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Identification of antibiosis and tolerance in rice varieties carrying brown planthopper resistance genes

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Abstract

Brown planthopper (BPH) [*Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae)] is a major pest in rice [*Oryza sativa* L. (Poaceae)] production. Identification of resistance genes and development of BPH-resistant varieties is an economical and effective way to control this pest. In this study, BPH honeydew excretion, survival rate, and emergence rate were used as indicators to detect the antibiotic level, whereas the relative growth rates of plant height (R_H) and fresh weight (R_W), and the number of days until yellowing were used to identify the level of tolerance to BPH in rice varieties. Rice varieties Swarnalata and B5, which showed high levels of antibiosis and tolerance to BPH, thus were highly resistant in the seedling bulk test; Mudgo and T12, which showed moderate resistance to the insects, had a high level of tolerance and moderate antibiosis to BPH. Varieties Rathu Heenati, ARC 10550, and Chin Saba were identified to be susceptible to BPH, showing a moderate level of tolerance and no antibiosis. In comparison to the evaluation methods of BPH resistance, the honeydew excretion and survival rate could be used to detect the antibiotic level, and the R_H, R_W, or leaf yellowing days could be employed as indicators to evaluate the rice varieties' tolerance. Overall, a combined application of these indicators can effectively identify the levels of antibiosis and tolerance to BPH in rice varieties, and BPH-resistance levels of the varieties were mainly determined by the antibiosis level. The results should help in understanding BPH-resistance categories of rice varieties and for resistance breeding.

Introduction

The brown planthopper (BPH) [*Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae)] is a typical piercing–sucking insect pest of rice (*Oryza sativa* L.; Poaceae), which feeds on phloem sap and thus affects the growth of rice and results in 'hopperburn' in rice fields (Watanabe & Kitagawa, 2000). Furthermore, BPH also transmits viruses, such as the ragged stunt virus and grassy stunt virus, and associated diseases to rice plants (Khush & Brar, 1991; Jena et al., 2006). Outbreaks of BPH are very frequent in tropical Asia and have caused heavy rice yield losses in recent years (Normile, 2008). To control this pest, the application of chemical insecticides has not been a satisfactory tactic in practical rice production, because insecticides can cause BPH resurgence and may play a major role in inducing outbreaks (Heinrichs et al., 1982; Tanaka et al., 2000). Alternatively, growing of resistant variety is an economical and efficient way for control of BPH pest. Lines showing BPH resistance are abundant in world rice germplasm collections (Zhang, 2007; Jena & Kim, 2010), and BPH-resistance genes have been identified in germplasm and some are used in resistant rice breeding programs (Cohen et al., 1997; Jairin et al., 2007; Rahman et al., 2009).

Plants may employ various resistance types to reduce insect damage in nature. Plant resistance to insects is generally differentiated in (1) antibiosis, a quality that reduces insect survival, growth rate, or reproduction following the ingestion of host tissue (2) tolerance, a capacity to produce a crop of high quality and yield despite insect infestation, and (3) antixenosis, a quality that repels or disturbs insects, causing a reduction in colonization or oviposition (Kennedy et al., 1987; Alam & Cohen, 1998). These three types of resistance are observed in rice plants against BPH.

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Panda & Heinrichs (1983) determined the levels of tolerance and antibiosis in rice varieties carrying the *Bph1* gene and showing moderate resistance to BPH. Similarly, Du et al. (2009) found that the resistance gene *Bph14* confers antibiosis that reduces the feeding and growth rate of BPH. More recently, we found that the resistance gene *Bph6* exerted antixenotic and antibiotic effects toward BPH in *Bph6*-near isogenic line plants (Qiu et al., 2010). However, the resistance types of most BPH-resistance genes identified remains largely unknown. Therefore, it is necessary to identify the levels of antibiosis and tolerance in germplasm carrying BPH-resistance genes, which should favor resistance breeding in rice.

