Inheritance and Mechanism of Resistance to Rice Stripe Disease in cv. Zhendao 88, a Chinese Rice Cultivar

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Abstract
Mechanisms of resistance to rice stripe disease in a Chinese rice cultivar (Oryza sativa L., cv. Zhendao 88) were determined, and molecular markers for the resistance gene were identified. Single tillers at the seedling stage were inoculated with Rice stripe virus (RSV) and its vector, the small brown planthopper (SBPH) Laodelphax striatellus Fallen, to test for non-preference and antibiosis. The inheritance of resistance in the F2 and F2:3 lines from the cross cvs Zhendao 88 × Wuyujing No. 3 was also examined by single-tiller inoculation. Cv. Zhendao 88 was highly resistant to RSV and weakly resistant to SBPH. The resistance gene was mapped by SSR and RAPD analyses to rice chromosome 11 within 4.7 cm of a SSR marker RM229 and a RAPD marker OPO11. Data and inheritance analysis indicated that rice stripe disease resistance in cv. Zhendao 88 was derived principally from resistance to RSV and controlled by a single dominant gene. Breeding for rice stripe resistance could be accelerated by using cv. Zhendao 88 as a resistant parent if the linked marker for virus resistance were used in a marker-assisted progeny selection programme.

Introduction
Rice stripe disease first occurred in Jiangsu, China in the 1990s and has since spread rapidly to become the most serious disease of rice (Oryza sativa L.) in the entire middle and lower regions of the Yangtze River (Co-operation Tackle Key Project of Rice Stripe Disease in Jiangsu Province 2003; Ordon et al. 2009). As most of the cultivars of O. sativa ssp. japonica have poor resistance to rice stripe disease in Jiangsu Province (Zhou et al. 2006), the development of RSV-resistant rice cultivars is a very high regional priority. The possible mechanisms of resistance to rice stripe disease can be derived from resistance to the virus, to the vector or to both. The resistance mechanisms to rice stripe disease vary among rice cultivars (Nemoto et al. 1994; Sun et al. 2006). An indica variety, IR50, was found to be moderately resistant to the virus and tolerant to the vector. Two japonica cultivars, Musashikogane and Minamihatamochi, were found to be highly resistant to virus infection but susceptible to the vector. These cultivars displayed very high resistance to rice stripe disease in mass inoculation tests, with cvs Musashikogane and Minamihatamochi displaying more disease resistance than IR50 (Nemoto et al. 1994).

The inheritance of rice stripe resistance within rice germplasm has been studied in natural field tests and by inoculation of plants with viruliferous SBPH. Resistance exhibited both qualitative (Washio et al. 1967,
1968a,b; Ikeda and Kaneda 1982; Xing et al. 1985; Ike et al. 2002; Zhou et al. 2007) and quantitative characters (Sun 2006; Ding et al., 2004). Resistance gene mapping revealed several different resistance loci on chromosomes 1, 3, 5, 7 and 11 in rice (Sun 2006; Ding et al., 2004; Hayano-Saito et al. 1998, 2000; Maeda et al., 2006), but only one resistance locus, located at the vicinity of Stvb-i on chromosome 11, was found. Rice stripe resistance occurred with some variation among the japonica rice cultivars in different years and locations (Sun 2006; Zhou et al. 2007). Some cultivars, such as cvs Zhendao 88, Lianjing No. 4 and Xudao No. 3, displayed high levels of resistance to rice stripe disease in field experiments (Zhou et al. 2007). However, little is known about the mechanism and heredity of rice stripe disease resistance in these cultivars, so its application was limited in crop improvement and breeding for disease resistance.

Our objectives were to investigate the mechanisms for resistance to rice stripe disease in cv. Zhendao 88 and to identify molecular markers related to the resistance gene(s).

Materials and Methods

Plant material
In 2006, F2 lines derived from the crossing of cvs Zhendao 88 and Wuyujing No. 3 were planted at the experiment station of Jiangsu Academy of Agricultural Sciences. Single tillers at the three-leaf stage were collected to identify rice stripe resistance. In 2007, The F2:3 lines were derived from harvested individual F2 plants for inoculation with RSV.

Vector populations
In 2005, viruliferous SBPHs were collected from Haian county, Jiangsu province, China and maintained in plants of cv. Wuyujing No. 3. One female SBPH was separated to oviposit after mating and assayed positive for RSV by ELISA (Zhou et al. 2004). The 2nd to 3rd generation offspring of these viruliferous females were designated the H population. The proportion of viruliferous SBPH in H was estimated to be 50% by an ELISA test for RSV and did not reduce in proportion after the 5th SBPH generation.

