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Analysis of metabolome and microbiome revealed the resistance mechanisms in sugarcane cultivars with high resistance to pokkah boeng disease

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Abstract

Background Endophytes are reported to play a role in resistance against plant pathogens. Understanding the metabolite-mediated endophytic microbiota composition in plants provides insights to improve plant stress resistance. In this study, via metabolome and microbiome analyses, we aimed to elucidate the resistance mechanism of sugarcane cultivars with high resistance to sugarcane pokkah boeng disease (PBD). The endophytic microbial composition and metabolites in the stems of various sugarcane cultivars with high resistance (HR) or high susceptibility (HS) to PBD were analyzed.

Results The results revealed that the endophytic fungi with biocontrol effects such as *Shinella*, *Dechloromonas*, and *Microbacter* were significantly enriched, and the abundance of pathogenic fungi such as *Fusarium*, *Ramichloridium*, *Scleroramularia*, *Phaeosphaeriopsis*, *Sarocladium*, *Zygophiala*, *Gibberella*, *Pseudocercospora*, *Cyphellophora*, *Monocillium*, *Apiotrichum*, *Microsphaeropsis*, and *Scleroramularia* significantly reduced in the stems of HR cultivars. Additionally, six metabolites [citric acid, isocitrate, malic acid, PC(16:0/0:0), phosphocholine, and lysoPC(16:0)] were significantly related to the endophytes in the stems of HR cultivars.

Conclusions These results suggested that more abundance of antagonistic microbes and highly active metabolic functions of endophytes in the HR cultivars were the important mechanisms underlying their higher resistance to PBD.

Keywords Sugarcane pokkah boeng disease (PBD), Endophytic, Microbial compositions, Metabolome

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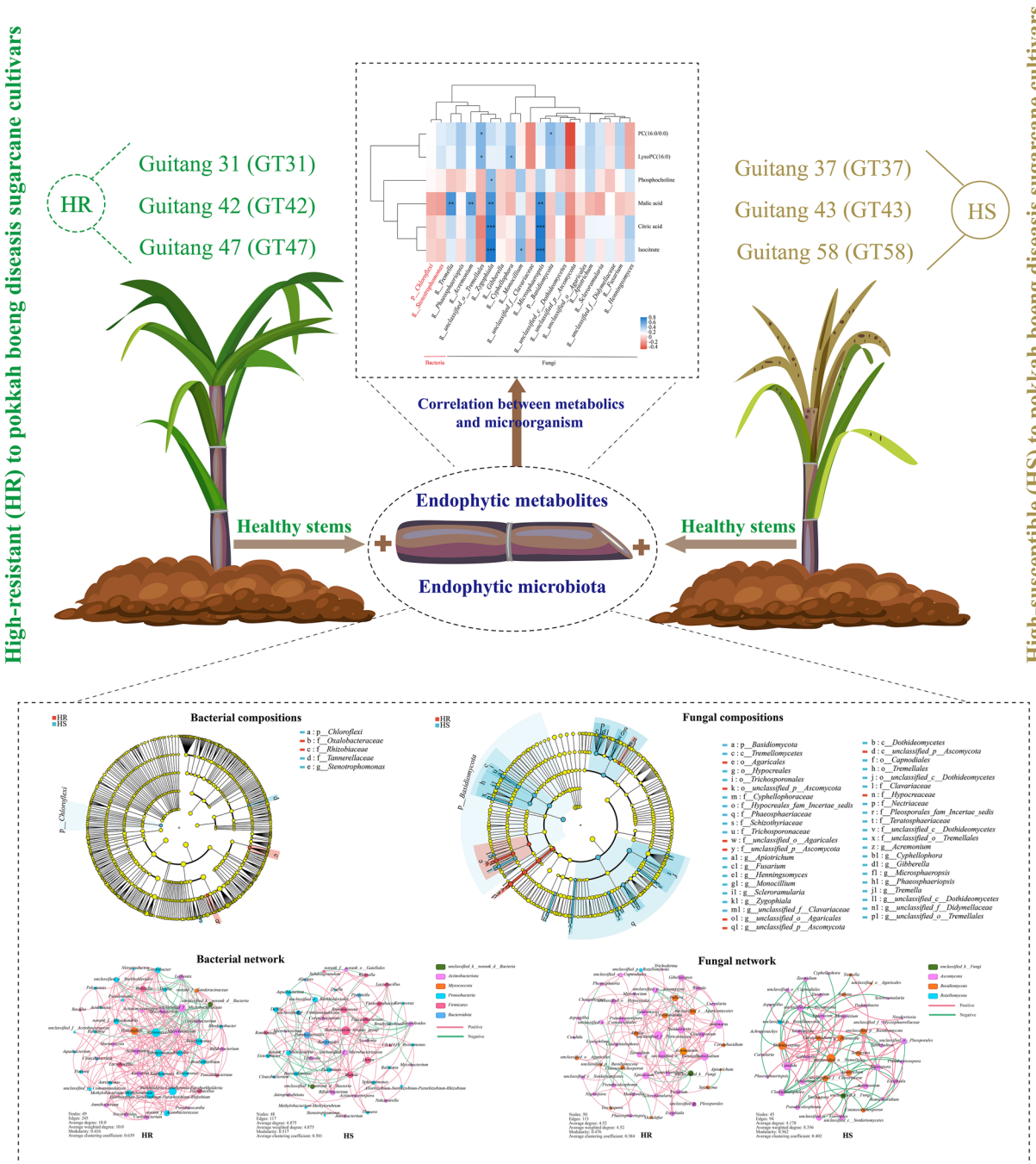
Graphical abstract



Background

Sugarcane (*Saccharum officinarum* L.) is an important renewable energy crop, contributing to 80% of sugar production worldwide [1–3]. Moreover, the byproducts of sugar production have diverse applications, e.g., ethanol is used as a solvent and disinfectant, and bagasse is used in paper making. During the whole growth process, sugarcane production can be affected by fungal, viral, and bacterial diseases. Among them, smut and pokkah boeng disease (PBD) are the most serious diseases [4].

PBD is a serious fungal disease in sugarcane worldwide. It is caused by various *Fusarium* spp., mainly *F. verticillioides* [5], *F. sacchari* [6], *F. proliferatum* [7], *F. subglutinans* [8], *F. oxysporum* [9], *F. fujikuroi* [10], and *F. andiyazi* [11]. Their intermediate hosts can be rice, sorghum, corn, banana, and pumpkin [12–14]. In 1896, PBD was reported in Java by Walker and Went for the first time [15]. Till now, it has been reported in various countries globally, including Brazil, Cuba, Egypt, India, United states, South Africa, Malaysia, and China [15, 16]. In recent years, because of the excessive application of chemical fertilizers, planting of susceptible cultivars, and climate change, the incidence of sugarcane PBD in China has become highly serious [17, 18]. Particularly, in Guangxi, which is the main sugarcane production area located in southwest China, high temperature and humidity from June to September promote the disease and lead to distortion of sugarcane top, brown necrotic spots, and even top rot in severe cases of PBD [19, 20]. It results in cane yield reduction (15–30 t ha^{-1} on an average) because of the lower cane height and smaller stems at the maturity stage [21].

Bavistin, blitox, copper oxychloride, dithane M-45 [19], and carbendazim [22] can be used as the chemical fungicides to manage PBD. However, the incidence of PBD is also related to the resistance abilities of sugarcane cultivars. Breeding and planting of PBD-resistant cultivars are the most common, economical, environment friendly, and effective methods for disease control [23]. In addition, application of biological control agents, which is another eco-friendly solution, needs to be studied considering the short- and long-term negative effects of fungicides on the environment and human health [20].

