

Plant hormones and metabolites related to the resistance response to feeding stimulation and leaf clipping control in Chinese pine (*Pinus tabuliformis* Carr.)

Yanan Zhao (✉ ya_nanzhao@126.com)

Hebei Agricultural University <https://orcid.org/0000-0001-8253-1427>

Guona Zhou

Hebei Agricultural University

Tianhua Sun

Hebei Agricultural University

Lifeng Wang

Hebei Agricultural University

Qiang Xu

Hebei Agricultural University

Junxia Liu

Hebei Agricultural University

Baojia Gao

Hebei Agricultural University <https://orcid.org/0000-0002-1316-240X>

Research Article

Keywords: feeding stimulation, resistant, plant hormone, flavonoid pathway, metabolome

Posted Date: September 1st, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1967628/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Plant hormones and metabolites related to the resistance response to feeding stimulation and leaf clipping control in Chinese pine (*Pinus tabulaeformis* Carr.)

Zhao Yanan, Zhou Guona, Sun Tianhua, Wang Lifeng, Xu Qiang, Liu Junxia, Gao Baojia*

*Corresponding author: baojiagao@163.com

Abstract: To define changes in metabolic pathways in response to mandibulate insect feeding and to provide a reference for further investigation of the molecular mechanisms underlying the development of conifer resistance, Chinese pine (*Pinus tabulaeformis* Carr.) in good growth status in Huangtuliangzi Forest Farm Pingquan city, Chengde city, Hebei Province, was stimulated by 10 pine caterpillars (*Dendrolimus tabulaefomis* Tsai et Liu) as feeding stimulation (FS) and leaf clipping control (LCC) as mechanical damage. The metabolome and total flavonoid content were measured in the needles at 0 (with no treatment, CK), 2, and 8 h after treatment. Plant hormones were measured with needles at 0, 0.5, 1, 1.5, 2, 4, 6, and 8 h after different treatments. The plant hormones jasmonic acid (JA) and auxin (IAA) were upregulated within a short time (2 h) after treatment, showing an expression trend consistent with the corresponding substances in the metabolome. A total of 30.8% of flavonoids were identified by metabolomics analysis. Analysis of KEGG metabolic pathways for the different accumulated metabolites (DAMs) showed that the top pathway annotated and enriched for DAMs in comparison to 2 h after FS (marked as 2 h) and 2 h after LCC (marked as LCC 2 h) was flavonoid biosynthesis; the top pathway mainly annotated and enriched for DAMs in comparison to 8 h and LCC 8 h was the same. Thus, the flavonoid pathway is one of the main pathways involved in resistance formation in conifers, and JA and IAA are involved in the development of resistance and growth during biotic stress.

Keywords: feeding stimulation; resistant; plant hormone; flavonoid pathway; metabolome

Introduction

Plants exposed to stress activate the related jasmonic acid (JA), salicylic acid (SA), and ethylene pathways to enable resistance (Berens, *et al.* 2017; Nascimento, *et al.* 2018; Pashkovskiy, *et al.* 2019; Yuan, M., *et al.* 2019; Zhang, *et al.* 2021d), and there are widespread interactions in plants similar to JA, methyl jasmonate (MeJA), SA, and abscisic acid (ABA) interactions (Bharath, *et al.* 2021; Ou, *et al.* 2022). Stress resistance is a balance between growth and defense in plants, with large amounts of reactive oxygen species (ROS) produced in chloroplasts. ABA is involved in the regulation of plant resistance by affecting the opacity and closure of stomata, which in turn regulate photosynthesis. Jingjun Ruan (2019) and Adriaan Verhage (2011) revealed the regulation of the JA mechanism under insect and pathogen stress. SA accumulation can be induced by an aphid elicitor (Cui, *et al.* 2019), and ABA depresses photosynthesis, altering the expression of 14-3-3 and GTP-binding proteins. Interrelationships between resistance conferred by closely related hormones JA and SA, as well as growth- and development-related hormones during resistance formation, remain unclear.

