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Identification of Antixenosis and Antibiosis in Two Newly Explored Brown Planthopper-resistance Rice Lines

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Abstract: The rice brown plant hopper (Nilaparvata lugens Stål, BPH) is one of the typical piercing-sucking insect pests to feed on rice phloem sap. Breeding and cultivation of resistant rice varieties is the most economical and efficient method for managing the BPH. In the present study, we aimed to detect the BPH-resistance level of two newly explored rice lines and to identify the BPH-resistance mechanism of the resistant lines with respect to physiological function. Consequently, rice lines Q660 and Q327 exhibited a highly resistant to the BPH insects with the average resistance scores of 2.8 and 3.2, respectively in the seedling bulk test. The settling BPH numbers were significant less in the Q660 plant at 48 h and in the Q327 plant at 120 h after release, indicating that antixenotic factors were presented in both rice lines. After treated with 4 days, the BPH population growth rates were significantly restrained in the resistant rice lines Q660 and Q327 indicated by the weight changes of the BPH on the different genotypic plants. Furthermore, the BPH survival rates on the rice line Q660 also significantly decreased compared with that on the rice line 93-11 after treated with 4 days. By contrast, there was no significant difference in the survival rate between Q327 and 93-11 plants. The identified resistant rice lines should be of great benefit for the BPH-resistant rice varieties.

Keywords: Antibiosis, antixenosis, brown plant hopper (nilaparvata lugens stål), oryza rufipogon, resistance resource

INTRODUCTION

The rice brown plant hopper (Nilaparvata lugens Stål, BPH) is one of the typical piercing-sucking insect pests to feed on rice phloem sap. Historically, BPH was considered an occasional pest of rice in tropical Asia; but it has become a severe constraint on rice production following the introduction of high-yielding varieties in the 1960s (Way *et al*., 1994). Conventional methods of controlling BPH are primarily dependent on chemical insecticides, which are costly and environmentally unfriendly. Previous studies have shown that breeding and cultivation of resistant rice varieties is the most economical and efficient method for managing the BPH (Alam and Cohen *et al*., 1998; Renganayaki *et al*., 2002), therefore it is imperative to explore and identify BPH resistance rice lines from diverse sources and incorporate the resistance trait into various cultivars.

Lines showing BPH resistance are abundant in world rice germplasm collections (Zhang, 2007; Jena and Kim, 2010). Until now, a number of BPH-resistance rice lines have been identified from various rice germplasm including five wild *Oryza* species (O. officinal is, O. *minuta*, O. *latifolia*, O. *rufipogon* and O. *australiensis*). Furthermore, parts of the resistance germplasm have been used in resistant rice breeding programs (Cohen *et al*., 1997; Jairin *et al*., 2007; Rahman *et al*., 2009). The common wild rice specie (O. *rufipogon* Griff) in Guangxi province, China, has been identified to be carried a number of merit agricultural traits, one of which is highly resistant to BPH. Previously, Li *et al*. (2006) collected 1400 germplasms of common wild rice lines from Guangxi province and applied to be BPH resistance screen. As a result, six rice lines were identified to be highly resistant to several kinds of BPH biotypes, such as biotypes 1 and 2, Bangladesh population.

In the present study, we aimed to detect the BPH-resistance level of two newly explored rice lines and to identify the BPH-resistance mechanism of the resistant lines with respect to physiological function.

MATERIALS AND METHODS

Plant materials and BPH insects: Three rice lines were used. Rice line Q660 was one of the backcrossing

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generations which derived from a cross of the common wild rice species (*Oryza rufipogon* Griff.) and the indicia rice line "NL188". Rice line Q327 was also from a backcrossing generation which derived from a cross of the common wild rice species and the indicia rice line "Xixiangruo". Another indicia rice line 93-11 was highly resistant to BPH biotype 2 (Qiu *et al*., 2010).

Brown plant hoppers were collected from rice fields in 2011 in Nanning (22°49'N, 108°19'E, where BPH populations of biotype 2 dominated), China and maintained at the rice research institute, Guangxi University, on TN1 plants to produce enough nymphs for infestation. Second and third instars were collected and used for experiments.

