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ORIGINAL PAPER

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Molecular mapping of a novel gene, *Grh5*, conferring resistance to green rice leafhopper (*Nephotettix cincticeps* Uhler) in rice, *Oryza sativa* L.

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Abstract The green rice leafhopper (GRH), *Nephotettix cincticeps* Uhler, is one of the most serious insect pests affecting cultivated rice (*Oryza sativa* L.) in temperate regions of East Asia. An accession of the wild rice species, *Oryza rufipogon* Griff. (W1962), was found to be highly resistant to GRH by an antibiosis test. To understand the genetic basis of the GRH resistance, a BC_1F_1 population derived from a cross between a susceptible Japonica variety, Taichung 65 (T65), and a highly resistant accession W1962 was analyzed by quantitative trait loci (QTL) mapping. A single major QTL for GRH resistance was detected on rice chromosome 8. A nearly isogenic population containing segments of the targeted QTL region derived from W1962 was then developed through advanced backcrossing with marker-assisted selection. Further molecular mapping using a BC_4F_2 population revealed that a new resistance gene, designated as Green rice leafhopper resistance 5 (*Grh5*), was located on the distal region of the long arm of chromosome 8 and tightly linked to the simple sequence repeat markers *RM3754* and *RM3761*. A nearly isogenic line (NIL) carrying *Grh5* was subsequently developed in the progeny of the mapping population. The resistance level of *Grh5*-NIL was compared with those of developed NILs for GRH resistance and was found to have the highest resistance. The DNA markers found to be closely linked to *Grh5* would be useful for marker-assisted selection for the improvement of resistance to GRH in rice.

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Introduction

The green rice leafhopper (GRH), *Nephotettix cincticeps* Uhler, is a major insect pest of cultivated rice and is distributed mostly in temperate regions of East Asia (Ghauri 1971). GRH is closely related to green leafhopper (GLH), *N. virescens* Distant, which is a major vector of Tungro, a destructive viral disease found in tropical rice fields in Asia. GRH sucks sap from both the xylem and phloem of susceptible rice varieties, leading to yield loss particularly in northeast Japan (Nirei and Nakazato 1975). In addition to direct plant destruction, the insect also causes damage to rice plants by transmitting other viral diseases, including the rice dwarf and waika viruses seen commonly in western Japan (Nakasuji and Nomura 1968).

The development of resistant cultivars is generally considered to be the most effective and economical means for defending against insect pests. Host plant resistance to insects has been classified into three mechanistic types: antibiosis, antixenothis and tolerance (Painter 1951). However, distinguishing between these mechanisms has been difficult using bulk seedling tests (Athwal et al. 1971). Kishino and Ando (1978) established a simple method for evaluating antibiosis to GRH, and the survival ratio of the GRH nymphs was examined on test varieties. Upon evaluation, several Indica varieties, including Rantj-emas 2, Tadukan, C203-1, Lepedumai and Pe-bi-hun, have been shown to be resistant to GRH, while all of the Japonica varieties were found to be susceptible. Genetic analyses of GRH resistance have been carried out, and several loci for GRH resistance have been identified (Kobayashi et al. 1980; Ikeda et al. 1989; Imbe and Iwasaki 1987). These GRH-resistance genes were then introduced into several cultivated rice varieties including Norin PL2, Norin PL6, Aichi 42 and Kanto PL10. The resistance genes *Grh1* from Pe-bi-hun (Tamura et al. 1999), *Grh3*(t) from Rantj-emas 2 (Saka et al. 1997), and two dominant genes, *Grh2* and

Grh4, both from Lepedumai and DV85 (Fukuta et al. 1998; Yazawa et al. 1998), have been commonly found in local rice varieties (Fujita et al. 2002) and were mapped on chromosomes using restriction fragment length polymorphism (RFLP) markers. Two additional loci conferring resistance to GRH were recently identified in two accessions of wild species and in a Surinam variety. They were tentatively named as *Grh5*(t), from *Oryza rufipogon* (Fujita et al. 2003), *Grh6*(t) from the Surinam variety (Tamura et al. 2004) and *Grh6-nivara*(t) from *Oryza nivara* (Fujita et al. 2004). In contrast, only one major resistance gene against GLH has been mapped near the *Grh6* region on chromosome 4 (Sebastian et al. 1995), despite the fact that more than 13 GLH resistance genes have been identified using the bulk seedling test.

The utilization of exotic germplasm enriched for genes conferring yield and resistance to stress is an effective approach to exploit cultivars. The genetic comparisons of modern cultivars and wild relatives using DNA markers have indicated that rice improvement is still practiced on a narrow genetic base (Tanksley and McCouch 1997). Thus, exotic rice germplasm has the genetic potential to be useful for detecting new genes/alleles not present in cultivated rice. The ancestral wild species of cultivated rice, *Oryza rufipogon* Griff., is an important germplasm for rice improvement. From comparison of the genetic diversity of *O. rufipogon* and *O. sativa* using RFLP markers, native populations of *O. rufipogon* were shown to harbor significantly greater genetic diversity than cultivated rice (Sun et al. 2001). Furthermore, *O. rufipogon* has more resources than that of local varieties for resistance to insect pests that include the brown planthopper (BPH), the whitebacked planthopper and the green and zigzag leafhoppers (Heinrichs et al. 1985).