To date, a number of methods have been surveyed to evaluate BPH resistance in rice (Paguia et al., 1980; Alam & Cohen, 1998; Huang et al., 2001; Hao et al., 2008; Myint et al., 2009). The seedling bulk test is the most popular and widely used method in determination of rice resistance to BPH, because it has the advantages of rapid and efficient screening of rice lines. These characteristics are especially valuable in resistance gene mapping and rice breeding (Shi et al., 2003; Jena et al., 2006; Li et al., 2006; Qiu et al., 2010). However, it is difficult to differentiate between antibiosis, tolerance, or antixenosis in a seedling bulk test. Therefore, finer techniques have been applied to investigate BPH resistance in rice. Paguia et al. (1980) detected the feeding activity of BPH on rice varieties with *Bph1* or *bph2* through measuring BPH honeydew excretion. Panda & Heinrichs (1983) took plant damage, plant weight loss, and yield reduction as 'tolerance' indicators, and insect biomass production, growth index, and population growth rate as 'antibiosis' indicators in rice varieties carrying the *Bph1* gene. Alam & Cohen (1998) studied rice variety IR64 carrying the *Bph1* gene and a doubled-haploid mapping population (Azucena/IR64) with six tests which measured varying combinations of antibiosis, tolerance, and antixenosis. As a result, a total of seven quantitative trait loci (QTL) associated with BPH resistance were identified. Recently, Myint et al. (2009) identified the virulence of long-term laboratory populations of BPH on rice varieties which carry different BPH-resistance genes, based on survival rates and the proportions of BPH females with swollen abdomens, indicating virulent females. All these studies have used two or more methods to characterize BPH resistance of rice varieties. However, only one or two BPH-resistance genes (Paguia et al., 1980; Panda & Heinrichs, 1983; Alam & Cohen, 1998), and only antibiosis (Paguia et al., 1980; Myint et al., 2009) has been characterized in these studies.

In considering the methods of determining the levels of antibiosis or tolerance in rice varieties, some are simple to conduct, such as the measurement of plant damage or plant weight loss (Panda & Heinrichs, 1983; Alam & Cohen, 1998), whereas others are more difficult to conduct in practical tests, such as the measurement of yield reduction (Panda & Heinrichs, 1983). Therefore, it was necessary to compare methods and identify an efficient and simple way to evaluate the levels of antibiosis and tolerance in rice. Rice varieties were selected with known resistance (i.e., antixenosis) to one or more BPH biotypes based on seedling bulk test (Athwal et al., 1971; Lakshminarayana & Khush, 1977; Khush et al., 1985; Kabis & Khush, 1988; Nemoto et al., 1989). However, antibiosis and tolerance of these varieties remain largely unknown. We identified the levels of antibiosis and tolerance in rice varieties carrying various BPH-resistance genes, and evaluated the adequacy of the commonly used bulk-screening method to determine the minimal set of experiments needed to sufficiently characterize BPH resistance in rice.

Materials and methods

Plant material and brown planthopper insects

Twelve rice varieties were used (Table 1). Rice varieties Mudgo (*Bph1*), ASD7 (*bph2*), Rathu Heenati (*Bph3*), ARC 10550 (*bph5*), Swarnalata (*Bph6*), T12 (*bph7*), Chin Saba (*bph8*), and Pokkali (*Bph9*) were reported to resist one or more BPH biotypes (Athwal et al., 1971; Lakshminarayana & Khush, 1977; Khush et al., 1985; Kabis & Khush, 1988; Nemoto et al., 1989). Rice line B5 is resistant to BPH biotypes 1 and 2 and carries the resistance genes *Bph14* and *Bph15* (Yang et al., 1999; Huang et al., 2001). Rice variety TN1, which is highly susceptible to all four BPH biotypes (1–4) and carried no BPH-resistance gene, was taken as susceptible control in the present study. Rice varieties 93-11 and Nipponbare showed high susceptibility to BPH (Qiu et al., 2010).

