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Full Length Research Paper

Impact of Rice Plant Secondary Metabolites on *Nilaparvata lugens* Stål Resistance

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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

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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

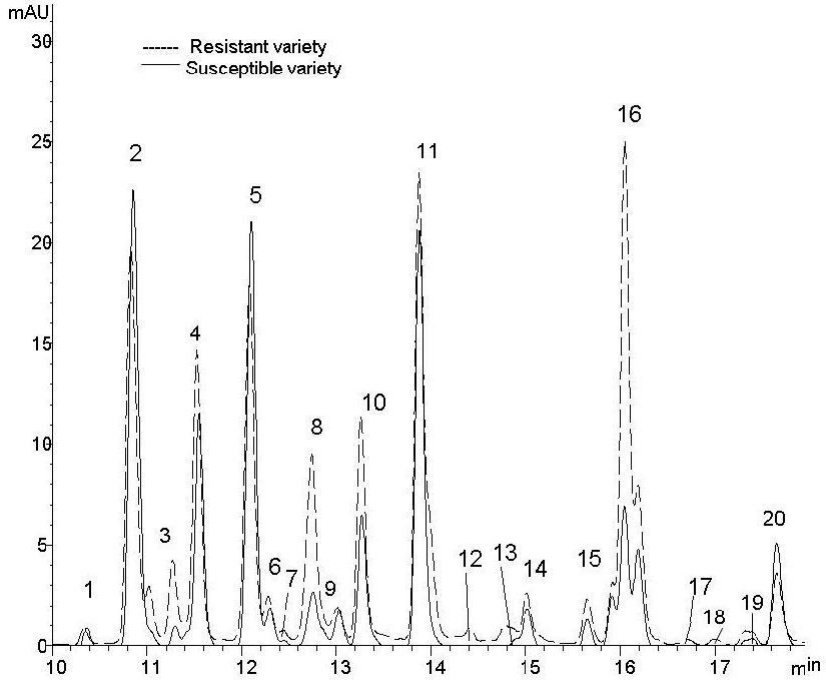

Figure 1. HPLC chromatograms of resistant and susceptible varieties to two population of BPH.

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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