Simple sequence repeat (SSR) marker loci are widely distributed throughout the genome and can be easily analyzed using the polymerase chain reaction (PCR) (McCouch et al. 2002). SSR markers have been extensively used to map agronomically important loci in rice, such as BPH resistance, leaf and neck blast resistance and bacterial blight disease resistance (Gu et al. 2004; Sirithunya et al. 2002; Yang et al. 2002).

The objectives of the present study were to explore the genetic basis for GRH resistance found in wild rice species, and to thereby facilitate the use of exotic germplasm for future rice improvements. First, quantitative trait loci (QTL) analysis for resistance to GRH was conducted by using an initial mapping population (BC_1F_1) derived from the cross between a susceptible cultivar and a highly resistant accession of *O. rufipogon*. Subsequently, a new locus for GRH resistance was mapped onto a molecular linkage map using a nearly isogenic population, which was developed by continuous backcrossing and marker-assisted selection (MAS) of the targeted QTL region. Finally, we described the rapid and successful development of a valuable nearly isogenic line (NIL) carrying resistance to GRH.

Materials and methods

Plant materials

A BC_1F_1 population was developed from the cross between *O. sativa*, a Japonica variety, Taichung 65 (T65) and a wild accession of *Oryza rufipogon* (W1962) from China. The BC_1F_1 population was evaluated for resistance to GRH and was subsequently used for QTL analysis. Several BC_1F_1 plants were repeatedly backcrossed with T65 as a recurrent parent to develop the BC_3F_1 generation. Among the 145 BC_3F_1 plants, 9 resistant BC_3F_1 plants were selected via the antibiosis test and were backcrossed with T65. A resistant BC_4F_1 plant was then self-pollinated and the BC_4F_2 population was used for mapping of a GRH resistance gene locus.

Evaluation of GRH resistance

The GRH population was collected in Fukuoka Prefecture in 1991 and was maintained by continuously rearing the insects on seedlings of the Japonica variety Nipponbare. Insects were kept at 25°±1°C and 16 h light: 8 h dark. The antibiosis test, as reported by Kishino and Ando (1978), was modified for use in the present study. In the antibiosis test of seedlings, the seedlings were infested with 7–10 first- or second-instar nymphs in test tubes approximately 2 weeks after sowing. In addition, the upper-most leaf blades under the flag leaves were excised and infested with 7–10 first- or second-instar nymphs. Nymph mortality was then calculated at 4 days after infestation. In this study, plants with nymph mortality found to be in the range of 0–40% were categorized as susceptible, and those with 60–100% nymph mortality were categorized as resistant. The seedlings of the BC_1F_1 population (46 individuals) and the leaf blades of BC_3F_1 (145 individuals) and BC_4F_2 populations (94 individuals) at the flag leaf stage were used for the antibiosis test. QTL analysis was performed given the results of the nymph mortalities in the BC_1F_1 population.

Genotyping using SSR markers

Total DNA of 46 BC_1F_1 and 94 BC_4F_2 individuals was extracted from fresh leaves of individual plants by the CTAB method (Murray and Thompson 1980). The genotypes of SSR loci in BC_1F_1 and BC_4F_2 individuals were then determined by PCR amplification in a PCR System-9700 (PerkinElmer, Massachusetts). The 15 µl PCR reaction mixture contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 200 µM dNTP, 0.2 µM primer, 1 unit of Taq polymerase (Takara, Japan) and 5–10 µg/ml of genomic DNA as template. The thermal cycler was programmed for a first denaturation step

of 5 min at 94°C, followed by 35 cycles, each of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s. The SSR products were resolved in 2.5% agarose gels by electrophoresis at 160 V for 1 h in 0.5XTBE buffer. The gels were stained with ethidium bromide and photographed under ultraviolet light.

Construction of a linkage map and QTL analysis

The genetic map for the BC_1F_1 population was constructed using a total of 100 SSR markers distributed across the 12 rice chromosomes. The resulting map spanned 1,504 cM, with an average interval of 15.1 cM between markers. Linkage of loci and map distances were determined using Mapmaker/Exp 3.0 (Lander et al. 1987). The nymph mortalities and the genotypic data of the BC_1F_1 population were used together for QTL analysis. Simple interval mapping was conducted using Windows QTL Cartographer V2.0 (Wang et al. 2004). A critical threshold value of the LOD score for QTL detection was calculated by conducting 1,000 permutation tests and was equivalent to 20.4 at a significance level of 0.05.

