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Comparative metabolomics of the interaction between rice and the brown planthopper

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Abstract

Introduction The interactions between plants and insect herbivores are complex and multifaceted. Rice and its specialist insect pest the brown planthopper (BPH), *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) constitute an ideal system for studying plant–insect interactions.

Objectives Combined metabolomics analyses of rice plant and BPH were conducted to understand the mechanism of host rice plant defense and BPH insect response.

Methods Metabolite dynamics in rice leaf sheath and BPH honeydew was investigated using the gas chromatography–mass spectrometry (GC–MS) method. The GC–MS data were analyzed by principal component analysis and partial least squares-discriminant analysis.

Results Twenty-six metabolites were detected in the leaf sheath extracts. Rice leaf sheath metabolomics analysis results show that BPH feeding induces distinct changes in the metabolite profiles of YHY15 and TN1 plants. These results suggest that BPH infestation enhance fatty acid oxidation, the glyoxylate cycle, gluconeogenesis and the GABA shunt in TN1 plants, and glycolysis and the shikimate pathway in YHY15. We propose that the *BPH15* gene mediates a resistance reaction that increases the synthesis of secondary metabolites through the shikimate pathway.

Thirty-three metabolites were identified in BPH honeydew. Honeydew metabolomics analysis results show that when BPH insects were fed on resistant YHY15 plants, most of the amino acids in honeydew were significantly decreased compared to those of BPH fed on TN1 plants. Based on metabolomics results, we propose that BPH feeding on resistant YHY15 plants would enhance amino acid absorption. At the same time, urea was significantly increased in BPH fed on YHY15.

Conclusion Metabolomics study is valuable in understanding the complex and multifaceted interaction between plants and insect herbivores and provide essential clue for development of novel control BPH strategies.

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1 Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. It is the staple food for half of the world's population, and it provides more than 20 % of the calories consumed by humans worldwide each year. It also has been regarded as one of the most important model plants (Cheng et al. 2013). Among rice pests, the brown planthopper (BPH), *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) is the most harmful one, causing great loss of yield every year (Cheng et al. 2013). Although chemical pesticides have been used to control the pests, environmental problems and pesticide resistance are not negligible issues. Cultivating rice resistant to BPH attack has proved to be an economic and environmentally friendly approach (Brar et al. 2009).

To date, more than 30 BPH resistance loci have been reported, of which four genes have been successfully cloned (Tamura et al. 2014). The first cloned BPH resistance gene, *BPH14*, encodes a coiled coil, nucleotide binding, leucine-rich repeat protein (CC-NB-LRR) belonging to the NB-LRR immune receptor family (Du et al. 2009). Other genes include: *BPH26*, which is similar to that of *BPH14* and also belongs to the CC-NB-LRR protein structure (Tamura et al. 2014), *BPH3*, which comprises lectin-like receptor kinases of three genes positioned in the cytoplasmic membrane, *BPH29*, a resistance gene containing a B3 DNA-binding domain (Liu et al. 2015; Wang et al. 2015). *BPH15* candidate genes do not belong to NB-LRR family of proteins, but may be involved in a unique resistance mechanism (Lv et al. 2014).

Resistance genes inhibit BPH feeding behavior and affect BPH physiology by, for example, lowering survival rates, prolonging nymphal periods, lowering weight gain, and reducing oviposition (Horgan 2009; Sōgawa and Pathak 1970). Under selection pressure, BPH may also gradually evolve a new biotype in response to a plant's resistance mechanisms (Claridge and Hollander 1980). Any new BPH biotype may threaten the deployment of resistant rice varieties. BPH Biotype 1 is unable to infest any resistant rice variety and usually only occurs on the TN1 rice varieties (Jena and Kim 2010). Biotype Y is an artificial biotype that flourishes on resistant rice YHY15, which contains *BPH15* (Jing et al. 2012).

Previous leaf sheath and phloem exudates proteomic analysis showed significant changes in expression in response to BPH feeding, defense signal transduction, redox regulation, carbohydrate and protein metabolism, as well as cell structural proteins (Wei et al. 2009; Du et al. 2015). The transcriptomic analysis of resistant *BPH15* introgression line and the susceptible recipient line showed BPH-responsive transcript profiles were distinct between resistant and susceptible plants and between early stage and late stage (Lv et al. 2014).

Transcriptomics and proteomics studies have only been able to predict changes in the functions of these genes or proteins. Metabolite changes brought about by these genes exerting their changed functions need to be examined by means of metabolomics (Nicholson et al. 1999; Nicholson and Lindon 2008). Metabolomics research is usually accomplished by means of nuclear magnetic resonance spectroscopy, gas chromatography–mass spectrometry (GC–MS), liquid chromatography–MS, and capillary electrophoresis–MS (Bjerrum 2015). GC–MS has the advantages of high resolution, high sensitivity, good reproducibility, a large number of standard metabolite spectra libraries, and its relatively low cost (Qi et al. 2015). Thus, GC–MS has been one of the main analytical platforms for studies in plant metabolomics (Qi et al. 2015).

In plant, the resistance to herbivore attack usually is thought to be principally determined by its secondary metabolism. Plant secondary metabolites are derived from primary metabolisms which affect growth and development of the plant as well as changes induced by herbivore attack (Schwachtje and Baldwin 2008). Plant–insect herbivores interactions represent one of the most biochemically complex and challenging scenarios that are currently being assessed by metabolomics approaches (Allwood et al. 2008). Previous metabolomics research has shown that insect feeding induced many systemic primary metabolic changes in plants and plant defenses, including changes in transamination, the GABA shunt, the TCA cycle, gluconeogenesis/glycolysis, and the pentose phosphate pathway. Changes to secondary metabolisms such as phenolics, glucosinolate, etc., are also known (Liu et al. 2010; Uawisetwathana et al. 2015; Leiss et al. 2009; Riach et al. 2015; Jansen et al. 2009). However, these studies were limited to examine only with regard to plant metabolites that had undergone changes that were induced by herbivorous insects. In the present study, we investigated metabolic profiles of both the rice leaf sheath and BPH excretions (honeydew). Our research goals were: (1) to elucidate the metabolite changes following BPH infestations in the resistant rice YHY15 with the resistance gene *BPH15*, and in the susceptible rice TN1, thus to reveal the resistance mechanism mediated by the *BPH15* gene, (2) to analyze the metabolite changes in honeydew of BPH Biotypes 1 and Y, respectively, after feeding on YHY15 and TN1, and so investigate the BPH's response mechanism to resistant rice, and (3) to investigate the characteristics of both interactions by a combined analysis of rice leaf sheath metabolites and BPH honeydew metabolites.

2 Materials and methods

2.1 Plant materials and insects

TN1 is a susceptible rice variety with no resistance gene. The resistant rice line YHY15 containing BPH-resistance gene *BPH15* was selected by marker-aided selection from locus from the population derived from TN1 × RI93 (a selected RIL carrying a single *BPH15* resistance gene), and *BPH15* was mapped to the short arm of chromosome 4 (Yang et al. 2004). The BPH Biotypes Y and 1 were full sib-matings for at least 40 generations and were maintained on YHY15 and TN1 rice plants, respectively. Biotype Y was a new lab biotype gained by forcing Biotype 1 to feed on YHY15 (Jing et al. 2012). BPH Biotype 1 was used for infesting rice in rice sheath metabolomics analysis and honeydew collection in honeydew metabolomics analysis. BPH Biotype Y was used for honeydew collection. BPH

and rice plants for the test were both kept and planted in the Institute of Genetics of Wuhan University. BPH and rice plants were grown on in a greenhouse with a controlled environment at 28 °C/14 h light (06:00–20:00) and 25 °C/ 10 h dark (20:00–06:00), with illumination and humidity conditions to ensure good growth of both rice and BPH.

2.2 BPH treatment

Ten well-germinated rice seeds were evenly sown in each experimental pot (10 cm in diameter, 15 cm in height). The four-leaf stage rice seedlings were used to conduct the experiment. TN1 and YHY15 plants were each divided into three groups. For both varieties, one group was used as controls without BPH infestation, the other two groups were treated with Biotype 1 BPH for 24 and 48 h, respectively. There were totally 30 TN1 and YHY15 rice leaf sheath samples used in this study, each sample containing a pool of five plants and each treatment containing five biological replicates. Fifteen 3rd–4th instar BPH nymphs were introduced to each plant of the treated groups and covered with gauze at 0, 24, and 48 h before the end of the experiments. After treatment, the outermost sheaths were quickly stripped and placed in liquid nitrogen for subsequent analysis.

