

Loss of Resistance to *Nilaparvata lugens* May Be Due to the Low-Level Expression of *BPH32* in Rice Panicles at the Heading Stage

Jirapong Jairin^{1*}, Phikul Leelagud¹, Thida Saejueng², Varapong Chamarerk³

¹Ubon Ratchathani Rice Research Center, Ubon Ratchathani, Thailand

²Maharakham University, Maha Sarakham, Thailand

³Rice Department, Bangkok, Thailand

Email: *jirapong.j@rice.mail.go.th

How to cite this paper: Jairin, J., Leelagud, P., Saejueng, T. and Chamarerk, V. (2017) Loss of Resistance to *Nilaparvata lugens* May Be Due to the Low-Level Expression of *BPH32* in Rice Panicles at the Heading Stage. *American Journal of Plant Sciences*, 8, 2825-2836.

<https://doi.org/10.4236/ajps.2017.811191>

Received: September 21, 2017

Accepted: October 24, 2017

Published: October 27, 2017

Copyright © 2017 by authors and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

The brown planthopper (BPH), *Nilaparvata lugens* (Stål), is the most important insect pest of rice in Asia. Host plant resistance is one of the strategies currently used to control BPH. The resistant rice cultivar Rathu Heenati (RH) carrying the *BPH3* gene (recently renamed as “*BPH32*”) remains effective despite more than 30 years of deployment. RH has been determined to be resistant against BPH at all growth stages. However, we observed that BPH could feed on panicles but not on the leaf sheaths of RH. The resistance gene *BPH32* was introduced into KDML105 through marker-assisted selection, and the introgression line UBN03078 was developed. This rice line was used to observe the patterns of target gene’s regulation. A low-level expression of *BPH32* on panicles has been hypothesized to cause susceptibility in UBN03078 at the heading stage. Findings from our gene expression analysis support the hypothesis that the resistance gene was down regulated in the uppermost internodes compared with the leaf sheaths of the heading rice plant. This phenomenon may allow BPH to feed on the panicles of the resistant plants, but this requires further investigation.

Keywords

Rice, Brown Planthopper, Gene Expression, Heading Stage, *BPH32*

1. Introduction

The brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) is the most important insect pest of rice production in Asia. Host-plant resistance strategy has been widely used for controlling BPH throughout Asia. Numerous

resistant varieties have been developed and released to farmers [1]. This strategy can lead to a reduction in insecticide usage and environmental pollution. However, BPH populations under resistance pressures can indeed adapt and survive well on the resistant plants, and a new biotype of the BPH population arises [2]. The occurrence of the new virulent biotypes has been a serious problem in using resistant rice varieties. Several resistant varieties carrying a single gene (*BPH1* and *BPH2*) have lost their resistance to BPH. An effective strategy to overcome this rapid breakdown of resistance genes is to identify broad-spectrum BPH resistance genes or pyramid the BPH resistance genes to develop varieties with durable resistance against BPH.

The Sri Lankan traditional rice variety 'Rathu Heenati' (RH) and the Indian landrace 'PTB33' carrying a major *BPH3* gene for BPH resistance [3] have been used in rice breeding programs for over three decades. The *BPH3* gene confers broad-spectrum resistance against BPH populations in Thailand [4]. The chromosomal location of *BPH3* from RH and PTB33 has been reported on the short arm of chromosome 6 [4]. The BPH resistance locus has been widely used in BPH resistance breeding via marker-assisted selection (MAS) [5] [6] [7] [8]. Recently, the *BPH3* gene was identified and renamed as *BPH32* [9] to avoid confusion with the cloned *BPH3* cluster genes located on the short arm of chromosome 4 [10]. The *BPH32* encodes a short consensus repeat domain-containing protein that confers an antibiosis resistance to BPH and is localized in the plasma membrane. This gene is highly expressed in the leaf sheaths but poorly expressed in the panicles of rice plants [9]. Jairin *et al.* [11] reported that the resistant cultivars RH and PTB33 were highly resistant to BPH at the vegetative stage (seedling to maximum tillering stages) of heavy BPH infestation, but showed susceptibility at the reproductive stage. BPH can feed on the phloem sap of the uppermost internode after elongation at the heading stage until the plants die. This phenomenon has not yet been verified.

The mechanism of plant resistance to phloem sap-feeding insects has been reported to involve the balance of the amino acid composition of the phloem sap [12] [13]. The remobilization of nitrogen in the rice panicles can increase the total free amino acids in the phloem sap [14] [15], which may affect BPH resistance. However, so far, there is no evidence to support and prove this hypothesis. Although the mechanism of losing resistance at the heading stage is not well understood, it has been hypothesized that the resistance gene *BPH32* may be poorly expressed in the uppermost internodes of heading rice plants [11]. To verify that the low-level expression of *BPH32* causes the loss of resistance in the uppermost internode, an introgression line UBN03078-101-342-4-1-141 (UBN03078) carrying *BPH32* from RH [6] was used for gene expression analysis in this study. The results from our study will provide a starting point for future studies aimed at understanding the factors or mechanisms that depress the expression of the *BPH32* gene in rice panicle. Furthermore, the results will provide useful information for the deployment of BPH resistance genes and development

of a screening method for BPH resistance in rice at the heading stage.

