

Metabolomics and transcriptomics reveal the response mechanisms of *Mikania micrantha* to *Puccinia spegazzinii* infection

Xinghai Ren ^{1,2*}, Guangzhong Zhang ^{1*}, Yujuan Gu ³, Mengjiao Jin ¹, Michael D. Day ⁴, Huai Liu ^{2†}, Wanqiang Qian ^{1†}, Bo Liu ^{1†}

¹ Shenzhen Branch, Guangdong Laboratory of Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture and Rural Affairs, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China

² Key Laboratory of Entomology and Pest Control Engineering, College of Plant Protection, Southwest University, Chongqing, China

³ Guangzhou customs district technology center, Guangzhou, China

⁴ Department of Agriculture and Fisheries, Ecosciences Precinct, GPO Box 267, Brisbane, Queensland 4001, Australia

* These authors contributed equally to this work.

† Corresponding author:

Huai Liu, Email: liuhuai@swu.edu.cn, Wanqiang Qian, Email: qianwanqiang@caas.cn, Bo Liu, Email: liubo03@caas.cn,

24 Abstract

25 *Mikania micrantha* is one of the 100 worst invasive species globally and can
 26 cause significant negative impacts to agricultural and forestry economics, particularly
 27 in Asia and the Pacific region. The rust *Puccinia spegazzinii* has been used
 28 successfully as a biological control agent in several countries to help manage *M.*
 29 *micrantha*. However, the response mechanisms of *M. micrantha* to *P. spegazzinii*
 30 infection have never been studied. To investigate the response of *M. micrantha* to
 31 infection by *P. spegazzinii*, an integrated analysis of metabolomics and
 32 transcriptomics was performed. The levels of 101 metabolites, including organic acids,
 33 amino acids and secondary metabolites in *M. micrantha* infected with *P. spegazzinii*,
 34 were significantly different compared to those in plants that were not infected. The
 35 relative amounts of L-leucine, L-isoleucine, L-tryptophan, histidine, L-phenylalanine
 36 and L-citrulline were increased under *P. spegazzinii* infection. These may convert to
 37 intermediates within the TCA cycle, participating in energy biosynthesis. In addition,
 38 phytoalexins, such as maackiain, nobiletin, vasicin, arachidonic acid and JA-Ile, were
 39 synthesized and accumulated in *M. micrantha* in response to *P. spegazzinii* infection.
 40 A total of 4,978 differentially expressed genes were identified in *M. micrantha*
 41 infected by *P. spegazzinii*. Many key genes of the Mitogen-activated protein kinase
 42 (MAPK) signalling pathway, showed significantly higher expression under *P.*
 43 *spegazzinii* infection. These results not only help us to understand the response of
 44 metabolites and gene expression in *M. micrantha* following infection by *P. spegazzinii*,
 45 but also help us understand the potential for the continuous biological control of *M.*
 46 *micrantha* using *P. spegazzinii*.

47
 48 **Key words:** transcriptome, metabolome, immune response, biological control,
 49 Pucciniales.

50

51 Introduction

52 *Mikania micrantha* Kunth (Asteraceae), commonly called ‘mile-a-minute’, is a
 53 rapidly growing vine native to tropical America (Day *et al.*, 2016; CABI, 2019). It is
 54 considered one of the world’s worst weeds (Love *et al.*, 2014), invading many
 55 countries in Asia and Oceania (Day *et al.*, 2016; CABI, 2019;) and has been listed in
 56 China as a plant species with national quarantine concerns by the Forestry
 57 Administration (Zhang *et al.*, 2004; Zhang *et al.*, 2019). *Mikania micrantha* has been
 58 reported in seven provinces in southern China, with the main infestations in
 59 Guangdong, Guangxi and Yunnan provinces (Shen *et al.*, 2018), and affects
 60 biodiversity and a wide range of agriculture and forestry enterprises, reducing
 61 productivity through competition (Day *et al.*, 2016). *Mikania micrantha* is also known
 62 as a plant killer because its rapid growth enables it to completely smother crops or
 63 trees and block sunlight preventing flowering and fruiting (Zhang *et al.*, 2004; Day *et*
 64 *al.*, 2016).

65 The rust fungus, *Puccinia spegazzinii* de Toni (Uredinales: Pucciniaceae) has
 66 been introduced into several countries, including China to help control *M. micrantha*
 67 (Day *et al.*, 2016). *Puccinia spegazzinii* is a microcyclic and autoecious rust, with a
 68 life cycle of 19-21 days (Ryan & Ellison, 2003). Teliospores and basidiospores of *P.*
 69 *spegazzinii* have only been recorded in the field (Ellison *et al.*, 2008). *Puccinia*
 70 *spegazzinii* is known to attack only *M. micrantha* and to a much lesser extent,
 71 *Mikania cordata* (Burm. F.) B. L. Robinson. Its potential as a biological control agent
 72 is because it is particularly damaging to the leaves, stems and petioles of *M.*
 73 *micrantha*, reducing growth rates and flowering and can tolerate a wide range of
 74 environmental conditions in which *M. micrantha* grows (Barreto & Evans, 1995; Day
 75 *et al.*, 2013a, b; Day & Riding, 2019).

