Deciphering the mechanism about the mycorrhizal-induced resistance and susceptibility in Populus alba × P. berolinensis seedlings from the perspective of gypsy moth larvae

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Research Article

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Abstract

The mechanism of arbuscular mycorrhizal fungi (AMF) affecting insect resistance in plants is well depicted at the physiological level. However, there is a lack of systematic research from the perspective of insects. In this study, we used AMF-*Populus alba × P. berolinensis* seedlings that could either increase or decrease the resistance to gypsy moth larvae, to elucidate the mechanism of mycorrhizal-induced resistance/susceptibility at the larval microbial and metabolic levels. The results showed that the larval growth was significantly inhibited in the AMF, *Glomus mossae* (GM) colonized seedlings, whereas enhanced in the *Glomus intraradices* (GI) colonized seedlings. Gut microbiome analysis elucidated that GM inoculation reduced the probiotic abundance (e.g., *Staphylococcus*, *Lactobacillus*, *Akkermansia*) in gypsy moth larvae and inhibited the detoxification and metabolic functions of the gut microbiota. However, GI inoculation improved the gut environment in gypsy moth larvae by reducing the abundance of pathogenic bacteria (*Chryseobacterium*, *Fusobacterium*, and *Neisseria*) and activating specific metabolic pathways (e.g., energy metabolism). Non-targeted metabolomics analysis further revealed that GM inoculation triggers a metabolic disorder in the larval fat body, accompanied by the down-regulation of metabolic pathways involved in detoxification and energy production. The levels of differentially accumulated metabolites related to amino acid synthesis and metabolism and exogenous toxin metabolism pathways were significantly increased in the GI group. Taken together, disturbance in the gut microbial function and fat body metabolism in gypsy moth larvae led to the induction of mycorrhizal-induced resistance after GM inoculation, and GI-induced susceptibility involved in the improvement of gut environment and energy metabolism.

1. Introduction

Arbuscular mycorrhizal fungi (AMF), belonging to the Glomeromycota phylum are widely distributed microorganisms associated with the plant roots. During the long-term co-evolution process, AMF have established symbiotic relationships with more than 80% of plant species on the earth (Meier and Hunter 2021). Generally, the host plant provides carbohydrates synthesized by photosynthesis to the AMF as carbon sources. Meanwhile, AMF colonization improves the absorption and utilization of nitrogen, phosphorus, and other nutrients in the soil by plants and significantly promotes the plants' growth (Xie et al. 2021). Kavadia et al. (2021) found that inoculation with the *Rhizophagus irregularis* significantly increased the biomass in aerial parts of *Vigna unguiculata*. In addition, AMF can also improve plant resistance to various environmental stresses, including salt stress, heavy metal stress, drought stress, and temperature stress, by promoting the physical and chemical properties of soil (Millar and Bennett 2016; Qiu et al. 2020; Rozpadek et al. 2014; Zhang et al. 2019a; Zhang et al. 2020).

As an important part of the terrestrial ecosystem, phytophagous insects easily form a complex interaction with the AMF and plants. Studies have shown that AMF, as a biological stimulator, can improve the plant resistance to phytophagous insects and weaken the growth of these insects by triggering the defense response in plants (Tomczak and Müller 2017). Formenti et al. (2019) showed that inoculation with the *R. irregularis* promoted jasmonic acid synthesis in tomatoes and inhibited the feeding of *Spodoptera*

Mauritia. Schoenherr et al. (2019) also reported that potatoes inoculated with *R. irregularis* could significantly inhibit the growth of *Trichoplusia ni*. Further, AMF inoculation has been shown to significantly increase the phenolic content in *Triticum aestivum* and inhibit the relative growth rate of *Helicoverpa punctigera* (Frew and Wilson 2021). With the deepening of research, Mycorrhizal-induced resistance (MIR) hypothesis was put forward (Pozo and Azcon-Aguilar, 2007). Mycorrhiza-induced resistance (MIR) refers to the induction of plant resistance to phytophagous insects by activating the salicylic acid or jasmonic acid signaling pathway following the establishment of a symbiotic relationship between AMF and plants. Due to the existence of MIR, AMF can be used as a potential alternative to chemical pesticides for pest control in agroforestry production.

Interestingly, MIR is not induced in all the AMF-plant systems. In recent years, few studies have shown that AMF increased the growth and development of insects after forming a symbiotic relationship with the plant. *Mamestra brasicae* gained weight and shortened development duration after feeding on *Plantago lanceolata* colonized by *R. irregularis* (Tomczak et al. 2016). In a similar study, Real-Santillan et al. (2019) reported that *Zea mays* inoculated with *Glomus spp.*, *Acaulospora spp.*, *Gigaspora spp.*, and *Intraspora spp* promoted the growth and development of *Spodoptera frugiperda* larvae. These phenomena are collectively termed mycorrhizal-induced susceptibility (MIS). In addition, the influence of mycorrhiza on the growth and development of insects also has a neutral effect. Minton et al. (2016) exhibited that *Solanum ptycanthum* and *S. dulcamara* inoculated with *R. irregularis* did not affect the growth and development of the phytophagous insect *Manduca sexta*. Therefore, it should be noted that the effects of AMF colonization on plant resistance to insects vary in the field and are dependent on various factors.

Based on the importance of AMF in agriculture and forestry, a large number of studies have systematically explored the interaction between AMF-agricultural and forestry plants and phytophagous insects and proposed different hypotheses related to describing plant resistance to the insects, such as MIR, MIS, and MIN. However, the studies available to date which decipher the mechanism of AMF affecting plant resistance to insects only focus on the physiological and cellular changes in the plants. The systematic investigation of AMF-induced plant resistance from the perspective of insects is still lacking. In our previous studies, we found that the inoculation with *Glomus mossae* (GM) improved the resistance to gypsy moth larvae in *Populus alba × P. berolinensis* seedlings, while inoculation with *Glomus intraradices* (GI) resulted in the opposite effect (Jiang et al. 2021a; Jiang et al. 2022). In the current study, leaves of *P. alba × P. berolinensis* inoculated with GM or GI were used to feed gypsy moth larvae, and the mechanism of mycorrhizal induced resistance/susceptibility was depicted at the microbial and metabolic level of gypsy moth larvae by combining the fat body metabolome and the gut microbiome. These findings will be beneficial in understanding how mycorrhiza-induced resistance/susceptibility is triggered from the insect perspective and further expand our knowledge of the AMF-plant-phytophagous insect interaction.