2. Materials and Methods

2.1. Plant Materials

An introgression line UBN03078 was developed through successive introgression backcrossing (BC3) and MAS with a single introgressed segment [6]. The BPH resistance gene *BPH32* from RH, the Sri Lankan landrace rice, was introgressed to KDML105 (KD), the aromatic rice known as Jasmine rice. The donor parent, RH, is highly resistant to BPH whereas the recurrent parent, KD, is extremely susceptible to BPH. RH, KD, PTB33, and TN1 were used for the evaluation of BPH resistance bioassays. UBN03078 carrying *BPH32* was used for gene expression analysis.

2.2. Insect Strain

A BPH population from a single colony was collected from the outbreak rice field in Ubon Ratchathani province, Thailand in 2016. The BPH strain was reared on seedlings of rice variety TN1 and maintained in the laboratory at $26^{\circ}\text{C} \pm 2^{\circ}\text{C}$ under a 14L:10D photoperiod. The BPH strain was maintained in a laboratory colony for more than ten generations before being employed for BPH bioassays.

2.3. Evaluation of BPH Resistance

For evaluating the BPH resistance of rice varieties and lines at the vegetative and heading stages, three phenotypic experiments were conducted including the standard seedbox screening test (SSST) [16], modified mass adult plant screening test (MAST) [11], and honeydew excretion test (HET) [17] [18].

The SSST was used to measure the levels of resistance of RH, KD, UBN03078, and TN1, at the seedling stage under greenhouse conditions. The pre-germinated seeds of the test lines were sown 5 cm apart in 20 cm rows in seedboxes. The susceptible control, TN1, was sown randomly in all seed boxes. Seven to ten days after sowing, the seedlings were infested with second- and third-instar nymphs of BPH at ten to twenty nymphs per seedling. When all TN1 plants had died, the degrees of seedling damage in the varieties or lines were recorded. Each experiment was replicated three times. The test lines were graded using the Standard Evaluation System (SES) for Rice (0 = no damage; 1 = very slight damage; 3 = first and second leaves partially yellowing; 5 = pronounced yellowing and stunting; 7 = mostly wilting, the plant still alive; and 9 = the plant completely wilted or dead) [19].

The MAST was used to evaluate the BPH resistance of seedling-stage to heading-stage plants. Seeds of the test lines were individually sown at a spacing of 10×20 cm with approximately 20 to 30 seeds in each row (10×20 cm) in 7×24 m² plots. Twenty-five days after sowing, the seedlings were infested with third- and fourth-instar nymphs of BPH at ten nymphs per seedling. We let the insects feed, mate, lay eggs and hatch freely. After infestation at 30, 40 and 50 days, and

at the heading stage, all test lines were evaluated and recorded as resistant or susceptible based on the degree of damage (Scale 1 - 9).

The HET for a measure of BPH feeding rate was conducted to determine the phenotypic reaction of the rice plants to BPH feeding using a parafilm sachet method [18]. At the vegetative stage, 30-day-old seedlings were infested with a newly emerged brachypterous female (1-day-old adult). The newly emerged BPH was released into the parafilm sachet and attached to the lower part of the seedling (**Figure 1(a)**) and kept in the laboratory at $26^{\circ}\text{C} \pm 2^{\circ}\text{C}$ under a light regime of 14/10-h light/dark. At the heading stage, twenty-day-old seedlings of RH, KD, and UBN03078 were transplanted in 30-cm-diameter pots at the density of one seedling per pot. At the heading stage, the rice plants were moved to a temperature control room. The leaf sheath of lowest leaf, node, the leaf sheath of flag leaf, and panicle neck of each plant were separately infested with a newly emerged adult female BPH. Before and two days after infestation, the weights of parafilm sachets were measured using a 0.01-mg sensitivity balance (ME204, Mettler Toledo). Resistant or susceptible plants were defined by the quantity of honeydew. The amount of honeydew of the BPH from each plant that was lower and greater than 10 mg was classified as resistant and susceptible, respectively.

