Research Article

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Biological effects of rice harbouring *Bph14* and *Bph15* on brown planthopper, *Nilaparvata lugens*

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Abstract

BACKGROUND: Brown planthopper (*Nilaparvata lugens* Stål; BPH) resistance genes *Bph14* and *Bph15* have been introgressed singly or pyramided into rice variety Minghui 63 (MH63). The antibiosis and antixenosis effects of these rice lines on BPH and the expression of five P450 genes of BPH regulated by these rice lines were investigated in this study.

RESULTS: The resistance level of rice lines harbouring resistance genes was improved compared with MH63. MH63::14 (carrying *Bph14*) had negative effects on the development of males, honeydew excretion of females, the female ratio and the copulation rate compared with MH63. MH63::14 also exhibited antixenosis action against BPH nymphs, female adults and oviposition. Besides these negative effects, MH63::15 (carrying *Bph15*) could also retard the development of females, lower the fecundity and shorten the lifespan of females. The antixenosis action of MH63::15 was stronger than that of MH63::14. When *Bph14* and *Bph15* were pyramided, antibiosis and antixenosis effects were significantly enhanced relative to single-introgression lines. Among the five P450 genes of BPH, expression of three genes was upregulated, one gene was downregulated and one gene was unchanged by resistant hosts.

CONCLUSION: Both *Bph14* and *Bph15* could improve resistance levels of MH63. MH63::15 and MH63::14&15 had greater potential to control BPH infestations than MH63::14.

Keywords: *Nilaparvata lugens*; *Bph14*; *Bph15*; antibiosis; antixenosis; P450; rice

1 INTRODUCTION

Rice, *Oryza sativa* L., is the staple food of more than three billion people of Asia. Brown planthopper (BPH), *Nilaparvata lugens* Stål, is one of the most destructive insect pests on rice. BPH extracts phloem saps of rice and causes 'hopper burn' in rice fields.[1,2] It also acts as a vector for rice grassy stunt virus and ragged stunt virus,[3] causing significant yield losses in rice production every year.[4] Traditionally, BPH has been controlled mainly by poisonous chemicals. Large amounts and long-term applications of pesticides have caused negative effects, e.g. resistance of insects to synthetic chemicals, resurgence, consumption of labour and money and environmental contamination.[5–7] All of these have prompted researchers to seek more economic and environmentally friendly strategies for BPH control. Rice varieties harbouring resistance genes have long been exploited as economic, effective and environmentally sound measures for BPH integrated management programmes. Up to now, a total of 21 BPH resistance genes have been identified from cultivated and wild rice species.[8,9] Much effort has been made over the years to introduce BPH resistance genes into cultivated rice varieties, and to develop high-resistance and high-yielding rice cultivars.

The biological, physiological and biochemical features of BPH are significantly affected by rice varieties with different resistance levels. When BPH was maintained on resistant plants, its growth was retarded, development was delayed, fecundity was lowered and population build-up was suppressed.[10–12] BPH made more frequent probes on resistant rice than on susceptible rice, but the mean duration of probes tended to be shorter than that on susceptible rice, and the percentage of probes that consisted of the primarily salivation phase was higher than that on susceptible rice.[13–17] *Bph14* and *Bph15* are two BPH resistance genes derived from the wild rice *O. officinalis* Wall ex Watt.[18] Using B5 (carrying *Bph14* and *Bph15*) and Taichung Native 1 (TN1, susceptible control) as plant material, it was found that the quantity of phloem sap of BPH ingested from the host was largest on TN1, then RI35 (carrying *Bph14*) and YHY15 (carrying *Bph15*), and it was smallest on B5.[17] Some genes of BPH involved in signalling, stress response, gene expression regulation, detoxification and metabolism were

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extensively regulated by B5.[19] Among the differentially expressed genes, those related to detoxification and defensive response such as *CYP6AX1, CYP6AY1*, NADH-quinone oxidoreductase and carboxylesterase were significantly upregulated in BPH by B5.[20,21]

