Artificial selection of biotypes of green rice leafhopper, *Nephotettix cincticeps* Uhler (Homoptera: Cixiidae), and virulence to resistant rice varieties

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**Abstract**

A population of green rice leafhopper, *Nephotettix cincticeps* Uhler (GRH), was artificially selected on 5 resistant rice varieties in the laboratory. The GRH lines selected on Saikai 164, Saikai 182, and Kanto-PL 6 were able to survive and reproduce on their respective varieties. In these lines, the developmental period of nymphs was shortened by continuous selection, although in the first generation it was longer than that of the line reared on Nipponbare carrying no resistance gene. We could not establish GRH lines virulent to Norin-PL 5 or Norin-PL 6. Six lines, reared on IR 24, Chugoku 105, Saikai 164, Saikai 182, Kanto-PL 6, and Aichi 80, were assessed for virulence among different resistant varieties by conducting a seedling test. All lines were highly virulent to the varieties on which they were selected. Similar virulence was obtained for combinations of IR 24 and Chugoku 105 lines, Saikai 164 and Saikai 182 lines, and Kanto-PL 6 and Aichi 80 lines. The Kanto-PL 6 and Aichi 80 lines were moderately virulent to Tadukan and Rantaj-emas 2. No lines were virulent to Norin-PL 5, Norin-PL 6, and Pe-bi-hun. The results of the leaf blade test were similar to those of the seedling test. The allele test confirmed that the GRH-resistance genes of Kanto-PL 6 and Aichi 80 are the same, and the resistance gene of Norin-PL 2 is located at a different locus from that of Kanto-PL 6 and Aichi 80. These results suggest that the virulence of GRH biotypes corresponds to the resistance genes of rice varieties and therefore, the use of biotypes allows the identification of groups of rice varieties that have similar resistance genes. We propose that the biotypes virulent to *Grh* 1-, *Grh* 2-, and *Grh* 3 (i)-carrying varieties be designated “Biotype 1”, “Biotype 2”, and “Biotype 3”, respectively.

**Key words:** *Nephotettix cincticeps*; rice; resistance; biotypes; selection

**INTRODUCTION**

The use of crop resistance is an effective method of integrated pest management. It offers the advantages of a relatively small effect on the environment, reduced labor, and lower costs. However, a serious problem is that the resistance of crops has broken down with the occurrence of biotypes of pest insects that are virulent to previously resistant crop varieties. To use plant resistance for insect pest control, we should clarify the potential of pests to overcome resistance and predict the occurrence of such biotypes.

The existence of resistance-breaking biotypes has been reported in many crops (Sogawa, 1983a, b; Diehl and Bush, 1984). Such a biotype of the brown planthopper, *Nilaparvata lugens*, was observed after resistant varieties of rice were cultivated for a few years (Sogawa, 1982; Gallagher et al., 1994). Several studies showed that *N. lugens* populations have genetic variation in virulence to resistant rice varieties using artificial selection in the laboratory (den Hollander and Pathak, 1981; Ito and Kisimoto, 1981; Claridge and den Hollander, 1982) and quantitative genetic analysis (Tanaka, 1999). The artificial selection of biotypes was also carried out in the green leafhopper, *Nephotettix virescens* (Kobayashi et al., 1983; Takita and Habibuddin, 1985).