Brown planthoppers were collected from rice fields in 2006 in Wuhan (30°31′S, 114°21′E; where BPH populations of biotype 2 dominated), China, and maintained at the Genetic Institute, Wuhan University, on TN1 plants for ca. 3 years. Second and third instars were collected and used for experiments.

Brown planthopper-resistance evaluation of rice varieties

The seedling bulk test was performed as described by Huang et al. (2001). Rice seeds were soaked in water and germinated at 30 °C. Sixty germinated seeds of a given variety were randomly sown in three 26-cm-long rows, with 2.5 cm between rows, in a plastic box (58 × 38 × 9 cm). Variety TN1 was randomly sown among the other rows as susceptible controls. At the third-leaf stage (ca. 13–14 days old), the seedlings were infested with second–third BPH instars at 10 insects per seedling and covered

Table 1 Performance of brown planthoppers (BPH) on rice varieties carrying different BPH-resistance genes

Variety	Resistance gene	Survival rate (%)[1]	Emergence rate (%)	L1[2]	L2
Mudgo	*Bph1*	52.2 ± 10.6c	40.7 ± 12.6b	4.2 ± 2.3b	11.6 ± 2.5a
ASD7	*bph2*	69.3 ± 9.5ab	50.8 ± 13.6ab	3.3 ± 1.3bc	10.0 ± 3.1a
Rathu Heenati	*Bph3*	68.0 ± 12.1ab	55.6 ± 10.8a	4.3 ± 1.2b	9.3 ± 2.8ab
ARC 10550	*bph5*	70.3 ± 6.8ab	46.7 ± 12.0ab	2.0 ± 1.0cd	7.1 ± 1.1bc
Swarnalata	*Bph6*	43.1 ± 13.2c	45.0 ± 13.4ab	5.2 ± 2.6ab	12.3 ± 3.9a
T12	*bph7*	61.7 ± 8.2bc	47.9 ± 16.3ab	5.9 ± 0.8a	11.1 ± 1.5a
Chin Saba	*bph8*	72.9 ± 6.8a	50.8 ± 12.7ab	2.6 ± 1.0c	7.4 ± 1.0bc
Pokkali	*Bph9*	65.6 ± 11.5ab	52.5 ± 8.8a	4.8 ± 1.2b	9.6 ± 2.1a
B5	*Bph14*, *Bph15*	45.0 ± 22.7c	46.1 ± 17.2ab	4.0 ± 1.2b	11.0 ± 1.7a
93-11	U	65.9 ± 9.6ab	46.9 ± 20.8ab	2.5 ± 0.6c	5.8 ± 2.6cd
Nipponbare	U	63.4 ± 14.0abc	50.8 ± 16.1ab	1.4 ± 0.5d	5.8 ± 1.6cd
TN1	None	67.8 ± 6.2ab	49.1 ± 17.1ab	1.3 ± 0.5d	5.5 ± 1.7d

[1]Mean (± SD) survival and emergence rates.
[2]Mean (± SD) time to leaf yellowing (days) of rice varieties since infested with BPH.
U, unknown. None, no resistance gene found in this rice variety. L1, L2, first (= lowest) and second leaf, respectively. Means with the same letter within a column are not significantly different (least significant difference test: $P>0.05$).

using fine, light-transmitting mesh. Each seedling was given a score of 0, 1, 3, 5, 7, or 9 according to Huang et al. (2001) when all of the TN1 seedlings had died (after ca. 9–10 days). Here, a 0 score indicated none of the leaves shrank and the plant was healthy, whereas 9 indicated the seedling was dead. Accordingly, 1, 3, 5, and 7 indicated 1 leaf yellowing, 1–2 leaves yellowing, 1 leaf shriveling, and 2–4 leaves shriveling, respectively. The lower scores indicate higher resistance to the insects. The resistance score of each variety was then inferred from the weighted average of the scores for all seedlings. The experiment was replicated three times and conducted in a greenhouse under natural light at 25–30 °C from May to September 2008 and 2009.

