

Characterization of Resistance to the Green Rice Leafhopper (*Nephotettix cincticeps* **Uhler) in a Core Collection of Landraces in Rice (***Oryza sativa* **L.)**

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Abstract

The green rice leafhopper (GRH; *Nephotettix cincticeps* Uhler) is one of the most devastating insect pests of cultivated rice (Oryza sativa L.) in temperate regions in Asia. Using the rice germplasms with biotic stress resistance is the most effective and environmentally-friendly way to control the insect pests in the paddy. Sixty accessions from a core set of worldwide collection of rice were characterized for resistance to the GRH by antibiosis test both at the seedling and at the booting stages. The positive correlations of average nymph mortality (ANM) were observed between at the seedling stage and at the booting stage on 3 days after infestation (DAI) ($r = 0.684**$), 5DAI ($r =$ 0.680**), and 7DAI ($r = 0.652$ **), respectively. This result will give us the opportunity to screen resistance to the GRH with the cost-efficient way using rice seedlings in a growth chamber. To classify the 60 accessions evaluated, the ANM of the GRH of each accession was compared to the respective ANM of resistant and susceptible controls with the least significant difference (LSD) value. Based on the statistical difference or similarity of the ANMs to the resistant and the susceptible controls, we proposed the four groups of resistance to the GRH, (I) high level of resistance, (II) considerable level of resistance, (III) moderate level of resistance, and (IV) susceptibility. At the seedling stage, a total of 26 accessions were highly resistant in addition to other 6 for considerable level of resistance and other 10 for moderate level of resistance. At the booting stage, on the other hand, a total of 18 accessions were highly resistant in addition to other 3 for considerable level of resistance and other 5 for moderate level of resistance. A total of 42 accessions with high to moderate level of resistance were distributed across 16 countries in Asia in addition to each one

for Madagascar and USA. The classification of landraces based on the present protocol for screening resistance to the insect provided fundamental information for genetics and breeding on resistance to the GRH in rice.

Keywords

Rice, Core Collection, Green Rice Leafhopper, Correlation, Germplasm

1. Introduction

The green rice leafhopper (GRH: Nephotettix cincticeps Uhler) is one of the most devastating insects of cultivated rice (Oryza sativa L.) and distributed in Asia [\[1\].](#page-8-0) The GRH not only causes damage by sucking sap from phloem and xylem of susceptible cultivars [\[2\]](#page-8-1) [\[3\],](#page-8-2) but also by transmitting viruses such as the rice dwarf and the yellow dwarf as a vector [\[1\]](#page-8-0) [\[4\].](#page-8-3) Rice plants are most sensitive to the GRH at both seedling and booting stages. The sucking and oviposition by the GRH often make plants dwarfish. It causes feasible condition to the plants for the infection by either fungi or bacteria, or both of them. Heavy infestation of the GRH around the booting to the heading stages makes the rice plants necrotic and often leads severe yield losses in rice.

Among the various kinds of pest managements, deployment of resistant cultivars is one of the most effective ways to control the GRH in the paddy without any environmental burden. Several GRH resistance genes derived from landraces and wild relatives have been introduced to the elite genetic background of rice cultivars [\[5\],](#page-8-4) such as Norin PL2 (*GRH1*) [\[6\],](#page-8-5) Aichi 80 (*GRH3*(t)) [\[7\],](#page-8-6) Norin-PL5 $(GRH1 + GRH2)$ [\[8\],](#page-8-7) and Norin-PL6 $(GRH2 + GRH4)$ [\[9\].](#page-8-8) One of the constraints to the deployment of resistant cultivars is the dominance of virulent insects against the resistance genes soon after the release of the resistant cultivars. Screening of a large-scale germplasm for GRH resistance is required to ensure useful resources of resistance genes and to utilize them for future breeding programs in rice. Screening "core collections" of crops is recommended to explore resistance resources to abiotic and biotic stresses [\[10\].](#page-8-9) In the concept of the core collection, it consists of a limited number of accessions represented as much as possible of the genetic diversity of the whole germplasms [\[10\]](#page-8-9) [\[11\].](#page-8-10) Core collections have been developed based on the genome-wide DNA markers as well as their agronomical traits in several crop species such as rice, maize, and sorghum [\[12\]](#page-8-11) [\[13\]](#page-8-12) [\[14\]](#page-8-13) [\[15\].](#page-9-0) Evaluations of core collections were performed in the tolerance or resistance to abiotic and biotic stresses and favorable resources have been effectively screened [\[16\]](#page-9-1) [\[17\].](#page-9-2)

