Genetic Analysis of Resistance to Green Rice Leafhopper (*Nephotettix cincticeps* UHLER) in Rice Parental Line, Norin-PL6, using RFLP Markers

Yoshimichi Fukuta, Katsunori Tamura, Masahiro Hira, and Shingo Oya

Hokuriku National Agricultural Experiment Station, Joetsu, Niigata 943-0193, Japan

Summary

We first characterized the graphical genotype of a *japonica* rice parental line, Norin-PL6 (Ou-PL1), which was bred to introduce the resistance genes to the green rice leafhopper (*Nephotettix cincticeps* UHLER: GRL) from an *indica* rice variety, Lepe-dumai, in order to identify the chromosome loci of resistance genes, using restriction fragment length polymorphism (RFLP) markers. It was observed that two chromosome segments introduced from Lepe-dumai were located on chromosomes 3 and 11, based on the Lepe-dumai RFLP patterns on chromosomes 3 and 11, respectively indicated by neighboring five and two RFLP markers. Moreover, two quantitative trait loci (QTL) with major genetic effects and interactive function, were detected in these regions on chromosomes 3 and 11, based on QTL analysis between the degree of resistance to GRL and the RFLP data in seventy-six *B* × *F* <sup>1</sup> plants derived from the combination between the *F* <sup>1</sup> hybrid of Toyonishiki/Norin-PL6 and Toyonishiki a *japonica* susceptible variety used as a recurrent parent. Although, It had been already reported that the resistance of Norin-PL6 was controlled by two or three dominant complementary genes, one of them being Grh<sub>2</sub>, the gene symbol and chromosome location for the other resistance gene(s) had not been determined. It is inferred that the two QTL on chromosomes 3 and 11 corresponded to the dominant complementary genes for resistance. Moreover, no other resistance genes to GRL have been reported on chromosomes 3 and 11. Based on these results, we designates Grh<sub>4</sub>(t) as one of the complementary resistance genes in Norin-PL6, and confirmed it as a new gene. We could not determine whether Grh<sub>2</sub> and/or Grh<sub>4</sub>(t) were located on chromosome 3 or 11, but we identified the RFLP markers which were linked to these resistance genes. These markers will enable would be useful to develop improved varieties or isogenic lines for resistance to GRL by the application of the marker-assisted breeding method. We will attempt to develop two kinds of isogenic lines which harbour independently each complementary gene for resistance using the RFLP markers, in order to elucidate the genetic mechanism of resistance to GRL and determine the location on chromosomes in Norin-PL6 in detail.

Key Words: green rice leafhopper (*Nephotettix cincticeps* UHLER), complementary resistance gene, RFLP marker, QTL analysis, graphical genotype.

Introduction

Green rice leafhopper (*Nephotettix cincticeps* UHLER: GRL) is a serious pest of rice (*Oryza sativa* L.) in the areas from Kanto to Kyushu, Japan where it transmits dwarf virus disease, and in areas from Hokuriku to Tohoku where it damages plants directly by sucking the plant sap. Since 1960, based on field tests and artificial inoculation, it has been reported that some *indica* rice varieties showed considerable differences in their degree of resistance to GRL, while most of the Japanese varieties were susceptible (Yasuo et al., 1960, Ishii et al. 1969, Koide et al., 1972, Kimura et al. 1969, Sakurai 1969, Oya and Sato 1980, Sekizawa and Ogawa 1980, Ando and Kishino 1986). The use of resistance genes harboured in *indica* varieties would be one of the methods to protect rice plants from GRL infestation. Development of improved varieties and lines for resistance to GRL and dwarf virus disease, by the introduction of resistance genes from indica rice varieties to Japanese *japonica* varieties, has been carried out at several breeding stations in Japan after successful introduction of the resistance genes of stripe virus disease into Japanese paddy varieties (Toriyama et al. 1966). Norin-PL2 (former name: Kanto-PL3) (Kobayashi, 1983, Kaneda et al., 1985), Norin-PL5 (former name: Saikai-PL2) (Imbe and Iwasaki, 1987), Kanto-PL6 (Kobayashi et al., 1980b), Norin-PL6 (old name: Ou-PL1) (Kishino et al. 1987) and Aichi 42 (Koumura et al. 1978, Syaku et al. 1979), have been bred from the *indica* rice varieties, Pe-bihun (Taiwan), C203-1 (India), Tadukan (Philippines), Lepe-dumai (India) and Rantaj-emas 2 (Indonesia) as donor parents, respectively.