Determination of antibiotic effect of rice to brown planthopper

Brown planthopper honeydew excretion, survival, and emergence rate were taken as indicators to detect antibiosis of rice varieties toward BPH. Brown planthopper honeydew excretion on the various rice varieties was surveyed following the method by Sogawa & Pathak (1970). Rice plants were used at the three-leaf stage (ca. 13–14 days old). Five third instars previously starved for ca. 2 h were placed on the plant in the chamber through a hole at the top of the cup. A cotton wad was then placed in the hole to prevent escape of the insects. Afterward, the insects were allowed to feed 48 h, then the filter paper was collected and treated with a 0.25% ninhydrin in acetone solution. After being oven-dried for 30 min at 60 °C, the honeydew stains appeared as dark to light violet due to their amino acid content. At last, the area of honeydew spot was traced on tracing paper and the squares were counted over millimeter-square graph paper as described by Paguia et al. (1980). The feeding chambers were arranged in a randomized complete block design, each plant serving as a replicate. The experiment was performed three times and each treatment was replicated 4–6 times.

To detect BPH survival and emergence rates on the various rice varieties, a plant at the two-leaf stage (ca. 9 days old) was transferred to individual glass test tubes (3 cm diameter, 18 cm high) previously added with Hoagland's nutrient solution, which was always maintained at a level of submerging the root during the duration of the experiment. At the three-leaf stage (ca. 13–14 days old), equally big seedlings were selected, and each tube/seedling was infested with five-second instars. Surviving and emerging insects were counted on days 6 and 9 after the start of infestation, respectively, and their corresponding rates were expressed as % surviving and emerging. On each variety we conducted 8–10 replicates.

Determination of tolerance of rice to brown planthopper

To determine tolerance to BPH insects, we measured relative growth rates of rice plant height (R_H) and weight (R_w), and monitored when the first and second leaves of the plants turned yellow (L1 and L2) after insect infestation. Treatment of the rice seedlings was identical to that described for the measurement of BPH-survival rate and BPH numbers were always maintained at five live insects for each tube/seedling. To determine the plant's growth changes (height, fresh weight), the plant (three-leaf stage, ca. 13–14 days old) cultured in Hoagland's solution was dried with filter paper, and plant height (from the residual part of the stem to the longest leaf tip) and plant fresh

weight were measured before and after BPH infestation for 6 days. R_H, the relative growth rate of plant height, was calculated using the following formula:

$$R_H = \frac{(H_{T1} - H_{T0})/H_{T0}}{(H_{C1} - H_{C0})/H_{C0}} \times 100,$$

where H_{T0} and H_{T1} are the treatment plant height before and after BPH infestation, respectively, and H_{C0} and H_{C1} are the control plant height without BPH infestation, measured at the same time as H_{T0} and H_{T1}. Calculation of R_W was similar to that of R_H. Each variety infested with BPH was replicated 9–15 times (five replicates for uninfested control).

The rice seedlings and BPH insects used in measuring the days until leaves yellowing were identical to that described for the R_H and R_W measurements. Here, the plant's lowest leaf was taken as the first, and the next one as the second leaf. The leaf was considered yellow if >95% of the leaf area turned yellow. Treatment was replicated 6–10 times per variety.

Statistical analysis

Data were analyzed using one-way ANOVA and means were compared using a least significant difference test with MicroSoft Excel. The rates of survival and emergence insects (%) were arcsine transformed prior to analysis. Correlation analysis was performed using SPSS 13.0 (SPSS Institute Inc, Chicago, IL, USA) and Pearson's correlation coefficient was used as a measure of the relationship between indicators.