The green rice leafhopper (GRH), *Nephotettix*
cincticeps Uhler, is a serious pest in temperate Asia. Populations of this insect, which multiply rapidly around the rice heading stage, can reach high levels and cause considerable losses of rice yield in northern Japan, though their peak density is relatively low in southwestern Japan (Kidokoro, 1979; Itô and Johraku, 1982; Satomi, 1993; Hirano and Fujii, 1995). GRH also causes damage to rice indirectly and through viral and phytoplasmal diseases such as rice dwarf disease, waika disease, and yellow dwarf disease (Fukushi, 1934; Shinkai, 1962; Hirao et al., 1974). As some indica varieties are resistant to GRH (Inoue, 1966), resistance genes from these varieties have been introduced into japonica rice for breeding resistant varieties and lines (Shumiya et al., 1984; Kaneda et al., 1985; Ono et al., 1986; Kishino et al., 1987; Tokura et al., 1995; Nakajima et al., 1998). In the field, no resistance-breaking biotypes of GRH were found after cultivation with the resistant varieties. In the laboratory, however, biotypes virulent to the rice breeding lines, Saikai 182 and Aichi 42, were selected using a GRH population collected from Chikugo, Fukuoka Prefecture (Takita and Nishiyama, 1989). At present, many GRH-resistant varieties and lines have different genetic sources (Table 1); therefore, it is necessary to examine the possibility that biotypes occur on those varieties. Virulence to IR 24 was different between populations of GRH from the southern district (Kagoshima, Chikugo and Kainan) and northern district (Mattou, Toyama, Joetsu and Teradomari) in Japan (Sato and Sogawa, 1981; Sogawa and Sato, 1981). Therefore, the possibility of biotype occurrence in the Joetsu (northern Japan) popula-
tion also needs to be estimated as well as the Chikugo (southern Japan) population. In this paper, we examine the development of virulence in GRH by subjecting the Joetsu population to artificial selection on several resistant varieties and lines. We also evaluate the virulence of selected GRH lines to clarify the differences of their virulence among resistant varieties and between rice growth stages. In addition, we analyze the relationship between resistance genes by conducting allele tests among some resistant varieties. Furthermore, we classify biotypes of GRH by examining the relationship between the resistance genes of rice varieties and the selected GRH lines.

**MATERIALS AND METHODS**

**Insects.** The GRH used in this study was collected in paddy fields cultivated with a japonica rice variety, Nipponbare, at the Hokuriku Research Center in October 1993. Nipponbare has no known GRH-resistance genes. The insects were maintained on Nipponbare seedlings by the method of Sugimoto (1969). The rearing of insects and the experiments described below were carried out in an air-conditioned insectary at 25°C with a 16L:8D photoperiod.

**Plants.** The rice varieties used in this study are listed in Table 1. Saikai 164, Saikai 182, Kanto-PL 6, Norin-PL 5 (former name: Saikai-PL 2), and Norin-PL 6 (former name: Ou-PL 1) carrying GRH-resistance genes were used for the selection of biotypes and Nipponbare was used as a control variety. In addition to these six varieties, IR 24, Norin-PL 2 (former name: Kanto-PL 3), Chugoku

<table>
<thead>
<tr>
<th>Variety</th>
<th>Donor parent for N. cincticeps resistance</th>
<th>Resistance gene</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nipponbare</td>
<td></td>
<td>No resistance gene</td>
<td>International Rice Research Institute (1972)</td>
</tr>
<tr>
<td>IR 24</td>
<td></td>
<td>Grh 1</td>
<td>Kaneda et al. (1985)</td>
</tr>
<tr>
<td>Chugoku 105</td>
<td>Norin-PL 2</td>
<td>Grh 1</td>
<td>National Agricultural Research Center (1995)</td>
</tr>
<tr>
<td>Saikai 164</td>
<td>C203-1</td>
<td>Unknown</td>
<td>Takita and Nishiyama (1989)</td>
</tr>
<tr>
<td>Saikai 182</td>
<td>Norin-PL 5</td>
<td>Grh 2</td>
<td>Kobayashi et al. (1980)</td>
</tr>
<tr>
<td>Kanto-PL 6</td>
<td>Tedukan</td>
<td>Unknown</td>
<td>Nakagome et al. (1989)</td>
</tr>
<tr>
<td>Aichi 80</td>
<td>Rantaj-emas 2</td>
<td>Grh 3 (l)</td>
<td>Ono et al. (1986)</td>
</tr>
<tr>
<td>Norin-PL 5</td>
<td>C203-1</td>
<td>Grh 2 + Grh 4</td>
<td>Kishino et al. (1987)</td>
</tr>
<tr>
<td>Norin-PL 6</td>
<td>Lepe dumai</td>
<td>Grh 2 + Grh 4</td>
<td></td>
</tr>
</tbody>
</table>
105, and Aichi 80, and three of their genetic sources, i.e. Pe-bi-hun, Tadukan, and Rantaj-emas 2, were used for testing the virulence of the selected GRH lines among different resistant varieties.

Selection of GRH lines virulent to resistant varieties. About 600 rice seeds of each variety were sown in a plastic petri dish (9 cm in diameter, 2.2 cm high) containing 60 ml of moist soil. After four days, during which the rice seedlings grew to the second leaf stage, 150 to 200 first-instar nymphs of GRH were released within 8 h of hatching on rice seedlings that were covered by a cylindrical vinyl chloride cage (8.5 cm in diameter, 15 cm high) equipped with three Tetoron® gauze windows. As a rule, seedlings were exchanged every five days. Emerged adults were moved to another cage that contained rice seedlings of the respective variety used for selection and allowed to oviposit to produce the next generation. These selection procedures were continued for several generations. In this paper, the name of the selected line of GRH, e.g. the “IR 24 line”, is based on the variety from which the line was selected.

