



Full Length Article

Optimum Screening Time for Improved WBPH-Associated QTL Analysis in Rice

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Abstract

Whitebacked Planthopper is a sucking phloem sap insect which can greatly affect quality of the grain and yield losses. Determination of the resistance and susceptibility to WBPH is very important to accurate QTL analysis. The main objective of the study was to examine the level of resistance to determine optimum screening time using 120 doubled haploid lines. CNDH populations were developed from anther culture of F₁, which was derived after a crossing WBPH, *Sogatella furcifera* resistance 'Cheongcheong' and susceptible 'Nagdong' lines. The genetic map with average 9.6 CM between markers was constructed from 120 CNDH populations, which included 217 SSR markers. Four QTLs were detected at different time and points based on the rate of seedling mortality after WBPH infestation. The markers were found to be comprehensive in identification of genotype and phenotype at coincident rate of 95% 14 days after infestation. These markers are efficiently very useful for MAS and develop new varieties in rice breeding program. © 2016 Friends Science Publishers

Keywords: WBPH; Doubled haploid; QTL; Rice

Introduction

Many traits are important for rice production, such as yield potential, quality characteristics, disease, resistance and stress tolerance. In Korea, the whitebacked planthopper (WBPH) *Sogatella furcifera* Horvath attacks and damages rice by sucking the phloem sap, resulting in hopper burns, diseasing transmission, which were reduce crop yield. The average rice annual losses in Korea were caused by diseases and insect infestation approximately 5.3% and 3.2%, respectively during 1971-1980 (Heu, 1983). Growing and development of resistant varieties to control diseases and insects is economically justified and minimize risk to human and environment. Identification and utilization of new genes approaching WBPH resistance has played important roles of rice breeding (Li *et al.*, 2003). Mapping of insect resistance gene has been intensively implemented by a wide array of molecular marker in plants. Therefore, scientists are working on the development of resistant varieties as this defines the most effective method of disease and pest control in rice. The resistant lines are derived from IR2035 that expresses the *Wbph1* and *Wbph2* genes by (Kim, 1978). There are six kinds of planthoppers that attack rice plants, (1) brown planthopper-BPH (*Nilaparvata lugens* Stål), (2) small brown planthopper-SBPH (*Laodelphax striatellus* Fallen), (3) green leafhopper-GLH (*Nephotettix virescens* Distant), (4) green rice leafhopper-GRH (*N. cincticeps*

Uhler), (5) whitebacked planthopper-WBPH (*S. furcifera* Horvath) and (6) zigzag leafhopper-ZLH [*Recilia dorsalis* Motschulsky] (Heong and Hardy, 2009)]. The WBPH *S. furcifera* Horvath is found in all rice plants and is one of the most serious sucking insect pests of rice. Using a mass screening technique, the genotypes of rice lines have been evaluated and donors for resistance have been identified. Since significant variation was observed between the two replications, data from each replication and the average data were independently used for QTL analysis (Chen *et al.*, 2010). Relative density of WBPH populations and damage scores in the DH populations indicated combined functions of both the major resistance genes as well as QTLs affecting the host plant response to WBPH infestations by (Liu *et al.*, 2001). Therefore, they have been used to develop of resistant rice varieties as an effective method of disease and insect pest control in rice crops. A vast number of breeding materials are grown and evaluated for various agronomic characteristics, grain quality, disease and insect resistance, and tolerance of abiotic stresses. The availability of high-quality data at the time of making plant selections is very important (Khush and Virk, 2005). The rice doubled-haploid (DH) populations derived from a cross between an improved *indica* variety, 'IR64' and a traditional tropical *japonica* variety, Azucena, has been used for mapping and analyzing major genes and QTL for numerous agronomic traits (Huang *et al.*, 1997; Fatah *et al.*, 2014). The