Table 1

<table>
<thead>
<tr>
<th>Disease severity values</th>
<th>Disease symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>1</td>
<td>Diseased leaves are light yellow-green and not twisted; growth is normal</td>
</tr>
<tr>
<td>2</td>
<td>Lesions are continuous with a scratch type of yellow-green symptom; the diseased leaves emerge somewhat twisted, but growth is essentially normal</td>
</tr>
<tr>
<td>3</td>
<td>Severely discoloured yellow-green lesions; a few diseased leaves wither</td>
</tr>
<tr>
<td>4</td>
<td>Most of the diseased leaves emerge folded and twisted; central leaves yellow and wither; growth terminates and the plant dies</td>
</tr>
</tbody>
</table>

Inoculation of seedlings

Twenty-five seeds of each line were sown in a 1-1 beaker filled with soil to a depth of c. 3 cm and grown at 22–26°C in a controlled-environment room with a 12-h photoperiod of fluorescent lighting. When the seedlings reached the 1.5–2 leaf stage, at least 20 asymptomatic seedlings of each line were used for inoculation tests. Second- to fourth-instar viruliferous SBPH were released into beakers, approximately four nymphs per seedling. Three days later, all seedlings from each beaker were transplanted individually to the field after SBPH nymphs were removed. The incidence of rice stripe disease symptoms was evaluated 7 days later as described earlier and classified according to symptom expression (Zhou et al. 2007). The rice stripe resistance of inoculated plants at the seedling stage was evaluated based on disease incidence (DI): plants with DI values of 0–10% were classified as ‘resistant’; DI values of 10–30% were classified as ‘moderately resistant’; and those with DI values above 30% were classified as ‘susceptible’.

Non-preference test

Germinated seeds were sown in rows in a 35 × 25 cm porcelain dish filled with soil. Each row of 10 seeds represented a line, and each line was replicated four times. Each porcelain dish was placed in an aluminium alloy mesh cage measuring 48 × 37.5 × 22 cm. When seedlings reached the 1.5–2 leaf stage, second- to fourth-instar viruliferous nymphs of SBPH were released into the cages with approximately five nymphs per seedling. Total number of settled SBPHs per variety per day and the average number of settled SBPHs were counted and calculated for 3 days, producing an index of non-preference for each plant line.

Antibiosis test

Six germinated seeds of each line were sown in a 6 × 40 cm beaker filled with soil to a depth of c. 3 cm; each line was replicated four times. When seedlings reached the 1.5–2 leaf stage, 20 viruliferous SBPH nymphs at second- to fourth-instar were released into each test beaker which was covered with tetrone gossamer. The survival rate of the introduced SBPH
nymphs was determined 5 days postintroduction, as the index of antibiosis for each line.

**Molecular marker and linkage analysis**

DNA samples of 200 F₂ lines and two parents were extracted from fresh leaves of each plant according to Dellaporta et al. (1983) with modifications. Following extraction, the DNA was dissolved in TE buffer (10 mM tris base, 0.1 mM EDTA). Twenty-one pairs of SSR markers, located on chromosome 11, were used in the analysis (http://www.gramene.org/microsat). Polymorphisms of the SSR markers were analysed following the procedure of Chen et al. (1997) with minor modifications. Each 10-μl PCR reaction contained 10–20 ng of template DNA, 1× PCR reaction buffer (Mg²⁺ free, TaKaRa, Dalian, China), 1.5 mM of MgCl₂, 50 μM of dNTPs, 20 μM of primers and 0.5 U of Taq DNA polymerase (TaKaRa Co.). Amplification profiles consisted of 5 min of denaturation at 94°C, 35 cycles of 30-s denaturation at 94°C, 30-s annealing (temperature determined by primer pair sequence) and 60-s extension at 72°C, followed by a final 10-min extension at 72°C. Denatured amplified products were electrophoresed through 8% (w/v) native polyacrylamide gels at 120 V for c. 3 h and visualized by silver staining. A RAPD marker OPO11 (Operon Technologies Inc., Huntsville, AL, USA) was used for the analysis to identify markers that were significantly associated with the resistant gene following the procedure of Williams et al. (1990). Genetic linkage maps were constructed using MAPMARKER 3.0, and centi-morgan values were calculated based on the Kosambi mapping function (Kosambi 1944; Lander and Green 1987; Lincoln et al. 1992).

