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Differences of endophytic microbial compositions and metabolites in roots between fusarium wilt resistant and susceptible melon varieties

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Abstract

Background Studies have shown that plant endophytic microbial communities are ubiquitous and closely related to plant growth and health. To clarify the mechanism of the melon varieties with high resistant to wilt, the endophytic microbial compositions and metabolites in roots of melon varieties with high resistant ability to wilt were analyzed.

Results The results showed that the abundances of Firmicutes, Ascomycota, *Bacillus*, *Bradyrhizobium*, *Amycolatopsis*, *Actinospica*, and *Catenulispora* all increased in roots of wilt high resistant melon varieties (MT) which compared to wilt susceptible melon varieties (MS). Meanwhile, *Ochrobactrum*, *Bordetella*, *Roseateles*, *Staphylococcus*, *Acidovorax*, *Amycolatopsis*, *Catenulispora*, *Promicromonospora*, and *Gymnopilus* were the unique endophytic microbes in roots of MT. Moreover, in comparison with the MS varieties, the functions of Defense mechanisms, Secondary metabolites biosynthesis, transport and catabolism, Nucleotide transport and metabolism, Signal transduction mechanisms, Coenzyme transport and metabolism, Carbohydrate transport and metabolism and Amino acid transport and metabolism all increased in roots of MT varieties. Additionally, the nucleotide metabolism and biosynthesis of cofactors metabolic pathways were also significantly increased in roots of MT varieties. On the other hand, the untargeted metabolome results showed that Biosynthesis of various plant secondary metabolites, Nucleotide metabolism and Biosynthesis of cofactors metabolic pathways were significantly increased in the expression of MT varieties; and the content of metabolic compounds such as flavonoids, Cinnamic acid compounds, Organic acid compounds, and Nucleotides were increased. In addition, the correlation between microbiome and metabolome indicates a significant correlation between the two.

Conclusions All above results suggested that higher abundant antagonistic microbes and metabolic functions of endophytes in roots of wilt high resistant melon varieties (MT) were the important mechanisms for their high resistance to wilt.

Keywords Melon, Wilt, Endophytes, Metabolites, Root

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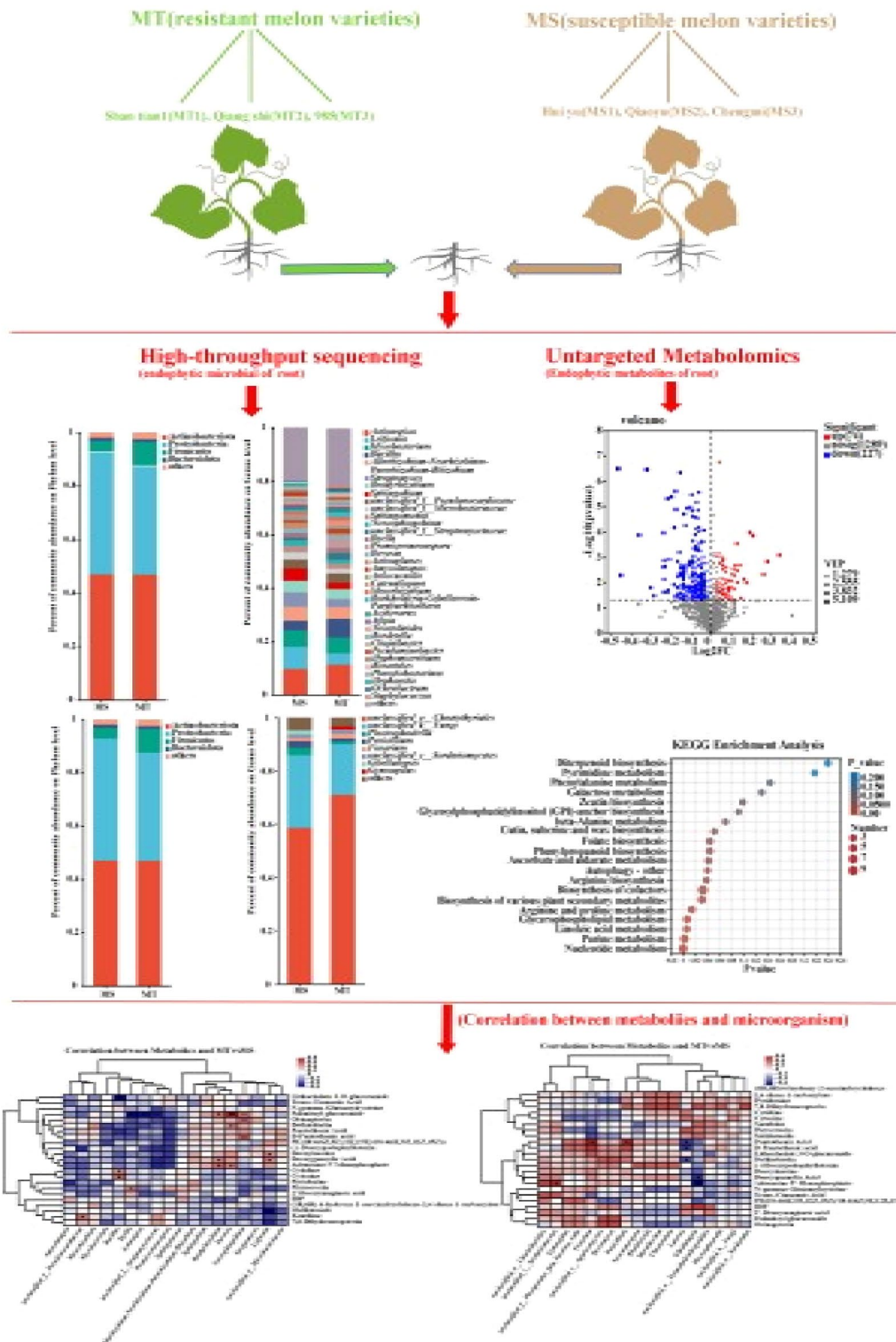Jinyan Huang
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Graphical Abstract

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Introduction

Melon (*Cucumis melo* L.), also known as cantaloupe, is an annual vine herb of the Melon (*Cucumis*) genus in the family Cucurbitaceae (Cucurbitaceae) [1]. Currently, it is widely cultivated around the world, for its unique flavor and high nutritional value [2]. However, melon diseases, such as wilt, powdery mildew, downy mildew and anthracnose seriously jeopardize the yield and quality [3–5]. Among them, melon wilt caused by the *Fusarium oxysporum* f. sp. niveum is the most serious melon disease [6]. For it was not easily detected in the early stage and it had caused seriously damage [7]. As fusarium wilt has been widely present in melon production around the world, particularly, serious incidence of fusarium wilt in melon always can be found in long-term cultivation regions [8, 9].

Previous studies had reported that crop rotation [10], wilt resistant varieties [11], grafting [12], chemicals pesticides [13], physical methods (soil solarization, etc.) [14] and beneficial microorganisms [15] utilization were all good method in reducing wilt incidence. Among them, although wilt-resistant melon varieties utilization is one of the good methods [9], but it always costs quite a lot of times in breeding melon varieties with higher resistant ability to stresses. At present, chemical pesticides, because of its simplicity, convenience and quick results, is still the main choice for farmers [16]. However, with the continuous use of chemical pesticides are not only gradually weakened (increasing resistances of pests and pathogens), but also induce the environment pollution (pesticide residues, heavy metal contamination, reduction of beneficial microorganisms, and soil compaction, etc.) [17–19]. Meanwhile, the cost of production is constantly increasing too. Additionally, human health, directly or indirectly can be negatively influenced by using chemical pesticides [20]. It is necessary to look for the eco-methods in controlling plant diseases.

Recently, researchers have found that exploiting beneficial microorganisms in nature for plant disease control is an environment friendly and sustainable control method [21]. As endophytic microorganisms widely distribute in plants, not only will not cause plant diseases, but also will promote the growth of plants [22]. Meanwhile, plant roots can secret a variety of compounds and metabolism and then soil microorganisms can be enriched around roots, the beneficial microorganisms only are selectively allowed entering the roots as endophytes [23]. However, if plant roots are invaded by pathogens, "help" should be called by plants and the beneficial microorganisms should change the physiological activities to enhance plants disease resistances [24].

Moreover, root endophytic microorganisms also can promote plants growth by promoting the absorption of mineral elements [25, 26], phytohormones or antibiotics production [27, 28], improving plant disease resistance [29] and plant genes expression and metabolic pathways regulations [30, 31]. Devi [32] found that tomato inoculation with endophytic *Bacillus* and *arbuscular mycorrhiza fungi* (AMF) significantly reduced the incidence of wilt, for pathogens could be inhibited through antibiotics, hydrolases, and secondary metabolites and increased tomato production. Khastini [33] also found that the incidence of wilt decreased 40–60% by inoculation with *Cadophora* sp., which could effectively inhibit the invasion of pathogens into adjacent cells. At present, *Pseudomonas*, *Streptomyces*, *Trichoderma*, *Penicillium*, and *Bacillus* have been used as the biocontrol strains [34, 35]. Additionally, as a plant can affect the plant microbiome by producing various metabolites, and in turn, the microbiome can also can affect the metabolome of the host plant [36, 37]. Plant disease resistance is closely related to metabolic activity in the body [38]. Microorganisms, which interact with the host plants are known to stimulate the production of abundant and diverse metabolites in the plant [39, 40]. However, the relationship between the endophytic microbiome and plant disease resistance is still unclear. Meanwhile, in field experiments, we also found that some melon varieties showed different resistant abilities against fusarium wilt.