Metabolites can most directly reflect changes in plants in vivo. With the development of omics technology, an intuitive understanding of plant-induced resistance can be gained by determining changes in the metabolome. Feeding stimulation by sticklebacks leads to the upregulation of substances

in the carbohydrate metabolic pathway (Farahbakhsh, *et al.* 2019; Rivas-Ubach, *et al.* 2016). Junji Takabayashi and Kaori Shiojiri demonstrated the impact of feeding stimulation-induced volatile organic compounds (VOCs) on insects with various feeding habits through field and indoor experiments (Takabayashi and Shiojiri 2019). Kandasamy Dineshkumar *et al.* (2019) demonstrated that VOCs induce defense in insects through the study of the Eurasian spruce bark beetle (*Ips typographus*) and its fungal symbionts. Many changing pathways—such as the flavonoid (Chen, *et al.* 2020; Wang, *et al.* 2019b; Zhang, *et al.* 2020a; Zhang, *et al.* 2020b), citric acid metabolism (Hamanishi, *et al.* 2015), and glutathione metabolism (Agudelo-Romero, *et al.* 2015; Choudhury, *et al.* 2018; Zhu, *et al.* 2020) pathways—in the stress response process have been discovered in a joint analysis of the transcriptome and metabolome, in which the most accumulated substances have been further studied to identify key genes regulating traits and resistance to be applied in breeding (Jeon, *et al.* 2020; Szymanski, *et al.* 2020). Different stressors induce changes in metabolic pathways, and the correlation between hormones and resistance substances has been mostly explored by applying exogenous hormones indoors. The correlation between the expression of endogenous hormones and resistance substances after plants perceive a stimulus is still unclear.

The composition and content of defense enzymes, secondary metabolites, and protease inhibitors in Chinese pine (*Pinus tabulaeformis* Carr.) in natural conditions are significantly changed after biotic stress, and the content of chemical substances, such as alkaloids, phenolic compounds, and tannins, increases, and there are also changes in the content of defense proteins (Qin Shijie, *et al.* 2021; Shi Yuanyuan, *et al.* 2017; Wang Yincui, *et al.* 2016; Zhang Bin, *et al.* 2017). However, these studies only described the changes in individual substances, and there is no holistic understanding of the metabolic pathway of insect resistance in conifers. With the completion of chromosome genome sequencing in Chinese pine, the role of complex regulatory elements on gene expression also provides a strong reference for the study of insect resistance mechanisms in conifers (Niu, *et al.* 2022).

Currently, the ecological environment is not optimistic, and an increasing number of scholars have noted the importance of endogenous resistance in stress response (Bailey-Serres, *et al.* 2019; Ferry, *et al.* 2004). The application of omics technology is increasingly extensive in mechanism recovery. Long lifecycles and complex ploidy make it difficult to study stress resistance in trees (Michael and VanBuren 2020). To quantify the overall changes in metabolites during the formation of insect resistance in conifers, we selected Chinese pine and Chinese pine caterpillars (*Dendrolimus tabulaeformis* Tsai et Liu) under natural conditions to determine the expression of endogenous phytohormones JA, SA, IAA, and ABA after different treatments. We also used clipping as mechanical damage control and conducted metabolome identification and validation for untreated and treated Chinese pine needles. The results revealed metabolite changes in the flavonoid pathway during conifers' response to insect stimulation and suggest antagonistic interactions between growth- and resistance-related hormones.

Materials and Methods

General Situation of the Research Area

A pure Chinese pine forest with the same elevation, aspect, and slope, as well as similar tree vigor and management level, was selected in Huangtuliangzi Forestry, Pingquan City, Chengde City, Hebei Province, China. A total of 75 healthy trees about 10 years old without any herbivorous insect stress were randomly selected and grouped into 15 groups at different times per treatment, with a replication of 5 trees in each group. The plants were maintained under natural conditions.