Using molecular-marker-assisted selection breeding based on backcross, *Bph14* and *Bph15* were introgressed into rice variety Minghui 63 (MH63), an elite indica restorer line for cytoplasmic male-sterile (CMS). The *Bph14* or *Bph15* singly introgressed, and two-gene pyramided lines were developed. For the purpose of application of these lines in rice hybrid production, it was essential to evaluate the resistance level of these rice lines and to illustrate the biological effects of these introgression lines on BPH. A comparative study was performed on antibiosis and antixenosis effects of these resistant rice lines on BPH and the expression of five genes (*CYP4C61, CYP4C621, CYP6CS1, CYP6CW1* and *CYP303A1*) of the P450 family in the authors' laboratory. This will aid the understanding of the resistance mechanism of these lines and shed light on their application in rice hybrid production.

2 MATERIALS AND METHODS

2.1 Plants and insects

MH63, an elite indica restorer line for CMS in China, does not harbour any resistance genes to BPH. MH63::14, MH63::15 and MH63::14&15 are three introgression lines originating from MH63 that harbour BPH resistance genes *Bph14, Bph15* or both *Bph14* and *Bph15* respectively. Three resistant lines, pure MH63 (gifted by Professor Yuqing He, National Key Laboratory of Crop Genetic Improvement, Wuhan, China) and susceptible control TN1 (Taichung Native 1) were employed in this study.

A colony of *N. lugens* was raised on TN1 in a growth room with a 14 : 10 h light : dark photoperiod, the day/night temperature was controlled at 25 ± 2 °C and relative humidity was around 80%. This insect-rearing protocol lasted 2 years.

2.2 Bioassay on evaluating the BPH resistance of rice lines

For evaluation of the BPH resistance of rice lines, a modified bulk seedling test was carried out as described by Pathak *et al*.[22] Each rice line was grown in a row 40 cm long, and the seedlings in each row were thinned to 15 plants at the 2.5-leaf stage and infested with second–third-instar BPH nymphs at a density of 10–12 nymphs per seedling. After all TN1 plants had died, the target seedlings were scored according to the Standard Evaluation Systems for Rice[23] and Huang *et al*.[18] The experiment was performed with three replications.

2.3 Nymph growth, adult reproduction and wing form

Fifty neonates that hatched out within 2 h were collected, and each three of them were reared on one rice seedling at tillering stage in a rearing cage. When they developed into third instars, the hoppers were transferred to fresh seedlings. When adults emerged, the survival rate from neonate to adult was calculated as the percentage of total number of adults emerging from the neonates divided by the total number of neonates released at the start of the experiment. At the same time, the duration of nymph development (the time needed for a neonate to develop into an adult), the brachyptery ratio of males (the number of brachyptery males/total number of males), the brachyptery ratio of females (the number of brachyptery females/total number of females) and the female ratio (number of females/total number of adults) were recorded. Three replications, i.e. 150 BPHs, were tested on each rice line. The newly emerged male and female adults were thereafter collected and coupled every day (one female plus one male), and reared on seedlings at tillering stage. There were about 15 families for each of three replications, in total 45 BPH families for each rice line. When the neonates of the new generation appeared, families were checked, and neonates were counted and removed daily. This procedure continued until 15 days after the female died. The lifespan of females was recorded, and the fecundity was evaluated as the total neonates produced by one copulated female. The females without progeny were considered to be unmated, and the copulation rate was calculated accordingly (the number of copulated females/total number of females coupled). All experiments were executed with a 14 : 10 h light : dark photoperiod, the temperature was 28 ± 1 °C and the relative humidity was around 80%.

