Content Variations of the Secondary Compounds in Rice Plants and Their Influence on Rice Resistance to Brown Planthopper, *Nilaparvata lugens*

ZHAO Ying [1], TONG Xiao-li [2], HUANG Feng-kuan [3]

([1]*Department of Applied Chemistry, College of Sciences, South China Agricultural University, Guangzhou 510642, China*; [2]*Department of Entomology, South China Agricultural University, Guangzhou 510642, China*; [3]*Institute of Plant Protection, Guangxi Academy of Agricultural Sciences, Nanning 530007, China*)

Abstract: Content variations of the four components in the plants of rice ASD7, IR36 (resistant to brown planthopper) and TN1 (susceptible to brown planthopper), and their influence on rice resistance to brown planthopper (BPH, *Nilaparvata lugens*) were investigated. The resistance diversity of rice to BPH biotype II at different plant ages was chiefly attributed to the content variability of the secondary compounds. The contents in the leaf sheath were the lowest as compared with other parts of rice plant. It might explain the reason that BPH has a preference for feeding on leaf sheath aggregately from chemical point of view.

Key words: rice; brown planthopper; secondary compounds; resistance; content variations

The brown planthopper (BPH), *Nilaparvata lugens* (Stål), is a monophagous pest that feeds only on rice, and sucks the juice from the phloem resulting in drying of the leaves and wilting of the tillers, causing ‘hopper burn’, as well as being an important virus vector [1-2]. The use of resistant varieties is the most economical and effective way for controlling BPH, so breeding of the insect-resistant varieties has become a priority in rice breeding. Numerous studies have revealed that the secondary compounds of plants play an important role in plant resistance [3-7]. In the last 40 years, knowledge of the plant chemistry in Gramineae including rice has mushroomed since 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) was found in *Zea mays* [8-9]. But the early studies mainly concentrated on the extraction, separation and identification of single key secondary compound (e.g. DIMBOA). In fact, the resistant activity in resistant crop varieties should be a result of the action of several secondary compounds rather than just one [3-4, 7, 10-11]. By using high-performance liquid chromatography (HPLC), Zhao et al [7, 11] reported that 13 distinct peaks were detected at retention time 10-16 min in the chromatogram from 130 samples of 20 rice varieties (including BPH-resistant and BPH-susceptible ones) and 6 varieties of *Oryza officinals*, and found that four peaks (i.e. Peak 1, Peak 2, Peak 8 and Peak 12) had high correlation with the resistance to BPH biotype II based on the combination of principal component analysis and multiple linear regression analysis. The resistance screening tests and BPH feeding behavior studies have indicated that resistance levels of rice varieties to BPH vary with rice growth stages [12-15], and BPH prefers to feed on leaf sheath aggregately whether the rice varieties are BPH-resistant or not. Our study, therefore, is based on following questions or hypothesis: Do the contents of these four peaks vary with the rice growth stages? Are there any relationship between BPH feeding behavior and the contents of these four secondary compounds in different parts of rice tissues? The aim of this study is to investigate the spatial and temporal variations of these four components in rice varieties resistant to BPH.

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Corresponding author: ZHAO Ying (zhaoying@scau.edu.cn)

MATERIALS AND METHODS

Rice varieties

Two BPH-resistant rice varieties ASD7, IR36 and a BPH-susceptible variety TN1 were chosen for the study. In order to obtain the fresh materials at different growth stages at the same time, seeds of different rice varieties were sowed successively at 2-3 days intervals for two months. The second leaf from the top of rice varieties were collected simultaneously to detect the content of the secondary compounds at different growth stages by HPLC analysis. To investigate the content variations in different parts of rice plant, the 1st leaf from the top (or flag leaf if the rice plant is at the booting stage), the 2nd to 4th leaf from the top and their leaf sheaths were sampled from IR36 and TN1 at 5-leaf stage and from IR36 and ASD7 at the booting stage.

Sample treatments

Fresh leaves were cut into pieces and mixed evenly, and 0.1067±0.0002 g of the leaves were soaked into 5 mL methanol for 12 h at room temperature. Then the extracts were filtered and stored in a refrigerator. Before HPLC determination, the filtrate was evaporated to dry, and then 5 mL 50% methanol water solution (*V*/*V*) was added to re-dissolved residue for analysis. Each sample was conducted with five replicates.