**Results**

**Analysis of the resistance mechanism in cv. Zhendao 88**

Reactions of cultivars to rice stripe disease infection using the four test methods are shown in Table 2. The disease incidences of cv. Wuyujing No. 3, the susceptible variety, were 73.3 and 76.7%, respectively, for the tiller and seedling inoculation. As the respective disease incidences were 6.7 and 10% for the tiller and seedling inoculation, cv. Zhendao 88 was classified as ‘resistant’. However, the antibiosis test showed a significant difference between IR 50 and the other two cultivars. The non-preference value of cv. Zhendao 88 fell between cvs Wuyujing No. 3 and IR 50. The result demonstrated that cv. Zhendao 88 is highly resistant to the RSV but weakly resistant to the vector.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Disease incidence:</th>
<th>Disease incidence:</th>
<th>Non-preference</th>
<th>Antibiosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tiller inoculation (%)</td>
<td>seedling inoculation (%)</td>
<td>(individuals/ plant)</td>
<td></td>
</tr>
<tr>
<td>Wuyujing No. 3</td>
<td>73.3</td>
<td>76.7</td>
<td>3.57a</td>
<td>92.5a</td>
</tr>
<tr>
<td>Zhendao 88</td>
<td>6.7</td>
<td>10.0</td>
<td>3.03b</td>
<td>86.3a</td>
</tr>
<tr>
<td>IR50</td>
<td>/</td>
<td>/</td>
<td>1.45c</td>
<td>68.8b</td>
</tr>
</tbody>
</table>

Different letters within columns represent a statistically significant difference (P < 0.5) by Duncan’s multiple range test; /, not detected.

**Genetic analysis of the resistance of cv. Zhendao 88 to rice stripe disease**

The inheritance of rice stripe resistance from the cross cvs Zhendao 88× Wuyujing No. 3 was studied by the analysis of reciprocals for single tillers; rice stripe resistance in cv. Zhendao 88 was controlled by a single dominant gene and the segregation of F₂ lines fits a ratio of 3 (resistant): 1 (susceptible) (Table 3).

To confirm the genetic basis for resistance in cv. Zhendao 88, we analysed the mode of inheritance biometrically for F₃ lines by inoculating them at the seedling stage. The rice stripe disease incidence distribution among F₃ lines was multimodal with three peaks (Fig. 1). The F₃ lines showed a good fit to an expected ratio of 1 (homozygous resistant): 2 (segregating phenotype): 1 (homozygous susceptible) for segregation of RSV resistance. Thus, the seedling and tiller inoculation agreed in determining that resistance was due to a single dominant gene.

**Mapping of rice stripe disease resistance gene in cv. Zhendao 88**

Only four markers showed polymorphisms between cvs Zhendao 88 and Wuyujing No. 3 out of twenty-one pairs of surveyed SSR markers distributed on chromosome 11. For a polymorphism index of 19.0%, 200 F₂ lines were analysed (Fig. 2) by SSR markers analysis along with graphical genotyping and linkage analysis to confirm the resistance gene on chromosome 11. The genetic distance between RM229 and the resistance gene, which was named Stv-zh, was 4.7 cm (Fig. 3). The RAPD marker OPO11 amplified a DNA fragment 2.5-kb band only from the resistant parent. This linkage analysis in the segregating population(s) showed OPO11 was completely or tightly linked to rice stripe disease resistance gene in cv. Zhendao 88 (Fig. 4).

**Discussion**

Rice stripe disease is transmitted by SBPH, and resistant rice cultivars are distinguished by their resistance to the virus or to the vector. Previous research revealed that the resistance mechanism to rice stripe disease varies among rice cultivars. Sun et al. (2006) found that the susceptible cv. Wuyujing No. 3 also showed no resistance to the vector. In our study, the antibiosis test showed no significant difference between cvs Wuyujing No. 3 and Zhendao 88, whereas the non-preference of cv. Zhendao 88 was slightly better than that of cv. Wuyujing No. 3. Because we found
high total resistance, we conclude that cv. Zhendao 88 is highly resistant to RSV but weakly resistant to the vector. These data can explain why cv. Zhendao 88 displayed stable resistance in various field tests.

Elucidating the resistance mechanism in cv. Zhendao 88 will also enhance our understanding of RSV resistance in other japonica varieties. Cv. Zhendao 88 was bred in 1993 and approved in 1997; it is a medium-maturing japonica variety with high yield, good quality and stress resistance. Cv. Zhendao 88 was the original resistance donor to several resistant japonica varieties, such as cvs 'Xudao No. 3', 'Xudao No. 4' and 'Lianjing No. 4', etc., which were grown in Jiangsu province contemporaneously (Zhou et al. 2007). At the same time, epidemics of rice black-streak dwarf virus disease also occurred in Jiangsu province, which is also transmitted by SBPHs. Thus, we recommend similar studies on Rice black-streaked dwarf virus disease resistance.

Genetic analysis indicated that the resistance to rice stripe disease in cv. Zhendao 88 is inherited as a single dominant trait. We previously verified the reliability and precision of tiller inoculation (National Invention Patent of China, Patent Number: ZL200610097816.6).