Mapping of GRH resistance gene

The BC_4F_2 population was used for linkage analysis of the GRH resistance gene and SSR markers. The genotypes of GRH resistance in the BC_4F_2 individuals were determined from the BC_4F_2 phenotypes and from each SSR marker genotype. Unconfirmed genotypes of GRH resistance in BC_4F_2 individuals were confirmed by an F_3 progeny test. Further detailed linkage analysis was conducted using additional SSR markers including *RM1615*, *RM502*, *RM3754*, *RM3761*, *RM6845*, *RM3155* and *RM4154* on chromosome 8.

Comparison of nymph mortality among NILs for GRH resistance genes

Five NILs, including *Grh5*-NIL, were evaluated for GRH resistance at the seedling stage with T65 used as a susceptible control. A NIL (BC_3F_4) for the GRH resistance gene, *Grh1*, was developed from the cross between the susceptible T65 carrying no GRH resistance genes and the resistant Indica variety IR24, which carries only *Grh1*, and through continuous backcrossing with T65 and MAS for *Grh1*. The other NILs (BC_6F_5) of single and combined *Grh2* and *Grh4* were developed from crosses between the susceptible T65 and the highly resistant Indica variety DV85 carrying both *Grh2* and *Grh4*, through continuous backcrossing with T65 and MAS. The *Grh5*-NIL was selected from the BC_4F_4 progeny through MAS. These NILs for GRH resistance were infested by GRH at the seedling stage, and their levels of resistance were compared by calculating nymph mortalities at 4 days after infestation. This experiment was repeated at least three times and separation of the means was statistically analyzed using the Tukey–Kramer test following analysis of variance.

Results

Detection of QTL for GRH resistance

QTL analysis using the BC_1F_1 population was performed to understand the genetic basis of resistance to GRH in W1962. W1962 showed high nymph mortality (95.8%), whereas T65 did not show any nymph mortality (0%). Twenty-four individuals in the BC_1F_1 population were resistant to GRH while 22 were susceptible (Fig. 1). This segregation ratio fit the expected 1:1 ratio for a single dominant gene model ($\chi^2 = 0.09$). The BC_1F_1 population was analyzed using a total of 100 SSR markers widely distributed throughout the genome. A single major QTL (LOD = 35.1) controlling GRH resistance was subsequently identified on chromosome 8 with a contribution of 95.2% to the phenotypic variation (Table 1; Fig. 2). This QTL, designated *qGRH-8*, was within a 7.0 cM interval flanked by *RM502-RM3155* on the long arm of chromosome 8. The W1962 allele at the *qGRH-8* locus showed a positive effect for resistance to GRH. No other QTLs of significance were detected.

Mapping of GRH resistance gene

A nearly isogenic population carrying *qGRH-8* was developed through advanced backcrossing and MAS. Among a total of 145 BC_3F_1 plants, 9 plants showing resistance to GRH were backcrossed with T65. A resistant plant eventually selected from the BC_4F_1 individuals was analyzed to confirm retained chromosomal segments using 437 SSR markers. The chromosomal segments derived from W1962 were confirmed in each one of the five regions of chromosomes 2, 3, 4, 8 and 9, involving *qGRH-8* (Fig. 3). In case of calculating the genetic distance of chromosome segments based on SSR marker position, a total size of the chromosomal segments introgressed from W1962 was approximately 49.7 cM. The BC_4F_1 plant was self-pollinated and analyzed for mapping the GRH resistance gene. The nymph mortality of the BC_4F_2 population showed discrete bimodal segregation

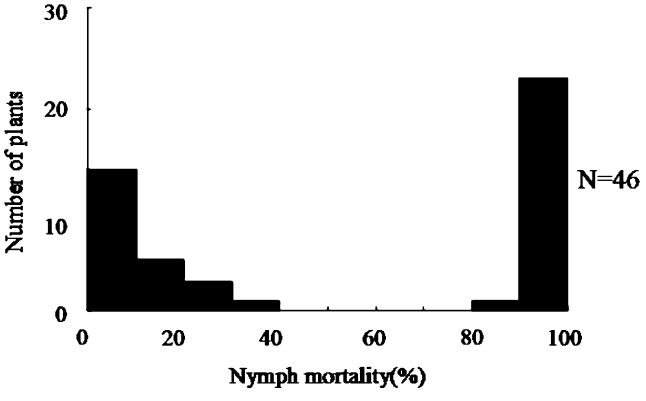

Fig. 1 Frequency distribution of GRH nymph mortality in the BC_1F_1 population derived from T65/W1962//T65

Table 1 Quantitative trait locus affecting resistance to green rice leafhopper, *Nephotettix cincticeps* Uhler, in the BC_1F_1 population derived from T65/W1962//T65

QTL	Chromosome	Marker interval	Interval distance (cM)	Peak LOD score[a]	PEV (%)[b]	Additive effect[c]
qGRH-8	8	*RM502–RM3155*	7.0	35.1	95.2	−92.4