2.4. RNA Isolation and RT-PCR Analysis

Twenty-day-old seedlings of UBN03078 were transplanted into 30-cm-diameter pots. At the heading stage, the plant pots were transferred to the laboratory. The leaf sheath of the flag leaf and the panicle of each variety were infested with 20 BPHs inside a 2 cm diameter glass tube. The leaf sheaths and panicles of each plant were collected and frozen immediately in liquid nitrogen at 0, 2, 8, 24 and 48 h after infestation and kept at -40°C before RNA extraction. All treatments, each with four biological replicates, were terminated at the same time. Total RNA was extracted using an RNeasy Plant mini kit (Qiagen) according to the manufacturer's instructions. The concentration of each RNA sample was measured

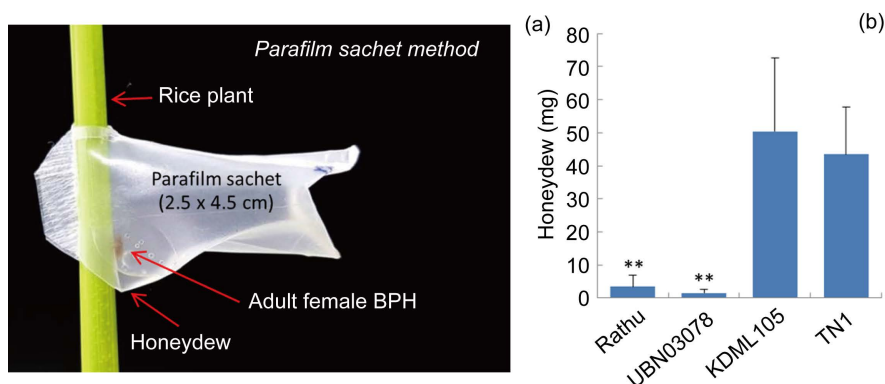


Figure 1. Evaluation of the brown planthopper feeding rate using the parafilm sachet method (a). Honeydew excretion of BPH feeding on seedlings of Rathu Heenati (Rathu), UBN03078-101-342-4-1-141 (UBN03078), KDML105 and TN1 at leaf sheaths (b). ** $P < 0.01$. One-way ANOVA was used to generate the P value.

using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). The integrity of RNA samples was also assessed by agarose gel electrophoresis. Total RNA (50 ng) was then converted into first-strand cDNA using an Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit following the manufacturer's instructions. The semi-quantitative RT-PCR analysis was performed to assess the expression levels of the resistance gene *BPH32*. The constitutively expressed actin gene was amplified as an internal control from rice leaf sheaths and panicles. The specific primers used in RT-PCR for *BPH32* and actin genes [9] were as follows: 5'-TGGGTTCGGTGGACCTGGG-3' (*BPH32* forward), 5'-GGACGTTGACCCTC-GCCGTG-3' (*BPH32* reverse) and 5'-GACGGAGCG TGGTTACTCATTC-3' (actin forward), 5'-GACCTCAGGGCAGCGGAAA-3' (actin reverse). The PCR reactions were carried out for 5 min at 94°C, followed by 35 cycles of 30 s at 95°C, 30 s at 55°C, 30 s at 72°C and a final extension of 10 min at 72°C. The PCR products were run on the QIAxcel capillary electrophoresis platform (Qiagen) and visualized on QIAxcelScreenGel software v1.0.2.0 (Qiagen). The ratio of the target band density to the actin band was used to represent the relative expression level of the target gene.

3. Results

3.1. Evaluation for BPH Resistance

BPH resistance evaluations at the vegetative and reproductive stages were conducted in the greenhouse. RH and UBN03078 expressed strong resistance against the Ubon Ratchathani BPH colony at the vegetative stage (seedling to tillering stages) of heavy BPH infestation, whereas KD and TN1 were highly susceptible to the BPH colony (Table 1). At the heading stage, RH and UBN03078 were susceptible when BPH moved to feed on the panicles and panicle necks until the plants died (Figures 2(a)-(c), Table 1). The result indicated that RH and the introgression lines were susceptible to BPH at the flowering stage, and BPH can feed and grow well on the panicle of the resistant plants carrying *BPH32* (Figure 2). We also observed that BPH could feed on any part of the uppermost internode (from node to panicle), which was not wrapped by the flag leaf sheath (Figure 2).

Table 1. The reaction of the rice line/varieties to the brown planthopper. The evaluation was conducted in the green house in wet season 2016.

Cultivar	30 DAI	40 DAI	50 DAI	Heading stage
Rathu Heenati	R	R	R	S
UBN03078	R	R	R	S
KDML105	S	S	S	S
TN1	S	S	S	S

DAI = Days after infestation; Average damage scores: 1.0 - 4.0 = resistant (R), 7.0 - 9.0 = susceptible (S).