2. Materials And Methods

2.1 Treatment of *P. alba × P. berolinensis* seedlings

Turfy soil, sand, and vermiculite were carefully mixed in a 3: 1: 1 ratio at the nursery of Northeast Forestry University and sterilized in an autoclave at 121℃ for 2h. The flowerpots with a diameter of 250 mm and a height of 230mm were divided into 3 groups. Among them, the CK group was only loaded with a 2kg soil mixture. The other two groups were filled with a homogeneous mixture of 2kg sterilized soil and 20g GM (denoted as GM) or GI (denoted as GI). Both AMF microbial inocula were provided by the Gansu Academy of Agricultural Sciences, China. The number of spores provided in 1g of AMF was about 15. Subsequently, the seedlings were planted into flowerpots at the end of April 2020. In total, 150 seedlings were planted in each group. After 90 days of AMF infection, leaves were collected to feed the gypsy moth larvae.

2.2 Insect rearing

Gypsy moth eggs were obtained from the campus of Northeast Forestry University (Harbin, China) in March 2020. At the beginning of August, eggs of the gypsy moth were sterilized with 10% formaldehyde solution for 1h and incubated for hatching at 25℃ with 16L: 8D photoperiod and 60 ± 1% relative humidity. After hatching, the eggs were fed an artificial diet in the same environment until the second instar. The newly molted 2nd instar larvae were divided into 3 groups, which were fed with leaves of the GM group, GI group, and CK group, respectively. A total of 100 larvae were kept in each group. During the experiment, the leaves were changed every two days. Thirty gypsy moth larvae were selected from each group, and their body weight and developmental period were recorded. Subsequently, the growth rate and relative growth rate of gypsy moth larvae were calculated according to the following formula: Growth Rate (GR) = (final weight-initial weight)/T; Relative Growth Rate (RGR) = (final weight-initial weight)/(initial weight × T) (Milanovic et al. 2020). Here, final weight and initial weight respectively indicate the weight of larvae at the end and beginning of instar; T represents the number of days between final weight and initial weight. SPSS 26.0 software was used to analyze GR and RGR by one-way ANOVA, and LSD (least significant method) was used to test the significant difference between the treatment group and the control group with a p-value > 0.05.

2.3 Gut microbiome analysis

The newly molted gypsy moth larvae at the 5th instar were anesthetized on ice, and their gut tissues were dissected. The gut tissue of 5 larvae was taken as one repeat. Similarly, 4 repeats were set for both the control and treatment groups. Gut flora DNA of gypsy moth larvae was extracted using EZN.A ® Stool kit. After qualitative detection on 0.8% agarose gel electrophoresis, all DNA samples were sent to the LianChuan Biotechnology Co., Ltd. (China, Hangzhou) for microbiome analysis. Briefly, using total DNA as a template, the V3-V4 region of 16S rDNA was amplified with universal primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGGGTATTACCC-3'). The amplified products were detected on 2% gel electrophoresis, and the amplified sequences were sequenced by the Ampre XT beads. The Overlap was used to splice the obtained original sequence. After quality control, sequences with below-average quality and a length less than 50 bp were eliminated. Subsequently, the chimeric

sequences were filtered by the Vsearch software (v2.3.4) to obtain high-quality clean data. The final sequence was obtained using DADA2 for de-repetition. The alpha diversity (Shannon and Simpson index) of gut microorganisms was calculated using QIIME2, and the beta diversity was calculated using principal coordinate analysis (PCoA) and principal component analysis (PCA). Linear discriminant analysis (LDA) and effect size (LEFSe) bifurcation diagram were used to identify the microbial groups with different relative abundance between control and treatment groups. PICRUSt2 (phylogenetic investigation of communities by reconstruction of unobserved states 2) was used to predict gut microbial function. Further, an independent sample t-test was used to evaluate the difference in the relative abundance of gut microorganisms with the same functions between CK and GM groups or CK and GI groups.

2.4 Metabolomic analysis of larval fat body

The newly molted gypsy moth larvae at the 5th instar stage were dissected on ice to obtain the fat body. The fat body collected from 5 larvae was regarded as one repeat. Similarly, each group was set with four repeats. Samples were sent to the China LianChuan Biotechnology Co., Ltd. for non-targeted metabolomics analysis. In brief, a 100mg tissue sample was weighed and ground in the liquid nitrogen. Subsequently, 150 μL 50% methanol was added, mixed evenly by shaking, and incubated at room temperature for 10min. The crude extract was placed in a refrigerator at -20 ℃ overnight for protein precipitation. The crude was centrifuged at 4000g to obtain the supernatant, which was used further as the metabolite extract. Isopropanol, acetonitrile, and water (2: 1: 1) were used to dilute the metabolite extract. The metabolite extracts were analyzed using a high-resolution mass spectrometer TripleTOF 5600plus (SCIEX, UK), and chromatographic separation was performed using an ultra-performance liquid chromatography (UPLC) system (SCIEX, UK). The LC-MS data was processed by the XCMS software (Ref.). The original data file was converted to mzXML format and then processed using the CAMERA and metaX toolbox tools (Ref.). The identified metabolites were annotated using KEGG databases. The partial least squares-Discriminant analysis (PLS-DA) was used to study the relationship between metabolite expression in CK, GM, and GI groups (Ref.). Differentially accumulated metabolites (DAMs) were identified with a fold change (FC) > 2 and *P* values < 0.05. Further, pathway analysis of differentially accumulated metabolites was performed using the Metaboanalysis (Ref.).