Therefore, to clarify the mechanism of why the various melon varieties showed different resistant abilities against fusarium wilt, the endophytic microbial compositions and metabolites in roots between fusarium wilt resistant and susceptible melon varieties were analyzed.

Table 1 Endophytic bacterial and fungal diversities and richness in roots of resistant (MT) and susceptible (MS) melon varieties

	Treatment	Shannon	Simpson	Ace	Chao	Coverage
Bacteria	MS	4.18±0.10a	0.039±0.0076b	832.95±24.40a	813.02±25.51a	0.99
	MT	4.00±0.11b	0.052±0.0072a	813.15±59.89a	793.29±58.86a	0.99
Fungi	MS	1.10±0.19a	0.51±0.10a	56.32±4.85a	58.93±8.37a	0.99
	MT	0.79±0.21a	0.67±0.12a	60.09±3.06a	62.20±0.99a	0.99

Note that all statistics are presented as the mean ± SD (standard deviation). Significant variations between treatments at $p < 0.05$ are indicated by different letters in the same column
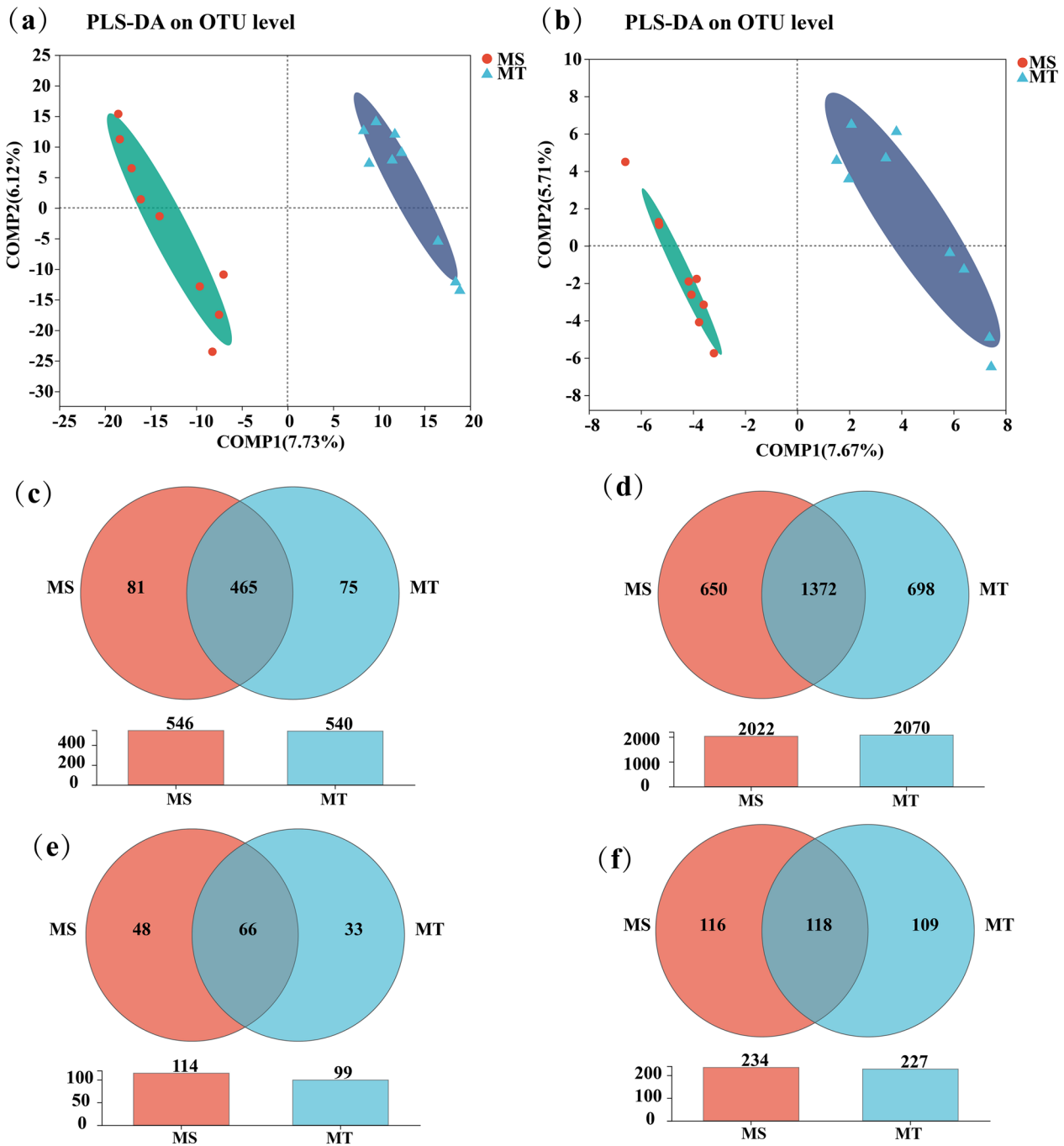
Fig. 1 Composition of root endophytic bacterial communities of melon resistant ((MT) and susceptible (MS) varieties. **a** PLS-DA score plot of the root endophytic bacterial communities. **b** PLS-DA score plot of the root endophytic fungal communities. **c** Venn plot of root endophytic bacterial communities at the genus level. **d** Venn plots of the root endophytic bacterial community at the OTU level. **e** Venn plots of the root endophytic fungal communities at the genus level. **f** Venn plots of the root endophytic fungal communities at the OTU level

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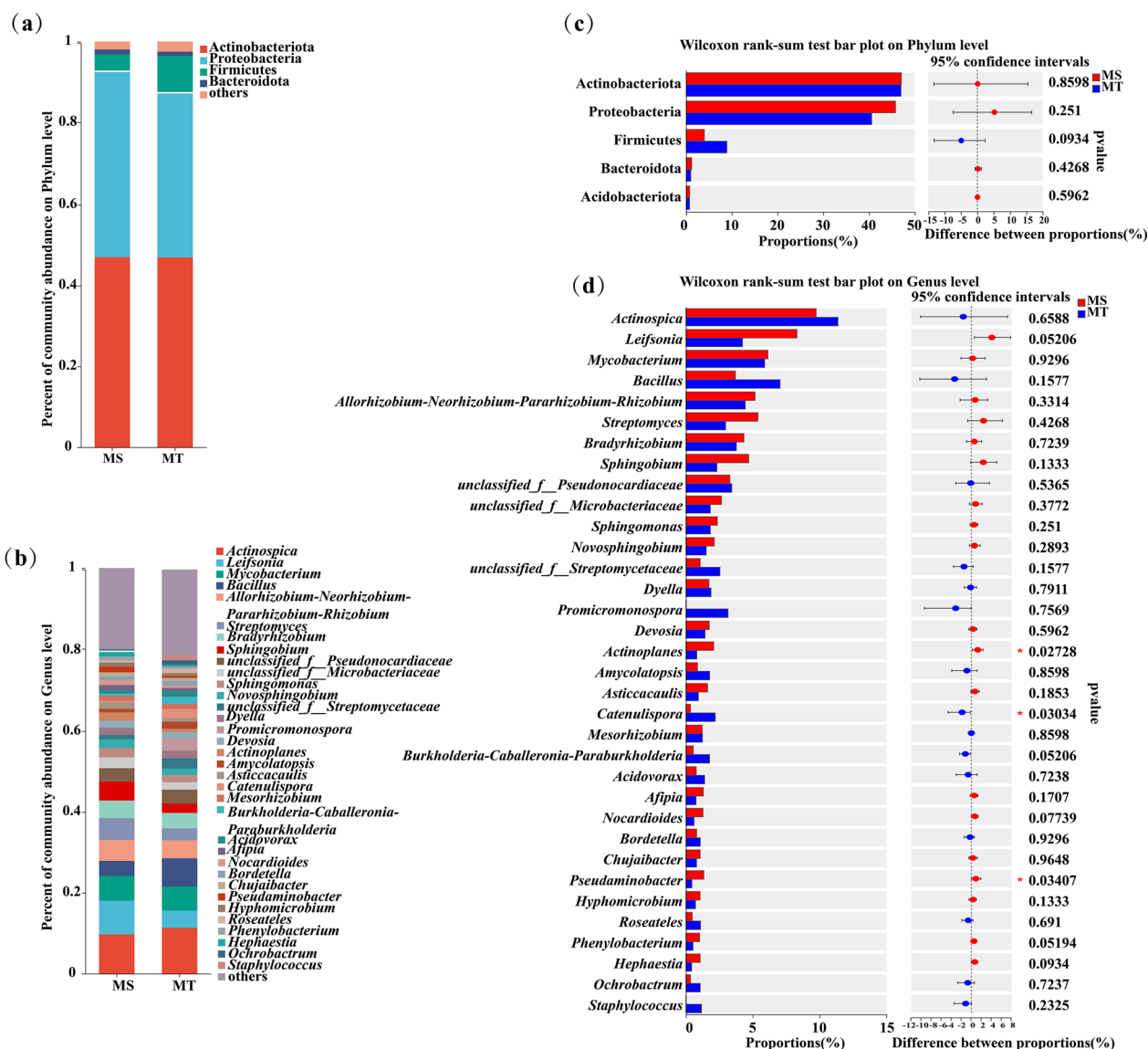
Fig. 2 Compositions of endophytic bacteria in roots of resistant varieties (MT) and susceptible melon varieties (MS) at phyla (**a**) and genus (**b**); test for significant difference in endophytic bacterial abundances at phyla (**c**) and genus (**d**) levels (*$P < 0.05$) between resistant (MT) and susceptible melon varieties (MS)