2.4 Quantification of honeydew excretion of BPH female adults

Honeydew collection followed a previous protocol.[24] Newly emerged brachypterous females (12–24 h old) were starved for 3 h but supplied with water in a petri dish via a moist filter paper. Individual insects were enclosed in a Parafilm sachet and attached to the lower part (0–5 cm from the base) of the main tiller of a six-week-old rice plant. After 24 h of feeding, the insects were removed from sachets, and the honeydew harvested from five insects was pooled and weighed. The experiment for each rice line was set up in a randomised complete block design with six replications.

2.5 Antixenosis test

2.5.1 Antixenosis of resistant rice against BPH nymphs

One plant each of MH63, MH63::14, MH63::15 and MH63::14&15 were randomly grown in individual plastic pots (14 cm length × 14 cm width × 15 cm height), so that one plant occupied each of the four corners. Each pot was placed in a cage covered with nylon net in a growth chamber, under conditions of 80% relative humidity, 25 ± 2 °C and 14 : 10 h light : dark photoperiod. At the 2.5–3.0-leaf stage, each seedling was infested with five second–third-instar BPH nymphs. The number of BPH that settled on each seedling was counted after 1, 2, 3, 4 and 5 days of infestation respectively. The total number of nymphs on each seedling for 5 days was calculated and treated as the value of the antixenosis test. The experiments were repeated 30 times.

2.5.2 Antixenosis of resistant rice against BPH female adults and oviposition

Plants were grown as above. At 30 days after sowing, for each plant, the main tiller was kept for the antixenosis test, and all other tillers were pruned. Each tiller was infested with two gravid females (5 days after emergence). The number of females settling on each tiller was counted after 1, 2 and 3 days of infestation respectively. The total number of females on each tiller was calculated and treated as the value of the antixenosis test. The number of BPH eggs on each tiller was also counted after 72 h of infestation. The experiments were repeated 30 times.

2.6 Quantitative real-time PCR

Rice seeds of MH63, MH63::14, MH63::15 and MH63::14&15 were planted in plastic pots separately. The fourth-instar nymphs of BPH were collected from TN1 and transferred to four-leaf-stage

Table 1. qRT-PCR primers of five genes of the P450 family

Accession number	Annotation	Primer sequence (5′–3′)
FN421126	Cytochrome P450s (*CYP6CW1*)	6CW1-F 5′-CGCCGACCTTCACATCCGGC-3′ 6CW1-R 5′-GATCGCGCACCTCGACAGCC-3′
FM994118	Cytochrome P450s (*CYP6CS1*)	6CS1-F 5′-GCCTTCTGCCTGCACGGGTT-3′ 6CS1-R 5′-ACGCCGGCTGGAGGGTACTT-3′
FM163384	Cytochrome P450s (*CYP4C61*)	4C61-F 5′-ATCGGCGATGGGAACCACGG-3′ 4C61-R 5′-AGTGGCTTCTCTACGCTCCACT-3′
FM163385	Cytochrome P450s (*CYP4C62*)	4C62-F 5′-GGCTGGACCTGTGCCGCAA-3′ 4C62-R 5′-TCCAGTGCCCAGCCCTGGTT-3′
FJ907954	Cytochrome P450s (*CYP303A1*)	303A1-F 5′-CATGGCTGCCAGTGGTGGGG-3′ 303A1-R 5′-CAGAAAGGGCCAGTCGGGCG-3′
EU179846.1	*actin1*	Actin1-F 5′-CCAACCGTGAGAAGATGACC-3′ Actin1-R 5′-GATGTCACGCACGATTTCAC-3′

seedlings of the four rice lines. A total of 30–40 insects were maintained on each seedling for 24 and 48 h, then collected and immediately frozen in liquid nitrogen for RNA extraction. The activities of five members of the P450 gene family were examined using quantitative real-time PCR (qRT-PCR) on an ABI 7500 real-time PCR system (Applied Biosystems, USA), and SYBR® Premix Ex Taq™ (perfect real time) (Takara Biotechnology Corporation Co. Ltd, Dalian, China) was used for the reactions according to the manufacturer's instructions. BPH *actin1* was used as a reference. The qRT-PCR primers for each gene are listed in Table 1. Three biological replicates were designed for each rice material. Triple independent reactions in each biological replicate were performed, and the signal intensity of the target gene was represented by the averaged value. The expression level for each gene was quantified relative to the value of the sample at 0 h, as previously described.[25]

2.7 Statistical analysis

One-way ANOVA analyses and LSD tests were performed with STATISTICA 7.[26] Percentage values were converted into arcsine before statistical analysis.