Treatment Modes

Chinese pine caterpillars underwent starvation for 10 h before conducting the experiment. Branches with consistent branch length and height above the ground were selected in four directions: east, west, north, and south of each tree. Meanwhile, the control (CK, recorded as 0 h) was not given any treatment. In the treatment group, 10 pine needles were collected from each branch as one parcel at 0.5, 1, 1.5, 2, 4, 6, and 8 h after treatment, and the control group contained 10 intact pine needles as one parcel. Needles were preserved in tinfoil, frozen in liquid nitrogen, and transferred to -80°C for storage. A total of 20 parcels of pine needles were acquired from 5 trees at different times under each treatment.

Metabolomic Profiling Analysis

Pine needles were freeze-dried using a vacuum freeze-dryer (Scientz-100F). The freeze-dried sample was crushed using a mixer mill (MM 400, Retsch) with a zirconia bead for 1.5 min at 30 hz. Lyophilized powder (100 mg) was dissolved in 1.2 mL of 70% methanol solution and vortexed six times for 30 s every 30 min. The samples were then refrigerated at 4°C overnight. Following centrifugation at 12000 rpm for 10 min, the extracts were filtrated (SCAA-104, 0.22 μm pore size; ANPEL, Shanghai, China, <http://www.anpel.com.cn/>) before UPLC-MS/MS analysis.

The analytical conditions were as follows: UPLC column, Agilent SB-C18 (1.8 μm , 2.1 mm $\times$ 100 mm). The mobile phase consisted of solvent A, pure water with 0.1% formic acid, and solvent B, acetonitrile with 0.1% formic acid. Sample measurements were performed with a gradient program that employed the starting conditions of 95% A, 5% B. A linear gradient to 5% A, 95% B within 9 min was programmed, and a composition of 5% A, 95% B was kept for 1 min. Subsequently, a composition of 95% A, 5.0% B was reached within 1.1 min and kept for 2.9 min. The flow velocity was set as 0.35 mL per minute; the column oven was set to 40°C ; and the injection volume was 4 μL . The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS.

LIT and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (Q TRAP), AB4500 Q TRAP UPLC/MS/MS system, equipped with an ESI Turbo Ion-Spray interface, operated in positive and negative ion modes, and controlled using Analyst 1.6.3 software (AB Sciex). The ESI source operation parameters were as follows: ion source, turbo spray; source temperature, 550°C ; and ion spray voltage (IS), 5500 V (positive ion mode)/ -4500 V (negative ion mode). Ion source gas I (GSI), gas II (GSII), and curtain gas (CUR) pressures were set at 50, 60, and 25.0 psi, respectively; the collision-activated dissociation (CAD) was high. Instrument tuning and mass calibration were performed with 10 and 100 $\mu\text{mol/L}$ polypropylene glycol solutions in QQQ and LIT modes, respectively. QQQ scans were acquired as MRM experiments with collision gas (nitrogen) set to medium. DP and CE for individual MRM transitions were done with further DP and CE optimization. A specific set of MRM transitions was monitored for each period according to the metabolites eluted within this period.

Determination of Plant Hormone Content

Pine needles (0.6 g) were well milled in liquid nitrogen. Then, 5.4 mL of PBS buffer ($\text{pH} = 7.3$) was added and centrifuged for 30 min at 5000 r/min at 4°C . The contents of JA, SA, IAA, and ABA were determined using Plant Hormone ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd.). The kit employed a double antibody sandwich method to determine the levels of plant hormones in the sample. The optical density values were measured at 450 nm with a microplate reader, and the standard curves were fitted in ELISA Calc by a four-parameter logistic model: $y = (A-D)/[1 + (x/C)^B] + D$. The corresponding parameters of the four plant hormones are shown in Table 1. All r^2 values were higher than 0.9, indicating a good correlation with the model.