Development and survival. Developmental periods and survival rates of GRH nymphs were observed to examine the changes in these traits by artificial selection. Fifty first-instar nymphs were released within 8 h of hatching into a cylindrical cage containing about 150 rice seedlings of the variety used for selection. The seedlings were exchanged every five days. Each selected line was tested in three cages (150 nymphs). The emerging adults were removed from the cage and the number of emerging adults and the nymphal period to emergence were recorded daily. The survival rate of nymphs was calculated as the average per cage in three replications.

Virulence of selected GRH lines among resistant varieties. The virulence of each GRH line selected for eight generations was evaluated by a seedling test (Hirae et al., 2002) and a leaf-blade test (Kishino and Ando, 1978) to clarify the differences in virulence among various resistant varieties and between rice growth stages. In the seedling test, one seedling at the second leaf stage (four days after seeding) was placed into a test tube (1.8 cm in diameter, 18 cm high) with 0.5 ml of water. Five first-instar nymphs were released within 8 h of hatching into the test tube, which was plugged with cotton covered with Tetoron gauze. Ten seedlings of each variety were tested. After four days, nymphs that had developed into second instars were counted. Virulence was evaluated as the percentage of second-instar nymphs among the 50 nymphs released. In addition to the Saikai 164, Saikai 182, and Kanto-PL 6 lines of GRH, selected in the present study, three more lines were established by selection on IR 24, Chugoku 105, and Aichi 80 for more than 11 generations. These six lines were tested for their virulence to the 13 resistant varieties described above (Table 1).

In the leaf-blade test, test leaves were collected from rice plants cultivated in paddy fields at the Hokuriku Research Center. Six rice varieties, Nipponbare, Chugoku 105, Saikai 182, Kanto-PL 6, Aichi 80, and Norin-PL 5, were transplanted on 16 May 1997 and the base of the most recently fully expanded leaf was cut at a length of 15 cm on 17 August (panicle formation stage to heading stage). The detached leaf was placed into a test tube (1.8 cm in diameter, 18 cm high) with 3 ml of water. Ten first-instar nymphs of each GRH line selected over more than 14 generations were released and the nymphs that had developed into the second instar were counted after 4 days. Five replications were made for each variety.

Allelic relationships between resistance genes. As the virulence test of the selected GRH lines suggests that the relationship of resistance between Norin-PL 2, Kanto-PL 6, and Aichi 80 is different from a previous report (Takita and Nishiyama, 1989) (see Results), allele tests between resistance genes were carried out by crossing the three varieties. F2 progeny from each cross of the three varieties was sown in a seedling cup (9 cm in diameter, 4.5 cm high) containing 60 ml of moist soil. Resistance of the individual seedlings was examined by the seedling test described above, using a non-selected line. We defined seedlings on which the proportion of nymphs developing into second instars was <40% and >60% as resistant and susceptible, respectively, according to Tamura et al. (1999) and Hirae et al. (2002). The relationship is expected to be non-allelic if the segregation ratio of resistance to susceptible progeny is consistent with 15:1, and allelic if all progeny shows resistance (Kobayashi, 1983; Ikeda, 1985).

Statistical analysis. The developmental period of nymphs was analyzed using ANOVA and the
Table 2. The number of *N. cincticeps* nymphs released and the percentage of adults emerged on resistant varieties and a control (Nipponbare)

<table>
<thead>
<tr>
<th>Rice variety</th>
<th>Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Nipponbare</td>
<td>95.0 (300)</td>
</tr>
<tr>
<td>Saikai 164</td>
<td>90.3 (300)</td>
</tr>
<tr>
<td>Saikai 182</td>
<td>88.3 (300)</td>
</tr>
<tr>
<td>Kanto-PL 6</td>
<td>37.8 (1,300)</td>
</tr>
<tr>
<td>Norin-PL 5</td>
<td>7.9 (1,900)</td>
</tr>
<tr>
<td>Norin-PL 6</td>
<td>7.9 (1,900)</td>
</tr>
</tbody>
</table>

The value shows the percentage of nymphs developing into adults. The numerical value in parentheses shows the number of first instar nymphs released on each variety.