development and application of NILs have greatly promoted QTL fine mapping in rice and other crop species. Taking the advantage of DNA marker data accumulated in the construction of genetic map using XB//XB/Dwr BIL populations, and facilitated by the segregation distortion towards the adapted rice line XB, a set of CSSLs were selected in their study. The chromosome segments were introgression from Dongxiang wild rice into the genetic background of CMS-DA maintainer line Xieqingzao B. As shown in the previous section, a number of lines have displayed high resistance to planthopper. Such lines could be used as NILs for QTL fine mapping or as intermediates for NIL development. They could also be used as intermediate to develop new breeding lines carrying beneficial genes from the wildrice (Chen *et al.*, 2006). QTLs associated with resistance to WBPH in this mapping population using a series of screening techniques quantifying three mechanisms of plant resistance to insects: antibiosis and tolerance by (Alam and Cohen, 1998). 120 ‘Cheongcheong/Nagdong’ doubled haploid (CNDH) populations were generated at the molecular breeding lab in Kyungpook National University, using conventional and *in vitro* anther culture. The parent plants selected for single crosses, which were used ‘Cheongcheong’ and ‘Nagdong’. The ‘Cheongcheong’ derived from YR675 (*japonica* type)/IR2035 (*indica* type), which is a Tongil type rice variety that has resistance to the WBPH. It is a high yield plant with a complete abscission layer originating from *Oryza sativa* L. ‘Nagdong’ is the main variety cultivated in our region and considered as positive and negative with WBPH resistance. In the present study, we objective was to examine the rice resistance optimum screening after infesting and to identify QTLs associated with rice resistance of 120 CNDH populations.

Materials and Methods

Plant Material

120 CNDH lines were generated at the molecular breeding lab in Kyungpook National University, using conventional and *in vitro* anther culture. The parent plants selected for single crosses were ‘Cheongcheong’ and ‘Nagdong’. The ‘Cheongcheong’ is a ‘Tongil’ type rice variety that is used for rice resistance with the WBPH. It is a high yield plant with a complete abscission layer originating from ‘Nagdong’ is the main variety cultivated in our region and considered as positive and negative with WBPH resistance. The rice varieties, ‘Cheongcheong’ and ‘Nagdong’, were used as parent plants. Taichung Native 1 (TN1) was grown in each tray with 120 CNDH populations for standard seed box screening.

Raising of WBPH

The WBPH were reared in room 18 cages (50 × 50 × 40

cm) in the greenhouse. 30 insects were put in six boxes each at maintenance section until ovipositor stage; they were then transferred into 12 cages for selection of 2nd and 3rd instars. The plants were used as food materials. Changing of food materials in the culture maintenance cages and other cages was done twice a week using plastic trays (20 x 14 x 5cm), the soil was 3 cm deep and fresh water was used to spray the cultures. The WBPH mass was added at the 1st instar, 2nd to 3rd instar and was changed to the 3rd to 5th instar and 5th to adults after 10-15 days (data not shown). This was in accordance with the life schedule of activities related to test insect culture and screening with the standard seed box screening test. Circled numbers indicate the procedural steps in chronological order (Heinrichs *et al.*, 1985) for genetic evaluation of insect resistance in rice. The 5th instar to adult insects were reared in separate cages, and then transferred to the new maintenance cage. All wilted plants were removed, and fresh plants were placed in the vacated area. The WBPH can redistribute themselves on to the fresh plants. All in all the insect were reared in oviposition stage, after 9-10 days the egg larvae become the first instar, and after 14 days the 2nd and 3rd instar nymphs were selected to infest the seedling stage.

Phenotype Test

All experiments were performed in greenhouses at Kyungpook National University. Temperature of the greenhouse was controlled constantly according to the seasons. The phenotypes of 120 CNDH populations were assessed to screen resistant lines with three kinds of plants, ‘Cheongcheong’, ‘Nagdong’ and TN1. All the lines were invaded from 2nd and 3rd instar of WBPH and the resistant lines were screened after 7 days, 14 days, and 21 days. Infestation occurred 11–12 days after sowing. We observed while performing the bio-scoring each week and the scoring for level of resistance was repeated twice. The phenotypes of the resistant varieties in the 120 CNDH populations were measured by bio-scoring plant damage using the Standard Evolution System (SES) for rice scale (Heinrichs *et al.*, 1985). Plant lines were identified using standard bio-scoring of the condition of rice during WBPH infestation. The contents of the seed-box were graded using grading plant damage that is the SES for rice scale (Heinrichs *et al.*, 1985). Therefore, all the plants lines were scored using a standard method to assess rice condition of WBPH resistant lines. Screening of the resistant lines was performed in a standard seed box (32 × 23 × 10 cm) in the greenhouse. All healthy seeds were collected and germinated in the incubator at 28 °C. In the standard seed box, soil was 3 cm deep and was sprayed with water. After watering the soil, CNDH lines were sowed in the standard screening box in 12 rows to score easily. All boxes were covered with plastic bag and clothes to maintain warmth and dark environment for four days (data not shown).