76 Rust fungi (Pucciniales) are one of the largest groups of plant pathogens and the
 77 most damaging to plants in the world (Kemen *et al.*, 2015; Lorrain *et al.*, 2019).
 78 Infected plants can produce a range of defence responses to a pathogen (Feng *et al.*,
 79 2015). The amount of adenosine triphosphate (ATP), which plays an important role in
 80 metabolite biosynthesis, signal transduction, and material transport can be increased.

These secondary metabolites play vital roles in performing or having passive physical and chemical barriers against pathogens. For instance, polyamines could change the mesophyll cell pre-penetration and penetration resistance mechanisms, e.g. as seen in oats in response to infection by *Puccinia coronata* f. sp. *avenae* W.P. Fraser & Ledingham (Pucciniaceae) (Montilla-Bascon *et al.*, 2016).

Other responses relate to the phenylpropanoid, flavonoid and isoflavonoid metabolic pathway genes, which are involved in the production of phytoalexins as seen in *Medicago truncatula* Gaertn (Fabaceae) following infection by *Phakopsora pachyrhizi* Syd. & P. Syd. (Phakopsoraceae) (Ishiga *et al.*, 2015). In addition, the accumulation of phenolic, rutin, glucosinolate, flavonoid, fatty acid and alkaloid phytoalexins in plants could promote host defence against pathogens (Maddox *et al.*, 2010; Amil-Ruiz *et al.*, 2011; Joosten & van Veen, 2011). Defence-signalling networks, such ethylene and the salicylic acid pathways, are also triggered when pathogens attempt to infect host plants (Yang *et al.*, 2014) and several gene families, such as the MCM1, WRKY, MYB, and bZIP families, are involved in the response of plants to pathogens (Al-Attala *et al.*, 2014; Wang *et al.*, 2017; Zhu *et al.*, 2018; Rathod *et al.*, 2020).

In this study, we have characterized *M. micrantha* as a model host pathosystem for *P. spegazzinii* to investigate the molecular mechanisms of host response to infection. Using transcriptome and metabolome analyses, we show how levels of essential amino acids, ATP and phytoalexins in *M. micrantha* change in response to infection by *P. spegazzinii*. In addition, we aim to show how genes related to biotic stress in *M. micrantha* were expressed following *P. spegazzinii* infection. This work will help understand the potential for the continuous use of *P. spegazzinii* in assisting in the management of *M. micrantha* in many countries.

Materials and Methods

Rust inoculation and sample collection

Puccinia spegazzinii IMI 393075 was imported into China from Australia in December 2019 and a culture was maintained on potted *M. micrantha* plants at the

111 Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural
112 Sciences. *Mikania micrantha* plants were grown in a greenhouse with lighting (day:
113 16 hours and night: 8 hours) and a daily temperature range of 20-35°C. For
114 inoculation, plant tissue (leaves, stems, petioles) with mature *P. spegazzinii* pustules,
115 indicated by a coppery-brown appearance, was suspended over healthy plants in a
116 sealed plastic chamber maintained at 22°C, 100% humidity, for 48 hours of darkness
117 (for details, see Ellison *et al.*, 2008; Day & Riding, 2019). After 48 h, the plants were
118 removed and placed in an insect cage in a greenhouse (25-35°C, relative humidity,
119 ambient lighting) for subsequent pustule development. After 17 days following
120 inoculation, infected leaves with obvious well-developed pustules, were collected in
121 three biological replicates. The pustules were cut from the leaves to eliminate the
122 metabolites of the rust contaminating the leaf samples and discarded. Leaves of
123 uninfected plants were also collected in three biological replicates to serve as controls.
124 The collected leaf samples were placed in liquid nitrogen for quick freezing and then
125 stored at -80°C for further experiments.

126

127 **Metabolite response of *M. micrantha* after infection by *P. spegazzinii***

128 Following freezing by liquid nitrogen, the samples of infected leaves and
129 uninfected leaves were ground into powder. A subsample of 100 mg powder from
130 each of the three samples of infected and uninfected leaves was weighed and the
131 homogenate was resuspended with prechilled 500µL 80% methanol and 0.1% formic
132 acid by vortexing well. The samples were incubated on ice for 5 min and were then
133 centrifuged at 15000 g, at 4°C for 10 min. The supernatant was diluted using LC-MS
134 grade water so the final concentration was 53% methanol. The samples were
135 subsequently transferred to fresh Eppendorf tubes and were centrifuged at 15000 g, at
136 4°C for 20 min. The supernatant was injected into the Liquid Chromatography–
137 Tandem Mass Spectrometry (LC-MS/MS) system analysis.

138 Based on novogene Database (novoDB, in-house database), the Multiple
139 Reaction Monitoring (MRM) was used to detect the metabolites in each sample. The
140 product ion (Q3) was used for metabolite quantification. The parent ion (Q1), Q3,

141 retention time (RT), declustering potential (DP) and collision energy (CE) were used
142 for metabolite identification. The data files generated by HPLC-MS/MS were
143 processed using the SCIEX OS Version 1.4 to integrate and correct the peaks. The
144 main parameters were set as follows: minimum peak height, 500; signal/noise ratio, 5;
145 gaussian smooth width, 1. The area of each peak represents the relative content of the
146 corresponding substance.

