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Whitebacked planthopper *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae) resistance in rice variety Sinna Sivappu

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Abstract Whitebacked planthopper (WBPH) along with brown planthopper (BPH) has emerged as a major pest of rice in several Asian countries. Development and cultivation of varieties resistant to both planthoppers is an ecologically acceptable strategy to manage these pests. Sinna Sivappu, a Sri Lankan landrace, was reported to be resistant to both planthoppers. While inheritance of BPH resistance has been reported, the genetics of WBPH resistance in this variety is not known. Using a mapping population of 255 $F_{2:3}$ families from Taichung Native (TN)1/Sinna Sivappu cross and 128 polymorphic simple sequence repeat (SSR) markers, genes or quantitative trait loci (QTLs) for WBPH resistance quantified in ten phenotypic tests were identified, adopting classical Mendelian segregation, correlation and QTL analyses. The inheritance pattern suggested that a single recessive gene controlled regulation of seedling damage score. Antixenosis or nymphal preference was influenced by two complementary recessive genes, whereas tolerance in terms of days to wilt was under the influence of a single dominant gene. Several of these phenotypic tests recorded high degree of positive or negative correlation between them, suggesting dependence or redundancy of the tests. QTL analysis revealed 13 loci associated with nine traits. Five major-effect QTLs were detected for damage score (chromosome 6), nymphal survival (chromosome 12), and days to wilt (three QTLs on chromosome 4). We suggest involvement of four WBPH resistance genes in Sinna Sivappu, designated as *wbph9(t)*, *wbph10(t)*, *wbph11(t)*, and *Wbph12(t)*. One of the recessive genes could be allelic to any of the recessive genes reported in cluster C on chromosome 6 which might confer resistance to both BPH and WBPH.

Keywords Whitebacked planthopper · SSR markers · Novel gene(s) · QTLs · *Oryza sativa* L.

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Introduction

The whitebacked planthopper (WBPH) *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae) is an important insect pest of rice in Asia. Along with the more serious brown planthopper (BPH) *Nilaparvata lugens* (Stål), this planthopper has again become a threat to rice production in the continent. Both nymphs

and adults suck phloem sap from rice plants, which become orange–yellow, wilt, dry, and finally die. Under favorable conditions, insect feeding results in plant death known as "hopperburn". BPH acts as a vector of virus diseases such as grassy stunt, ragged stunt, and wilted stunt (Khush and Brar 1991), while WBPH acts as a vector of southern rice black-streaked dwarf virus (Zhou et al. 2008). The common method for controlling BPH and WBPH resorted to by farmers is application of insecticides, which is undesirable on several counts. Alternatively, development and cultivation of resistant cultivars has generally been considered to be the most economic and environmentally sound strategy for management of these planthoppers. However, it is very important to consider the genetic nature of resistance while developing such cultivars, as vertical resistance (controlled by one or a few major genes) provides short-lived resistance due to quick development of virulent biotypes. On the other hand, horizontal resistance (controlled by many quantitative trait loci) ensures durable pest resistance (Santhanalakshmi et al. 2010). The genetic nature of host plant resistance to WBPH has been reported to be both qualitative as well as quantitative (Fujita et al. 2013). Earlier studies on inheritance of resistance revealed 14 genes, i.e., *wbph 1* in Nagina 22 (see Fujita et al. 2013), *wbph 2* in ARC 10239, *wbph 3* in ADR 52, *wbph 4* in Podiwi-A8, *wbph 5* in N'diang Marie, *wbph6* in Guiyigu, and *wbph 7(t)* and *wbph 8(t)* in B5, an introgressed line from *O. officinalis*. Six more genes have been tentatively identified as *wbphM1* and *wbphM2* in Mudgo, *wbphAR* in ARC 11367, *wbphN* in NCS 2014, *wbphO* in MO1 (Sidhu et al. 2005), and *Ovc* in Asominori (Yamasaki et al. 2003). Several studies have been conducted to identify genomic regions/loci/alleles controlling WBPH resistance, and a total of 75 quantitative trait loci (QTLs) have been reported so far for WBPH resistance in rice (Fujita et al. 2013). Of these, 14 QTLs pertaining to days to wilt and 6 QTLs for damage score in standard seedbox screening test (SSST) were reported by Geethanjali et al. (2009) in a doubled haploid (DH) population derived from IR64/Azucena. Sogawa et al. (2009) reported five QTLs for honeydew secretion in DH lines of Zaiyeqing8/Jingxi17. Chen et al. (2010) reported three QTLs for damage score in SSST in backcross inbred lines (BILs) of Xieqingzao B/Dwr. Several other QTLs have also been reported (Yamasaki et al. 1999, 2003) for different components of resistance which do not pertain to the traits covered in the present study.