In Norin-PL6 (Ou-PL1), among these lines, Ikeda et al. (1986) and Kishino et al. (1987) suggested that the resistance to GRL was controlled by two dominant complementary genes at the seedling stage and that three dominant complementary genes were responsible for the resistance at the adult stage of the plants. However, the detailed genetic mechanism of the resistance to GRL which is expressed complementarily by the interactions of two or three genes in Norin-PL6, and the location of
the complementary genes for resistance have not been clarified yet.

In this paper, the genetic mechanism of the resistance to GRL was elucidated and the location of the genes for resistance in Norin-PL6 was determined, based on the analysis of the graphical genotype and QTL analyses using the RFLP markers, because it was very difficult to identify the loci by classical linkage analysis in the case of the quantitative traits. Based on these results, the relationships between the genes for resistance to GRL in Norin-PL6 and other identified genes were examined.

Materials and Methods

Plant materials

Seventy six B1F1 plants derived from the cross between the F1 hybrid of a japonica susceptible rice variety, Toyonishiki, with a japonica resistant parental line, Norin-PL6, and Toyonishiki as a recurrent parent, were used for the investigations of the degree of resistance to green rice leafhopper (GRL) and RFLP analyses. Norin-PL6 is a resistant line to GRL with japonica genetic background. It was bred from a cross between Toyonishiki and an indica resistant variety, Lepe-dumai. The F1's were backcrossed with a japonica line, Ou-284, and their B1F2's was further backcrossed twice with Toyonishiki.

Resistance test against GRL

Using the Joetsu population of GRL collected in 1994 and preserved in the Laboratory of Insect Pest Control of Hokuriku National Agricultural Experiment Station, the resistance to GRL in the B1F1 plants, F1's, Norin-PL6 and Toyonishiki was investigated at three stages of the rice plant, seedling, and early tillering stages and flag leaf stage before heading of panicule.

In the seedling stage test, one seedling plant at the first or second leaf stage was caged with five newly hatched nymphs in a test tube and the number of surviving insects was examined three days after infestation. In the tillering stage test, one seedling was caged in a plastic cylinder with ten newly hatched nymphs. In the flag leaf stage test, the number of surviving insects among in the ten newly hatched nymphs caged with one piece of flag leaf in a test tube was determined after ten and four days.

Graphical Genotype of Norin-PL6 by RFLP analysis

The graphical genotype of Norin-PL6 was characterized by comparing the RFLP patterns among Norin-PL6, Toyonishiki and Lepe-dumai using 123 RFLP markers which were distributed throughout the genome on the chromosomes. The RFLP markers were developed and located on the linkage maps by Saito et al.(1991) and Kurata et al.(1994).

Total DNA of these varieties was extracted from 10 gram each of fresh green leaves by the CTAB method (Murry and Thompson 1980). The DNA samples were digested with eight restriction enzymes, Apal, BamHI, BglII, DraI, EcoRI, EcoRV HindIII and KpnI. Digested DNA was then separated by 0.8% agarose gel electrophoresis and transferred to a Nylon membrane (Boehringer Mannheim). Southern hybridization and RFLP analyses were carried out following the procedures previously described by Kurata et al. (1994).

In the analysis of the graphical genotype of Norin-PL6, the RFLP markers which indicated the Lepe-dumai genetic pattern were surveyed, and the chromosome regions introduced from Lepe-dumai were estimated. The boundaries of chromosome regions were fixed halfway between two neighboring RFLP markers.

QTL analysis

Using the RFLP markers indicating the Lepe-dumai genetic pattern, linkage analysis among the RFLP markers was performed with the computer program MAPMAKER/EXP ver. 3.0 (Lincoln et al. 1992). To detect the linkage among the resistance genes to GRL and the RFLP markers, QTL analysis was performed based on the RFLP linkage maps and the degree of resistance in the seventy-six B1F1 plants according to the method of interval mapping using a computer program qGene ver. 2.29a. In the QTL analysis, the LOD values were calculated at 1.0 cM intervals. A LOD score of 3.0 was selected as the threshold for confirming the presence of the resistance genes to GRL, and it was assumed that the QTL were located at the peak of LOD score curve in interval mapping. Two way analysis in qGene was used to evaluate digenic interactions between the identified QTL for resistance genes to GRL represented by the RFLP markers most closely linked to them.