The sustainable mutually beneficial interactions between plants and microorganisms lead to change in important biological actives, as well as defense strategies against various abiotic and biotic stresses on plant growth and development [24]. In particular, endophytes may play a role in promoting host plant survival e.g., endophytes promote host plant growth and development by synthesizing and secreting certain metabolites [24] and play a role in protecting host plant from plant pathogens or other destructive agents [25]. Previous studies reported that numerous endophytes (including bacteria and fungi) could prevent plant diseases by producing potent antibacterial and antifungal substances [24, 26, 27]. Chlebek et al. [28] reported that endophytic *Pseudomonas* produces various antifungal metabolites, such as hydrogen cyanide, pyrrolnitrin, pyoluteorin, phenazine-1-carboxylic acid, and 2, 4-diacetylphloroglucinol, which could significantly slow down the growth and development of harmful fungi. Bolivar-Anillo et al. [29] reported that several endophytic *Bacillus* species produce diverse secondary metabolites that are physiologically active against numerous phytopathogenic fungi. In addition, Fontana et al. [30] reported that endophytic fungi could produce active compounds that protect plants from the damage. Microbial metabolic functions would further be affected by the changes in the microbial community [31].

At present, managing and preventing sugarcane PBD without any chemical agent has become increasingly important in sugar production. In this study, differences of endophytic microbial communities and the characteristics of their metabolites and metabolic functions in the stems of sugarcane cultivars with varying resistance to PBD were analyzed. This study will enhance the understanding of endophytic functions related to anti-PBD action and their underlying mechanisms.

Materials and methods

Experimental site, plant materials, and sample collection

Sugarcane samples were collected from the Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences (SRIGAAS), Longan County (107°598′′ E and 23°637′′ N), Nanning City, Guangxi Zhuang Autonomous Region, China. The experimental location had subtropical monsoon climate, with an altitude of 94.0 m, average annual temperature of 21.7 ℃, and annual precipitation of 1227–1691 mm. The experimental plot was flat, with good drainage and irrigation conditions. The prevalent soil type was quaternary red clay [1]. Before planting, the method [1, 3] previously described was used to measure the chemical properties of soil as follows: soil pH 5.87, soil organic matter (SOM) 28.03 g kg^{-1}, total nitrogen (TN) 1.48 g kg^{-1}, total phosphorus (TP) 0.87 g kg^{-1}, total potassium (TK) 9.54 g kg^{-1}, alkaline nitrogen (AN) 123.67 mg kg^{-1}, available phosphorus (AP) 47.67 mg kg^{-1}, and available potassium (AK) 272 mg kg^{-1}.

The test materials included three sugarcane cultivars with high resistance (HR) to PBD, namely, 'Guitang 31' (GT31), 'Guitang 42' (GT42), and 'Guitang 47' (GT47), and three sugarcane cultivars with high susceptibility (HS) to PBD, namely, 'Guitang 37' (GT37), 'Guitang 43' (GT43), and 'Guitang 58' (GT58). These sugarcane cultivars were bred by SRIGAAS with different genetic component. These six cultivars were cultivated in triplicate plots. Therefore, the study included 18 plots in total. All field managements including fertilization, irrigation, and weeding were as per our previous conventional field management program [1].

Sugarcane stem samples were collected from the 18 plots in December 2021. They were sampled and disinfected as described previously [1, 2].

Analysis of endophytic microbial diversity in sugarcane stems

From the sugarcane stems, whole DNA was extracted [32], amplified using PCR with primers for endophytic bacteria and fungi (Table 1), and sequenced as described in our previous study [32]. PCR was conducted using the GeneAmp® 9700 PCR thermocycler (ABI, Foster City, CA, United States). Sequencing data were processed and analyzed as described in our previous study [1, 2, 33, 34] and deposited in the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA998735).

Untargeted metabolomic analysis using LC–MS

In total, 50 mg sugarcane stem sample was accurately weighed in a 2-mL centrifuge tube. A frozen tissue grinder was used for grinding the sample for 6 min at − 10 °C and 50 Hz, followed by ultrasonic extraction for 30 min at − 5 °C and 40 kHz. Further, the sample was placed at − 20 °C for 30 min and centrifuged for 15 min at 13,000*g* and 4 °C. The supernatant was transferred to an injection vial. From each sample, 20 mL of the supernatant was taken and combined as the quality control sample.

The metabolomic analysis was performed using ultra-high-performance liquid chromatography tandem Fourier transform mass spectrometry (UHPLC-Q Exactive HF-X) system from Semefeld with the following chromatographic conditions: injection volume 2 L, column temperature 40 °C, mobile phase A: 95% water + 5% acetonitrile (containing 0.1% formic acid), mobile phase B: 47.5% acetonitrile + 47.5% isopropanol + 5% water (including 0.1% formic acid), and chromatographic column: the Waters ACQUTTY UPLC HSS T3 (100 mm × 2.1 mm I). Full scan mode was used to acquire the data, and the m/z range was 50–600. Chroma TOF software (version 4.34, LECO, St Joseph, MI) was used to process the GC–MS data after converting from ChemStation analysis files (version E.02.02.1431, Agilent, CA, United States) to net CDF format files. Chroma TOF was used to filter and calibrate baseline data, to extract raw peaks, and to detect and integrate peaks. The metabolites were identified using Tracerfinder 3.2 (Thermo Fisher Scientific). Multivariate analysis was performed using Majorbio Cloud Platform (https://www.majorbio.com).

Statistical analyses

Alpha-diversity, non-metric multidimensional scaling analysis (NMDS), principal co-ordinates analysis (PCoA) (Bray–Curtis distance), partial least squares discriminant analysis (PLS-DA), microbial community composition, Venn diagram analysis, linear discriminant analysis (LDA), and LDA effect size (LEfSe) analysis of microbial communities were conducted as described in our previous studies [1, 2, 32–34]. Networkx was used to calculate the correlation among the top-50 abundant microbial genera and construct the correlation network based on the Spearman coefficient ($r \geq 0.5$, $P < 0.05$). Gephi (v 0.10.2) was used to simplify and modify the network analysis graph. Wilcoxon rank-sum test was used to analyze the significant differences ($P < 0.05$). The Majorbio Cloud Platform (www.majorbio.com) was used to conduct online data analysis. Kyoto Encyclopedia of Genes and Genomes (KEGG, www.kegg.jp/kegg/kegg1.html) was used to analyze metabolic group data.

Results

Microbial alpha- and beta-diversities of the endophytes in sugarcane stems

The endophytic bacterial and fungal coverage rates reached 99.75% and 99.99% (Fig. 1a and i), respectively, indicating that the sequencing data were reliable. The endophytic bacterial diversity (Shannon and Simpson indices, Fig. 1b, c) and richness (Ace and Chao1 indices, Fig. 1d, e) exhibited no significant difference between the HR and HS cultivars ($P > 0.05$). In addition, the endophytic fungal diversity (Fig. 1j, k) was significantly lower in the stems of the HR cultivars than in those of the HS cultivars ($P < 0.05$). However, the richness and diversity of endophytic fungi exhibited no significant difference between the HR and HS cultivars ($P > 0.05$) (Fig. 1l, m). Moreover, the results of Bray-Curties PCoA and NMDS analyses indicated that the endophytic bacterial communities were similar (Fig. 1f, g) but the endophytic fungal communities were significantly different between both