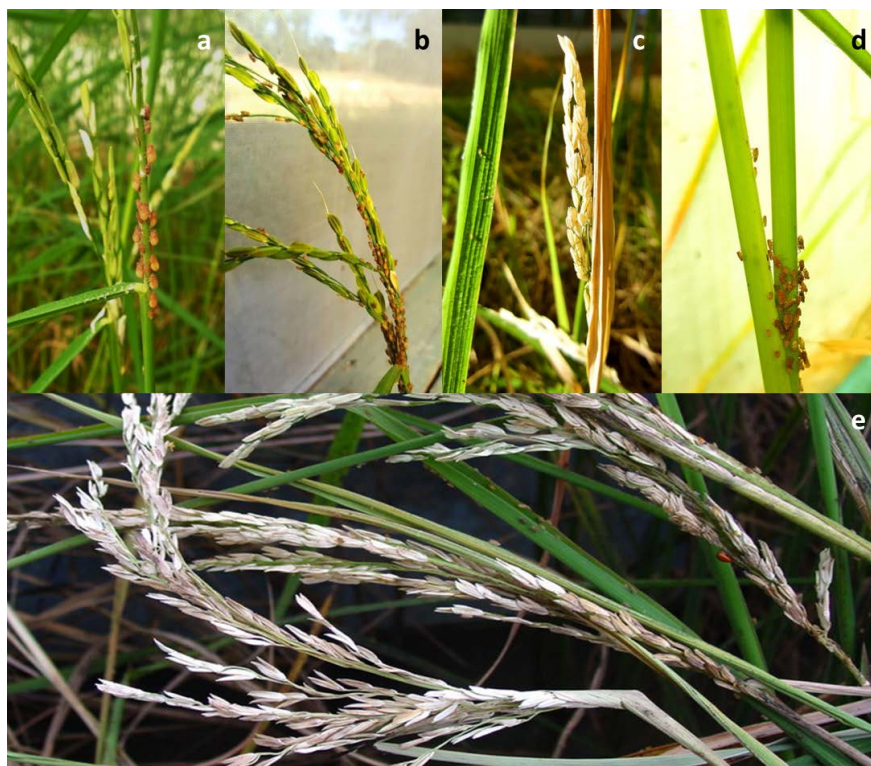


Figure 2. The nymphs and adults brown planthopper can feed on the panicles, panicle neck (a-b) and the last node bears the panicle (d) at the heading stage of resistant varieties. The feeding of BPH causes the unfilled grains and the rice plants die (c, e).

3.2. Honeydew Excretion Analysis

The parafilm sachet method (**Figure 1(a)**), which was developed for determining the feeding rate of BPH, was used to evaluate the resistance of rice varieties at the seedling and heading stages. At the seedling stage, the mean values of the honeydew quantity excreted by the adult females BPH on RH, UBN03078, KD, and TN1 ($n = 10$) were 3.5 ± 3.4 , 1.5 ± 1.2 , 50.4 ± 22.3 , and 43.5 ± 14.3 (mg \pm s.e.), respectively (**Figure 1(b)**). Based on the quantity of honeydew, RH and UBN03078 were resistant to the BPH at the seedling stage, whereas KD and TN1 were susceptible. At the heading stage, four parts of rice plants including the leaf sheath of the lowest leaf (LLS), uppermost or the last node (UN), the leaf sheath of the flag leaf (FLS), and the panicle neck (PN) were infested with the adult female BPHs (**Figure 3**). The mean values of honeydew excreted by the BPH of each rice variety and each part of rice plants were significantly different (**Figure 3**). The volume of honeydew from leaf sheaths of resistant plants (RH and UBN03078) was lower than 10 mg, however, the amount of honeydew collected from the node and panicle neck was greater than 10 mg of the resistant plants. Large amounts of honeydew were collected from all parts of the susceptible plant (KD). The result indicated that BPH cannot feed on leaf sheaths of the resistant rice varieties carrying *BPH32*, but can normally feed on any part of the uppermost internode, which was not wrapped by the leaf sheaths (**Figure 2**, **Figure 3**).