WBPH and BPH often occur at the same time, though in varying proportions across time and space. It is thus imperative that breeding for resistance should target both planthoppers (Bentur and Viraktamath 2008). Extensive screening of germplasm reported several sources possessing resistance to both planthoppers, i.e., BPH and WBPH (Kalode et al. 1977). By using such resistance sources, some resistance loci conferring resistance to both planthoppers have been detected (Tan et al. 2004). Earlier studies reported BPH and WBPH resistance in Sinna Sivappu, a landrace from Sri Lanka (Heinrichs et al. 1985, DRR 1999). Sidhu and Khush (1978) reported two genes conferring BPH resistance in Sinna Sivappu, one dominant and one recessive; one of the two genes is allelic to either *Bph3* or *bph4*, while the second gene might be a new gene. However, the genetics of WBPH resistance in Sinna Sivappu has not been studied yet. The present study was conducted to analyze the genetics and location of WBPH resistance genes or QTLs, using $F_{2:3}$ families from the cross of Taichung Native (TN)1 and Sinna Sivappu. We suggest involvement of three recessive and one dominant gene controlling different resistance traits and suggest one of the recessive gene to be allelic to *bph4*.

Materials and methods

Plant material

The mapping population consisted of 255 single F_2 plant-derived $F_{2:3}$ families from the cross between WBPH-susceptible parent TN1 and resistant parent Sinna Sivappu. A single confirmed F_1 plant was self-pollinated to obtain 255 F_2 seeds, which were raised as individual plants. Leaf sample of each plant was collected for DNA isolation and genotyping with polymorphic markers. Selfed seeds (F_3 seeds) from these plants were harvested separately. F_3 seeds were used to raise $F_{2:3}$ families and were used for phenotyping against WBPH. MO1 variety harboring *wbphO* gene (Sidhu et al. 2005) was also included in disease screening experiments along with resistant (Sinna Sivappu) and susceptible (TN1) checks.

Insect population

At the Directorate of Rice Research, Hyderabad, WBPH is being reared under controlled greenhouse conditions on the susceptible rice variety TN1 (Kalode et al. 1975). Freshly hatched nymphs or adults of specified age were utilized for various screening tests. Necessary precautions were also taken to keep the culture away from predators and other natural enemies and to prevent contamination with BPH.

Greenhouse evaluation for WBPH resistance

Phenotyping experiments were conducted in the greenhouse at 25 ± 5 °C and 50 ± 10 % relative humidity (RH) under natural light/dark conditions. The $F_{2:3}$ families along with parents TN1 and Sinna Sivappu, and R check MO1 were evaluated for different resistance traits following standard protocols. Recommended protocols for seedling reaction in SSST, nymphal preference at 24, 48, and 72 h, nymphal survival, nymphal duration, honeydew area on plants 30 and 60 days after sowing (DAS) (Heinrichs et al. 1985), and days to wilt 30 DAS and 60 DAS (Geethanjali et al. 2009) were adopted. Seedling reaction in SSST was recorded on a 0–9 scale as per the Standard Evaluation System (SES) of Rice (IRRI 1996).