Table 1 The primer sequence of endophytic bacteria and fungi

Type of microorganism	Primer name	Sequence	Regions	Amplification rounds	References
Endophytic bacterial	799F	5′-AACMGGATTAGATACCCKG-3′	V5-V7	First round	[2]
	1392R	5′-ACGGGCGGTGTGTRC-3′			
	799F	5′-AACMGGATTAGATACCCKG-3′		Second round	
	1193R	5′-ACGTCATCCCCACCTTCC-3′			
Endophytic fungal	ITS1F	5′-CTTGGTCATTTAGAGGAAGTAA-3′	ITS1	–	[2]
	ITS2R	5′-GCTGCGTTCTTCATCGATGC-3′			

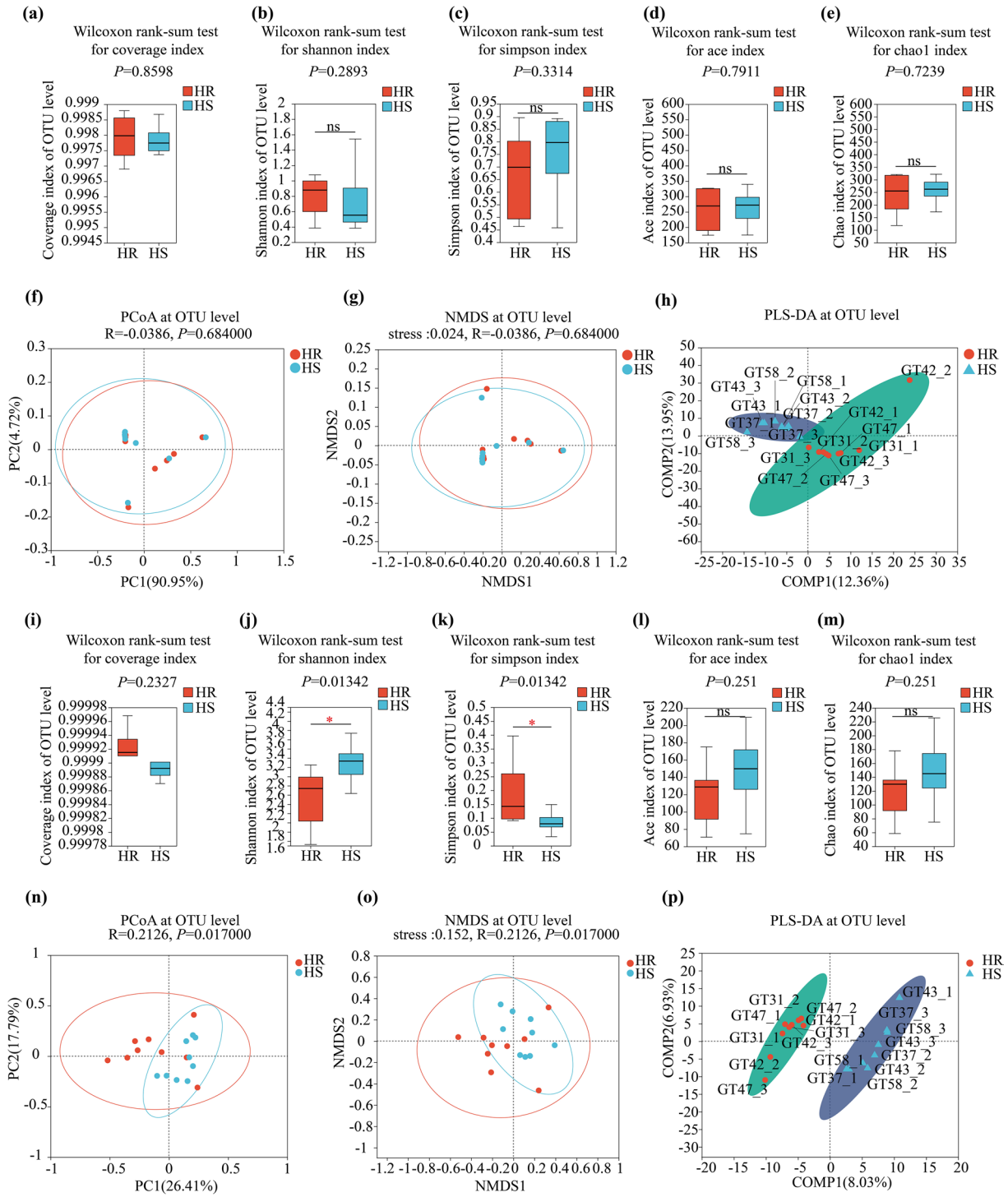


Fig. 1 Analysis of endophytic microbial alpha- and beta-diversities at the operational taxonomic unit level in the stems of sugarcane cultivars with high resistance (HR) and high susceptibility (HS) to pokkah boeng disease (PBD). **a** Endophytic bacterial coverage index; **b** endophytic bacterial Shannon index; **c** endophytic bacterial Simpson index; **d** endophytic bacterial Ace index; **e** endophytic bacterial Chao1 index; **f** endophytic bacterial PCoA analysis; **g** endophytic bacterial NMDS analysis; **h** endophytic bacterial PLS-DA analysis; **i** endophytic fungal Coverage index; **j** endophytic fungal Shannon index; **k** endophytic fungal Simpson index; **l** endophytic fungal Ace index; **m** endophytic fungal Chao1 index; **n** endophytic fungal PCoA analysis; **o** endophytic fungal NMDS analysis; **p** endophytic fungal PLS-DA analysis. *, $P < 0.05$; ns indicates no significant difference (Wilcoxon rank-sum test)

group of cultivars (Fig. 1n, o). Moreover, PLS-DA analysis revealed that the endophytic bacteria and fungi from both cultivars were separately clustered (Fig. 1h, p).

Endophytic microbial community compositions in sugarcane stems

The numbers of total endophytic bacterial operational taxonomic units (OTUs) in the stems of the HR and HS cultivars were 764 and 753, respectively. The proportions of special endophytic bacterial OTUs in the stems of the HR and HS cultivars were 25.52% and 24.43%, respectively (Fig. 2a). However, an opposite trend was observed for endophytic fungi. In the stems of the HR and HS cultivars, the number of total endophytic fungal OTUs was 475 and 502, and the proportion of special endophytic fungal OTUs was 28.69% and 32.53%, respectively (Fig. 2f).

At the phylum level, the dominant (proportion > 1%) endophytic bacterial phyla were *Proteobacteria*, *Actinobacteriota*, and *Firmicutes* in stems of the HR (average of 73.60%, 24.54%, and < 1%, respectively) and the HS (average of 79.84%, 18.10, and 1.11%, respectively) cultivars (Fig. 2b). At the genus level, the dominant endophytic bacterial genera were *Delftia*, *Leifsonia*, *unclassified_o__Burkholderiales*,