3 RESULTS

3.1 Severity scores of different rice lines due to BPH

The severity scores of plants of rice lines from low to high were MH63::14&15 < MH63::15 < MH63::14 < MH63 < TN1 (Fig. 1). The severity scores of MH63::14, MH63::15 and MH63::14&15 were <3.0, and much lower than that of MH63. This indicated that the resistance level of introgression lines was obviously improved. The *Bph14* and *Bph15* pyramided line MH63::14&15 showed more enhanced resistance to BPH compared with either of the single-introgression lines MH63::14 and MH63::15. Additionally, MH63::15 exhibited higher resistance to BPH than MH63::14.

3.2 Effects on honeydew production of newly emerged female adults

Newly emerged brachypterous females (12–24 h old) were starved for 3 h and singly enclosed in a Parafilm sachet to infest rice main tiller for 24 h, and honeydew excretion of five females was collected together and weighed. Honeydew production of BPH on TN1 and MH63 was significantly higher than on introgression lines. A medium amount of honeydew was generated on MH63::14, which was significantly higher than that on MH63::15 and MH63::14&15 (Table 2). There was no significant difference between MH63::15 and MH63::14&15. Thus, both *Bph14* and *Bph15* had remarkable negative effects on female feeding behaviour, with the influence of *Bph15* being greater than that of *Bph14*.

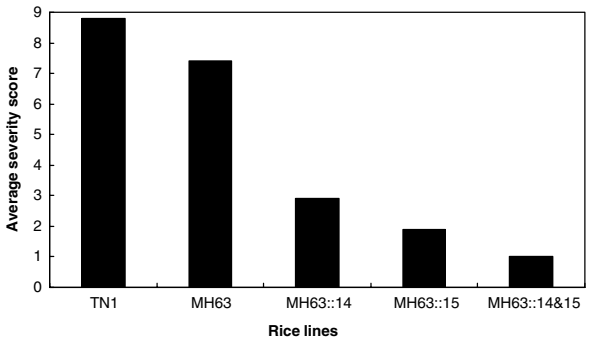

Figure 1. Average severity score of plants being tested ($n = 45$). The severity score caused by BPH was evaluated according to the Standard Evaluation Systems for Rice[23] and Huang *et al.*[18] 0, none of the leaves shrank and the plant was healthy; 1, one leaf was yellowing; 3, 1–2 leaves were yellowing or one leaf shrank; 5, 1–2 leaves shrank or one leaf shrivelled; 7, 3–4 leaves shrank or 2–4 leaves shrivelled, the plant still alive; 9, the plant died.

Table 2. Honeydew production of females on different rice lines

Rice lines	Honeydew (± SD) (mg)[a]
TN1	114.5 9 (±10.1) a
MH63	109.6 (±6.2) a
MH63::14	70.7 (±6.4) b
MH63::15	28.4 (±2.3) c
MH63::14&15	37.0 (±4.7) c

[a] Means were average honeydew production of five newly emerged brachypterous females for 24 h. Means in the same column followed by different letters are significantly different at the $P < 0.05$ level.