Results

Endophytic microbial diversities and richness in roots between fusarium wilt resistant and susceptible melon varieties

As shown in Table 1, only the endophytic bacterial diversity, i.e., their Shannon and Simpson indexes in roots of fusarium wilt resistant (MT) and susceptible (MS) melon varieties were significantly different between each other ($P < 0.05$). However, the endophytic bacterial richness, fungal diversity and richness were all not significantly different between each other.

Endophytic microbial compositions in roots between fusarium wilt resistant and susceptible melon varieties

Partial least squares discriminant analysis (PLS-DA) was performed to assess the endophytic bacterial and fungal compositions in roots between fusarium wilt resistant (MT) and susceptible (MS) melon varieties at the OUT level. The results showed that the endophytic bacterial and fungal communities in roots between MS and MT were clustered separately, it suggested that the endophytic bacterial and fungal compositions were significant

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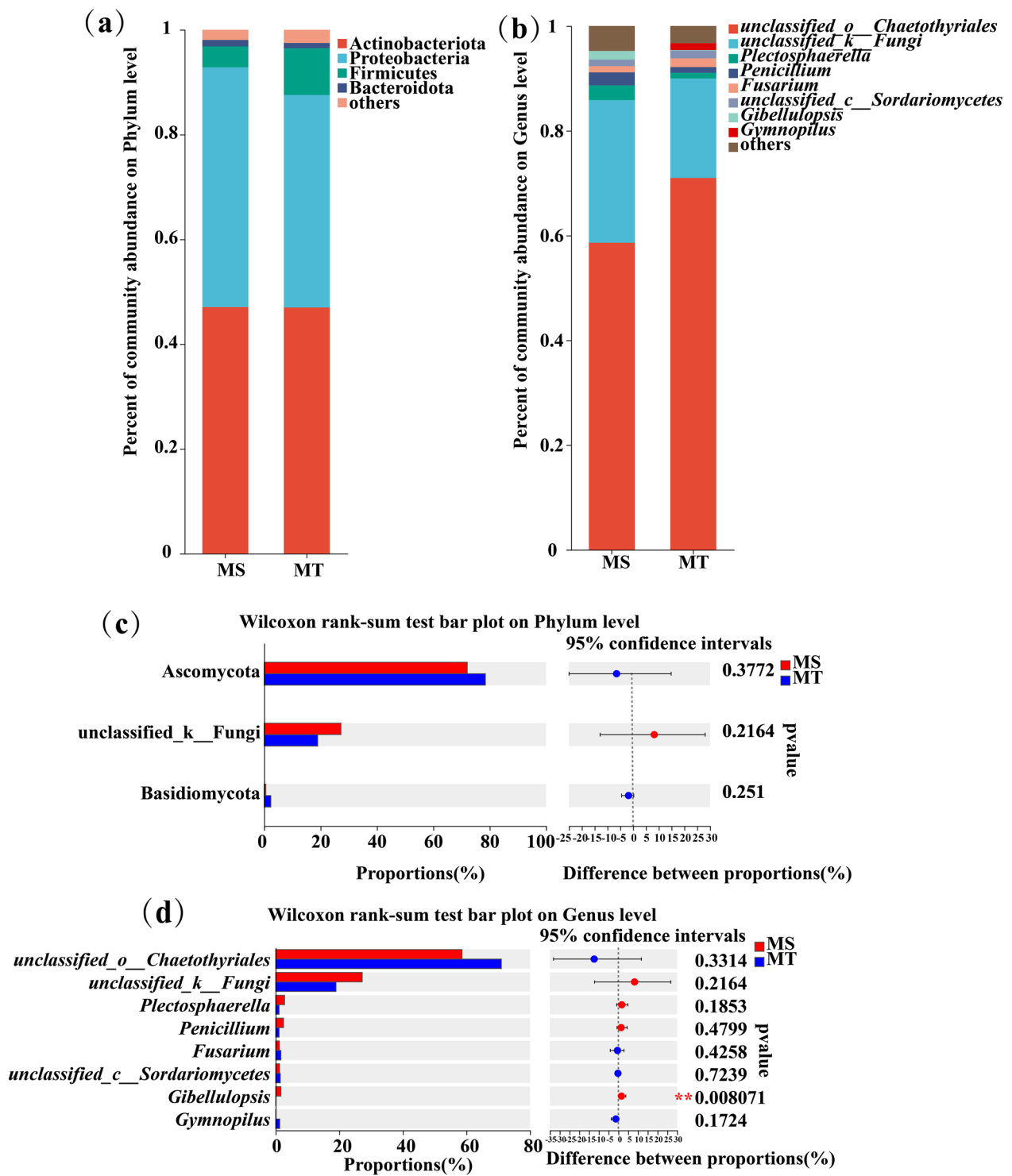
Fig. 3 Compositions of endophytic fungi in roots of resistant varieties (MT) and susceptible melon varieties (MS) at phyla (**a**) and genus (**b**); test for significant difference in endophytic fungal abundances at phyla (**c**) and genus (**d**) levels (*$P < 0.05$) between resistant (MT) and susceptible melon varieties (MS)

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differences between MS and MT varieties ($P < 0.05$) (Fig. 1a, b).

In addition, Venn plot analysis also showed that the numbers of endophytic bacteria at the genus level in MS and MT were 546 and 540, respectively. Meanwhile, the numbers of special bacterial genera in MS and MT were 81 and 75, respectively (Fig. 1c). Moreover, the numbers of endophytic bacteria at the OTU level in MS and MT were 2022 and 2070, respectively; and the numbers of unique bacterial OTUs in MS and MT were 650 and 698, respectively (Fig. 1d).

Additionally, the numbers of endophytic fungi at the genus level in MS and MT were 114 and 99, respectively; meanwhile, the numbers of special fungal genera in MS and MT were 48 and 33, respectively (Fig. 1e). Moreover, the numbers of endophytic fungal at the OTU level in MS and MT were 234 and 227, respectively; and the numbers of unique bacterial OTUs in MS and MT were 116 and 109, respectively (Fig. 1f).

At the phylum level, the numbers of dominant endophytic bacterial phyla (the relative abundances are greater than 1%, the same below) in roots of MT and MS were all 4. Bacteroidota, Firmicutes, Proteobacteria and Actinobacteriota were all the common dominant endophytic bacterial phyla in roots of MT and MS. In comparison with MS only the abundance of Firmicutes increased in roots of MT, and the abundances of Bacteroidota, Proteobacteria and Actinobacteriota were all decreased (Fig. 2a).

At the genus level, 25 dominant endophytic bacterial genera could be detected in roots of MT and MS. Among them, 16 common dominant bacterial genera were found (Fig. 2b). In comparison with MS, *Ochrobactrum, Bordetella, Roseateles, Staphylococcus, Acidovorax, Burkholderia−Caballeronia−Paraburkholderia, Amycolatopsis, Catenulispora* and *Promicromonospora* were the unique dominant endophytic bacterial genera in roots of MT. By contrast, *Phenylobacterium, Hyphomicrobium, Hephaestia, Chujaibacter, Nocardioides, Afipia, Pseudaminobacter, Asticcacaulis,* and *Actinoplanes* were the special dominant endophytic bacterial genera in roots of MS (Fig. 2c, d).

At the phylum level, the numbers of dominant endophytic fungal phyla in roots of MT and MS were 2 and 3, respectively. i.e., Ascomycota, Basidiomycota, and unclassified_k__Fungi (Fig. 3a). In comparison with MS, the proportion of Ascomycota increased, but the proportion of unclassified_k__Fungi decreased in roots of MT. And Basidiomycota was the unique dominant endophytic fungal phylum in roots of MT.

