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RESEARCH ARTICLE

Genetics of novel brown planthopper *Nilaparvata lugens* (Stål) resistance genes in derived introgression lines from the interspecific cross *O. sativa* var. Swarna × *O. nivara*

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Abstract. The brown planthopper (BPH) *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) is considered a threat to rice (*Oryza sativa* ssp.) crop in many parts of the world including India. Among the BPH-resistance (R) genes so far reported in rice, most of them are ineffective against BPH biotype 4 predominant in the Indian sub-continent. In this study, we show the introgression line RPBio4918-230S was identified as BPH resistant after five years of rigorous screening at seedling stage and two years at tillering and reproductive stages. The inheritance of resistance indicated that two recessive genes are involved at seedling and reproductive stages. The allelic relation with known genes using linked reported markers suggested that the genes present in RPBio4918-230S are different. We report here the genetics of the two newly introgressed BPH resistance genes from *O. nivara* in the background of Swarna which are effective at all the important growth stages. The genes have been tentatively named as *bph39(t)* and *bph40(t)*. The honeydew area (feeding rate) and days to wilt parameters observed at 30 days after sowing in BC_1F_3 indicated that newly introgressed genes have both antibiosis and tolerance mechanisms for resistance. The BPH resistance genes identified in this study would facilitate the breeding of broad spectrum and durable resistance in rice against BPH biotype 4.

Keywords. brown planthopper; novel gene introgression; reproductive stage resistance; rice; *Nilaparvata lugens*; *Oryza nivara*.

Introduction

More than half of the world's population including 2.70 billion people in Asia feed on rice and is one of the most important staple food crops (Sarao and Mangat 2014). Among the various factors limiting rice production, insect pests are of prime importance, which reduce yield up to 60% under epidemic conditions (Heong 2009). The BPH, *Nilaparvata lugens* (Stål), is a typical phloem sap feeder and has emerged as important insect-pest limiting rice production in Asia (Normile 2008; Heong 2009). Both nymphs and adults of BPH suck the phloem sap from the lower portion of the plant, which results in yellowing of leaves, reduced tillers and plant height, and increased unfilled grains. Under severe attack, it causes extensive plant mortality known as 'hopper burn' (Watanabe and Kitagawa 2000; Liu *et al*. 2009; Ram *et al*. 2010). BPH also transmits viral diseases of rice like grassy stunt, ragged stunt (Ling *et al*. 1978) and wilted stunt. Frequent and indiscriminate use of insecticides has led to a rapid increase in BPH resistance to several insecticides in Asia causing resurgence in BPH triggering its establishment as a major insect pest (Reissing *et al*. 1982). Hence, cultivation of resistant rice varieties is the better and environmentally safe substitute (Song *et al*. 2002).

Rice crop is vulnerable to BPH at seedling as well as reproductive stages. Hence, it is important to identify resistant donors and genes for seedling as well as tillering and booting stages. Using seedling stage screening, 38 BPH resistance genes have been identified (Fujita *et al*. 2013; Ren *et al*. 2016; Naik *et al*. 2018; Kumar *et al*. 2018) from cultivated and wild species of rice but their effectiveness at tillering and reproductive stage is not known. Many of the

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resistance genes/QTLs such as *Bph1*, *bph2*, *Bph3*, *bph4*, *Bph6*, *Bph9*, *Bph19*, *Bph25*, *Bph26*, *Bph27*, *Bph28*, *Bph31* and *Bph32* have been mapped with molecular markers from *O. sativa* accessions and *Bph10*, *bph12*, *Bph13*, *Bph14*, *Bph15*, *bph16*, *Bph17*, *Bph18*, *Bph20, Bph21, Bph27*, *bph29*, *bph30* and *Bph31(t)* from wild species of rice. Most of the genes for BPH resistance have been identified against the BPH biotypes 1, 2 and 3; however, their effectiveness against biotype 4 is limited. Some of the genes derived from wild species are effective against major biotypes including biotype 4, which is most destructive and distributed across Indian subcontinent (Heinrichs *et al*. 1985; Ram *et al*. 2010). In our previous report, we showed that among the gene donors, only *Bph3*+*Bph17*, *Bph6*, *Bph20*+*Bph21*, *Bph22*, *Bph23* and *bph24* provide resistance or moderate resistance against BPH biotype 4 at seedling stage but are ineffective at tillering and reproductive stage (Akanksha *et al*. 2017). Only the introgression lines/germplasm RPBio4918-230S, OM4498, RP2068-18-3-5 and PTB33 with unidentified genes showed resistance to BPH at seedling as well as tillering and reproductive stages. We identified novel BPH resistance genes from the line derived from interspecific cross of *O. sativa* var. Swarna and *O. nivara,* which is effective against the BPH biotype 4 at both seedling and adult plant growth stages. The genetics of these novel introgressed genes is reported in the present study.