Genotyping

DNA was isolated from the leaf samples of 255 F_2 plants along with those of parents using the modified method of Zheng et al. (1995) and then used for polymerase chain reaction (PCR) amplification following the protocol of Chen et al. (1997). A set of 514 SSR markers uniformly spread over 12 rice chromosomes was screened for parental polymorphism survey, which resulted in identification of 128 polymorphic markers. The entire mapping population was genotyped with these 128 polymorphic markers, and alleles were scored on agarose gel. Some of the reported markers linked to BPH and WBPH resistance genes (Fujita et al. 2013) were also screened for polymorphism. The original sources and motifs for all SSR markers used in the present study are available in the Gramene database at http://www.gramene.org. PCR amplification of SSRs was performed in 10 µl reaction volume containing template DNA (20–25 ng), 250 µM each of dNTPs, PCR buffer (1×), 0.6 U/µl of Taq DNA polymerase (Genei, Bangalore, India), and ≥0.2 µM of both forward and reverse primers. PCR amplifications were performed in 96-well plates on a thermal cycler (Eppendorf, Hamburg, Germany) using the following PCR conditions: hot start at 94 °C for 7 min followed by denaturing at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min for 35 amplification cycles, with final extension at 72 °C for 7 min. The PCR products were resolved on 3 % agarose gel (SeaKem; Rockland, USA) stained with ethidium bromide (0.5 µg/ml; Sigma-Aldrich, St. Louis, USA) in 0.5× Tris/borate/ethylenediaminetetraacetic acid (TBE) buffer at 100–150 V for 2 h using a submarine electrophoresis unit (Genei, Bangalore, India) and photographed under ultraviolet (UV) light. The size of the amplified fragments was calculated using Alphaease software (Alpha Innotech, San Leandro, USA) with a 100-bp ladder (MBI Fermentas, Vilnius, Lithuania) as size reference standard.

Data analysis

Phenotypic data for each of the tests recorded for the 255 $F_{2:3}$ families were subjected to goodness-of-fit analysis with expected Mendelian ratios for simple inherited traits. For this purpose, resistance was considered as a simple qualitative trait; all the $F_{2:3}$ families with values around the mean value recorded for the resistant parent Sinna Sivappu were treated as resistant, and the rest as susceptible. Thus, $F_{2:3}$ families were grouped as R and S to test F_2 ratio and into R, MR (segregating with intermediate values), and S (with values around the mean value recorded for the susceptible parent TN1) groups to test $F_{2:3}$ ratio. The frequency distribution of $F_{2:3}$ families across levels of phenotypic values was plotted, and data were analyzed for normal distribution using the Anderson–Darling test (Theodorsson 1988). Mean, range, standard deviation, standard error, paired t test, χ^2 test, correlation analysis, and probability estimates for null hypothesis rejection using these tests for all the phenotypic values of WBPH resistance were obtained using MS Excel software.

Resistance against WBPH was also investigated by QTL analysis. Genotype data assembled for all the polymorphic makers among all the 255 F_2 plants were subjected to linkage analysis using JoinMap version 4.0 (Van Ooijen 2006). Map distances were

calculated using the Kosambi (1944) mapping function. Placement of markers into different linkage groups (LGs) was done with "LOD groupings" and "Create group using the mapping tree" commands. Mean χ^2 contributions or average contributions to the goodness of fit of each locus were also checked to determine the best fitting position for markers in genetic maps. Markers showing negative map distances or a large jump in mean χ^2 value were discarded. Final maps were drawn with the help of MapChart version 2.2 (Voorrips 2002). QTL analysis of $F_{2:3}$ families was performed using a composite interval mapping (CIM) method (Zeng 1994) in Windows QTL Cartographer version 2.5 (Wang et al. 2007). A permutation number of 1,000 was applied for each trait in QTL analysis, and a LOD threshold of 2.5 was adjusted for identification of significant QTL. The relative contribution of each QTL towards phenotype was calculated as the percentage of phenotypic variation explained (PVE, %). The percentages of variation explained by a QTL and the additive effect were also estimated using the software.

Results

Inheritance of WBPH resistance

For all ten phenotypic traits, values recorded for the susceptible parent TN1 were significantly different from those recorded for the resistant parent Sinna Sivappu (Table 1). However, Sinna Sivappu differed significantly from the standard resistant check MO1 only in terms of damage score and days to wilt for plants 30 DAS. Thus, Sinna Sivappu was mostly as resistant to WBPH as the check MO1. Mean values and range of all ten phenotypic traits for the F_3 families are also presented in Table 1. Transgressive segregants were observed in $F_{2:3}$ families in terms of damage score (Fig. 1), nymphal survival, nymphal duration, and honeydew area on plants 30 and 60 DAS, suggesting multigene influence on the trait. However, the Anderson–Darling test for normality in distribution of values recorded for the 255 $F_{2:3}$ families across the observed range for these phenotypic traits confirmed normal distribution in respect of only honeydew area on plants

Table 1 Mean phenotypic trait values of the two parents TN1 and Sinna Sivappu, the resistant check MO1, and F_3 families recorded in greenhouse tests against WBPH