147 These metabolites were annotated using the Kyoto Encyclopedia of Genes and
148 Genomes (KEGG) database (<http://www.genome.jp/kegg/>), Human Metabolome
149 Database (HMDB) database (<http://www.hmdb.ca/>) and Lipid maps database
150 (<http://www.lipidmaps.org/>). We applied univariate analysis (t-test) to calculate the
151 statistical significance (P-value). The metabolites with a P-value < 0.05 and a fold
152 change (FC) ≥ 2 or ≤ 0.5 were considered to be differential metabolites.

153

154 **Establishment of cDNA library and differentially expressed gene (DEG) analysis**

155 The total RNA of *M. micrantha* leaves infected by *P. spegazzinii* and leaves from
156 uninfected plants was extracted using an RNA extraction kit, and the quality of the
157 extracted RNA was checked using a Nanodrop 2000 spectrophotometer. The integrity
158 of total RNA was checked using formamide denaturing gel electrophoresis. mRNA
159 was isolated from the total RNA using Dynabeads Oligo (dT) 25 isolation beads. The
160 RNA of the extracted sample was used to build a cDNA library by a reverse
161 transcription kit based on the manufacturer's instruction (NEBNext® Ultra™ RNA
162 Library prep Kit for Illumina®).

163 After establishing a cDNA library, the concentration of purified cDNA was
164 preliminarily quantified by a Qubit2.0 fluorometer. The insert size of the cDNA
165 library was checked by an Agilent 2100 bioanalyser. To ensure a quality library, the
166 concentration of purified cDNA was accurately quantified using Q-PCR. The cDNA
167 library was sequenced on the Illumina sequencing platform, using paired-end (PE)
168 technology, in which 150 bp PE reads were generated. The quality control of
169 RNA-Seq reads was assessed by Trinith (Chen *et al.*, 2019).

To obtain localization information for the reads in the reference genome, clean reads were compared with the reference genome of *M. micrantha* (GCA_009363875.1) (Liu *et al.*, 2020), using HISAT2-2.0.5 (Pertea *et al.*, 2016), and the expression level was calculated using the fragments per kilobase million (FPKM) method. The differentially expressed genes (DEGs) were analysed using the DEseq2 package version 3.8.6 (Love *et al.*, 2014). Genes with a $|\log_2 \text{fold change}| > 1$ and false discovery rate (FDR) < 0.05 were considered DEGs. The KEGG enrichment analysis of functional significance terms based on the KEGG (<http://www.kegg.jp/kegg/pathway/html>) database was conducted using a hypergeometric test to find significant KEGG terms in DEGs for comparison with the genome background.

181

182 **Contents of ATP determination following *P. spegazzinii* infection**

Following freezing by liquid nitrogen, the samples of infected leaves and uninfected leaves were ground into powder. A subsample of 100 mg powder from each of the three biological samples of infected and uninfected leaves was weighed and 1ml buffer solution was added and homogenized in an ice bath using an electric tissue grinder. The mixed extracts were then vortexed and centrifuged at 8000 g and 4°C for 10 min. The supernatant was placed into a new Eppendorf tube, and 500 µL trichloromethane was added before being mixed thoroughly, and centrifuged at 10000 g and 4°C for 3 min. The upper suspension of each sample was kept and incubated on ice for subsequent detection of ATP content in infected and uninfected leaves using an ATP content detection kit (Beijing Solarbio Technology Co., Ltd.).

193

194 **Results**

195 **Metabolite response of *M. micrantha* after infection by *P. spegazzinii***

A total of 101 significant differentially abundant metabolites (23.88% of total metabolites) were identified in the leaves of *M. micrantha* in this study. Of these, 39 metabolites had significantly higher levels in infected leaves compared with the

199 uninfected *M. micrantha* samples, while 62 metabolites had significantly lower levels
200 following infection by *P. spegazzinii*, compared to the uninfected *M. micrantha*
201 samples (**Figure 1A**). The 101 significant differentially metabolites could be grouped
202 into 24 major classes (**Supplementary Table S1**). Flavonoids were the most abundant
203 metabolites among the significantly differential metabolites, accounting for 13.86% of
204 the total significantly metabolites (**Supplementary Figure S1**).

205 Based on the KEGG database, a total of 19 significantly increased metabolites
206 are enriched in essential amino acid synthesis and metabolic pathways, such as
207 phenylalanine, tyrosine and tryptophan biosynthesis; phenylalanine metabolism;
208 valine, leucine and isoleucine degradation; glycine, serine and threonine metabolism
209 (**Figure 1B**). The co-joint KEGG enrichment analysis of the significantly decreased
210 metabolites, showed 17 co-mapped pathways, such as those related to neomycin,
211 kanamycin and gentamicin biosynthesis, citrate cycle (TCA cycle), phosphonate and
212 phosphinate metabolism, thiamine metabolism, and glyoxylate and dicarboxylate
213 metabolism (**Figure 1C**).