Materials and methods

Parental material and developing introgression lines

The resistant material RPBio4918-230S is an introgression line at BC_2F_6 generation developed from *O. nivara* (acc no. IRGC81848) in the background of a high yielding popular rainfed lowland variety Swarna which is susceptible. The line RPBio4918-230S was selected based on its stable resistance to BPH Biotype 4, over five years of screening in the greenhouse and two years in field condition from tillering to reproductive stage under hopperburn conditions at ICAR-Indian Institute of Rice Research (IIRR), Hyderabad. The line RPBio4918-230S has the weedy traits like grain shattering, awns, and seed dormancy inherited from wild species.

RPBio4918-230S was crossed with Swarna, as a female parent (recurrent parent), and backcrossed to generate 47 BC_1F_1 populations. All the BC_1F_1 were grown in field of ICAR-IIRR, Hyderabad to develop BC_1F_2 generation. All the plants in BC_1F_1 were bagged with butter paper bag at flowering stage to avoid outcrossing. The seed harvested from each BC_1F_1 plants were used for screening against BPH, and was grown as BC_1F_2 population. In the BC_1F_2 population, which were segregating for BPH resistance, three to seven plants from each population with morphologically similar to Swarna were selected and bagged at flowering. As many as 203 BC_1F_2 plants were harvested separately for further studies in BC_1F_3 generation. All the BC_1F_2 populations were screened for resistance reaction (damage score) of BPH at seedling stage in greenhouse to study the inheritance while part of the BC_1F_2 seeds were used to screen BPH tolerance in field condition at tillering and reproductive stage under severe infestation condition. The 203 BC_1F_3 families were also screened for damage score in greenhouse to study the genetics. The 21 selected plants from resistant, segregating and susceptible families along with TN1, RPBio4918-230S and Swarna were screened for parameters like honeydew and days to wilt to understand the mechanism of resistance.

Rearing of BPH biotype 4

The BPH insects were maintained in the greenhouse of Entomology Department at ICAR-IIRR, by feeding them on TN1 plants (susceptible check) in cages. Mass rearing of BPH was done in 70 cm × 75 cm wooden cages having glass panels on one side and wire mesh on all other sides. The suitable temperature (30 ± 5°C), relative humidity (60 ± 10%) and light–dark photoperiod were also maintained for the multiplication of BPH. Premated gravid females collected from IIRR were allowed to oviposit on 30 days old TN1 plants for two days and freshly hatched second and third instar nymphs were used for infestation in the experiment. After a period of 20 days from the day of insect release, when the plants started drying, the newly emerged male and female adults were shifted to another wooden cage for fresh cycle of insect culture.

Screening for BPH resistance

Phenotypic evaluations of 50–200 plants in each of 47 BC_1F_2 families were carried out to know the segregation pattern using standard seedbox screening test (SSST). Each family was again evaluated at adult plant growth stage under field conditions in Andhra Pradesh Rice Research Institute, Maruteru, India, which is a hot spot location for BPH. Screening at seedling stage was conducted following SSST developed at IRRI, Philippines (Heinrichs *et al*. 1985). Experiments were performed in the greenhouse at 30 ± 5°C with 60 ± 10% relative humidity (RH) under natural light/dark conditions. The seeds were presoaked and sown in the rows in 60 × 45 × 10 cm seed boxes along with resistant and susceptible checks. Each box was planted with one BC_1F_2 population having 10 rows along with one middle row of PTB33 (resistant) and one row of TN1 (susceptible check) in the borders with 20 to 25 plants in a row. While in BC_1F_3 generation, each line was sown with one family of 25–30 plants and the experiment was repeated thrice. Twelve-day-old seedlings at two to three–3 leaf stage were infested with first instar nymphs at the rate of six to eight nymphs per seedling. Approximately one week after

infestation, when 100% plants died in the susceptible check (TN1), the damage scores were recorded in 0–9 scale following the standard evaluation system (SES) of rice of each plant of every row. While at the adult stage screening, 20-day-old seedlings of backcross populations were planted in three rows with 30 plants in each row with 20 × 15 cm spacing (row × plant) at APRRI & RARS Maruteru, India. The experiment was conducted following randomized block design with two replications. Single seedling per hill was planted with count of 30 to 90 plants in the families. After every five families of test entries, the resistant and susceptible checks along with both parents were planted. TN1 was also planted all around the replications and experiment. At tillering stage, crop was artificially infested with second instar nymphs to increase the insect load to create hopperburn situation. Observations on the tolerance and susceptible plants were taken when 90% TN1 plants were dead in all the experiments at tillering stage. The plants that became yellow having most of the leaves dried were considered as susceptible. The plants with green leaves and healthy stem, and seed set at maturity were considered as resistance at reproductive stage.

