

Differential Expression of Whitebacked Planthopper Resistance in the japonica/indica Doubled Haploid Rice Population under Field Evaluation and Seedbox Screening Test

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Abstract: Whitebacked planthopper (WBPH) -resistance in a japonica / indica doubled haploid (DH) rice population established from a cross between WBPH-resistant japonica Chunjiang 06 and susceptible indica TN1, was comparatively evaluated through a field experiment based on the WBPH immigrant density and standardized seedbox screening test (SSST). All the susceptible DH lines in the field experiment behaved accordingly in SSST. However, 35 of resistant 66 lines (53%) in the field, were categorized to susceptible groups in SSST. Likewise, there were no significant differences in WBPH immigrant densities among 70 DH lines that were highly resistant to susceptible in SSST. The results revealed that SSST could not evaluate properly WBPH resistance in the DH lines. Four QTLs for WBPH-resistance phenotyped by the immigrant density were detected on chromosomes 2, 3, 4, and 11. Of them, the QTL on chromosome 4 was the most effective (LOD 21.8, variance 78%). Five QTLs associated with seedling mortality were mapped on chromosomes 2, 3, 4, 5 and 6. In addition to the QTL (LOD 10.5, variance 68%) on chromosome 4, there was another major QTL (LOD 12.7, variance 71%) located on chromosome 5, which was SSST-specific but might be irrespective of the WBPH resistance traits.

Key words: *Sogatella furcifera*; rice; doubled haploid population; varietal resistance; sucking inhibitory resistance; seedbox screening test

The whitebacked planthopper (WBPH), *Sogatella furcifera*, is an important economic insect pest of rice during early vegetative growth period. A Chinese japonica rice Chunjiang 06 (CJ-06) has been found to have dual mechanism of varietal resistance to WBPH, namely ovicidal and sucking inhibitory resistance, which are independently conferred by single dominant genes^[2, 3]. For molecular mapping of the WBPH-resistance genes, we have established a new japonica/indica DH population from a cross between CJ-06 and TN1 through anther culture method^[4].

In the current experiment, WBPH-resistance expressions in the DH population were comparatively examined by the field evaluation and standard seedbox screening test (SSST), which had been widely employed to evaluate and phenotype the planthopper resistance in rice^[1].

MATERIALS AND METHODS

DH rice lines

A DH population established by anther culture of F₁ plants from a cross between WBPH-resistant japonica CJ-06 and WBPH-susceptible indica TN1 was employed. CJ-06 and TN1 were also considered as positive and negative controls, respectively.

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 natural infestations of WBPH. WBPH-resistance based on density of macropterous female immigrants of WBPH in 152 DH lines was evaluated in the center 9 hills of each plot on July 2. Subsequent population development and plant

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damages caused by the WBPH infestations were also observed to confirm WBPH-resistance.

Evaluation of WBPH-resistance by SSST

Modified SSST^[6] was used to evaluate WBPH-resistance in 170 DH lines. Seedling mortality of each line was recorded as susceptible TN1 seedlings were almost killed. Experiments were designed with three replications. Arcsine transformation of average percentage seedling mortality was subjected to QTL and statistical analyses.

QTL analysis

A molecular-marker linkage map containing a total of 109 SSR markers distributed evenly on 12 rice chromosomes was constructed by using Mapmaker / EXP ver. 3.0 (ZENG Da-li unpublished). Mapmaker / QTL ver. 1.1 was used for QTL analysis for WBPH-resistance traits evaluated by both methods described above.

RESULTS

Field evaluation

WBPH immigrated to susceptible TN1 with a density of 5.6 females per hill. Their progeny established a high enough density of WBPH populations to cause serious damage to TN1 plants. On the other hand, only 0.3 females per hill were recorded in resistant counterpart CJ-06, where no WBPH population developed. There was a continuous variation ranging from 0 to 11.4 females per hill in the density of WBPH females immigrated to 145 DH lines. The frequency distribution was skewed to the lower density side (Fig. 1). The 46 DH lines (32%) in which the immigrant density was below one female per hill, had the highest frequency.

WBPH population growth was suppressed far below the damage-causing level in the 66 DH lines (46%), where the immigrant density was below 2 females per hill (0.8 ± 0.5 females). Of them, there were no visible damages in 49 lines, and only yellowing of the lower leaves was observed in the remaining 17 lines. On the other hand, the

non-ovicidal 26 lines among 50 DH lines (35%) where 5 or more females invaded each hill (7.6 ± 1.6 females) had suffered serious damages including complete destruction.