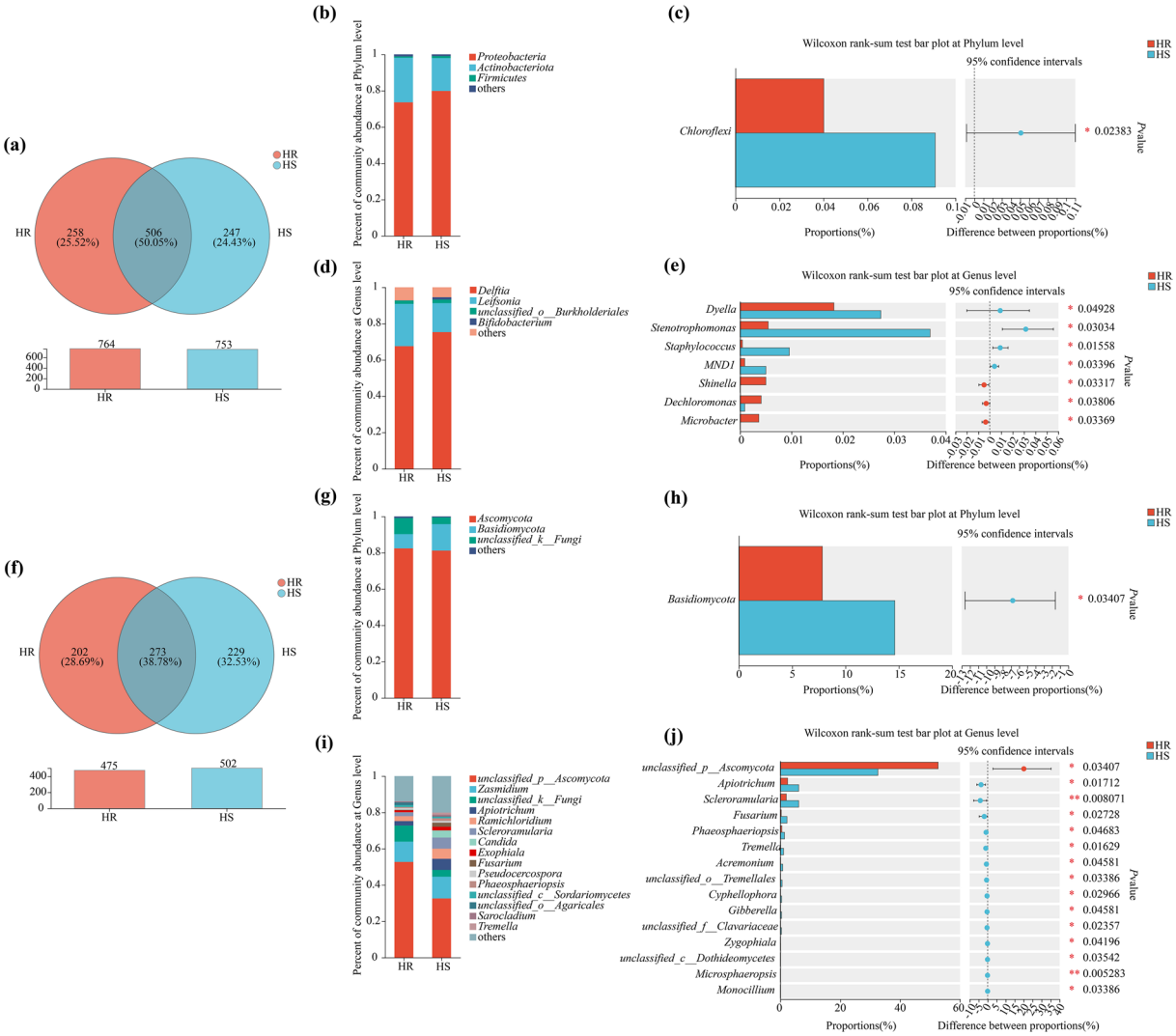


Fig. 2 Analysis of endophytic microbial community compositions at the phylum and genus levels in the stems of the HR and HS sugarcane cultivars. **a** Venn diagram analysis of endophytic bacteria; **b** endophytic bacterial phyla; **c** endophytic bacterial phyla were significantly different between both cultivars; **d** endophytic bacterial genera; **e** endophytic bacterial genera were significantly different between both cultivars; **f** Venn diagram analysis of endophytic fungi; **g** endophytic fungal phyla; **h** endophytic fungal phyla were significantly different between both cultivars; **i** endophytic fungal genera; **j** endophytic fungal genera were significantly different between both cultivars

and *Bifidobacterium* (Fig. 2d) in the stems of the HR (average proportion 67.55%, 23.33%, 1.83%, and < 1%, respectively) and HS (average proportion 75.32%, 15.95%, 1.97%, and 1.31%, respectively) cultivars.

The dominant (proportion > 1%) endophytic fungal phyla were *Ascomycota*, *Basidiomycota*, and *unclassified_k__Fungi* in the stems of the HR (average proportion 82.41%, 7.84%, and 8.88%, respectively) and HS (average proportion 81.15%, 14.63%, and 3.74%, respectively) cultivars (Fig. 2g). At the genus level, the dominant endophytic fungal genera in the stems of the HR and HS cultivars were *unclassified_p__Ascomycota* (average proportion 52.72% and 32.59, respectively), *Zasmidium* (11.17% and 11.98%), *unclassified_k__Fungi* (8.88% and 3.74%), *Apiotrichum* (2.45% and 6.14%), *Ramichloridium* (2.70% and 5.56%), *Scleroramularia* (2.02% and 6.14%), *Candida* (< 1% and 3.92%), *Exophiala* (1.05% and 2.02), *Fusarium* (< 1% and 2.25%), *Pseudocercospora* (< 1% and 1.02%), *Phaeosphaeriopsis* (< 1% and 1.42%), *unclassified_c__Sordariomycetes* (1.09% and < 1%), *unclassified_o__Agaricales* (1.49% and < 1%), *Sarocladium* (< 1% and 1.03%), and *Tremella* (< 1% and 1.10%) (Fig. 2i).

The Wilcoxon rank-sum test was performed on the endophytic bacteria and fungi in the stems of the HR and HS cultivars. The results revealed significant differences between the HR and HS cultivars in 1 bacterial phylum (Fig. 2c), 7 bacterial genera (Fig. 2e), 1 fungal phylum (Fig. 2h), and 15 fungal genera (Fig. 2j).

A total of 5 endophytic bacterial (Fig. 3a) and 43 fungal (Fig. 3c) clades in the stems of the HR and HS cultivars exhibited significant differences (LDA scores ≥ 2.0). However, no significant enrichment of endophytic dominant bacteria could be detected at the phylum or genus levels in the HR cultivars. Compared with the HR cultivars, the stems of the HS cultivars exhibited significantly enriched *Chloroflexi* phylum and *Stenotrophomonas* genus (Fig. 3b).

Furthermore, at the phylum level, except *Basidiomycota*, no other endophytic dominant fungi could be detected as significantly enriched in the stems of HR and HS cultivars. However, at the genus level, *unclassified_p__Ascomycota* and *unclassified_o__Agaricales* were significantly enriched in the HR cultivars. In the HS cultivars, *Apiotrichum*, *Scleroramularia*, *unclassified_f__Didymellaceae*, *Fusarium*, *Henningsomyces*, *Tremella*, *Phaeosphaeriopsis*, *Acremonium*, *unclassified_o__Tremellales*, *Zygophiala*, *Gibberella*, *Cyphellophora*, *Monocillium*, *unclassified_f__Clavariaceae*, *Microsphaeropsis*, and *unclassified_c__Dothideomycetes* were significantly enriched (Fig. 3d).

The results of single factor correlation network analysis revealed that the edges, nodes, and average degree of endophytic bacterial (Fig. 3e) and fungal (Fig. 3f) networks were higher but the bacterial and fungal modularities were lower in the HR cultivars than in the HS cultivars (Fig. 3e, f). In addition, *Actinobacteriota* and *Proteobacteria* accounted for the largest proportions in the endophytic bacterial network (Fig. 3e), whereas *Ascomycota* and *Basidiomycota* accounted for the largest proportions in the endophytic fungal network (Fig. 3f).

Analysis of endophytic metabolites

The expression of metabolites in the stems of various sugarcane cultivars was analyzed and demonstrated in a Venn plot. The results revealed that the special metabolites were higher in the stems of the HR cultivars than in those of the HS cultivars (Fig. 4a).