3.3 Effects on nymphs' survival rate, development and wing form

The influence of different rice lines on the survival rate, development and wing formation of BPH nymphs was investigated

Table 3. Survival rate, development and wing formation of nymphs on different rice lines

Rice lines	Survival rate from neonate to adult (%)[a]	Duration of nymph development (days)[a]		Brachyptery ratio (%)[a]		Female ratio (%)[a]
		Male	Female	Male	Female	
TN1	100.0 (±0.0) a	13.4 (±0.1) bc	14.2 (±0.1) c	4.3 (±1.3) a	100.0 (±0.0) a	52.2 (±1.1) a
MH63	98.0 (±1.0) a	13.2 (±0.1) c	14.0 (±0.2) c	4.3 (±1.3) a	100.0 (±0.0) a	52.2 (±1.1) a
MH63::14	100.0 (±0.0) a	13.6 (±0.1) ab	14.2 (±0.1) bc	3.3 (±2.0) a	99.0 (±1.0) a	47.8 (±1.1) b
MH63::15	98.0 (±1.0) a	13.7 (±0.1) ab	14.5 (±0.2) ab	0.0 (±0.0) b	100.0 (±0.0) a	41.1 (±1.1) c
MH63::14&15	98.0 (±1.0) a	13.9 (±0.2) a	14.7 (±0.1) a	0.0 (±0.0) b	100.0 (±0.0) a	36.7 (±1.9) d

[a] Means in the same column followed by different letters are significantly different at the $P < 0.05$ level.

Table 4. Copulation rate, lifespan and fecundity of adults on different rice lines

Lines	Copulation rate (%)[a]	Lifespan of female adults (days)[a]	Fecundity (neonates/female)[a]
TN1	100 (±0.0) a	20.3 (±0.8) a	623 (±41) a
MH63	98.9 (±1.1) a	18.7 (±1.0) a	561 (±40) ab
MH63::14	92.2 (±1.1) b	18.5 (±0.7) a	504 (±33) b
MH63::15	77.8 (±2.2) c	14.0 (±0.4) b	297 (±25) c
MH63::14&15	67.0 (±2.0) d	13.2 (±0.3) b	292 (±28) c

[a] Means in the same column followed by different letters are significantly different at $P < 0.05$ level.

Table 5. Antixenosis of resistant rice lines against BPH[a]

Lines	Number of BPH nymphs per seedling	Number of BPH female adults per tiller	Number of egg per tiller
MH63	33.6 (±1.6) a	11.3 (±0.7) a	175 (±14) a
MH63::14	27.6 (±1.1) b	4.5 (±0.5) b	54 (±6) b
MH63::15	11.3 (±1.4) c	4.4 (±0.5) b	47 (±6) b
MH63::14&15	5.8 (±0.6) d	0.7 (±0.1) c	20 (±3) c

[a] Means in the same column followed by different letters are significantly different at the $P < 0.05$ level.

(Table 3). There was no difference in survival rate from neonate to adult among rice lines, with about 98–100% survival rate.

There was a significant difference in development duration of nymphs among rice lines. Compared with MH63, the development duration of males was significantly delayed by MH63::14, MH63::15 and MH63::14&15, with the influence of *Bph15* and MH63::14&15 being greater than that of *Bph14*. The development duration of females was obviously retarded by MH63::15 and MH63::14&15, and no obvious differences were caused by MH63::14.

Nearly all females were brachyptous, with no significant difference in brachyptery ratio of females exhibited among rice lines. The brachyptery ratio of males on all rice lines was very low (<6%), and MH63::15 and MH63::14&15 slightly but significantly decreased the brachyptery ratio of males versus other rice lines.

There was a significant difference in female ratio among rice lines. The female ratio on MH63::14&15 was the lowest (36.7%), then on MH63::15 (41.1%), followed by MH63::14 (47.8%). This showed a strong dosage effect of the pyramiding of *Bph14* and *Bph15*.