There are two main methods of resistance identification for rice cultivars against rice stripe disease. One is an artificial inoculation test, which is complicated, expensive and unsuited for early generation selection. The other is a field test, whose precision and accuracy are affected by climate, cultivation conditions and SBPH quantity. Because of the limitations of the two selection methods, the development of breeding for rice stripe disease resistance has been hindered. With the identification of our molecular marker, mark-

<table>
<thead>
<tr>
<th>Parent and/or cross</th>
<th>Number of resistant plants</th>
<th>Number of susceptible plants</th>
<th>Expected ratio</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 Wuyujing No. 3</td>
<td>8</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 P1 × P2</td>
<td>18</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 Zhendao 88× Wuyujing No. 3</td>
<td>158</td>
<td>42</td>
<td>3 : 1</td>
<td>1.50</td>
</tr>
<tr>
<td>F1 P2 × P1</td>
<td>19</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>28</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Resistance segregation of cross-combinations and $\chi^2$ test

Fig. 1: Frequency distribution of F2:3 lines ($n=200$) with different rice stripe disease incidence, derived from the cross between cvs Wuyujing No. 3 and Zhendao 88. Disease incidence for the parents cvs Wuyujing No. 3, Zhendao 88 and F1 is indicated by arrows.

Fig. 2: Segregation of RM229 in F2 population of F2 lines derived from Zhendao 88× Wuyujing No. 3. Lane identities from left to right were DNA ladder (M), cv. Wuyujing No. 3 (W), cv. Zhendao 88 (Z), F1 (F) and 25 F2 lines (1–25).

Fig. 3: RAPD analysis showing the co-segregation pattern of a RAPD marker (OPO11) with rice stripe disease resistance in the population of F2 lines derived from cvs Zhendao 88× Wuyujing No. 3. Lane identities from left to right were DNA ladder (M), cv. Wuyujing No. 3 (W), cv. Zhendao 88 (Z), F1 (F) and 13 F2 lines (1–13). Arrow indicates the polymorphic band distinguishing parents.
er-assisted selection can be used to improve rice resistance against RSV.

Although there were many reports on resistance gene mapping for this disease, one locus located at the vicinity of \textit{Stvb-i} on chromosome 11 was found fairly consistently across various cultivars (Hayano-Saito et al. 1998, 2000; Maeda et al. 2006; Sun 2006). The rice stripe disease resistance gene in cv. Asanohikari was located on the chromosome 11, collocated with restriction fragment length polymorphism marker ST10 (10). Two QTLs controlling the resistance to rice stripe disease were identified in near-isogenic lines which were developed from a Japanese upland rice line, Kanto 72. One of the QTLs was located between RM209 and RM287 on the chromosome 11 by SSR marker and linkage analysis (Maeda et al. 2006). Using IR36/Nekken2 RIL population, one putative QTL was also located in the region of marker RM202–RM287 on chromosome 11 by Sun (2006). Therefore, twenty-one pairs of SSR markers distributed on chromosome 11 were developed to map the resistance gene in cv. Zhendao 88. We located the resistance gene on chromosome 11, 4.7 cm from a SSR marker RM229. Compared by marker location, the resistance gene in cv. Zhendao 88 might be \textit{Stvb-i}, which needs further study.

\textit{Stvb-i} was applied in Japan in the 1970s and has since displayed stable resistance to rice stripe disease. This offers direct molecular evidence that cv. Zhendao 88 was a fairly good resistance donor for breeding resistant \textit{japonica} rice varieties to rice stripe disease. However, the polymorphisms of the SSR marker between cvs Zhendao 88 and Wuyujing No. 3 were relatively lower, as the parents were both \textit{japonica} varieties. Thus, the mapping analysis is still considered imprecise. A RAPD marker OPO11 was located on the same locus with the \textit{Stvb-i} in a DH population obtained from a cross between HR10624-AC5 and Milyang123 by Cho et al. (2004). RAPD analysis was performed in the segregating population, and the linkage analysis in the segregating population showed that OPO11 was completely or tightly linked to rice stripe disease–resistance gene in cv. Zhendao 88. The amplification results of OPO11 primer in several resistant \textit{japonica} cultivars, such as Xudao No.3, originated from cv. Zhendao 88, showed the same as that of Zhendao 88 (data not published). This is the first report about markers tightly linked to rice stripe disease resistance gene in a resistant \textit{japonica} rice cultivar (National Invention Patent of China, application number: 2007101909280). The aforementioned marker linked to the rice stripe resistance gene in cv. Zhendao 88 can be used in marker-assisted selected programme for rice stripe resistance breeding. As a dominant marker, the marker OPO11 should be converted into a SCAR marker in the future, which can be used more conveniently in the identification and marker-assisted progeny selection programme.

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