3. Results

3.1 Analysis of growth and development indexes

To evaluate the effect of GM or GI colonized-*Populus* on the growth and development of gypsy moth larvae, the growth rate and relative growth rate of 2nd-4th instar larvae were measured. As shown in Fig. 1A-C, the growth rates of 3rd and 4th instar larvae of gypsy moth in the GM inoculation group were significantly lower than those in the CK group, while the growth rates of 2nd-4th instar larvae in the GI treatment group were higher as compared to the CK group. After GM and GI treatments, the relative growth rate of larvae was consistent with the growth rate. The relative growth rate of 3rd and 4th instar

larvae was significantly lower and higher in the GM and GI groups as compared to the untreated group, respectively.

3.2 Diversity analysis of gut microbial community

Based on the 16S rDNA sequencing, about 888230 high-quality transcripts were obtained from 12 samples of CK, GM, and GI groups. After classification, 598 operational taxonomic units (OTU) were identified. Venn diagram showed that 65 OTUs were common among the CK, GM, and GI groups, 23 OTUs were common between CK and GM groups, 25 OTUs were common between CK and GI groups, and 18 OTUs were common between GM and GI groups. In addition, 224, 145, and 98 unique OTUs were identified in the CK, GM, and GI groups, respectively. Venn diagram showed that the OTU composition of gut microflora of gypsy moth larvae was significantly changed by feeding on *P. alba × P. berolinensis* inoculated with GM or GI. (Fig. S1)

The total sequences obtained by 16S rDNA sequencing were aligned, and the richness and species differences within the gut microbial community of gypsy moth larvae were evaluated by comparing α and β diversity. The results showed that compared to the untreated group, the Shannon and Simpson indexes of gut microflora in the GM treatment group had no significant change, whereas these indexes were decreased markedly in the GI group (Fig. 2A and B). PCA showed that the gut microflora in CK, GM, and GI groups were well isolated, indicating that the similarity coefficient of species community among the three groups was low (Fig. 2C). At the same time, PCoA analysis also revealed a significant difference in the gut microbiota composition among the three groups (Fig. 2D).

3.3 Differential gut microflora analysis

A total of 18 bacterial phyla were identified in the CK, GM, and GI groups. LefSe analysis showed that the abundance of Firmicutes in the untreated group was higher than that in the GM group, while the abundance of Cyanobacteria in the GM group was higher than in the CK group (Fig. S2A/B). The relative abundance of 4 phyla was significantly different between the untreated and GI groups. The untreated group was characterized by a higher abundance of Proteobacteria, Firmicutes, and Bacteroidetes, while the GI group was characterized by a higher abundance of Cyanobacteria (Fig. S3A/B). At the genus level, 251 bacterial genera were identified in the CK, GM, and GI groups. There were significant differences in the relative abundance of 15 genera between the CK and GM groups. The abundance of *Staphylococcus*, *Klebsiella*, *Lactobacillus*, *Lachnospirnceae_ND3007*, *Ruminococcaceae_UCG005*, *Fusicatenibacter*, *Akkermansia* and *Ruminococcacea_UCG014* increased significantly in the CK group, whereas the abundance of Subgroup_unclassified, *Methylibium*, TRA3_unclassified, *Acinetobacter*, *Ralstonia*, *Sphingopyxis*, and Oxyphotobacteria_unclassified increased markedly in the GM group (Fig. S2A/B). There were noticeable differences in the relative abundance of 28 genera between CK and GI groups. Among them, the abundance of *Chryseobacterium*, *Ruminococcus*, *Bifidobacterium*, Actinobacteria_unclassified, *Delftia*, *Bradyrhizobium*, *Bacteroides*, *Alloprevotella*, *Fusobacterium*, *Lachnospiraceae*, *Neisseria*, *Fusicatenibacter*, *Brevundimonas*, *Lactobacillus*, *Klebsiella*, *Streptococcus*,

Ruminococcaceae, *Ralstonia*, *Staphylococcus* and *Pseudomonas* was found to be increased in the CK group, whereas the abundance of Alphaproteobactia_unclassified, *Rosenbergiella*, *Mesorhizobium*, *Thiobacillus*, Oxyphotobacteria_unclassified, Mitochondria_unclassfifed, Subgroup_unclassified *Sphingosinicella* and Oxyphotobacteria_unclassified was increased in the GI group (Fig. S3A/B).

3.4 Analysis of gut microbial function

The KEGG database was used to evaluate the functional alterations in the gut microbial community among control/treatment groups, and an independent sample t-test was used for significance analysis. As shown in Fig. S4A, glycan biosynthesis and metabolism and digestive system pathways were significantly down-regulated, whereas cardiovascular diseases, amino acid metabolism, metabolism of other amino acids, endocrine system, neurodegenerative diseases, cancers, transport and catabolism, circulatory system and metabolism pathways were up-regulated in the GM group as compared to the CK group. The expression levels of biosynthesis of other secondary metals, cellular processes and signaling, lipid metabolism, transport and catabolism, transcription, membrane transport, and metabolism pathways in the GI treatment group were significantly lower than those in the CK group, while cell growth and death, genetic information processing, metabolic diseases, metabolism of other amino acids, metabolism of terpenoids and polyketides, immune system, excretory system, digestive system, folding, sorting and degradation, metabolism of cofactors and vitamins, enzyme families, immune system diseases, energy metabolism pathways were markedly higher than those in the CK group (Fig. S4B).