Honeydew/feeding rate (30 days after sowing (DAS))

Twenty-one lines with resistance, susceptible and segregating reaction to BPH were used for honeydew measurement, as honeydew quantity is an indirect measure of feeding preference to understand the mechanism of resistance. It was measured by the amount of honeydew excreted by the insects indicating the feeding preference and efficiency of BPH. Whatman No-1 filter paper was dipped in a 0.02% bromocresol green solution prepared in ethanol, allowed to dry for 1 h and dipped again until the filter paper turned yellowish orange. The treated filter paper was placed at the bottom of 30 day-old plants, planted in small plastic pots. A small plastic cup with a hole was placed over the filter paper and five prestarved adult insects were released in the cup. Cotton was plugged in the hole to prevent the escape of the insects. The adults were allowed to feed for 24 h at the base of stem. The honeydew droplets excreted by the adults turned into blue spots when exposed to the filter paper. The relative area (mm^2) of the spots produced by honeydew excreted on bromocresol green treated filter paper was determined using Image-J software. Amount of feeding by the insect on test entry was expressed as area of honeydew excreted in mm^2. The progenies were statistically compared based on mean value obtained from three replications.

Days to wilt (30 DAS)

Seventeen lines in BC_1F_3 generation with resistance, susceptible and segregating reaction to BPH were used for analysing days to wilt and to understand the mechanism of tolerance. Thirty-day-old seedlings of each line were infested with 25 first or second instar nymphs in the mylar tube cage and the open end of the tube was covered with a muslin cloth and tied with a rubber band. The plants were observed for their health on daily basis. The day on which the test plant wilts were complete, it was recorded and the damage was estimated as the number of days required to kill the seedlings. The experiment was repeated thrice.

Marker analysis to understand the allelic relation with known genes

Allelic relations with the known mapped genes were studied using gene-linked markers of the genes showing resistance/moderate resistance reaction. Fresh leaves of two-week-old seedlings of different gene donors, RPBio4918-230S and Swarna were used for DNA extraction according to CTAB method (Saghai Maroof *et al*. 1994). A total of 14 genes were selected and primers were synthesized for polymorphism test from a total of 25 SSR markers. Polymerase chain readction (PCR) conditions were as described in Chen *et al*. (1997). In summary 10 μL PCR reactions contained, 10× PCR buffer 1 μL, dNTPs (2.5 mM) 0.5 μL, SSR primers 1 μL, *Taq* polymerase (1U/μL) 0.5 μL, Genomic DNA template (50 ng) 2 μL and sterile distilled water 4 μL. The PCR was performed with a profile of 94°C for 5 min, followed by 30 cycles at 94°C for 30 s, at 55°C for 30 s, at 72°C for 1 min, and finally for 7 min at 72°C for the final extension.

Results

Screening of introgression lines

Among the gene donors, Rathuheenati (*Bph3*+*Bph17*), Swarnalatha (*Bph6*), IR 71033-121-15 (*Bph20*+*Bph21*), IR 71033-121-15 (*Bph23*), IR 73678-6-9-B (*bph24*) and ADR52 (*Bph25* + *Bph26*) showed resistant reaction, while IR 54751-2-44-15-24-3 (*Bph11*) and IR 65482-7-216-2 (*Bph18*) showed moderate resistance. Others were susceptible during the seedling stage. RPBio4918-230S, OM4498, RP2068-18-3-5 and PTB33 were resistant at both seedling and reproductive stages (table 1; figure 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/).

The allelic relationship of the genes in RPBio4918-230S with other known genes of BPH resistance

The PCR amplification pattern of the alleles of donors, RPBio4918-230S and Swarna with the closely linked SSR markers showed that all the markers of the genes under study produce monomorphic bands in Swarna and RPBio4918-230S and polymorphic bands between gene donors and RPBio4918-230S, except for the flanking markers of *Bph17*.

Table 1. Reaction of gene donors and introgression lines to BPH biotype 4 at seedling and tillering/reproductive stage.

Donor/ introgression line	Gene	Reaction at tillering/ reproductive	Reaction at seedling stage
IR64	*Bph1*	S	S
ASD7	*bph2*	S	S
IR62	*Bph3*	S	S
ARC10550	*bph5*	S	MR
T12	*Bph7*	S	S
CHINSABA	*Bph8*	S	S
IR 65482-7-216-2	*Bph18*	S	MR
IR 71033-121-15	*Bph20+21*	S	R
ADR52	*Bph25+26*	S	R
RPBio4918-230S (introgression line)	–	R	R
OM4498	–	MR	R
RP 2068-18-3-5	–	R	R
SINASIVAPPU	–	S	R
MUDGO	*Bph1*	S	S
IR36	*bph2*	S	S
IR40	*bph2*	S	S
1R70	*Bph3*	S	S
IR74	*Bph3*	S	S
POKKALI	*bph9*	S	S
PTB33	–	R	R