2.3 BPH honeydew collection

Parafilm-M were cut and folded to form a bag with 3 cm in length and 5 cm in width. These bags were then fixed to the stem of the rice seedlings 2 cm above the soil, leaving a small opening to allow placement of the BPHs. Five similarly sized fifth instar female BPHs were placed in each bag, which was then sealed. After the BPH had been allowed to feed on TN1 or YHY15 for 24 h, the bag was removed and any honeydew collected with a micropipette, then transferred into a centrifuge tube and kept on ice. Each bag as one biological replicate, there were six biological replicates for each biotype treating with TN1 or YHY15. There were total 24 honeydew samples used in this study.

2.4 Evaluation of BPH weight change and honeydew excretion

To quantify the amount of honeydew, BPH honeydew was collected as described above, except one bag contained one newly brachypterous female and collected honeydew after 48 h. Honeydew weight was measured using a microbalance.

To quantify changes in body weight, newly brachypterous females were selected and measured using a microbalance. The insects were then placed on a 4-week-old YHY15 or TN1 plant. After 72 h, each individual was

weighed again. The BPH weight gain was calculated as the proportional change in weight relative to the initial weight. Each treatment of the honeydew excretion and body weight experiments was replicated for 15 times.

2.5 Metabolite extraction and derivatization

Leaf sheath extract was prepared according to the protocol reported by Lisec et al. (2006). Briefly, a 100 mg sample of leaf sheath was ground to a powder and extracted with 1400 mL methanol (pre-cooled to -20 °C) and 60 µL ribitol (0.2 mg/mL aqueous solution) as an internal standard. After extraction the sample was shaken for 10 min in a thermomixer and centrifuged at $11,000 \times g$ for 10 min. The supernatant was transferred to a 1.5 mL centrifuge tube and mixed vigorously with 750 µL chloroform (-20 °C) and 1400 µL of pre-cooled double-distilled water (-20 °C), vortexed for 10 s and centrifuged at $2200 \times g$ for 10 min. The 150 µL (polar phase) of supernatant was then transferred into another clean 1.5 mL tube and then dried under vacuum. Supernatants of honeydew were obtained by centrifuging at $10,000 \times g$ for 5 min. 10 µL supernatant were used for GC–MS detection, with 1 µL ribitol (0.2 mg/ mL aqueous solution) as the internal standard, and then dried with a flow of nitrogen in preparation for the next step of derivatization. The dried residue was re-dissolved in 40 µL methoxyamination reagent (methoxy-amino-hydrochloride, 20 mg/mL solution of pyridine) and derivatized in 70 µL MSTFA at 37 °C for 30 min. The derivatized sample was then transferred to a liner tube suitable for GC–MS analysis.

2.6 GC–MS analysis conditions

The derivatized sample was analyzed on a GC–MS (Thermo Trace GC Ultra-ISQ mass spectrometer, Thermo Fisher Scientific, USA) with a Rtx-5MS capillary column (30 m × 0.25 mm). Helium was used as the carrier gas at a flow rate of 1 mL/min; column initial oven temperature was held at 100 °C for 3 min, ramped to 280 °C at 5 °C/ min, and then held for 5 min. Sample size was maintained at 1 µL with an AS-3000 auto sampler, and set for taking splitless injections; injection temperature was maintained at 250 °C. The mass spectrometer was tuned according to the manufacturer's recommendations using tris-(perfluorobutyl)-amine (CF43). 70 eV electro impact mode was used for the ionization. The recorded mass range was 50–650 m/z.

2.7 GC–MS data analysis

Peaks with a signal-to-noise ratio (S/N) lower than 30 were rejected, which was an acceptable level to avoid false

positives. Also, artifact peaks were excluded with the help of a blank sample that was prepared and analyzed along with the samples. The compounds were identified by searching NIST MS Search 2.0 software from the NIST library. We selected target compounds that gave more than 70 % matching with the database. We also identified compounds by comparing their mass spectra and retention time with those of standard substances (Tables S1, S2, S3). Furthermore, three masses were selected as qualifier ions to assist in identifying compounds (Tables S1, S2). The determined target compounds were automatically integrated with the peak area by using the automatic integrating function of the Xcalibur software, and manual correction. The relative peak area of each compound was determined by normalization to the ribitol internal standard. We then used the corrected retention time, sample information and relative peak area to generate a three-dimensional matrix.

2.8 Statistical analyses

The metabolites' three-dimensional matrix information was then input into the SIMCA-P software (V11.0, Umetrics, Umeå, Sweden). Before principal component analysis (PCA) is performed, the data file was scaled with unit variance scaling. SIMCA-P was applied to perform PCA and partial further square-discrimination analysis (PLS-DA). MeV_4_6_0 (multiple experiment viewer) was used to draw the heat map plot and perform hierarchical cluster analyses. T test and ANOVA analyses were performed with SPSS17.0 software (SPSS, Inc., Chicago, IL, USA).

3 Results

3.1 Biotypes 1 and Y BPH performance on TN1 and YHY15 rice plants

The honeydew weight and body weight changes of Biotypes 1 and Y feeding on TN1 and YHY15 rice plants, respectively, are shown in Fig. 1. After Biotype 1 had fed on TN1 and YHY15, the mean of BPH honeydew excretion was 38.7 and 6.8 mg, respectively. The equivalent values for Biotype Y were 57.1 and 42.5 mg, respectively. The honeydew excretion of Biotype 1 feeding on YHY15 was significantly lower than other treatments ($p < 0.05$, ANOVA, Tukey-HSD).

The results of BPH body weight change were similar to those of the honeydew excretion. The mean BPH weight changes of Biotype 1 after having fed on TN1 and YHY15 were 1.0 and -0.2 mg, respectively; while the equivalent results for Biotype Y were 1.2 and 0.88 mg, respectively.
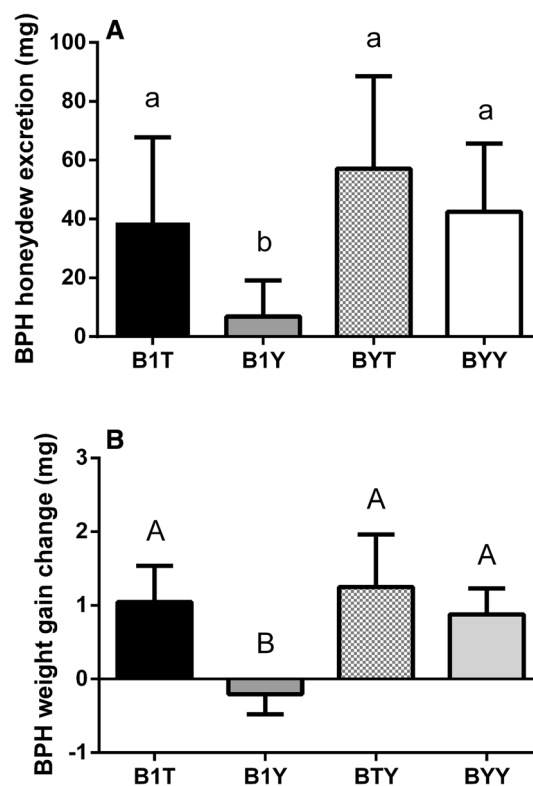
Fig. 1 Means ($\pm$SD) of BPH honeydew excretion quantity (**a**) and BPH weight gain change quantity (**b**). Each treatment of the honeydew excretion and body weight experiments was replicated for 15 times ($n = 15$). *B1T* Biotype 1 BPH feeding on TN1, *B1Y* Biotype 1 feeding on YHY15, *BYT* Biotype Y feeding on TN1, *BYY* Biotype Y feeding on YHY15. *Different letters above bars* indicate significant differences among treatments by the Tukey test: $p < 0.01$, *capital letters*, or $p < 0.05$, *lower case letters*

The weight change undergone by Biotype 1 feeding on YHY15 was significantly lower than other treatments ($p < 0.01$, ANOVA, Tukey-HSD). These results suggest that YHY15 can inhibit Biotype 1 feeding, while Biotype Y was well adapted to the resistance mechanism of YHY15.