3.5 Metabonomics analysis

To compare the metabolic differences between treated and untreated groups, the metabolites isolated from the fat body of gypsy moth larvae were analyzed under the positive ion (ESI +) mode of UPLC-QTOF MS. A total of 20687 target peaks were detected, of which 153 were annotated using KEGG. The PCA analysis showed that there was a good separation among the three groups. Principal components PC1 and PC2 accounted for 88.6% and 4.1% of the changes, respectively (Fig. 3A). PLS-DA analysis further revealed considerable segregation between the CK, GM, and GI groups (Fig. 3B). FC analysis followed by the t-test led to the identification of 25 DAMs in the GM and GI groups (Fig. 4A/B). Compared to CK, the contents of pantothenic acid, uridine, cytarabine, 5,6-dihydroxyindole-2-carboxylic acid, isoleucine, (-)-epicatechin, penicillamine, pirbuterol, daidzin, hydroxykynurenine, 2-furancarboxaldehyde, pyruvic acid, L-kynurenine, alpha-ionone, linustatin, N, N-dimethylformamide, DL-ornithine, and 5-hydroxy indole-3-acetic acid decreased in GM treated group, while the contents of inosine 5'-monophosphate, L-propionylcarnitine, phosphatidylcholine, 2-lysophosphatidylethanolamine, phosphatidylethanolamine, corticosterone, and acyl1-monogalactosyl-diacylglycerol increased. For the GI treated group, the contents of pirbuterol, uridine, dodecanoic acid, (-)-epicatechin, pantothenic acid, isonicotinic acid, daidzin, pyridine, N(6)-(1,2-dicarboxyethyl)AMP, beta-nicotinamide mononucleotide, and trigonelline was significantly reduced, while the contents of camptothecin, phenylalanine, L-propionylcarnitine, 9,10-dihydroxy-12z-octadecenoic acid, allantoic acid, trans-cinnamic acid, (-)-riboflavin, arginine, salicylic acid, indole, 1H-indole-3-carboxaldehyde, kynurenic acid, L-tryptophan, and 3,4-dihydroxy-L-phenylalanine was markedly increased than that of CK. KEGG enrichment analysis combined with the pathway topological

characteristics was used to evaluate the influence of DAMs on KEGG pathways. Post GM treatment, glycerophospholipid metabolism, glycosylphatidylinositol (GPI)-anchor biosynthesis, pantothenate and CoA biosynthesis, citrate cycle (TCA cycle), and pyruvate metabolism were found to be affected (Fig. 5A). Further, riboflavin metabolism, phenylalanine metabolism, arginine biosynthesis, nicotinate, and nicotinamide metabolism, and pantothenate and CoA biosynthesis pathways were significantly altered after GI treatment (Fig. 5B).

4 Discussion

As proposed by the MIR hypothesis, the insect resistance of plants colonized with AMF is increased by activating the defense signaling pathways. For example, Wang et al (2020) found that AMF colonization significantly increased the phenolic content in wheat and reduced the number of wheat aphids. It is generally believed that the "AMF-plant" symbiotic system has ecological specificity (Razak and Gange 2021; Simon et al. 2017). In some cases, this ecological specificity inhibits plant resistance and promotes the growth of harmful insects. In line with our previous studies, the present study revealed that GM and GI treatments respectively induced and decreased the resistance of *P. alba × P. berolinensis* seedlings to gypsy moth (Jiang et al. 2021a; Jiang et al. 2022). These results, together with the previous studies, demonstrated that specific AMF colonization could induce alterations (either increase or decrease) in the plant resistance to insects. Therefore, for the utilization of AMF in agriculture and forestry production, AMF-plant combinations concerning insect resistance should be selected carefully to avoid the positive effects of AMF colonization on the growth of herbivorous insects.

During the long-term evolution process, insects and their gut microbiota have established a mutually beneficial symbiotic relationship (Engel and Moran 2013; Feng et al. 2011; Jiang et al. 2021b). As one of the main factors to maintain homeostasis in host insects, the gut microbiota is regarded as an important index to measure the growth of host insects. In the present study, different gut microbiota at the genus level were identified between the untreated and treated groups by LefSe analysis, and subsequently, their functions were elucidated. The results showed that the relative abundance of 8 genera in gypsy moth larvae gut in the GM group was decreased as compared to the untreated group. Of these, *Staphylococcus*, *Klebsiella*, *Lachnospirnceae_ND3007*, *Ruminococcaceae*, *Akkermansia*, and *Lactobacillus* are major probiotics that participate in the energy metabolism, detoxification metabolism, or improvement of the gut barrier (Chouaia et al. 2019; Dabbou et al. 2020; Dai et al. 2014; Feng et al. 2011; Liang et al. 2020; Muhammad et al. 2017; Osawa et al. 2006). A decrease of these genera in the GM group indicated that the digestion of food materials and the degradation ability of plant secondary substances were reduced in the gypsy moth larvae. Surprisingly, the abundance of some probiotics (*Acinetobacter* and *Ralstonia*) that can promote digestion and absorption in the host significantly increased in the GM group (Briones-Roblero et al. 2017; Paulson et al. 2014), indicating that gypsy moth larvae try to compensate for the nutrition obtained from low-quality food with the help of gut microbiota. The abundance of 20 genera in the GI group decreased significantly as compared to those in the untreated group, including 7 pathogenic bacteria (*Chryseobacterium*, *Fusobacterium*, *Neisseria*, *Klebsiella*, *Streptococcus* and *Pseudomonas*) (Bog et al. 2020; Dijokaite et al. 2021; He et al. 2019; Moman et al. 2021; Tzec-Interian et al. 2020; Teoh et al.

2021; Yip et al. 2021). These results indicate that GI treatment seems to improve the gut environment in gypsy moth larvae by reducing the abundance of pathogenic bacteria in the larval gut. In addition, some probiotics associated with the nutritional metabolism and detoxification were also found to decrease in the GI group, including *Bifidobacterium*, *Bacteroides*, *Lachnospirnceae* and *Ruminococcaceae*, *Brevundimonas*, *Ralstonia* and *Lactobacillus* (Chen et al. 2021; Chouaia et al. 2019; Dijokaite et al. 2021; Jiang et al. 2021c; Li et al. 2022; Liang et al. 2020; Muhammad et al. 2017; Osawa et al. 2006; Paulson et al. 2014). In our previous studies, GI colonization was shown to improve the leaf quality significantly, as evident by a marked increase in the nutrient content and a decrease in the secondary metabolites content. Therefore, the improvement in leaf quality post GI colonization is responsible for lowering the need for a lot of gut probiotics in gypsy moth larvae to assist nutrition metabolism and the detoxification process.