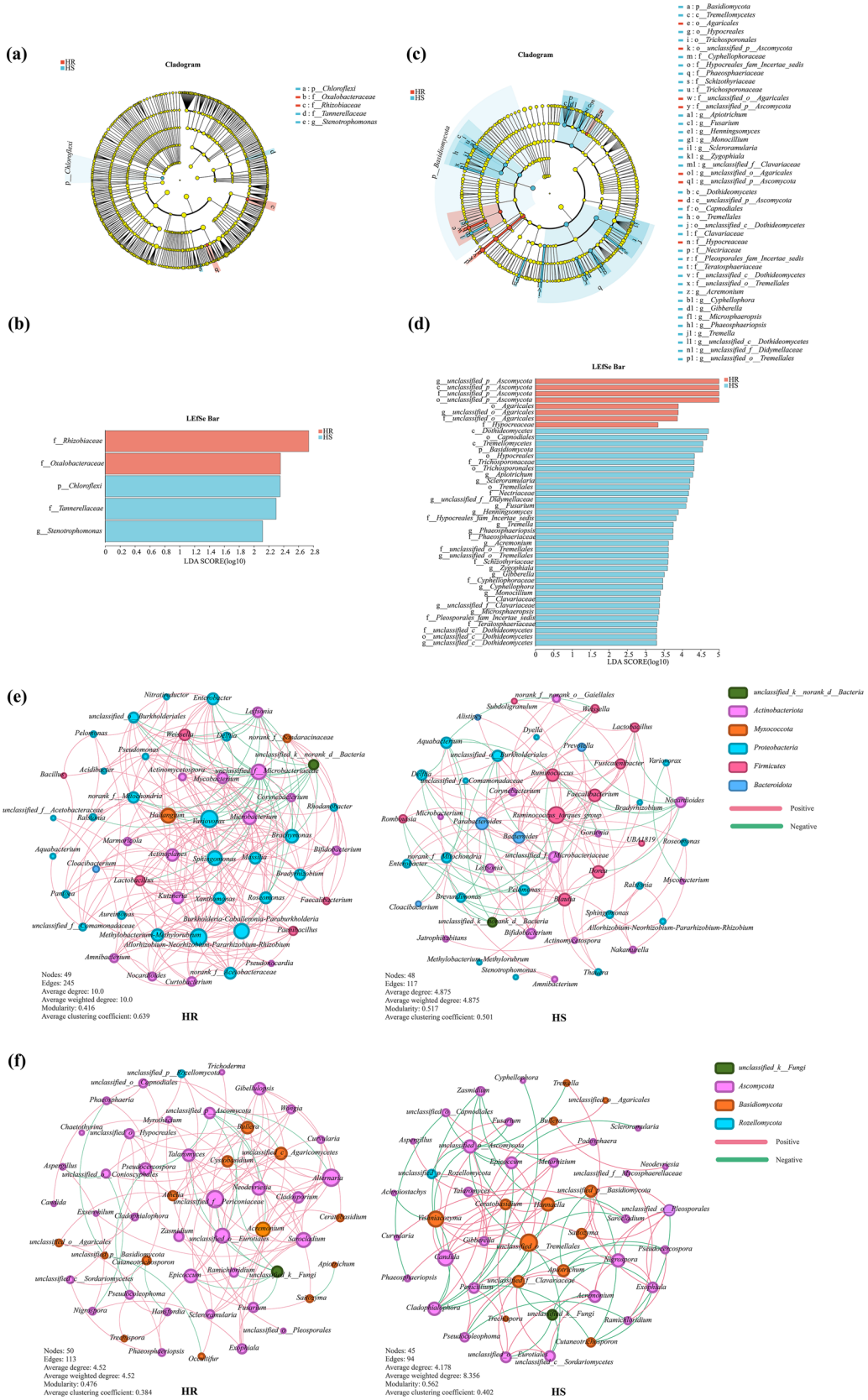
OPLS-DA was used to analyze the differences of metabolites between the HR and HS cultivars. The results revealed that the two groups were separately clustered (Fig. 4b). Meanwhile, based on the prediction ability of OPLS-DA model, total interpretation variation (R^2X), category segregation variation (R^2Y), and predictive ability of cross-validation (Q^2) were 0.535, 0.908, and 0.671 cum, respectively (Fig. 4c).

In addition, the results of differential volcano map revealed that 18 and 48 metabolites were up- and downregulated in the HR cultivars which compared to the HS cultivars, respectively (Fig. 4d). According to the *P*-value, the top-8 metabolites with significant expression were identified; there were D-myoinositol 4-phosphate, gingerglycolipid B, D-erythro-eritadenine, scutellarein 7-methyl ether 6-rhamnosyl-(1- > 4)-xyloside, caryoptosidic acid, wogonin, PE(18:2(9Z,12Z)/P-16:0), and octanoylglucuronide (Fig. 4d). Moreover, the top-50 metabolites were classified into 10 clusters (Fig. 4e). The endophytic metabolites in the stems were significantly different between the HR and HS cultivars. Compared with the HS cultivars, subclusters 3, 4, 5, 7, 8, and 9 were downregulated, and sub-clusters 1, 2, 6, and 10 were upregulated in the HR cultivars.

The variable importance in projection (VIP) score was evaluated to assess the abundance of metabolites in the stems of the HR and HS cultivars (based on the

(See figure on next page.)

Fig. 3 LEfSe and network analyses of endophytic microbial community compositions in the stems of the HR and HS sugarcane cultivars ($P < 0.05$, LDA scores ≥ 2). **a** Endophytic bacterial cladogram; **b** Endophytic bacterial LEfSe bar; **c** Endophytic fungal cladogram; **d** Endophytic fungal LEfSe bar; **e** Endophytic bacterial network analyses; **f** Endophytic fungal network analyses. Phylum, class, order, family, and genus are indicated by p, c, o, f, and g, respectively



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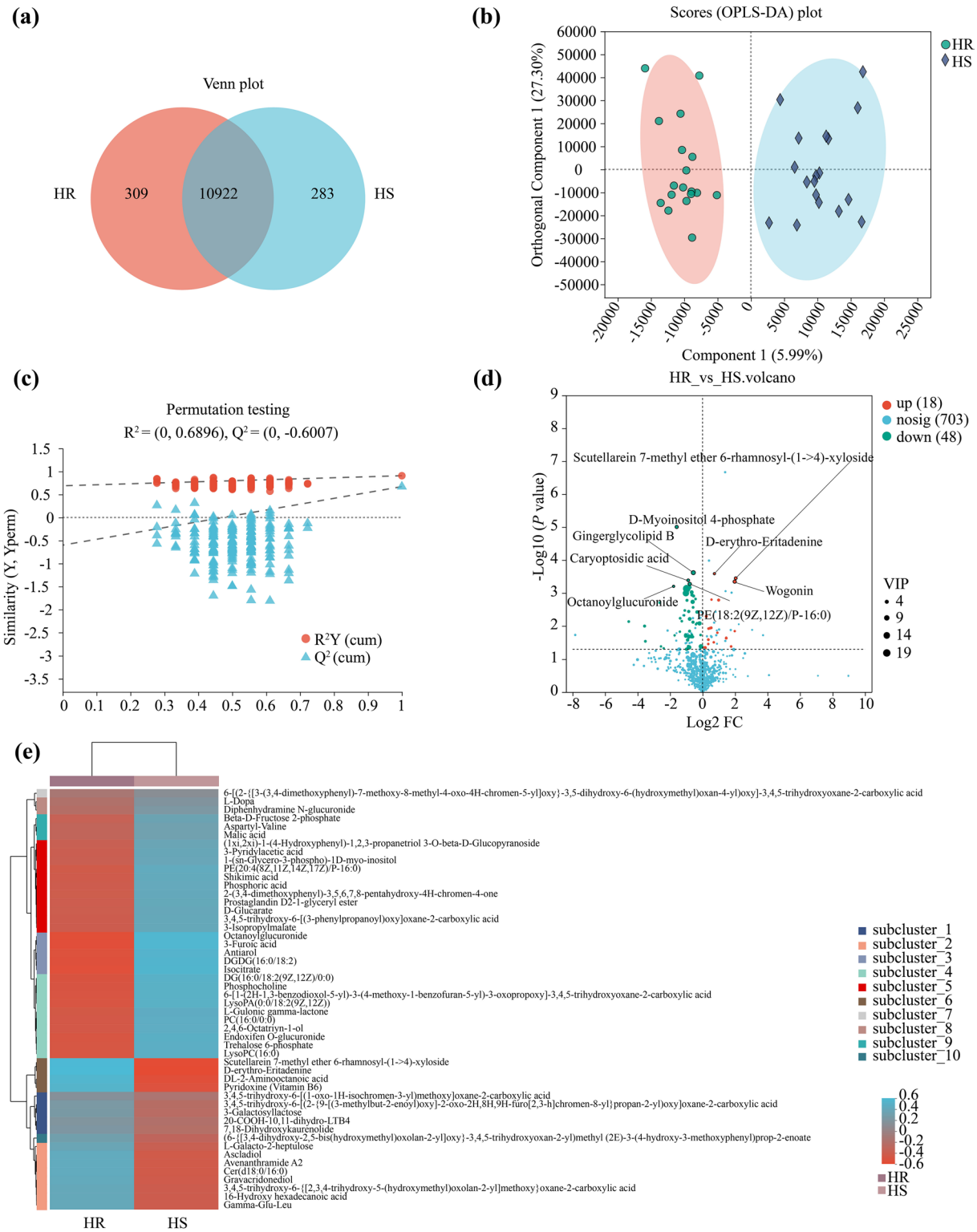