Trait	Parents check			F_3 families	
	TN1 (susceptible), mean ± SE ($n = 5$)	Sinna Sivappu (resistant), mean ± SE ($n = 5$)	MO1 (resistant), mean ± SE ($n = 5$)	Mean ± SE ($n = 255$)	Range
Damage score	8.9 ± 0.07[a]	2.8 ± 0.05[b]	1.8 ± 0.07[c]	4.7 ± 0.15	1.1–9.0
Nymphal preference (24 h) (no. WBPH/plant)	12.8 ± 0.27[a]	3.8 ± 0.15[b]	3.3 ± 0.22[b]	7.9 ± 0.08	4.5–9.9
Nymphal preference (48 h) (no. WBPH/plant)	12.7 ± 0.26[a]	3.5 ± 0.22[b]	2.92 ± 0.15[b]	7.7 ± 0.08	4.3–9.7
Nymphal preference (72 h) (no. WBPH/plant)	12.3 ± 0.21[a]	3.24 ± 0.23[b]	2.6 ± 0.13[b]	7.2 ± 0.08	4.3–9.4
Nymphal survival (%)	96.0 ± 0.17[a]	35.5 ± 0.17[b]	33.0 ± 0.31[b]	66.0 ± 0.09	37–93.3
Nymphal duration (days)	10.6 ± 0.25[a]	19.2 ± 0.31[b]	20.2 ± 0.30[b]	14.7 ± 0.167	10–19
Honeydew area (mm^2) (30 DAS)	234.6 ± 2.96[a]	82.7 ± 1.30[b]	75.4 ± 1.51[b]	159.0 ± 2.29	81.3–240*
Honeydew area (mm^2) (60 DAS)	198.7 ± 6.91[a]	74.4 ± 2.20[b]	68.5 ± 1.86[b]	151.0 ± 2.49	72–204*
Days to wilt (30 DAS)	7.3 ± 0.15[a]	12.3 ± 0.20[b]	14.2 ± 0.30[c]	9.7 ± 0.11	7.3–12.3
Days to wilt (60 DAS)	9.4 ± 0.19[a]	17.9 ± 0.25[b]	18.3 ± 0.28[b]	13.5 ± 0.16	9.3–18.3

Means in a row not followed by the same superscript letter are different at $P < 0.05$, paired t test; * not significant in Anderson–Darling test of normal distribution; other trait values significantly differed from normal distribution; *SE* standard error

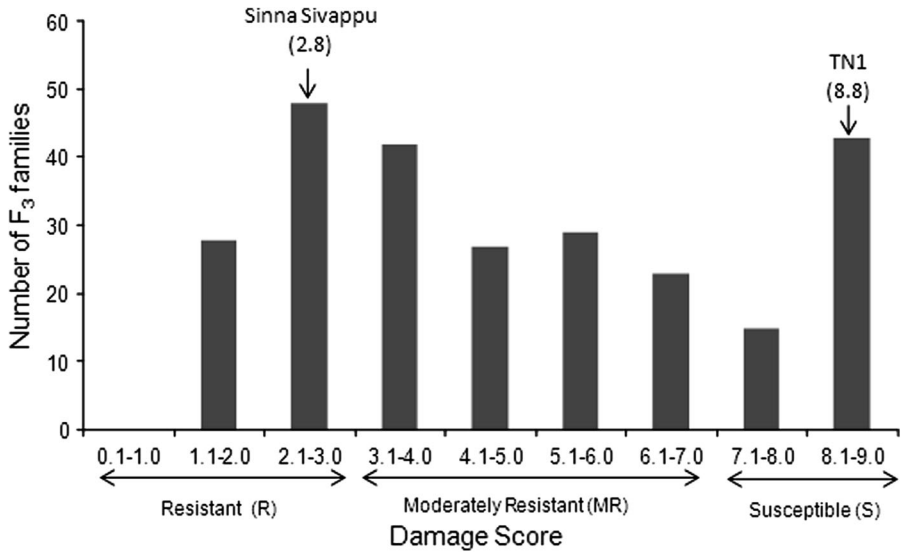

Fig. 1 Frequency distribution of F_3 families derived from TN1/Sinna Sivappu cross across the range of damage score in standard seedbox screening test (SSST) in greenhouse against WBPH, *S. furcifera*. Transgressive segregants with values beyond the range displayed by the parents were observed

Table 2 Inheritance of resistance in terms of different phenotypic traits recorded in F_3 families of the cross TN1/Sinna Sivappu