In the present study, 60 out of 69 accessions of a core set of rice landraces were characterized for the response to the GRH [\[13\].](#page-8-12) The objectives of this study were to develop the efficient technique for the characterization of the response to the GRH in rice and to identify the resources with resistance to the GRH in a core set of rice landraces.

2. Materials and Methods

2.1. Plant Materials

A core set of rice cultivars consists of 69 accessions, which was provided by National Institute of Agrobiological Sciences, Japan, was used in the present study [\[13\].](#page-8-12) Among them, sixty-three are landraces and remaining 6 are modern cultivars. This core set of rice cultivars retained 91% of the alleles detected in the original 332 accessions and covered the variation of the initial set of accessions in terms of several agro-morphological traits. This core collection distributed across 16 counties in Asia and one accession in each USA, Brazil and Madagascar. Due to the limitation of the seed stocks, nine accessions were excluded for the evaluation of the resistance to the GRH. Those were WRC54, WRC60, WRC62, WRC64, WRC67, WRC97, WRC98, WRC99 and WRC100. The culti-var "DV85" (Oryza sativa L. ssp. indica) carrying GRH2 and GRH4 [\[18\]](#page-9-3) [\[19\]](#page-9-4) was used as a resistant control and "Taichung 65" (T65; Oryza sativa L. ssp. japonica) with none of the resistance gene to the GRH was used as a susceptible control.

2.2. The GRH Strain

The GRH strain was collected in Fukuoka (N33˚38' E130˚28') Japan in 1991. The GRH strain has been maintained by continuous rearing more than 200 generations on seedlings of the susceptible japonica cultivar Nipponbare under the condition of 25° C \pm 1 $^{\circ}$ C and 16 h light: 8 h dark.

2.3. Evaluation of GRH Resistance

Antibiosis test for GRH resistance reported by Kishino and Ando [\[20\]](#page-9-5) was modified and used in the present study. Seven-day-old rice seedlings of each accession were infested with 10 first-in star nymphs in a test tube with five replications for the seedling test. The average nymph mortality (ANM) of each accession was then calculated as the mean of the five replications ($(ANM = (Rep.1 +$ Rep.2 + Rep.3 + Rep.4 + Rep.5)/5) at 3 days after infestation (DAI), 5DAI, and 7DAI, respectively. The flag leaves of each accession were similarly infested with 10 first-instar nymphs in a test tube with three replications for the booting test. The ANM of each accession was calculated as the mean of the three replications $((ANM = (Rep.1 + Rep.2 + Rep.3)/3)$ at 3DAI, 5DAI, and 7DAI, respectively.

2.4. Data Analysis

Analysis of variance (ANOVA) was performed to test the difference in the response to the GRH between the accessions and controls, T65 and DV85. Least significant difference (LSD) was computed and used as a basis for grouping the accessions into different kinds of resistance categories. To classify 60 accessions evaluated in the present study, the ANM of the GRH of each accession was compared to the respective means of resistant and susceptible controls with the LSD value. Finally, the classification was conducted based on the statistical difference to the ANM of DV85 as the resistant control, and the difference to the

ANM of T65 as the susceptible control. Correlation and regression analysis was conducted between the ANMs of the GRH at the seedling stage and at the booting stage.