Fig. 4 Comparison of endophytic metabolites in the stems of the HR and HS sugarcane cultivars. **a** Venn plot; **b** OPLS-DA scores plot; **c** HR vs HS comparison cultivar response permutation test of the OPLS-DA model; **d** Volcano map. The X-coordinate is the multiple change value of the difference in metabolite expression between the two groups, named $\log_2$FC; the Y-coordinate is the statistical test value of the difference in metabolite expression, named $-\log_{10}$(*P*-value); the higher the value, the more significant the difference in expression. Each dot represents a specific metabolite, and the size of the dot represents the Vip value. The left and right points indicate the metabolites with down- and upregulated expressions, respectively. **e** Clustering heat map

PLS-DA model). Higher VIP score can be considered as indicting higher abundance of metabolites. Compared with the HS cultivars, 17 and 13 metabolites in the stems of the HR cultivars were significantly down- and upregulated, respectively. Among the top-5 abundant metabolites, only DL-2-aminooctanoic acid (VIP = 4.7155) was significantly upregulated in the stems of the HR cultivars. However, beta-D-fructose 2-phosphate (VIP = 4.9188), 1-(sn-Glycero-3-phospho)-1D-myo-inositol (VIP = 4.3494), cyclocalopin B (VIP = 4.1129), and PE(20:4(8Z,11Z,14Z,17Z)/P-16:0) (VIP = 3.5723) were significantly downregulated in the stems of the HR cultivars (Fig. 5a).

KEGG analysis revealed that one, three, two, and one metabolites were annotated to the amino acids (peptides as the biological function), carboxylic acids (organic acids as the biological function), phospholipids (lipids as the biological function), and neurotransmitters (hormones and transmitters as the biological function), respectively (Fig. 5b).

Additionally, based on the KEGG pathway database, all metabolites derived from the HR and HS cultivars were classified into the second category of 10 metabolic pathways. i.e., Carbohydrate metabolism, Amino acid metabolism, Lipid metabolism contains the largest number of metabolites. In the first category of metabolic pathway, 22 metabolites were classified into Metabolism; 2 metabolites were classified into Environmental Information Processing; 1 metabolite was classified into Human Diseases (Fig. 5c).

Compared with the HS cultivars, the KEGG pathway enrichment analysis revealed that Citrate cycle (TCA cycle), Glyoxylate and dicarboxylate metabolism, Glycerophospholipid metabolism, AGE-RAGE signaling pathway in diabetic complications, and Photosynthesis were significantly enriched in the stems of HR cultivars (Fig. 5d). Additionally, the KEGG topology analysis results with *P*-value calibration (BH) revealed that citrate cycle (TCA cycle) (citric acid, isocitrate, malic acid), glyoxylate and dicarboxylate metabolism (citric acid, isocitrate, malic acid), and glycerophospholipid metabolism [PC(16:0/0:0), phosphocholine, lysoPC(16:0)] were the significantly relevant metabolic pathways (with relevant metabolites involved in this pathway) in the stems of HR cultivars (Fig. 5e). Furthermore, the endophytic bacterial PICRUSt function prediction results revealed that citrate cycle (TCA cycle) and glyoxylate and dicarboxylate metabolism functions were upregulated in the stems of the HR cultivars compared with those of the HS cultivars (Fig. 5f).

Correlation analysis of endophytic microorganisms with metabolites revealed that PC(16:0/0:0) was significantly positively correlated with *unclassified_o__Tremellales* and Basidiomycota. Meanwhile, LysoPC(16:0) was significantly positively correlated with *unclassified_o__Tremellales* and *Cyphellophora*. Moreover, phosphocholine was significantly positively correlated with *Zygophiala*, and malic acid was significantly positively correlated with *Tremella*, *Acremonium*, *Zygophiala*, and *Microsphaeropsis*. Furthermore, citric acid was significantly positively correlated with *Zygophiala* and *Microsphaeropsis*. Isocitrate was significantly positively correlated with *Zygophiala*, *Monocillium*, and *Microsphaeropsis* (Fig. 6a). Additionally, network analysis of endophytic microorganisms with metabolites revealed that citric acid, isocitrate, *Zygophiala*, and *Microsphaeropsis* were strongly correlated with each other and more closely associated with other microorganisms or metabolites (Fig. 6b).