Results

Brown planthopper-resistance scores of rice

The 12 rice varieties were scored as 2.1–8.9 in the seedling bulk test, varying from highly resistant to highly susceptible (Figure 1). Rice varieties TN1, 93-11, and Nipponbare, with no BPH-resistance gene, displayed high susceptibility to BPH and almost all seedlings died in the test; their average resistance scores were 8.9, 8.6, and 8.9, respectively. Among the varieties previously reported to carry BPH-resistance genes, B5 (*Bph14* and *Bph15*), Pokkali (*Bph9*), and Swarnalata (*Bph6*) displayed high resistance to BPH and had average resistance scores of 2.1, 2.2, and 3.0, respectively. Mudgo (*Bph1*) and T12 (*bph7*) were moderately resistant, with scores of 4.2 and 4.9, respectively. Four varieties that were reported to carry resistance genes, including ASD7 (*bph2*), Rathu Heenati (*Bph3*), ARC 10550 (*bph5*), and Chin Saba (*bph8*) proved moderately or highly susceptible to BPH in this experiment. This result revealed that rice varieties carrying different resistance genes vary in resistance level to BPH in China.

Brown planthopper honeydew excretion on rice varieties

Honeydew excretion measured by color area ranged from 128 mm^2 on Pokkali to 1 340 mm^2 on TN1 (Figure 2). Much of the honeydew excretion (>952 mm^2) was quantified for BPH nymphs on susceptible varieties 93-11, Nipponbare, TN1, and a previously reported resistant variety, Chin Saba. Honeydew excretion on Mudgo, ASD7, Rathu Heenati, ARC 10550, and T12 was 372–670 mm^2, on rice line B5 it was <300 mm^2, and on Swarnalata and Pokkali it was <200 mm^2. Apparently the honeydew excreted by BPH differed among rice varieties carrying different resistance genes.

Survival and emergence rates of brown planthopper

The average BPH-survival rates ranged 43.1–72.9% (Table 1). High survival rates (>65%) were observed for nymphs on ASD7, Rathu Heenati, ARC 10550, T12, Chin Saba, Pokkali, 93-11, Nipponbare, and TN1; survival was highest on Chin Saba (72.9%). Low survival rates were found on Mudgo (52.2%), B5 (45.0%), and Swarnalata (43.1%).

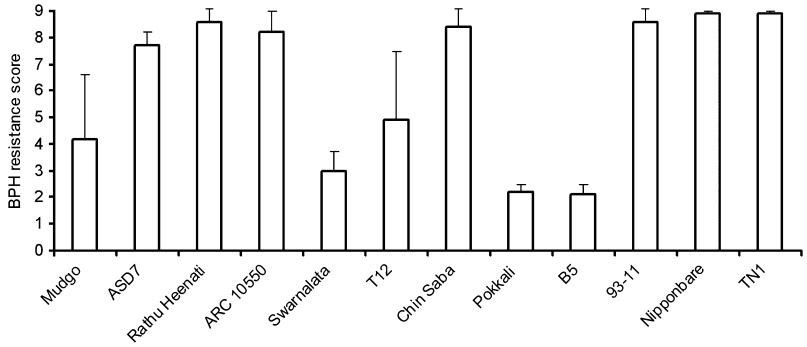

Figure 1 Mean (+ SD) resistance scores of rice varieties carrying different resistance genes detected by a seedling bulk test with infestation of brown planthoppers (BPH).