Trait	No. of F_2 plants, χ^2				No. of F_3 families, χ^2				
	S	R	(3S:1R)	*P* value	S	MR	R	(1:2:1)	*P* value
Damage score	179	76	3.139	0.076	58	121	76	3.204	0.202
Nymphal survival (%)	200	55	1.601	0.206	92	108	55	16.702	<0.001
Nymphal duration (days)	199	56	1.256	0.262	25	174	56	41.454	<0.001
Honeydew area (30 DAS)	213	42	9.894	0.002	38	175	42	35.518	<0.001
Honeydew area (60 DAS)	199	56	1.256	0.262	31	168	56	30.631	<0.001
Trait	No. of F_2 plants, χ^2				No. of F_3 families, χ^2				
	S	R	(15S:1R)	*P* value	S	MR	R	(7:8:1)	*P* value
Nymphal preference (24 h)	239	16	0.004	0.950	90	149	16	7.793	0.020
Nymphal preference (48 h)	238	17	1.133	0.287	93	145	17	5.561	0.062
Nymphal preference (72 h)	238	17	1.133	0.287	94	144	17	4.971	0.083
Trait	No. of F_2 plants, χ^2				No. of F_3 families, χ^2				
	S	R	(1S:3R)	*P* value	S	MR	R	(1:2:1)	*P* value
Days to wilt (30 DAS)	76	179	3.319	0.077	76	140	39	13.188	0.001
Days to wilt (60 DAS)	49	206	4.550	0.033	49	148	58	7.227	0.027

30 and 60 DAS (Table 1). Generally, such a distribution did not support polygenic control of many of these phenotypic traits tested. This prompted us to test $F_{2:3}$ segregation data to fit simple Mendelian ratios (Table 2). The inheritance pattern noted in respect of damage score was controlled by a single recessive gene, and antixenosis, i.e., number of nymphs settled after 24, 48, and 72 h of release, was controlled by two complementary recessive genes. However, tolerance, i.e., days to wilt for plants 30 and 60 DAS, was