This indicates that the genes for BPH resistance in RPBio4918-230S and the donors under test are different. While the markers of *Bph17* (RM8213 and RM5953) showed polymorphic bands in Rathu Heenathi and RPBio4918-230S indicating that the resistant gene/s present in RPBio4918-230S is different from *Bph17* reported in Rathu Heenathi. The amplification pattern with RM309 (closely linked marker of *Bph25*), RM8213, RM5953 (closely linked markers of *Bph17*), RM16994 (closely linked marker of *Bph6*) and one of the flanking marker for *Bph31(t)* clearly indicate that RPBio4918 carries different allele(s) from the known gene donors responsible for BPH resistance (table 2; figure 2, a–c in electronic supplementary material). The other gene donors showed susceptible reaction to BPH biotype-4 while RPBio4918-230S showed resistance reaction at seedling as well as at maximum tillering stage indicating that it is a new gene and effective against biotype 4.

Inheritance of BPH resistance in the introgression line RPBio4918 -230S

The introgression line RPBio4918-230S expressed strong resistance (damage score 1.64) to BPH biotype 4 at seedling stage and also at tillering and reproductive stages, whereas Swarna was completely susceptible (figure 3 in electronic supplementary material). The χ^2 test showed that the segregation ratio of resistant and susceptible plants in the BC_1F_2 families fitted into 7:9 and 1:3 ratio, indicating that the two pairs of recessive genes controlled the BPH resistance in RPBio4918-230S. Of the 47 families, 28 segregated for resistance and susceptible plants while 19 showed only susceptible reaction. Among the segregating families, 11 segregated in 1:3 (R:S) and 17 in 7:9 (R:S) ratio (table 3).

The adult plant stage screening also indicated that, of the 47 families 18 were susceptible, 11 segregated in 1:3 (R:S) and 18 families segregated in 7:9 (R:S) ratio indicating involvement of two recessive genes for resistance at reproductive stage (table 3). The χ^2 test revealed goodness of fit among the ratios obtained with $\chi^2 = 0.04$ to 0.9, $P < 0.85$ (tables 1–3 in electronic supplementary material). Further, the 200 BC_1F_3 plants derived from the randomly selected plants were also phenotyped using SSST. The results indicated that among the 200 progenies in BC_1F_3 generation, 114 were susceptible, 23 resistant and 63 segregated. Among the segregating progenies, 38 segregated in 7:9 (R:S) and 25 in 1:3 (R:S) ratio (table 3). These results confirmed that two recessive genes were involved for resistance to BPH in the introgression line RPBio4918-230S. χ^2 test revealed goodness of fit among the ratios obtained with χ^2 =0.03 – 1.2, (P<0.9) in all the families confirmed the involvement of two recessive genes controlling BPH resistance (tables 1–3 in electronic supplementary material). Frequency distribution of damage score of the BC_1F_3 families skewed towards the donor parent RPBio4918 (figure 1).

Feeding rate

Honeydew excretion area of the resistant BC_1F_3 plants was significantly lower than that of the susceptible plants; parent Swarna and susceptible check TN1. The honeydew excretion area was 147.9 mm^2 for TN1, 135 mm^2 for Swarna and 30.5 mm^2 for RPBio4918-230S and 20.2 mm^2 for PTB33. The honeydew excretion area in BC_1F_3 lines ranged from 20.8 mm^2 in resistant plant to 161.3 mm^2 in susceptible plant. In susceptible entries, the honeydew excretion area varied from 82.6 mm^2 in SN-134 to 161.3 mm^2 in SN-194, while in resistant entries, it was from 20.8 mm^2 in SN-141 to 39.8 mm^2 in SN-234. Among the segregating progenies, the honeydew excretion area ranged from 41.6 mm^2 in SN-160 to 60.1 mm^2 in SN-162 (table 4).

Honeydew area for xylem and phloem sap spots

Dark and light spots were observed in honeydew spotted on bromocresol green treated filter paper disc. Darker spots which indicates the phloem sap, were observed more in TN1 (147.9 mm^2) and all the susceptible progenies (figure 2; table 4 in electronic supplementary material). In the susceptible BC_1F_3 families, higher rate of phloem sap consumption and phloem spots was observed with no xylem

Table 2. Allelic relationship between parents RPBio4918-230S, Swarna and gene donors with closely linked markers of different genes known for BPH resistance.