To further explore the functional diversity of gut microbiota in gypsy moth larvae between AMF-treated and untreated groups, PICRUSt2 function prediction and significance analysis was performed. We observed that glycan biosynthesis and metabolism and digestible system pathways in the GM group were down-regulated, indicating the inhibition of digestion, absorption, and energy metabolism in gut microbiota. In addition, the pathways related to cardiovascular diseases, neurodevelopmental diseases, and cancer were also up-regulated in the GM group, which suggests that the *P. alba × P. berolinensis* seedlings with GM colonization provide a better environment for the reproduction of pathogenic bacteria in the larval gut. However, the energy metabolism and detoxification pathways in the GI group were significantly up-regulated, including the metabolism of other amino acids, metabolism of terpenoids and polyketides, metabolism of cofactors and vitamins, and energy metabolism. It can be deduced that the ability of larvae to utilize nutrients and adapt to host plants increased in the GI treatment group. In addition, immune-related pathways such as immune system and enzyme families were significantly up-regulated in the GI group, which might be the reason behind the decrease of pathogenic bacteria in the GI group. Altogether, the decrease in gut probiotics and microbial function disorder is one of the main reasons for the growth retardation of gypsy moth larvae in the GM group, while the improved gut environment and the enhanced metabolic functions of gut flora are responsible for the increased larval growth in GI group.

Metabolites, as important markers associated with various physiological and biochemical activities, are closely related to the growth and development of insects. The metabolic levels of gypsy moth larvae in the treated and untreated groups were analyzed by untargeted metabonomics. Our results revealed that 25 DAMs were identified in both GM and GI groups. In the comparative analysis of GM and CK groups, it was observed that several DAMs that are critical for growth, reproduction, and detoxification metabolism were significantly reduced in the GM group, including pantothenic acid, uridine, 5, 6-dihydroxyindole-2-carboxylic acid, isoleucine, penicillamine and hydroxykynurenine (Abolaii et al. 2020; Barek et al. 2018; Li et al. 1999; Li et al. 2020; Ren et al. 2021; Zhang et al. 2019b). These results indicated that the metabolic disorder of gypsy moth larvae in GM treatment group occurred, consistent with the growth retardation of gypsy moth larvae mentioned above. In addition, Corticosterone (a metabolite), which can lead to developmental disorder and a repressed immune system (Cabor et al. 2019), was increased significantly in the fat body of GM larvae, highlighting the possible reason underlying decreased adaptability of larvae

to the leaves of *P. alba × P. berolinensis* colonized with GM. However, GI colonization significantly enhanced the metabolism in gypsy moth larvae. Some DAMs related to growth and development (e.g. (-)-Riboflavin, 3, 4-Dihydroxy-L-phenylalanine, phenylalanine, arginine, and L-tryptophan) showed a marked increase in the GI group, while other DAMs with toxic effects decreased significantly, such as Dodecanoic acid and Pyridine (Du et al. 2021; Ninomiya et al. 2008; Reis et al. 2020; Vatanparast et al. 2020). Among these, amino acids were the majorly up-regulated metabolites in the GI group. Previous studies have demonstrated that amino acids are positively correlated with the digestive level or growth of insects (Pan et al. 2014). Therefore, the increase of amino acid content in the GI treatment group may account for the improvement of larval growth. Functional analysis of DAMs showed that the metabolites affected by GM were mainly involved in the energy metabolism and detoxification pathways, such as citrate cycle (TCA cycle), pyruvate metabolism, and glycerophospholipid metabolism. Combined with the analysis of DAMs, it was found that the energy metabolism and detoxification ability disorder was another reason for the growth inhibition of gypsy moth larvae in the GM treatment group. DAMs increased by GI treatment were mainly involved in the amino acid synthesis and metabolism (e.g., Phenylalanine metabolism, Arginine biosynthesis), and exogenous toxin metabolism (e.g., Nicotinate and nicotinamide metabolism, and Pantothenate and CoA biosynthesis), elucidating that GI colonization further increased the ability of gypsy moth larvae to digest food and adapt to the chemical defense of plants.

5. Conclusion

Two mycorrhizal fungi (GM and GI), *P. alba × P. berolinensis* seedlings and gypsy moth larvae, were successfully used to investigate the basis of mycorrhiza-induced resistance/susceptibility at the physiological level in insects. The gut microbial function and fat body metabolism disorder in gypsy moth larvae caused by GM colonization of *P. alba × P. berolinensis* is one of the main reasons for GM-induced resistance, while the improvement of gut environment and fat body metabolism in larvae results in the GI-induced susceptibility. These findings may provide new insights for understanding how physiological alterations in herbivorous insects affect AMF-plant-herbivorous insect interactions.\

Declarations

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Contributions

Dun Jiang conceived the experiments. Dun Jiang and Mingtao Tan participated in the design of the experiments. Mingtao Tan performed the experiments. Dun Jiang and Mingtao Tan wrote and revised the manuscript. All authors approved the final manuscript.

Conflict of interest statement

The authors declare that they have no competing interests.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

Availability of data and material

All the data that support the findings of this study are available in the manuscript.