High-performance liquid chromatography conditions

The samples were filtered through a 0.45 µm filter and determined at HP1100 HPLC system with Hypersil C_{18} reversed column (250 × 4.0 mm, ODS 5 µm). The gradient used 1% acetic acid (*V*/*V*) (A) and HPLC grade acetonitrile (B). The HPLC conditions were modified from Mattice et al [4] and Kong et al [3] as follows: The program was 8% B at 1.5 mL/min for 3 min, increased to 35% B over 22 min at 1.5 mL/min, increased to 80% B for 4 min, decreased to 8% B over 11 min. The total run time was 40 min, the injection volume was 10 µL and the wavelength of the UV detector was 320 nm. The data were collected for the first 30 min and the retention time and integral of related specific chromatographic peaks were determined.

RESULTS

The major chromatographic peaks associated with rice varietal resistance to BPH

The results of the early study demonstrated that peak 1, peak 2, peak 8 and peak 12 (Fig. 1) were the key resistance-related secondary compounds in rice variety resistant to BPH biotype II, which suggested that the resistance in BPH-resistant varieties was a result of the action of several compounds rather than just one [7, 11].

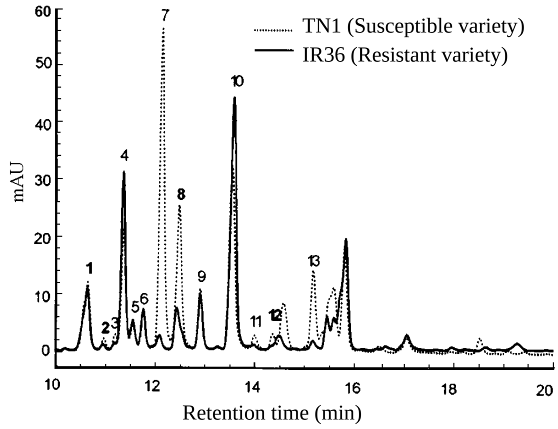

Fig. 1. HPLC chromatograms of resistant and susceptible rice varieties to BPH biotype II.

Content variations of the secondary compounds at different rice growth stages

The contents (i.e. the peak areas in chromatogram) of peak 1 and peak 2 were illustrated without significant difference between IR36 and TN1 at rice growth stages except at 5-leaf stage (Fig. 2). The contents of peak 1 and peak 8 which were positively correlated with the resistance to BPH biotype II maintained higher levels in IR36 during the whole growth stage in comparison with the other two peaks which were negative contribution to resistance to biotype II. Contrary to IR36, the content of peak 12 maintained much higher levels than that of the other three peaks in TN1.

Table 1 presented the simulated resistance grades of IR36 and TN1 using the prediction model established by Zhao et al [11]. The result indicated that it was the content change

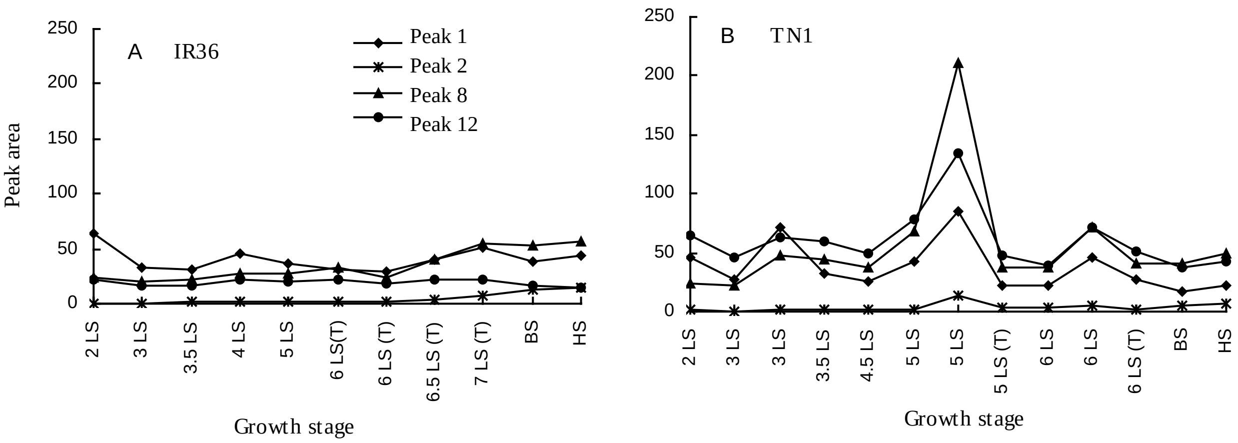

Fig. 2. Content variations of the secondary compounds at different growth stages of rice.

2 LS, 2-leaf stage; 3 LS, 3-leaf stage; 3.5 LS, 3.5-leaf stage; 4 LS, 4-leaf stage; 4.5 LS, 4.5-leaf stage; 5 LS, 5-leaf stage; 5 LS (T), 5-leaf stage (Tiller); 6 LS, 6-leaf stage; 6 LS (T), 6-leaf stage (Tiller); 6.5 LS (T), 6.5-leaf stage (Tiller); 7 LS (T), 7-leaf stage (Tiller); BS, Booting stage; HS, Heading stage.