Marker name	Gene	Chromosome	Donor used	Polymorphism between RPbio4918 and donor	Polymorphism between RPbio4918 and Swarna
RM463	*bph2*	12	ASD-7	Polymorphic	Monomorphic
RM1702				Polymorphic	Monomorphic
RM8072	*Bph3*	6	Rathuheenathi	Polymorphic	Monomorphic
RM588				Polymorphic	Monomorphic
RM589				Polymorphic	Monomorphic
RM586	*bph4*	6	Babawee	Polymorphic	Monomorphic
RM589				Polymorphic	Monomorphic
RM6997	*Bph6*	4	Swarnalata	Polymorphic	Monomorphic
RM5742				Polymorphic	Monomorphic
RM5341	*Bph9*	12	Pokkali	Polymorphic	Monomorphic
RM463				Polymorphic	Monomorphic
RM8213	*Bph17*	4	Rathuheenathi	Polymorphic	Polymorphic
RM5953				Polymorphic	Polymorphic
RM1305	*Bph12*	4	*O. officinalis*		Monomorphic
RM16459	*Bph 15*	4			Monomorphic
RM261					Monomorphic
RM16846	*Bph27*	4			Monomorphic
RM16853					Monomorphic
RM6775	*Bph25*	6	ADR-52	Monomorphic	Monomorphic
RM6273				Polymorphic	Monomorphic
MSSR1				Polymorphic	Monomorphic
S00310				Monomorphic	Monomorphic
RM204				Polymorphic	Monomorphic
RM8101				Monomorphic	Monomorphic
RM309	*Bph26*	12	ADR-52	Polymorphic	Monomorphic
RM5479				Monomorphic	Monomorphic
MSSR2				Polymorphic	Monomorphic
RM17007	*Bph31(t)*	4			Monomorphic
RM16994					Monomorphic
RM435	*bph20*	6	RBPH54 (introgression line of *O. rufipogon*)		Monomorphic
RM540					Monomorphic
RM222	*bph21*	10	RBPH54 (introgression line of *O. rufipogon*)		Monomorphic
RM244					Monomorphic

spots. RPBio4918-230S and SN-141 (resistant entry) were identified with the lowest phloem sap consumption with an excreted area (phloem spot) of 3.8 and 3.5 mm^2, respectively. Among the segregating BC_1F_3 families, lower consumption of phloem sap was observed in terms of phloem spots ranging from 9.5 in SN-161 to 22.8 mm^2 in SN-162. These results showed that the feeding rate in the resistant and susceptible families differed indicating that the level of antixenosis in segregating families also varies.

Tolerance (days to wilt 30 DAS)

The susceptible line TN1 took four days while PTB33 took 16 days to wilt. Swarna wilted in three days, RPBio4918-230S wilted in 14 days and BC_1F_3 lines took 3 to 10 days to wilt (table 4). The mean and range of damage score, honeydew area, days to wilt and damage score of parents, BC_1F_3 progenies and checks PTB33, TN1 indicated that all the traits were segregating (table 5 in electronic supplementary material).

Correlations among traits

Highly significant positive correlations were observed between damage score and feeding rate (honeydew area) at 24 h after infestation while highly significant negative correlations were observed between damage score and days to wilt (figure 3, a & b). Days to wilt at 30 DAS were also negatively correlated with feeding rate (honeydew area) at 24 h (table 6 in electronic supplementary material).

Discussion

Brown planthopper has become the major threat to rice production in most of the rice growing areas of India. Host plant resistance is ecological friendly and durable way to combat the impact of this major pest. Most of the genes so for identified are based on the reaction to BPH at seedling stage but their reaction to tillering and reproductive stages which are the most crucial crop stage determining the yield, are not explored well. Of the 38 BPH resistance genes

Table 3. Segregation of BPH resistance in BC_1F_2 (seedling and adult plant stage) and BC_1F_3 populations.

Generation of materials screened	Families evaluated	Susceptible families	Resistant families	Families segregating in ratio (resistance/susceptible)		
				Total	1:3	7:9
BC_1F_2 (seedling stage glass house)	47	19	–	28	11	17
BC_1F_2 (adult plant stage)	47	18	–	29	11	18
BC_1F_3 (seedling stage)	200	114	23	63	25	38

Details are presented in electronic supplementary tables 1, 2 and 3.

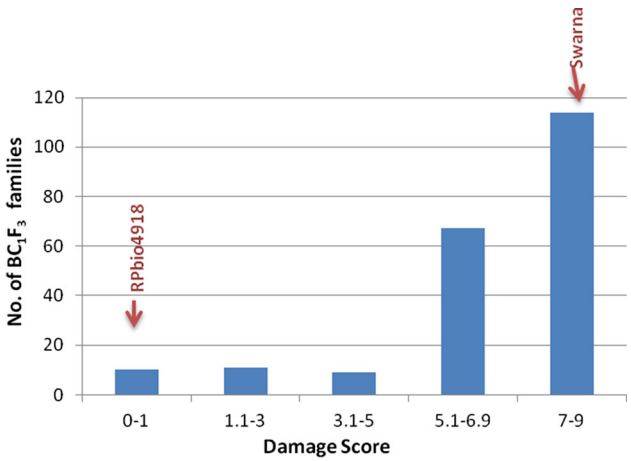

Figure 1. Frequency distribution of damage score of BC_1F_3 families for reaction to BPH.

identified, only 17 have been mapped (Lv *et al*. 2014; Wu *et al*. 2014; Liu *et al*. 2015; Wang *et al*. 2015; Hu *et al*. 2016; Balachiranjeevi *et al*. 2019; Yuexiong *et al*. 2019). However, only seven of them have been cloned and characterized (Du *et al*. 2009; Tamura *et al*. 2014; Liu *et al*. 2015; Wang *et al*. 2015; Hu *et al*. 2016; Ren *et al*. 2016; Zhao *et al*. 2016), but their effectiveness on adult plant growth stages is not well understood.