Discussion

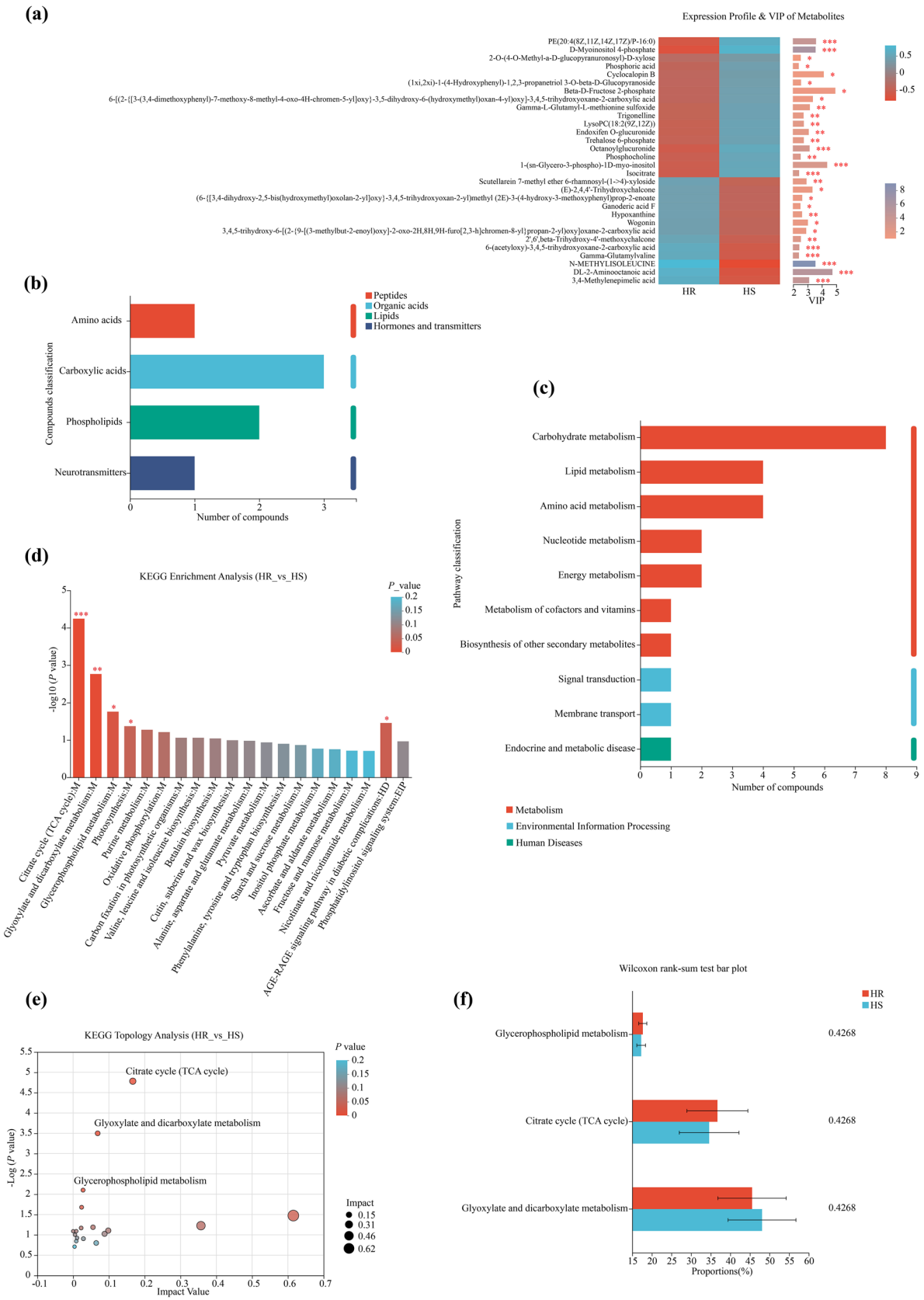
PBD, caused by *Fusarium* spp., is one of the main sugarcane diseases in the world [10, 18]. In PBD, the fungal conidia can be spread via wind; therefore, new leaves are easily infected under high temperature, humidity, and rainfall conditions [35]. Chemical fungicides used against PBD cannot penetrate into the waxy layer of sugarcane stems; however, they exhibit adverse effects on the environment and human health [20]. Therefore, the use of antagonistic microorganisms, known as biological control agents, can effectively control plant diseases [36] and promote plant growth by providing nutrients, inhibiting pathogenic bacteria, producing various hydrolytic enzymes, and inducing stress and disease resistance in plants [37]. Tiwari et al. [20] suggested that diseases caused by *Fusarium* spp., such as wilt, neck rot, and *Fusarium* head blight, in several crops could be effectively managed by *Trichoderma* spp.

Endophytic microbial diversity in the stems of the HR and HS sugarcane cultivars

It is well known that endophytic microorganisms in plants can promote plant growth and resist pathogens [23, 38]. The assembly of beneficial or harmful microorganisms in the host is influenced by the host genotype and environmental conditions [39]. Qiao et al. [40] reported that both

(See figure on next page.)

Fig. 5 Analysis of endophytic metabolites in the stems of the HR and HS sugarcane cultivars. **a** Variable importance in projection (VIP) scores of metabolites. **b** KEGG classification of metabolites. The ordinate is the secondary class of KEGG compounds, and the abscissa is the number of metabolites annotated to this class. **c** KEGG functional pathways. **d** KEGG enrichment analysis. **e** KEGG topology analysis. **f** Endophytic bacterial PICRUSt function prediction



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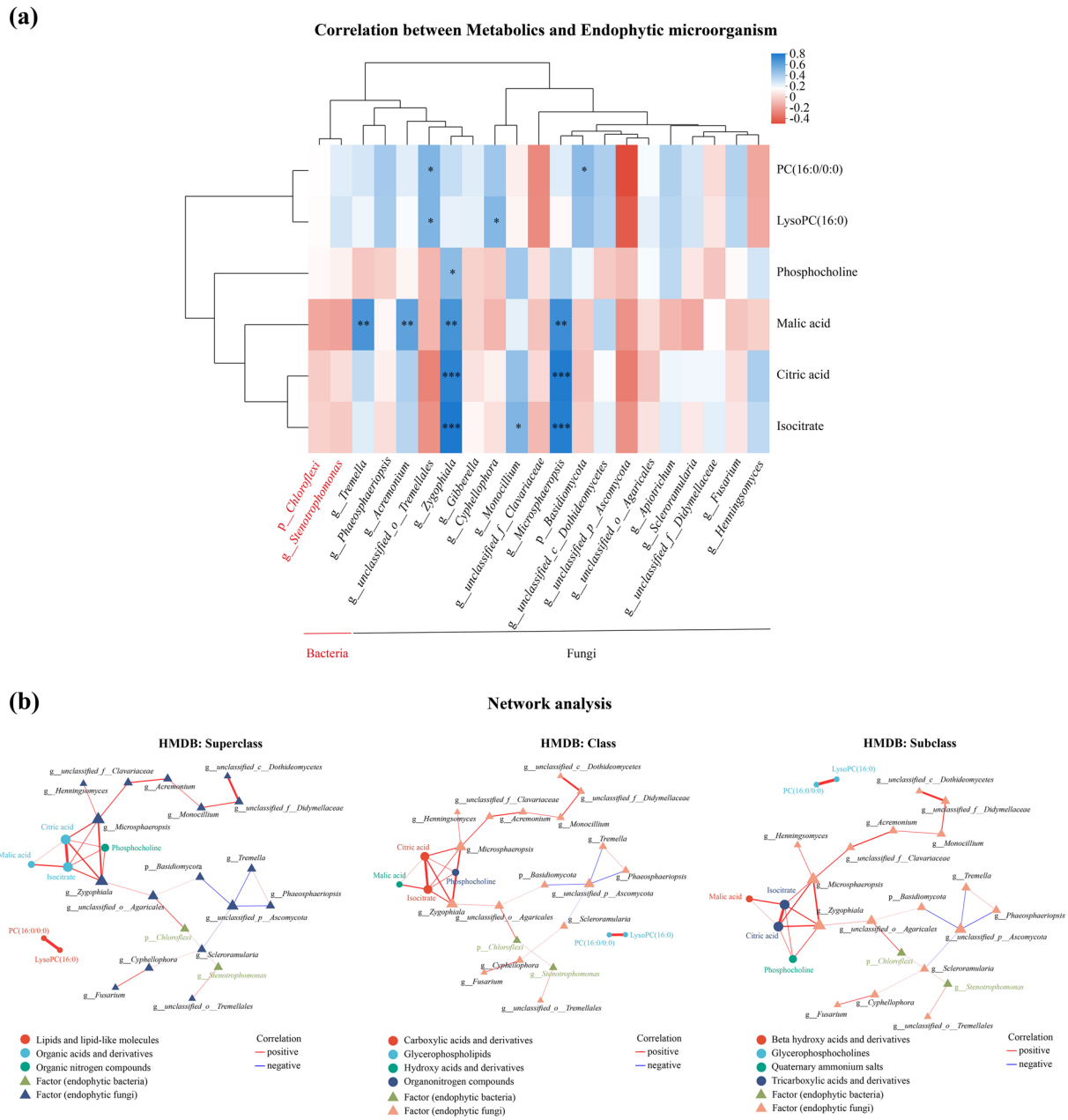

Fig. 6 Correlation **a** and Network **b** analyses of endophytic metabolites and microorganisms in the stems of the HR and HS sugarcane cultivars

the internal (i.e., plant cultivar or genotype in this study) and external environment of plants could affect the endophytic diversity.

