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*Full Length Research Paper*

# Correlation of resistance to *Nilaparvata lugens* Stål with secondary metabolites of rice plants

**Lang Yang[1,2], Guang-Wen Liang[1]*, Feng-Kuan Huang[2], Ling Zeng[1] and Bing Lin[1]**

[1]Laboratory of Insect Ecology, South China Agriculture University, Guangzhou 510642, China.
[2]Plant Protection Research Institution, Guangxi Academy of Agricultural Science, Nanning 530007, China.

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**It is very difficult and complex to distinguish and estimate rice varieties' resistance. Thus, it is necessary to built up a simple, nicety, steady and speedy method of resistant appraisement. Secondary metabolite is the important basic substance of rice resistance. The correlation of rice plant resistance to brown planthopper (BPH), *Nilaparvata lugens* Stål, with 20 distinct secondary metabolite high performance liquid chromatography (HPLC) peaks were investigated. Two resistance prediction models were established through multiple regression analysis. Model A was established for the resistance of brown planthopper (BPH) field population II, and model B was established for the resistance of field population Bangladesh. The correlations between the BPH resistance levels (Y) of rice varieties and the peak areas (X) were significant (R' = 0.961 and 0.942 for model A and model B respectively, p<0.01). The results showed that in model A, peak 2, 5, 6, 7, 11, 12, 13, 15, 16, 17 and 18 were the secondary metabolites that affected the resistance to BPH. In model B, peak 1, 2, 5, 6, 7, 8, 10, 12, 13, 14, 15, 16, and 17 were the metabolite peaks that affected resistance. It was demonstrated that the resistant activity of rice varieties to BPH was closely associated with quantitative combinations of many secondary metabolites, which suggested that the BPH resistance of rice plants was the results of actions of several secondary metabolites that varied in contributions. The validation results showed that field bioassay scores agreed with the simulated scores well, indicating that these models were useful and accurate. And these models can be used as fast assistant-method to evaluate the resistance of rice plant to BPH and assist the selection of resistant rice plants for breeding.**

**Key words:** Brown planthopper, *Nilaparvata lugens* Stål, field population, high performance liquid chromatography, model.

## INTRODUCTION

The brown planthopper (BPH) (*Nilaparvata lugens* Stål), with its characteristic migration pattern, paroxysm and rampancy, is one of the most destructive insect pests in the rice-producing areas, which employs heavy insecticides, killing off natural enemies of BPH. The severity of damage and the frequency of outbreaks have increased since 1960s, due to several reasons, firstly is the planting of short-stature and heavy-tillering cultivars (Dyck and Thomas, 1979), the second is the wider use of nitrogenous fertilizers and insecticides (Pathak, 1972; Sogawa, 1982; Holt et al., 1996; Sogawa et al., 2003), another reason BPH multiplies so rapidly is its high reproductive potential (Loevinsohn et al., 1988, 1993). In China, this insect has caused losses of over 500,000 tons of rice annually (Zhu et al., 2004). In China and Japan, BPH migrates each spring from Vietnam and south China descending in large numbers onto rice fields where natural enemies are low in number. Compounded by insecticide usage which further reduces natural enemy allowing BPH to multiply rapidly. This same phenomenon was observed in Indonesia in areas where synchronous planting was carried out over large areas in the dry season which has the effect of reducing natural enemy numbers and when the wet season crop is planted high BPH populations result (Sawada et al., 1992; Holt et al., 1996). Using resistant cultivars has been proven to be one of the most effective ways to control this pest (Pataki, 1969; Sogawa, 1982). It is reported that each resistant

*Corresponding author. E-mail: ylyanglang@gxaas.net or yang2001lang@163.com. Tel: 86-0771-3245626. Fax: 86-0771-3243874.

cultivar showed different response when faced with different field population of BPH (Gallagher et al., 1994). There are many and different field populations of BPH, and field population Ⅱ became the major field population in south China since 1990s (Li et al., 1999b). Bangladesh BPH as a new field population showed up in south and southeast China in recent years. This field population causes more harm to rice plants than field population Ⅱ (Luo et al., 1995).

In the past two decades, the resistance mechanisms of rice to BPH have been studied (Panda and Heinrichs, 1983). Many researchers believe that the difference in secondary metabolites between resistant and susceptible variety is the major factor (Yoshihara, 1979a, 1979b; Zhang et al., 1998; Zhao et al., 2004). The major functions of secondary metabolites in plants are to act as chemical signals in the ecosystem and as antibiosis agents against insects and pathogens (Kong et al., 2002; Hu et al., 2003). Liu et al. (1995) found that non-volatile secondary metabolites control insect behaviors, such as continued feeding, growth and development, and oviposition. Many researchers studied the active components that are toxic to insects (Panda et al., 1983; Khan and Saxena, 1985, 1986; Liu et al., 1990). For example, oxalic acid is one important factor that controls BPH resistance. It affects the metabolism and synthesis of proteins in insect body and inhibits metabolism in BPH (Yoshihara et al., 1979a, 1979b). Shigematsu et al. (1982) found thatβ-paddy sterol, legumina sterol and rape oil sterol can restrain BPH strongly. Zhang et al. (1998) identified several secondary metabolites, such as rape oil sterol, legumina sterol, paddy sterol and 3-nitrophthalic acid, from the resistant rice variety. They believed that 3-nitrophthalic acid was the major secondary metabolite that conferred resistance to BPH.

Kong et al. (2002) identified nine secondary metabolites in rice plant that were related to resistance to weed. Their results showed that different components and contents of secondary metabolites were related with the rice resistance to *Echinochloa crus-galli*. It is widely believed that the rice resistance to diseases is connected with the secondary metabolites in rice plants (Xu et al., 1997; Wang, 1999a; Hu et al., 2003). Moreover, results by Zhao et al. (2005a) indicated that the rice resistance to BPH was related with components and contents of secondary metabolites, and the resistant secondary components was highest in the second leaf of rice young plant (Zhao et al., 2005b) .

In this paper, the correlation between BPH resistance and the secondary metabolites in rice were studied. The characteristic secondary metabolites that were related with rice resistance to BPH were investigated. The purpose of this research was to set up and validate a model between rice resistance and secondary metabolites. This model will be used to screen the resistant parents and identify the resistant offspring.