PCR Protocol

7 SSR markers, RM 3482 (TTGTTGTCAGCTACGGTGG, CTGCTTCGTGATGTTGTTGG), RM11966 (TGGTCCACACGTGATGAGTC, GAAGATAGGGGAGTGGGGAG), RM8235 (GCTGTACAGTGTCTTCGTTTC, TGCCTATTGCCTACTCACTC), RM3709 (TATATTGAGGGAGCAAGCCG, CATCCCAAAGCTAGAACCCC), RM11694 (GCGTCTATGCGTATCTTCATCTTACC, CATCCCAAAGCTAGAACCCC), RM11669 (AAACCGTTCCAGGGAGACTGACC, TCGTCTGATCCATCCATCCATCC), RM17699 (GAGGTGTTTATAGAAGTA, AATTAGCTTATCTTGTGTTTC), were used to construct a genetic linkage map. A total of 24 μ L consisting of 2 μ L of 10–20 ng/ μ L template DNA, 1 μ L of 5–10 pM of each primer, 0.1 μ L of *Taq* polymerase (Imclone Biotech Co., IN5001), 0.375 μ L of dNTP mixture, 2.4 μ L of 10 \times Ex buffer, and 17.125 μ L of nuclease-free water (Qiagen, Cat. No. 129114). Analyses were conducted using a GeneAmp PCR System 2700 (Applied Biosystems, USA). The PCR reaction consisted of an initial period of denaturation for 5 min at 96°C followed by 34 cycles of 30 sec at 96°C, 30 sec at 55°C and 1 min at 72°C, followed by a final extension period of 8 min at 72°C and storage at 4°C. Finally, the amplified products were separated by electrophoresis (Qiagen QIAxcel) for subsequent genotyping.

Ratio of Coincidence between the Phenotype and Genotype using Other Lines

Plant materials were sampled at 14 days after WBPH infestation to extract DNA. A total of 35 lines consisting of 9 SNM (Samgang/Nagdong/Ilmi) lines and 26 F₁ populations derived from cross between JSNDH (Junam/Samgang/Nagdong doubled haploid) and CNDH lines were employed for genotyping using 7 SSR markers. The markers were selected from high rate of coincidence and constructed sequences of the markers as a pair of primers for PCR. Comparison analysis between phenotype and relative expression were conducted after scoring resistance to WBPH and PCR.

Statistical Analysis and Quantitative Trait Loci (QTLs)

The experiment was completely randomized block design with three replicates per treatment. Phenotypic data analyzed statistically by using the SAS program (ver. 9.4). Total 217 SSR markers by (McCouch *et al.*, 2002) were used to construct a genetic linkage map (Yun *et al.*, 2014). This is a genetic and bioinformatics program, Win QTL Cart 2.5, which is used for powerful tool to present analysis results and can import and export data in a variety of formats. We used the Windows QTL Cartographer V2.5, QTL to analyze the traits linking phenotype and genotype

data. QTL analyses were performed by composite interval mapping (CIM) by Windows QTL cartographer 2.5 (Wang *et al.*, 2006). A permutation test (1000 iterations) was used to establish experiment wise significance at a 0.05 confidence level defined as a minimum logarithm of odds (LOD) threshold for each trait in CIM (Doerge and Churchill, 1996). LOD values were computed from the likelihood ratio (LR) values based on the empirical relationship of one LOD being equal to 0.217LR. We used a candidate gene map of the 120 CNDH populations with a set of resistance related candidate gene markers (217 markers loci). Main window of Win QTL Cart 2.5 allows movement between open files, control of analysis parameters and display of chromosome graphics. Display parameters were set to show LOD profile as a block graph view, and the ratio between effect window size and LOD window size.