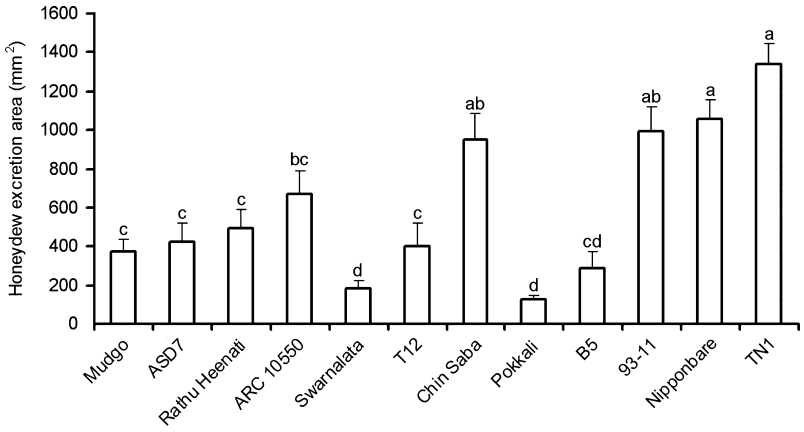

Figure 2 Mean (+ SEM) amount of honeydew excreted by brown planthoppers on rice varieties carrying different resistance genes, expressed as area of honeydew excreted by five third instars in 48 h. Means with the same letter are not significantly different (least significant difference test: P>0.05).

Emergence rates varied from 45.0 to 55.6% and did not differ significantly among varieties. Survival rate was correlated with emergence rate (r = 0.63, P = 0.03), suggesting that BPH-survival rate is a more effective indicator of the level of antobiosis than the emergence rate.

Plant height and fresh weight of rice after brown planthopper infestation

After infestation, the relative growth rate in terms of plant height (R_H) of rice varieties Mudgo, Swarnalata, T12, Pokkali, and B5 increased by 59.1, 64.3, 49.7, 68.7, and 65.3%, respectively (Figure 3). R_H for the other resistant varieties ASD7, Rathu Heenati, ARC 10550, and Chin Saba was 33.0–47.9%, whereas it was 9.9, 7.5, and 9.0% for the susceptible varieties 93-11, Nipponbare, and TN1, respectively. The variation in the relative growth rate in terms of plant fresh weight (R_W) was similar to that of R_H (Figure 3), and the two were highly correlated (r = 0.95, P<0.001). R_W of the rice varieties Mudgo, ASD7, ARC 10550, Swarnalata, T12, Pokkali, and B5 was 57.2, 26.2, 17.3, 52.6, 26.1, 72.6, and 65.7%, respectively. R_W was negative for varieties Rathu Heenati (−19.2%) and Chin Saba (−5.4%) and for the susceptible varieties 93-11, Nipponbare, and TN1 it was −40.5, −92.3, and −48.6%, respectively. R_W of the latter three varieties was significantly lower than that of the other varieties carrying BPH-resistance genes. Thus, R_H and R_W could be used to evaluate tolerance of rice varieties to BPH.

Days until yellowing after brown planthopper infestation

Generally, after 6 days of BPH infestation, the first (= lowest) leaf had turned yellow, and after 13 days also the

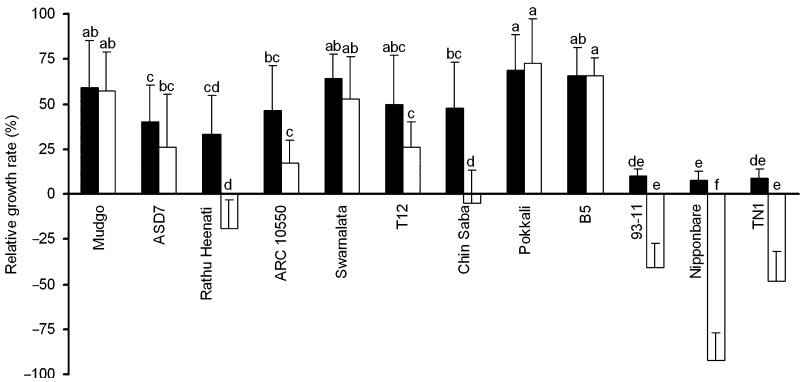

Figure 3 Mean (+ SD) relative growth rates (%) of plant height (R_H, black bars) and fresh weight (R_W, white bars) of rice varieties carrying different resistance genes, after being infested with brown planthoppers. Means with the same letter are not significantly different (least significant difference test: P>0.05).

second leaf (Table 1). However, the traits were highly variable among varieties. The first leaf of Nipponbare, TN1, and ARC 10550 turned yellow within 2 days of infestation, followed by 93-11, Chin Saba, and ASD7 (3 days), and B5, Rathu Heenati, Mudgo, and Pokkali (<5 days). The second leaf of the susceptible varieties 93-11, Nipponbare, and TN1 turned yellow in 6 days, followed by ARC 10550 and Chin Saba (8 days); the second leaf of the other resistant varieties maintained green after 10 or more days. The rates of yellowing of first and second leaves were correlated among varieties ($r = 0.88$, $P<0.001$).