[a] Critical threshold value of LOD score was equivalent to 20.3 at an experiment-wise significance level of 0.05
[b] Percentage of explained phenotypic variation
[c] Negative value indicates effect from W1962

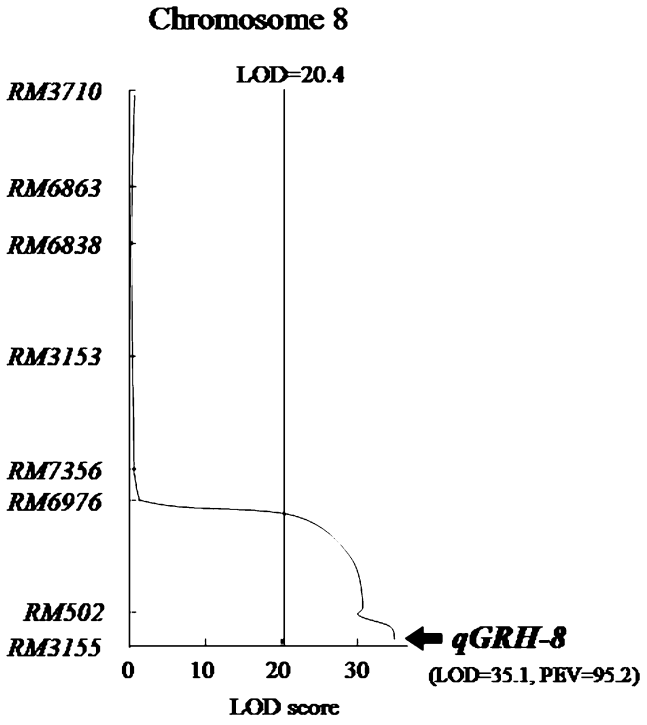

Fig. 2 Location of *qGRH-8* conferring resistance to GRH on the linkage map of rice chromosome 8 constructed by SSR markers using a BC_1F_1 population derived from T65/W1962//T65

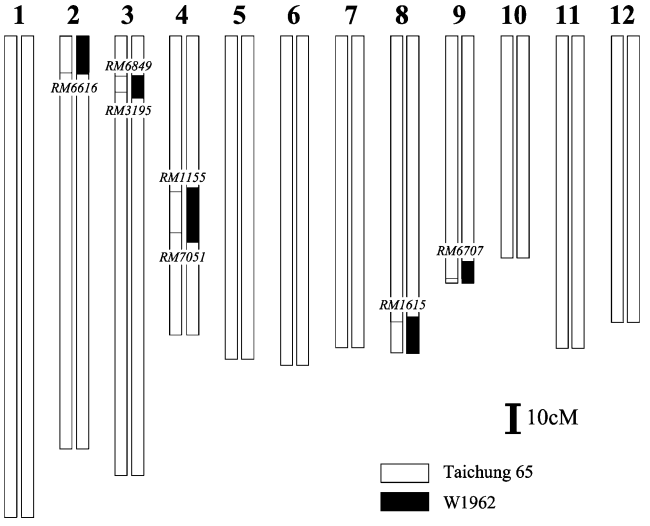

Fig. 3 The graphical genotype of a selected BC_4F_1 plant postulated by bulked DNA of BC_4F_2. The *white squares* show the chromosomal segment of Taichung 65 while the *black squares* show the chromosomal segment of W1962. Only the co-segregation between GRH resistance and the retained segments of rice chromosome 8 was detected

with 0–40% mortality in 29 susceptible individuals and 60–100% mortality in 65 resistant individuals (Fig. 4). This segregation ratio fit a 3:1 ratio ($\chi^2 = 1.72$), indicating that a single dominant gene controlled GRH resistance in the BC_4F_2 population. We designated this new resistance gene as *Grh5* (Green rice leafhopper resistance 5), since no genes for resistance to GRH have yet been reported on chromosome 8. The BC_4F_2 population was subsequently analyzed for GRH resistance using SSR markers near the *qGRH-8* region of chromosome 8. GRH resistance completely co-segregated with the SSR marker loci, *RM3754* and *RM3761*, on chromosome 8. As shown in Fig. 4, 29 individuals having T65 homozygous alleles (white area) at *RM3754* exhibited susceptibility with low nymph mortality (0–40%), while 65 individuals, having either W1962 homozygous alleles (black area) or heterozygous (diagonal area) at *RM3754*, exhibited resistance with high nymph mortality (60–100%). The unconfirmed *Grh5* genotypes in the BC_4F_2 individuals were confirmed through phenotypic evaluation of the BC_4F_3 progeny. Thus, F_3 tests served to distinguish the heterozygous allele from the W1962 homozygous allele at *Grh5* in the BC_4F_2 individuals showing resistance. Linkage analysis was conducted using the *Grh5*, *RM1615* and *RM6845* genotypes. Table 2 shows that *Grh5* was linked to the SSR markers, *RM1615* and *RM6845*. A total of ten individuals were found to be recombinant in the interval from *RM1615* to *RM6845*, one of which was a double recombinant. Seven individuals were found to be recombinants in the interval from *RM1615* to *Grh5*, and four individuals were found to be recombinants in the interval from *Grh5* to *RM6845*. The genetic distances between *RM1615* and *RM6845*, *RM1615* and *Grh5* and *Grh5* and *RM6845* were 4.9, 3.8 and 2.2 cM, respectively. Finally, the resistance gene, *Grh5*, was located between *RM1615* and *RM6845* and co-segregated with the SSR marker loci, *RM3754* and *RM3761* (Fig. 5).