In addition to major genes, more than 70 quantitative trait loci (QTLs) associated with resistance to BPH were also reported. These QTLs/gene clusters might involve multiple genes and alleles, which mediate different resistance mechanisms to various BPH biotypes (Qiu *et al*. 2010). It is also necessary to use the donors/genes responsible for seedling as well as reproductive stage tolerance. The gene donors and the introgression lines screened for adult plant stage in field under hopper burn conditions indicated that only RPBio4918-230S, OM4498, RP2068-18-3-5 and PTB33 were resistant at maximum tillering and reproductive stages. The remaining genotypes were susceptible, although a few of them were resistant at seedling stage in greenhouse conditions. The effectiveness of evaluation of insect responses depends on preferred crop growth stage and environment (Fujita *et al*. 2013). There are few reports indicating significant correlation between greenhouse and field choice tests (Sogawa 1994; Alam and Cohen 1998) but the gene expressions are likely to change over the later plant growth stages under the influence of environmental factors and insect pressure. Jairin *et al*. (2007) reported that BPH resistance in Rathuheenati is ineffective at later plant stages in Thailand. The introgression line RPBo4918-230S developed from *O. nivara* was screened for five years at seedling stage and two years at tillering and reproductive stages and found to be resistant (damage score 1.2–2.0). The germplasm lines identified as resistant sources for BPH biotype-4 are the valuable material for identifying new gene/QTLs.

Several tests such as field screening, days to wilt, antixenosis (settling), honeydew estimation were also suggested to confirm resistance/tolerance to BPH (Liu *et al*. 2009; Sarao and Bentur 2016; Akanksha *et al*. 2017). The correlation analysis in this study suggested a positive association of resistance in terms of damage score with feeding rate (honeydew excretion) and negative association with days to wilt. These results suggest that for mass screening of segregating populations, damage score would be adequate for identifying resistant plants but the correlation between damage score at seedling stage and tillering/reproductive stage tolerance is equally important.

BPH feeding varies from genotype to genotype, which includes probing response and duration of feeding (Sarao and Bentur 2016; Ramdeen *et al*. 2017). The remarkable fact, which we observed in the honeydew spotted on bromocresol green treated filter paper disc were some dark and lighter spots. More number of darker spots were seen in TN1 and the susceptible lines than in the resistant lines and RPBio4918-230S. In the susceptible BC_1F_3 families, higher rate of phloem sap consumption was observed with no xylem spots (figure 4 in electronic supplementary material). The honeydew spotted is usually dark if the phloem sap is rich in amino acids, and is light if the source is low in amino acids, such as xylem (Heinrichs *et al*. 1985). These results showed that the feeding in the resistant, segregating and susceptible families vary indicating differences in antibiosis. We observed variation in the level of antibiosis in the populations. The resistant lines have lesser honeydew spots with lower consumption of phloem sap as compared to the susceptible while the segregating lines have moderate expansion of honeydew area. Jena *et al*. (2017) also found that most of the pyramided NILs having two to three gene combinations showed higher consumption of xylem sap but

Table 4. Screening of parents and populations for honeydew excretion and days to wilt in selected BC_1F_3.

Population	Honeydew area (mm^2)		Days to wilt		Damage score	
	Range	Mean±SD	Range	Mean±SD	Mean	Range
Parents and checks						
Swarna	129.1–137.6	135 ± 9.1	2–4	3 ± 0.3	9	
RPBio4918-230S	26.2–33.6	30.5 ± 4.2	12–15	14 ± 1.2	1.6	
TN1	142.0–154.2	147.9 ± 6.5	3–6	5 ± 1.4	9	
PTB33	18.4–23.8	20.2 ± 3.7	14–18	16 ± 1.7	1.3	
BC_1F_3 population						
Susceptible	82.6–161.35	123.05 ± 24.9	4–7	5.25 ± 1.4	8.4	7.9–9
Segregating	41.6–61.1	50.6 ± 7.24	7.1–8	7.4 ± 0.5	5.82	4.8–6.5
Resistant	20.8 – 40.8	32.07 ± 8.6	8.1–10	9.3 ± 0.6	1.8	1.0–1.9