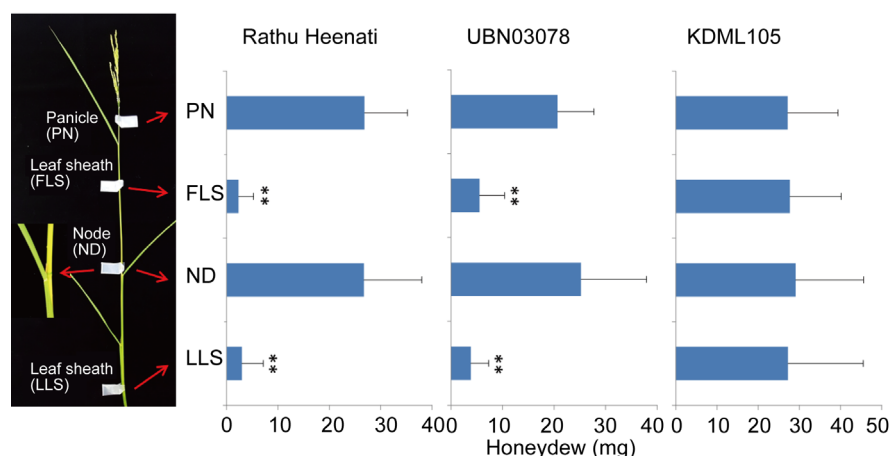


Figure 3. Locations for honeydew droplet collecting on rice panicle neck (PN), flag leaf sheath (FLS), node (ND), and leaf sheath of the lowest leave (LLS) and the average amount of honeydew excretions from individual female brown planthopper on Rathu Heenati, UBN03078-101-342-4-1-141, and KDML105 at the heading stage. ** $P < 0.01$. One-way ANOVA was used to generate the P value.

We confirmed this result by evaluating BPH resistance of RH, UBN03078, and KD at the heading stage using honeydew test again, and the same result was verified. The BPH could feed on the panicle neck but could not feed on flag leaf sheath of the resistant plants, while the BPH could feed on the panicle neck and leaf sheath of KD, a highly susceptible variety (Figure 4). The mean values of the honeydew quantity excreted by BPH on the panicles of RH, UBN03078, and KD ($n = 15$) were 38.0 ± 16.6 , 40.1 ± 15.0 , and 39.6 ± 19.1 (mg \pm s.e.), respectively, while on the flag leaf sheath were 1.2 ± 2.4 , 0.2 ± 0.3 , and 35.1 ± 9.6 (mg \pm s.e.), respectively (Figure 4). The result showed that the amount of the honeydew from the panicles of resistant and susceptible rice varieties was not significantly different. Conversely, the amounts of honeydew from flag leaf sheaths were significantly different among resistant and susceptible varieties. We have evaluated some BPH resistant varieties such as PTB33, RD49, PSL2, and Babawee for BPH resistance at the heading stage by allowing BPH to feed on flag leaf sheath and panicle neck using the parafilm sachet method. We found that BPH could not feed on the leaf sheath but the panicle neck as RH, and UBN03078 (data not shown).

3.3. Expression Analysis of *BPH32*

To reveal the molecular mechanism of BPH resistance in UBN03078, we examined the expression of the *BPH32* gene. Total RNA was isolated from flag leaf sheath and panicle (without spikelets) of UBN03078 at 0, 2, 8, 24 and 48 h after infestation. Although the amount of total RNA isolated from the panicle was significantly higher than in the leaf sheath, the mean level of expression of *BPH32* gene of the leaf sheath was greater than the panicle (Figure 5(b)). It may indicate that the mRNA level of *BPH32* gene that was quantified by reverse

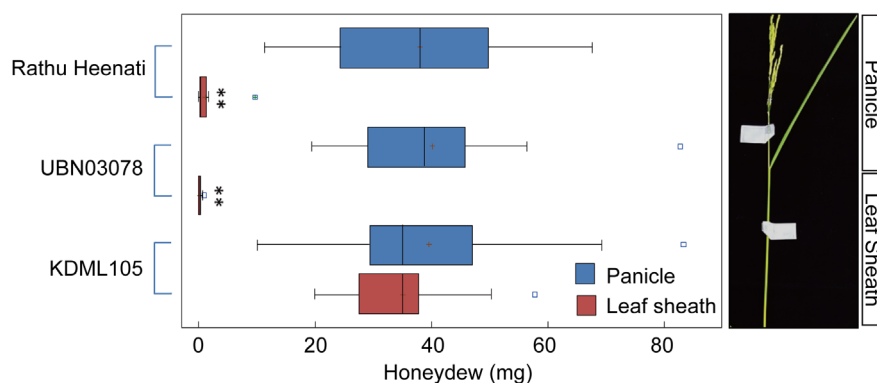


Figure 4. Honeydew excretions of BPH feeding on panicle necks and flag leaf sheaths of Rathu Heenati, UBN03078-101-342-4-1-141, and KDML105 at the heading stage. ** $P < 0.01$. One-way ANOVA was used to generate the P value.