## MATERIALS AND METHODS

### Insects

The BPH colonies used for the research were field population Ⅱ and field population Bangladesh, which were identified by using a set of testers (Li et al., 1999a). They were kindly provided by Dr. Huang FK at Guangxi Academy of Agricultural Science, China. The field population Ⅱ was recognized as the predominant field population in most of the rice fields in China since 1990s (Wang et al., 1999b), and the field population Bangladesh was a new field population in Nanning, Guangxi province at the present time (Luo et al., 1995). The insect was fed on TN1 for about 5 weeks to produce a sufficiently large population, and first-to-second-instar nymphs were selected for infestation. The rest were maintained with alternative plants of TN1 for subsequent infestation.

### Plant materials

The plant materials consisted of two groups (Tables 1 and 5), they came from different country and area. One was used to set up the resistant model; the other was used to verify the model. The evaluation of BPH resistance to the rice plant was conducted by Dr. Huang FK, which was conducted using the standard seed-box screening technique (IRRI, 1979) with some modifications. At the 3.5 to 4 leaf stage, the tray was transferred into an iron sheet case. Water at 2 to 3 cm deep was flowed into the tray to keep humidity high and ants off the seedlings. Then, the plants were infested with the selected nymphs at a density of 10 nymphs per seedling as evenly as possible. After infestation, each tray was covered with a nylon-net cage immediately. The scoring system proposed by the International Rice Research Institute (IRRI, 1979) with some modifications was used to rate each seedling: 0 is no visible damage; 1 is partial yellowing of first leaf; 3 = first and second leaves partially yellow; 5 = pronounced yellowing or some stunting; 7 = mostly wilting, the plant was still alive; 9 = the plant completely wilted or died. Since the severity of damage of plants is dynamic, the score of each seedling rated on a day when more than 90% of the susceptible parents died. For data analysis, plants with a scale of 0-5.9 and 6-9 were designated as resistant and susceptible, respectively. Each treatment was replicated 3 times.

### Sample preparation and high-performance liquid chromatography (HPLC) analysis

All rice young plants grow without fertilizer. At the stage of three leaves, the rice leaves were collected and seven duplicates of each sample were used. About 0.0200±0.0002 g sample in each treatment was weighed, torn into pieces, soaked in 2 ml methanol, and filtered after 12 h. The filtrate was then evaporated to remove methanol and redissolved in 1 ml 1:1 (v/v) methanol and water, which was placed to stand for 6 h and filtered with a 0.45 μm filter membrane. The filtrate was directly analyzed by reversed-phase HPLC.

HPLC analysis was performed with a HP1100 HPLC system (Agilent Technologies) equipped with a $C_{18}$ column (Hypersil ODS 5 μm, 4.0 x 300 mm). The chromatography conditions were set according to the methods by Mattice et al. (2001) Kong et al. (2002) and Zhao et al. (2004) with modifications. Mobile phase A was 1% acetic acid solution and mobile phase B was acetonitrile. The gradient started with 8% B at a flow rate of 1.5 ml/min for 3 min, followed by increasing B to 35% in 25 min with a flow rate of 1 ml/min. Mobile phase B was then reduced to 20% within 6 min and finally to 8% B. The UV detector G1314A VWD was set at 320 nm. All the solvents were of HPLC or analytical grade. Water was

**Table 1.** Resistant scales of rice varieties to BPH population II and population Bangladesh.

| Population II | | | Population Bangladesh | | |
|---|---|---|---|---|---|
| **No.** | **Varieties** | **Resistant score** | **No.** | **Varieties** | **Resistant score** |
| 1 | $IR_{36}$ | 3 | 1 | $IR_{36}$ | 8 |
| 2 | $TN_1$ | 9 | 2 | $TN_1$ | 9 |
| 3 | IR18350-93-2 | 2.9 | 3 | IR18350-93-2 | 9 |
| 4 | IR35410-50-2-2-1 | 3.1 | 4 | IR35410-50-2-2-1 | 8.9 |
| 5 | IR8608-82-1-3-1-3 | 2.9 | 5 | IR8608-82-1-3-1-3 | 8.8 |
| 6 | IR15314-30-3-1-3 | 2.9 | 6 | IR13429-196-1-2-1 | 8.8 |
| 7 | IR71604-4-1-4-10-8-3-3-1 | 3 | 7 | IR15314-30-3-1-3 | 9 |
| 8 | IR71718-161-2-2-3 | 3 | 8 | IR71604-4-1-4-10-8-3-3-1 | 3.4 |
| 9 | IR73885-1-4-1-4-3-6 | 3 | 9 | IR71718-161-2-2-3 | 2.7 |
| 10 | IR71604-4-4-3-8-7-3-3-3 | 3 | 10 | IR73885-1-4-1-4-3-6 | 3 |
| 11 | IR71727-90-2-3-3 | 3 | 11 | IR71604-4-4-3-8-7-3-3-3 | 2.6 |
| 12 | IR717718-85-3-2-3 | 3 | 12 | IR71727-90-2-3-3 | 2.9 |
| 13 | IR1718-59-1-2-3 | 3 | 13 | IR717718-85-3-2-3 | 3.1 |
| 14 | IR54742-38-13-15-2-3 | 3.4 | 14 | IR1718-59-1-2-3 | 3.8 |
| 15 | Sinna sivappu | 2.8 | 15 | IR54742-38-13-15-2-3 | 4.6 |
| 16 | IR56422-109-2-1-2-3 | 3.7 | 16 | Sinna sivappu | 4.8 |
| 17 | IR54742-1-18-12-11-2 | 2.9 | 17 | IR56422-109-2-1-2-3 | 5.5 |
| 18 | IR13257-46-1E-P1 | 5.9 | 18 | IR54742-1-18-12-11-2 | 4.3 |
| 19 | YNAUBPHR841963 | 5.2 | 19 | IR13257-46-1E-P1 | 8.1 |
| 20 | IR54742-11-10-13-21-2 | 5 | 20 | YNAUBPHR841963 | 9 |
| 21 | IR65482-4-136-2-2 | 4 | 21 | IR54742-11-10-13-21-2 | 9 |
| 22 | Jin Gang-30 | 9 | 22 | IR65482-4-136-2-2 | 9 |
| 23 | GuangXuan-Ⅲ | 9 | 23 | IR49688-167-1-3-1 | 7.8 |
| | | | 24 | Jin Gang-30 | 9 |
| | | | 25 | GuangXuan-Ⅲ | 9 |

redistilled.