Tukey-Kramer method. The survival rate of nymphs and the percentage of nymphs developing into second instars were analyzed using ANOVA and the Tukey method after arcsine transformation of the data. In the allele test, chi-square tests were performed using the expected segregation ratio of 15:1 or 1:0.

**RESULTS**

**Selection of GRH lines virulent to resistant variety**

The results of artificial selection experiments are shown in Table 2. In the GRH lines reared continuously on Saikai 164 and Saikai 182, a great number of nymphs survived to adulthood from the first generation. In the line reared on Kanto-PL 6, the number of emerging adults was not large in the first two generations, but became large from the third generation; however, the number of adults was small in the first generation reared on Norin-PL 5 and third generation raised on Norin-PL 6. Accordingly, we did not continue selection using these two varieties.

**Development and survival**

The developmental period and survival rate of nymphs for each generation of selection are shown in Table 3. In the Saikai 164 line and Saikai 182 line, the developmental period was longer by 2-4 days than that of the Nipponbare line in the first generation. However, the developmental period was shortened by continuous selection, and was not significantly different from that of the Nipponbare line in the sixth generation. In these two lines, the survival rate was almost the same as that of the Nipponbare line in the first generation. In the Kanto-PL 6 line, the developmental period was prolonged in the first generation but did not differ significantly from that of the Nipponbare line in the fourth generation. In this line, although the survival rate was lower than that of the Nipponbare line in the first generation, it rose from the second generation. The Norin-PL 5 and Norin-PL 6 lines showed low survival rates in the first generation.

**Virulence of selected GRH lines among resistant varieties**

In the seedling test, the virulence of the selected GRH lines among resistant varieties was evaluated based on the percentage of nymphs developing into second instars (Fig. 1). All 6 GRH lines (IR 24, Chugoku 105, Saikai 164, Saikai 182, Kanto-PL 6 and Aichi 80) were highly virulent to the varieties on which they were selected. The IR 24 and Chugoku 105 lines were also highly virulent to other varieties, i.e. IR 24, Norin-PL 2, and Chugoku 105, but were not virulent to Pe-bi-hun which is the source of the resistance of Norin-PL 2. The Saikai 164 and Saikai 182 lines were highly virulent to Saikai 164 and Saikai 182; however, these lines had low virulence in Norin-PL 5, which is the source of the resistance of Saikai 182. The lines selected on Kanto-PL 6 and Aichi 80 were virulent to both Kanto-PL 6 and Aichi 80, and moderately virulent to Tadukan and Rantaj-emas 2 which are the sources of the resistance of Kanto-PL 6 and Aichi 80, respectively. No lines were virulent to Norin-PL 5, Norin-PL 6, and Pe-bi-hun.

The results of the leaf blade test are shown in...
Table 3. Changes in the developmental period and survival rate of *N. cincticeps* nymphs on resistant varieties and Nipponbare for each generation of selection