BPH-resistance evaluation of rice lines: The seedling bulk test was performed as described by Qiu *et al*. (2010). Rice seeds were soaked in water and germinated at 30°C. Eighty germinated seeds of a given rice line were randomly sown in a tray (52×37×6 cm) in three 30-cm-long rows, with ca. 2.5 cm between lows. At the third-leaf stage (ca. 13-4 days old), the seedlings were infested with 2^{nd}-3^{rd} BPH instars at 10 insects/seedling. The trays were covered with a fine gauge nylon-net cage (42×32×45 cm) after infestation. Rice variety TN1 was randomly sown among the other rows as susceptible controls. Each seedling was given a score of 0, 1, 3, 5, 7, or 9 according to Qiu *et al*. (2010) when all of the TN1 seedlings had died (after ca. 9-10 days). The lower scores indicate higher resistance to the BPH insects. The resistance score of each line was then inferred from the weighted average of the scores for all seedlings. The experiment was replicated two times and conducted in a greenhouse under natural light at 25-30°C from May to October 2011 in Guangxi University.

Host selection behavior: The experiment was conducted as described by Qiu *et al*. (2010). Two 14-day old seedlings of Q327 or Q660 and 93-11 were transplanted in a plastic bucket (15 cm diameter, 14 cm height) with the same genotypic seedlings at opposite ends of roughly perpendicular diagonals. The bucket was then completely covered with fine, light-transmitting mesh; and a total of six buckets were used for each pair of genotypes. To observe the host selection of the BPH, 60 second-instars nymphs were placed in each bucket and allowed to choose host plants (42 days old) on which to feed and reproduce over a 120-h period. The BPH insects which settled on each plant were counted at 3, 6, 24, 48, 72, 96 and 120 h after release. Finally, the percent of settled BPH of each plant was applied to evaluate the antixenosis.

BPH development on rice plants: To measure BPH survival and growth on the Q327, Q660 and 93-11 plants, seedlings (14 days old) were transplanted in individual 0.4 L plastic cups and cultured in a greenhouse (constant temperature 25-29°C). The BPH growth was measured after 4 days on the tested rice lines using ten pre-weighed, second-in star nymphs. Ten replicates of 42-day-old seedlings were established for each genotype treatment of Q327, Q660 and 93-11. During the period of BPH infestation, the surviving insects in each cup/plant were recorded every day for 4 days to examine the BPH survival rate on plants. Four days after the treatment, the surviving nymphs on each plant were collected and the weight was recorded. The Population Growth Rate (PGR) of surviving nymphs was calculated according to Klingler *et al*. (2005) and Qiu *et al*. (2010).

Statistical analysis: Data were analyzed with one-way ANOVA and means were compared using a Least Significant Difference (LSD) test with MS-Excel.

RESULTS AND DISCUSSION

BPH-resistance scores of rice lines Rice lines Q660 and Q327 exhibited a highly resistant to the BPH insects with the average resistance scores of 2.8 and 3.2, respectively; while 93-11 showed high susceptibility to the BPH and scored 8.9 in the seedling bulk test. One-way ANOVA analysis showed that there were significant differences in the BPH-resistance scores between 93-11 and resistant rice lines ($F = 378.6$, $p<0.001$ for Q660 and 93-11; $F = 316.4$, $p<0.001$ for Q327 and 93-11). But no significant difference was observed in the resistance scores between Q660 and Q327 ($F = 2.1$, $p = 0.17$).

Antixenotic effect of resistance lines toward BPH insects: The resistant plants of Q660 and Q327 were used to detect the host choice of BPHs. The settled BPHs on the Q660 and Q327 plants were more than that on the 93-11 plants over the 6 and 48 h observation periods, respectively, in the BPH host choice test. After that, the percent of settled BPHs decreased on the resistant plants; whereas it increased on the 93-11 plants over the same observation period. One-way ANOVA analysis showed that the BPH insects had significant preference for Q660 and Q327 at 48 h ($F = 13.4$, $p = 0.006$) and 120 h ($F = 14.3$, $p = 0.005$) after release, indicating that antiemetic factors were presented in both resistant rice lines (Fig. 1).