Table 1. Simulated resistance grades of rice to brown planthopper biotype Ⅱ at different growth stages.

Plant age	Simulated grade
IR36	
2-leaf	3.6
3-leaf	3.9
3.5-leaf	3.8
4-leaf	3.8
5-leaf	3.8
Early 6-leaf (Tiller)	4.0
Late 6-leaf (Tiller)	3.9
6.5-leaf (Tiller)	3.8
7-leaf (Tiller)	3.1
Booting	3.4
Heading	2.9
TN1	
2-leaf	9.0
Early 3-leaf	7.2
Late 3-leaf	7.2
3.5-leaf	7.9
4.5-leaf	7.1
Early 5-leaf	9.0
Late 5-leaf	9.5
5-leaf (Tiller)	7.1
Early 6-leaf	6.0
Late 6-leaf	8.2
6-leaf (Tiller)	7.3
Booting	6.1
Heading	6.4

patterns of the secondary compounds that made IR36 maintain high levels of resistance to BPH biotype II during the whole rice growth stage, while TN1, on the contrary, was always susceptible to BPH biotype II. It suggested that the changes in the levels of resistance to BPH biotype II with rice plant age is largely attributed to the content changes of these four peaks with rice growth stages.

Content variations of the secondary compounds in different parts of rice plant

The contents of these four peaks in five different parts and at two growth stages of rice plant were detected by HPLC based on the fresh tissues from the typical resistant varieties (IR36 and ASD7) and typical susceptible variety TN1. The results indicated that, whether at the seedling stage (5-leaf stage) or at the booting stage, contents of the secondary compounds, particularly in peak 1 and peak 8, were ranked as follows: the 2nd leaf from the top (functional leaf) > old leaf and 1st leaf from the top > leaf sheath (Fig. 3). It is quite evident that the contents of these four peaks were the lowest in leaf sheath irrespective of growth stage or rice variety. This finding might explain the reason that BPH has a preference for feeding aggregately on leaf sheath from chemical point of view.

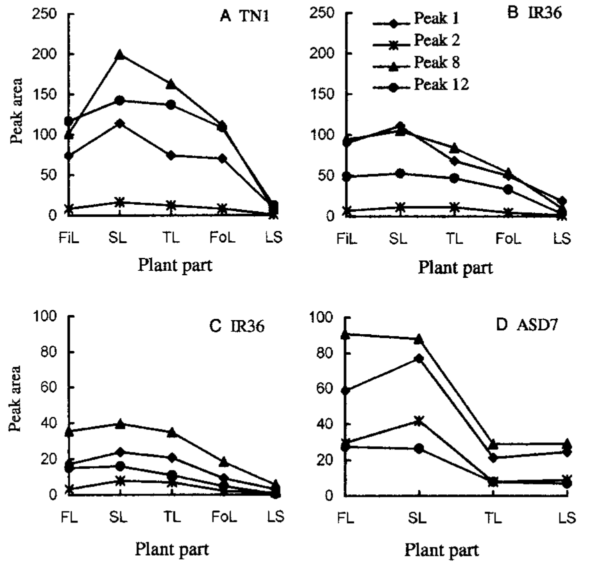

Fig. 3. Content variations of the secondary compounds in different parts of rice plant (A & B, at the 5-leaf stage; C & D, at the booting stage).

FiL, The first leaf from the top; FL, Flag leaf; SL, The second leaf from the top; TL, The third leaf from the top; FoL, The fourth leaf from the top; LS, Leaf sheath.

DISCUSSION

Although BPH feeds by phloem abstraction and is often found to feed on leaf sheath aggregately whether the rice varieties are BPH-resistant or not, BPH could disperse to other parts of rice for feeding (e.g. culm or other leaves) only when the density of BPH population is much higher. Our study suggests that the feeding behavior of BPH associates not only with the ecological factors (such as the physical characteristics or nutrition of rice, microclimate of paddy field, etc) [16-19], but also with the contents and distribution of the secondary compounds in different parts of rice plant.

In fact, the function and contribution weight of these four peaks (i.e. peak 1, peak 2, peak 8 and peak 12) to resistance of rice were different. The resistance level of rice to BPH biotype II were positively correlated with contents of peak 1 and peak 8, but decreased with the increase of the contents of peak 2 and peak 12. In our study, furthermore, the resistance grades of IR36 and TN1 at different growth stages simulated by the prediction model [11] were consistent with the bioassay scores of Liu et al [20], showing that the changes of resistance to BPH during the rice growth stages depend upon the content variations of several secondary compounds rather than one or two secondary compounds.

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