Comparison of nymph mortality among NILs for GRH resistance genes

The NIL for *Grh5*/*Grh5* was selected from the BC_4F_4 population using MAS. Individuals carrying *grh5*/*grh5* in the BC_4F_4 population were segregated into moderately resistant and susceptible plants when the plants were

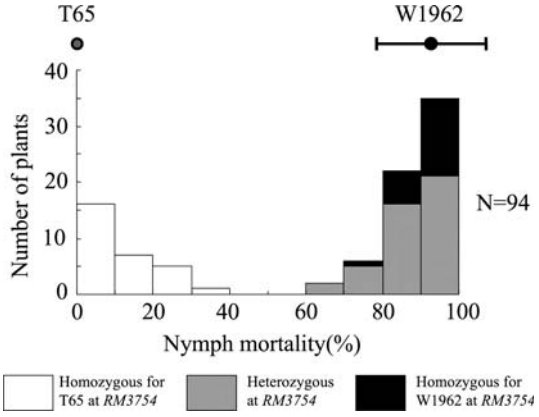

Fig. 4 Frequency distribution of GRH nymph mortality in the BC_4F_2 population derived from a cross between W1962 and Taichung 65. The original resistant accession W1962 showed a nymph mortality of 90.9%, whereas the susceptible variety T65 showed a nymph mortality of 0%. *Black*, *diagonal* and *white areas* show the homozygous allele for W1962, the heterozygous allele and the homozygous allele for T65 at *RM3754*, respectively

kept at more than 5 days after infestation. The moderately resistant plants showed susceptibility at 4 days after infestation; however, the plants changed phenotype to moderate resistance (30–70%) at 5 days after infestation (data not shown). This result suggested that an original accession of *O. rufipogon* (W1962) has another resistance gene with a minor effect in addition to *Grh5*, because W1962 showed high resistance at less than 4 days after infestation. The chromosomal segments introduced from W1962 were retained on chromosomes 2, 3, 4, 8 and 9 in the selected BC_4F_1 plant (Fig. 3). The individuals carrying *grh5*/*grh5* in the BC_4F_4 population were analyzed for retained segments on chromosomes 2, 3, 4 and 9 using SSR makers. A minor resistance gene co-segregated with the SSR markers on chromosome 4. This result indicated that the moderately resistant plants must have a minor gene on chromosome 4 in the genetic background of *grh5*/*grh5*. Although there was no QTL with a LOD score beyond 1.0 on chromosome 4, the position showed a peak LOD in the BC_1F_1 population. In the genetic background of *grh5*/*grh5*, the plants carrying the minor resistance gene showed moderate resistance with comparatively higher nymph mortality (55.7%) than T65. In the genetic background of *Grh5*/*Grh5*, however, the effect of the minor resistance gene was totally masked by the major resistance gene, *Grh5*, and no phenotypic difference was found among the genotypes at a locus for the minor resistance gene. A *Grh5*-NIL, excluding the minor resistance gene, was selected in the BC_4F_4 population using MAS. To compare the *Grh5*-NIL with the NILs of GRH resistance genes, all the available NILs for GRH resistance genes were evaluated using the antibiosis test. The average nymph mortalities of the NILs are shown in Fig. 6. The nymph mortality of the NIL for *Grh2*/*Grh2*, *Grh4*/*Grh4* was highest (100.0%) among the NILs for GRH resistance genes. Nymph mortality was categorized as highly resistant for both *Grh5*/*Grh5* (86.9%) and *Grh1*/*Grh1* (82.2%). The average nymph mortality of the NILs for *Grh2*/*Grh2* was 74.6%. Nymph mortality of the NIL for *Grh4*/*Grh4* (3.9%) was low and almost equal to that of the susceptible variety T65 (6.9%).