<table>
<thead>
<tr>
<th>Generation</th>
<th>Selected line</th>
<th>Period of development (mean±SD) (days)</th>
<th>Nymphs developing into adults (%±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>1</td>
<td>Nipponbare</td>
<td>19.8±1.0 (68) a</td>
<td>18.4±1.1 (71) a</td>
</tr>
<tr>
<td></td>
<td>Saikai 164</td>
<td>22.9±2.2 (70) b</td>
<td>20.8±1.6 (62) b</td>
</tr>
<tr>
<td></td>
<td>Saikai 182</td>
<td>23.8±2.8 (64) b</td>
<td>21.7±2.5 (62) b</td>
</tr>
<tr>
<td></td>
<td>Kanto-PL 6</td>
<td>33.2±3.0 (52) c</td>
<td>29.3±3.2 (45) c</td>
</tr>
<tr>
<td></td>
<td>Norin-PL 5</td>
<td>(0)</td>
<td>32.0 (1)</td>
</tr>
<tr>
<td></td>
<td>Norin-PL 6</td>
<td>34.0±2.7 (11) c</td>
<td>33.1±2.0 (9) d</td>
</tr>
<tr>
<td>2</td>
<td>Nipponbare</td>
<td>20.5±0.9 (68) a</td>
<td>18.9±0.8 (71) a</td>
</tr>
<tr>
<td></td>
<td>Saikai 164</td>
<td>22.8±1.9 (71) b</td>
<td>20.9±1.5 (64) b</td>
</tr>
<tr>
<td></td>
<td>Saikai 182</td>
<td>24.9±2.6 (61) c</td>
<td>23.0±2.5 (56) c</td>
</tr>
<tr>
<td></td>
<td>Kanto-PL 6</td>
<td>29.6±2.4 (61) d</td>
<td>26.7±1.8 (45) d</td>
</tr>
<tr>
<td>3</td>
<td>Nipponbare</td>
<td>18.5±0.9 (68) a</td>
<td>17.5±0.9 (71) a</td>
</tr>
<tr>
<td></td>
<td>Saikai 164</td>
<td>20.5±1.8 (85) b</td>
<td>19.1±1.3 (54) b</td>
</tr>
<tr>
<td></td>
<td>Saikai 182</td>
<td>20.5±1.8 (78) b</td>
<td>18.8±1.3 (56) b</td>
</tr>
<tr>
<td></td>
<td>Kanto-PL 6</td>
<td>21.1±3.1 (65) b</td>
<td>19.0±2.5 (57) b</td>
</tr>
<tr>
<td>4</td>
<td>Nipponbare</td>
<td>19.1±1.3 (90) a</td>
<td>17.9±0.9 (54) a</td>
</tr>
<tr>
<td></td>
<td>Saikai 164</td>
<td>19.8±1.7 (82) a</td>
<td>18.4±1.4 (42) a</td>
</tr>
<tr>
<td></td>
<td>Saikai 182</td>
<td>21.3±2.3 (76) b</td>
<td>18.9±1.4 (53) a</td>
</tr>
<tr>
<td></td>
<td>Kanto-PL 6</td>
<td>19.4±1.5 (50) a</td>
<td>18.0±1.2 (75) a</td>
</tr>
<tr>
<td>6</td>
<td>Nipponbare</td>
<td>19.4±1.2 (87) a</td>
<td>17.8±0.7 (57) a</td>
</tr>
<tr>
<td></td>
<td>Saikai 164</td>
<td>19.3±1.1 (92) a</td>
<td>17.9±0.9 (39) a</td>
</tr>
<tr>
<td></td>
<td>Saikai 182</td>
<td>19.5±1.0 (73) a</td>
<td>18.2±1.2 (64) a</td>
</tr>
<tr>
<td></td>
<td>Kanto-PL 6</td>
<td>19.0±1.0 (71) a</td>
<td>17.8±0.8 (71) a</td>
</tr>
<tr>
<td>8</td>
<td>Nipponbare</td>
<td>19.3±0.9 (59) a</td>
<td>18.0±1.0 (74) a</td>
</tr>
<tr>
<td></td>
<td>Saikai 164</td>
<td>19.4±1.2 (91) a</td>
<td>18.0±1.5 (47) a</td>
</tr>
<tr>
<td></td>
<td>Saikai 182</td>
<td>19.4±0.9 (86) a</td>
<td>18.1±1.1 (54) a</td>
</tr>
<tr>
<td></td>
<td>Kanto-PL 6</td>
<td>19.1±0.9 (66) a</td>
<td>17.7±1.0 (72) a</td>
</tr>
</tbody>
</table>

The numerical value in parentheses shows the number of nymphs developing into adults.  
\(a\) Means followed by the same letter are not significantly different among rice varieties within a generation and sex (*p* > 0.05, Tukey-Kramer method).  
\(b\) Means followed by the same letter are not significantly different among rice varieties within a generation (*p* > 0.05, Tukey method).

Fig. 2. The Chugoku 105, Saikai 182, and Aichi 80 lines were highly virulent to the varieties used for their selection and the Aichi 80 line was also virulent to Kanto-PL 6. The non-selected control line (Nipponbare) showed rather high virulence in Saikai 182, though its virulence was significantly lower than that in Nipponbare. No lines were virulent to Norin-PL 5. The leaf blade test gave similar results to the seedling test, although the level of resistance of Saikai 182 was lower in the leaf blade test.