214 The levels of phytoalexin-related metabolites, e.g. maackiain, nobiletin, vasicin
215 and arachidonic acid in *M. micrantha* leaves infected with *P. spegazzinii* were 1.73,
216 2.14, 3.69 and 5.98 times higher respectively than those in uninfected leaves (**Figure**
217 **1D**). The level of the stress-related compound, JA-Ile was 6.40 times higher in
218 infected leaves than that in uninfected leaves (**Figure 1D**).

219

220 Differentially expressed gene (DEG) analysis

221 Based on the transcriptome analysis between the *P. spegazzinii*-infected group (A)
222 and non-infected (control) group, a total of 4,978 DEGs (15.20% of total genes) were
223 identified, including 2,054 significantly up-regulated genes and 2,924 significantly
224 down-regulated genes (**Figure 2A**). Among these DEGs, a total of 2,078 DEGs are
225 enriched in 133 KEGG pathways (**Supplementary Table S2**). The most significantly
226 enriched pathways of up-regulated DEGs are related to protein processing in
227 endocytosis and plant-pathogen interaction (**Figure 2B**). The expression of genes
228 associated with biosynthesis of terpenoid and flavonoid, such as in terpenoid

backbone biosynthesis, isoflavonoid biosynthesis, monoterpene biosynthesis, sesquiterpene and triterpene biosynthesis and flavone and flavonol was significantly suppressed (**Figure 2C**).

A total of 95 WRKY genes were identified in the *M. micrantha* genome, in which the gene expression of 22 WRKY (23.16% of total WRKY genes) were significantly different following infection by *P. spegazzinii* as determined by the RNA-seq analysis. Among these genes, 12 WRKY genes were significantly up-regulated, including one WRKY1 gene, seven WRKY22 genes, three WRKY33 genes and one WRKY4/5 gene. The gene expression of the three WRKY33 genes were significantly up-regulated by 2.2 times following *P. spegazzinii* infection (**Figure 3** and **Supplementary Table S3**). A total of 6 BAK1 genes were identified in the *M. micrantha* genome, in which the gene expression of 2 BAK1 genes were significantly up-regulated by 1.5 times following *P. spegazzinii* infection (**Figure 3** and **Supplementary Table S3**).

Contents of ATP determination following *P. spegazzinii* infection

The gene expression of key amino acids synthesis, such as branched-chain amino acid aminotransferase and 2-isopropylmalate synthase, was significantly up-regulated when *M. micrantha* was infected by *P. spegazzinii* (**Figure 4A** and **4C**). Moreover, the amounts of these amino acids, such as L-leucine, L-isoleucine, L-tryptophan, histidine, L-phenylalanine, and L-citrulline, which can turn into succinyl coenzyme A and acetyl-CoA by multistep transamination and decarboxylation, were also significantly increased after *P. spegazzinii* infection (**Supplementary Figure S2**). Furthermore, the expression of almost all TCA cycle-related genes (6 of 8 key genes) was significantly up-regulated (**Figure 4B, D** and **Supplementary Table S3**), causing the amounts of these amino acids to be significantly increased, promoting the production of ATP following infection by *P. spegazzinii* (**Figure 4E**).

Discussion

There were both positive and negative responses in terms of levels of metabolites

and gene expression when *M. micrantha* was infected with *P. spegazzinii*. While most of the metabolites and gene expressions decreased following infection, there were numerous metabolites and genes that had increased, suggesting that infection by *P. spegazzinii* had stimulated compensatory or defence responses. Over one third of the metabolites detected, had significantly higher levels than that seen in uninfected plants. Most of these metabolites pertain to amino acid synthesis and metabolic pathways, suggesting that *M. micrantha* is able to produce more amino acids to maintain growth following infection by *P. spegazzinii*.

The amounts of essential amino acids, ATP and phytoalexins (e.g. nobiletin, maackiain and JA-Ile) were significantly increased following infection by *P. spegazzinii* infection. The stress related compound JA-Ile which plays an important role in response to the biotic or abiotic stressors (Wasternack, 2014), was significantly increased more than 6.4 times following infection by *P. spegazzinii*, again suggesting some sort of defence mechanism.

About 40% of genes identified were up-regulated and most of these were related to protein processing, suggesting the plant may be trying to maintain growth while infected. There were numerous genes e.g. WRKY33 and WRKY22, relating to plant resistance (Jiang *et al.*, 2017) that had also increased following infection, suggesting the plant is trying to overcome infection. ATP production also increased following infection, as seen in other studies (e.g. Montilla-Bascon *et al.*, 2016).

In addition, the key genes in the TCA cycle and MAPK signalling pathways presented high expression following *P. spegazzinii* infection. These results suggested that when *M. micrantha* was infected by *P. spegazzinii*, it activated a defence response, but this does not necessarily imply resistance.