At the genus level, the numbers of dominant endophytic fungal genera in roots of MT and MS were all 8. *Fusarium, Penicillium, unclassified_k__Fungi,* unclassified_o__Chaetothyriales, *Plectosphaerella,* unclassified_c__Sordariomycetes, *Gibellulopsis, Gymnopilus* and others (Fig. 3b). In comparison with MS, the proportions of *Fusarium,* unclassified_o__Chaetothyriales, unclassified_c__Sordariomycetes increased, but *Penicillium,* unclassified_k__Fungi, and *Plectosphaerella* decreased in roots of MT. *Gymnopilus* and *Gibellulopsis* were the special dominant fungal genera in roots of MT and MS, respectively ($P < 0.05$) (Fig. 3c, d).
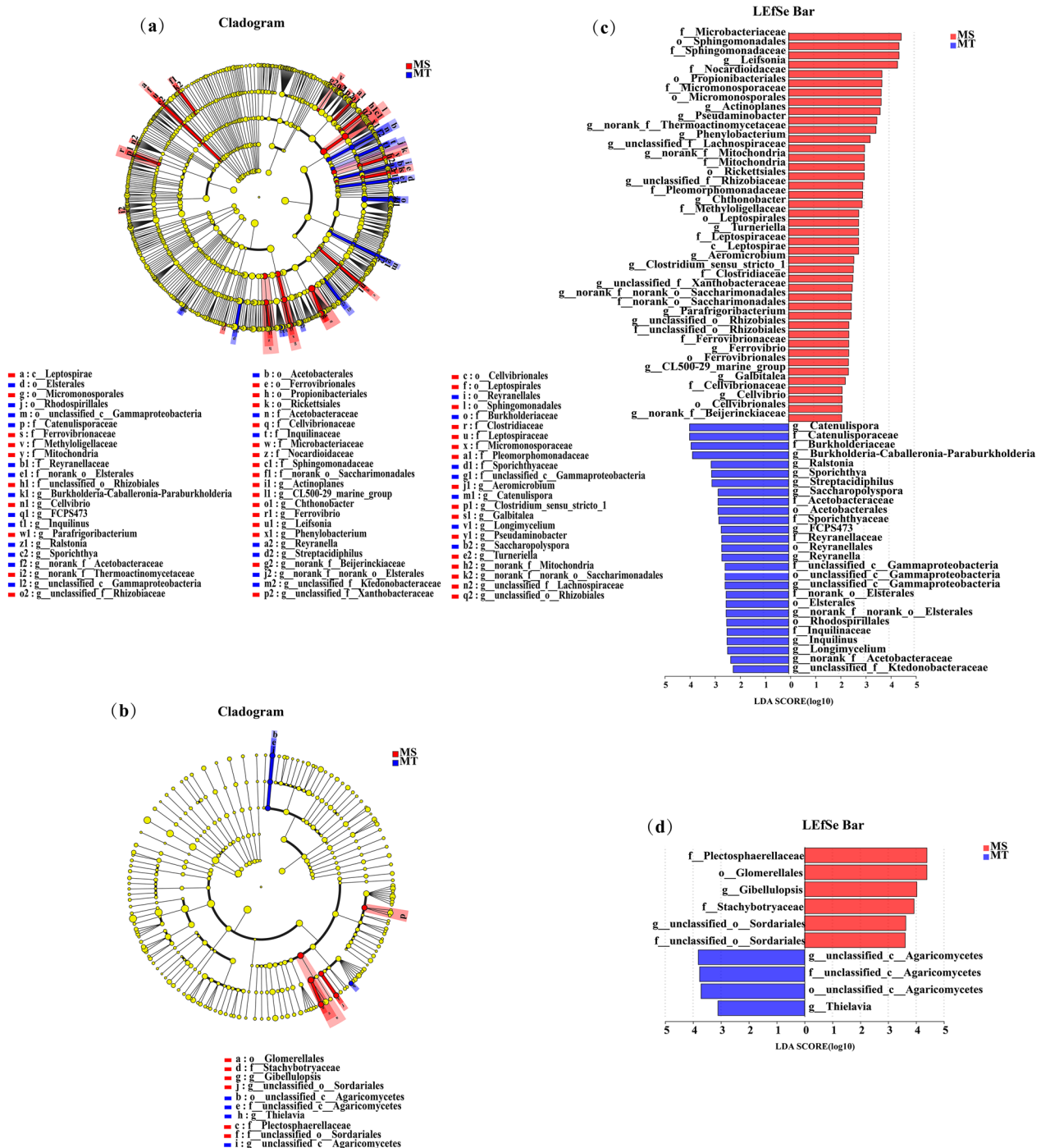
LEfSe analysis of endophytic bacterial and fungal communities in roots of melon at the phylum and genus levels

The Linear discriminant analysis Effect Size (LEfSe) analysis was also performed to identify the definitive values of endophytic bacteria in roots of MT and MS, respectively. As shown in Fig. 4a, c a total of 69 bacterial clades showed significant differences (LDA $\geq$ 2.0) (Fig. 4a, c). At the genus level, *Leifsonia, Actinoplanes, Pseudaminobacter,* norank_f__Thermoactinomycetaceae, *Phenylobacterium,* unclassified_f__Lachnospiraceae, norank_f__Mitochondria, unclassified_f__Rhizobiaceae, *Chthonobacter, Turneriella, Aeromicrobium,* Clostridium_sensu_stricto_1f__Clostridiaceae, unclassified_f__Xanthobacteraceae, *Cellvibrio, Galbitalea,* norank_f__norank_o__Saccharimonadales, *Parafrigoribacterium,* unclassified_o__Rhizobiales, *Ferrovibrio,* CL500-29_marine_group, *Cellvibrionaceae,* and norank_f__Beijerinckiaceae were significantly enriched in MS varieties; In contrast, *Catenulispora, Burkholderia−Caballeronia−Paraburkholderia, Ralstonia, Sporichthya, Streptacidiphilus, Saccharopolyspora,* unclassified_c__Gammaproteobacteria, norank_f__norank_o__Elsterales, *Longimycelium,* rank_f__Acetobacteraceae, unclassified_f__Ktedonobacteraceae were significantly enriched in roots of MT varieties.

Meanwhile, as shown in Fig. 4b, d, a total of 10 fungal clades also showed significant differences (LDA $\geq$ 2.0). At the genus level, *Gibellulopsis* and unclassified_o__Sordariales significantly enriched in roots of MS varieties; by contrast, unclassified_c__Agaricomycetes and *Thielavia* significantly enriched in roots of MT varieties.

Functional predictive analysis

In addition, based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, the PICRUSt function prediction method was used to predict the function of endophytic bacteria in the root system, in which a total of 19 functional types of endophytic bacteria were detected between MT and MS. Although the analysis revealed that although the functional types of root endophytic bacteria were very similar between MS and MT, however, 10 functional types of MT were higher, and 9 functional types of MT were lower than those of MS (Fig. 5a, c).

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Fig. 4 LEfSe analysis of root endophytic bacteria (**a**) and fungi (**b**) in roots of wilt resistant (MT) and susceptible melon varieties (MS); LDA analysis of endophytic bacteria (**a**) and fungi (**b**) ($P < 0.05$, LDA score = 2.0). The diameter of each circle is proportional to the abundance of that group. Different prefixes indicate different levels (p, phylum; c, class; o, order; f, family; g, genus). (MT) Wilt resistant varieties; (MS) Wilt susceptible varieties

The FUN Guild function was also conducted to predict the root endophytic fungal functions, and 5 function types of endophytic fungi were detected between MT and MS. Among them, 3 function types of MT were higher, and 2 function types of MT were lower than those of MS (Fig. 5b, d). i.e., in comparison with MS, Wood Saprotroph, Animal Pathogen-Endophyte-Lichen, Parasite-Plant, Pathogen-Soil, Saprotroph-Wood, Saprotroph

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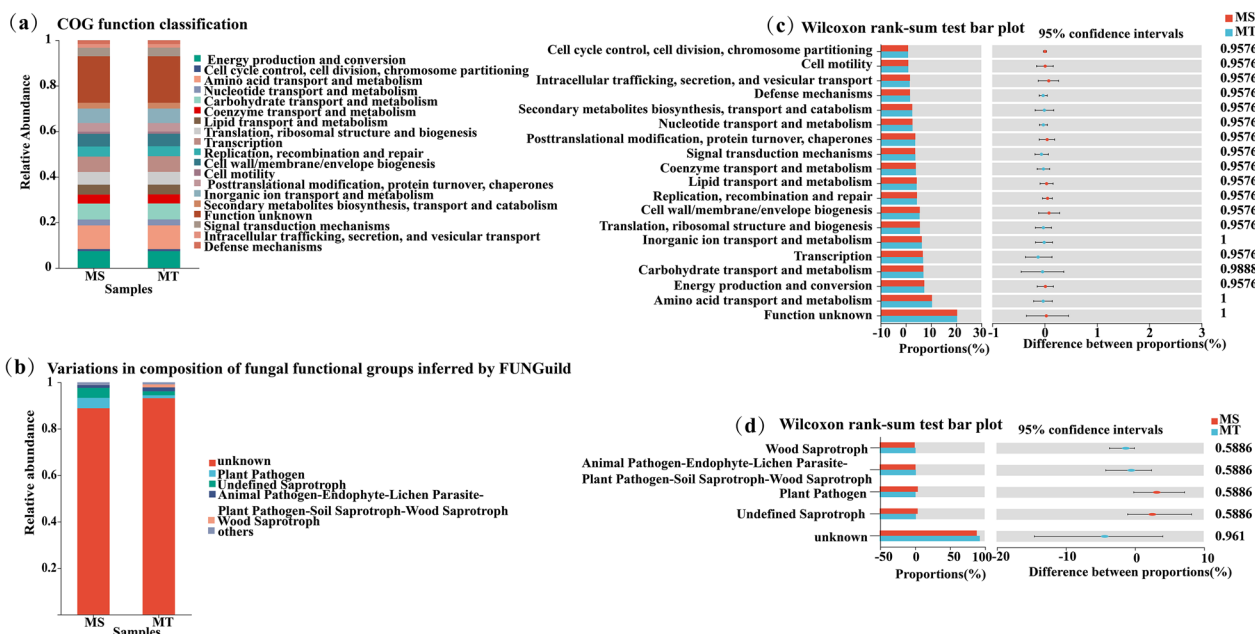
Fig. 5 Functional predictions of endophytic bacterial (**a**) and fungal (**b**) communities in the roots of melons. Compositional variability test for endophytic bacterial (**c**) and fungal (**d**) level communities. (MT) Wilt resistant varieties; (MS) Susceptible varieties

