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Phenotypic Expression of Whitebacked Planthopper Resistance in the Newly Established japonica / indica Doubled Haploid Rice Population

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Abstract: A new doubled haploid (DH) rice population was established from a cross between WBPH-resistant japonica Chunjiang 06 (CJ-06) and susceptible indica TN1. Sucking inhibitory and ovicidal resistance of the DH rice lines were evaluated on the basis of non-preference response of WBPH immigrants and honeydew excretion by WBPH females, and appearance of watery lesions in the necrotic discoloration of leaf sheaths ovipositied by WBPH,respectively. Both the major gene resistance to WBPH, sucking inhibitory and ovicidal resistance, showed 1 (resistant): 1 (susceptible) segregation ratio in the DH population. Relative density of WBPH populations and damage scores in the DH population indicated combined functions of both the major resistance genes as well as QTLs affecting the host plant response to WBPH infestations. Thus, the newly developed CJ-06/TN1 DH population could be a useful material to analyze major genes and QTLs for WBPH resistance in japonica rice.
Key words: *Sogatella furcifera*; rice; doubled haploid population; varietal resistance; sucking inhibitory resistance; ovicidal resistance

The whitebacked planthopper (WBPH), *Sogatella furcifera*, is a typical monsoon-dependent migratory paddy pest in East Asia. In China, WBPH has emerged as an economic insect pest of rice at early vegetative growth stage since the nation-wide cultivation of F_1 hybrid rice in 1980s [1]. A Chinese japonica rice “Chunjiang 06” (CJ-06) has been found to have dual mechanisms of varietal resistance to WBPH, namely ovicidal resistance and sucking inhibitory resistance [2]. Genetic analysis indicated that the both resistance traits were independently conferred by single dominant gene[3]. Preliminary mapping of the WBPH-resistance loci have been conducted by using an indica/japonica doubled haploid (DH) rice population from a cross between Zhaiyeqing 8 and Jinxi 17, and also by using segregating F_2 populations from reciprocal crosses between CJ-06 and a WBPH-susceptible indica variety TN1. Major ovicidal and sucking inhibitory loci have been mapped to the chromosomes 6 and 11 by means of linkage analysis between phenotypes and molecular markers, respectively [4, 5].

For further molecular mapping of WBPH-resistance genes, we have established a new japonica / indica DH population from a cross between CJ-06 and TN1 by anther culture method. Phenotypic expressions of the new DH population were examined in the present studies.

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MATERIALS AND METHODS

New DH population

A DH population consisted of 179 lines was established by anther culture of F_1 plants from a cross between WBPH-resistant japonica CJ-06 and WBPH-susceptible indica TN1, and by subsequent spontaneous chromosome doubling.

Field evaluation of WBPH-resistance in DH population

Twenty-five seedlings of each DH line were transplanted in a square-meter plot on June 16, 2003, and grown under the natural infestations of WBPH without spraying any pesticides. WBPH-resistance in the DH lines was evaluated on the basis of WBPH population performance, ovicidal necrotic symptoms, and plant damages caused by WBPH infestations.

WBPH population performance

The number of macropterous female immigrants of WBPH to 9 hills at the center of each plot was counted on July 2. Relative density of WBPH population on each DH line was estimated by counting WBPH nymphs and adults that were tapped down into a tray of 29 cm×41 cm from 2 hills in each plot on July 22, when the first generation nymphs reached the maximum density.

Necrotic symptoms of leaf sheaths

Necrotic discoloration of rice leaf sheaths due to oviposition by WBPH and ovicidal response of rice plants was recorded on July 8. Intensity of the necrotic symptoms was visually rated from 0 to 3 based on the following categories.

0, no visible symptom;
1, brownish oviposition damages, but no watery lesions;
2, discontinuous watery lesions;
3, vertically elongated conspicuous watery lesions.

Plant damage

Plant damage caused by WBPH infestations was recorded on August 4 after the WBPH populations emigrated. The level of plant damage was scored on the plot basis dividing into five grades as given below.

0, no damage;
1, only lower leaves of plants died;
2, lower half of plant died;
3, three fourths of plant died;
4, whole plant died.