#### Data analysis

The peaks that were separated by HPLC were used to set up the model. The model was used to correlate to the HPLC peak area and BPH resistant value. The peaks in the model were associated with the plant resistance to BPH by using the SAS software (SAS Institute Inc., U.S.).

## RESULTS

### HPLC separation

Typical overlapped HPLC chromatograms of resistant at susceptible samples are shown in Figure 1. The peaks were labeled numerically. Twenty peaks, numbered 1 to 20, were used in the model construction. Retention times of the peaks were in the range of 10 to18 min.

### Establishing models

Twenty peaks represented 20 secondary metabolites were separated. The area of each peak indicated the content of the secondary metabolites, which was described as $X_1$, $X_2$, $X_3$, $X_4$, $X_5$, $X_6$, $X_7$, $X_8$, $X_9$, $X_{10}$, $X_{11}$, $X_{12}$, $X_{13}$, $X_{14}$, $X_{15}$, $X_{16}$, $X_{17}$, $X_{18}$, $X_{19}$, $X_{20}$, respectively. The areas of these 20 peaks are listed in the Tables 2 and 3.

From the Tables 2 and 3, it showed that the concentrations of metabolites were different between samples. The peaks that showed no statistical difference were marked by the same small letter.

The correlations between the 20 secondary metabolite contents (peak areas) and the resistant scores were analyzed using SAS software to set up the relationship model of varieties resistant score (Y) and secondary metabolite content (X). Model A was used for the field population Ⅱ and model B was used for the field population Bangladesh. The correlation model (equation) of the resistant score (Y) and secondary metabolite (X) of field population II (model A) was:

$$Y = 5.3578-0.0302X_2+0.0577X_5-0.0312X_6-0.3293X_7-0.0182X_{11}-0.1014X_{12}+0.2470X_{13}+0.1264X_{14}+0.0352X_{15}-0.0179X_{16}+0.3858X_{17}-0.1212X_{20}$$

The correlation equation of field population Bangladesh (model B) was: $Y = 2.1873+0.2204X_1+0.0564X_2-0.0478X_5+0.0411X_6-0.2642X_7$

**Table 2.** The HPLC peak areas of 23 rice varieties that infected with BPH population II.

| Samples | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 17.1b | 179.1cde | 10.3efg | 231.5a | 127.8bc | 20.9f | 10.4a | 66.1ghijkl | 9.1defghi | 91.8bc |
| 2 | 6.5efg | 134.1efgh | 7.6efgh | 48.1fg | 110.3cdef | 126.8cde | 0.0c | 82.5ef | 6.6fghi | 51.6ijk |
| 3 | 9.7cde | 211.1ab | 9.3efg | 90.7de | 182.4a | 15.4f | 4.7b | 25.5n | 17.7d | 52.3fghij |
| 4 | 2.6g | 98.4ij | 4.1ghi | 26.7h | 69.3m | 100.5e | 0.0c | 52.9klm | 4.9ghikj | 42.0jk |
| 5 | 9.0cde | 143.1efgh | 7.6efgh | 40.5gh | 87.8ijklm | 118.2de | 0.0c | 60.6hijkl | 5.6fghij | 33.7kl |
| 6 | 14.4bcd | 164.5def | 9.7efg | 60.9efg | 108.5cdef | 121.6cde | 0.0c | 98.3de | 11.2cdefgh | 50.5ghij |
| 7 | 6.3efg | 115.1hij | 22.7d | 112.1cd | 71.9lm | 17.1f | 0.0c | 74.2fghij | 8.2efg | 91.7bc |
| 8 | 19.1b | 136.6fgh | 29.4c | 102.3cd | 93.7ghij | 28.2f | 0.0c | 70.5fghijk | 12.1cdef | 89.8c |
| 9 | 7.9de | 154.6defg | 26.7cd | 88.2de | 120.8bcde | 20.4f | 0.0c | 77.7fgh | 16.8ab | 83.9cd |
| 10 | 5.1fg | 110.2hij | 4.6ghi | 114.1cd | 98.9efg | 15.1f | 4.4b | 48.7lm | 5.4fghij | 66.9efg |
| 11 | 3.6g | 115.5hij | 4.2ghi | 129.8c | 103.8defghi | 15.6f | 4.1b | 52.1klm | 4.9ghijk | 72.2de |
| 12 | 3.7g | 91.9ij | 2.8hi | 93.2de | 89.6ijkl | 11.6f | 5.8b | 34.8mn | 4.3ghijk | 70.5de |
| 13 | 6.2efg | 188.4bcd | 6.1fgh | 171.1b | 118.1cde | 27.9f | 5.3b | 77.5fgh | 5.9fghij | 61.6efgh |
| 14 | 3.3g | 226.4a | 6.5fgh | 183.7b | 137.1b | 26.2f | 0.0c | 75.1fgh | 11.4cdefg | 60.1efghi |
| 15 | 5.1fg | 125.4ghi | 22.6d | 107.5cd | 83.1jklm | 19.6f | 0.0c | 75.1fghi | 7.3efghi | 99.6bc |
| 16 | 6.2efg | 162.7def | 11.3ef | 175.1b | 111.3cdefg | 33.2f | 0.0c | 75.1fghi | 7.9dfghi | 94.5bc |
| 17 | 7.1def | 151.1efg | 5.5fghi | 136.3c | 101.9efghi | 24.5f | 0.0c | 54.1ijklm | 4.5ghijk | 61.7dfghi |
| 18 | 14.2bcd | 205.4abc | 65.8a | 30.5gh | 114.7cdef | 147.1bc | 0.0c | 119.2bc | 10.3defg | 39.6jk |
| 19 | 10.4cde | 157.6defg | 5.3fghi | 12.3h | 90.5hijk | 163.6ab | 0.0c | 106.0cd | 1.7ij | 19.7lm |
| 20 | 15.0bc | 232.1a | 8.8efgh | 76.8def | 127.6bc | 185.3a | 0.0c | 112.2bcd | 9.5defghij | 47.6hijk |
| 21 | 8.7de | 175.2cde | 51.8b | 79.6def | 138.9b | 139.2bcd | 0.0c | 157.3d | 10.1defghij | 83.5bc |
| 22 | 27.1a | 83.4j | 13.5e | 93.2def | 101.9efghi | 113.7de | 0.0c | 75.9fghi | 11.9bcd | 114.0a |
| 23 | 18.5b | 138.8fgh | 0.0c | 90.4de | 122.2bcd | 146.7bc | 0.0c | 82.3efg | 15.2abc | 99.0b |