Table 2 Total ion numbers and identification statistics in roots of different wilt resistant melon varieties

Ion mode	All peaks	Identified metabolites	Metabolites in library	Metabolites in KEGG
pos	3762	1006	918	578
neg	4703	581	564	276
Total	8465	1587	1482	804

could be detected higher in roots of MT than those of MS; by contrast, Plant Pathogen and Undefined Saprotroph could be found lower in roots of MT than those of MS. In addition, Wilcoxon rank sum test was also performed to evaluate the root endophytic bacterial and fungal functions between MT and MS. The results showed that there were no significant differences in root endophytic bacterial (Fig. 5c) and fungal (Fig. 5d) functions between MT and MS.

Metabolome analysis of the roots

Based on Untargeted Metabolomics (LC–MS) analysis, the overall metabolome compositions of different melon samples were examined (Table 2). A total of 8465 metabolite ion peaks and 1587 metabolites were obtained. Among them, 1482 metabolites in the public database and 804 metabolites in the KEGG database were identified.

Partial least squares discriminant analysis (PLS-DA) was also performed on the root metabolites of different

wilt resistant melon varieties. As shown in Fig. 6a, b, the QC samples were well grouped, indicating that the bioanalytical quality and data quality were highly reliable. There were significant differences ($P < 0.05$) indicating that of root metabolites between wilt resistant (MT) and susceptible melon varieties. In addition, the PLS-DA model was analyzed by 200 replacement tests, the intercept of the Q^2 regression line was less than 0, and the R^2 and Q^2 regression lines showed an upward trend model, which indicated that the replacement test was over determined, and that the model was not over fitted (Fig. 6c, d).

Additionally, a total of 1590 metabolites, with 301 metabolites showed significant differences ($P < 0.05$) (Fig. 6e). In comparison with the MS, 74 metabolites were significantly upregulated and 227 metabolites were significantly down regulated in roots of MT.

Based on the PLS-DA model, the variable importance of predicted (VIP) scores described the order of differential metabolite abundance in the root system between MS and MT (VIP > 1, $P < 0.05$) (Fig. 6f). Particularly, for 30 most abundant metabolites, 5 metabolites, such as 22-Hydroxydocosanoicacid, 8-Prenylnaringenin, Melilotoside, Dinitrobenzenesulfonicacid, Cerebronicacid, and ʟ-Glutamicacid5-phosphate significantly up-regulated and 25 metabolites were significantly down-regulated.in roots of MT compared to MS.

Based on the HMDB database, 302 differential metabolites could be detected in roots between MT_and _MS varieties. Among them most highly enriched categories
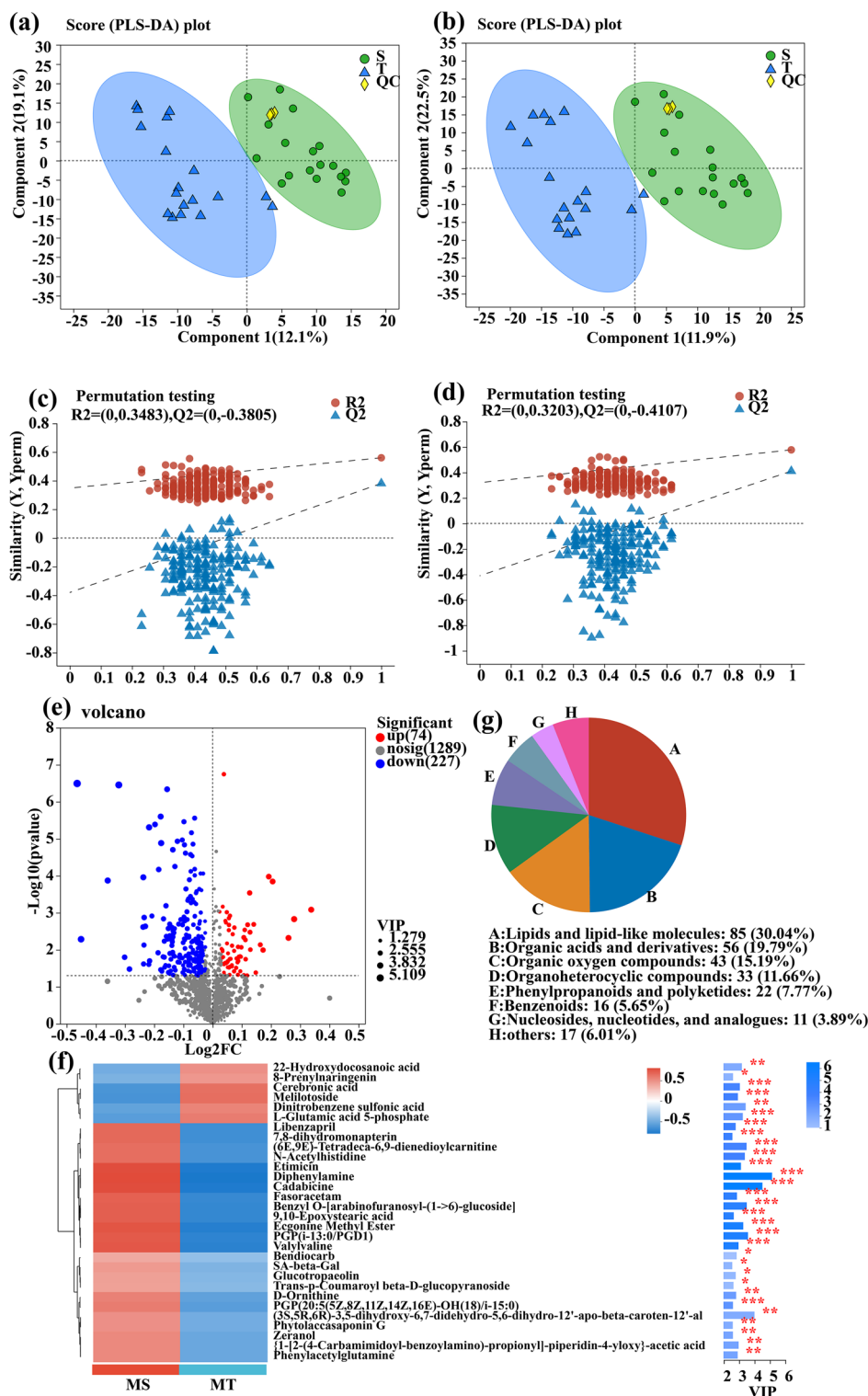
Fig. 6 Positive ion mode (**a**) and negative ion mode (**b**) PLS-DA analysis plots positive ion mode. Positive ions mode (**c**) and negative ions mode (**d**) PLS-DA substitution test plots. Volcano plot analysis of (MT) resistant and (MS) susceptible varieties (**e**); root differential metabolite VIP analysis plot (**f**); HMDB classification of total differential metabolites in roots (**g**). (MT) Wilt resistant melon varieties; (MS) Wilt susceptible melon varieties; (QC) quality control samples
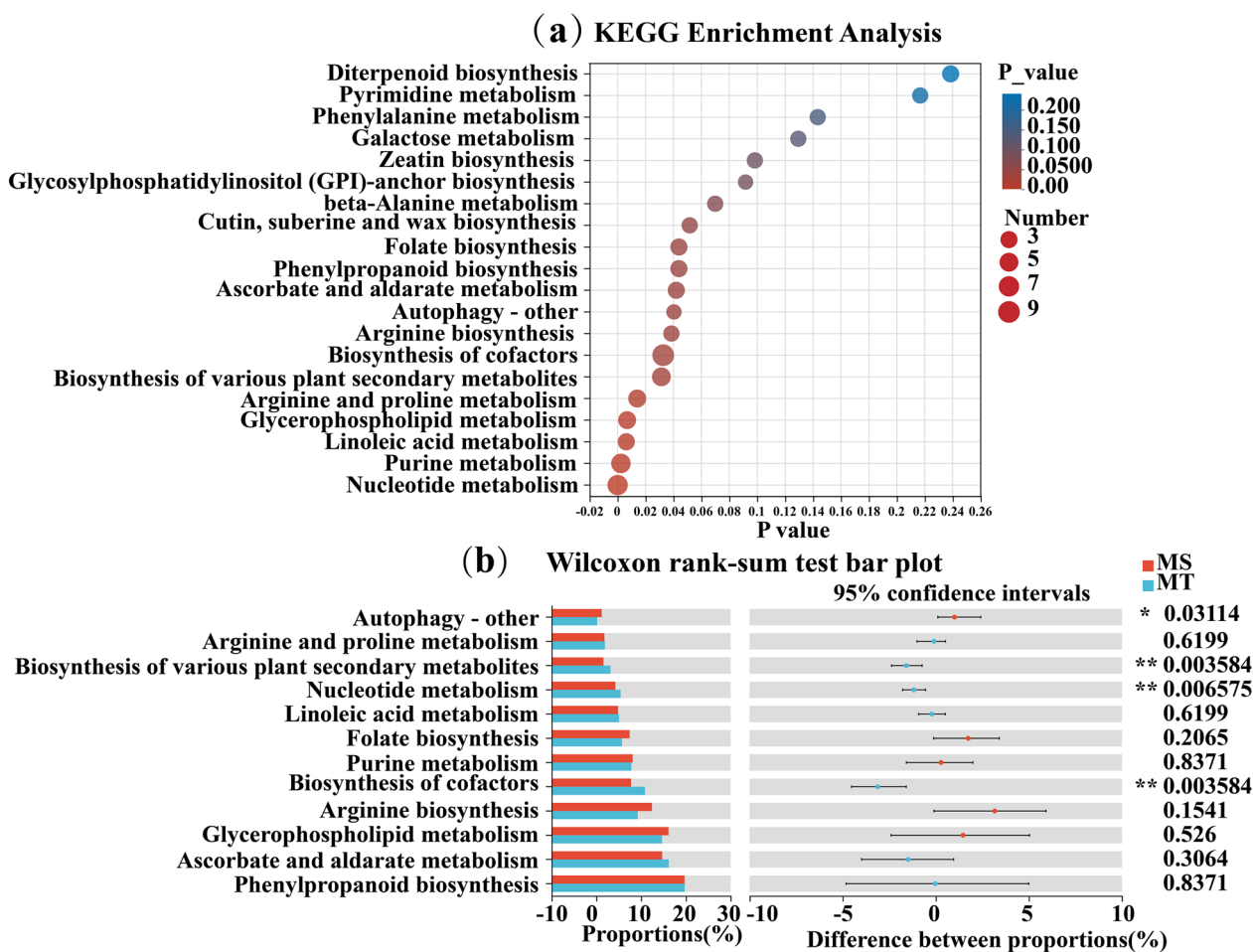
Fig. 7 Differential metabolite KEGG pathway enrichment bubble plots of wilt resistant (MT) and (MS) susceptible varieties (**a**); KEGG pathway difference test (**b**). *Indicates $0.01 < P < 0.05$, **indicates $0 < P < 0.01$