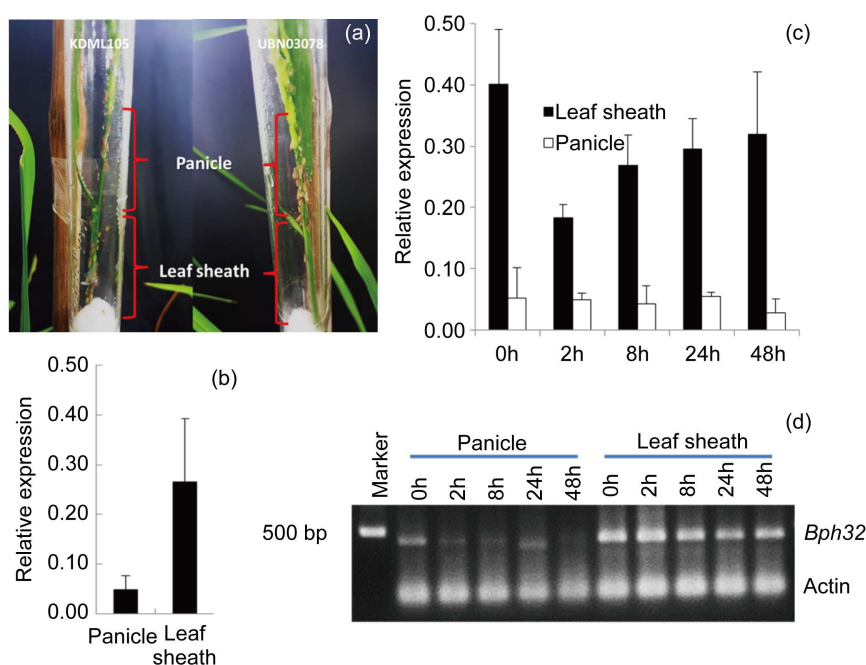


Figure 5. Glass tubes were used for brown planthopper infestation on leaf sheaths and panicles. Almost all brown planthoppers fed only on panicle of UBN03078, while they fed equally on the flag leaf sheath and the panicle in KDML105 (a). The average relative expression of resistance gene *BPH32* in panicles and leaf sheaths of UBN03078-101-342-4-1-141 (b). Semi-quantitative RT-PCR analysis of *BPH32* and actin genes in panicle and leaf sheath of UBN03078 at 0, 2, 8, 24 and 48 h after infestations (c-d).

transcription-PCR was low in the panicle and high in the leaf sheath. Semi-quantitative RT-PCR analysis showed that *BPH32* displayed differential expression patterns in response to BPH infestation between leaf sheaths and panicles of the resistant line UBN03078. Expression analysis revealed that *BPH32* carried by UBN03078 was highly and stably expressed in the leaf sheaths (**Figure 5(c)**). This result confirmed that *BPH32* is a stable BPH resistance gene and provides a valuable gene for rice resistant against BPH at the vegetative stage.

However, low-level expression of *BPH32* was observed in the rice panicles at the heading stage (**Figure 5(c)**, **Figure 5(d)**). The repression of the gene might allow BPH to feed on the uppermost internode of the resistant plants. We forced the BPHs to feed on the leaf sheath and panicle of resistant (UBN03078) and susceptible (KD) plants. We found that most BPH fed only on the panicle of the resistant plants but fed equally on both flag leaf sheath and panicle in susceptible plants (**Figure 5(a)**).

4. Discussion

Currently, there has been remarkable progress in the genomic studies of BPH resistance in rice. Some BPH resistance genes were detected in rice germplasms and used in breeding programs throughout Asia [1] [6] [7]. The understanding of the molecular basis of resistance to BPH was greatly advanced with the cloning and characterization of several BPH resistance genes. Among these, a broad spectrum *BPH32* gene originally derived from PTB33 and RH has been extensively studied and introgressed into numerous commercial rice varieties with desirable backgrounds [6] [8]. New rice varieties with BPH resistance have been developed and introduced to farmers [1]. The *BPH32* gene is still used in rice breeding programs [20], and the functional DNA markers of this gene have been developed for MAS [21].

BPH32 which encloses a unique short consensus repeat domain protein and is located in the plasma membrane, has been reported to inhibit BPH feeding [9]. The structure and function of the *BPH32* protein conferring BPH resistance may be different between susceptible and resistant plants. Real-time PCR analysis showed that *BPH32* was expressed in all investigated tissues at the flowering stage, and its expression level was the highest in leaf sheaths followed by leaf blades, culms, and roots but not the panicle [9]. The result of our study was consistent with previous studies that showed high *BPH32* expression in the leaf sheaths but a poor expression in the uppermost internode at heading stage of the same plant. As expected, BPH can feed on any part of the uppermost internode which emerges beyond the leaf sheaths but cannot feed on the leaf sheaths of resistant plants. When we removed the flag leaf sheath wrapping the last internode, we observed that BPH could feed and excrete a large amount of honeydew. This occurrence has previously been reported in rice varieties carrying the *BPH32* gene [11]. There are some factors, currently unknown, which influence the loss of resistance or repress the expression of the resistance gene during flowering. We believe that the loss of resistance in UBN03078 (also in RH and PTB33) might be due to the low-level expression of resistance genes in the uppermost internode at the heading stage. Before and after BPH infestation, the average expression level of *BPH32* constantly remained high in the leaf sheath tissue indicating that the gene function may not be induced by the BPH infestation.