Correlation analysis among resistance indicators

Resistance scores of the tested varieties identified in the seedling bulk test were correlated with all indicators except emergence rate ($r = 0.82$, $P = 0.001$ for honeydew excretion; $r = 0.76$, $P = 0.004$ for survival rate; $r = 0.39$, $P = 0.21$ for emergence rate; $r = -0.84$, $P = 0.001$ for R_H; $r = -0.85$, $P = 0.001$ for R_W; $r = -0.75$, $P = 0.005$ for L1; and $r = -0.79$, $P = 0.002$ for L2). Thus, the resistance score from the rice seedling bulk test was a comprehensive indicator of antibiosis and tolerance to BPH. Also, the separate indicators used in the present study could effectively identify the antibiosis and tolerance levels of rice varieties toward BPH.

Honeydew excretion was positively, but not significantly, correlated to the survival ($r = 0.54$, $P = 0.07$) and emergence rates ($r = 0.16$, $P = 0.63$), whereas it was significantly negatively correlation to the tolerance indicators ($r<-0.86$, $P<0.001$). Afterward, BPH-survival rate on the tested varieties was significantly correlated with emergence rate ($r = 0.63$, $P = 0.03$) and L2 ($r = -0.68$, $P = 0.02$), but not with R_H ($r = 0.50$, $P = 0.1$), R_W ($r = 0.51$, $P = 0.1$), or L1 ($r = 0.50$, $P = 0.1$). As for pairs of tolerance indicators of the tested varieties, they were significantly positively correlated ($r>0.73$, $P<0.01$). Hence, honeydew excretion and survival rate were the most effective indicators of rice antibiosis, and R_H, R_W, and L2 most effectively indicated rice tolerance to BPH.

Discussion

Methods of brown planthopper-resistance evaluation in rice

The indicators honeydew excretion, survival rate, and emergence rate were applied to detect the level of antibiosis to BPH in rice varieties. Consequently, BPH-survival rates varied from 45 to 73% of the rice varieties as measured by the 6th day in this study. This result fitted well with that of Du et al. (2009) and Qiu et al. (2010). However, the studies conducted by Myint et al. (2009) showed that the survival rates of *N. lugens* females were 0% on several rice varieties carrying resistance genes. Also, it contrasts with *Meu1*-mediated resistance against potato aphid [*Macrosiphum euphorbiae* (Thomas)] in tomato, which caused 100% mortality within 10 days in a study performed by Kaloshian et al. (1997). Afterward, we noted that BPH-survival rate positively correlated with BPH emergence rate, and rice varieties with lower quantities of honeydew excreted usually had lower survival rates. In conclusion, the BPH-resistance levels of the rice varieties were mainly determined by the antibiotic effect conferred by the resistance genes. Furthermore, the quantity of BPH honeydew excreted and the survival rate could be used to identify levels of antibiosis in rice varieties.

R_H, R_W, and leaf yellowing day appeared effective indicators of (levels of) tolerance to BPH in rice varieties. Plant dry weight loss had a similar function in determining the tolerance of rice varieties to BPH (Panda & Heinrichs, 1983; Alam & Cohen, 1998). The detection of BPH tolerance of varieties through measuring plant height changes and/or leaf yellowing day is both easy and keeps the plants alive. The 'leaf yellowing' method has seldom been used to evaluate BPH tolerance levels in rice.