were lipids and lipid-like molecules: 85 (30.04%), Organic acids and derivatives: 56 (19.79%) organic oxygen compounds: 43 (15.19%), organoheterocyclic compounds: 33 (11.66%) and Phenylpropanoids and polyketides: 22 (7.77%) (Fig. 6g).

Based on KEGG database, the metabolites derived from MT and MS melon varieties were significantly enriched in autophagy-other, arginine and proline metabolism, biosynthesis of various plant secondary metabolites, nucleotide metabolism, linoleic acid metabolism, folate biosynthesis, purinemetabolism, biosynthesis of cactors, arginine biosynthesis, glycerophospholipid metabolism, ascorbate and aldarate metabolism, phenylpropanoid biosynthesis, and 12 other metabolic pathways (Fig. 7a).

Wilcoxon rank sum test was performed for KEGG pathways, could be detected significantly different in roots of MT which compared to those in roots of MS. Meanwhile, phenylpropanoid biosynthesis, biosynthesis

of cofactors, biosynthesis of various plant secondary metabolites and nucleotide metabolism, biosynthesis of various plant secondary metabolites, nucleotide metabolism and biosynthesis of cofactors metabolic pathways were significantly greater in roots of MT than those in roots of MS (Fig. 7b).

In addition, 12 significant differential metabolic pathways, and 42 differential metabolites, including 32 differential metabolites increasing and 10 differential metabolites decreasing were detected (Appendix 1).

Correlation analysis of endophytic microorganisms and metabolites in melon roots

The correlations between root endophytic microorganisms (bacteria and fungi) and the 23 most abundant metabolites were calculated and analyzed using the Spearman correlation algorithm and the Bray–Curtis distance algorithm.

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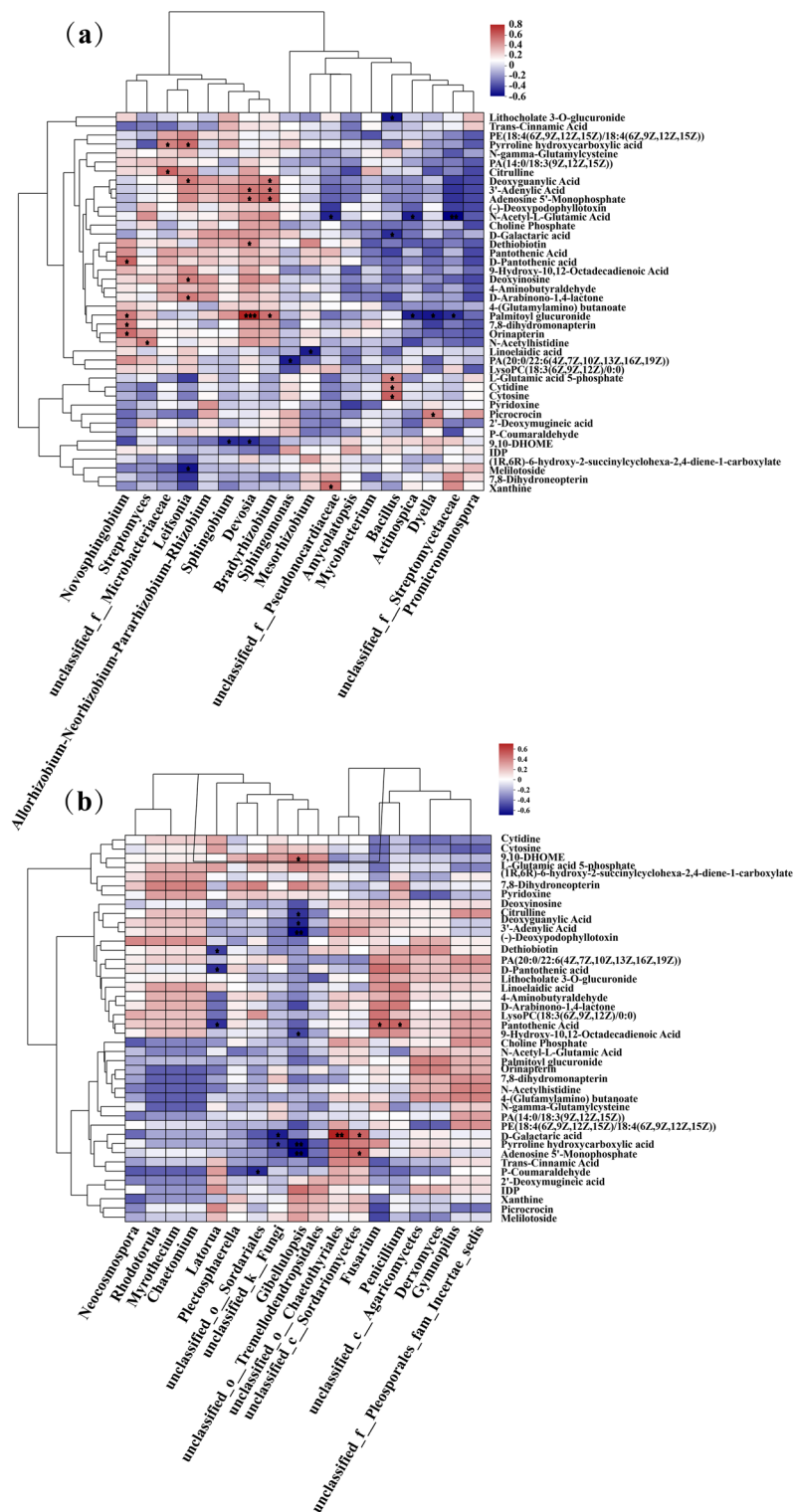
Fig. 8 Correlation of endophytic bacteria (**a**) and fungi (**b**) with metabolites in melon roots genus level

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At the bacterial level, such as *unclassified_f__Microbacteriaceae* and *Citrulline* were significantly positive correlation with Pyrroline hydroxycarboxylic acid; *Bacillus* was significantly positive correlation with L-glutamic acid 5-phosphate, cytidine and cytosine; Bradyrhizobium, Novosphingobium, and Devosia were significantly positive correlation with Palmitoyl glucuronide; *Devosia* and *Bradyrhizobium* were significantly positive correlation with Adenosine 5′-Monophosphate and 3′-Adenylic Acid. Moreover, *Sphingobium* and *Devosia* were significantly negative correlation with 9,10-DHOME; *Actinospica*, *Dyella* and *unclassified_f__Streptomycetaceae* were significantly negative correlation with Palmitoyl glucuronide and *N*-Acetyl-L-Glutamic Acid (Fig. 8a).