Results

The phenotypes of the 120 CNDH populations were evaluated when the seedlings of the susceptible control line TN1 were damaged following WBPH infestation. The resistant and susceptible rice in the 120 CNDH populations was measured by grading following the Standard Evaluation System for Rice (Heinrichs *et al.*, 1985). Cheongcheong was found to carry a resistance trait, while Nagdong carried a moderately susceptible trait. Ten resistant rice in 120 CNDH populations were under the same conditions and produced similar results. The distribution of resistant rice at each point in time was not significantly different (Table 1). The average severity scores of 120 CNDH populations were determined. The ‘Cheongcheong’ line carried the trait of high resistance to WBPH. The severity scores of the 120 CNDH populations varied greatly depending on the seedling stage and analysis of variance was highly significant among the observed the lines. This suggests that genetic factors for WBPH resistance were segregated in that population. ‘Cheongcheong’ is a resistant variety with the resistance bio-score of 0.2 while ‘Nagdong’ had a score of 0.4 following 7 days of infestation (Table 1). The mean bio-score of 120 CNDH populations was 1.8 with a standard deviation (SD) of 1.00. Bio-scoring following 14 days of infestation was 1.3 for ‘Cheongcheong’ and 2.2 for ‘Nagdong’ with a mean bio-score for all 120 CNDH populations of 2.5 with an SD of 1.75. Following 21 days of infestation, the bio-scores for ‘Cheongcheong’ was 5.0, ‘Nagdong’ 6.2, with the 120 CNDH population mean of 2.10 and an SD of 2.10.

Four QTLs affecting WBPH resistance were identified in chromosome 1 and chromosome 8, which were detected in both replications and average data (Table 2) with marker significant of LOD values of 3.3–4.0. Among them, 3 QTLs, qWBPH1-1, qWBPH1-2 and qWBPH1-3 were located on the same regions of chromosome 1 and qWBPH8 was located on the chromosome 8.

Table 1: Phenotypic value of 120 CNDH lines and parental ‘Cheongcheong/Nagdong’ for WBPH resistance

Length of infestation (day)	Parental lines			DH populations		
	Cheongcheong	Nagdong	Mean± SD ¹	Min.	Aver.	Max.
7	0.2	0.4	1.8±1.10	0.00	2.27	4.55
14	1.3	2.2	2.5±1.75	0.20	3.30	6.40
21	5.0	6.2	2.1±2.10	1.20	5.10	9.00

¹Mean ± Standard deviation; Min: Minimum, Aver: Average, Max: Maximum

Table 2: The QTLs associated with WBPH resistance in rice

Effect days	QTL ¹	Chr ²	Marker interval	LOD ³	Add.	R ² (%)	Increase allele
7	qWBPH1-1	1	RM3482-RM11966	4.0	- 0.3	29	Nagdong
	qWBPH1-2	1	RM3709-RM11694	3.5	0.3	29	Cheongcheong
14	qWBPH1-3	1	RM11694-M11669	3.3	0.5	28	Cheongcheong
21	qWBPH8	8	RM17699-RM264	3.3	0.7	25	Cheongcheong

¹Quantitative trait loci; ²Chromosome; ³Logarithm of odds; Add.: additive effect, R²: percentage of phenotypic variation

Table 3: Coincident rate of QTL markers from comparison with phenotype and genotype of resistant and susceptible lines among 120 CNDN lines