Measurement of honeydew excretion

Five seedlings for each DH line were grown in a galvanized iron case (27 cm×6 cm×9 cm) until early tillering stage under open conditions. Gravid WBPH females were individually confined onto the upper portion of leaf sheaths of each plant with parafilm sachets (2 cm×2 cm), and allowed to suck for one day at room temperature. Amount of honeydew excreted in each sachet was quantified by weighing.

RESULTS

Density of WBPH immigrants

WBPH immigrated to CJ-06 and TN1 at densities of 0.3 and 5.6 females per hill. There was a great variation ranging from 0 to 11.4 females per hill in the density of WBPH females immigrated to the 151 DH lines (Fig. 1). However, frequency distribution of immigrant density was not normal. Sixty-eight DH lines (45.0%) distributed to a class of less than 2 females per hill.

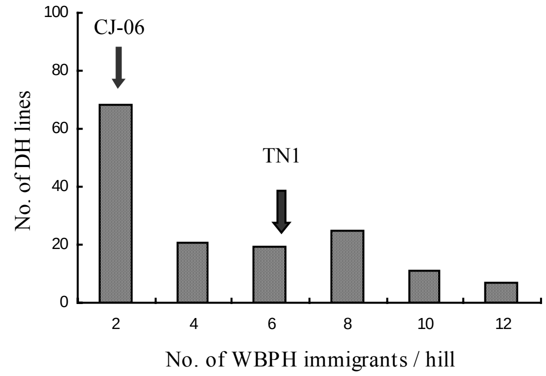

Fig. 1. Frequency distribution of density of WBPH immigrants in the DH lines.

Necrotic discoloration of leaf sheaths

There were pronounced variations in the necrotic discoloration of leaf sheaths due to oviposition by WBPH and subsequent ovicidal response of rice plants in the DH lines (Fig. 2). CJ-06 showed sporadic but distinct ovicidal symptoms (score 2), while no discoloration appeared in TN1 (score 0). Likewise, entirely no discoloration (score 0) appeared in 37 of 151 lines (24.5%). On the other hand, 29 lines (19.2%) expressed severe necrotic ovicidal symptoms (score 3). Remaining 85 lines showed differential discoloration scored from 0.5 to 2.5. Eighty-five DH lines (56.3%) showed discoloration associated with watery lesions (score 2–3), indicating the induction of ovicidal activity. On the other hand, 66 lines (43.7%) had no discoloration or only discoloration due to oviposition damages (score 0–1.5).

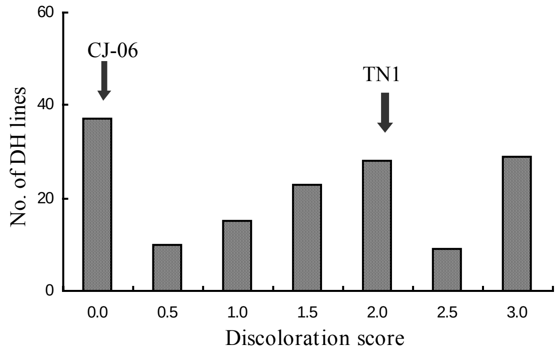

Fig. 2. Frequency distribution of the DH lines that showed different scores of necrotic discoloration of leaf sheaths under the field conditions.

Relative density of WBPH population

The numbers of WBPH recorded from CJ-06 and TN1 were 7 and 681, respectively. Among the 151 DH lines, WBPH numbers varied widely ranging from 6 to 2,918 (Fig. 3). Frequency distribution of DH lines

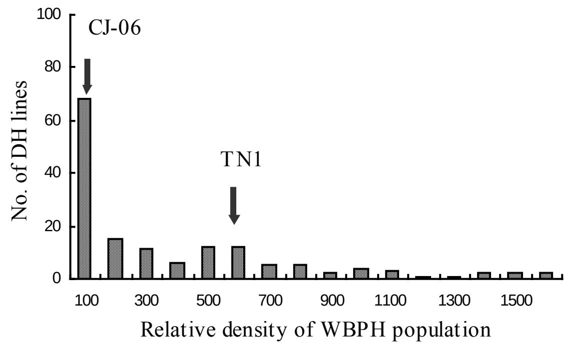

Fig. 3. Frequency distribution of relative density of WBPH populations in the DH lines.

displayed that 68 lines (45.0%) were categorized into the class of less than 100 insects.