| $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ | $X_{15}$ | $X_{16}$ | $X_{17}$ | $X_{18}$ | $X_{19}$ | $X_{20}$ |
|---|---|---|---|---|---|---|---|---|---|
| 287.3a | 0.0j | 14.0b | 39.0ij | 20.1ijk | 182.7fhij | 8.6cde | 9.0abcd | 11.8efg | 34.1efgh |
| 146.3ghi | 19.0def | 12.9bc | 57.1gh | 69.1cd | 169.0hijk | 6.1efg | 6.8abcdef | 12.6de | 23.7ij |
| 136.6hij | 4.5ij | 3.1de | 22.5lmn | 11.7kl | 97.4m | 3.5g | 4.6ef | 7.9ghij | 42.4bcd |
| 126.3ijk | 13.9fgh | 1.9de | 51.6h | 63.3de | 200.1defg | 4.5fg | 5.4def | 10.8fg | 30.2fghi |
| 135.1hij | 14.9efg | 7.6cd | 40.9i | 38.0f | 156.0jkl | 5.9defg | 5.0ef | 7.5ghij | 30.5fghi |
| 164.5fgh | 25.6d | 0.0e | 64.8fg | 57.0e | 187.9efgh | 9.5c | 9.2abc | 12.6def | 37.5cdef |
| 154.5ghi | 12.5gh | 14.5b | 21.9lmn | 19.5hijk | 203.3def | 7.7cdef | 6.2cdef | 7.748ghij | 40.8bcde |
| 165.9fgh | 21.4de | 4.3de | 35.4ijk | 26.9gh | 226.1cd | 13.0b | 10.4a | 17.121c | 42.4bcd |
| 172.3efg | 13.9fgh | 11.8bc | 27.5jklm | 18.9ijk | 240.2bc | 5.7defg | 5.2ef | 11.1efg | 35.4defg |
| 195.7cdef | 5.3hij | 2.1de | 23.0lmn | 12.6jkl | 142.2l | 5.9defg | 5.4def | 6.4hij | 23.0 ij |
| 96.0cdef | 5.8hi | 3.1de | 24.2lmn | 17.0ijkl | 148.6kl | 5.9defg | 6.6bcdef | 7.4ghij | 20.5j |

**Table 2.** Contd.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 187.4cdef | 7.8ghi | 12.3bc | 16.0n | 17.8ijkl | 137.0l | 4.1fg | 4.5f | 4.3j | 21.1j |
| 244.0b | 7.6ghi | 18.1b | 18.4lmn | 20.3hij | 132.4l | 5.0efg | 4.7ef | 4.6ij | 28.1ghij |
| 180.5defg | 9.1ghi | 16.0bc | 16.6mn | 11.7kl | 56.8n | 5.5efg | 7.0abcdef | 5.2ij | 46.4b |
| 152.5ghi | 13.3fgh | 2.2de | 27.7jklm | 21.1hi | 172.1hijk | 9.1cd | 4.8ef | 9.1gh | 33.0efgh |
| 246.1b | 10.0ghi | 18.6b | 25.1klm | 30.1g | 203.0def | 8.4cde | 9.8ab | 11.2efg | 34.7defgh |
| 243.6b | 6.7hi | 15.1b | 15.0n | 16.1ijkl | 184.6fghi | 5.3efg | 6.8bcdef | 8.3ghi | 27.4hij |
| 88.6l | 24.9d | 15.0b | 70.5ef | 64.6d | 221.9cd | 8.2cde | 8.8abcd | 15.2cd | 44.4bc |
| 99.2kl | 33.8c | 0.0e | 90.2bc | 73.2c | 130.1l | 7.4cdef | 7.4abcdef | 4.2j | 36.7cdef |
| 205.6cd | 23.3d | 27.9a | 51.9h | 66.1cd | 175.4fghijk | 7.7cdef | 7.0abcdef | 13.7cde | 33.7efgh |
| 139.7hij | 25.1d | 0.0e | 77.0de | 37.9f | 212.4de | 6.2cdefg | 5.8cdef | 15.7cd | 36.5def |
| 202.2cde | 47.1b | 0.0e | 99.5b | 109.5b | 259.8ab | 19.2a | 9.4abc | 25.3b | 57.8a |
| 217.6bc | 54.7a | 0.0e | 114.1a | 132.3a | 276.3a | 20.1a | 0.0g | 41.6a | 58.3a |

The same small letters in a column indicate no statistical difference (p>0.05) (DMRT's SSR).