3. Results

3.1. Response of Rice Landraces to the GRH at the Seedling Stage

The resistant control DV85 had a high ANM with 91.0% at 3DAI that reached to 100% at 7DAI [\(Table 1\)](#page-4-0). On the other hand, the susceptible control T65 had an extremely low ANM with 2.5% at 3DAI that was still low (5.0%) at 7DAI [\(Table](#page-4-0) [1\)](#page-4-0). Broad variation of the ANMs ranged from 0% to 100% was observed in the core collection of rice landraces in the response to the GRH at the three evaluation times (Figures $1(a)$ –(c)). The frequency distribution of the ANMs of the GRH on rice landraces was discrete at 3DAI where 26 accessions showed high ANMs and the remaining 34 accessions showed low to medium ANMs [\(Figure](#page-3-0) [1\(a\),](#page-3-0) [Table 1\)](#page-4-0). The frequency distribution became continuous and it was skewed to the high ANMs at 5DAI and 7DAI [\(Figure 1\(b\), Figure 1\(c\)\)](#page-3-0). The accessions were grouped as (I) highly resistant (26 accessions), (II) considerably resistant (6 accessions), (III) moderately resistant (10 accessions) and (IV) susceptible (18 accessions) based on the comparison to the respective susceptible and resistant controls [\(Table 1,](#page-4-0) Supplementary [Table S3\)](#page-11-0). The each ANM of highly resistant accessions was distinctly higher than that of T65 throughout the period evaluated

Figure 1. Frequency distribution of the ANMs of the GRH on 60 rice accessions at the seedling stage at 3 days after infestation (DAI) (a), 5DAI (b) and 7DAI (c), and at the booting stage at 3DAI (d), 5DAI (e) and 7DAI (f).

Table 1. Classification of the rice landraces based on the response to the GRH by the seedling test

ns, not significant different; HR, high resistance; CR, considerable resistance; MR, moderate resistance; S, susceptible. "Nymph mortality of total accessions in the group. CR: distinctly higher than that of T65 and slightly lower than that of DV85. MR: higher than that of T65 but distinctly lower than that of DV85.

and was the same as DV85 at 7DAI. The ANMs of considerably resistant accessions were the same as that of T65 at 3DAI, but became distinctly higher than that of T65 at 7DAI. These ANMs were slightly lower than that of DV85 one landrace from Bangladesh (WRC55) which showed an equivalent level of resistance on DV85 at 7DAI. Similarly, the ANMs of moderately resistant accessions were the same as that of T65 at 3DAI, but became significantly higher than that of T65 at 7DAI. These ANMs were distinctly lower than that of DV85 throughout the period evaluated. The ANMs of susceptible accessions were the same as that of T65, and distinctly lower than that of DV85 throughout the period evaluated. Among the 26 highly resistant accessions, 9 were from India, each 3 were from China, Cambodia, and Philippines, 2 were from Nepal and each one was from Korea, Thailand, Myanmar, Malaysia, Indonesia, and Madagascar, respectively (Supplementary [Table S1\)](#page-10-0).

3.2. Response of Rice Landraces to the GRH at the Booting Stage

The resistant control DV85 had a considerably high ANM of 66.7% at 3DAI that reached to high ANM of 80.0% at 7DAI. On the other hand, the susceptible control T65 had an ANM of 0.0% throughout the period evaluated [\(Table 2\)](#page-5-0). Broad variation of the ANMs ranged from 0% to 96.7% was observed in sixty accessions of rice landraces in the response to the GRH at the three evaluation times as similarly as at the seedling stage (Figures $1(d)-(f)$). The frequency distribution of the ANMs of the GRH on rice landraces was continuous and skewed toward the susceptible throughout the period evaluated [\(Figures 1\(d\)-\(f\)\)](#page-3-0). The accessions were grouped as (I) highly resistant (18 accessions), (II) considerably resistant (3 accessions), (III) moderately resistant (5 accessions) and (IV) susceptible (35 accessions) [\(Table 2,](#page-5-0) Supplementary [Table S4\)](#page-16-0). Among the 18 highly resistant accessions, 5 were from India, each 3 were from China and Cambodia, each 1 was from Korea, Myanmar, Philippines, Malaysia, Bhutan, and Madagascar, respectively (Supplementary [Table S2\)](#page-10-1).