In previous studies, other plant species have responded to the presence of pathogens by up-regulating metabolites and DEGs (Pitino *et al.*, 2017; Tang *et al.*, 2018; Wang *et al.*, 2018). The innate immune system (Gu *et al.*, 2019) and phytoantitoxin (Stevenson & Haware, 1999; Kliebenstein, 2004) of plants are used to respond to pathogen infection through cell permeability, programmed cell death and defence responses (Benaziz, 1967; Savchenko *et al.*, 2010; Yao *et al.*, 2012; Zlotek &

289 Wojcik, 2014; Zaynab *et al.*, 2018). When plants are under biotic and abiotic stress,
290 the WRKY family plays vital roles in stress-induced defensive responses (Jiang *et al.*,
291 2017; Wang *et al.*, 2017; Satapathy *et al.*, 2018; Wang *et al.*, 2019; Rathod *et al.*, 2020;
292 Ramos *et al.*, 2021).

293 This study has shown that levels of some genes and metabolites increased
294 following infection by *P. spegazzinii*, suggesting that the plant is trying to compensate
295 or overcome infection. Some of these positive responses relate to resistance which
296 then raises the question of whether *M. micrantha* could ever become resistance to *P.*
297 *spegazzinii* and therefore, will the rust's impact as a biological control agent lessen?

298 Despite these positive changes in levels of metabolites and gene expression, this
299 study, also showed that *P. spegazzinii* can have a negative impact on gene expression,
300 metabolites and metabolic pathways. Most of the metabolites (>60%) and genes (60%)
301 had lower levels following infection, suggesting that infection of *P. spegazzinii* is
302 having some negative impact on *M. micrantha*.

303 *Puccinia spegazzinii* is currently being used as an effective biological control
304 agent and has been released against *M. micrantha* in many countries (Ellison *et al.*,
305 2008; Winston *et al.*, 2014; Day *et al.*, 2016; Kumar *et al.*, 2018; Day & Riding,
306 2019). The management of *M. micrantha* using *P. spegazzinii* is seen as a benefit to
307 many land holders, as plants can reshoot after slashing and herbicides are harmful to
308 humans and the environment (Choudhury, 1972; Day *et al.*, 2016; Day & Riding,
309 2019). In both laboratory and field trials, *P. spegazzinii* has been shown to reduce
310 growth rates and flowering of *M. micrantha* (Day *et al.*, 2013b). The results in this
311 study at least partially explain what is being observed in the field where growth rates
312 of *M. micrantha* have decreased, offering hope that *P. spegazzinii* will continue to be
313 able to suppress populations of *M. micrantha* in the field.

314 In this study, we characterized *M. micrantha* as a model host pathosystem for *P.*
315 *spegazzinii* to investigate the molecular mechanisms of host responses to a pathogen.
316 We have provided the first description genome wide, of the different gene expressions
317 and metabolite levels in the leaves of *M. micrantha* following *P. spegazzinii* infection.
318 Interactions and coevolution between *M. micrantha* and *P. spegazzinii* may influence

the overall pathogenicity of rust. Over time, *M. micrantha* may evolve a mechanism to resist *P. spegazzinii*, and the plant could once again become a problem for countries that already have released rust. Alternatively, the rust may develop ways to overcome the plant's defence responses or resistance. Further studies may provide more insight. The results here certainly provide a better understanding of the interactions between *M. micrantha* and *P. spegazzinii* and the potential of *P. spegazzinii* as a long-term biological control agent for *M. micrantha*.

Conclusion

In this study, we revealed the response and interaction mechanism of *M. micrantha* to *P. spegazzinii* based on the analysis of plant metabolites and gene expression. The relative levels of essential amino acid, ATP and phytoalexins were synthesized and accumulated in *M. micrantha* following *P. spegazzinii* infection. In addition, many key genes of the MAPK signalling pathway, showed significant high expression under the *P. spegazzinii* infection.

Data availability statement

Publicly available datasets were analyzed in this study. The RNA-seq data can be found in the NCBI under the accession numbers SRR17139378–SRR17139383.

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Author contributions

X.H.R. and Y.J.G. carried out the sample collection and the inoculation of rust. X.H.R. and G.Z.Z. provided technical support for the bioinformatics analysis. M.J.J. detected the contents of ATP in plant leaves. X.H.R. and B.L. wrote the manuscript. M.D.D.

349 revised the paper and provided context in terms of biological control. B.L. W.Q.Q.and
350 H.L. designed the experiments and coordinated the project.