Zeng et al. [41] reported that the endophytic bacterial composition was different in various cotton cultivars. Previous studies reported that the endophytic bacterial alpha-diversity was higher in the susceptible cultivars than in the resistant cultivars [41, 42]. On the contrary, our results revealed that the endophytic bacterial alpha-diversity exhibited no significant difference between the HR and HS cultivars. Meanwhile, there was also no significant difference in the ecological niche of endophytic bacteria in the stems between the HR and HS cultivars.

Xu et al. [23] reported that the type of cultivars (with particular genotypes) could significantly affect the composition of the endophytic fungal community. Similar observations were reported for Mediterranean pine forests [43], apple [44], mulberry [23], *Alnus* [45], *Quercus ilex* L. [46], and black spruce (*Picea mariana*) [47]. In the present study, although the endophytic fungal

alpha-diversity and richness were lower in the stems of the HR cultivars than in those of the HS cultivars, the fungal diversity was significantly different between the HR and HS cultivars. This was consistent with previous studies [23].

Endophytic microbial community composition in the stems of the HR and HS sugarcane cultivars

Plant disease resistance drives the changes in microbial communities residing inside the plant [48]. Plant can recruit some disease-resisting and growth-promoting beneficial microbes to resist pathogens by changing their endophytic microbial composition [41].

Leifsonia can produce cellulose [49] with bacteriostatic activity [50], promote plant growth, and produce gibberellin and auxin [51]. In our study, in comparison with HS cultivars, the relative abundance of *Leifsonia* in the stems of HR cultivars was higher, which could help sugarcane to resist PBD (Fig. 2d). *Shinella*, a novel diazotroph, promotes sugarcane growth [52] and has antibiotic [53], biodegradable, and bioremediation effects [54]. *Dechloromonas* is a phosphorus-accumulating organism with biodegradation function [55] and could enhance biological phosphorus removal [56, 57]. *Microbacter* has plant growth–promoting properties [58]. In the present study, *Shinella*, *Dechloromonas*, and *Microbacter* were significantly enriched in the stems of HR cultivars, suggesting that the enrichment of these beneficial microbes might be the main reason underlying higher resistance of HR cultivars to PBD (Fig. 2e).

The pathogenicity and toxicity of *Fusarium* is well known [59]. Previous studies reported that *Fusarium* causes PBD in sugarcane [5–11]. In addition, *Ramichloridium*, *Scleroramularia*, *Phaeosphaeriopsis*, *Sarocladium*, *Zygophiala*, and *Gibberella* are reported as pathogens in bananas [60], apple and pawpaw [61], Butcher's Broom (*Ruscus aculeatus*) [62], rice [63], apple [64], and maize [65]. *Pseudocercospora*, a plant pathogenic fungus, commonly causes spotting and wilting of the leaves and fruits of the host plants [66]. *Cyphellophora* [67], *Monocillium* [68], *Apiotrichum* [69], *Microsphaeropsis* [70], and *Scleroramularia* [23] are reported as pathogens in plants or humans. All these fungal genera were significantly enriched in the stems of HS cultivars. Therefore, higher proportion of plant pathogens was enriched in the HS cultivars than in the HR cultivars. This phenomenon suggested that host genotypes (resistance or susceptibility) can influence the presence or colonization of beneficial microbes or pathogens in the host plant, and the ability of PBD resistance is related to the endophytic microbial composition.

Metabolomic profiling of the stems of HR and HS sugarcane cultivars

Previous studies reported that the metabolic pathways could affect the stress resistance of plants [71, 72]. Wang et al. [73] reported that cysteine was related to PBD symptoms. Meanwhile, alanine, lysine, proline, and glutamic acid were reported to play a role in regulating and protecting processes of PBD. Additionally, proline, polyamines, and glutamate played a significant role in defense of sugarcane against pathogens [73]. The metabolism of cyanamide, glutamate, proline, tyrosine, and arachidonic acid actively contributes to plant stress tolerance and responsiveness [73].

In the TCA cycle, nutrients are oxidized to produce key metabolites for reductive equivalents, energy production, and biosynthetic reactions [74]; and the products in the TCA cycle also contribute to cell viability and proliferation [74]. Organic acid, particularly citric acid, has enhancing effect on antioxidant defense system [75] and metal ion clearance [76]. Isocitrate is associated with energy production, nitrogen metabolism, fatty acid synthesis, glyoxylic acid cycle, and light respiration [77]. Malic acid can remove free radicals, maintain membrane stability, enhance root vitality, and improve plant resistance (such as drought and oxidation resistances). Citric acid and malic acid can promote the growth and development of new young cells [78]. As a variant of the TCA cycle, the glyoxylic cycle could interfere with the oxidized cell potential, converting glyoxylate into malate [79]. Bio-membranes contain a significant amount of glycerophospholipids, which are known to control several cellular signaling events involving ion channels or G-protein coupled receptors. Protein translocation, apoptosis, inflammation, and neurogenesis are crucial in calcium homeostasis [80]. These studies suggested that active metabolic pathways and high abundance of metabolite contents may be important mechanisms for the resistance to PBD by the HR cultivars.

Conclusion

In summary, endophytic microbial composition and metabolites in the stems of various sugarcane cultivars resistant or susceptible to PBD were analyzed. The results revealed that the endophytic fungi with biocontrol effects such as *Shinella*, *Dechloromonas*, and *Microbacter* were significantly enriched, and the abundance of pathogenic fungi such as *Fusarium*, *Ramichloridium*, *Scleroramularia*, *Phaeosphaeriopsis*, *Sarocladium*, *Zygophiala*, *Gibberella*, *Pseudocercospora*, *Cyphellophora*, *Monocillium*, *Apiotrichum*, *Microsphaeropsis*, and *Scleroramularia* significantly reduced

in the stems of sugarcane cultivars with higher resistance to PBD. Additionally, six metabolites [citric acid, isocitrate, malic acid, PC(16:0/0:0), phosphocholine, lysoPC(16:0)], were significantly related to the endophytes in the stems of various sugarcane cultivars with higher resistance to PBD. These results suggested that more abundance of antagonistic microbes and highly active metabolic functions of endophytes in the HR cultivars were the important mechanisms underlying their higher resistance to PBD.

Abbreviations

PBD	Sugarcane pokkah boeng disease
HR	High resistance sugarcane cultivars
HS	High susceptibility sugarcane cultivars
SRIGAAS	Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences
SOM	Soil organic matter
TN	Total nitrogen
TP	Total phosphorus
TK	Total potassium
AN	Alkaline nitrogen
AP	Available phosphorus
AK	Available potassium
NMDS	Non-metric multidimensional scaling analysis
PCoA	Principal co-ordinates analysis
PLS-DA	Partial least squares discriminant analysis
LDA	Linear discriminant analysis
LEfSe	LDA effect size analysis
KEGG	Kyoto encyclopedia of genes and genomes
OTU	Operational taxonomic unit
VIP	Variable importance in projection

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Author contributions
S. Y. and H. T. conceived and designed research. J. X., Z. C., and T. L. conducted experiments. Z. C. and T. L. contributed to new reagents or analytical tools. J. X. analyzed data. J. X. wrote the manuscript. All authors read and approved the manuscript.

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Availability of data and materials
Sequencing data were deposited in the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA998735).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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