**Table 3.** The HPLC peak areas of 25 rice varieties that infected with BPH population Bangladesh.

| Samples | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 17.1b | 179.0cde | 10.3efg | 231.5a | 127.8bc | 20.9f | 10.4a | 66.0ghijkl | 9.1defghi | 91.8bc |
| 2 | 6.5efg | 134.0efgh | 7.6efgh | 48.0fg | 110.3cdef | 126.8cde | 0.0c | 82.5ef | 6.6fghi | 51.6ijk |
| 3 | 9.7cde | 211.0ab | 9.3efg | 90.7de | 182.4a | 15.4f | 4.7b | 25.5n | 17.7d | 52.3fghij |
| 4 | 2.6g | 98.4ij | 4.0ghi | 26.7h | 69.3m | 100.5e | 0.0c | 52.9klm | 4.9ghikj | 42.0jk |
| 5 | 9.0cde | 143.0efgh | 7.6efgh | 40.5gh | 87.8ijklm | 118.2de | 0.0c | 60.6hijkl | 5.6fghij | 33.7kl |
| 6 | 10.9cd | 154.0defg | 50.6b | 40.3gh | 127.8bc | 135.2bcd | 0.0c | 126.7b | 18.3a | 66.7def |
| 7 | 14.4bcd | 164.5def | 9.7efg | 60.9efg | 108.5cdef | 121.6cde | 0.0c | 98.3de | 11.2cdefgh | 50.5ghij |
| 8 | 6.3efg | 115.0hij | 22.7d | 112.0cd | 71.9lm | 17.0f | 0.0c | 74.2fghij | 8.2efg | 91.7bc |
| 9 | 19.1b | 136.6fgh | 29.4c | 102.3cd | 93.7ghij | 28.0f | 0.0c | 70.5fghijk | 12.1cdef | 89.8c |
| 10 | 7.996de | 154.6defg | 26.7cd | 88.2de | 120.8bcde | 20.4f | 0.0c | 77.7fgh | 16.8ab | 83.9cd |
| 11 | 5.164fg | 110.2hij | 4.6ghi | 114.0cd | 98.9efg | 15.1f | 4.4b | 48.7lm | 5.4fghij | 66.9efg |
| 12 | 3.636g | 115.5hij | 4.2ghi | 129.8c | 103.8defghi | 15.6f | 4.0b | 52.1klm | 4.9ghijk | 72.2de |
| 13 | 3.716g | 91.9ij | 2.8hi | 93.2de | 89.6ijkl | 11.6f | 5.8b | 34.8mn | 4.3ghijk | 70.5de |
| 14 | 6.2efg | 188.4bcd | 6.1fgh | 171.0b | 118.1cde | 27.9f | 5.3b | 77.5fgh | 5.9fghij | 61.6efgh |
| 15 | 3.3g | 226.4a | 6.5fgh | 183.7b | 137.1b | 26.2f | 0.0c | 75.3fgh | 11.4cdefg | 60.1efghi |
| 16 | 5.1fg | 125.4ghi | 22.6d | 107.5cd | 83.1jklm | 19.6f | 0.0c | 75.1fghi | 7.3efghi | 99.6bc |
| 17 | 6.2efg | 162.7def | 11.3ef | 175.0b | 111.3cdefg | 33.2f | 0.0c | 75.1fghi | 7.9dfghi | 94.5bc |
| 18 | 7.0def | 151.1efg | 5.5fghi | 136.3c | 101.9efghi | 24.5f | 0.0c | 54.0ijklm | 4.5ghijk | 61.7dfghi |
| 19 | 14.2bcd | 205.4abc | 65.8a | 30.5h | 114.7cdef | 147.1bc | 0.0c | 119.2bc | 10.3defg | 39.6jk |

**Table 3.** Contd.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 10.4cd | 157.6defg | 5.3fghi | 12.3h | 90.5hijk | 163.6ab | 0.0c | 106.0cd | 1.7ij | 19.7lm |
| 21 | 15.0bc | 232.1a | 8.8efgh | 76.8def | 127.6bc | 185.3a | 0.0c | 112.2bcd | 9.5defghij | 47.6hijk |
| 22 | 8.7de | 175.2cde | 51.8b | 79.6def | 138.9b | 139.2bcd | 0.0c | 157.3d | 10.1defghij | 83.5bc |
| 23 | 5.6fg | 200.9abc | 4.6ghi | 192.3b | 74.5klm | 26.3f | 1.1c | 57.2hijkl | 12.4de | 52.4fghij |
| 24 | 27.1a | 83.4j | 13.5e | 93.2def | 101.9efghi | 113.7de | 0.0c | 75.9fghi | 11.9bcd | 114.0a |
| 25 | 18.5b | 138.8fgh | 0.0c | 90.4de | 122.2bcd | 146.7bc | 0.0c | 82.3efg | 15.2abc | 99.0b |
| | | | | | | | | | | |
| **$X_{11}$** | **$X_{12}$** | **$X_{13}$** | **$X_{14}$** | **$X_{15}$** | | **$X_{16}$** | **$X_{17}$** | **$X_{18}$** | **$X_{19}$** | **$X_{20}$** |
| 287.3a | 0.0j | 14.1b | 39.1ij | 20.1ijk | | 182.7fghij | 8.6cde | 9.1abcd | 11.8efg | 34.1efgh |
| 146.3ghi | 19.0def | 12.9bc | 57.1gh | 69.1cd | | 169.0hijk | 6.1cdefg | 6.8abcdef | 12.6de | 23.7ij |
| 136.6hij | 4.5ij | 3.1de | 22.5lmn | 11.7kl | | 97.4m | 3.5g | 4.6ef | 7.9ghij | 42.4bcd |
| 126.3ijk | 13.9fgh | 1.9de | 51.6h | 63.3de | | 200.0defg | 4.5fg | 5.4def | 10.8fg | 30.2fghi |
| 135.3hij | 14.9efg | 7.6cd | 40.9i | 38.0f | | 156.0jkl | 5.9efg | 5.0ef | 7.5ghij | 30.5fghi |
| 109.2ikl | 22.9d | 0.0e | 85.4cd | 65.6d | | 255.4ab | 7.3cdef | 6.9bcdef | 16.2cd | 38.2cdef |
| 164.5fgh | 25.6d | 0.0e | 64.8fg | 57.1e | | 187.9efgh | 9.5c | 9.2abc | 12.6def | 37.5cdef |
| 154.5ghi | 12.518gh | 14.5b | 21.9lmn | 19.5hijk | | 203.3def | 7.7cdef | 6.2cdef | 7.7ghij | 40.8bcde |
| 165.9fgh | 21.4de | 4.3de | 35.4ijk | 26.9gh | | 226.1cd | 13.0b | 10.4a | 17.1c | 42.4bcd |
| 172.3efg | 13.9fgh | 11.8bc | 27.5jklm | 18.9ijk | | 240.2bc | 5.7defg | 5.2ef | 11.0efg | 35.4defg |
| 195.7cdef | 5.3hij | 2.1de | 23.0lmn | 12.6jkl | | 142.2l | 5.9defg | 5.4def | 6.4hij | 23.0ij |
| 96.0cdef | 5.8hi | 3.1de | 24.2lmn | 17.0ijkl | | 148.6kl | 5.9defg | 6.6bcdef | 7.4ghij | 20.5j |
| 187.4cdef | 7.8ghi | 12.3bc | 16.0n | 17.8ijkl | | 137.0l | 4.1fg | 4.5f | 4.3j | 21.1j |
| 244.0b | 7.9ghi | 18.1b | 18.4lmn | 20.3hij | | 132.4l | 5.0efg | 4.7ef | 4.6ij | 28.1ghij |
| 180.5defg | 9.1ghi | 16.0bc | 16.6mn | 11.7kl | | 56.8n | 5.5efg | 7.0abcdef | 5.2ij | 46.4b |
| 152.5ghi | 13.3fgh | 2.2de | 27.7jklm | 21.1hi | | 172.1hijk | 9.1cd | 4.8ef | 9.1fgh | 33.0efgh |
| 246.1b | 10.0ghi | 18.6b | 25.1klm | 30.1g | | 203.0def | 8.4cde | 9.8ab | 11.2efg | 34.7defgh |
| 243.6b | 6.7hi | 15.1b | 15.0n | 16.1ijkl | | 184.6fghi | 5.3efg | 6.8bcdef | 8.3ghi | 27.4hij |
| 88.6l | 24.9d | 15.0b | 70.5ef | 64.6d | | 221.9cd | 8.2cde | 8.8abcd | 15.2cd | 44.4bc |
| 99.2kl | 33.8c | 0.0e | 90.2bc | 73.2c | | 130.1l | 7.4cdef | 7.4abcdef | 4.2j | 36.7cdef |
| 205.6cd | 23.3d | 27.9a | 51.9h | 66.1cd | | 175.4fghijk | 7.7cdef | 7.0abcdef | 13.7cde | 33.7efgh |
| 139.7hij | 25.1d | 0.0e | 77.0de | 37.9f | | 212.4de | 6.2cdefg | 5.8cdef | 15.7cd | 36.5def |
| 240.8b | 9.6ghi | 0.0e | 29.2jkl | 9.98l | | 157.4ijkl | 7.1cdef | 4.4f | 6.6hij | 33.6efgh |
| 202.2cde | 47.1b | 0.0e | 99.5b | 109.5b | | 259.8ab | 19.2a | 9.4abc | 25.3b | 57.9a |
| 217.6bc | 54.7a | 0.0e | 114.1a | 132.3a | | 276.3a | 20.1a | 0.0g | 41.6a | 58.3a |