	Phenotype and markers	Parents		Resistant lines										Susceptible lines										Rate (%)	
		C	N	42-1	42-2	45	55	63	65	78	87	90	103-1	3	6	14-2	26	27	34-1	36	37	48-3	52		
DAI	7	Score	0.2	0.4	0.3	0.1	0.1	0.3	0.2	0.2	0.1	0.1	0.2	0.4	0.9	2.2	0.7	4.0	1.6	0.6	2.2	1.4	1.0	0.7	
	14		1.3	2.2	0.8	1.0	1.1	1.3	1.0	0.4	0.9	1.4	1.7	0.8	5.8	4.5	2.4	4.5	3.0	2.7	4.4	2.3	3.1	4.2	
	21		5.0	6.2	5.5	5.5	5.9	2.2	4.9	4.8	5.6	5.6	4.7	3.5	9.0	9.0	8.1	8.4	7.9	8.1	8.4	6.4	9.0	9.0	
Chr.	1	RM3482	O ⁴	X ⁵	O	O	O	O	O	O	O	O	O	O	X	X	X	O	X	X	X	X	X	X	95
	1	RM11966	O	X	O	O	O	O	O	-	-	-	-	-	X	X	X	X	X	X	X	X	X	X	75
	1	RM8235	O	X	O	O	O	O	O	O	O	O	-	X	X	X	X	X	X	X	X	X	X	X	90
	1	RM3709	O	X	O	O	O	X	O	O	-	-	-	-	X	X	X	X	X	X	X	X	X	X	75
	1	RM11694	O	X	O	O	O	X	O	O	O	O	O	O	X	X	X	X	X	X	X	X	X	X	95
	1	RM11669	O	X	O	O	O	-	-	-	-	-	-	-	X	O	O	X	O	O	X	O	O	X	40
	8	RM17699	O	X	-	O	O	X	O	O	O	O	O	O	X	X	X	X	X	X	X	X	X	X	90

Note: C = Cheongcheong; N = Nagdong; Rate = Coincidence rate of markers; DAI = days after infestation; Chr. = Chromosome; O = allele of Cheongcheong; X = allele of Nagdong

The ‘Cheongcheong’ allele contributed 3 QTLs for bio-scoring following 7, 14, 21 days of infestation, whereas the remaining 1 QTL was contributed by ‘Nagdong’ allele for bio-scoring following 7 days. Two QTLs, qWBPH1-1 and qWBPH1-2 on 7 days of infestation located in the interval RM3482-RM11966 and RM3709-RM11694, showed an additive effect of -0.3 and 0.3 on the long arm of the chromosome 1 accounted for 30% of the phenotypic variations with the mean LOD values of 4.0 and 3.5, respectively. The qWBPH1-3 on 14 days of infestation in the region RM11694-RM11669 on the chromosome 1 with a LOD value of 3.3 showed an additive effect of 0.5 on the trait and explained 30% of the phenotypic variation. The qWBPH8 of 21 days of infestation was detected on the short arm of the chromosome 8 with flanking markers of RM17699-RM264, showed an additive effect of 0.7 and accounted for 30% phenotypic variation with a LOD value of 3.3. 83.3% WBPH resistance bio-score was reported 7 days after infestation, 11.7% at 14 day and 8.3% at 21 days.

One QTL was also detected using the average data after 14 days of infestation, it was detected on chromosome 1 located in the intervals RM11694-RM11669. The markers in the interval RM3482-11966 and RM3709-RM11694 were also detected on the long arm of chromosome 1. To avoid the detection of artificial QTL effects due to real QTL in surrounding intervals, chromosome 1 was divided into

two segments, one extending from RM3482-RM11966 and the other most important one is in the interval RM11694-RM11669. One QTL at the interval RM11694-RM11669 on chromosome 1 was the most effective one after 14 days of infestation.

The coincident rate of the phenotype and genotype was determined in 10 lines of resistant and susceptible rice controlled by ‘Cheongcheong’ and ‘Nagdong’ lines, respectively (Table 3). The phenotypic variation was assessed with a genotype of seven markers. The marker RM3482, RM11966, RM8235, RM3709, RM11694 and RM11669 on chromosome 1 represented a coincident ratio of 95, 75, 90, 75, 95 and 40%, respectively. RM17699 on chromosome 8 represented 90% coincident ratio.

The WBPH resistant candidate lines (34 lines including JSNDH13/CNDH32-9-2-3-3) was applied for selected 7 markers from the DH population by way of QTLs as follows: The coincident rates were 74.3% (RM3482: Nagdong allele), 81.6% (RM11966: Nagdong allele), 73.7% (RM8235: Nagdong allele), 81.6% (RM3709: Nagdong allele), 81.6% (RM11694: Nagdong allele), 26.3% (RM11669: Nagdong allele) and 81.6% (RM17699: Nagdong allele) (Table 4). However, the efficiency of the markers (RM11966, RM3709 and RM11694: Nagdong wild type alleles) shown high ratio of coincidence of 81.6%, the same as the phenotype result.