**Allelic relationships between resistance genes**

The frequency distributions of the resistance of F2 progeny from the crosses of three GRH-resistant varieties are given in Table 4. All F2 plants derived from the cross of Kanto-PL 6/Aichi 80 exhibited a resistant phenotype, indicating that the GRH-resistance genes of Kanto-PL 6 and Aichi 80 are allelic. The ratio of resistant: susceptible individuals in the F2 progeny from the crosses of Kanto-PL 6/Norin-PL 2 and Aichi 80/Norin-PL 2 was not significantly different from the expected ratio of 15:1,
Fig. 1. Virulence of selected lines of *N. cincticeps* among rice varieties in the seedling test. Columns and vertical bars show the means and standard errors of the percentage of nymphs which developed to second instars. For each line, columns with the same letter are not significantly different in virulence among the rice varieties at 0.05 (Tukey method).

confirming independent segregation of the two dominant genes. These results show that the GRH-resistance gene of Norin-PL 2 is certainly located on a different locus from that of Kanto-PL 6 and Aichi 80.

**DISCUSSION**

It was reported that GRH populations from Kagoshima, Chikugo, and Kainan (Tokushima Prefecture) were easily reared for successive generations on IR 24, whereas populations from Mattou (Ishikawa Pref.), Toyama, Joetsu, and Teradomari (Niigata Pref.) could not be raised on IR 24 (Sogawa and Sato, 1981). Takita and Nishiyama (1989) established two selected lines of GRH, one virulent to Aichi 42 and the other virulent to Saikai 182, using the Chikugo population. In the present study, GRH lines which could survive and reproduce on several resistant varieties controlled by single resistance gene were established using the Joetsu population. This result shows that the GRH population from Joetsu has genetic variations in virulence to resistant varieties. It also suggests that
GRH resistance could be broken down by the occurrence of biotypes virulent to resistant varieties in the Joetsu district. It is important to note that we have not been able to establish GRH lines virulent to Norin-PL 5 or Norin-PL 6 controlled by two complementary resistance genes. Further investigations are needed to examine whether a biotype virulent to these varieties will occur.

Based on their virulence among resistant varieties in the seedling test, we can classify the selected GRH lines into three groups: IR 24 and Chugoku 105 lines, Saikai 164 and Saikai 182 lines, and Kanto-PL 6 and Aichi 80 lines, because lines in the same group were similarly virulent to rice varieties.

The IR 24 and Chugoku 105 lines were highly virulent to IR 24, Chugoku 105, and Norin-PL 2, suggesting that the same genes confer the resistance of these varieties. Chugoku 105 was bred from Norin-PL 2 as a resistance donor, and Norin-PL 2 was bred from Pe-bi-hun (Table 1). We found that the IR 24 and Chugoku 105 lines were not virulent to Pe-bi-hun. These results show that the resistance to GRH in Pe-bi-hun is controlled by two or more genes: one of these resistance genes is the same as that of IR 24 and Norin-PL 2. In fact, the resistance in Norin-PL 2 is controlled by a single dominant gene (Kaneda et al., 1985), and the resistance gene of IR 24 was reported to be allelic to one of the two resistance genes of Pe-bi-hun (Kobayashi, 1983). Also, Tamura et al. (1999) confirmed that the resistance gene of IR 24 is the same as that of Norin-PL 2.

**Table 4. Frequency distribution of phenotypes of F$_2$ progeny from crosses of N. cincticeps-resistant varieties**

<table>
<thead>
<tr>
<th>Cross</th>
<th>Resistant (No.)</th>
<th>Susceptible (No.)</th>
<th>Expected ratio (R:S)</th>
<th>$\chi^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanto-PL 6×Aichi 80</td>
<td>285</td>
<td>0</td>
<td>285:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanto-PL 6×Norin-PL 2</td>
<td>279</td>
<td>25</td>
<td>285:19</td>
<td>2.02</td>
<td>0.16</td>
</tr>
<tr>
<td>Aichi 80×Norin-PL 2</td>
<td>266</td>
<td>23</td>
<td>271:18</td>
<td>1.44</td>
<td>0.23</td>
</tr>
</tbody>
</table>
as that of Norin-PL 2. The results of this study are consistent with these reports.

We also found that the Saikai 164 and Saikai 182 lines were virulent to Saikai 164 and Saikai 182 but not to Norin-PL 5. Saikai 182 was bred from Norin-PL 5, and Saikai 164 and Norin-PL 5 were bred from C203-1 (Table 1). The resistance in Norin-PL 5 is controlled by two complementary genes (Imbe and Iwasaki, 1987). These genes, designated Grh 2 and Grh 4, are located on chromosome 11 and chromosome 3, respectively, of the rice plant (Tamura et al., 1997; Fukuta et al., 1998). Resistance in Saikai 182 is controlled by a single gene (Grh 2) (Tamura et al., 1998). Saikai 164 probably has the single resistance gene Grh 2, the same as Saikai 182. Thus, the Saikai 182 and Saikai 164 lines of GRH may adapt to the resistance conferred by Grh 2 but not to the resistance of Norin-PL 5, which is controlled by a combination of Grh 2 and Grh 4. It is necessary to confirm this tentative conclusion by conducting allele tests.