The same small letters in a column indicate no statistical difference ($p>0.05$) (DMRT's SSR).

**Table 4.** The biased correlation coefficients between the HPLC peak area (X) and the resistance (Y) in two models

| Model A | | | | Model B | | | |
|---|---|---|---|---|---|---|---|
| | Biased correlation coefficient | t | p | | Biased correlation coefficient | t | p |
| $r(Y,X_2)=$ | -0.7361 | 3.4419 | 0.0055 | $r(Y,X_1)=$ | 0.5764 | 2.3399 | 0.0373 |
| $r(Y,X_5)=$ | 0.8811 | 5.8928 | 0.0001 | $r(Y,X_2)=$ | 0.8103 | 4.5869 | 0.0006 |
| $r(Y,X_6)=$ | -0.7274 | 3.3524 | 0.0064 | $r(Y,X_5)=$ | -0.6448 | 2.798 | 0.0161 |
| $r(Y,X_7)=$ | -0.7669 | 3.7795 | 0.0031 | $r(Y,X_6)=$ | 0.6407 | 2.7678 | 0.017 |
| $r(Y,X_{11})=$ | -0.7671 | 3.7815 | 0.003 | $r(Y,X_7)=$ | -0.5084 | 1.9585 | 0.0538 |
| $r(Y,X_{12})=$ | -0.5816 | 2.2609 | 0.045 | $r(Y,X_8)=$ | -0.7246 | 3.4874 | 0.0044 |
| $r(Y,X_{13})=$ | 0.9116 | 7.0154 | 0.0001 | $r(Y,X_{10})=$ | 0.7595 | 3.8725 | 0.0022 |
| $r(Y,X_{14})=$ | 0.8292 | 4.6912 | 0.0006 | $r(Y,X_{12})=$ | -0.6321 | 2.7058 | 0.0191 |
| $r(Y,X_{15})=$ | 0.5931 | 2.3293 | 0.0399 | $r(Y,X_{13})=$ | -0.4278 | 1.5701 | 0.0423 |
| $r(Y,X_{16})=$ | -0.8069 | 4.3211 | 0.0012 | $r(Y,X_{14})=$ | 0.4792 | 1.8109 | 0.0452 |
| $r(Y,X_{17})=$ | 0.7591 | 3.6874 | 0.0035 | $r(Y,X_{15})=$ | 0.3156 | 1.1034 | 0.0514 |
| $r(Y,X_{20})=$ | -0.7528 | 3.6171 | 0.004 | $r(Y,X_{16})=$ | -0.4899 | 1.8639 | 0.0469 |
| | | | | $r(Y,X_{17})=$ | -0.6187 | 2.612 | 0.0227 |

$-0.0779X_8+0.1110X_{10}-0.1836X_{12}-0.1053X_{13}+0.1014X_{14}$ $+0.0517X_{15}-0.0137X_{16}-0.7197X_{17}$.

In the model A, F value was 25.224, which was high (P = 0.00) <0.05; d was 2.563, which was near 2. Duplicate correlation coefficient (R) was 0.984, and adjustable correlation coefficient (R') was 0.961. Y represented the resistant sore of rice variety, and $X_j$ represented the peak area. When Y is smaller, the resistance is stronger. In the model B, F was F = 15.454, which was high too (P = 0.00) <0.05; d was 1.949, which was close to 2. Duplicate correlation coefficient (R) of model was 0.974, and adjustable correlation coefficient R' = 0.942. Y and $X_j$ were the same as in model A. Biased correlation coefficient (P) of each two regression coefficient was under 0.05 (Table 4). The parameters in Table 4 showed that the reliability and applicability of the two models. There were 12 secondary metabolites ($X_j$) in model A that were closely related with the resistance of rice plants (Y), and they were peak 2 , 5, 6, 7, 11, 12, 13, 14, 15, 16, 17, and 20. There were 13 secondary metabolites in model B that were closely related with the resistance of rice plants, and they were peak 1, 2, 5, 6, 7, 8, 10, 12, 13, 14, 15, 16, 17. The biased correlation coefficients (P) between Y and Xj were less than 0.05, suggesting that these models can reliably describe the relationship between secondary metabolite content (Xj) and resistance level (Y) of rice varieties.

From model A, it was shown that some peaks had positive effects on the rice resistance (Y) to BPH, such as peak 2, 6, 7, 11, 12, 16, and 20, but others showed negative effects on the resistance, such as peak 5, 13, 14, 15, and 17. In model B, peak 5, 7, 8, 12, 13, 16, and 17 showed positive effects on the rice resistance to BPH, and peak 1, 2, 6, 10, 14, and 15 showed negative effects on the rice resistance.

## Model validation

Model A and B were validated using 72 rice varieties and the results are shown in Table 5, where the field bioassay scores and the simulated scores are compared. For data analysis, plants with a scale of 0-5.9 and 6-9 were designated as resistant and susceptible, respectively. If simulated scores were less than 0 or higher than 9, they were also designated as resistant and susceptible, respectively. Following these conditions, the model A accord ratio was 94.34% and the model B was 90.14%.