Table 4: Ratio of coincidence between the phenotype and genotype using Tongil type populations

Lines	Phenotype scoring	Rating ¹	Chr. 1						Chr. 8
			RM3482	RM11966	RM8235	RM3709	RM11694	RM11669	RM17699
Cheongcheong	2.0	R	+	+	+	+	+	+	+
Nagdong	5.4	S	-	-	-	-	-	-	-
TN1	7.9	S	-	-	-	-	-	-	-
JSNDH13 ² /CNDH32-9-2-3-3 ³	1.9	R	-	-	+	-	-	+	-
JSNDH13/CNDH32-9-1-2-4	2.1	R	-	-	-	-	-	+	-
JSNDH13/CNDH32-9-1-3-3	2.6	R	-	-	+	-	-	+	-
JSNDH13/CNDH32-9-2-3-3	2.8	R	-	-	-	-	-	+	-
JSNDH13/CNDH32-9-4-2-1	2.9	R	-	-	-	-	-	+	-
SNM-5-B-2-2-B-B ⁴	3.0	R	-	-	-	-	-	-	-
JSNDH13/CNDH32-9-1-5-1	3.0	R	+	-	-	-	-	+	-
JSNDH13/CNDH32-9-1-1-2	3.2	S	-	-	-	-	-	+	-
SNM-5-B-2-2-4-B	3.3	S	-	-	-	-	-	-	-
JSNDH13/CNDH32-9-4-2-2	3.4	S	-	-	+	-	-	+	-
JSNDH13/CNDH32-11-2-5-3	3.4	S	+	-	-	-	-	+	-
JSNDH13/CNDH32-9-2-1-5	3.4	S	+	-	-	-	-	+	-
JSNDH13/CNDH32-9-2-4-3	3.6	S	-	-	-	-	-	+	-
JSNDH13/CNDH32-9-1-3-4	3.7	S	+	-	-	-	-	+	-
JSNDH13/CNDH32-9-1-1-2	3.8	S	-	-	-	-	-	+	-
JSNDH13/CNDH32-9-1-3-1	4.0	S	-	-	+	-	-	+	-
JSNDH13/CNDH32-9-2-3-4	4.2	S	+	-	-	-	-	+	-
JSNDH13/CNDH32-11-1-2-5	4.3	S	-	-	-	-	-	+	-
JSNDH13/CNDH32-9-2-5-5	4.5	S	-	-	-	-	-	+	-
JSNDH13/CNDH32-9-1-2-2	4.6	S	+	-	-	-	-	+	-
JSNDH13/CNDH32-11-2-1-3	4.7	S	+	-	-	-	-	+	-
JSNDH13/CNDH32-9-1-2-4	4.9	S	-	-	-	-	-	+	-
SNM-7-1-B-B-B-B	5.1	S	-	-	-	-	-	+	-
JSNDH13/CNDH32-11-1-2-1	5.3	S	-	-	-	-	-	+	-
JSNDH13/CNDH32-11-1-2-3	5.9	S	+	-	-	-	-	+	-
SNM-5-B-2-2-2-B-B	6.0	S	-	-	-	-	-	+	-
SNM-7-1-B-3-B-B	6.0	S	-	-	-	-	-	+	-
JSNDH13/CNDH32-11-3-3-2	6.3	S	+	-	-	-	-	+	-
JSNDH13/CNDH32-9-4-2-1	6.5	S	-	-	+	-	-	+	-
SNM-7-1-B-3-B-B	6.6	S	-	-	+	-	-	+	-
SNM-5-B-2-2-2-B-B	6.6	S	-	-	+	-	-	+	-
JSNDH13/CNDH32-9-2-4-4	6.8	S	-	-	-	-	-	+	-
SNM-12-3-3-B-B-B	6.9	S	-	-	-	-	-	+	-
SNM-7-1-B-5-B-B	7.1	S	-	-	-	-	-	+	-
JSNDH13/CNDH32-11-2-1-4	7.5	S	-	-	-	-	-	+	-
Coincident rate (type)			74.3(N)	81.6(N)	73.7(N)	81.6(N)	81.6(N)	26.3(C)	81.6(N)