Additionally, at the fungal level, *Penicillium* and *Fusarium* were significantly positive correlations with pantothenic acid; *Gibellulopsis* was significantly positive correlations with 9,10-DHOME; *unclassified_o__Chaetothyriales* and *unclassified_c__Sordariomycetes* were significantly positive correlations with Adenosine 5′-Monophosphate, D-Galactaric acid. Moreover, *Latorua* was significantly negative correlations with Dethiobiotin, D-Pantothenic acid, and Pantothenic Acid; *unclassified_o__Sordariales* was significantly negative correlations with *P*-Coumaraldehyde; unclassified_k__Fungi was significantly negative correlations with Pyrroline hydroxycarboxylic acid and D-Galactaric acid; *Gibellulopsis* was significantly negative correlations with 9-hydroxy-10, 12-octadecadienoic acid, adenosine 5′-monophosphate, 3-pyrroline hydroxycarboxylic acid, 3′-adenylic acid, deoxyguanylic acid and citrulline (Fig. 8b).

Discussion

As endophytic microorganisms form a good symbiotic relationship with plants through interaction [41]. For instance, plants provide endophytic microorganisms with food and shelter, and endophytic microorganisms not only do not harm their hosts, but also can significantly promote the growth and disease resistance and adaptability through various forms of life activities [42]. Such as plant roots were invaded by pathogens, endophytic microorganisms in roots should sensitively provide timely defense against pathogens [43].

Endophytic microbial communities and functional prediction of wilt resistant (MT) and susceptible melon varieties (MS)

Although *Bacillus*, *Mesorhizobium*, *Fusarium*, *unclassified__o_Chaetothyriales*, *unclassified__c__Sordariomycetes*, *Dyella*, *Actinospica*, *unclassified__f__Pseudonocardiaceae* and *unclassified__f__Streptomycetaceae* were all detected as the dominant microbial genera in roots of MT and MS varieties. However, the abundances of them in roots of MT varieties were all higher than those of MS varieties.

Meanwhile, previous studies had demonstrated that *Bacillus* could effectively control melon wilt by promoting a significant increase in salicylic acid and antibiotic-like compounds in melon plants after inoculation [35]. Meanwhile, Quach et al. [44] found that unclassified_o__Chaetothyriales could produce active metabolites, such as ergot alkaloids, diterpenoid alkaloid and termarin in plants, which could significantly enhance the adaptability of plants. Nagpal et al. [45] also found that the occurrence of wilt could be significantly reduced by inoculation with *Mesorhizobium*, for it could promote the activities of soil enzymes, the absorptions of mineral elements and total phenolic contents. Moreover, *Catenulispora*, also could produce antibiotics for enhancing plant disease resistance [46]. Furthermore, previous studies also showed that *Bacillus* and *Pseudomonas* infested plants significantly increased of phenolics (gallic, cinnamic, ferulic, and tannic acids, among others) levels in plants, and they could significantly reduce plant morbidity even though plants were attacked by the pathogens [47, 48]. *Bradyrhiza* and *Methylobacterium* could promote the alkaloid compounds production in plants, which could significantly enhance the adaptability of plants to the environment [49, 50]. Also, *Aspergillus* and *Bacillus* could induce the gene expression of host plant terpene synthase and promote the accumulation of terpenes, which could significantly alleviate drought stress [51, 52]. i.e., microorganisms not only could increase the levels of specific bioactive metabolites in hosts, but also could convert less bioactive forms of the metabolite into active derivatives [53, 54].

In comparison with MS, *Ochrobactrum*, *Roseateles*, *Staphylococcus*, *Acidovorax*, *Burkholderia–Caballeronia–Paraburkholderia*, *Amycolatopsis*, *Catenulispora* and *Promicromonospora* were the unique dominant endophytic bacterial genera in roots of MT. Previous studies had shown that *Ochrobactrum* could effectively reduce the content of heavy metals (copper ions) in soil through surface adsorption, extracellular chelation and biological reduction, which can significantly enhance the adaptability of plants [55]. Roseateles and Acidovorax also had been reported that they could degrade plastics and polycyclic aromatic hydrocarbons pollutants in the environment [56, 57]. Younas et al. [58] found that inoculation with *Staphylococcus* endophytes significantly increased branch length, dry weight and chlorophyll content of plants, while promoting the absorption of nitrogen, phosphorus and potassium. *Burkholderia–Caballeronia–Paraburkholderia* as a common endophytic bacterium that can effectively alleviate vegetative

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and abiotic stresses (drought and high temperature) [59, 60]. Meanwhile, *Amycolatopsis* could promote plant growth and enhance plant resistance by producing novel secondary metabolites [61]. Busti et al. [46] also found that *Catenulispora* played an important role in mediating plant resistance through producing antibiotics. *Promicromonospora* was also found that it could promote plant growth and exhibit phosphate solubilization potential by producing plant hormones (gibberellin and salicylic acid) [62].

Additionally, in comparison with MS, defense mechanisms, secondary metabolites biosynthesis, transport and catabolism, transcription, nucleotide transport and metabolism, signal transduction mechanisms, carbohydrate transport and metabolism, coenzyme transport and metabolism, translation, ribosomal structure and biogenesis, inorganic ion transport and metabolism, amino acid transport and metabolism, Wood Saprotroph and Animal Pathogen-Endophyte-Lichen Parasite-Plant Pathogen-Soil Saprotroph-Wood Saprotroph were enhanced in roots of MT. As microorganisms can regulate plant growth and development through a variety of metabolic modalities. Particularly, when the plant is subjected to external environmental stresses, the synthesis of secondary metabolites can enhance its adaptation by regulating metabolic activities, such as the synthesis of secondary metabolites [63, 64], the synthesis of different kinds of amino acids [65], and the expression of disease-resistance genes or synthesis of signaling substances [66], etc.

Metabolome of wilt resistant (MT) and susceptible (MS) melon varieties roots of melon

Plant resistance is also closely associated with in vivo metabolism. Previous studies have confirmed that plant metabolites are important chemical compounds (phenolic compounds, terpenoids, nitrogen-containing compounds, etc.). It is produced by plants to adapt to changes in external conditions [67]. Plant secondary metabolites play multiple roles, including defense against pathogens, pests, and herbivores; respond to environmental stresses, and mediate organism interactions [68]. Changes of metabolic activities in plants are not only regulated by their own genes, but also significantly influenced by the external environment [69]. Also, it is well known that metabolic pathways and metabolite synthesis can be significantly affected by endophytic microorganisms [70].

In comparison with the MS varieties, not only Folate biosynthesis, Phenylpropanoid biosynthesis, Ascorbate and aldarate metabolism, Autophagy-other, Arginine biosynthesis, Biosynthesis of cofactors, Arginine and proline metabolism, Glycerophospholipid metabolism, Linoleic acid metabolism, Purine metabolism and Nucleotide metabolism metabolic pathways, but also Pyrroline hydroxycarboxylic acid, 4-aminobutyraldehyde, Citrulline, 4-(Glutamylamino) butanoate, Pantothenic Acid, Linoelaidic acid and Pyridoxine were all significantly increased in roots of MT varieties; However, 7,8-dihydroneopterin, cytidine, trans-cinnamic acid, picrocrocin and xanthine were significantly decreased in roots of MT varieties.

As previous studies had reported that Pyrroline hydroxycarboxylic acid was the intermediate compound in arginine and proline syntheses, and arginine and proline metabolisms could improve plants resistant to stresses by enhancing the signal transduction and regulation of plants under stress [71]. Also, 4-aminobutyraldehyde and polyamines could significantly enhance plant adaptability under stress conditions [72]. Meanwhile, citrulline was a non-coding protein amino acid, which played an important role in nitrogen metabolism and stress resistance. For example, it could play the roles in long-distance nitrogen nutrient transport [73], maintaining cell osmotic pressure during stress [74] and acting as a scavenger of photorespiratory NH_4^+ [75], etc. Moreover, 4-(glutamylamino) butanoate, could significantly enhance the ability of plants to respond to adverse external conditions [76]. Pantothenic Acid and Pyridoxine also played the important roles in plants, such as hormone synthesis, gene expression, cell breakdown, DNA repair for regulating plant growth and environmental response capacity [77, 78]. Linoelaidic acid, an important unsaturated fatty acid in plants, acted as a signaling molecule in controlling the defense-related genes and the disease resistance-related proteins [79, 80]. i.e., significant differences of root metabolites exactly could be found in roots between MT and MS varieties.