Based on the above field evidence, the DH lines to which less than 2 and more than 5 WBPH females immigrated to each hill, were regarded to be resistant and susceptible ones, respectively. The 29 DH lines (20%), where 2 to 5 females immigrated to each hill, were tentatively categorized as an intermediate group.

Seedbox screening test

Of 170 DH lines, 131 lines (77%) were declared as moderately to highly susceptible, where 30% or more seedlings were killed. Among the susceptible lines, the highly susceptible ones, whose seedling mortality was higher than 70%, occupied as much as 60% of the DH lines tested. Most of the highly susceptible lines died earlier than TN1. Thirty-nine DH lines (23%) with seedling mortality below 30% were considered to be moderately to highly resistant. Of them, 31 lines (79%) were moderately resistant. Only 8 DH lines (5%) were found to be resistant and highly resistant. Those lines were at par with CJ-06 in terms of resistance. Frequency distribution of seedling mortality displayed a bi-modal pattern (Fig. 2).

QTL analysis

Four QTLs for WBPH immigrant density (*qIMG*) were mapped on chromosomes 2, 3, 4 and 11 (Table 1). The most effective *qIMG-4* (LOD 21.8, variance 78%) was located on chromosome 4. Five QTLs associated with seedling mortality in SSST (*qSST*)

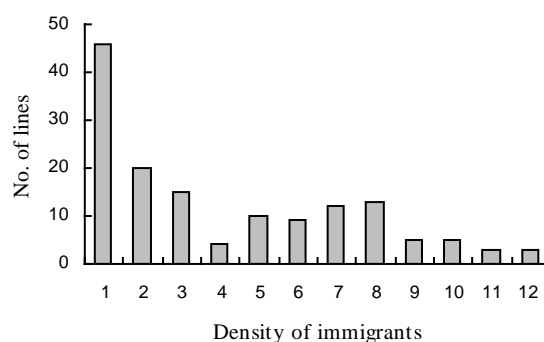


Fig. 1. Frequency distribution of WBPH immigrant density (no. of macropterous female adults / hill) in the DH lines.

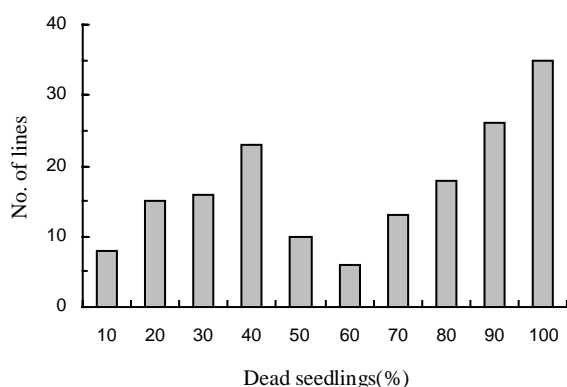


Fig. 2. Frequency distribution of percentages of seedling mortality.

were detected on chromosomes 2, 3, 4, 5 and 11 (Table 1). Of them, *qSST-4* and *qSST-5* were highly effective (LOD 10.5 and 12.7, variance 68% and 71%, respectively). The QTLs associated with immigrant density and seedling mortality on chromosomes 2, 3 and 4 were localized at the identical marker intervals.

DISCUSSION

Expression of WBPH resistance in the DH lines in the paddy fields

WBPH-resistance in the DH lines has been inherited from a parent variety CJ-06, which has sucking inhibitory- and ovicidal-resistance conferred

by independent single major genes^[2,3]. Thus, there are four different sets genotype with different combinations for these two resistance traits in the DH lines in the present experiments^[4]. Under field conditions, sucking inhibitory resistance caused a distinctive non-preference response of WBPH immigrants to the resistant host plants^[2,4]. Restricted immigration resulted in a failure of population establishment. Therefore, the density of macropterous females of WBPH in the transplanted paddy fields during the immigration period could be a reliable parameter for the sucking inhibitory resistance in the rice plants. Ovicidal resistance has little chance to be functional in the sucking inhibitory plants, because of restricted oviposition. However, it works as an effective resistance trait to suppress the WBPH population development in the non-sucking inhibitory plants^[4].

Expression of WBPH resistance in the DH lines in SSST

Evaluation of WBPH-resistance in SSST relies upon the interactions between young rice seedlings (2 to 3 leaf stage) and newly hatched nymphs of WBPH. Preferential sucking by nymphs and differential tolerance (vigorousness) of seedlings are the main factors responsible for differential mortality among DH line seedlings. However, such an interaction between WBPH and the rice plant does not exist under

Table 1. QTLs associated with WBPH immigrant density in DH lines planted in the field and mortality of DH line seedlings due to infestations with WBPH nymphs in SSST.