BPH performance on resistance plants: The BPH PGR on the Q660, Q327 and 93-11 plants was compared to determine whether the resistant plants affect BPH growth and development. After treated with 4 days, the resistant trait caused a 1.5 and 1.1-fold reduction in PGR of the BPH in Q660 and Q327,

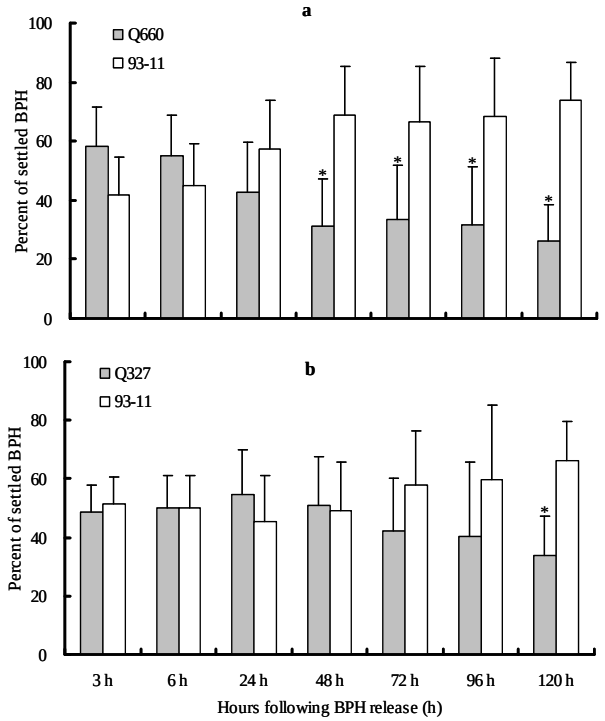

Fig. 1: Results of BPH host choice test, (a) Q660 and 93-11, (b) Q327 and 93-11
Bars represent means of six replicates; Error bars represent the S.D.; Means labeled with asterisks are significantly different ($p<0.05$)

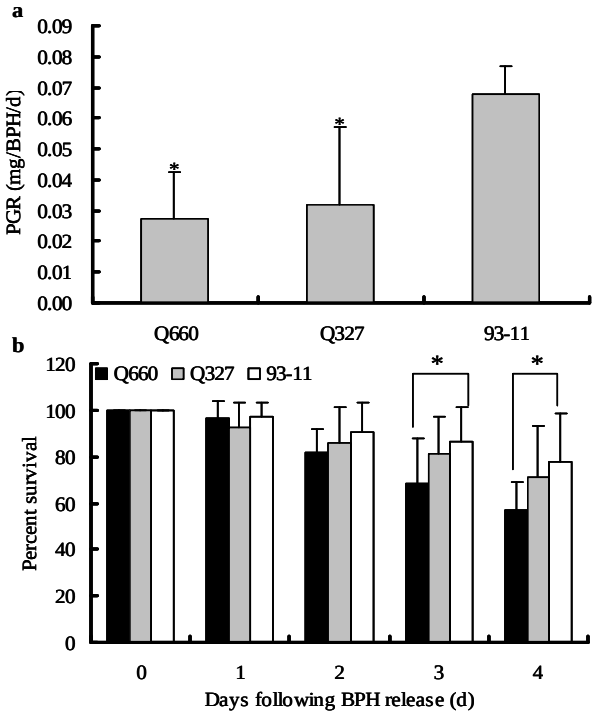

Fig. 2: Effects of plant genotype on the BPH population growth rate (mg/BPH/day, PGR) and BPH survival, (a) PGR of the Q660, Q327 and 93-11, PGR was measured as described by Edwards (2001), (b) BPH survival rates on Q660, Q327 and 93-11
Bars represent means of 11 replicates for a and b; Error bars represent the S.D.; Means labeled with asterisks are significantly different ($p<0.05$)

respectively, comparing with 93-11 ($F = 52.5$, $p<0.001$ for Q660; $F = 18.0$, $p<0.001$ for Q327; (Fig. 2a). The result indicates that the BPH growth and development were significantly inhibited on the resistant plants.