The Kanto-PL 6 line exhibited almost the same virulence to the rice varieties tested as the Aichi 80 line, suggesting that Kanto-PL 6 and Aichi 80 have the same resistance genes. Takita and Nishiyama (1989) reported that a GRH line selected on Aichi 42, which is a parent of Aichi 80, was highly virulent to both Kanto-PL 6 and Norin-PL 2, and classified Aichi 42 and Norin-PL 2 into the same group. This result was different from that of our examination of the virulence of selected GRH lines. Allelic analysis showed that the GRH-resistance genes of Kanto-PL 6 and Aichi 80 are allelic and differ from the gene of Norin-PL 2. Our conclusion is consistent with the fact that the resistance gene (Grh 3 (t)) of Aichi 80 is located on chromosome 6 (Saka et al., 1997), while that of Norin-PL 2 is located on chromosome 5 (Tamura et al., 1999). The Chikugo population used by Takita and Nishiyama (1989) was certainly virulent to Grh 1-carrying varieties, e.g. IR 24 and Norin-PL 2 (Sato and Sogawa, 1981; Sogawa and Sato, 1981), and therefore, the line selected on Aichi 42 was able to adapt to Grh 1-carrying varieties as well as Grh 3 (t)-carrying varieties.

In the case of the brown planthopper (BPH), Nilaparvata lugens, resistance genes of rice varieties have been identified on the basis of the relationship between resistance gene and BPH biotypes (International Rice Research Institute, 1976; Kaneda et al., 1981; Ikeda, 1985; Nemoto et al., 1989). A similar method can be applied in the case of the characteristics of GRH biotypes and resistant rice varieties. We propose that biotypes that have virulence to Grh 1-, Grh 2-, and Grh 3 (t)-carrying varieties be designated “Biotype 1”, “Biotype 2”, and “Biotype 3”, respectively (Table 5).

The resistance to GRH fluctuated with the growth stage of rice plants; it was highest at the young seedling stage and approximately 20 days before rice heading (Kishino and Ando, 1979; Takeda and Nagata, 1987; Ando, 1998). In the

<table>
<thead>
<tr>
<th>Rice variety</th>
<th>Source of resistance</th>
<th>Resistance gene</th>
<th>Biotype 1</th>
<th>Biotype 2</th>
<th>Biotype 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IR 24</td>
<td>Chugoku 105</td>
<td>Saikai 164</td>
</tr>
<tr>
<td>Nipponbare</td>
<td>None</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IR 24</td>
<td>Grh 1</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Norin-PL 2</td>
<td>Pe-bi-hun</td>
<td>Grh 1</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Chugoku 105</td>
<td>Pe-bi-hun</td>
<td>Grh 1</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Pe-bi-hun</td>
<td>Grh 1+a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saikai 164</td>
<td>C203-1</td>
<td>Unknown</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saikai 182</td>
<td>C203-1</td>
<td>Grh 2</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Norin-PL 5</td>
<td>C203-1</td>
<td>Grh 2+Grh 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norin-PL 6</td>
<td>Lepe dumai</td>
<td>Grh 2+Grh 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanto-PL 6</td>
<td>Tadukan</td>
<td>Grh 3 (t)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aichi 80</td>
<td>Rantai-emas 2</td>
<td>Grh 3 (t)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+: Virulent, -: Avirulent.
present study, varietal resistance at the rice seedling stage was similar to that at the reproductive stage (panicle formation stage to heading stage). This result indicates that a GRH biotype selected on resistant rice seedlings is able to adapt to that variety from the seedling stage to maturity.

This study revealed that artificial selection could shift the GRH to a biotype virulent to a previously resistant rice variety, and hence, suggests the occurrence of such biotypes in the field. To predict and prevent the occurrence of resistance-breaking biotypes, there is a need for further examination of the mode of inheritance of the biotypes, genetic relationships between biotypes, and relationships between the virulence and life-history traits of GRH.

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