3.4 Effects on copulation rate, longevity and fecundity of adults

Compared with MH63 and TN1, the copulation rate of adults was significantly decreased by all of the resistant lines. The copulation rate on MH63::14&15 (67.0%), MH63::15 (77.8%) and MH63::14 (92.2%) decreased 32.3, 21.3 and 6.7% in comparison with MH63. MH63::14&15 reduced the copulation rate more than MH63::15, and MH63::15 reduced the copulation rate more than MH63::14 (Table 4).

The female lifespan of BPH on MH63::14&15 and MH63::15 was significantly shortened by 5.5 and 4.7 days respectively versus MH63. No negative effects on female lifespan were detected when BPH was maintained on MH63::14 (18.5 days) (Table 4).

The fecundity of females on MH63::14&15 and MH63::15 was about 50% of that on MH63, whereas there was no significant difference in fecundity of BPH on MH63::14 and MH63 (Table 4).

3.5 Antixenosis action of resistant rice against BPH nymphs

The average number of nymphs settled on each rice seedling was significantly different among the four rice lines (Table 5). It was significantly higher on MH63 than on the other three resistant rice lines. The numbers from high to low were MH63 (33.6) > MH63::14 (27.6) > MH63::15 (11.3) > MH63::14&15 (5.8). These results indicated that all three resistant rice lines were antixenosis resistant to BPH nymphs. The antixenosis action of MH63::14&15 was stronger than that of MH63::15, and the antixenosis action of MH63::15 was stronger than that of MH63::14.

3.6 Antixenosis of rice plants to female adults and oviposition

MH63::14, MH63::15 and MH63::14&15 were all antixenosis resistant to BPH females and oviposition (Table 5). BPH female adults most preferred settlement and oviposition on MH63, with 11.3 insects and 174.5 eggs per tiller. MH63::14&15 was the least preferred rice line, with 0.7 insects and 19.7 eggs per tiller. There was no difference in antixenosis action against BPH females between MH63::14 and MH63::15, while BPH eggs per tiller of MH63::14 was more than the number on MH63::15.

3.7 Effects on expression characteristics of three P450 genes in BPH

CYP4C61, *CYP4C62*, *CYP6CS1*, *CYP6CW1* and *CYP303A1* were annotated as P450 members in BPH belonging to the CYP4, CYP6 and CYP303 family. The expression pattern of the five genes