3.2 Characterization of metabolites by GC–MS

Thirty TN1 and YHY15 leaf sheath samples that were infested by BPH at different time points, and the respective controls were investigated. We identified a total of 26 metabolites by searching the mass spectra for matches with standards in the MS library (NIST) and combining the findings with the retention times matched with those of standard substances (Tables 1, S2). The metabolites what were identified using standards and were annotated using database were listed in Tables S1 and S2. Most of these identified compounds were primary metabolites, such as sugars, organic acids, and amino acids. Twenty-four honeydew samples from Biotypes 1 and Y feeding on TN1 and YHY15 were investigated. We identified 33

Table 1 Metabolites relative peak area (mean ± SEM) measured by GC–MS from rice leaf sheath samples and the changes of metabolites induced by BPH Infestations

Metabolites	Changes in TN1			Changes in YHY15		
	T0h	T24h	T48h	Y0h	Y24h	Y48h
Sugars						
17 Fructose	1.04 ± 0.05	2.89 ± 0.33 ↑	5.00 ± 0.67 ↑	1.48 ± 0.06	1.29 ± 0.12	1.72 ± 0.08 ↑
18 Glucose	1.71 ± 0.08	3.25 ± 0.28 ↑	4.74 ± 0.58 ↑	1.97 ± 0.05	1.56 ± 0.12 ↓	2.14 ± 0.07
Organic acids						
1 Oxalic acid	0.47 ± 0.09	0.44 ± 0.03	0.39 ± 0.04	0.44 ± 0.04	0.64 ± 0.1	0.54 ± 0.06
5 Succinic acid	0.052 ± 0.005	0.103 ± 0.013 ↑	0.115 ± 0.01 ↑	0.073 ± 0.005	0.057 ± 0.007	0.102 ± 0.011 ↑
6 Glyceric acid	0.19 ± 0.02	0.28 ± 0.02 ↑	0.30 ± 0.02 ↑	0.17 ± 0.04	0.19 ± 0.02	0.18 ± 0.02
9 Malic acid	0.97 ± 0.08	1.88 ± 0.13 ↑	2.12 ± 0.14 ↑	1.17 ± 0.06	0.76 ± 0.09 ↓	1.07 ± 0.07
10 Salicylic acid	0.10 ± 0.01	0.15 ± 0.02 ↑	0.15 ± 0.02 ↑	0.19 ± 0.02	0.17 ± 0.02	0.19 ± 0.02
14 2,6-Dihydroxybenzoic acid	0.028 ± 0.004	0.030 ± 0.001	0.050 ± 0.017	0.029 ± 0.004	0.030 ± 0.004	0.034 ± 0.002
15 Shikimic acid	0.15 ± 0.01	0.20 ± 0.05	0.15 ± 0.02	0.15 ± 0.02	0.30 ± 0.04 ↑	0.38 ± 0.02 ↑
16 α-Ketoglutaric acid	0.20 ± 0.02	0.23 ± 0.06	0.26 ± 0.04 ↑	0.25 ± 0.02	0.19 ± 0.02 ↓	0.46 ± 0.05 ↑
Fatty acids						
20 Hexadecanoic acid	4.36 ± 0.42	3.18 ± 0.29 ↓	3.92 ± 0.45	4.07 ± 0.15	3.58 ± 0.14 ↓	4.24 ± 0.10
23 9,12-Octadecadienoic acid	0.64 ± 0.17	0.41 ± 0.10	0.86 ± 0.35	0.75 ± 0.10	0.67 ± 0.11	1.11 ± 0.13
25 Hexadecanoic acid, (2S)-2,3-dihydroxypropyl ester	0.80 ± 0.11	0.52 ± 0.11	0.85 ± 0.15	0.67 ± 0.04	0.72 ± 0.04	0.76 ± 0.03
24 Octadecanoic acid	3.42 ± 0.19	2.66 ± 0.37	3.15 ± 0.26	3.10 ± 0.11	2.78 ± 0.14	3.28 ± 0.08
26 Octadecanoic acid, 2,3-dihydroxypropyl ester	0.90 ± 0.12	0.57 ± 0.14	0.92 ± 0.2	0.73 ± 0.07	0.63 ± 0.16	0.84 ± 0.05
Amino acids						
2 Valine	0.095 ± 0.011	0.068 ± 0.017	0.154 ± 0.008 ↑	0.102 ± 0.006	0.069 ± 0.009 ↓	0.080 ± 0.022
4 Isoleucine	0.031 ± 0.003	0.015 ± 0.002 ↓	0.071 ± 0.01 ↑	0.021 ± 0.001	0.013 ± 0.003 ↓	0.012 ± 0.002 ↓
12 GABA	0.11 ± 0.01	0.14 ± 0.01 ↑	0.13 ± 0.01 ↑	0.13 ± 0.01	0.093 ± 0.006 ↓	0.16 ± 0.02
7 Serine	0.093 ± 0.014	0.094 ± 0.007	0.124 ± 0.015 ↑	0.080 ± 0.005	0.037 ± 0.004 ↓	0.074 ± 0.008
8 Threonine	0.078 ± 0.006	0.066 ± 0.008 ↓	0.115 ± 0.025 ↑	0.066 ± 0.005	0.029 ± 0.004 ↓	0.046 ± 0.003 ↓
11 Proline	0.12 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.083 ± 0.004	0.041 ± 0.006 ↓	0.060 ± 0.016
13 Glutamic acid	0.17 ± 0.01	0.28 ± 0.02 ↑	0.28 ± 0.015 ↑	0.13 ± 0.01	0.074 ± 0.007 ↓	0.14 ± 0.01
19 Aspartic acid	0.042 ± 0.011	0.054 ± 0.012	0.125 ± 0.029 ↑	0.060 ± 0.002	0.057 ± 0.003	0.065 ± 0.003
Polyols						
3 Glycerol	0.98 ± 0.23	0.97 ± 0.24	0.65 ± 0.04	0.89 ± 0.11	0.68 ± 0.05	0.96 ± 0.14
21 Myo-inositol	0.21 ± 0.03	0.20 ± 0.03	0.25 ± 0.02	0.16 ± 0.01	0.13 ± 0.02	0.15 ± 0.01
22 Phytol	0.125 ± 0.032	0.077 ± 0.014	0.174 ± 0.059	0.109 ± 0.005	0.099 ± 0.010	0.138 ± 0.016

↑, Significant increase compared with control (t-test, $p < 0.05$); ↓, significant decrease compared with control (t-test, $p < 0.05$)

T0h control of TN1 without BPH infestation, *T24h* TN1 infested by BPH for 24 h, *T48h* TN1 infested by BPH for 48 h, *Y0h* control of YHY15 without BPH infestation, *Y24h* YHY15 infested by BPH for 24 h, *Y48h* YHY15 infested by BPH for 48

metabolites (Tables 2, S2), which were also mainly primary metabolites. All identified leaf sheath metabolites and honeydew metabolites had relative peak area values and the %RSD of each treatment group is shown in Tables S1 and S2. Figures S1 and S2 show typical total ion chromatograms of the rice plant leaf sheath and honeydew, respectively.

3.3 Metabolic profiling with BPH-induced metabolic changes for both BPH-resistant (YHY15) and BPH-susceptible (TN1) rice plants

In order to visualize the classification of BPH which induced metabolites in the rice plants of BPH-susceptible (TN1) and BPH-resistant (YHY15) rice plants, we firstly performed a