Discussion

Six GRH resistance genes have been precisely mapped on rice chromosomes to date. *Grh1* was identified near the centromeric region of chromosome 5 (Tamura et al. 1999). *Grh2* and *Grh4* have been reported as a pair of dominant resistance genes to GRH with complementary expression and located on chromosomes 11 and 3, respectively (Fukuta et al. 1998; Yazawa et al. 1998). *Grh3* was found to be located proximal to the centromeric region on chromosome 6 (Saka et al. 1997). We identified a new GRH resistance gene, *Grh5*, located on the long arm of chromosome 8. *Grh6*, derived from a cultivar SML 17, was identified on the short arm of chromosome 4 (Tamura et al. 2004). At the same chromosomal location, a new allele of *Grh6*, *Grh6-nivara*, was identified from *O. nivara* (Fujita et al. 2004), which we will describe in another paper. In this study, SSR markers linked to *Grh5*, *RM3754* and *RM3761* were found and used to develop *Grh5*-NIL. These SSR markers will facilitate selection of resistant genotypes through MAS.

Virulent insect pests, the so-called new biotypes, often appear after the release of modern improved varieties of rice that carry a single major gene for resistance to insect pests. These pests represent a serious threat to rice paddies, because they have acquired virulence to the specific resistance gene, which will have subsequently lost its effectiveness in insect pest management. For example, the BPH population immigrating into Japan began to become virulent to the Brown planthopper resistance 1 (*Bph1*) in the

Table 2 Linkage relationship between green rice leafhopper resistance and SSR markers in the BC_4F_2 population derived from a cross between W1962 and Taichung 65

Locus[a]		Number of plants										Recombination value (%)	Genetic distance (cM)
A(a)	B(b)	AABB	AABb	Aabb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb	Total		
RM1615	*Grh5*	28	0	0	1	40	2	0	4	19	94	3.8±1.4	3.8±1.4
RM1615	*RM6845*	27	1	0	3	38	2	0	3	20	94	4.9±1.6	4.9±1.6
Grh5	*RM6845*	28	1	0	2	41	1	0	0	21	94	2.1±1.1	2.2±1.1

[a] Large letter showed Taichung 65 allele, while small letter showed W1962 allele

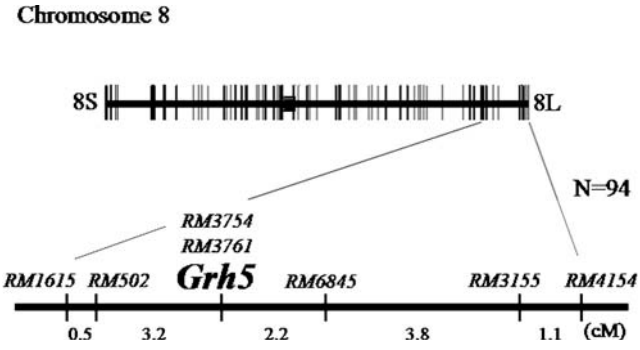

Fig. 5 Linkage map indicating the position of *Grh5*, a gene for resistance to GRH, on rice chromosome 8. Framework map in the upper portion of the figure is taken from Harushima et al. (1998)

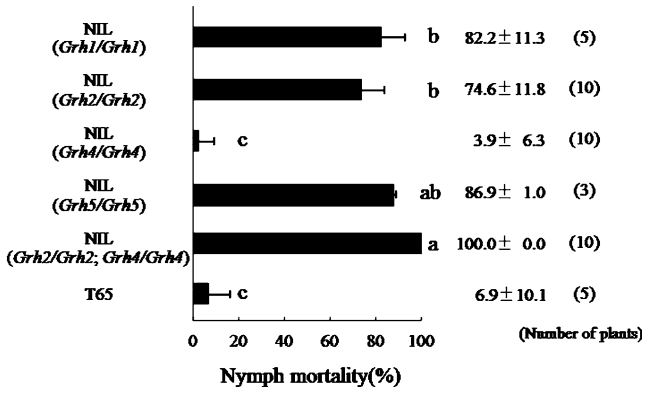

Fig. 6 GRH nymph mortality of NILs carrying *Grh1*/*Grh1*, *Grh2*/*Grh2*, *Grh4*/*Grh4*, *Grh5*/*Grh5* and *Grh2*/*Grh2*; *Grh4*/*Grh4* at 4 days after infestation. Means denoted with different letters are significantly different at the 5% level according to Tukey–Kramer test