¹R: resistance, S: susceptible. +: expressed band of ‘Cheongcheong’ type, -: expressed band of ‘Nagdong’ type; ²Junam//Samkang/Nagdong13; ³‘Cheongcheong/Nagdong’ Doubled haploid 32-9-2-3-3; ⁴Samkang/Nagdong//Ilmi-5-Bulk-2-2-Bulk-Bulk

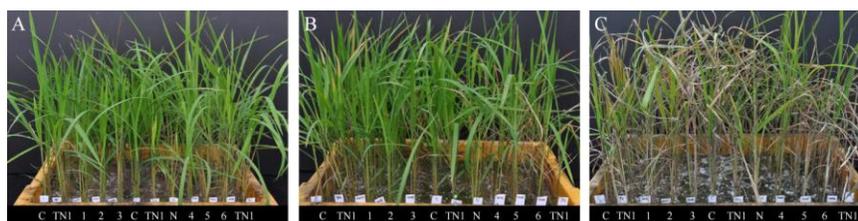


Fig. 1: Grading the phenotype of resistant rice after whitebacked planthopper infestation with in Cheongcheong/Nagdong double haploid population, (A) The resistance score after 7 days of infestation, (B) The resistance score after 14 days of infestation, (C) The resistance score after 21 days of infestation

The efficiency of marker RM11669 for WBPH resistance on chromosome 1 showed low ration of coincidence of 26.3% when comparing phenotype and genotype. The frequency of the ‘Cheongcheong’ allele was more than the ‘Nagdong’ allele when the segregating populations were derived from Tongil/*japonica* cross. All in all the genetics showed that for the RM3482, RM11966,

RM8235, RM3709, RM11694, RM17699, frequencies ‘Nagdong’ was the only donor of increasing allele.

Discussion

Resistant rice varieties are required to withstand WBPH infestation. The WBPH is a serious pest that damages rice

crops by sucking phloem sap. The 120 CNDH populations was developed from F₁ derived from a crossing WBPH resistance 'Cheongcheong' and susceptible 'Nagdong' lines. The population was fixed genetically in seventh generation. The genetic map with average 9.6 cM between markers was constructed from 120 CNDH populations using 217 SSR markers. In this study, we identified the QTLs associated with resistance in 120 CNDH populations for breeding insect-resistant rice. Linkage maps have been utilized for identifying chromosomal regions that contain genes controlling simple traits and quantitative traits using QTL analysis (Mohan *et al.*, 1997). QTLs associated with WBPH resistance in rice were introduced (Yamasaki *et al.*, 2000). Therefore, the density of WBPH in transplanted paddy fields during the immigration period could be a reliable parameter for sucking inhibition in rice plants. Functional resistance is limited in the plants that inhibit sucking because of restricted oviposition. Many QTL contributed by IR64 for resistance to WBPH were detected in different phenotypic measuring seedling resistance, antibiosis and tolerance mechanisms, using IR64/Azucena DH populations by (Geethanjali *et al.*, 2009). However, identification of varieties of WBPH resistant rice has been performed and their particular differences were found between the resistant varieties. A number of rice genes are very important for WBPH resistance. The variety 'Cheongcheong' was moderately resistant rice to WBPH in the seedling bulk test by (Kim, 1978) and this resistance was inherited from IR2035 in the form of *Wbph1* and *Wbph2* genes. Three QTLs associated with WBPH resistance were mapped on chromosomes 2, 6 and 11. The QTLs were *qwbp2b*, *qwbp6d*, and *qwbp11* located between RM2770-RM12532 on chromosome 2. RM527-RM528 on chromosome 6 and RM4469-RM26981 on chromosome 11 with a LOD score of 5.2 and 2.8, respectively. These three QTLs explained 56.0% of the total phenotypic variance detected by resistance score (Kim *et al.*, 2013). The present study showed that WBPH resistance could be quantitatively assessed at three different durations. All of these examples demonstrate that it is possible for the plants to achieve a broad range of resistance to different species of insects via a multiple resistance gene. The interactions between herbivorous insects and plants are rather complex. There are some very interesting biological questions concerning, how the resistances against WBPH are controlled by the same resistant genes, how rice-resistant varieties and susceptible varieties are recognized by different WBPH pieces and so forth (Tan *et al.*, 2004). Also two additional QTLs, *WBPH7(t)* and *WBPH8(t)*, is located on chromosome 3 and 4, respectively (Tan *et al.*, 2004; Sidhu *et al.*, 2005). The results have significant implications in studying the interactions between Kim *et al.* (2013) findings and this study just on chromosome 1 and 8 including sucking insects, plants and in breeding programs of resistance to rice. Molecular markers have been widely used to detect QTL