Although honeydew excretion, survival rate, R_H, R_W, and/or L2 have been identified as effective indicators of antibiosis and tolerance of rice varieties to BPH, they are probably difficult to use in practical breeding programs or BPH-resistance gene mapping because of their labor intensiveness. Therefore, a synthesis of these methods would be welcome. Specifically, the seedling bulk test was favorable for resistance germplasm screening, resistance rice breeding, and gene mapping, whereas honeydew excretion, survival rate, R_H, or R_W could be used to detect antibiotis or tolerance levels.

Brown planthopper-resistance types in rice

Rice varieties Swarnalata and B5 both displayed high levels of antibiosis and tolerance to BPH, resulting in a high resistance level in the seedling bulk test (average resistance scores of 3.0 and 2.1, respectively). High BPH resistance had already been reported for Swarnalata and B5, based on antibiosis and antixenosis (Qiu et al., 2010) and antibiosis (Huang et al., 2001; Du et al., 2009), respectively. The high level of tolerance has not been published before. The moderately resistant varieties Mudgo and T12, with average seedling-bulk-test resistance scores of 4.2 and 4.9, respectively, showed high tolerance and moderate/low antibiosis. No studies on BPH antibiosis or tolerance have been reported for T12, but many rice cultivars containing the *Bph1* gene (such as Mudgo) are known to have moderate levels of antibiosis and tolerance, or high tolerance plus no antibiosis (Panda & Heinrichs, 1983; Cohen et al., 1997; Jena & Kim, 2010).

Although varieties Rathu Heenati, Chin Saba, and ARC 10550 – previously reported to carry BPH-resistance genes – were susceptible to BPH (average seedling-bulk-test resistance scores of 8.6, 8.4, and 8.2, respectively), they were more tolerant than 93-11, Nipponbare, and TN1 (varieties without BPH-resistance genes): for instance, plant height and fresh weight of the first three varieties increased more, and their first and second leaves stayed green longer, compared with the latter three varieties. One possible explanation for the apparent change of BPH resistance of rice varieties (reported to carry resistance genes, yet susceptible according the seedling bulk test) is a change of BPH biotypes, with different virulence levels to rice varieties carrying resistance genes (Panda & Heinrichs, 1983; Tang et al., 2010). For example, rice varieties carrying the gene *bph1* displayed resistance to BPH biotype 1, but they were highly susceptible to BPH biotype 2 and lacked antibiosis or tolerance (Panda & Heinrichs, 1983; Myint et al., 2009). Plant age might be another factor affecting resistance expression; resistance levels can vary between seedling and adult stages. Thirdly, the tolerance levels in these varieties may have been too weak to be identified in the seedling bulk test, which suggested that the BPH-resistance level was mainly determined by the level of antibiosis.

The variety Pokkali had high BPH resistance (2.2 score), but it differed in the levels of antibiosis and tolerance: honeydew excretion was very little (128 mm^2 tracing spot area), whereas BPH nymph survival rate was as high as 65.6% – higher than that of the moderately resistant varieties Mudgo (52.2%) and T12 (61.7%) or the highly susceptible Nipponbare (63.4%). Possibly, BPH can slow down its development on Pokkali by ingesting less phloem sap. This should be tested in further experiments.

It should be noted that this study examined only the levels of antibiosis and tolerance in rice varieties carrying major BPH-resistance genes. We did not identify whether resistance mechanisms (antibiosis, tolerance) in the various varieties were only conferred by these major resistance genes. Alam & Cohen (1998) found two QTLs were investigated to be predominantly associated with antixenosis and tolerance in IR64 which carried the major resistance gene *Bph1*. Hence, possibly other loci conferred tolerance or antibiosis to BPH in varieties such as Mudgo, Pokkali, or B5. The best way to test this will be to map the associated loci and construct their near isogenic lines, and then characterize the BPH-resistance mechanisms (Inukai et al., 1996; Qiu et al., 2010). Solving these problems should improve our understanding of BPH-resistance mechanisms in rice varieties carrying resistance genes, thus allowing better application of resistance genes in breeding programs.

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