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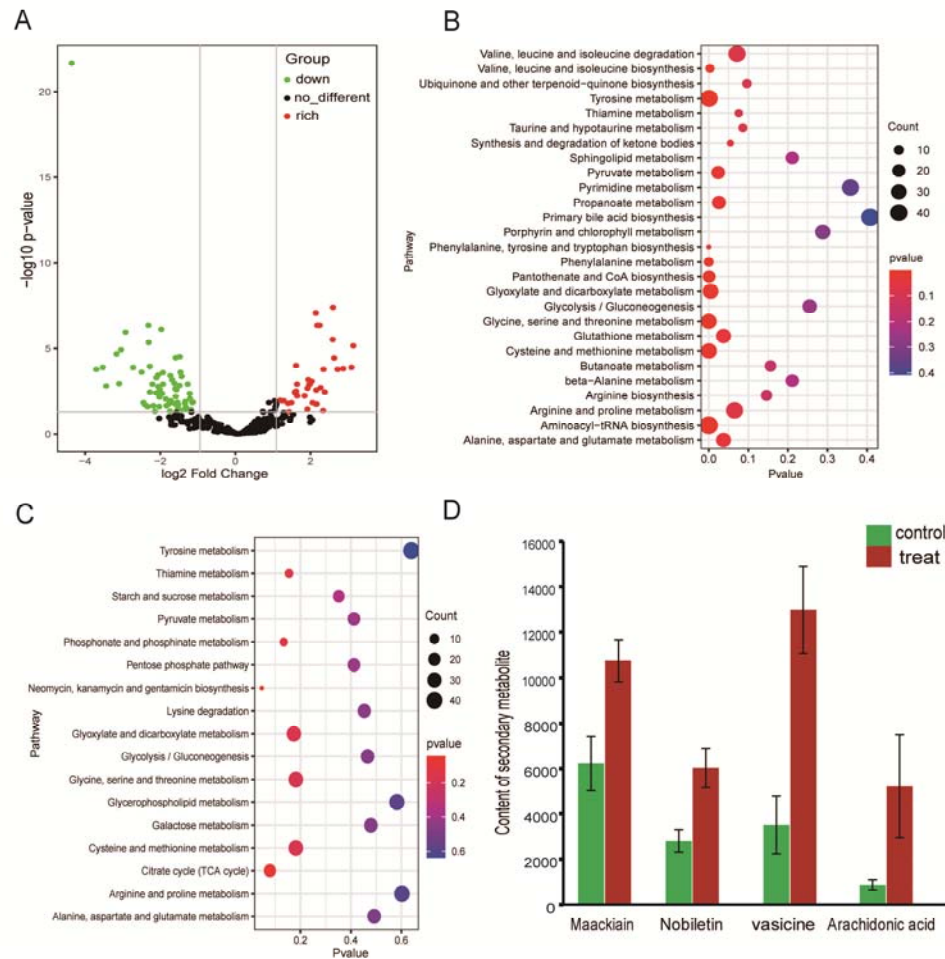
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529

530 **Figure 1. Volcano map and enrichment of differential metabolites.** (A) Volcano
531 map of differential metabolites. Red plots indicate the up-regulated metabolites;
532 Green plots indicate the down-regulated metabolites; Black plots indicate No
533 significant difference. (B) KEGG enrichment pathway of up-regulated metabolites. (C)
534 KEGG enrichment pathway of downregulated metabolites. (D) Contents of secondary
535 metabolites of *M. micrantha* under *P. spegazzinii* stress. Different letters indicate the
536 level of difference, $p < 0.05$.

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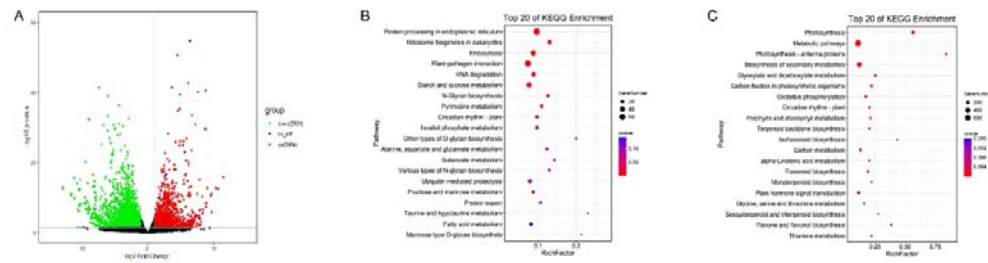
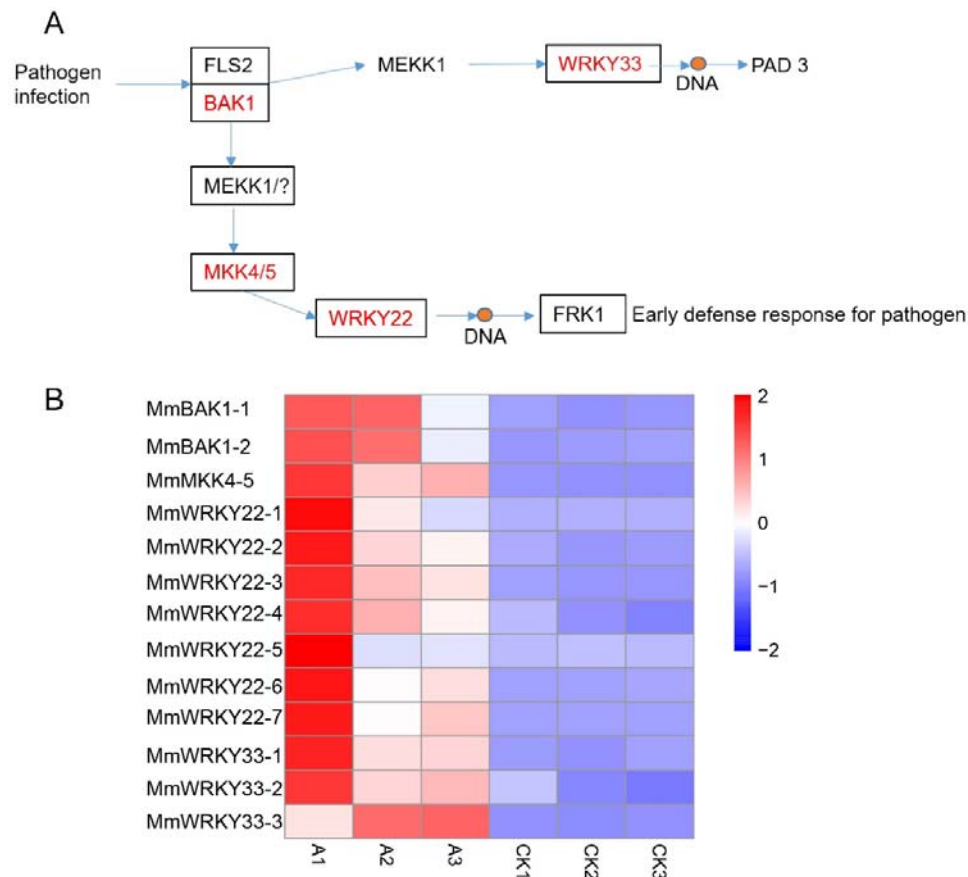