Tab 1. Parameters of a four-parameter logistic model in establishment of the standard curve for four plant

130

	hormones				
	A	B	C	D	r ²
JA	2.92	-1.02	1555.91	0.03	0.99
SA	76.41	-0.89	108678.75	0.04	0.94
IAA	45.50	-0.60	33667.39	-0.05	0.99
ABA	93.83	-0.86	36697.58	0.04	0.99

131 **Determination of Flavonoid Content**

132 Pine needles (0.6 g) were ground in liquid nitrogen, and 6 ml of extraction solution was added.
 133 Samples were then submitted to 300 W ultrasonication extract (60°C) in 8 s intervals every 5 s for 30
 134 min and then centrifuged at 12,000 rpm for 10 min at 25°C. The flavonoid content was determined
 135 using a plant flavonoid content assay kit (Beijing Boxbio Science & Technology Co., Ltd.). The optical
 136 density values were measured at 510 nm with a microplate reader, and the flavonoid content in plants
 137 was calculated. The standard curve was $y = 0.6457x - 0.0059$, and the r^2 was higher than 0.99.

138 **Data Processing**

139 The data involved in this article were the mean $\pm$ SD from three biological replications. The plant
 140 hormone results were tested using Duncan's new complex polar difference method, and the total
 141 flavonoid assay results were tested using the LSD method. Significance was determined at $P < 0.05$.
 142 One-way ANOVA was performed using DPS, and two-way ANOVA and correlation analysis were
 143 performed using SPSS.

144 **Results**145 **Expression of Metabolites of Feeding Stimulation and Leaf Clipping Control**

146 To further explain metabolite changes in Chinese pine after feeding stimulation, primary and
 147 secondary metabolites were identified in the needles at 0, 2, and 8 h after feeding stimulation (marked
 148 as 2 h and 8 h) and leaf clipping control (marked as LCC 2 h and LCC 8 h) using an ultra-performance
 149 liquid chromatography tandem mass spectrometry (UPLC-MS/MS) platform with extensive targeted
 150 metabolism techniques. A total of 999 metabolites were identified. Principal component analysis
 151 (PCA) was then conducted, as shown in Fig 1a, and the first principal component (PC1) explained
 152 33.3% of the original data. The top three were flavonoids (30.8%), phenolic acid (17.2%), and lipids
 153 (13.15%) (Fig. 1b). A total of 93 flavonols, 90 flavonoids, 37 dihydroflavones, 19 flavonoid glycosides,
 154 17 flavanols, 15 chalcones, 15 isoflavones, 13 dihydroflavones, 7 anthocyanins, and 2 diflavones were
 155 detected.

156 Variable Importance in Projection (VIP), based on the OPLS-DA model combined with the
 157 P -value and fold change (FC) from univariate analysis, was used to further identify differential
 158 metabolites. Substances with an $FC \geq 2$ and ≤ 0.5 and $VIP \geq 1$ were selected as differentially
 159 accumulated metabolites (DAMs). At 2 and 8 h after feeding stimulation, there were 248 (158
 160 upregulated and 90 downregulated) and 202 (139 upregulated and 63 downregulated) DAMs. A total of
 161 297 DAMs were detected 2 h after treatment, of which 126 metabolites were upregulated and 171 were
 162 downregulated. In the comparison at 8 h after treatment, a total of 239 DAMs were detected, of which
 163 62 were upregulated and 177 were downregulated (Fig. 1c). Forty-five DAMs accumulated only in the
 164 comparison of 0 h and FS, and 11 DAMs accumulated only in the comparison of FS and LCC (Fig. 1d).
 165 Notably, plant hormone expression was downregulated in both time-comparison groups with different
 166 treatments.