We measured the BPH survival rates on the Q660, Q327 and 93-11 plants every day for 4 days to test whether antibiotic factor was observed on the two resistant rice lines. As shown in Fig. 2b, the percent survival of BPHs on the resistant plants and 93-11 decreased gradually with the days of the BPH infestation. However, it decreased more quickly on the resistant plants and showed a significant difference in number between Q660 and 93-11 at 4 days after release ($F = 5.3$, $p = 0.03$ at 3 days; $F = 7.1$, $p = 0.02$ at 4 days). By contrast, there was no significant difference in the survival rate between Q327 and 93-11 plants by the fourth day after release ($F = 0.52$, $p = 0.48$ at 4 days).

DISCUSSION

Wild rice species are rich in resistant sources to BPH. As of, today, a number of resistance rice lines have been identified from several wild rice species. For instance, the rice line B14 carrying the resistance gene BPH12 was derived from O. *latifolia* (Yang *et al*., 2002; Qiu *et al*., 2012). Moreover, several highly BPH resistant rice lines, such as IR54741-3-21-22, IR54745-2-21-12-17-6 and B5 were all from the introgression lines of O. officinal is (Huang *et al*., 2001; Renganayaki *et al*., 2002; Jena *et al*., 2002) Fig. 2. In the present research, two rice lines, Q660 and Q327, derived from the backcross generations of Guangxi common wild rice specie, were detected to be highly resistant to the BPHs. Both lines could be applied to BPH-resistant rice breeding. In previous studies, several BPH resistant rice sources have been detected in Guangxi wild rice specie and assigned to be gene mapping. For example, the rice line RBPH54, derived from O. *rufipogon* (Griff.) in Guangxi province, was indicated to be highly resistant to BPHs and carried the resistance genes bph20 (t) and bph21 (t) (Yang *et al*., 2012). With crossing and backcrossing techniques, rice lines Q660 and Q327 have been introduced into several merit rice varieties, restorer lines and sterile lines to obtain the highly resistance gene-inserted lines in the practical breeding program. However, it still remained unknown whether there are one or more BPH-resistance genes controlling the BPH resistance in the rice lines Q660 and Q327. Further experiments are needed to construct the mapping populations and locate the corresponding

resistance genes and then explored and applied the tightly linked molecular markers for the marker-assisted selection in resistant rice breeding.

Understanding the mechanisms underlying rice resistance to BPH is very essential for developing appropriate breeding strategies. Generally, the insect-resistance types with regard to the physiological function were classified into antixenosis (non-preference), antibiosis and tolerance (Painter, 1951). Based on the tests of the BPH host choice and performance on the resistant and susceptible plants, rice lines Q660 and Q327 showed significant antixenosis to the BPH insects. Moreover, the BPH PGR and survival rate were significantly decreased on the rice line Q660, indicating the antibiotic factors were exited. Cohen *et al*. (1997) found that rice variety IR64 showed antixenosis, antibiosis and tolerance to the BPH. Recent studies conducted by Qiu *et al*. (2011, 2012) also indicated that several BPH-resistant varieties, e.g., Mudgo, Pokkali, Swarnalata and B14, exhibited antibiosis and tolerance or antixenosis to the tested BPH populations. However, all the tested rice varieties or lines were identified to carry one or more BPH resistance genes and the resistance characteristics were conferred by the associated resistance genes. To test whether the antibiosis and antixenosis were conferred by the resistance genes in Q660 and Q327, it should construct the near-isogonics lines of the resistance gene or clone the resistance genes.

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REFERENCES

Alam, S.N. and M.B. Cohen, 1998. Detection and analysis of QTLs for resistance to the brown planthopper, Nilaparvata lugens, in a doubled-haploid rice population. Theor. Appl. Genet., 97: 1370-1379.

Cohen, M.B., S.N. Alam, E.B. Medina and C.C. Bernal, 1997. Brown planthopper, Nilaparvata lugens, resistance in rice cultivar IR64: Mechanism and role in successful N. lugens management in Central Luzon, Philippines. Entomol. Ex. Appl., 85: 221-229.

Edwards, O.R., 2001. Interspecific and intraspecific variation in the performance of three pest aphid species on five grain legume hosts. Entomol. Ex. Appl., 100: 21-30.

Huang, Z., G.C. He, L.H. Shu, X.H. Li and Q.F. Zhang, 2001. Identification and mapping of two brown planthopper resistance genes in rice. Theor. Appl. Genet., 102: 929-934.