Table 2 Metabolites relative peak area (mean ± SEM) measured by GC–MS from BPH honeydew and the changes of metabolites in honeydew of Biotypes 1 and Y, respectively, when fed on TN1 and YHY1

| Metabolites	Changes in Biotype 1		Changes in Biotype Y	
	B1T	B1Y	BYT	BYY
Sugars				
20 D-Ribofuranose	0.16 ± 0.11	0.74 ± 0.42	1.16 ± 0.69	2.08 ± 1.12
21 Xylopyranose	0.89 ± 0.46	0.46 ± 0.19	1.01 ± 0.44	1.05 ± 0.35
24 Fructose	149.6 ± 41.20	168.3 ± 61.49	178.2 ± 50.47	214.4 ± 71.08
25 Glucose	66.91 ± 21.92	45.75 ± 17.70	127.4 ± 43.64	149.1 ± 49.86
32 α-D-Glucopyranoside	155.5 ± 50.37	167.7 ± 73.26	276.8 ± 79.19	287.6 ± 100.3
Organic acids				
5 Phosphate	20.28 ± 3.96	31.83 ± 10.70	20.36 ± 5.70	30.15 ± 11.13
7 Succinic acid	0.47 ± 0.41	1.40 ± 0.86 ↑	5.52 ± 1.63	3.23 ± 1.00
8 Glyceric acid	0.17 ± 0.01	0.26 ± 0.11	0.47 ± 0.10	0.36 ± 0.04
9 Fumaric acid	0.073 ± 0.07	0.24 ± 0.13	0.57 ± 0.10	0.50 ± 0.13
12 Malic acid	0.62 ± 0.32	5.43 ± 2.28 ↑	13.52 ± 4.31	22.52 ± 6.00
14 α-Hydroxypyruvic acid	0.33 ± 0.07	0.42 ± 0.13	0.33 ± 0.03	0.42 ± 0.07
18 Trans-aconitic acid	0.061 ± 0.019	0.071 ± 0.017	0.077 ± 0.015	0.090 ± 0.005
22 Shikimic acid	3.03 ± 1.35	8.54 ± 4.76	6.76 ± 1.75	11.63 ± 3.87
23 1,2,3-Propanetricarboxylic acid	2.93 ± 0.71	5.50 ± 2.66	13.50 ± 2.36	13.32 ± 1.41
26 Ribonic acid	2.29 ± 1.10	3.12 ± 0.39	0.18 ± 0.05	0.40 ± 0.14
Fatty acids				
27 Hexadecanoic acid	6.91 ± 1.51	5.88 ± 1.37	5.70 ± 1.33	11.99 ± 2.56
31 Hexadecanoic acid,(2S)-2,3-dihydroxypropyl ester	0.89 ± 0.17	0.82 ± 0.21	0.89 ± 0.12	1.51 ± 0.19 ↑
30 Octadecanoic acid	4.48 ± 1.36	5.44 ± 1.29	5.59 ± 1.34	12.31 ± 2.50 ↑
33 Octadecanoic acid, 2,3-dihydroxypropyl ester	3.59 ± 0.83	3.47 ± 1.0	2.21 ± 0.79	4.25 ± 1.40
Amino acids				
1 Valine	2.63 ± 0.64	0.43 ± 0.17 ↓	1.54 ± 0.34	0.21 ± 0.08 ↓
3 Leucine	1.34 ± 0.54	0.19 ± 0.08 ↓	1.92 ± 0.51	0.20 ± 0.04 ↓
6 Glycine	0.30 ± 0.13	0.40 ± 0.30	0.75 ± 0.14	0.14 ± 0.12 ↓
10 Serine	7.19 ± 3.08	0.34 ± 0.28 ↓	1.55 ± 0.72	0.045 ± 0.008 ↓
11 Threonine	6.08 ± 1.82	0.30 ± 0.23 ↓	1.37 ± 0.46	0.055 ± 0.027 ↓
13 Proline	19.75 ± 4.06	3.80 ± 1.66 ↓	17.25 ± 4.83	0.41 ± 0.16 ↓
15 Phenylalanine	6.71 ± 2.39	1.59 ± 1.24	3.52 ± 0.70	0.20 ± 0.06 ↓
16 Asparagine	0.12 ± 0.03	0.31 ± 0.24	0.45 ± 0.16	0.042 ± 0.007 ↓
17 α-Aminoadipic acid	0.095 ± 0.022	0.039 ± 0.004	0.30 ± 0.09	0.063 ± 0.017 ↓
19 Glutamine	8.77 ± 3.16	0.66 ± 0.24 ↓	3.84 ± 1.22	0.71 ± 0.13 ↓
29 Tryptophan	0.72 ± 0.31	0.43 ± 0.06	1.30 ± 0.29	0.28 ± 0.03 ↓
Polyols				
4 Glycerol	0.19 ± 0.10	0.22 ± 0.06	0.0 ± 0.0	1.15 ± 0.49 ↑
28 Myo-inositol	4.61 ± 1.27	5.09 ± 1.79	6.84 ± 1.65	6.77 ± 1.17
Others				
2 Urea	0.023 ± 0.012	0.084 ± 0.024 ↑	0.044 ± 0.011	0.081 ± 0.011 ↑

↑, Significant increase compared with control (t-test, $p < 0.05$); ↓, significant decrease compared with control (t-test, $p < 0.05$)

B1T Biotype 1 BPH fed on TN1 (control of Biotype 1), *B1Y* Biotype 1 fed on YHY15, *BYT* Biotype Y fed on TN1 (control of Biotype Y), *BYY* Biotype Y fed on YHY15

PCA. Figure 2a showed the PCA scores of the metabolites of TN1 and YHY15 that was infested with BPH for 0 h (i.e., without BPH infestation as control, T0h), 24 h (T24h) and 48 h (T48h). In Fig. 2a, 57.2 % of the total variation were explained by PC1 (36.6 %) and PC2 (20.6 %). Each point represented the rice metabolite composition (i.e.,

Fig. 2 PCA scores plots rice leaf sheath and honeydew metabolites. **a** PCA scores plots of rice leaf sheath metabolites of TN1 and YHY15 infested by BPH for 0 h (control), 24 h and 48 h. *Symbols filled squares* TN1 plants with BPH treatments for 0 h, *filled circles* TN1 plants with BPH treatments for 24 h, *filled diamonds* TN1 plants with BPH treatments for 48 h, *up pointing triangles* YHY15 plants with BPH treatments for 0 h, *down pointing triangles* YHY15 plants with BPH treatments for 24 h, *asterisks* YHY15 plants with BPH treatments for 48 h. **b** PCA scores *plots of* metabolites in honeydews of Biotypes 1 and Y after feeding on TN1 and YHY15. *Symbols filled squares* Biotype 1 fed on TN1, *filled circles* Biotype 1 fed on YHY15, *filled diamonds* Biotype Y fed on TN1, *up pointing triangles* Biotype Y fed on YHY15
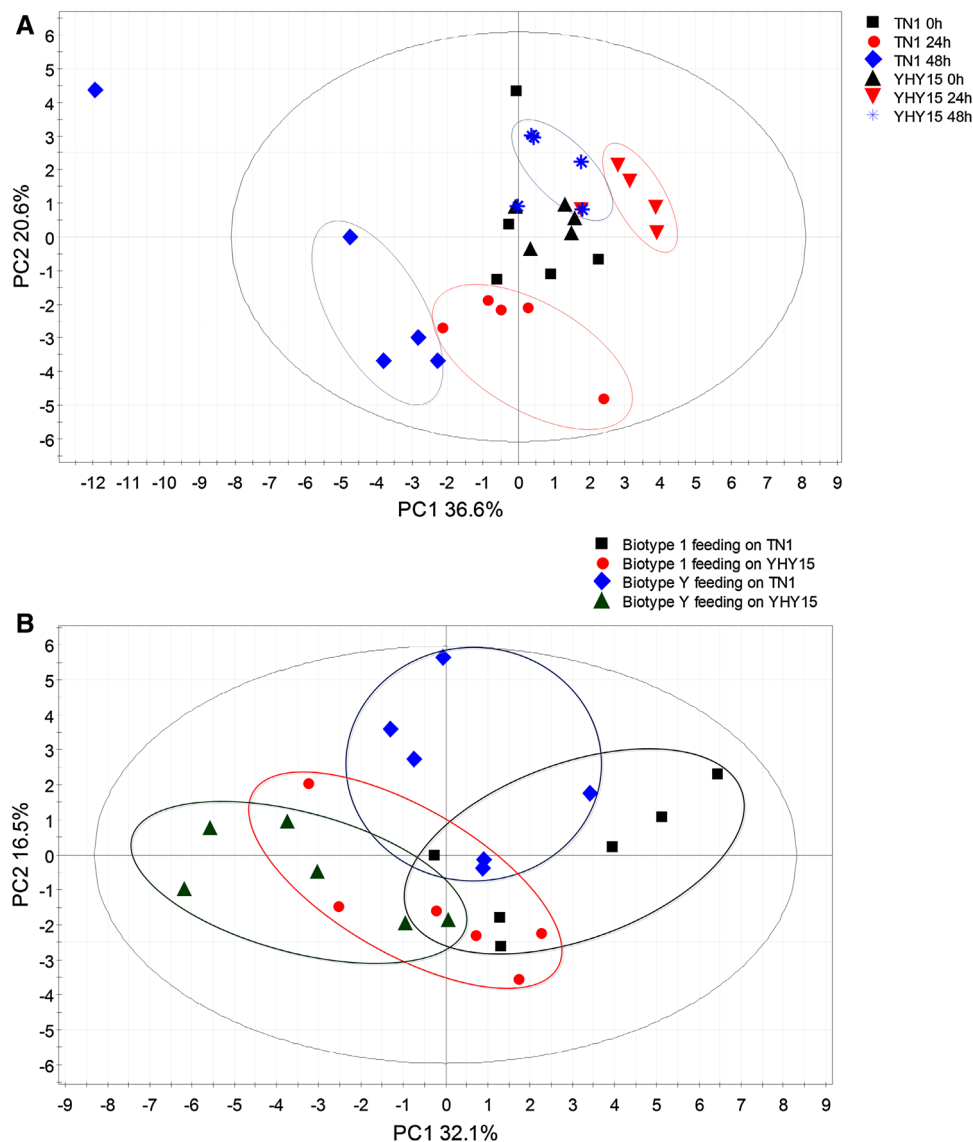
metabonome) of each sample. The metabolites that had the largest positive loading values were oxalic acid, glycerol, shikimic acid, salicylic acid (SA). The largest negative loading values were glucose, myo-inositol, fructose, threonine, isoleucine (Fig. S3). PCA scores plot showed that the best discrimination was due to PC1 of the PCA scores plot, with the most obvious discrimination being between T48h and Y24h. It can be seen from Fig. 2a that T24h and T48h were obviously discriminated from Y24h and 48 h, while it was not obviously discriminated between T0h and Y0h. These results showed that the difference of metabolites change between TN1 and YHY15 was mainly caused by BPH feeding instead of genetic background difference between two varieties. Resistance gene may through unique resistance mechanism in response to BPH infestation.