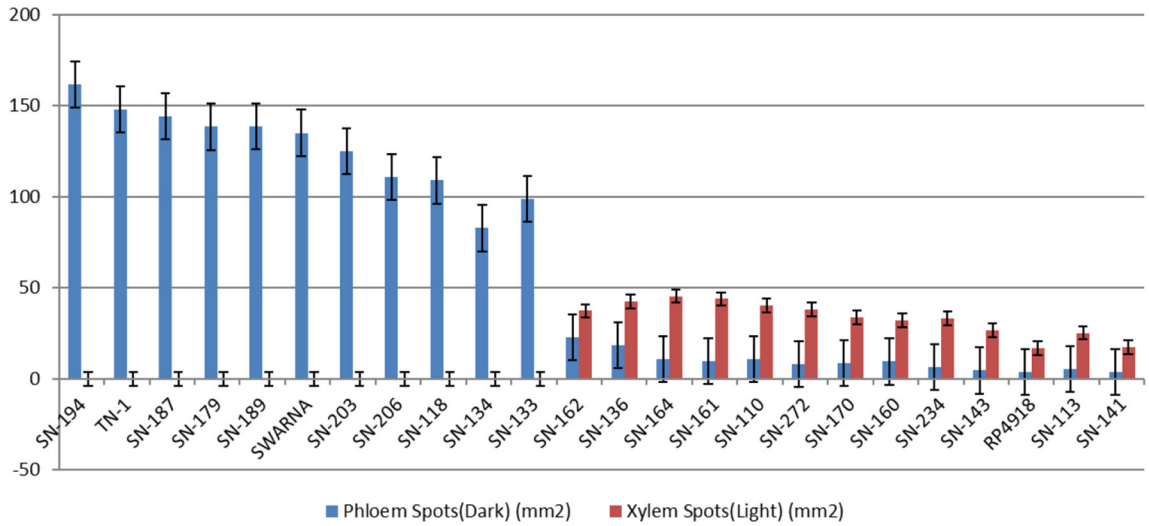

Figure 2. Area of xylem and phloem honeydew excretion spots in selected resistant, susceptible and segregating BC_1F_3 families.

reduced consumption of phloem sap compared with the NILs having single R genes. Correspondingly, the lower levels of defensive chemicals in the sap of susceptible lines could have encouraged planthopper-feeding rates, which caused the earlier wilting of the susceptible lines compared with RPBio4918-230S and PTB33. The results of honeydew excretions indicated that the antixenosis was one of the mechanisms involved in the BPH resistance in introgression line RPBio4918-230S. Similarly, in the case of *Bph14*, mainly resistance was due to antibiosis, which reduces the feeding and growth rate of BPH (Du *et al*. 2009). Qiu *et al*. (2010) reported that Bph6 exerted antixenotic and antibiosis effects while conferring BPH resistance.

Tolerance is the ability of a variety to produce high yield despite insect infestation; and this factor of host plant resistance is less exploited (Sarao and Bentur 2016). Geethanjali *et al*. (2009) proposed a simple test of days to wilt for tolerance parameter, which was accepted for BPH and WBPH screening (Alagar and Suresh 2007; Ramesh *et al*. 2014; Ramdeen *et al*. 2017). The introgression line from RPBio4918-230S and the derived resistant backcross lines showed a high level of tolerance. Earlier, Bae and Pathak (1970) reported antibiosis and tolerance as the major factors of resistance while their study on 20 selected rice varieties, which were less preferred by BPH. Alagar and Suresh (2007) reported that 30 and 60-day-old plants of ARC10550, KAU1661 and ARC6650 took significantly longer period for wilting than TN1. Antibiosis mechanism is mainly operated in Dagad Deshi for BPH resistance (Sonali *et al*. 2011). Similarly, Qiu *et al*. (2014) found *Bph7* mainly accounts for tolerance component of resistance against BPH. However, the mechanisms of resistance in most of the genes are still unknown. Therefore, it is necessary to identify the level of antibiosis and tolerance in donors carrying BPH resistance gene/QTLs which will help in incorporating durable resistance in rice varieties (Huang *et al*. 2001; Hao *et al*. 2008; Myint *et al*. 2009a, b). The introgression line RPBio4918-230S displayed high levels of antibiosis and tolerance to BPH. This will provide better option for plant breeders and entomologists to use wild species of rice to detect resistant genes and develop suitable varieties to fight against many of the destructive insect pest like BPH.