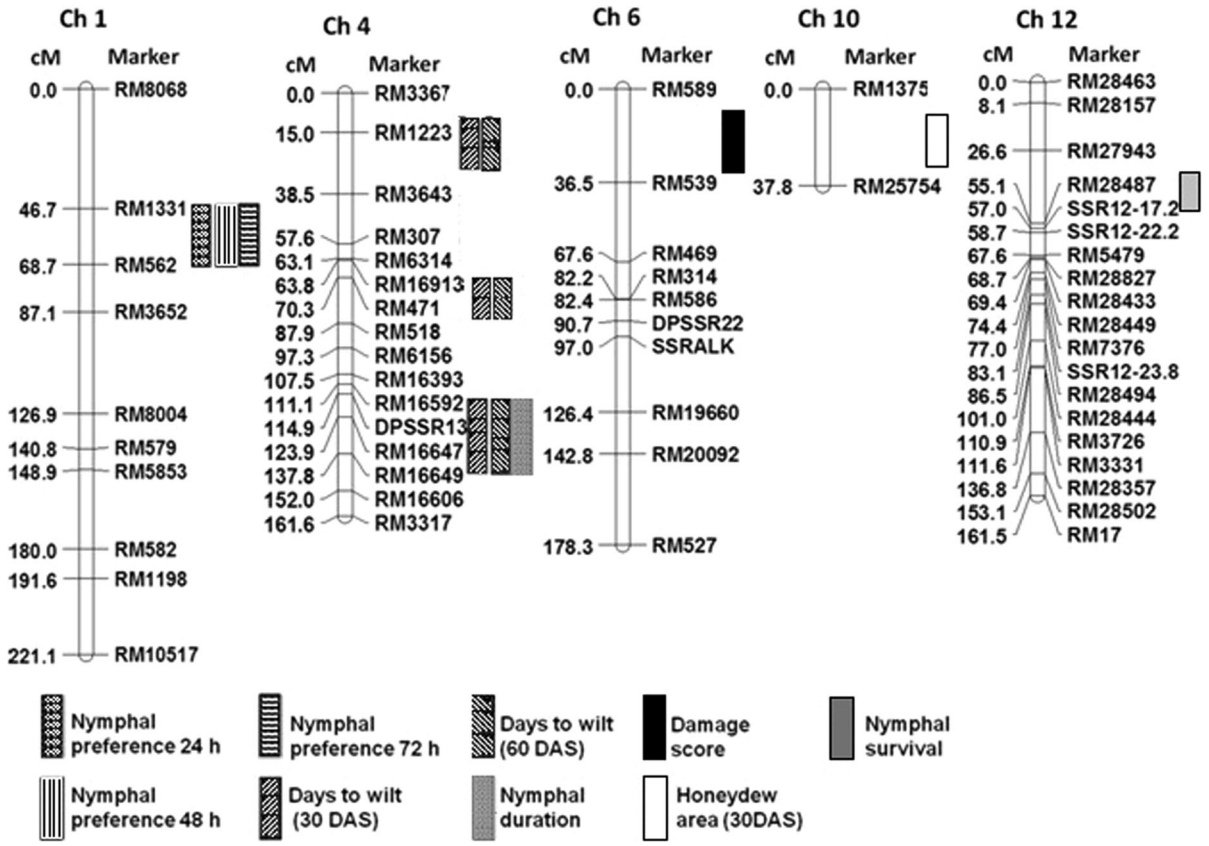

Fig. 2 Molecular linkage map constructed by simple sequence repeat (SSR) markers assayed on the TN1/Sinna Sivappu F_2 population and quantitative trait loci (QTLs) conferring resistance to WBPH using different methods

controlled by a dominant gene. Data pertaining to other traits listed in Table 1 did not show significant fitness to simple Mendelian ratio. Thus, a minimum of one recessive gene, one dominant gene, and two complementary recessive genes may influence the inheritance of WBPH resistance in Sinna Sivappu. To determine whether the recessive gene(s) implicated for different traits could be the same, linkage analysis with markers was carried out.

Construction of SSR linkage map and QTL identification

Of 514 SSR markers tested for polymorphism between TN1 and Sinna Sivappu, 128 (25 %) were found to be polymorphic. All 255 F_2 plants were genotyped with these polymorphic markers. Twenty-eight of these markers were dropped from genetic mapping due to nonsignificant linkages and high map distances with adjacent markers in the linkage group. Thus, the final molecular linkage map was constructed with 100 polymorphic markers to identify QTLs conferring WBPH resistance. The map covered 1,747.5 cM on all 12 chromosomes, with average interval of 17.4 cM. QTL analysis using Windows QTL Cartographer version 2.5 with an LOD threshold of 2.5 and significance level of 0.01 detected 13 QTLs for WBPH resistance (Fig. 2). Five of these were major-effect QTLs accounting for 24.9–87.7 % of the phenotypic variation (Table 3), and the remaining eight were minor-effect QTLs (Table 4).

Since clusters of QTLs were detected on chromosomes 6 and 12 in regions already reported to be rich in planthopper resistance genes, some of the available gene-linked SSR markers were tested for polymorphism between the parents (Table 5). RM586 linked to *bph4* gene on chromosome 6 showed polymorphism between the parents, suggesting its involvement in

Table 3 Localization on three rice chromosomes of five major-effect QTLs accounting for phenotypic variation in damage score, nymphal survival, and days to wilt 30 and 60 DAS, recorded in greenhouse against WBPH, *S. furcifera*

Trait	QTL	Chr. no.	Marker interval	LOD	Additive effect	PVE (%)
Damage score	*qWDS-6*	6	RM589-RM539	35.3	3.00	87.7
Nymphal survival	*qWNS-12*	12	SSR12-17.2-RM28487	49.2	2.17	64.0
Days to wilt (30 DAS)	*qWDW(30)-4.1*	4	RM3643-RM1223	6.0	−1.31	24.9
Days to wilt (30 DAS)	*qWDW(30)-4.2*	4	RM16913-RM471	11.5	−1.60	36.1
Days to wilt (60 DAS)	*qWDW(60)-4.1*	4	RM3643-RM1223	7.1	−2.18	28.1

LOD logarithm of odds, *PVE (%)* percentage of phenotypic variance explained

Table 4 Localization on three rice chromosomes of minor-effect QTLs accounting for phenotypic variations noted in seven tests in greenhouse against WBPH, *S. furcifera*