In order to further understand how leaf sheath metabolites are affected by different BPH treatments, we performed partial least squares-discriminant analysis (PLS-DA), which showed obvious differences among the various treatments (Fig. 3). In TN1 (Fig. 3a), the PLS results showed that the model had good quality, with each group being obviously distinguished [R2X(cum) = 0.663, R2Y(cum) = 0.885, Q2(cum) = 0.701]. The three different BPH-treated periods were clearly separated, mainly in the PLS1 dimension, with the difference between T0h and T48h being the most obvious (PLS1 score = 32.8 %). In the PLS2 dimension, the separation between T0h and T24h was the most obvious (PLS2 score = 26.2 %). The PLS-DA loadings plot (Fig. 3b) showed the variable influence on the separation.
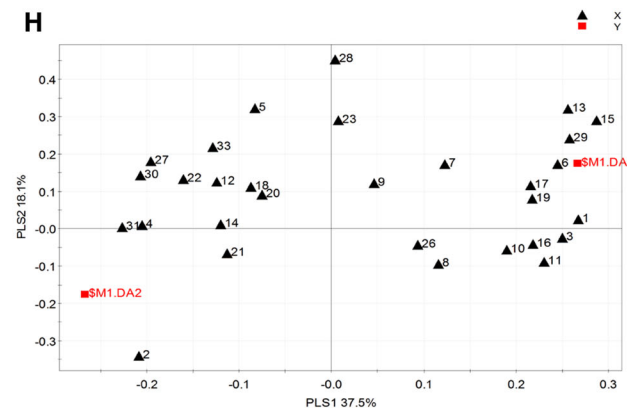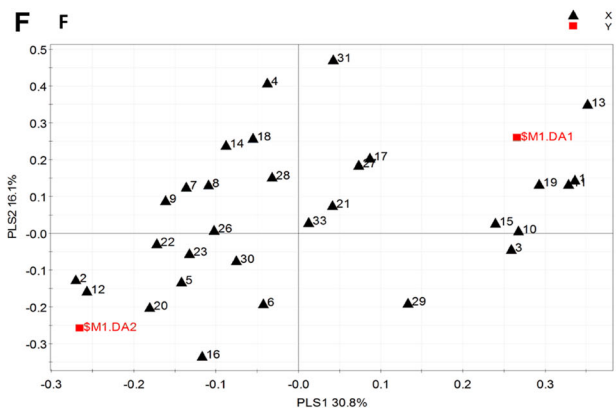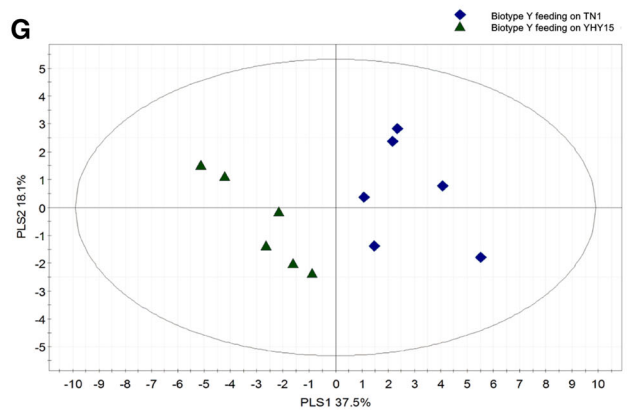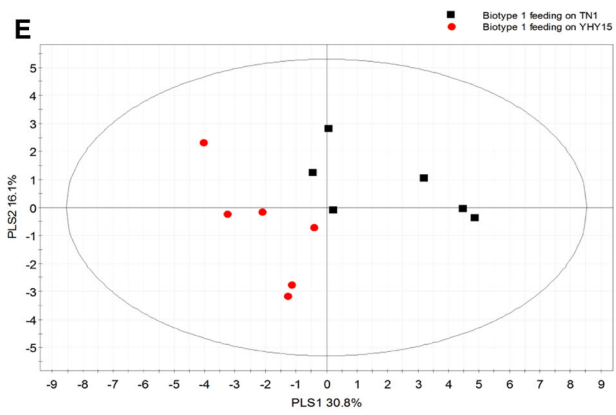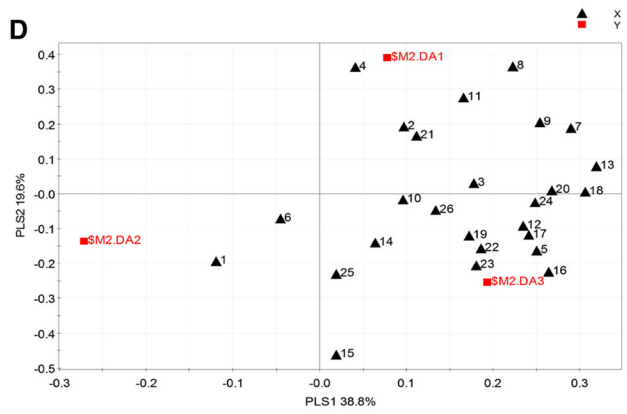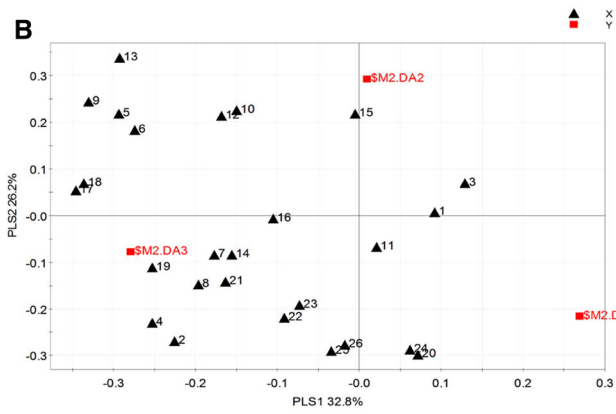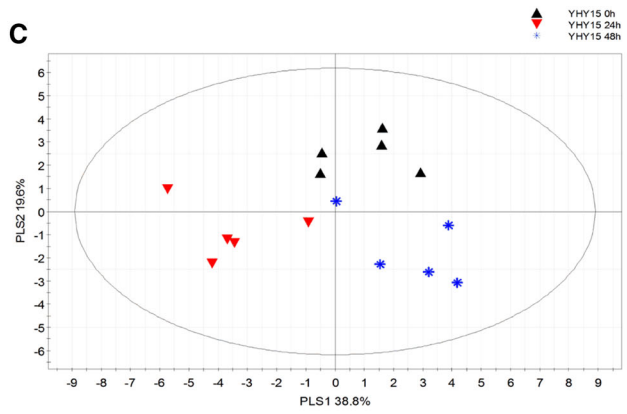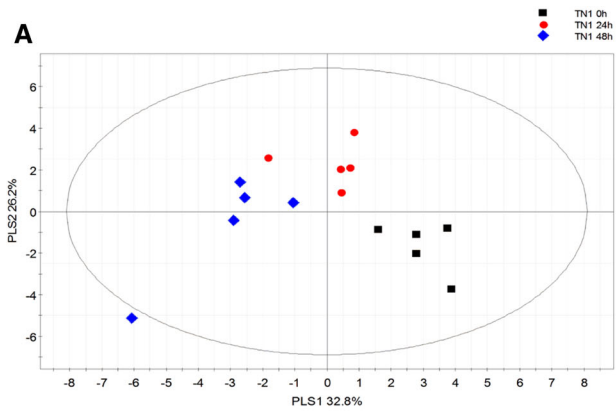
Fig. 3 PLS-DA scores *plots* and loading *plots* of rice leaf sheath and honeydew metabolites. **a** TN1 PLS-DA scores plot obtained from metabolic profiles of leaf sheaths of TN1 plants with BPH treatments for 0 h (control), 24 h and 48 h, *symbols filled squares* TN1 plants with BPH treatments for 0 h, *filled circles* TN1 plants with BPH treatments for 24 h, *filled diamonds* TN1 plants with BPH treatments for 48 h. **b** TN1 PLS-DA loading plot. **c** YHY15 PLS-DA scores *plot* obtained from metabolic profiles of leaf sheaths of YHY15 plants with BPH treatments for 0 h (control), 24 h and 48 h, *symbols up pointing triangles* YHY15 plants with BPH treatments for 0 h, *down pointing triangles* YHY15 plants with BPH treatments for 24 h, *asterisks* YHY15 plants with BPH treatments for 48 h. **d** YHY15 PLS-DA loading *plot*. **e** Biotype 1 PLS-DA scores *plot* obtained from metabolic profiles of honeydews of Biotype 1 after feeding on TN1 and YHY15, *symbols filled squares* Biotype 1 fed on TN1, *filled circles* Biotype 1 fed on YHY15. **f** Biotype 1 PLS-DA loading *plot*. **g** Biotype Y PLS-DA scores plot obtained from metabolic profiles of honeydews of Biotype Y after feeding on TN1 and YHY15, *symbols filled diamonds* Biotype Y fed on TN1, *up pointing triangles* Biotype Y fed on YHY15. **h** Biotype Y PLS-DA loading *plot*