## DISCUSSION

In this paper, the correlations between the secondary metabolites and the resistance of rice plant brown plant hopper were investigated. The secondary metabolites were analyzed by HPLC and the resistance scores were determined by a scoring system proposed by the International Rice Research Institute (IRRI, 1979). The results showed that there was a strong correlation between the resistance to BHP and the contents of certain secondary metabolites. Among the 20 secondary metabolites examined, peak 2, 5, 6, 7, 11, 12, 13, 14, 15, 16, 17, and 20 affected the rice resistance to BPH field population Ⅱ, and peak 1, 2, 5, 6, 7, 8, 10, 12, 13, 14, 15, 16, and 17 affected the rice resistance to BPH field population Bangladesh. For these peaks that showed effects on BPH resistance, some showed positive effects, others showed negative effects. Respective models (equations) to BPH field population II and field population Bangladesh were generated and used to predict the resistance scores of 72 rice varieties. The predicted scores agreed well with the assay scores. Therefore,

**Table 5.** The bioassay scores and simulated scores of BPH resistance of 72 rice varieties.

| No. | Varieties | Population Ⅱ | | Population Bangladesh | | No. | Varieties | Population Ⅱ | | Population Bangladesh | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Bioassay score | Simulated score | Bioassay score | Simulated score | | | Bioassay score | Simulated score | Bioassay score | Simulated score |
| 1 | IR19753-5-2-3-2-1 | 2.7 | 6.83 | 9 | 9.81 | 37 | Gui-99 | 3.5 | -0.85 | 7 | 7.02 |
| 2 | IR13427-45-IMR-5 | 2.8 | 3.5 | 3.8 | 3.31 | 38 | BKNBR1030-11-2 | 3.6 | 2.05 | 9 | 8.41 |
| 3 | IR68 | 3.6 | 4.69 | 4.2 | 3.87 | 39 | IR26 | 5.2 | 2.28 | 9 | 9.22 |
| 4 | TeTep | 9 | 9.13 | 9 | 8.99 | 40 | Double Gui-1 | 6.8 | 6.41 | 9 | 11.04 |
| 5 | IR13429.196-1-2-1-1 | 2.8 | 3.52 | 5.1 | 8.29 | 41 | IR19774-23-2-2-1-3 | 2.4 | 5.01 | 9 | 7.64 |
| 6 | IR11418-19-2-3 | 2.9 | 2.28 | 5.7 | 2.01 | 42 | IR13564-95-1 | 2.8 | 3.05 | 9 | 10.36 |
| 7 | IR29658-43-3-2-1 | 3.5 | 2.97 | 4.8 | 5.58 | 43 | C1321-9 | 2.3 | 5.42 | 9 | 10.46 |
| 8 | IR13429.299-2-1-3 | 2.9 | 3.27 | 5.4 | 4.21 | 44 | IR19660-46-1-3-2-2 | 2.9 | 1.49 | 9 | 9.79 |
| 9 | Baengunghalbyeo | 3.8 | 3.12 | 9 | 7.47 | 45 | IR32453-20-3-2-2 | 3.5 | 3.31 | 5.8 | 5.21 |
| 10 | C691019 | 2.5 | 2.13 | 8.7 | 11.69 | 46 | IR13419.31-3 | 2.9 | 6.75 | 9 | 7.45 |
| 11 | IR15496-219-2-3 | 2.2 | 4.23 | 9 | 6.16 | 47 | IR29692-65-2-3 | 3.1 | 3.09 | 5.1 | 6.65 |
| 12 | Gui-33 | 5 | 5.37 | 7 | 6.99 | 48 | IR13564.109-1 | 2.4 | 3.88 | 9 | 8.8 |
| 13 | IR20878-1-P1 | 3.4 | 3.45 | 9 | 8.09 | 49 | IR4619-57-1-1-1-1 | 3.7 | 0.74 | 5.8 | 5.81 |
| 14 | IR15847-135-1-1 | 2.4 | 3.21 | 9 | 7.25 | 50 | RP1015-2-11-1 | 4.6 | 6.46 | 4.8 | 0.01 |
| 15 | IR49707-1-3-2-3 | 3.4 | 4.22 | 3.1 | 3.89 | 51 | BG90-2 | 9 | 6.05 | 9 | 7.44 |
| 16 | IR18599-68-1 | 2.9 | 1.53 | 9 | 9.95 | 52 | Bknbr76026-3-25-1-1klg-2 | 3.5 | 3.6 | 8.6 | 6.17 |
| 17 | IR13240.39-3-3-3-P1 | 2.9 | 3.52 | 3.6 | 7.45 | 53 | IR29723-88-2-3-3 | 3.9 | 4.04 | 4.4 | -0.31 |
| 18 | IR21018-97-1 | 2.4 | 2.88 | 9 | 8.51 | 54 | IR13427.45-3-1-2-2-2 | 2.9 | 2.09 | 2.6 | 2.13 |
| 19 | IR13419-31-1 | 3.3 | 4.12 | 9 | 7.19 | 55 | IR13240.82-2-3-2-3-1 | 2.6 | 1.42 | 3.4 | 2.64 |
| 20 | C681032 | 2.5 | 3.19 | 8.7 | 6.15 | 56 | BG367-1 | 3.6 | 4.66 | 9 | 10.55 |
| 21 | DATA | 4.5 | 4.01 | 9 | 8.28 | 57 | IR62 | 2 | 2.72 | 4.6 | 5.88 |
| 22 | Nan geng-15 | 9 | 7.06 | 9 | 8.85 | 58 | IR52 | 3.3 | 1.26 | 4.4 | 5.24 |
| 23 | IR13240-108-2-2-3 | 4 | 5.18 | 4.6 | 4.35 | 59 | IR31892-46-3-2 | 3.9 | 1.11 | 3.2 | 3.9 |
| 24 | C1322-28 | 2.3 | 4.1 | 9 | 6.58 | 60 | IR13429-109-2-2-1 | 2.7 | 3.86 | 4.3 | 6.87 |
| 25 | IR13423.17-1-2-1 | 3.3 | 3.93 | 9 | 11.59 | 61 | IR978-51-1-2 | 1.8 | 0.91 | 3.5 | 3.4 |
| 26 | IR15847-215-2-1 | 3.4 | 5.73 | 9 | 5.81 | 62 | B2850-B-SI-2-2 | 3.7 | 4.84 | 5.8 | 5.27 |
| 27 | IR19743-25-2-2-3-1 | 2.6 | 1.79 | 9 | 10.39 | 63 | IR31429-14-2-3 | 2.2 | 5.88 | 4.3 | 4.46 |
| 28 | B2980B-SR-2-1-1-1-2-1 | 2.7 | 5.74 | 9 | 6.72 | 64 | BKNBR1030-28-1-5 | 3.1 | 4.62 | 9 | 10.45 |
| 29 | IR15498-167-3-2-2 | 2.8 | 4.05 | 9 | 6.05 | 65 | IR13427-45-2-3-3 | 3.2 | 3.21 | 2.6 | 4.7 |
| 30 | Hong nan | 3.1 | 3.02 | 3 | -0.59 | 66 | Jing gang-30 | 9 | 9.44 | 9 | 9.18 |
| 31 | IR10781-75-2-2 | 3 | 4.17 | 9 | 6.89 | 67 | IR15324-117-2-2-3 | 2.7 | 3.71 | 3.3 | -0.66 |
| 32 | IR10781-143-2-3 | 2.6 | 5.23 | 9 | 5.28 | 68 | IR29723-17-3-2-1 | 3.3 | 4.86 | 3.3 | 4.51 |
| 33 | IR13240-39-3 | 2.8 | 3.44 | 4.5 | 5.63 | 69 | BG367-4 | 2.1 | 5.34 | 5.4 | 7.87 |