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Figures

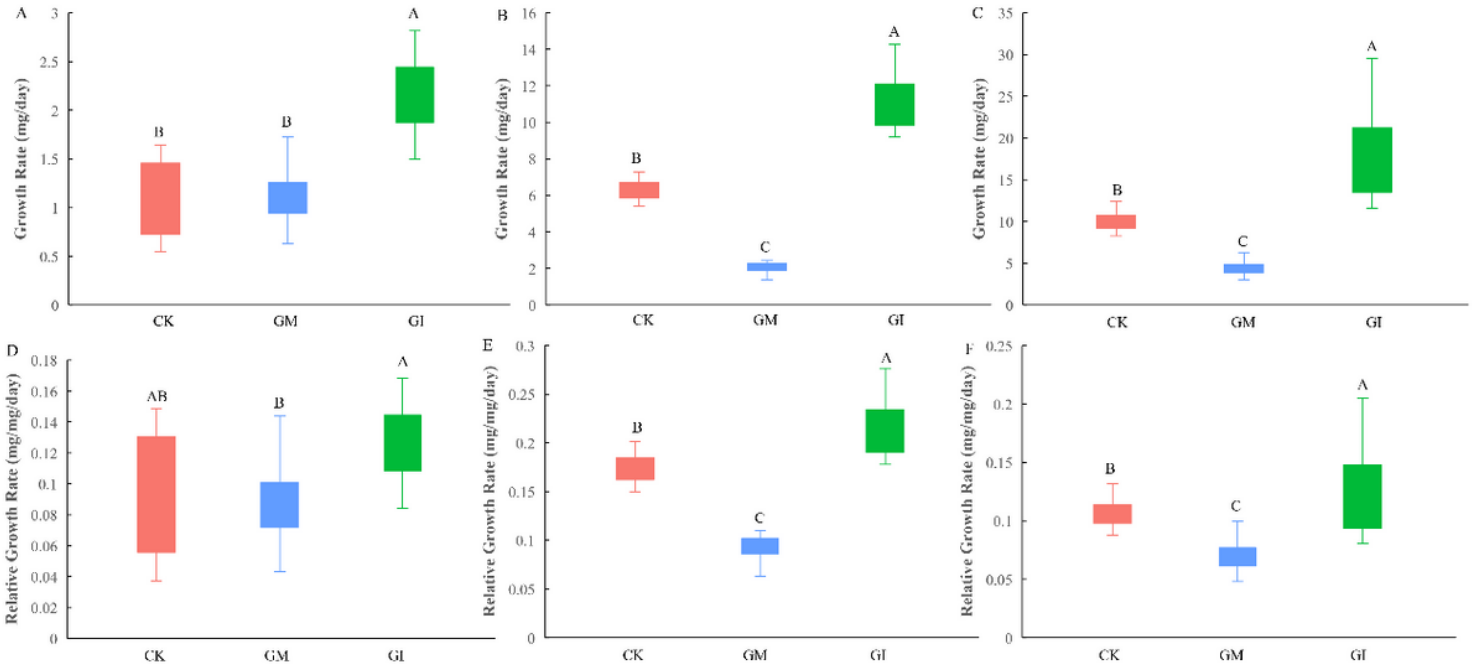

Figure 1

The growth rate(A-C) and relative growth rate(D-F) of the 3rd, 4th and 5th instar gypsy moth larvae fed on the leaves of GM, GI or nonmycorrhizal-colonized poplar plants. Different capital letter indicates significant differences among groups (P<0.05).