Results

Graphical genotype

In Norin-PL6, we detected the five RFLP markers C198, C1186, Y3870L, XNpb144 and C1677 and twoC1003A and G1456 markers which indicated same the RFLP patterns as those of the Lepe-dumai genotype on chromosomes 3 and 11, respectively. These RFLP markers had been located in the same regions on each chromosome by Saito et al.(1991) and Kurata et al. (1994). Nine among 123 RFLP markers used did not detect the polymorphism among the three varieties, Norin-PL6, Lepe-dumai and Toyonishiki, while the other 106 RFLP markers detected the Toyonishiki genotype pattern (Fig.1).

These results suggest that the resistance genes may be located on chromosomes 3 and 11, since the two segments introduced from the indica donor, Lepe-dumai, were present on these chromosomes in Norin-PL6.

Segregation of the degree of resistance to GRL in the B1F1 population

In Toyonishiki the survival ratios of the insects were
Fig. 1. Graphical genotype of Norin- PL6.

Southern analyses were carried out among a japonica line, Norin- PL6, an indica variety, Lepe dumai, and a japonica variety, Toyonishi.

Each bar on the chromosome shows the position of the 123 RFLP markers used.

The RFLP linkage map reported by Kurata et al. (1994) is shown with slight modifications.

The RFLP markers indicated by XNpb followed by a number were derived from Saito et al. (1991) and the other markers from Kurata et al. (1994).

Numbers at the top indicate chromosome numbers.

The boundaries of the chromosome regions were fixed midway between the RFLP markers.

QTL analysis

For the characterization of the genotype of the seventy-six B,F1 plants, five RFLP markers on chromosome 3 and two RFLP markers on chromosome 11 were used. The resulting map spanned over 12.0 cM and 5.3 cM on chromosomes 3 and 11, respectively. On chromosome 3, since the two markers C1186 and Y3870L, and the two markers XNpb144 and C1677 were located at the same positions, it was considend that the linkage map consisted of three loci (Fig. 3).

It was observed that all the RFLP markers linked the resistance genes to GRL in all the resistance tests except for the combination of the seedling test and the RFLP marker C1003A on chromosome 11, where a LOD score of 3.0 was selected as a threshold for confirming the presence of the resistance genes to GRL by single point QTL analysis. On chromosome 3, the highest values of LOD scores were detected at the loci of the two markers XNpb144 and C1677 (Fig. 3). The values of the LOD score of the QTL were 5.12, 4.48 and 4.99 in the seedling stage test, early tillering stage test and flag leaf test, respectively. These values were high compared

higher than in Norin- PL6 for all the resistance tests, indicating that Toyonishi and Norin- PL6 were susceptible and resistant to the GRL of the Joetsu population, respectively. The F1s derived from the cross between Toyonishi and Norin- PL6 were resistant to GRL, but the degree of resistance was different between the seedling and flag leaf stages. The F1s as well as Norin- PL6 showed a high degree of resistance based on the survival ratio of the insects in the seedling test, which the F1 showed at intermediate deforce of resistance between that of the parents in the flag leaf test. (Fig. 2). Suggesting that the resistance to GRL in Norin- PL6 is controlled by dominant genes, but the expression of the resistance depends on the plant growth stages.

In all the tests of the B,F1 population, it was shown that the segregation of the survival ratio of the insects was distributed in a wide range from 0 % to 100%. In the early tillering and flag leaf stage tests, the distribution was separated into two groups which low and high survival ratios of insects at the boundary of 60%, respectively. However, the segregation in the seedling stage test showed a continuous distribution (Fig. 2)

Higher than in Norin- PL6 for all the resistance tests, indicating that Toyonishi and Norin- PL6 were susceptible and resistant to the GRL of the Joetsu population, respectively. The F1s derived from the cross between Toyonishi and Norin- PL6 were resistant to GRL, but the degree of resistance was different between the seedling and flag leaf stages. The F1s as well as Norin- PL6 showed a high degree of resistance based on the survival ratio of the insects in the seedling test, which the F1 showed at intermediate deforce of resistance between that of the parents in the flag leaf test. (Fig. 2). Suggesting that the resistance to GRL in Norin- PL6 is controlled by dominant genes, but the expression of the resistance depends on the plant growth stages.

In all the tests of the B,F1 population, it was shown that the segregation of the survival ratio of the insects was distributed in a wide range from 0 % to 100%. In the early tillering and flag leaf stage tests, the distribution was separated into two groups which low and high survival ratios of insects at the boundary of 60%, respectively. However, the segregation in the seedling stage test showed a continuous distribution (Fig. 2).
Fig. 2. Segregation of resistance to GRL in B1;F1 population.