**Table 3. Contd.**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 34 | Hong gu-zhan | 9 | 10.02 | 9 | 7.18 | 70 | DV85 | 9 | 12.82 | 9 | 8.22 |
| 35 | Chianung sen yu13 | 4.6 | 8.27 | 8.9 | 6.42 | 71 | IR28224-3-2-3-2 | 3.3 | 5.44 | 2.9 | 1.45 |
| 36 | IR14497-15-2 | 3.4 | 4.47 | 9 | 6.13 | 72 | IR13525.5-2-3-3 | 2.7 | 3.25 | 3.3 | 5.54 |

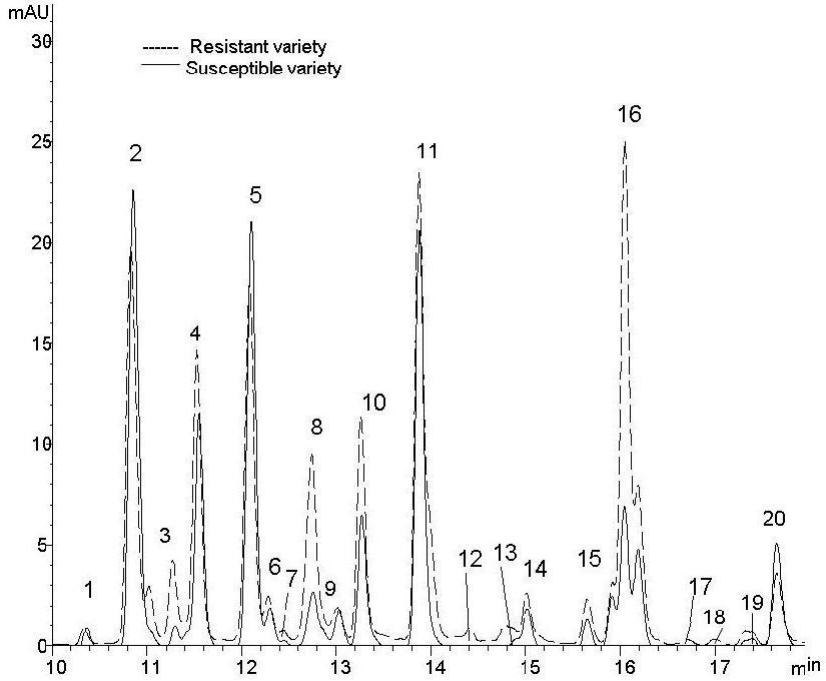


**Figure 1.** HPLC chromatograms of resistant and susceptible varieties to two population of BPH.

these models can be used to predict the resistance abilities of rice plants and facilitate the screening of resistant rice varieties. However, some varieties' simulated scores were larger than 9 and some were less than 0, which lay outside the scope of standard range of 0-9, suggesting these models need to be improved further in future studies.

The secondary metabolites of rice plants were detected by UV absorption at 320 nm, which is the specific absorption region of aromatic metabolites. According to the study of Kong (2002), among the 20 peaks, peak 4, 5, 6 and 8 likely belong to indican phenol compounds with long aliphatic chains, peak 10, 11, and 12 likely belong to indican flavone, and peak 14 and 15 likely belong to indican hydroxamic acids. These compounds contribute to the allelopathic effects of rice.

It was also reported that hydroxybenzene compounds show effects on the metabolism of hormones in plants (Wang et al., 1992). Indican hydroxamic acids and hydroxybenzene compounds are important secondary metabolites that confer resistance to insects in gramineous plants (Liu et al., 2002). We are in the process to identify the structure of each secondary metabolites and investigate the activities of these secondary metabolites in BPH resistance.

Previous researches had focused on the qualitative and quantitative analysis of a single anti-pest compound (Gutierrez et al., 1988; Barria et al., 1992). However, the plants' chemical resistance to insects is likely the results of the combined activities of many metabolites (Mattice et al., 2001; Kong et al., 2002). The activities of many components have not been illustrated because of the difficulties in the separation and identification of these low levels of metabolites. Therefore, qualitative and quantitative analysis of a single compound cannot explain the whole resistance of rice plant and it cannot be used to screen the resistance to insects reliably. Zhao et al. (2005) attempted to estimate the rice resistance to BPH through analysis of metabolites. In this study, the resistant models to two types of BPH were established through metabolites quantification by HPLC. Twenty metabolite peaks were used to construct the models. Even though the natures of these metabolite peaks have not been fully identified, we showed that BPH resistance can be predicted accurately by the peaks areas of specific metabolites.

The HPLC method used in this research was simple and fast. It did not harm the plant because only a small amount of plant tissue was needed. This method can be used to evaluate the resistance of rice plant to BPH and assist the selection of resistant rice plants for breeding. However, this method is still in the early stage of research. Care should be taken in the use of the prediction models. More studies are needed to identify the structures and functions of secondary metabolites that are responsible for resistance. Moreover, more information needs to be gathered to improve the appraisal system.

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