associated with quantitative resistance to insects in many crops (Yencho *et al.*, 2000). Thus, Four QTLs were detected on chromosome 1 and chromosome 8. Following infestation, the analysis of the results was repeated and showed that one QTL was detected at interval, RM11694-RM11669 of chromosome 1. Quantitative resistance was observed in the CNDH populations following WBPH infestation for 7 days, and there were 100 lines with 83.3% resistant rice lines. Following 14 days of WBPH infestation, there were 11.7% resistant rice lines and after 21 days infestation, only 8.3% of resistant rice remained in all 120 lines. The QTLs detected four regions of DNA on two chromosomes, chromosome 1 and chromosome 8. After 7 days, the dominance effects were almost the same. The knowledge of heterozygosity, dominance and recessive alleles allow us to imperfectly predict the value of genotype. The QTLs were located on chromosome 1 with the marker interval RM3482-RM11966 at LOD of 4.0 and RM3709-RM11694 at LOD of 3.5. After 14 days, QTLs were detected on chromosome 1, *qWBPH1* at the marker interval RM3709-RM11694 with LOD of 3.3 on additive effect of 0.5 and phenotypic variance of 30%. This indicates that the same location was involved in resistance after 7 days of infestation. Finally, after 21 days, QTL were found to be located on chromosome 8. *qWBPH8* located at the interval RM17699-RM264 of LOD 3.3 on additive effect 0.7 and phenotypic variance of 30%. Comparing the three different stages of the optimum screening time (raw data not shown), the distribution of WBPH resistance at each point in time was not significantly different, however damage score at 14 days after infestation with WBPH assumed a continuous distribution, this suggested that there was no overall bias toward either parent. In addition, the damage score at 14 days after infestation was evaluated when the seedlings of the susceptible control 'TN1' were completely killed (Ave. 8.9) by WBPH at 12 days after infestation. Taking the population as a whole, it has been identified that RM11694-RM11669, LOD 3.3 of 'Cheongcheong' allele in QTL detection (Table 2, 3) is very important in rice molecular breeding. One QTL, RM11694, at 14 days after infestation confirms the role of this QTL in regulatory screening resistant WBPH. When used in appropriate situations, it is a tool that can help plant breeders select more efficiently for desirable crop trait. MAS for SSR selection were performed in 'Cheongcheong' allele, and resistance to WBPH was detected in several phenotypic tests measuring seedling resistance, antibiosis and tolerance mechanisms. A preliminary association of candidate genes involved in defense was made using QTL. Furthermore, the results suggest the possibility of common loci conferring resistance to WBPH in the 'Cheongcheong'. Following this development, these new varieties should be further validated through fine-mapping and candidate gene analysis for their use in rice cultivars with improved resistance to WBPH.

Conclusion

Genetic map with average 9.6 cM between markers was constructed from 120 CNDH populations, which were used 217 SSR markers. Four QTLs were detected on four regions of the chromosomes 1, qWBPH1 optimum 7 days found in the interval RM3482-RM11966, RM3709-RM11694, 14 days RM11694-RM11669 and chromosome 8, qWBPH found in the interval RM17699-RM264. The markers found to be comprehended in identification comparison rate 95% the genetic and phenotypic after 14 days.

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