Trait	QTL	Chr. no.	Marker interval	LOD	Additive effect	PVE (%)
Honeydew area (30 DAS)	*qWHDA(30DAS)-10*	10	RM1375-RM25754	2.6	−19.9	9.82
Nymphal preference (24 h)	*qWNP(24h)-1*	1	RM562-RM1331	16.4	0.517	2.34
Nymphal preference (48 h)	*qWNP(48h)-1*	1	RM562-RM1331	10.9	0.61	3.24
Nymphal preference (72 h)	*qWNP(72h)-1*	1	RM562-RM1331	2.7	0.47	2.25
Nymphal duration	*qWND-4*	4	RM16592-RM16649	3.1	−1.68	13.4
Days to wilt (30 DAS)	*qWDW(30DAS)-4.3*	4	RM16592-RM16649	3.4	−0.77	10.1
Days to wilt (60 DAS)	*qWDW(60DAS)-4.2*	4	RM3643-RM518	3.59	−1.00	13.5
	qWDW(60DAS)-4.3	4	RM16913-RM16649	3.83	−1.47	11.03

LOD logarithm of odds, *PVE (%)* percentage of phenotypic variance explained

Table 5 Amplification pattern noted for the parents TN1 and Sinna Sivappu when tested with reported planthopper resistance gene-linked SSR markers

Resistance gene	Chr. no.	SSR marker	Status	Donor
Bph3	6	RM588	Monomorphic	Ptb33
Bph3	6	RM19291	Monomorphic	Rathu Heenathi
Bph3	6	RM8072	Monomorphic	Rathu Heenathi
bph4	6	RM586	Polymorphic	Babawee
Bph6	4	RM16994	Monomorphic	Swarnalatha
Bph6	4	RM119	Monomorphic	Swarnalatha

WBPH resistance. However, three other markers linked to *Bph3* gene on the same chromosome did not show polymorphism.

Correlation among traits

Correlation among the ten traits was analyzed to understand their interdependence (Table 6). Significant positive correlations were observed among values for nymphal preference 24, 48, and 72 h after release, between honeydew area on plants 30 and 60 DAS, and between days to wilt 30 and 60 DAS, suggesting redundancy of multiple observations. Significant positive correlations between damage score and honeydew area on plants 30 and 60 DAS, and damage score and nymphal preference 24, 48, and 72 h after release, suggested that the damage score reflected nymphal settling and feeding response. Also, nymphal survival values showed significant correlations with honeydew area on plants 30 and 60 DAS and nymphal preference 24, 48, and 72 h after release. Nymphal duration showed positive correlation with days to wilt

Table 6 Correlation matrix among different phenotypic trait values observed for F_3 families of the cross TN1/Sinna Sivappu

Trait	Nymphal duration (days)	Nymphal survival (%)	Honeydew area (30 DAS)	Honeydew area (60 DAS)	Days to wilt (30 DAS)	Days to wilt (60 DAS)	Nymphal preference (24 h)	Nymphal preference (48 h)	Nymphal preference (72 h)
Damage score	0.0581	0.0184	0.2896**	0.3205**	0.0229	−0.0097	0.3082**	0.2910**	0.2757**
Nymphal duration		−0.0980	−0.0943	−0.0143	0.6805**	0.5184**	−0.1026	−0.1303*	−0.1947**
Nymphal survival			0.1870**	0.1759**	−0.0849	−0.0372	0.1647**	0.1442*	0.1341*
Honeydew area (30 DAS)				0.7823**	−0.1245*	−0.0741	0.6292**	0.5925**	0.5364**
Honeydew area (60 DAS)					−0.0376	−0.0172	0.6653**	0.6382**	0.5779**
Days to wilt (30 DAS)						0.7400**	−0.0988	−0.1013	−0.1532*
Days to wilt (60 DAS)							−0.0810	−0.0841	−0.1491
Nymphal preference (24 h after release)								0.9040**	0.7834**
Nymphal preference (48 h after release)									0.8746**
Nymphal preference (72 h after release)									

Significant at * $P < 0.05$ or ** $P < 0.01$, d.f. = 253

30 and 60 DAS and negative correlation with nymphal preference 48 and 72 h after release.