Traits examined	QTLs	Chromosome	Distance (cM) ^a	Peak LOD	Variance explained	Additive effect ^b
Immigrant density	<i>qIMG-2</i>	2	40.5	3.07	14.6	-21.4
	<i>qIMG-3</i>	3	24.5	2.63	16.4	24.5
	<i>qIMG-4</i>	4	11.6	21.76	78.4	56.1
	<i>qIMG-11</i>	11	11.5	3.87	21.5	28.1
Seedling mortality	<i>qSST-2</i>	2	40.5	3.12	70.7	-38.7
	<i>qSST-3</i>	3	24.5	5.69	28.3	25.2
	<i>qSST-4</i>	4	11.6	10.54	88.1	38.1
	<i>qSST-5</i>	5	29.5	12.07	71.4	38.8
	<i>qSST-6</i>	6	11.8	3.77	17.9	-20.1

^a Distance of each QTL region between the nearest markers.

^b Positive and negative additivity indicate that contributing alleles originate from DJ-06 and TN1, respectively.

natural conditions. Thus, the relationship between seedling mortality and WBPH resistance in the paddy plant must be investigated carefully.

Fifty DH lines susceptible in the field were too susceptible in SSST. However, 35 of 66 DH lines (53%) that showed obvious resistance in the field were classified as moderately to highly susceptible in SSST (Table 2). Likewise, 21 of 29 DH lines (72%) categorized tentatively to the intermediate group in the field were rated as susceptible to highly susceptible in SSST. There were no statistically pronounced differences among the average densities of WBPH immigrants in 70 DH lines (51%) divided into the categories ranging from highly resistant to susceptible in SSST (Table 3). The results disclosed that WBPH-resistance in the DH lines was not evaluated properly in SSST. Such improper evaluation could be attributable to 1) differential expressions of resistance traits between young seedlings in a seedbox and rice plants growing in a paddy field, 2) differential responses of young nymphs and adults of WBPH to the resistant host plants, and 3) differential tolerance against WBPH infestations at seedling stage.

It has been known that all the IR rice varieties are not incorporated with any major genes for resistance to WBPH and are susceptible to WBPH based on the SSST at IRRI. However, 16 of 27 IR varieties tested have been found to express practical resistance to WBPH in the field, where WBPH population growth

Table 2. Differences in expression of WBPH-resistance in DH lines between field evaluation and SSST.

Evaluation method		No. of DH lines	%
Field test	SSST ^a		
Resistant	HR	2	1.4
	R	5	3.4
	MR	24	16.6
	MS	21	14.5
	S	6	4.1
	HS	8	5.5
Intermediate	MR	1	0.7
	MS	7	4.8
	S	3	2.1
	HS	18	12.4
	S	5	3.4
Susceptible	HS	45	31.0

^a HR, Highly resistant; R, Resistant; MR, Moderately resistant; MS, Moderately susceptible; S, Susceptible; HS, Highly susceptible.

Table 3. WBPH immigrant density in the DH lines of six different resistance categories in SSST.

Resistance in SSST ^a	No. of data	Immigrant range	Density/hill ^b
HR	2	— ^c	— ^c
R	5	0.0–0.8	0.7±0.7 a
MR	25	0.1–2.7	0.8±0.6 a
MS	28	0.2–4.3	1.2±0.0 a
S	14	0.3–11.4	3.7±0.7 ab
HS	71	0.2–11.2	5.9±0.6 b

^a See Table 2;

^b Kruskal-Wallis test ($P < 0.0001$);

^c Data are too few for statistical analysis.

were strongly suppressed [5]. This evidence also indicates that WBPH-resistance in IR rice varieties was not evaluated properly by SSST.

Differential QTLs for WBPH resistance identified under field evaluation and SSST

Based on WBPH immigrant density in the DH lines, a highly effective QTL was detected on chromosome 4. The most effective QTLs for the other traits associated with WBPH-resistance such as amount of honeydew excretion, population density of WBPH and host plant damages were also mapped on the same chromosomal position (our unpublished data). Those QTLs indicated the localization of major gene for sucking inhibitory resistance to WBPH at the identical position in chromosome 4.

In the QTL analysis based on SSST, two different major QTLs were found to be located on chromosomes 4 and 5. The QTL on chromosome 4 was identical to the major QTL for WBPH immigrant density and other WBPH-resistance traits. However, the other major QTL on chromosome 5 was specific to SSST, and possibly irrespective of the WBPH resistance traits, because no other QTLs associated with WBPH resistance were detected at the same chromosomal position.

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