B1;F1 (Toyonishiki/Norin;PL6//Toyonishiki) were used for the investigations of the degree of resistance to the green rice leafhopper (GRL).

A: Test at seeding stage, n=71
B: Test at early tillering stage, n=71
C: Test at flag leaf stage before heading of panicle, n=76

PL6/Norin; PL6
Toy=Toyonishiki

: Average and range in parents and F1

with the threshold of 3.0. The variances (R²) of the QTL ranged from 23.3% to 26.7% in the three kinds of tests.

On chromosome 11, the highest values of LOD score were detected at the position of the RFLP marker G1465 in all the tests. The values of LOD scores of QTL in the seedling stage test, tillering stage test and flag leaf test, were 3.82, 5.09 and 4.99, respectively. The variance (R²) of the QTL was in the range of 20.7% to 28.4% (Table 1).

Based on the two-way interaction analysis, the most significant interaction was also detected between the RFLP markers, XNpb144 on chromosome 3 and G1465 on chromosome 11. Only the B1;F1 plants which showed the heterozygous genotypes of XNpb144 and G1465 at the same time, were highly resistant to GRL. The values of the F test which ranged from 82.8 to 327.0 were very high compared with those of single-point analysis (Table 1).

These results indicate that the two QTL for resistance to GRL were located in the vicinity of the RFLP markers, XNpb144 and C1677 on chromosome 3 and G1465 on chromosome 11, respectively, and expressed a complementarity with major genetic effects at all the plant stages, i.e., seedling, early tillering and flag leaf stages.

Discussion

We confirmed that two major QTL for resistance to GRL in Norin-PL6 behaved complementarily in the resistance tests using the Joetsu population of GRL. On the other hand, Kishino et al. (1987) had reported that the number of dominant complementary genes in Norin-PL6 varied from two at the seedling stage to three at the adult stage. Although it was assumed that the two QTL for resistance to GRL in the present study corresponded to the dominant complementary genes reported by Kishino et al. (1987), there was a disagreement of in the results on the number of genes for resistance. In our analysis, the indica chromosome segments introduced from a donor variety, Lepe-dumai, were detected only on chromosomes 3 and 11, and the QTL analysis between the RFLP markers and resistance genes to GRL was restricted to these chromosome regions. Assuming that more than two genes controlled the resistance to GRL in Norin-PL6, it is possible that we were not able to find the other Lepe-dumai segment in the chromosome regions between the RFLP markers which detected the Toyonishiki genotype or non-poly-morphism in the analysis of the graphical genotype. It is also offered that the disagreement between the results in the two studies may be due to the differences in the GRL populations used or methods applied in the resistance tests. The expression of the resistance gene(s) may vary.
Table 1. Effects of putative loci on resistance to GRL

<table>
<thead>
<tr>
<th>Nearest marker locus to QTL</th>
<th>Chr.</th>
<th>Test</th>
<th>Means of survival ratio in insects (%)</th>
<th>F</th>
<th>P</th>
<th>LOD</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aa(n)</td>
<td>aa(n)</td>
<td>Bb(n)</td>
<td>bb(n)</td>
<td>AaBb(n)</td>
</tr>
<tr>
<td>Single point analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XNpb144</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1465</td>
<td></td>
<td>11</td>
<td>Seedling</td>
<td>69.8(43.97.6(33)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tilling</td>
<td>65.1(41)98.7(30)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Flag leaf</td>
<td>55.8(43.93.9(33)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two - way interactions</td>
<td></td>
<td></td>
<td>Seedling</td>
<td>34.8(19)97.6(24) 96.2(21)100.0(12)128.2</td>
<td>82.8</td>
<td>0.000 -</td>
<td>-</td>
</tr>
<tr>
<td>analysis</td>
<td></td>
<td></td>
<td>Tilling</td>
<td>30.5(19)95.0(22) 98.9(19)98.2(11)173.9</td>
<td>0.000 -</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>XNpb144 - G1465</td>
<td></td>
<td></td>
<td>Flag leaf</td>
<td>10.5(19)91.7(24) 94.3(21)93.3(12)327.0</td>
<td>0.000 -</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

1). Test at seedling stage, 2). Test at early tilling stage, 3). Test at flag leaf stage before heading of panicle.
F - threshold for significance of a single marker and two-way interaction: 10.0
LOD - threshold for significance of a single marker: 3.0
A and B indicate the Norin' - PL6 (indica variety, Lepe - dumai,) genotypes of XNpb144 on chromosome 3 and G1465 on chromosome 11, respectively.
a and b indicate the Toyonishiki (japonica) genotypes.
P value corresponds to F.

depending on the biotypes of GRL. To confirm in detail the expression of resistance to GRL depending on the plant growth stages, it is necessary to use different populations or biotypes of GRL.