For YHY15 (Fig. 3c), the PLS model also differentiated between each group [R2X(cum) = 0.780, R2Y(cum) = 0.955, Q2(cum) = 0.677]. PLS1 and PLS2 values were 38.8 and 19.6 %, respectively. Y0h was most clearly separated from Y24h and Y48h in the PLS2 dimension, while Y24h and Y48h were most clearly separated in the PLS1 dimension. The PLS-DA loadings plot (Fig. 3d) showed the variable influence on the separation.

Combining with VIP value (VIP >1) of PLS-DA model and t-tests, we screened metabolites which levels changed significantly comparing with 0 h (i.e., without BPH infestation as control) if t-tests gave p < 0.05. The relative levels of the identified metabolites with VIP >1 were selected to show in Fig. S7. The metabolite heat map of Fig. S5 was generated to study the variable differences between the control and BPH treatments. We first screened any significantly changed metabolites in TN1 (Table 1; Fig. S7). Proteinaceous isoleucine and threonine were reduced in TN1 after being fed for 24 h, but levels of glutamic acid increased. Isoleucine, valine, glutamic acid, serine, threonine and aspartic acid contents increased at 48 h. Levels of the non-protein amino acid GABA increased at 24 and 48 h. The organic acids, succinic acid, glyceric acid, malic acid and SA showed trends of similar changes that increased gradually at 24 and 48 h, the α-ketoglutaric acid content increased at 48 h. Among the fatty acids, levels of hexadecanoic acid were reduced at 24 h. The sugars, glucose and fructose were significantly raised after 24 and 48 h.

Next, we distinguished changes of metabolites in YHY15 (Table 1; Fig. S7). Valine, isoleucine, serine, proline, threonine, and glutamic acid levels were reduced at 24 h. At 48 h, valine, proline, glutamic acid, serine levels returned to those found at 0 h, while isoleucine and threonine contents at 48 h decreased further. The non-protein

amino acid GABA, though reduced at 24 h, returned to the 0 h level at 48 h. The organic acids malic acid, and α-ketoglutaric acid showed the similar trends of a reduction at 24 h, with succinic acid and α-ketoglutaric having increased at 48 h. The shikimic acid was on the rise at 24 and 48 h, and glyceric acid and SA did not change significantly.

3.4 Metabolic profiling with metabolic changes for Biotypes 1 and Y honeydews

Twenty-four honeydew samples were collected including from Biotype 1 feeding on TN1 (B1T), Biotype 1 feeding on YHY15 (B1Y), Biotype Y feeding on TN1 (BYT) and Biotype Y feeding on YHY15 (BYY). We conducted a PCA for these honeydew samples. In the PCA scores plot (Fig. 2b), the 24 samples clearly separated into four groups according to the different biotypes and their feeding on different rice plants. The first two axes, PC1 (32.1 %) and PC2 (16.5 %), together explained 48.6 % of the variation. Samples from the same biotype and feeding on same rice plant variety were clustered into the same group. According to the PCA scores plot, BYY was most clearly discriminated from B1T along the PC1 dimension, while the PC2 dimension separated B1Y and BYT. The honeydew metabolites that have the largest positive loading values are valine, threonine, serine, leucine, glutamine, phenylalanine, proline, tryptophan, asparagine and glycine. The largest negative loading values are hexadecanoic acid,(2S)-2,3-dihydroxypropyl ester, octadecanoic acid, shikimic acid, malic acid, hexadecanoic acid, urea, fumaric acid, 1,2,3-propanetricarboxylic acid, glycerol and α-hydroxypyruvic acid (Fig. S4).

We continued to construct a PLS-DA model for analyzing different metabolites. For Biotype 1 (Fig. 3e), the PLS model discriminated between B1T and B1Y in the PLS1 dimension: R2X(cum) = 0.468, R2Y(cum) = 0.779, Q2(cum) = 0.145. For Biotype Y (Fig. 3g), the PLS model also discriminated between BYT and BYY in the PLS1 dimension: R2X(cum) = 0.556, R2Y(cum) = 0.87, Q2(cum) = 0.655. Different metabolites of Biotypes 1 and Y from the PLS-DA loading plot are shown in Fig. 3f, h.

The relative levels of the identified metabolites with VIP >1 were partly selected to be shown in Fig. S8. The trends of changing metabolites are shown in Table 2 and Fig. S6. Metabolites changes were deem as significantly when B1Y comparing with B1T and BYY comparing with BYT if p value <0.05 of t-tests. When Biotype 1 feeding on YHY15 is compared with its feeding on TN1, the levels of most amino acids in honeydew decreased, for example, valine, leucine, serine, threonine, proline, phenylalanine and glutamine. But the levels of glycine, asparaginate and tryptophan showed little change. Among the organic acids, the

levels of succinic acid and malic acid were elevated; changes in the fatty acids hexadecanoic acid and octade-canoic acid were not obvious, while the level of urea increased. When Biotype Y feeding on YHY15 is compared with feeding on TN1, the levels of most amino acids in honeydew (valine, leucine, glycine, serine, threonine, proline, phenylalanine, asparagine, glutamine and trypto-phan) were reduced. The non-protein amino acid, α-aminoadipic acid was decreased too; hexadecanoic acid,(2S)-2,3-dihydroxypropyl ester and octadecanoic levels were increased; the level of urea was also raised.