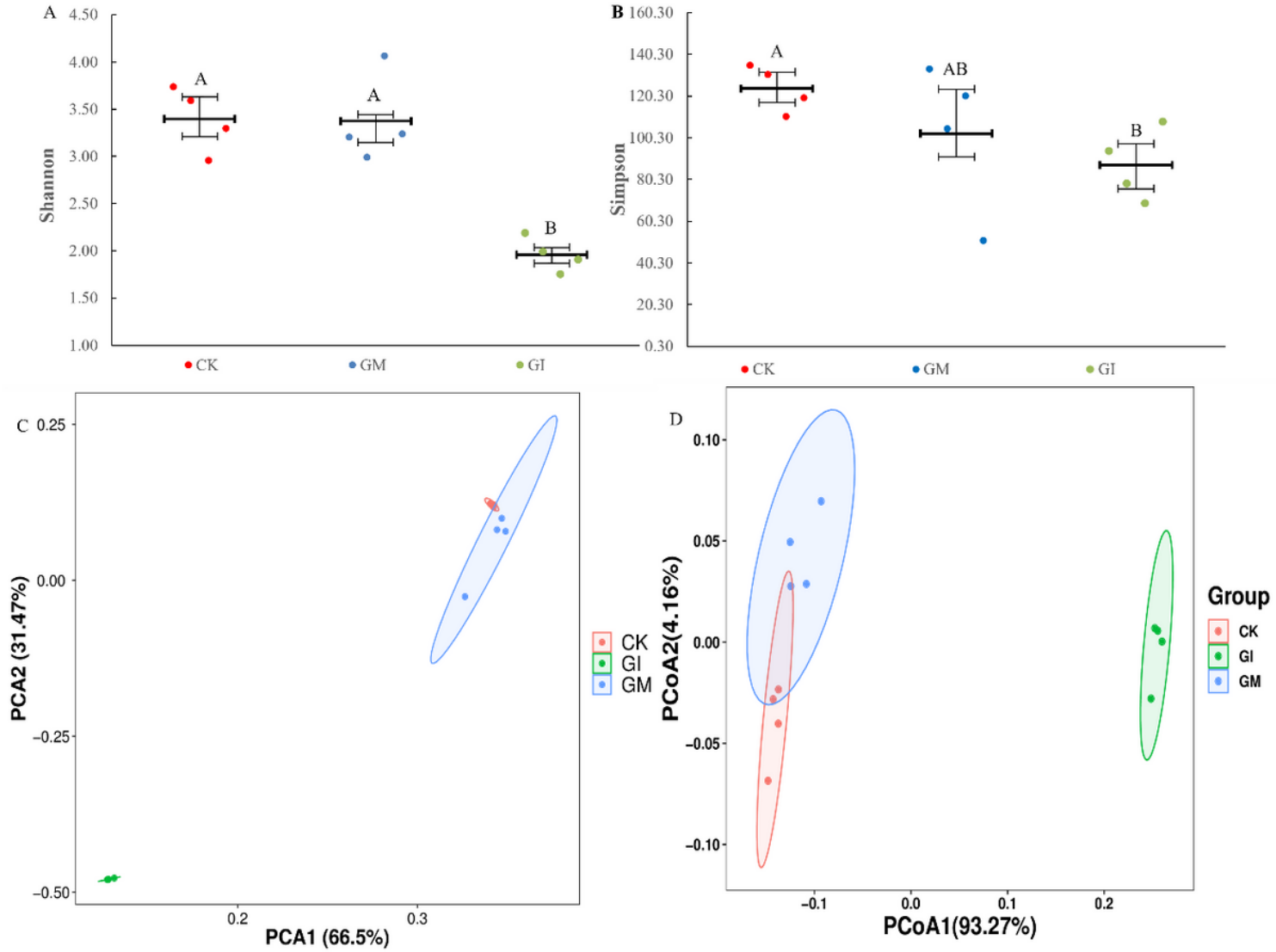

Figure 2

The gut microbial diversity in the gypsy moth larvae at the 5th instar after rearing on the leaves of GM, GI or n nonmycorrhizal-colonized plants. (A): Shannon index. (B): Simpson index. (C): Principal components analysis (PCA). (D): PCoA plot of the gut microbiota structures based on the unweighted UniFrac analysis. Different capital letter indicates significant differences between treatment groups or control group(P<0.05).

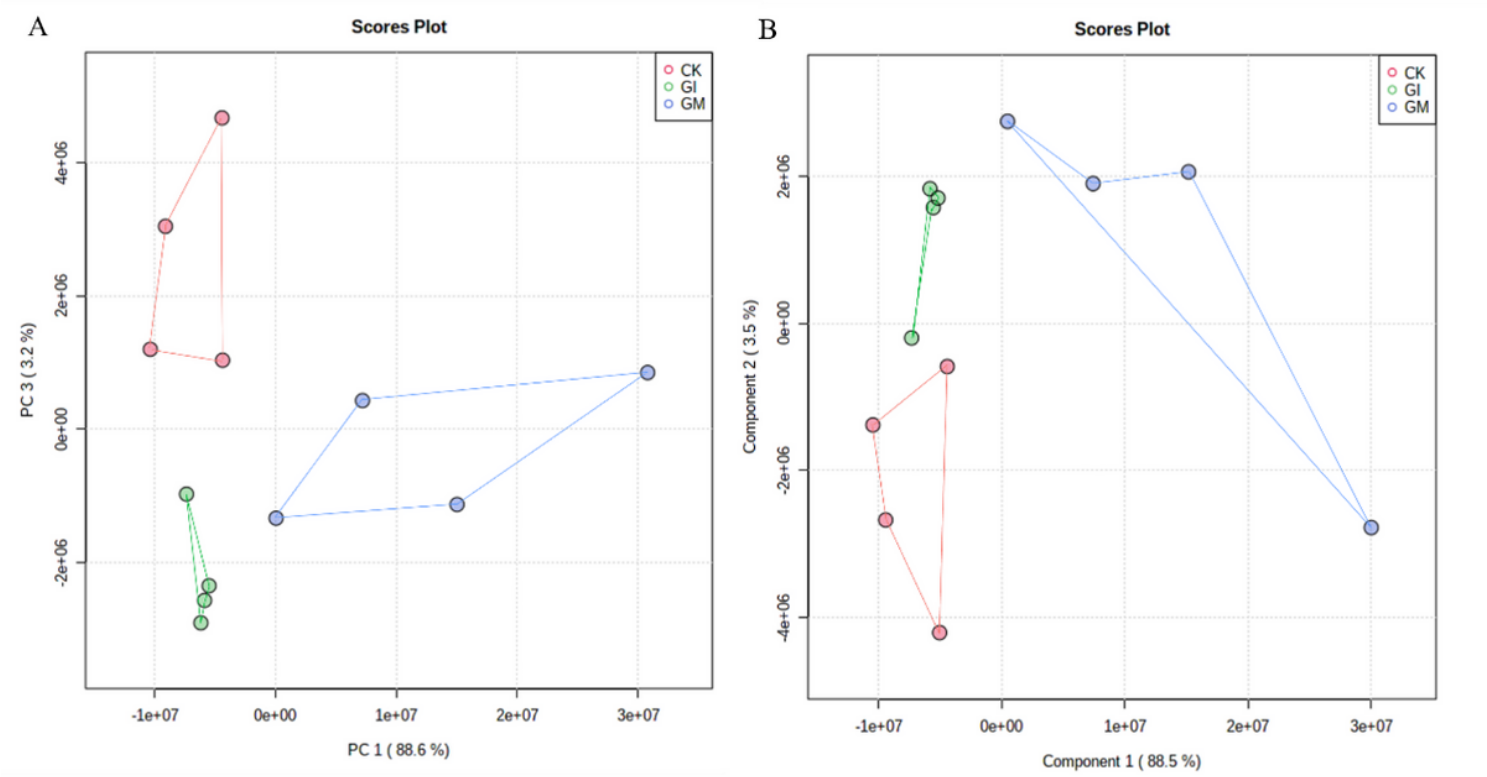

Figure 3

The principal components analysis (PCA, A) and supervised partial least squared discriminant analysis (PLS-DA, B) of metabolite profiles in fat body of gypsy moth larvae.