It was also found that the two major QTL for resistance to GRL in Norin-PL6 were located in the vicinity of the RFLP markers, XNpb144, C1677 and C848 on chromosome 3 and, G1465 on chromosome 11, respectively. No other resistance genes to GRL have been reported in these regions of chromosomes 3 and 11. Kobayashi et al. (1980a) reported that the resistance to GRL in Norin-PL2 (Kanto-PL3) was controlled by one of the two dominant genes derived from Pe-bihun, and then Takita (1990) stated that Grh1 was the resistant gene. Tamura et al. (1997) mapped the gene Grh1 in Norin-PL2 on chromosome 5 using RFLP markers. In Aichi 42, Nishioka et al. (1981) reported that the resistance to dwarf disease, which was caused indirectly by the transmission of the virus of GRL in Rantaj-emas 2, was controlled by an incomplete dominant gene. Sogawa et al. (1982) estimated that the same genetic mechanism operated in the resistance to rice dwarf disease and GRL. Thus, it has been assumed that the resistance to GRL in Aichi 42 was also controlled by a single dominant gene. Saka et al. (1996), Saka et al. (1997) reported that the resistance gene in Aichi 42 was located on chromosome 6 based on the results of linkage analysis using RFLP markers and they designated the gene as Grh3(t). In Kanto-PL6, although the genetic mechanism of the resistance and the relationships with other resistant varieties have not been clarified in detail, it was found that the resistance in the donor variety, Tadukan, was controlled by two dominant genes (Kobayashi et al., 1980a). The data of Takita (1990) suggested that Kanto-PL6 and Aichi 42 harboured the same resistance gene, implying that one of the two resistance genes in Tadukan is Grh3(t) and that Kanto-PL6 harbours the gene. In Norin-PL5 (Saikai-PL2), Imbe and Iwasaki (1987) had recognized that the resistance to GRL was controlled by two dominant complementary genes. Takita and Nishiyama (1989) reported that a japonica resistant line, Saikai-182, which was bred from Norin-PL5, harboured one of the two genes in Norin-PL5. And then, Takita (1990) stated that the gene in Saikai-182 and Norin-PL5 was Grh2. Ikeda et al. (1986) and Kishino et al. (1987) found that both Norin-PL6 and Norin-PL5 carried the same two dominant complementary genes for resistance. These results indicate that one of the dominant complementary resistance genes in Norin-PL6 is Grh2, and that it is located on chromosome 3 or 11. Therefore, we designated one of the two dominant complementary genes in Norin-PL5 and Norin-PL6 as Grh4(t) and considered it as a new gene. However, we could not determine whether Grh2 and/or Grh4(t) were located on chromosome 3 or 11, respectively. It is necessary to identify the chromosome segment harbouring the resistance gene Grh2 in Saikai-182, Norin-PL5 and Norin-PL6.

In this paper, we identified molecular (RFLP) markers which were closely linked with the two resistance genes to GRL on chromosomes 3 and 11. These RFLP markers will be utilized to develop resistant varieties and iso-
genic lines by the marker-assisted selection (MAS) method. Further RFLP analysis and application of MAS method are necessary for the development of two kinds of isogenic lines which harbor independently each dominant complementary gene for resistance, Grh2 and Grh4(t), in Norin-PL6, in order to analyze the genetic relationship of the two genes and determine the location in detail.

Acknowledgments

We thank Drs. J. Mochizuki and M. Takeda, Tohoku National Agricultural Experiment Station, MAFF, Japan for kindly supplying the seeds of Norin-PL6, and Drs. T. Yagi, T. Ogawa and I. Ashikawa, Hokuriku National Agricultural Experiment Station, MAFF, Japan for their helpful advice and valuable discussions.

Literature Cited


— (1983) Inheritance of resistance to green rice leafhopper (Nephotettix cincticeps UHLER) and dwarf virus disease.

Fukuta, Tamura, Hiraue and Oya