Plant damages due to WBPH infestation

Damage scores of CJ-06 and TN1 were 0 and 3, respectively. Of 151 DH lines tested, 65 lines (43.0%) did not suffer from any visible damages (score 0) (Fig. 4). Only lower leaves of 49 lines (32.5%) were discolored and wilted by WBPH infestations (score 1). More than half of the plants of 37 DH lines (24.5%) were killed by higher density of WBPH populations (score 2 to 4). Of them, five DH lines were completely destroyed (score 4).

Honeydew excretion

WBPH females excreted only 0.7 mg of honeydew per day on CJ-06, while 13.9 mg on TN1. Honeydew excretion varied from 0 to 34.4 mg per day beyond the parental range among 109 DH lines tested (Fig. 5). WBPH females excreted less than 5 mg of honeydew per day on 53 DH lines (48.6 %).

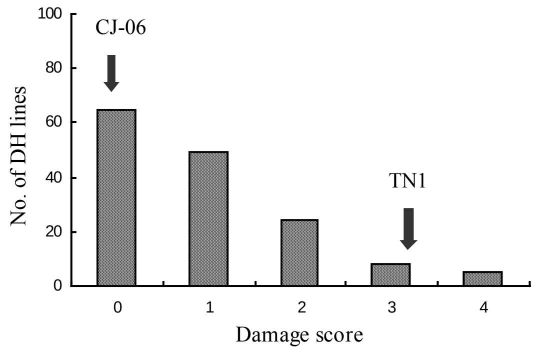

Fig. 4. Frequency distribution of the DH lines that suffered from different degrees of damage by WBPH infestation.

DISCUSSION

Phenotyping for WBPH resistance in the DH lines

Sucking inhibitory resistance to WBPH in rice plants is defined by restricted honeydew excretion. Honeydew measurement experiments with the parafilm sachet method have indicated that WBPH females excrete honeydew of only 5 mg/(female·day) on WBPH-resistant CJ-06, and a majority of the individuals excrete less than 10 mg/(female·day) [2]. On the contrary, they excrete honeydew of 15.6 mg/(female·day) on the susceptible TN1, and almost all the individuals excrete 10 mg/(female·day) or more.

Under the field conditions, sucking inhibitory resistance is expressed as a distinct non-preference behavioral response to the resistant host plant [2]. There was a close positive correlation (r=0.80**) between the amount of honeydew excreted by WBPH females and field density of WBPH immigrant females in the CJ-06/ TN1 DH lines (Fig. 6). On about half of the DH lines (44.0%), honeydew excretion was less than 5 mg per day and immigrant density was below 3 females per hill. Based on the above findings, the DH lines on which the honeydew excretion was less than 5 mg/(female·day) or immigrant density was less than 3 females/hill, were tentatively phenotyped as sucking inhibitory resistance.

Ovicidal resistance could be evaluated by the appearance of necrotic discoloration at oviposition sites in the leaf sheaths. However, the expression of ovicidal symptoms is strongly suppressed in the sucking inhibitory DH lines under natural infestations with WBPH in the fields, because oviposition takes place at a lower frequency in the sucking inhibitory lines due to their strong antixenosis against WBPH females. In the current experiments, the DH lines that had necrotic symptoms associated with watery lesions (score 2 to 3), were all categorized as ovicidal lines regardless of intensity of the symptoms.

Sucking inhibitory and ovicidal traits segregated

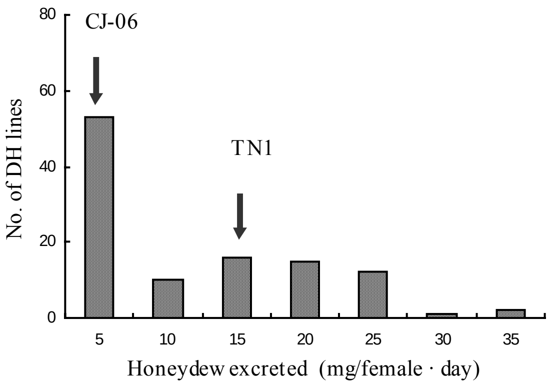

Fig. 5. Frequency distribution of honeydew excretion in the DH lines.