4 Discussion

In the present study, we analyzed the changes in metabo-lites of leaf sheaths from the BPH-susceptible rice TN1, and the BPH-resistant rice YHY15, either without BPH infestation (control) or after being treated with BPH for 24 and 48 h. The results showed that the metabolite changes depended on the feeding time, which were obviously dif-ferent between TN1 and YHY15. The metabolites of TN1 changed to a greater extent and over a wider range of compounds than did those of YHY15. For example, changes of amino acids and some sugars and organic acids from TN1 were greater than YHY15. Transcriptome anal-yses of the *BPH15* introgression line and the susceptible recipient line response to BPH infestation showed that the resistant rice exhibited fewer changes in the expression of its *BPH15*, suggesting that the resistant rice was indeed more tolerant than susceptible rice (Lv et al. 2014). PCA for rice plants metabolites showed that it was not obviously discriminated between T0h and Y0h. PLS-DA results showed that TN1 and YHY15 metabolite changed at dif-ferent times after being infested by BPH. These results indicated that without BPH infestation the genotype dif-ference did not cause significant metabolites difference between TN1 and YHY15. However, after BPH infestation, resistance gene activating resistance response induced metabolites difference between TN1 and YHY15. In the PCA and PLS-DA plots of honeydew metabolites we can see that the difference (discrimination) in different biotype and meanwhile in feeding on different rice varieties. Overall, Biotype 1 get together with Biotype Y feeding on TN1 are discriminated from Biotypes 1 and Y feeding on YHY15.

4.1 BPH-induced changes in gluconeogenesis, GABA shunt, β-oxidation and glyoxylate cycle for TN1 rice plant

A previous study suggests that BPH feeding will enhance gluconeogenesis in BPH-susceptible and BPH-resistant rice plants, probably through the GABA shunt and TCA cycle, and so increase the GABA bypass (GABA shunt) to relieve ROS-induced cytotoxicities (Liu et al. 2010). Under insect attack, excess ammonia would accumulate. Since plant cells often convert ammonium ions into asparagine or glutamine, etc., these transamination products can be metabolized through the citrate cycle via α-ketoglutaric acid, oxaloacetic acid, and succinic acid (i.e., the GABA shunt) (Skopelitis et al. 2006). Our present results have shown that BPH infestation caused fructose and glucose levels to rise sharply in TN1, while causing accumulations of aspartic acid and glutamic acid (Table 1; Fig. S7). This result clearly indicates that gluconeogenesis was promoted (Fig. 4). Under BPH attack, the TN1 GABA level was significantly increased and accompanied elevations of glutamic acid and α-ketoglutaric acid levels. It was sug-gested that BPH infestation activated the GABA shunt in TN1. SA content in 24 and 48 h samples were above those in the control group (0 h), which showed that BPH attack also activated the SA-mediated defense in TN1.

After BPH attacked for 48 h, hexadecanoic acid con-tents in TN1 were decreased compared with the control (Table 1; Fig. S7), indicating consumption of fatty acids. When plants are attacked, constitutive defense traits have costs that outweigh their benefits. This suggests that defense-related products are autotoxic (Baldwin and Cal-lahan 1993) or that resistance is energetically costly (Heil et al. 2000; Smedegaard-Petersen and Tolstrup 1985; Zangerl et al. 1997). Thus, the degradation of fatty acids during β-oxidation is a potential energy source during plant defense (Bolton 2009). The BPH treatments on TN1 for 24 and 48 h led to an elevation of TCA intermediates such as malic acid, glyceric acid and succinic acid. We speculate that fatty acids, via β-oxidation, produce an amount of acetyl-CoAs, and that these acetyl-CoAs, are then metab-olized via the glyoxylate cycle to produce succinic acid, and malic acid. Succinic acid and malic acid is then con-verted to oxaloacetic acid that, through the TCA cycle, enters the gluconeogenesis pathway to form glucose (Fig. 4). Meanwhile, β-oxidation of fatty acids can provide ATP for gluconeogenesis. We speculate that BPH feeding promoted β-oxidation, and that this β-oxidation caused hexadecanoic acid contents to decrease. The pathway oxidation of a fatty acid has been shown to be upregulated during the resistance response to several pathogens (Bolton et al. 2008; Schenk et al. 2003). In plants, the β-oxidation of fatty acids can produce H_2O_2, which plays an important role in plant defenses (Kawano 2003). A previous study has shown that pathogenic attack by the fungus *Botrytis cinerea* on soybean leaves induces the carbon reallocation mechanism based on the re-initiation of the glyoxylate cycle (pseudo-senescence of the infected leaves) (Cots et al. 2002).
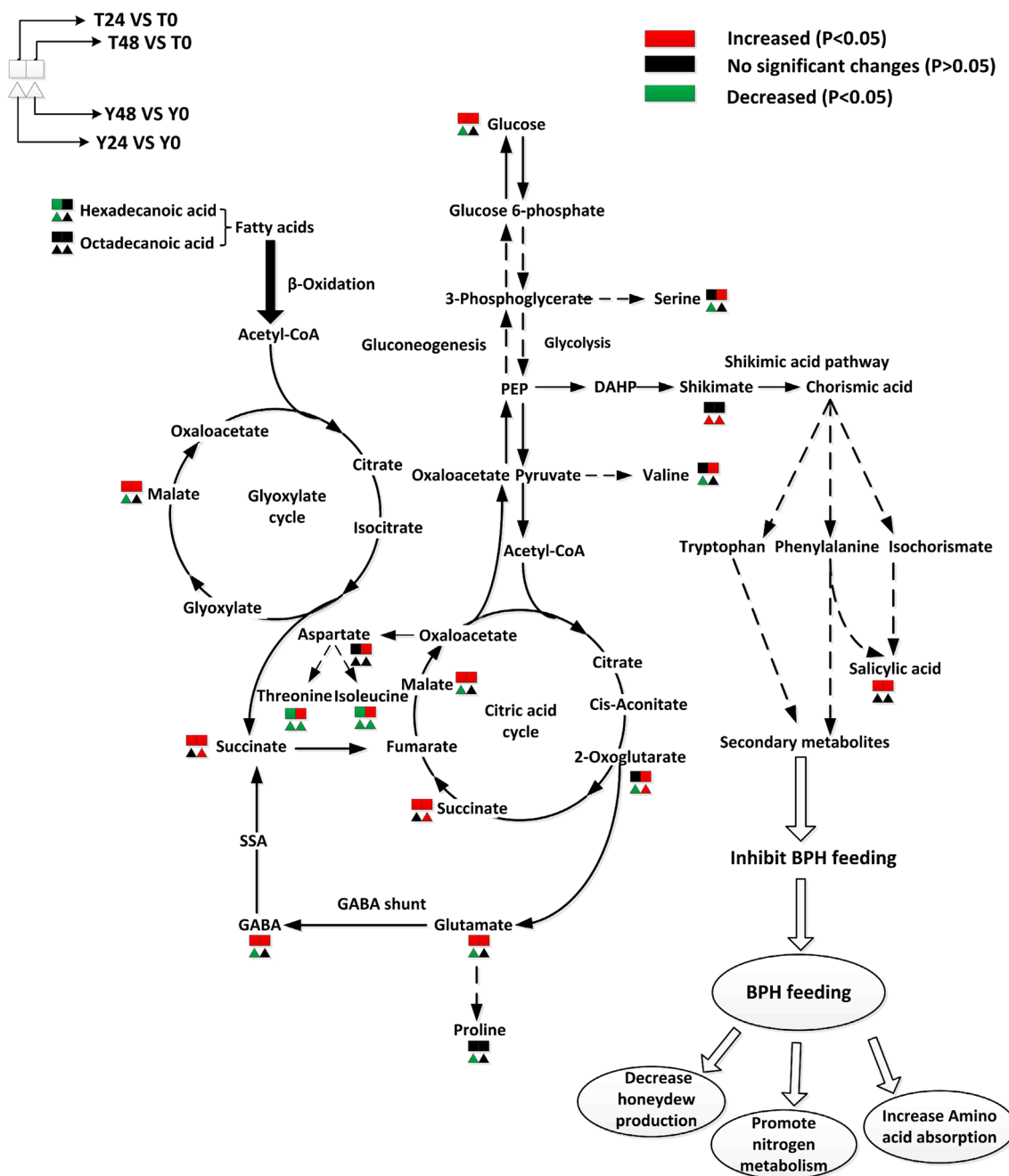
Fig. 4 Changes in the metabolic pathways of TN1 and YHY15 induced by BPH feeding. T24 versus T0 means the comparison between TN1 treated with BPH for 24 h and for 0 h (control), T48 versus T0 means the comparison between TN1 treated with BPH for 48 h and for 0 h (control), Y24 versus Y0 means the comparison between YHY15 treated with BPH for 24 h and for 0 h (control), Y48 versus Y0 means the comparison between YHY15 treated with BPH for 48 h and for 0 h (control). *Red symbols* denote significant increases (p < 0.05), *black symbols* denote no significant changes (p > 0.05), *green symbols* denote significant decreases (p < 0.05)

4.2 BPH-induced changes in glycolysis and shikimic acid pathway for YHY15 rice plant

During the first 24 h of BPH feeding on YHY15, glucose, hexadecanoic acid, malic acid, and α-ketoglutaric acid levels all decreased (Table 1; Fig. S7). It is suggested that BPH feeding promoted β-oxidation and glycolysis rather than gluconeogenesis (Fig. 4). Meanwhile, the shikimic acid level steadily increased within 24 and 48 h. This result suggests that YHY15 resistance to BPH feeding may operate through the shikimic acid synthesis of secondary metabolites. Shikimate is the first identified intermediate of the shikimate pathway. In the shikimate pathway, the primary metabolites *p*-hydroxybenzoate, phenylalanine,

tyrosine, tryptophan and *p*-aminobenzoate, are all precursors of secondary metabolites (Herrmann 1995; Maeda and Dudareva 2012; Weaver and Herrmann 1997).