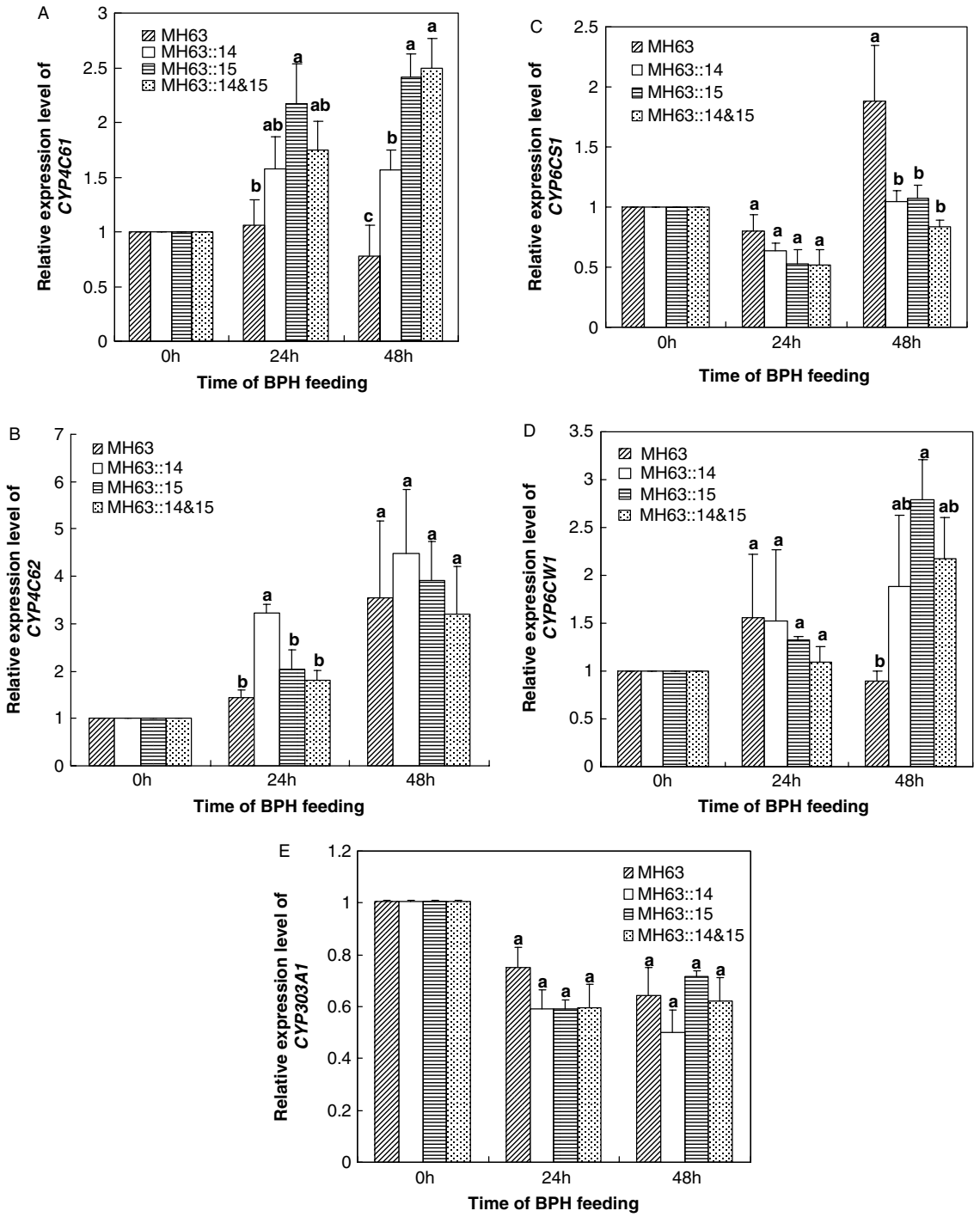

Figure 2. Expression of *CYP4C61*, *CYP4C62*, *CYP6CS1*, *CYP6CW1* and *CYP303A1* of BPH on different rice lines; different bars are used to represent the gene expression level in BPH on different rice lines. (A) The expression pattern of *CYP4C61*; (B) the expression pattern of *CYP4C62*; (C) the expression pattern of *CYP6CS1*; (D) the expression pattern of *CYP6CW1*; (E) the expression pattern of *CYP303A1*. The expression level was quantified relative to the value obtained from samples at 0 h. The average expression level on each rice line was based on three biological replicates. Error bars indicate standard errors. BPH *actin1* was used as reference control. Bars labelled with different letters differed significantly ($P < 0.05$).

of BPH responding to BPH resistance genes *Bph14* and *Bph15* was investigated with qRT-PCR (Fig. 2). *CYP4C61*, *CYP4C62*, *CYP6CS1*, *CYP6CW1* and *CYP303A1* exhibited a distinct expression pattern responding to resistant rice lines.

After feeding on MH63::15 for 24 h, *CYP4C61* in BPH had significantly increased its abundance compared with that on MH63. After feeding for 48 h, *CYP4C61* abundance in BPH on all the resistant rice lines was significantly enhanced relative to that on MH63, and *CYP4C61* in BPH reached its expression peak at 48 h on MH63::15 and MH63::14&15, moreover, its expression level on MH63::15 and MH63::14&15 was significantly higher than on MH63::14 (Fig. 2A).

After feeding on MH63::14 for 24 h, *CYP4C62* in BPH significantly increased its abundance compared with that on MH63. However,

there was no significant difference in *CYP4C62* expression level among the susceptible and resistant rice lines after 48 h of feeding (Fig. 2B).