Table 2. Classification of the rice landraces based on the response to the GRH by the booting test.

Groups	No. of accessions		Nymph mortality ^a	P values Difference from		Response to the GRH
			(%)	T65	DV85	
$\mathbf I$	18	3DAI	71.1 ± 5.0	< 0.001	ns	
		5DAI	80.4 ± 4.5	< 0.001	ns	HR
		7DAI	81.6 ± 4.2	< 0.001	ns	
\mathbf{I}	3	3DAI	36.6 ± 8.3	< 0.01	< 0.05	
		5DAI	43.3 ± 3.3	< 0.001	< 0.05	CR
		7DAI	50.3 ± 1.9	< 0.001	< 0.05	
Ш	5	3DAI	19.6 ± 5.1	ns	< 0.001	
		5DAI	25.5 ± 5.3	< 0.05	< 0.001	MR
		7DAI	32.2 ± 3.1	< 0.05	< 0.001	
IV	34	3DAI	2.6 ± 0.3	ns	< 0.001	
		5DAI	3.9 ± 0.7	ns	< 0.001	S
		7DAI	5.7 ± 0.8	ns	< 0.001	
Total	60					
DV85 (Resistant control)		3DAI	66.7 ± 4.3	< 0.001		
		5DAI	73.3 ± 5.4	< 0.001		HR
		7DAI	80.0 ± 3.7	< 0.001		
T65 (Susceptible control)		3DAI	0.0 ± 0.0		< 0.001	
		5DAI	0.0 ± 0.1		< 0.001	S
		7DAI	0.0 ± 0.1		< 0.001	

ns, not significant different; HR, high resistance; CR, considerable resistance; MR, moderate resistance; S, susceptible. ^aNymph mortality of total accessions in the group. CR: distinctly higher than that of T65 and slightly lower than that of DV85. MR: higher than that of T65 but distinctly lower than that of DV85.

3.3. Correlation between ANMs of the GRH at the Seedling and at the Booting Stages

The relationship between ANMs of the GRH at the seedling and at the booting stages was demonstrated (Figures $2(a)-(c)$). Significant positive correlations were found between the ANMs of the GRH at seedling and booting stages at significant level of 1%. The correlation coefficients between ANMs of the seedling and the booting stages were 0.684 at 3DAI, 0.680 at 5DAI and 0.652 at 7DAI.

The growth stage-specific resistance was observed at the seedling stage but not at the booting stage (Supplementary [Table S3,](#page-11-0) Supplementary [Table S4\)](#page-16-0). The four accessions (WRC25, WRC34, and WRC40) were highly resistant and one (WRC55) was considerably resistant at the seedling stage but obviously susceptible at the booting stage. There was nothing to be resistant only at the booting stage.

4. Discussion

4.1. Phenotypic Coverage of the Rice Core Collection

Core collection of several crops has been developed based on phenotypic and passport data [\[12\].](#page-8-11) In the present study, we used a core collection that was developed by using DNA markers. This core set of rice landraces covered 80% - 100% of agronomical traits [\[13\].](#page-8-12) Sixty accessions originated across 19 countries including 16 countries in Asia, 1 country in Africa and 2 countries in North and South America. Sixty accessions covered a whole range of the resistance to the GRH, from susceptible to highly resistant. Total of 26 out of 60 accessions were highly resistant at the seedling stage. Among the eight loci conferring resistance to GRH, four loci GRH1, GRH2, GRH3 and GRH4 were found in rice landraces [\[9\]](#page-8-8) [\[21\]](#page-9-6) [\[22\]](#page-9-7) [\[23\].](#page-9-8) GRH6 was found in both rice landraces and wild relative of rice, Oryza sativa and Oryza nivara [\[24\]](#page-9-9) [\[25\].](#page-9-10) Rice landraces are sources of useful genetic resources for GRH resistance. The three resistance genes GRH1, GRH2 and *GRH3* were lost its effectiveness by the GRH strains known as biotype 1, biotype 2 and biotype 3 in the laboratory condition [\[26\].](#page-9-11) Therefore, it is necessary to screen large-scale germplasm in order to identify new genes/alleles that contribute to resistance to GRH in rice.