The outbreaks of BPH in rice fields have frequently been observed at the heading stage. It is necessary to identify the genotype resistant to BPH at the

heading stage for use as a genetic resource to develop resistant varieties. It is also necessary to develop a screening method for the evaluation of BPH resistance at the heading stage in rice germplasm. Recently, twenty-five near-isogenic lines (NILs) carrying ten BPH resistance genes and their pyramids were developed in the background of the *indica* variety IR24 [21]. The NILs will be useful as new genetic resources for BPH resistance breeding. Therefore, it is interesting to know the NILs that will confer resistance against BPH at the flowering stage. Moreover, further studies are needed to clarify the factors or mechanisms that depress the expression of the *BPH32* gene in the uppermost internode of the resistant plants.

5. Conclusion

The resistance gene *BPH32* from RH and PTB33 has been used in rice breeding programs in Thailand for over three decades. This gene confers broad-spectrum resistance against BPH populations in Thailand. The results from this study revealed that BPH could not feed on leaf sheaths of resistant varieties carrying *BPH32*, but it could feed on the uppermost internodes that elongate and emerge from the flag leaf sheath during the flowering period. The expression analysis of *BPH32* gene revealed that losing of resistance at the heading stage may be due to the low-level expression of *BPH32* in the introgression line. This finding provides new insights into BPH resistance at the flowering time. It will provide useful information to deploy BPH resistance genes, develop novel BPH resistant varieties and improve the screening method for BPH resistance at the heading stage.

Acknowledgements

This study was supported by grants from the Rice Department, Ministry of Agriculture and Cooperatives, Thailand (Project No. 231473). We thank our colleagues from the plant molecular biology and entomology laboratories at Ubon Ratchathani Rice Research Center who greatly assisted the research.

References

- [1] Khush, G.S. and Virk, P.S. (2005) IR Varieties and Their Impact. Los Baños (Philippines). International Rice Research Institute.
<http://irri.org/resources/publications/books/item/ir-varities-and-their-impact>
- [2] Cruz, A.P., Arida, A., Heong, K.L. and Horgan, F.G. (2011) Aspects of Brown Planthopper Adaptation to Resistant Rice Varieties with the *Bph3* Gene. *Entomologia Experimentalis et Applicata*, **141**, 245-257.
<https://doi.org/10.1111/j.1570-7458.2011.01193.x>
- [3] Lakshminarayana, A. and Khush, G.S. (1977) New Genes for Resistance to the Brown Planthopper in Rice. *Crop Science*, **17**, 96-100.
<https://doi.org/10.2135/cropsci1977.0011183X001700010028x>
- [4] Jairin, J., Phengrat, K., Teangdeerith, S., Vanavichit, A. and Toojinda, T. (2007) Mapping of a Broad-Spectrum Brown Planthopper Resistance Gene, *Bph3*, on Rice Chromosome 6. *Molecular Breeding*, **19**, 35-44.
<https://doi.org/10.1007/s11032-006-9040-3>