Figure 2. Volcano map and enriched of differential genes. (A) Volcano map of differentially expressed genes; Red plots indicate the up-regulated metabolites; Green plots indicate the down-regulated metabolites; Black plots indicate No significant difference. (B) KEGG pathway enrichment of up-regulated genes; (C) KEGG pathway enrichment of down-regulated genes.



545

546 **Figure 3. MAPK signaling pathway-plant in *M. micrantha* under infection by *P.***

547 ***spgazzinii*.** The gene expression levels were denoted in blue (down-regulated) and

548 red (up-regulated). (A) MAPK signaling pathway-plant; (B) expression pattern of

549 MAPK signalling pathway-plant key genes in *M. micrantha*. The red box indicates

550 upregulated expression.

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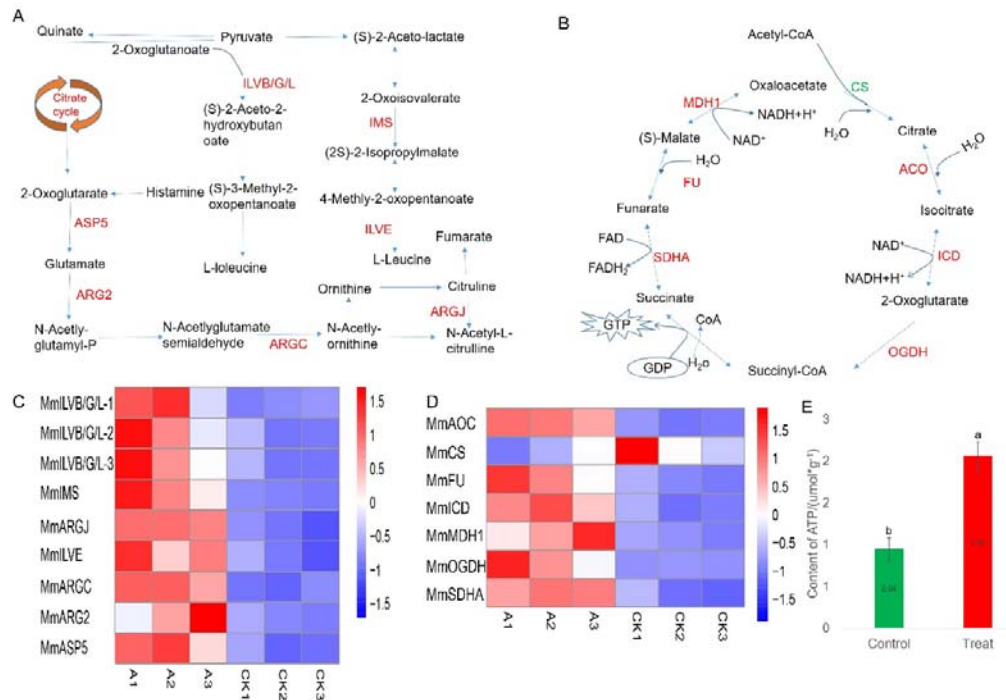
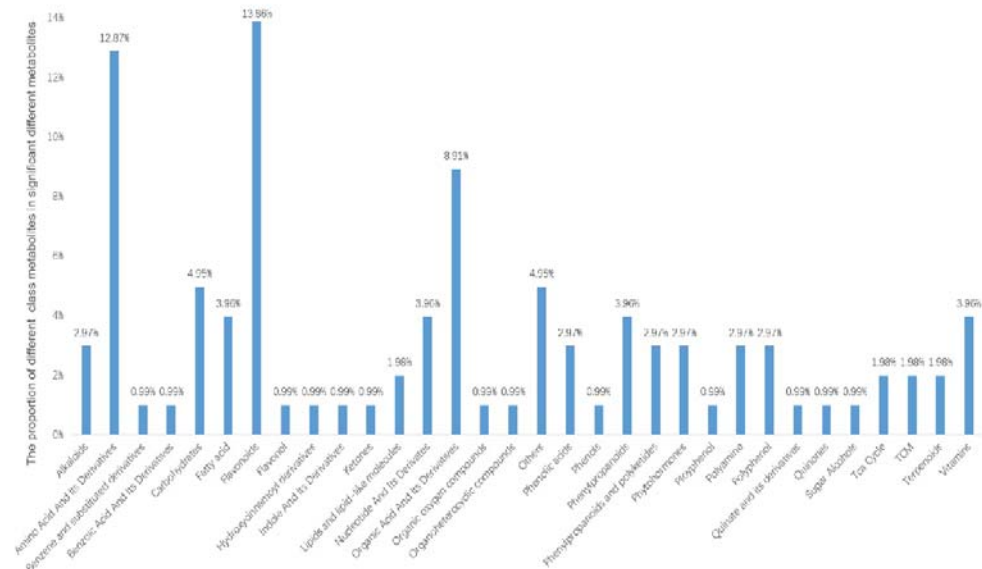