Little difference in expression level of *CYP6CS1* was detected at 24 h among BPHs after feeding on either resistant or susceptible rice seedlings. It is important to note that the activity of *CYP6CS1* in BPH after 48 h of feeding on resistant rice lines decreased significantly compared with MH63 (Fig. 2C).

After feeding for 24 h, the abundance of *CYP6CW1* in BPH on different rice lines was very similar; however, the transcript amount of *CYP6CW1* in BPH feeding for 48 h on MH63::15 was significantly increased compared with MH63 (Fig. 2D).

The expression pattern of *CYP303A1* in BPH was not influenced by *Bph14* and *Bph15*. After feeding for 24 and 48 h, there was no significant difference in *CYP303A1* abundance among the susceptible and resistant rice lines (Fig. 2E).

4 DISCUSSION

After BPH resistance genes *Bph14* and *Bph15* were introduced into MH63, the single-introgression lines and the two-gene pyramided line exhibited high resistance to BPH, with a severity score of <3.0 in the seedling bulk test. The resistance level of the pyramided line was higher than that of the single-introgression lines, reflecting the dosage effects of resistance gene pyramiding.

The antibiosis and antixenosis effects of the three resistant rice lines on BPH were examined in this study. The results showed that all three resistant rice lines had antibiosis and antixenosis effects on BPH. Rice lines carrying *Bph15* significantly delayed the development of nymphs, reduced honeydew excretion of females, decreased female ratio and copulation rate, reduced fecundity and shortened the lifespan of females. Moreover, they had strong antixenosis resistance against BPH nymphs, female adults and oviposition. Antibiosis effects of *Bph14* were very weak; MH63::14 only delayed the development of males and decreased the female ratio and copulation rate compared with MH63. MH63::14 exhibited antixenosis action against BPH nymphs, female adults and ovipositon. When *Bph14* and *Bph15* were pyramided, antibiosis and antixenosis effects were significantly enhanced relative to the single-introgression lines. Thus, MH63::15 and MH63::14&15 had greater potential to control infestation of BPH in rice production than MH63::14.

Cytochrome P450s are a large and important gene superfamily present in nearly all organisms. They catalyse the NADPH-associated reductive cleavage of oxygen to produce a functionalised product and water. In insects, cytochrome P450s contribute to numerous physiological processes including growth, development, feeding, resistance to pesticides and tolerance to plant toxins.[27–29] Some P450 members of the CYP4 family and CYP6 family have been reported to be inducible by host allelochemicals.[20,30,31] In BPH, the transcripts of *CYP6AX1* and *CYP6AY1* accumulated rapidly, responding to B5 (rice harbouring *Bph14* and *Bph15*), and the expression level of *CYP6AX1* and *CYP6AY1* on B5 was much higher than that on TN1.[20] *CYP4C61*, *CYP4C62*, *CYP6CS1*, *CYP6CW1* and *CYP303A1* are another five cDNAs that were annotated as P450 members in BPH belonging to the CYP4, CYP6 and CYP303 family. In the present study, the response of these five BPH P450 genes at gene expression level to resistant hosts was examined by qRT-PCR. Both *CYP4C61* and *CYP6CW1* in BPH maintained on resistant rice lines were upregulated, but the time course and intensity of inductive expression were different. However, after feeding for 48 h, the expression level of *CYP6CS1* in BPH was significantly downregulated by the resistant hosts, which was quite different from the expression pattern of *CYP6AX1*, *CYP6AY1*, *CYP4C61* and *CYP6CW1* responding to resistant hosts. *CYP4C62* of BPH was upregulated by MH63::14 after 24 h of feeding, but there was no significant difference in *CYP4C62* abundance among the susceptible and resistant rice lines after 48 h of feeding. The expression level of *CYP303A1* in BPH was not influenced by resistant rice lines. This may reflect flexible response mechanisms of BPH in adapting to host resistance.

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