207.

9 Chen F, Temnykh S, Xu Y, Cho Y G, McCouch S R. Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theor Appl Genet*, 1997, **95**: 553-567.

10 Sanguinetti C J, Dias Neto E, Simpson A J G. Rapid silver staining and recover of PCR products separated on polyacrylamide gels. *Biotechniques*, 1994, **17**: 915-919.

11 Lincoln S, Daly M, Lander E. Constructing genetics maps with MAPMAKER/EXP3.0. Cambridge, M A: Whitehead Institute Technical Report, 1992.

12 Wang S C, Zeng B Z. Windows QTL Cartographer V 2.0. Raleigh, N C: Bioinformatics Research Center, North Carolina State University, 2003. http://statgen.ncsu.edu/ qtlcart/Cartographer.html.

13 SAS Institute. SAS/STAT User Guide: version 6. Vol. 2. 4th ed. Cary, North Carolina: SAS Institute Inc, 1989.

14 McCouch S R, Cho Y G, Yano M, Paul E, Blinstrub M. Report on QTL nomenclature. *Rice Genet News*, 1997, **14**: 11-13.

15 Temnykh S, Park W D, Ayres N. Mapping and genome organization of microsatellite sequence in rice (*Oryza sativa* L.). *Theor Appl Genet*, 2001, **100**: 697-712.

16 Yasufumi K, Qian Q, Hiroyuki S, Teng S, Zeng D L, Kan F, Zhu L H. QTL Analysis of sheath blight resistance in rice (*Oryza sativa* L.). *Acta Genet Sin*, 2002, **29**(1): 50-55.

17 Ding X L, Jiang L, Liu S J, Wang C M, Chen L M, Cheng Z B, Fan Y J, Zhou Y J, Wan J M. QTL analysis for rice stripe disease resistance gene using recombinant inbred lines (RILs) derived from crossing of Kinmaze and DV85. *Acta Genet Sin*, 2004, **31**(3): 287-292. (in Chinese with English abstract)

18 Maeda H, Sugisawa T, Nemoto H, Sunohara Y. QTL analysis for rice stripe resistance in the Japanese upland rice Kanto 72. *Breeding Sci*, 2004, **54**: 19-26.

19 Ding X L, Jiang L, Zhang Y X, Sun D Z, Zhai H Q, Wan J M. Detection and analysis of QTL for resistance to stripe disease in rice, using backcross inbred lines. *Acta Agron Sin*, 2005, **31**(8): 1041-1046. (in Chinese with English Abstract)

20 Sun D Z, Jiang L, Liu S J, Zhang Y X, Huang P H, Cheng X N, Zhai H Q, Wan J M. Detection of QTLs for resistance to rice stripe virus and small brown planthopper in rice (*Oryza sativa* L.). *Acta Agron Sin*, 2006, **32**(6): 805-810. (in Chinese with English abstract)

21 Hayano-Saito Y, Tsuji T, Fujii K, Satio K, Iwasaki M, Saito A. Localization of the rice stripe disease resistance gene, *Stv-b*^{i}, by graphical genotyping and linkage analysis with molecular markers. *Theor Appl Genet*, 1998, **96**: 1044-1049.

22 Maeda H, Nemoto H, Yagi T, Fukuta Y. QTL analysis for rice stripe disease resistance using recombinant inbred lines (RILs) derived from crossing between Milyang and Akihikari. *In:* China Association of Agricultural Science, China National Rice Research Institute, China National Hybrid Rice Research and Development Center, China Foundation for Agricultural Science and Education. Prospects of Rice Genetics and Breeding for the 21st Century: Paper Collection of International Rice genetics and Breeding Symposium. Beijing: China Agricultural Science and Technology Press, 1999: 53-57.

23 Lin S C, Min S K. Rice Varieties and Genealogy in China. Shanghai: Shanghai Scientech Press, 1991: 315. (in Chinese)