Figure 2. Correlation and regression between the ANMs of the GRH at the seedling stage and the ANMs at the booting stage in a rice core collection at 3DAI (a), 5DAI (b) and 7DAI (c). ANM; average nymph mortality.

4.2. Positive Correlation of GRH Resistance between the Rice Growth Stages

We found significant positive correlations of GRH resistance between at the seedling and at the booting stages (Figures $2(a)-(c)$). This indicated that genetic components existed in landraces are commonly stable from the seedling to the booting stages. In addition, the correlation of GRH nymph mortality between at the seedling stage at 3DAI and at the booting stage at 7DAI was observed $(r =$ 0.683, data was not shown). The evaluation at the seedling stage, therefore, promises us to identify the genetic resources with GRH-resistance accurately.

4.3. Seedling Specific Resistance Found in Rice Landraces

In rice, growth stage plays an important role against the insect's attack. It seems to be that damages to the rice plant caused by the GRH are most serious at the seedling and the booting stages. In the present study, we evaluated the resistance to the GRH of the core collection at the seedling and the booting stages. Four highly resistant accessions (WRC25, WRC34, WRC37, and WRC40), four considerable resistant accessions (WRC6, WRC7, WRC48, and WRC55) and eight moderate resistant accessions (WRC4, WRC9, WRC13, WRC15, WRC44, WRC45, WRC46, and WRC50) at the seedling stage. But all of the above-mentioned accessions were obviously susceptible at the booting stage. These accessions are useful for exploitation of new genes/alleles confer seedling-specific resistance to the GRH at the seedling. On the other hand, there were no accessions with booting-specific resistance among the 60 accessions.

5. Conclusion

In this study, we identified a large number of accessions that were a wide range of resistance to the GRH at both seedling and booting stages. Based on positive correlations of the GRH resistance between seedling and booting stages, we suggested that seedling stage is an effective stage to screen resistance to the GRH with the cost-efficient way using rice seedlings in a growth chamber. The four groups of resistance to the GRH were identified as (I) high level of resistance, (II) considerable high level of resistance, (III) moderate level of resistance, and (IV) susceptibility. In addition, we identified the unique phenomenon in some accessions, which showed resistance at the seedling stage but susceptible at booting stage. The exploitation of resistant landraces based on the present protocol will provide fundamental information for genetics and breeding on resistance to GRH in rice.

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Supplements

Table S1. Geographical origin and classification of resistance to the green rice leafhopper (Nephotettix cincticeps Uhler) of a core collection at the seedling stage.

a Classification of accessions based on their nymph mortality of the GRH at the seedling stage by comparing their mean and LSD with resistant cultivar (DV85) and susceptible cultivar (T65). HR: highly resistant, R: resistant, MR: moderate resistant, S: susceptible.

Table S2. Classification of resistance to the green rice leafhopper (Nephotettix cincticeps Uhler) of a core collection at the booting stage.

a Classification of accessions based on their nymph mortality of the GRH at seedling stage by comparing their mean and LSD with resistant and susceptible controls. HR: highly resistant, R: resistant, MR: moderate resistant, S: susceptible

Continued

Table S4. The nymph mortality of the green rice leafhopper on the rice landrace accessions at booting stage.

a Susceptible at the seedling stage.

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