late 1980s (Sogawa 1992) and has become highly virulent to rice varieties carrying both *Bph1* and *bph2* since the late 1990s (Tanaka and Matsumura 2000). Recently, both *Bph1* and *bph2* have completely lost their effectiveness in insect pest management in East Asia. The virulent biotypes of BPH were experimentally identified by continuous rearing of BPH on resistant lines, each carrying a single major gene for BPH resistance (Ketipearachchi et al. 1998). By a similar methodology, virulent biotypes against each of the three resistance genes *Grh1*, *Grh2* and *Grh3* were isolated (M. Hirae, personal communication). These data suggested that natural strains of GRH are likely to feed on rice plants having a single major gene for resistance. In contrast, virulent biotypes against pyramiding lines carrying both *Grh2* and *Grh4* did not occur experimentally (M. Hirae, personal communication). In line with these findings, we have demonstrated that, although the nymph mortality of the *Grh4*-NIL showed susceptibility to GRH, the NIL carrying *Grh2* and *Grh4* showed higher nymph mortality than *Grh2*-NIL alone. Additionally, both *Grh2* and *Grh4* have been essential to express resistance to GLH, which is a related species of GRH (Yasui and Yoshimura 1999). The pyramiding lines carrying *Grh2* and *Grh4* may thus have an important roll in expressing durable resistance to the rice leafhoppers. It suggests that gene pyramiding that combines multiple resistance genes with different mechanistic types will suppress the dominancy of virulent biotypes in the insect population. In this study, we found that the high resistance of W1962 was dependent on two loci conferring resistance to GRH, *Grh5* and the minor resistance gene on chromosome 4. The pyramiding lines carrying these resistance genes may suppress the dominancy of virulent biotypes and show durable resistance to GRH. To study durability of the resistance, the development of pyramiding lines for GRH resistance is essential. The development of pyramiding lines carrying multiple resistance genes through phenotype alone is difficult when the difference between a single gene and multiple resistance genes cannot be distinguished phenotypically. As such, SSR markers that are linked to resistance genes will become important tools in the development of these pyramiding lines. SSR markers tightly linked to resistance genes enable the selection of pyramiding lines carrying multiple resistance genes without the need for evaluating the resistance to insect pests. The novel resistance gene, *Grh5*, and its tightly linked SSR markers, *RM3754* and *RM3761,* will serve to increase durability of the resistance against virulent biotypes.

The development of multiple resistant lines from different origins may be advantageous for increasing overall resistance. Wild species have more sources of resistance to the insect pests, BPH, whitebacked planthopper, GLH and zigzag leafhopper than landraces do (Heinrichs et al. 1985). The BPH resistance genes from the wild species *O. officinalis*, *O. australiensis* and *O. latifolia* have been transferred to cultivated rice varieties and resistant introgression lines have been developed (Ishii et al. 1994; Jena and Khush 1990; Yang et al. 2002). BPH resistant lines derived from *O. officinalis* were identified as having multiple resistance genes through genetic analysis (Huang et al. 2001), suggesting that several resistant wild species may carry multiple genes for resistance to insect pests. Thus, W1962 was analyzed by QTL analysis in order to understand the genetic basis of GRH resistance. The SSR markers *RM3754* and *RM3761*, which are tightly linked to *Grh5*, will facilitate selection of resistant genotypes for the purpose of rice improvement. Transferring GRH resistance from wild species can be facilitated by using MAS with advanced backcrossing with the recurrent parent. The NILs for resistance genes derived from exotic germplasm are thus useful for the improvement of GRH resistance in rice breeding programs.

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References

Athwal DS, Pathak MD, Bacalangco EH, Pura CD (1971) Genetics of resistance to brown planthoppers and green leafhoppers in *Oryza sativa* L. Crop Sci 11:747–750

Fujita D, Yoshimura A, Yasui H (2002) Detection of QTLs associated with antibiosis to green rice leafhopper, *Nephotettix cincticeps* Uhler, in four Indica rice varieties. Rice Genet Newslett 19:90–91

Fujita D, Doi K, Yoshimura A, Yasui H (2003) Mapping of a new resistance gene for green rice leafhopper introgressed from *Oryza rufipogon* Griff. into cultivated rice, *Oryza sativa* L. Rice Genet Newslett 20:79–80

Fujita D, Doi K, Yoshimura A, Yasui H (2004) Introgression of a resistance gene for green rice leafhopper from *Oryza nivara* into cultivated rice, *Oryza sativa* L. Rice Genet Newslett 21:64–66

Fukuta Y, Tamura K, Hirae M, Oya S (1998) Genetic analysis of resistance to green rice leafhopper (*Nephotettix cincticeps* Uhler) in rice parental line, Norin-PL6, using RFLP markers. Breed Sci 48:243–249

Ghauri MSK (1971) Revision of the genus *Nephotettix* Matsumura (Homoptera:Cicadelloidea:Euscelidae) based on the type material. Bull Entomol Res 60:481–512

Gu K, Tian D, Yang F, Wu L, Sreekala C, Wang D, Wang G-L, Yin Z (2004) High-resolution genetic mapping of *Xa27*(t), a new bacterial blight resistance gene in rice, *Oryza sativa* L. Theor Appl Genet 108:800–807

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboki Y, Yamamoto T, Lin SY, Antonio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush GS, Sasaki T (1998) A high-density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics 148:479–494

Heinrichs EA, Medrano FD, Rapusas HR (1985) In: Heinrichs EA, Rapusas H, Medrano F (eds) Genetic evaluation for insect resistance in rice. International Rice Research Institute, Los Banos, pp 1–356

Huang Z, He G, Shu L, Li X, Zhang Q (2001) Identification and mapping of two brown planthopper resistance genes in rice. Theor Appl Genet 102:929–934