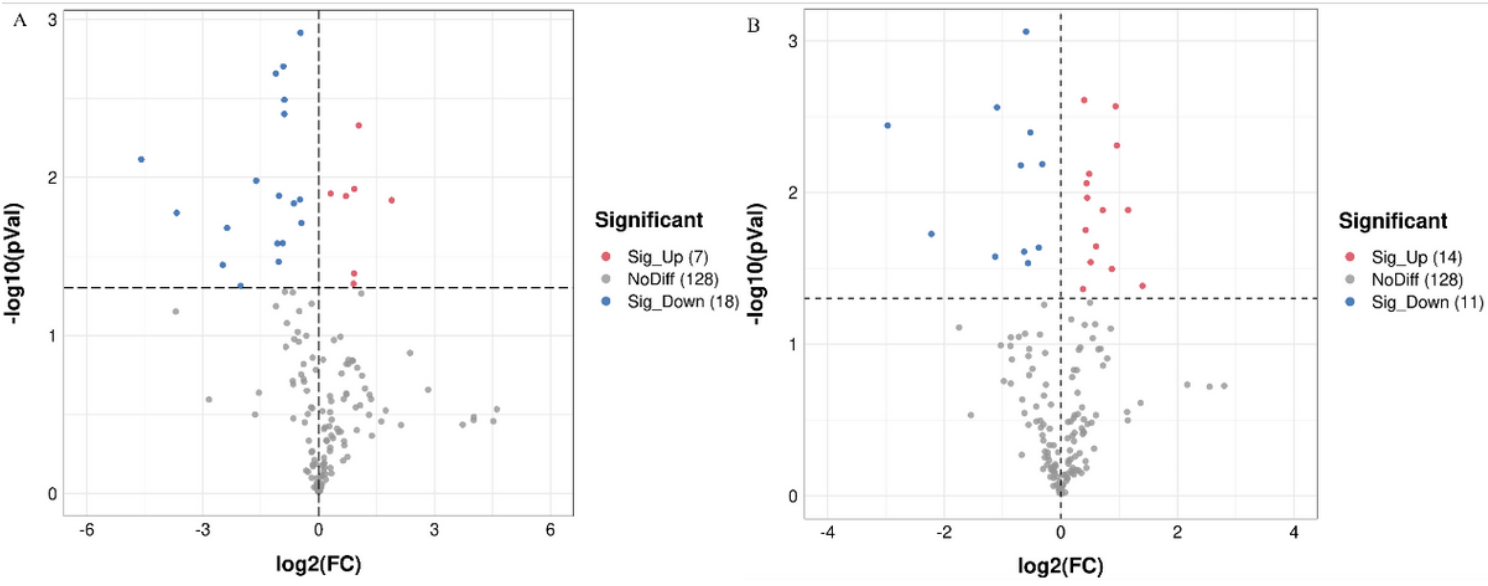

Figure 4

Volcano plots showing the differentially expressed metabolites among GM and CK groups (A) or among GI and CK groups (B). All metabolites presented in volcano plots were annotated through the KEGG database.

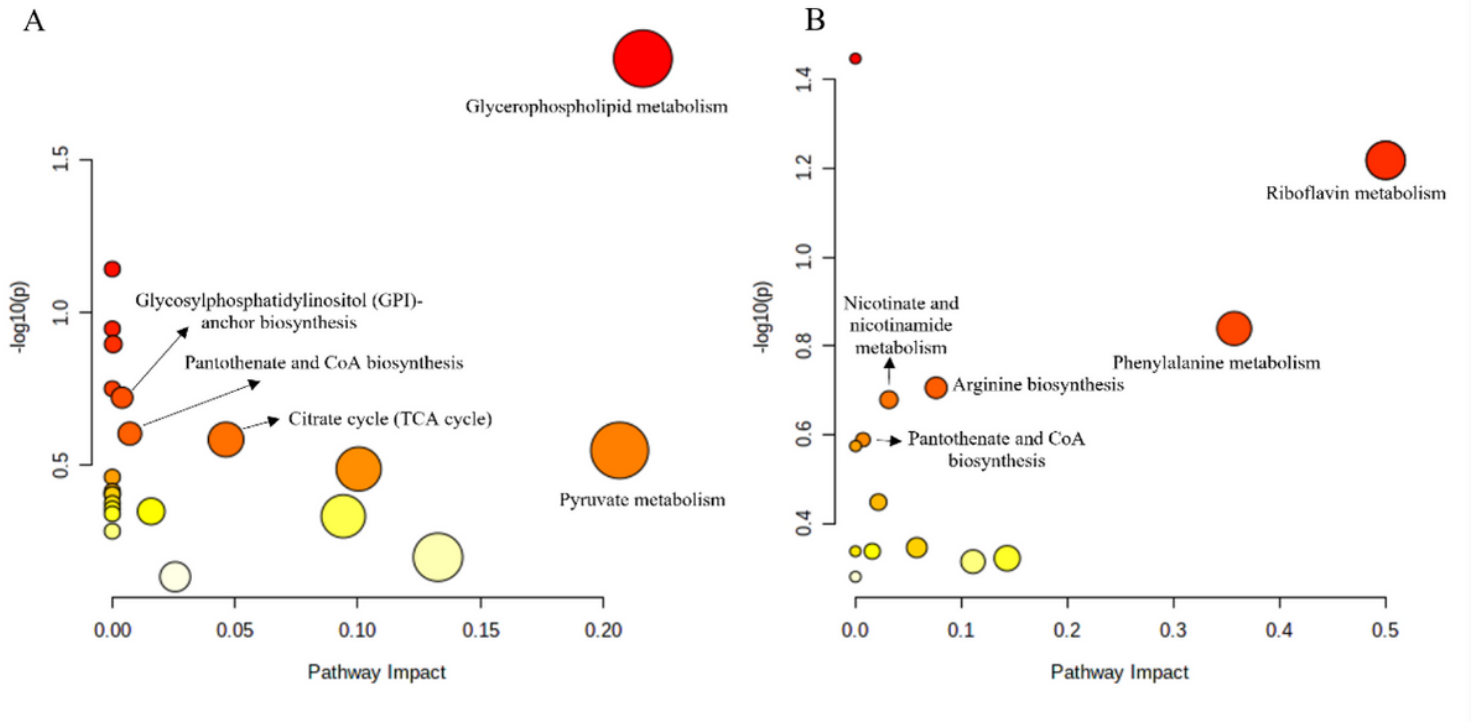

Figure 5

Pathway analysis of the differentially expressed metabolites between CK group and GM group (A) or CK group and GI group (B).

Supplementary Files

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- Supplementarymaterial.docx