All above results suggested that not only different endophytic microbial compositions, but also various metabolites in roots of wilt resistant and susceptive melon varieties were all significantly different. Our findings will help us reshaping the endophytic microbial compositions by regulating metabolites in melon roots for enhancing wilt resistances in future.

Materials and methods
Field site description and experimental designs

The experiment was conducted at the Experimental Base of the Horticulture Research Institute, Guangxi Academy of Agricultural Sciences, (22° 46′ N and 108° 10′ E). The soil physicochemical properties of the experimental base were as follows: pH 5.31, the contents of organic matter total nitrogen, phosphorus and potassium were 13.9 g kg^{-1}, 0.81 g kg^{-1}, 0.39 g kg^{-1}, and 4.68 g kg^{-1}, respectively. Meanwhile, the contents of available nitrogen, phosphorus, and potassium were 53.71 mg kg^{-1}, 20.12 mg kg^{-1} and 82.34 mg kg^{-1}, respectively.

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Table 3 Sequence type and primer sequences

Sequence type	Primer name	Primer sequence	Length	Sequence
Bacterial 16SrRNA	799F	5'-AACMGGATTAGATACCCKG-3'	394 bp	MiseqPE250
	1193R	5'-ACGTCATCCCCACCTTCC-3'		
Fungal ITS	ITS1F	5'-CTTGGTCATTTAGAGGAAGTAA-3'	350 bp	MiSeq PE300
	ITS2F	5'-GCTGCGTTCTTCATCGATGC-3'		

Based on the previous observations in fields experiment, three fusarium wilt resistant melon varieties (Shan tian1, Qiang shi, 985; abbreviated MT group) and three fusarium wilt susceptible melon varieties (Hui yu, Qiaoyu, Chengmi, abbreviated MS group) were used in this experiment, respectively. All the melon varieties were provided by the Horticulture Research Institute of Guangxi Academy of Agricultural Sciences. And all melon varieties were planted in the same filed and grew under the identical managements.

Root samples collection
Root samples were collected on May 19, 2023. Firstly, plants were randomly selected and melon roots were collected carefully; secondly, root samples were rinsed six times using sterile water and wiped up with sterile papers. And then root samples were put into the sealed sterile bags taking back to the lab immediately as possible as. Root samples in lab were treated as describes as Xiao et al. [81]. That is, root samples were washed with 75% ethanol for 1 min, and then were dipped into 1% NaClO solution for 3 min and were rinsed with sterile water for 0.5 min.

To determine the sterilization of the melon roots surface, 100 μL sterile water from each washed stem was placed on a Luria–Bertani (LB) agar plate (g/L) (NaCl-10, tryptone-5, yeast extract-5, and agar-20) and incubated at 25 °C for 7 days. No colonies developed on the plates and it indicated that root surfaces were thoroughly sterilized. The sterilizations of the root samples were completed before detection and analysis of the endophytic microorganisms [82]. The root samples were placed in sterile bags and stored at −80 °C for DNA extraction.

Determination of the root endophytic microbiome
Extraction, PCR amplification and sequencing of total DNA from the samples were performed by Majorbio-Bio-PharmTechnologyCo., Ltd. (Shanghai, China). High-throughput sequencing was performed using the MiSeq platform on the basis of E.Z.N. Total DNA was extracted from A.DNAKit (OmegaCompany, Norwalk, CT, USA) instructions. DNA concentration and purity were measured using a NanoDrop2000 spectrophotometer (ThermoCompany, Waltham, NJ, USA), and the purity and

quality of the genomic DNA was checked on a 1% agositol gel. PCR amplification was performed on a ABIGeneAmp® 9700 with specific primers and sequence types that are shown in Table 3.

Ilumina MiSeq sequencing was performed as follows: PCR products from the same sample were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and mixed, followed by detection on and recovery from a 2% agarose gel. The recovered products were quantified using a Quantus™ Fluorometer (Promega, USA). Library construction was carried out using the NEXTFLEX® Rapid DNA-Seq Kit. The PCR amplification process for the 16S rRNA gene was as follows: initial denaturation at 95 °C for 3 min, followed by three cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s, a single extension at 72 °C for 10 min, and termination at 4 °C. DNA gel extraction kits from AXY (Axygen Biosciences, Union City, California, USA) were used according to the manufacturer's instructions to extract and purify PCR products from a 2% agarose gel and quantify them by a quantum fluorimeter (Promega, USA). Sequence data processing involved the following steps: original 16S rRNA gene sequencing read demultiplexing, quality filtering with fastp version 0.20.0, and merging with Flash version 1.2.7, using the maximum mismatch rate for the overlapping region in Fast P0.20.0. Uparse 7.1 was used for clustering operational taxonomic units (OTUs) at a similarity of 97%, and chimeric sequences were identified and deleted. RDP Classifier version 2.2 was used to classify and analyze the 16S rRNA sequences; the confidence threshold was 0.7, and the classification of each representative OTU sequence was analyzed [83]. Sequencing was performed using Illumina's MiSeqPE250 and MiSeqPE300 platforms (Shanghai Majorbio Bio-pharm Technology Co., Ltd.). Raw data were uploaded to the NCBI database for comparison.

Untargeted metabolomic assay
100 μL liquid sample was added to a 1.5 mL centrifuge tube with 400 μL solution [acetonitrile: methanol=1:1 (v:v)] containing 0.02 mg/mL internal standard (L-2-chlorophenylalanine) to extract metabolites. The samples were mixed by vortex for 30 s and low-temperature

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sonicated for 30 min (5 ℃, 40 kHz). The samples were placed at −20 ℃ for 30 min to precipitate the proteins. Then the samples were centrifuged for 15 min (4 ℃, 13,000×g).The supernatant was removed and blown dry under nitrogen. The sample was then re-solubilized with 100 μL solution (acetonitrile: water = 1:1) and extracted by low-temperature ultrasonication for 5 min (5 ℃, 40 kHz), followed by centrifugation at 13,000×g and 4 ℃ for 10 min. The supernatant was transferred to sample vials for LC–MS/MS analysis. The LC–MS/MS analysis of sample was conducted on a Thermo UHPLC-Q Exactive system equipped with an ACQUITY HSS T3 column (100 mm×2.1 mm i.d., 1.8 μm; Waters, USA) at Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).The mobile phases consisted of 0.1% formic acid in water:acetonitrile (95:5, v/v) (solvent A) and 0.1% formic acid inacetonitrile:isopropanol:water (47.5:47.5, v/v) (solvent B). The flow rate and column temperature were 0.40 mL/min and 40 ℃, respectively.

Statistical analysis

Data were statistically analyzed using Microsoft Excel 2019 and SPSS 26.0. The alpha diversities of the bacterial and fungal communities were calculated using Mothur (version 1.30.2, https://mothur.org/.com/calculators/; accessed on 15 June 2023). Principal coordinate analysis was performed using the R language (version 3.3.1) tool. For the analysis of microbial community composition and Venn diagrams, OTU tables with 97% similarity were selected and analyzed using the R language (version 3.3.1) tool. The pre-processed metabolite data matrix was subjected to partial least squares discriminant analysis (PLS-DA) using the 'ropls' package in R (version 1.6.2). The LEfSe analysis's LDA score was set to 2, and the Wilcoxon rank sum test was performed to see whether there were any differences between the groups. Additionally, the LDA Score was utilized to analyze and lessen the impact of species with substantial differences. PICRUSt was used to remove the effect of the number of copies of the 16S marker gene in the genome of the species and to standardize the OTUs abundance table, using the green gene ID corresponding to each OTUs. Each OTU's matching KEGG Orthology (KO) information and COG family information were acquired, and the abundance of each COG and KO could then be computed. The functional and descriptive data for each COG were obtained by parsing the COG database against the eggNOG database [84]. The FunGuild annotation tool was used to identify the different functional groups in the fungal community, categorizing the fungal taxa into three trophic modalities–saprotrophy, symbiotrophy and pathotrophy. These modes were further subdivided into specific guilds comprised of fungi that share similar lifestyle modes

[85]. Model stability was assessed using seven cycles of cross-validation. The selection of significantly different metabolites was determined based on the variable weight values (VIP) and Student's *t*-test *P*-values obtained from the PLS-DA model; metabolites with VIP > 1 and *P* < 0.05 were classified as significantly different metabolites. Using high-quality KEGG metabolic pathways as the reference, pathway enrichment and topology analyses were performed using Metabolic Analyst 3.0. A heat map was used to correlate the top 15 dominant microorganisms in terms of their abundance with the root metabolites [86]. Online data analysis was performed using the free online Mayobio Cloud Platform (http://www.majorbio.com, accessed on 14 August 2023) developed by Mayobio Biomedical Technologies Ltd., Shanghai, China.

Supplementary Information

Supplementary Material 1.

Declarations

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare that there are no competing interests.

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