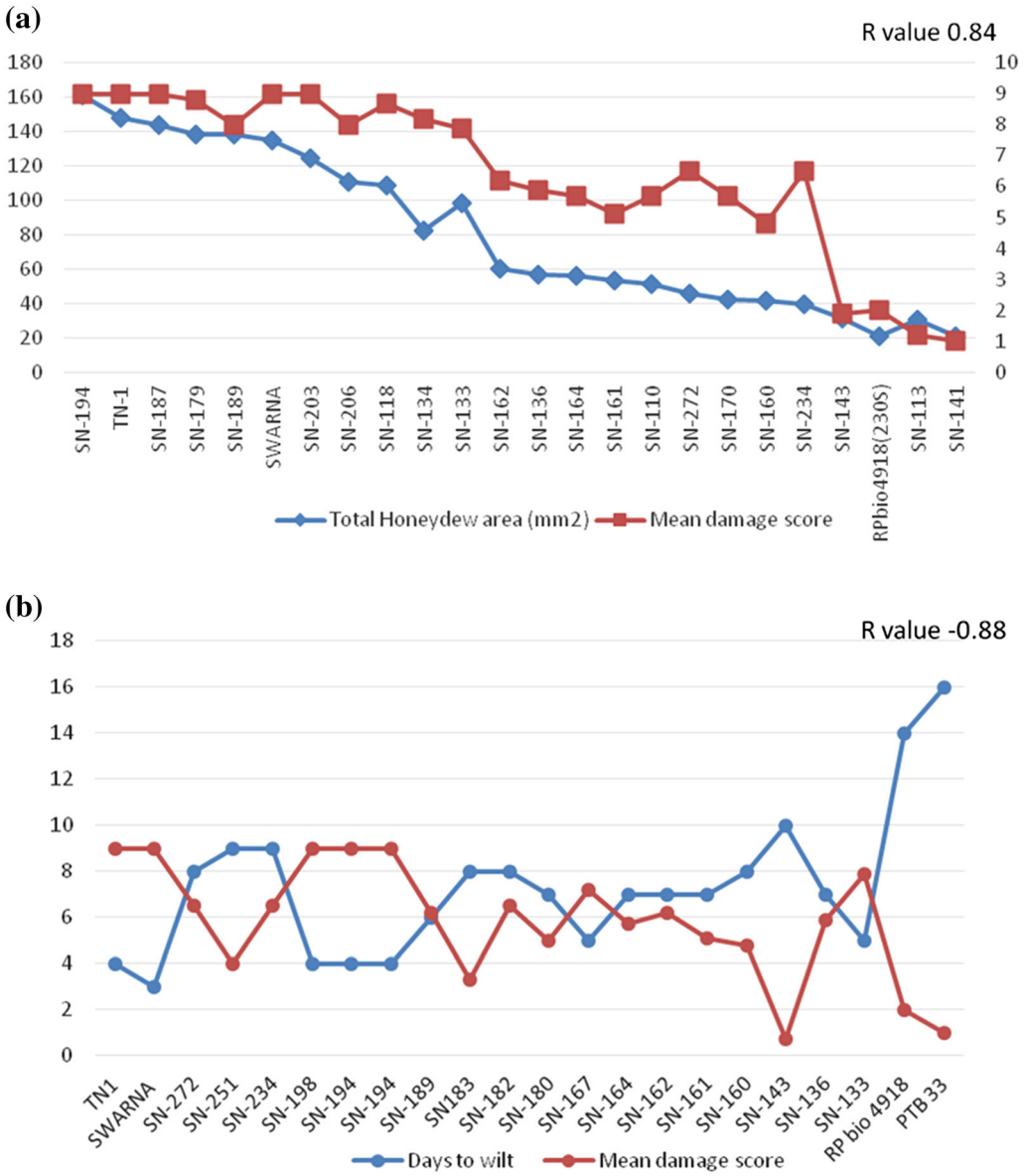

Figure 3. (**a**) Graph representing correlation between honeydew excretion and damage score. (**b**) Graph representing correlation between days to wilt and damage score.

Many of the resistance genes are ineffective against biotype-4 (Ram *et al*. 2010; Akanksha *et al*. 2017); hence, we screened the linked markers of effective genes to understand allelic relationship in resistant parent RPBio4918-230S. The amplification pattern of the alleles indicated that the detected genes are different from the known genes, hence tentatively proposed as bph39(t) and bph40(t). These genes showed resistance to BPH biotype-4 at seedling, tillering and reproductive stage of rice crop. Further, the mapping of this novel gene will confirm its position on chromosome and its novelty to use in breeding programme for better and durable resistance. Kim *et al*. (2004) used STS and RFLP markers linked to brown planthopper resistance gene *Bph1* to classify the source of resistance in rice cultivars.

The skewness of the segregating population for damage score towards the donor parent was due to segregation distortions, which is a common feature in interspecific or intersubspecific crosses in rice (Xu *et al*. 1997; Harushima *et al*. 2002) due to predominance of weedy traits like shattering, dormancy and sterility. Considering the change of BPH biotypes and their outbreak resulting in the breakdown of resistance in the varieties, identification of novel genes is essential to ensure the durability of resistance especially in field conditions. In this study, the inheritance of resistance in populations derived from RPBio4918-230S and Swarna clearly indicated that two recessive genes controlled BPH resistance in RPBio4918-230S at seedling and reproductive stage under field conditions. The exploitation of the

identified introgression line for the dissection of genetic mechanisms underlying the resistance will provide fundamental basis for breeding BPH resistance genotypes in rice.

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