Jairin, J., K. Phengrat, S. Teangdeerith, A. Vanavichit and T. Toojinda, 2007. Mapping of a broad-spectrum brown planthopper resistance gene, Bph3, on rice chromosome 6. Mol. Breeding., 19: 35-44.

Jena, K.K. and S.M. Kim, 2010. Current status of Brown Planthopper (BPH) resistance and genetics. Rice, 3: 161-171.

Jena, K.K., I.C. Pasalu, Y.K. Rao, Y. Varalaxmi, K. Krishnaiah, G.S. Khush and G. Kochert, 2002. Molecular tagging of a gene for resistance to brown planthopper in rice (*Oryza sativa* L.). Euphytica, 129: 81-88.

Klingler, J., R. Creasy, L.L. Gao, R.M. Nair, A.S. Calix, H.S. Jacob, O.R. Edwards and K.B. Singh, 2005. Aphid resistance in Medicago truncatula involves antixenosis and phloem-specific, inducible antibiosis and maps to a single locus flanked by NBS-LRR resistance gene analogs. Plant Physiol., 137: 1445-1455.

Li, R.B., L.S. Li, S.M. Wei, Y.P. Wei, Y.Z. Chen, D.L. Bai, L. Yang, F.K. Huang, W.L. Lv, X.J. Zhang, X.Y. Li, X.Q. Yang and Y.W. Wei, 2006. The evaluation and utilization of new genes for brown planthopper resistance in common wild rice (Oryza rufipogon Griff.). Mol. Plant Breeding, 4(3): 365-371.

Painter, 1951. Insect Resistance in Crop Plants. The Macmillan Company, New York, pp: 520.

Qiu, Y.F., J.P. Guo, S.L. Jing, L.L. Zhu and G.C. He, 2010. High-resolution mapping of the brown planthopper resistance gene Bph6 in rice and characterizing its resistance in the 9311 and Nipponbare near isogenic backgrounds. Theor. Appl. Genet., 121: 1601-1611.

Qiu, Y.F., J.P. Guo, S.L. Jing, M. Tang, L.L. Zhu and G.C. He, 2011. Identification of antibiosis and tolerance in rice varieties carrying brown planthopper resistance genes. Entomol. Ex. Appl., 141: 224-231.

Qiu, Y.F., J.P. Guo, S.L. Jing, L.L. Zhu and G.C. He, 2012. Development and characterization of japonica rice lines carrying the brown planthopper resistance genes BPH12 and BPH6. Theor. Appl. Genet., 124(3): 485-494.

Rahman, M.L., W. Z. Jiang, S.H. Chu, Y.L. Qiao, T.H. Ham, M.O. Woo, J.H. Lee, M.S. Khanam, J.H. Chin, J.U. Jeung, D.S. Brar, K.K. Jena and H.J. Koh, 2009. High-resolution mapping of two rice brown planthopper resistance genes, Bph20(t) and Bph21(t), originating from Oryza minuta. Theor. Appl. Genet., 119(7): 1237-1246.

Renganayaki, K., A.K. Fritz, S. Sadasivam, S. Pammi, S.E. Harrington, S.R. McCouch, S.M. Kumar and A.S. Reddy, 2002. Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from Oryza officinalis into cultivated rice, O. sativa. Crop Sci., 42: 2112-2117.

Way, M.J. and K.J. Heong, 1994. The role of biodiversity in the dynamics and management of insect pests of tropical irrigated rice-a review. Bull. Entomol. Res., 84: 567-587.

Yang, H.Y., X. Ren, Q.M. Weng, L.L. Zhu and G.C. He, 2002. Molecular mapping and genetic analysis of a rice brown planthopper (Nilaparvata lugens Stål) resistance gene. Hereditas, 136(1): 39-43.

Yang, L., R.B. Li, Y.R. Li, F.K. Huang, Y.Z. Chen, S.S. Huang, L.F. Huang, C. Liu, Z.F. Ma, D.H. Huang and J.J. Jiang, 2012. Genetic mapping of bph20(t) and bph21(t) loci conferring brown planthopper resistance to Nilaparvata lugens Stål in rice (*Oryza sativa* L.). Euphytica, 183: 161-171.

Zhang, Q.F., 2007. Strategies for developing green super rice. Proceedings of the National Academy of Sciences of the USA, 104: 16402-16409.