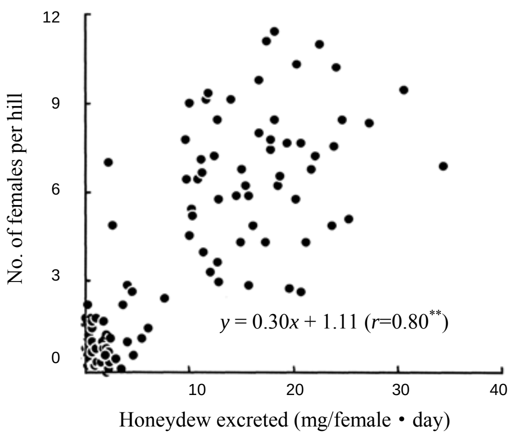

Fig. 6. Correlation between the amount of honeydew excretion and immigrant density in the DH lines.

independently among the DH lines. The ratio of resistant and susceptible lines was almost 1:1 for each resistance trait (Table 1). The DH lines were also segregated almost evenly into the four phenotypes with different combinations of the sucking inhibitory and ovicidal traits. These results indicated that the established DH lines retained the original ratio of WBPH-resistance traits in the haploid pollen of F_1 plants. Also, they implied that each major gene locus independently confers the both WBPH resistance traits.

Table 1. Segregation of WBPH resistance traits in the DH lines.

Resistance	No. of plants	R	S	χ^2 (1:1)[a]
Sucking inhibitory	151	80	71	0.54
Ovicidal	151	85	66	2.40

Resistance	No. of plants	R/R	R/S	S/R	S/S	χ^2 (1:1:1:1)[b]
Sucking inhibitory/Ovicidal	151	31	49	35	36	4.84

[a] Significant limit of χ-squared value = 3.84 (P=0.05, df=1);
[b] Significant limit of χ-squared value = 7.81 (P=0.05, df=3);
R, Resistant, S, Susceptible.

Necrotic discoloration in the DH lines with different WBPH-resistance traits

Appearance of necrotic discoloration due to oviposition and ovicidal reaction was compared among the DH lines with different combinations of WBPH-resistance traits (Table 2). Sucking inhibitory but non-ovicidal DH lines produced the lowest average score (0.21) for discoloration. All the DH lines without any discoloration (score 0) belonged to this phenotype. On the contrary, ovicidal but non-sucking inhibitory DH lines exhibited the most conspicuous discoloration (average score, 2.73). Most of the DH lines scored 3 fell into this phenotype. The susceptible DH lines that have neither sucking inhibitory nor ovicidal traits and the resistant ones that have both sucking inhibitory- and ovicidal traits showed intermediate grades of discoloration, the most frequently scored 1.5 and 2.0, respectively. However, the causes of discoloration were different between the two groups of DH lines. Discoloration in the susceptible lines was made by intensive oviposition damages, while that of resistant ones was due to a sporadic ovicidal response.

Relationship between immigrant density and subsequent population growth

There were remarkable differences in the WBPH immigrant density among the DH lines of different resistance phenotypes (Table 3). Average densities of WBPH immigrants in the sucking inhibitory DH lines were significantly lower, as low as about one-sixth of those in the non-sucking inhibitory ones regardless whether they had ovicidal resistance or not.

Relative density of WBPH population in the sucking inhibitory lines was also significantly lower than that in the non-sucking inhibitory lines, which was only about 8 to 13% of the latter. Furthermore, WBPH population

Table 2. Oviposition and ovicidal discoloration in the DH lines of different phenotypes for WBPH resistance.