Discussion

It is evident from the above results that resistance to WBPH in Sinna Sivappu inherited as three discrete traits. One recessive gene (damage score), one dominant gene (days to wilt), and two complementary recessive genes (nymphal preference) controlled these traits (Table 2). Trait correlation analysis (Table 6) also suggested positive correlation between damage score and honeydew area on plants 30 and 60 DAS, and damage score and nymphal preference. Thus, it appears that these phenotypic traits are interdependent and could be manifestations of single gene action. QTL analysis also suggested localization of QTLs in one region on chromosome 6 (Table 3). The QTL *qWDS-6* accounted for 87.7 % of phenotypic variation noted in SSST as damage score. Thus, it is logical to assume that one of the recessive genes detected in the present segregation analysis, proposed as *wbph9(t)* for damage score, is the same as *qWDS-6*. Fujita et al. (2013) noted a cluster of BPH and WBPH resistance genes (cluster C) on chromosome 6 around this region, including *Bph3*, *bph4*, *qBph6(t)*, *Bph25(t)*, and *Ovc*. The mode of action of this gene can be hypothesized to be gustatory mediated through presence of feeding deterrents or absence of feeding stimulants.

Extending a similar argument, days to wilt, which reflected the tolerance trait of WBPH resistance in Sinna Sivappu, was noted to be under the influence of a single dominant gene. Corroboratively, this trait did not correlate with damage score, nymphal preference 24 and 48 h after release or, generally, amount of honeydew excreted. However, tolerance was related to nymphal duration. Three major-effect QTLs, namely *qWDW(30DAS)-4.1*, *qWDW(30DAS)-4.2*, and *qWDW(60DAS)-4.1*, were localized on chromosome 4, accounting for 24.9–36.1 % of phenotypic variation. In addition, another three minor QTLs were also localized on chromosome 4, namely *qWDW(30)-4.3*, *qWDW(60)-4.2*, and *qWDW(60)-4.3*, accounting for 10.1–13.5 % of phenotypic variation. Thus, it is reasonable to assume that the dominant gene, proposed as *Wbph12(t)* for days to wilt, would be at this locus on chromosome 4. Interestingly, *qWND4* associated with nymphal duration was also mapped to this region. Thus, these two traits may reflect a single gene action. Fujita et al. (2013) noted two clusters of planthopper resistance genes on chromosome 4: cluster B with *Bph17*, *Bph12(t)*, *Bph15*, and *Bph20(t)*, and cluster D with *bph12(t)*, *Bph6*, and *bph18* genes. Based on the map position of the SSR markers, the *Wbph12(t)* locus lies within cluster D. However, *Bph6*-linked SSR markers RM16994 and RM119 did not show polymorphism between the parents TN1 and Sinna Sivappu. It is imperative to understand the nature and function of the *Wbph12(t)* class of genes, since they elicit a plant loss compensation mechanism to overcome insect-inflicted damage without, probably, exerting selection pressure on the insect. This feature is likely to confer durable resistance and would be compatible with natural biological control in combination with the action of effective fauna of predators and parasitoids. The possible association of this gene with influence on prolonging nymphal duration would assist biocontrol agents to be more effective.

A single major-effect QTL was localized on chromosome 12, accounting for 64 % of the phenotypic variation in the nymphal survival trait. Interestingly, nymphal survival was clearly detected in no-choice test rather than in free-choice tests. Thus, antibiotic component of resistance neither determined the damage score in SSST nor influenced short-term honeydew test. Cluster A with six BPH resistance genes [*Bph9*, *Bph10*, *Bph26(t)*, *Bph16(t)*, *Bph18(t)*, and *Bph21(t)*] has been reported on chromosome 12 (Fujita et al. 2013). Most of the early studies reporting planthopper resistance as major genes (Khush and Brar 1991) were based on seedling damage score in SSST and could have missed identification of factors responsible for both tolerance and antibiotic effects. Likewise, ovicidal effects reported for WBPH resistance (Yamasaki et al. 2003) were neglected in these studies. On the contrary, recent studies based on QTL analysis (Alam and Cohen 1998; Yamasaki et al. 1999, 2003; Sogawa et al. 2009; Geethanjali et al. 2009; Chen et al. 2010) did not consider these loci as Mendelian entities and did not apply segregation analysis for these traits. Several components of resistance phenotype dissected and analyzed in these studies are often treated as independent parameters. Our study tried to integrate these approaches and identified four major genes (one recessive, one

dominant, and two complementary recessive) responsible for WBPH resistance in Sinna Sivappu variety. It is obvious that some residual resistance, as suggested by other QTLs, remains unaccounted for. Also, more closely linked markers are needed for pyramiding these four genes/loci through marker-assisted selection (MAS) to ensure durable WBPH resistance in rice.

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