- [5] Hu, J., Cheng, M., Gao, G., Zhang, Q., Xiao, J. and He, Y. (2013) Pyramiding and Evaluation of Three Dominant Brown Planthopper Resistance Genes in the Elite *indica* Rice 9311 and Its Hybrids. *Pest Management Science*, **69**, 802-808. <https://doi.org/10.1002/ps.3437>
- [6] Jairin, J., Teangdeerith, S., Leelagud, P., Kothcharerk, J., Sansen, K., Yi, M., Vanavichit, A. and Toojinda, T. (2009) Development of Rice Introgression Lines with Brown Planthopper Resistance and KDML105 Grain Quality Characteristics through Marker-Assisted Selection. *Field Crops Research*, **110**, 263-271. <https://doi.org/10.1016/j.fcr.2008.09.009>
- [7] Liu, Y., Chen, L., Liu, Y., Dai, H., He, J., Kang, K., Pan, G., Huang, J., Qiu, Z., Wang, Q., Hu, J., Liu, L., Chen, Y., Cheng, X., Jiang, L. and Wan, J. (2016) Marker Assisted Pyramiding of Two Brown Planthopper Resistance Genes, *Bph3* and *Bph27(t)*, into Elite Rice Cultivars. *Rice*, **9**, 27. <https://doi.org/10.1186/s12284-016-0096-3>
- [8] Wan, B., Zha, Z., Li, J., Xia, M., Du, X., Lin, Y. and Yin, D. (2014) Development of Elite Rice Restorer Lines in the Genetic Background of R022 Possessing Tolerance to Brown Planthopper, Stem Borer, Leaf Folder and Herbicide Through Marker-assisted Breeding. *Euphytica*, **195**, 129-142. <https://doi.org/10.1007/s10681-013-0988-x>
- [9] Ren, J., Gao, F., Wu, X., Lu, X., Zeng, L., Lv, J., Su, X., Luo, H. and Ren, G. (2016) *Bph32*, a Novel Gene Encoding an Unknown SCR Domain-Containing Protein, Confers Resistance Against the Brown Planthopper in Rice. *Scientific Reports*, **6**, 37645. <https://doi.org/10.1038/srep37645>
- [10] Liu, Y., Han, W., Hong, C., Yanling, L., Jun, H., Haiyan, K., Zhiguang, S., Gen, P., Qi, W., Jinlong, H., Feng, Z., Kunngeng, Z., Xiaoming, Z., Yulong, R., Liangming, C., Yihua, W., Zhigang, Z., Qibing, L., Fuqing, W., Xin, Z., Xiuping, G., Xianian, C., Ling, J., Chuanyin, W., Haiyang, W. and Jianmin, W. (2015) A Gene Cluster Encoding Lectin Receptor Kinases Confers Broad-Spectrum and Durable Insect Resistance in Rice. *Nature Biotechnology*, **33**, 301-305. <https://doi.org/10.1038/nbt.3069>
- [11] Jairin, J., Phengrat, K., Teangdeerith, S., Vanavichit, A. and Toojinda, T. (2007) Physical Mapping of *Bph3*, a Brown Planthopper Resistance Locus in Rice. *Maejo International Journal of Science and Technology*, **1**, 166-177. <http://www.mijst.mju.ac.th/vol1/166-177.pdf>
- [12] Douglas, A.E. (1993) The Nutritional Quality of Phloem Sap Utilised by Natural Aphid Populations. *Ecological Entomology*, **18**, 31-38. <https://doi.org/10.1111/j.1365-2311.1993.tb01076.x>
- [13] Sandstrom, J. (2000) Nutritional Quality of Phloem Sap in Relation to Host Plant-Alternation in the Bird Cherry-Oat Aphid. *Chemoecology*, **10**, 17-24. <https://doi.org/10.1007/s000490050003>
- [14] Hayakawa, T., Yamaya, T., Mae, T. and Ojima, K. (1993) Changes in the Content of Two Glutamate Synthase Proteins in Spikelets of Rice (*Oryza sativa*) Plants during Ripening. *Plant Physiology*, **101**, 1257-1262. <https://doi.org/10.1104/pp.101.4.1257>
- [15] Tabuchi, M., Abiko, T. and Yamaya, T. (2007) Assimilation of Ammonium Ions and Reutilization of Nitrogen in Rice (*Oryza sativa* L.). *Journal of Experimental Botany*, **58**, 2319-2327. <https://doi.org/10.1093/jxb/erm016>
- [16] Heinrichs E.A., Medrano F.G. and Rapusas H.R. (1985) Genetic Evaluation for Insect Resistance in Rice. IRRI, Los Baños, Philippines. http://books.irri.org/9711041103_content.pdf
- [17] Kobayashi, T., Yamamoto, K., Suetsugu, Y., Kuwazaki, S., Hattori, M., Jairin, J., Sanada-Morimura, S. and Matsumura, M. (2014) Genetic Mapping of the Rice Resis-

tance-Breaking Gene of the Brown Planthopper *Nilaparvata lugens*. *Proceedings of the Royal Society B*, **281**, Article ID: 20140726.

- [18] Pathak, P.K., Saxena, R.C. and Heinrichs, E.A. (1982) Parafilm Sachet for Measuring Honeydew Excretion by *Nilaparvata lugens* (Homoptera: Delphacidae) on Rice. *Journal of Economic Entomology*, **75**, 194-195. <https://doi.org/10.1093/jee/75.2.194>
- [19] IRRI. (2002) Standard Evaluation System for Rice (SES). International Rice Research Institute, Manila.
<http://www.knowledgebank.irri.org/images/docs/rice-standard-evaluation-system.pdf>
- [20] Jairin, J., Kotchasatit, U., Saleeto, S., Jearakongman, S., Srivilai, K., Chamarek, V., Kothcharerk, J., Pattawatang, P., Korinsak, S., Wongsaprom, C. and Toojinda, T. (2017) Application of Marker-Assisted Breeding to Improve Biotic Stress Resistance for Rainfed Lowland Rice in Northeastern Thailand. *SABRAO Journal of Breeding and Genetics*, **49**, 168-178.
<http://www.sabao.org/journals/SABRAO492JuneIssue/SABRAO-J-Breed-Genet-49-2-168-178%20-Jairin.pdf>
- [21] Jena, K.K., Hechanova, S.L., Verdeprado, H., Prahalada, G.D. and Kim, S.R. (2017) Development of 25 Near-Isogenic Lines (NILs) with Ten BPH Resistance Genes in Rice (*Oryza sativa* L.): Production, Resistance Spectrum, and Molecular Analysis. *Theoretical and Applied Genetics*, **130**, 2345-2360.
<https://doi.org/10.1007/s00122-017-2963-8>