Ikeda R, Takeda M, Higashi T (1989) Genetic analysis of resistance to green rice leafhopper, *Nephotettix cincticeps*, on rice Norin-PL6 (In Japanese with English abstract). Ann Rept Plant Prot North Japan 40:84–86

Imbe T, Iwasaki M (1987) Inheritance of resistance to the green rice leafhopper *Nephotettix cincticeps* UHLER and dwarf disease in rice Norin-PL5 (In Japanese with English summary). Jpn J Breed 37:177–184

Ishii T, Brar DS, Multani DS, Khush GS (1994) Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice, *O. sativa*. Genome 37:217–221

Jena KK, Khush GS (1990) Introgression of genes from *Oryza officinalis* Well ex Watt to cultivated rice, *O. sativa* L. Theor Appl Genet 80:737–745

Ketipearachchi Y, Kaneda C, Nakamura C (1998) Adaptation of the brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), to BPH resistant rice cultivars carrying *bph8* or *Bph9*. Appl Entomol Zool 33:497–505

Kishino K, Ando Y (1978) Insect resistance of the rice plant to green rice leafhopper *Nephotettix cincticeps*, UHLER. 1. Laboratory technique for testing the antibiosis (In Japanese with English abstract). Jpn J Appl Entomol Zool 22:169–177

Kobayashi A, Kaneda C, Ikeda R, Ikehashi H (1980) Inheritance of resistance to green rice leafhopper, *Nephotettix cincticeps*, in rice (In Japanese). Jpn J Breed 30(suppl 1):56–57

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D, Stein L (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res 9:199–207

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Nakasuji F, Nomura S (1968) A study on the injury by the green rice leafhopper, *Nephotettix cincticeps* Uhler (In Japanese). Proc Assoc Pl Prot Sikoku 3:21–26

Nirei M, Nakazato T (1975) Some notes on the decrease of rice yield caused by green rice leafhopper (In Japanese). Proc Assoc Pl Prot Hokuriku 23:41–43

Painter H (1951) Insect resistance in crop plants. Macmillan Company, New York, pp 1–520

Saka N, Toyama T, Tuji T, Nakamae H, Izawa T (1997) Fine mapping of green ricehopper resistant gene *Grh-3(t)* and screening of *Grh-3(t)* among green ricehopper resistant and green leafhopper resistant cultivars in rice (In Japanese). Breed Sci 47(suppl 1):55

Sebastian LS, Ikeda R, Huang N, Imbe T, Coffman WR, McCouch SR (1995) Molecular mapping of resistance genes to rice tungro spherical virus and green leafhopper in rice. Phytopathology 86:25–30

Sirithunya P, Tragoonrung S, Vanavichit A, Pa-In N, Vongsaprom C, Toojinda T (2002) Quantitative trait loci associated with leaf and neck blast resistance in recombinant inbred line population of rice (*Oryza sativa*). DNA Res 9:79–88

Sogawa K (1992) Rice brown planthopper (BPH) immigrants in Japan change biotype. Int Rice Res Newslett 17(2):26–27

Sun CQ, Wang XK, Li ZC, Yoshimura A, Iwata N (2001) Comparison of the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*O. sativa* L.) using RFLP markers. Theor Appl Genet 102:157–162

Tamura K, Fukuta Y, Hirae M, Oya S, Ashikawa I, Yagi T (1999) Mapping of the *Grh1* locus for green rice leafhopper resistance in rice using RFLP markers. Breed Sci 49:11–14

Tamura K, Fukuta Y, Hirae M, Oya S, Ashikawa I, Yagi T (2004) RFLP mapping of a new resistance gene for green rice leafhopper in Kanto PL10. Rice Genet Newslett 21:62–64

Tanaka K, Matsumura M (2000) Development of virulence to resistant rice varieties in the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae), immigrating into Japan. Appl Entomol Zool 35:529–533

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Wang S, Basten CJ, Zeng Z-B (2004) Windows QTL Cartographer 2.0. Raleigh, NC, Department of Statistics, North Carolina State University

Yang H, Ren X, Weng Q, Zhu L, He G (2002) Molecular mapping and genetic analysis of a rice brown planthopper (*Nilaparvata lugens* Stål) resistance gene. Hereditas 136:39–43

Yasui H, Yoshimura A (1999) QTL mapping of antibiosis to green leafhopper, *Nephotettix virescens* Distant and green rice leafhopper, *Nephotettix cincticeps* Uhler in rice, *Oryza sativa* L. Rice Genet Newslett 16:96–98

Yazawa S, Yasui H, Yoshimura A, Iwata N (1998) RFLP mapping of genes for resistance to green rice leafhopper (*Nephotettix cincticeps* Uhler) in rice cultivar DV85 using near isogenic lines (In Japanese with English summary). Sci Bull Fac Agr Kyushu Univ 52:169–175