Figure 4. Gene expression of amino acid metabolism and TCA cycle pathway in *M. micrantha* under *P. spegazzinii* stress. (A) Pathway of amino acid metabolism; (B) pathway of the TCA cycle; (C) Expression pattern of key amino pathway genes in *M. micrantha*; ILVB/G/L: acetolactate synthase I/II/III large subunit; IMS: 2-isopropylmalate synthase; ILVE: branched-chain amino acid; ARGJ: glutamate N-acetyltransferase; ARGC: N-acetyl-gamma-glutamyl-phosphate reductase; ARG2: amino-acid N-acetyltransferase; ASP5: aspartate aminotransferase, chloroplastic. (D) Expression pattern of key TCA cycle genes in *M. micrantha*; SDHA: succinate dehydrogenase (ubiquinone) flavoprotein subunit; FU: fumarate hydratase, class II; MDH1: malate dehydrogenase 1; CS: citrate synthase; ACO: aconitate hydratase; ICD: isocitrate dehydrogenase; OGDH: 2-oxoglutarate dehydrogenase E1 component. (E) Changes in the contents of ATP by the *P. spegazzinii* infection; Different letters indicate the level of difference, $p < 0.05$.

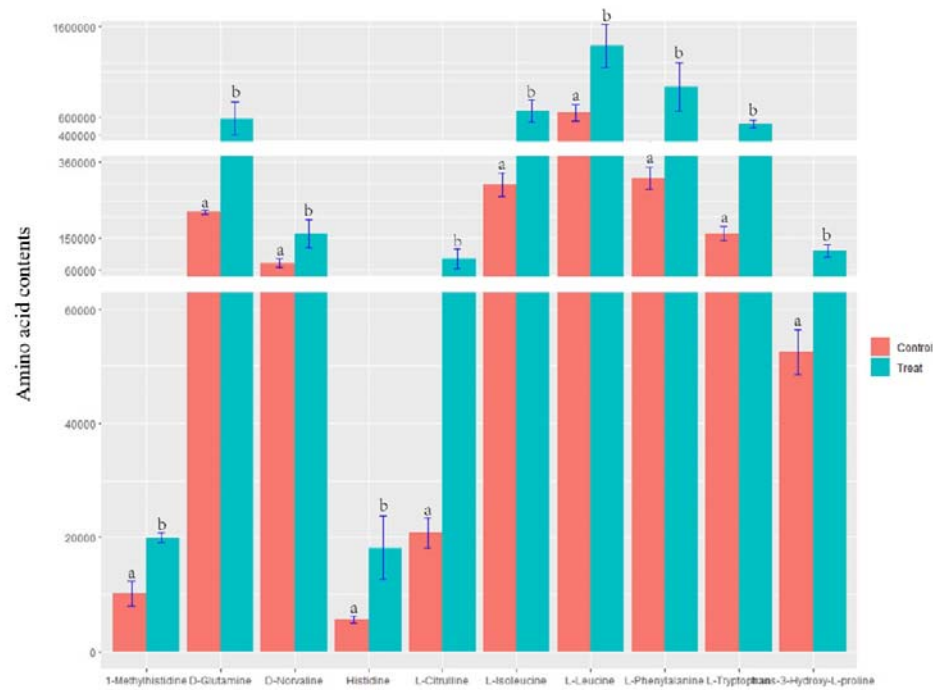


567

568 **Supplementary Figure S1 The proportion of significant different compounds in**

569 ***M. micrantha* under infection by *P. spegazzinii*.**

570



571
572 **Supplementary Figure S2 The difference of amino acid contents in *M. micrantha***
573 **leaves between the uninfected and infected by *P. spegazzinii*.**
574