In the initial phase of BPH feeding, the breaking down of glucose into molecules that may enhance glycolysis can provide a large number of phosphoenolpyruvic acids as the initial substrate to produce shikimic acid. In the early phase of BPH infestation, i.e., within 24 h, YHY15 may need energy to activate its defensive reaction. After BPH had been feeding for 48 h, the sugar and fatty acid contents were restored to the 0 h level, but the content of shikimic acid was high, suggesting that synthesis of secondary metabolites continued.

4.3 The difference of BPH response mechanism between TN1 and YHY15

At the beginning of BPH infestation in TN1, we deduced energy comes from fatty acid oxidation that provides acetyl-CoA to the glyoxylate cycle, which produces oxaloacetic acid for gluconeogenesis. The TN1 response to BPH infestation may be related to the GABA bypass and SA. Plants accumulate GABA to resist insect infestation (Narayan and Nair 1990; Bown et al. 2006; Shelp et al. 2012; Bouche and Fromm 2004; Kinnersley and Turano 2000). The GABA shunt is associated with the Ca^{2+} signaling pathway for regulating genes related to the Ca^{2+} signaling pathway and affects Ca^{2+} concentrations in response to abiotic stresses (Gut et al. 2009; Bouché et al. 2003). A previous study indicated that *BPH14* is closely related to BPH resistance and SA (Du et al. 2009). SA as a signal molecule plays a critical role in local defenses and in systemically acquired resistance (Shah 2003; Arimura et al. 2005; Park et al. 2007).

The shikimic acid content in YHY15 increased significantly, which indicated the importance of the shikimic acid pathway to its resistance mechanism. It is well known that the shikimate pathway is critical for producing secondary metabolites as a defense against insect herbivores (Bennett and Wallsgrove 1994; Paré and Tumlinson 1999). Secondary metabolites in rice play an important role in various stress responses and resistance mechanisms. Chen et al. (2014) detected hundreds of metabolites in rice by applying widely targeted metabolomics. They identified two major classes of subspecies-specific metabolites: C-glycosylated flavonoids and phenolamides (Chen et al. 2014). It is interesting that both of these classes have been reported to have indispensable roles in chemical defenses against biotic and abiotic stresses (Kaur et al. 2010; Luo et al. 2009). Investigation of the rice transcriptome following BPH feeding revealed the activation of a wide and complex response. The expression of genes related to secondary metabolites resulted in there being a clear difference

between resistant the *BPH15* introgression line and the susceptible recipient line (Lv et al. 2014).

The defense mechanism that depends on SA is extensive, and in susceptible rice, SA may also be involved in basal defense. Liu et al. indicated that resistant rice B5 (which contains both *BPH14* and *BPH15*) and susceptible rice TN1 have some similarities in their response mechanisms, which we deduce may be mediated by SA (Du et al. 2009). The major resistance gene in YHY15 is *BPH15*. According to fine-scale mapping results (Lv et al.) there is no NB-LRR gene. Furthermore, that they found no clear accumulation of SA is in agreement with our present study (Lv et al. 2014). On the basis of our study, we conclude that the *BPH15* mediated resistance mechanism may not be consistent with *BPH14*, and that *BPH15* is closely associated with the shikimic acid pathway. Its resistance depends on the shikimic acid pathway directly synthesizing secondary metabolites inimical to BPH.

4.4 Metabolite changes in honeydew of BPH Biotypes 1 and Y after being fed on YHY15

As BPH suck phloem saps of rice during the process of feeding, they continually absorb nutrients such as amino acids and sugars present in the sap, and continually excrete unused amino acids, sugars and water in the form of honeydew (Sōgawa 1970). A qualitative and quantitative analysis of honeydew can therefore offer valuable information about the sucking sites and the rate of sucking (Sōgawa 1982). The deposition of honeydew on host plants also has a significant effect on plant defenses and herbivore recognition, and can be an important factor in the interaction between a herbivore and its host plant (Schwartzberg and Tumlinson 2014). Thus, changes of metabolites in honeydew can dynamically reflect the utilization of rice phloem saps in the BPH digestive tract and BPH metabolic physiology when feeding on different rice varieties.

Our study has shown that changes in the metabolite composition of honeydew differed between Biotypes 1 and Y, and depended on whether feeding occurred on TN1 or YHY15. It indicated that the proportions of various metabolites were not static, but changed with the host plant. The most obvious change of metabolites in honeydew of Biotypes 1 and Y when feeding on YHY15 compared with feeding on TN1 was the amino acid contents. After feeding on YHY15, the levels of most amino acids declined (Table 2; Fig. S8). We deduced that this was due to BPH feeding being suppressed. Because the plant's resistance response may produce anti-feedant substances that might reduce the rate at which BPH sucked phloem saps, BPH might compensate by enhancing their utilization of the amino acids they had acquired from those phloem saps, rather than excrete them.

In BPH, yeast-like symbionts (YLSs) can synthesize all essential amino acids (Xue et al. 2014). Previous research has indicated that there exists a diversity of symbionts in BPHs which is probably related to the range of different host biotypes (Tang et al. 2010). Thus, there are other possible mechanisms that resistant rice may have on BPH: for example by causing its YLS to impede the synthesis of essential amino acids.

Most terrestrial animals convert ammonia to either urea or uric acid compounds that can be concentrated in body fluids to a greater extent than ammonia with no toxic effect (Wright 1995). Most insects excrete nitrogenous waste as uric acid (or as its urate salts, such as ammonium urate); some excrete it as hypoxanthines, other purines, allantoin, allantoic acid, urea, certain amino acids, tryptophan derivatives, or pteridines (Cochran 1975, 1985). Some insects' detoxification, for example *Aedes aegypti* mosquito, usually are through glutamic acid and proline synthesis, along with the excretion of ammonia, uric acid, and urea (Scaraffia et al. 2005, 2008; Esquivel et al. 2014). In our study we found that when Biotypes 1 and Y BPHs fed on YHY15 the urea level in their honeydew was up-regulated compared with those fed on TN1. This result suggested that after BPH feeding on YHY15, the ammonia metabolism may be promoted for detoxification. It may due to absorb secondary metabolism products form resistance rice YHY15 which are toxic or repellant to BPH.

5 Conclusion

By adopting a metabolomics approach, we investigated the differences between the response mechanisms of YHY15 and TN1. Our results have shown that metabolites change according to the duration of feeding time, and that the extent of metabolite variation in such compounds as amino acids, sugars and organic acids, was greater in the susceptible TN1 than in the resistant YHY15. BPH infestation of TN1 resulted in fatty acid oxidation, the glyoxylate cycle, gluconeogenesis, and the GABA shunt being enhanced, while BPH infestation of YHY15 enhanced glycolysis and the shikimate pathway (Fig. 4). Our results indicate that susceptible and resistant rice plants with *BPH15* gene use different metabolic pathways to respond to BPH infestation. We also analyzed the metabolites in honeydew of BPH Biotypes 1 and Y insects fed on TN1 and YHY15 rice plants, respectively, which indicated that BPH feeding on YHY15 enhanced amino acid absorption and increased cerebral ammonia metabolism to relieve the toxic stress from the plant's secondary metabolites.

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