Phenotype[a]	No. of lines	Discoloration score							Mean±sd[b]
		0.0	0.5	1.0	1.5	2.0	2.5	3.0	
R/S	49	37	6	3	3	0	0	0	0.21±0.43 a
S/S	36	0	4	12	20	0	0	0	1.22±0.34 b
R/R	31	0	0	0	0	20	6	5	2.26±0.38 c
S/R	35	0	0	0	0	8	3	24	2.73±0.42 c
Total	151	37	10	15	23	28	9	29	

[a] Phenotype: Sucking inhibitory/Ovicidal. R, Resistant; S, Susceptible.
[b] Means followed by the same letter are not significantly different (P=0.05, Kruskal-Wallis test). The same as in tables below.

Table 3. Densities of immigrants and subsequent populations in the DH lines of different phenotypes for the WBPH-resistance.

Phenotype (Sucking inhibitory/Ovicidal)	No. of lines	Immigrant density[a] (Mean±sd)	Population density[b] (Mean±sd)
R / R	31	1.3±0.8 a	65.7±91.6 a
R / S	49	1.0±0.8 a	107.1±138.5 a
S / R	35	6.9±2.1 b	377.8±309.3 b
S / S	36	6.5±2.4 b	822.8±561.9 c

[a] No. of macropterous female adults per hill;
[b] No. of nymphs and adults tapped down into a tray (29 cm×41 cm) from two hills;

Table 4. WBPH population density in the DH lines gave different damage scores.

Damage score	No. of lines	Population density [a] (Mean ± sd)
0	65	89 ± 111 a
1	49	245 ± 230 b
2	24	614 ± 274 c
3	8	1230 ± 429 c
4	5	1559 ± 718 c

[a] No. of nymphs and adults tapped down into a tray (29 cm × 41 cm) from 2 hills;
Means followed by the same letter are not significantly different (P=0.05, Kruskal-Wallis test).

Table 5. Damage scores in the DH lines with different resistance phenotypes.

Phenotype [a]	No. of lines	Damage score					Mean ± sd
		0	1	2	3	4	
R/S	31	22	9	0	0	0	0.29 ± 0.45 a
R/S	49	33	15	1	0	0	0.35 ± 0.51 a
S/R	35	8	14	8	4	1	1.31 ± 1.04 b
S/S	36	2	11	15	4	4	1.92 ± 1.04 b
Total	151	65	49	24	8	5	

[a]Phenotype: Sucking inhibitory/Ovicidal.

density in the non-sucking inhibitory but ovicidal lines was significantly suppressed by about 46% in the susceptible (non-sucking inhibitory and non-ovicidal) lines. There was a significant positive correlation (r=0.59**) between the immigrant density and subsequent population density in the non-ovicidal DH lines, but not in the ovicidal lines (r=0.30^{ns}).

The above findings indicated that WBPH population development was primarily restricted by the sucking inhibitory resistance, and subsequently suppressed to a lesser extent by the ovicidal resistance.

Damage scores of the DH lines with different phenotypes

The average densities of WBPH populations increased lineally as elevation of damage scores (Table 4). There were significant differences among the average population densities at damage scores 0, 1, and 2 or more. However, no significant differences were found among the average population densities at damage scores 2, 3, and 4. It indicated that the damage intensity is not affected only by density of WBPH populations, but also by differential tolerance to WBPH infestation and biomass size among the DH lines.

Among 65 DH lines that had entirely no damage (score 0), 55 (84.6%) were sucking inhibitory lines (Table 5). Likewise, of 80 sucking inhibitory lines, 79 (98.8%) belonged to the category of damage score 1. On the other hand, 4 of 5 DH lines destroyed completely were regarded as susceptible lines, which had neither sucking inhibitory nor ovicidal resistance. The DH lines that had only ovicidal resistance were mostly categorized to the damage scores 0–2 possibly due to ovicidal resistance. The non-sucking inhibitory and non-ovicidal (susceptible) DH lines were damaged with WBPH infestations at various extents, covering all the damage scores from 0 to 4. This evidence indicated that damage expression is governed by minor or polygenic traits as well as such major gene traits as sucking inhibitory and ovicidal resistance.

CONCLUSION

The newly established DH population from a cross between WBPH resistant japonica CJ-06 and susceptible indica TN1 provided an ideal material for mapping the major genes and QTLs for WBPH resistance in japonica rice.

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