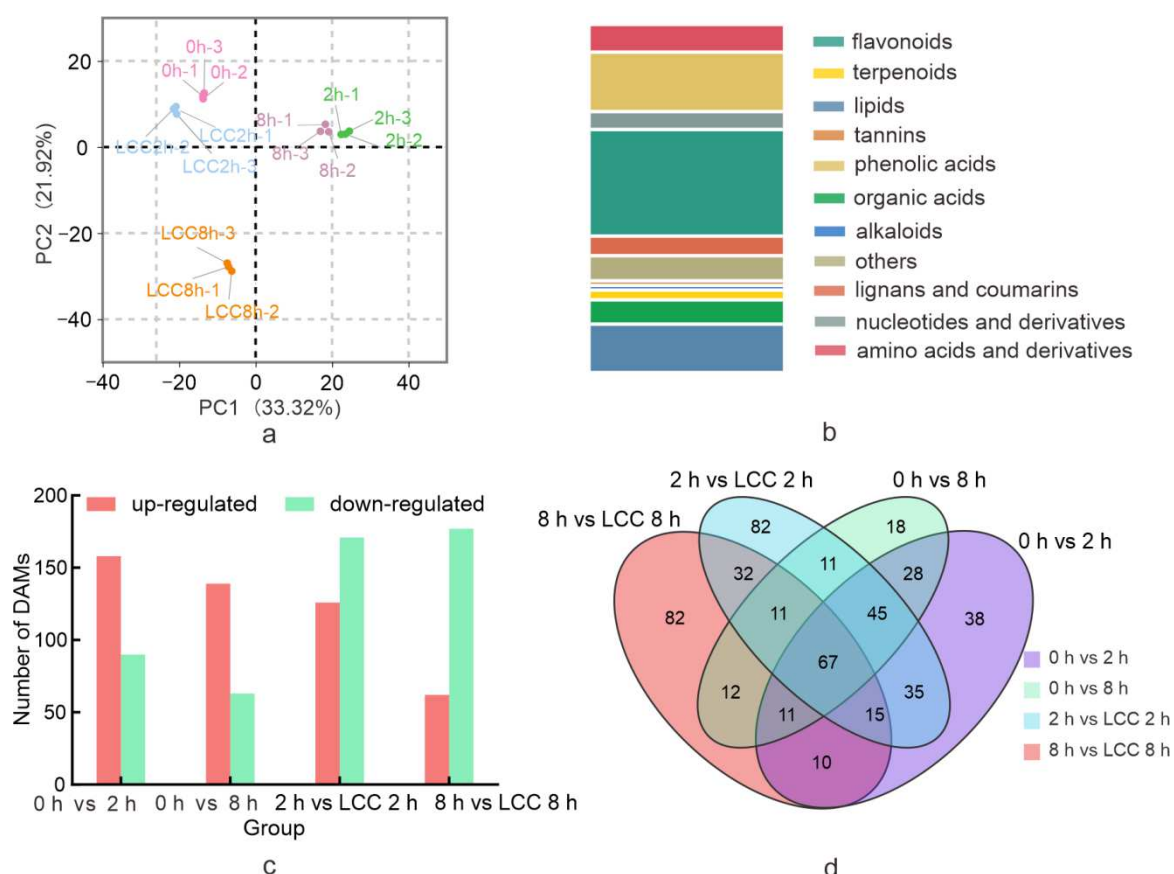


Fig 1. (a) Principal component analysis (PCA) of metabolites in different groups. PC1 is the first principle component, and PC2 is the second principle component. The percentage indicates the extent to which that principal component explains the dataset. Each point represents one sample, and the same color represents the same treatment. (b) Proportion of different metabolite types in all metabolites. Each color represents one type of metabolite. (c) Histogram of the number of differentially accumulated metabolites (DAMs) in the different comparison groups. (d) Venn diagram of DAMs in different comparison groups.

Functional Enrichment Analysis of DAMs

We mapped all DAMs from different comparison groups into the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. After anchoring to the pathways in which metabolites are involved, the annotation results were clustered to identify the pathways in which more DAMs were enriched. As shown in Fig. 2, except for the metabolic pathways and biosynthesis of secondary metabolites, the top three pathways annotated and enriched for DAMs at 2 h vs. LCC 2 h were flavonoid biosynthesis (12), amino acid synthesis (8), and arginine and proline metabolism pathways (6). The top three pathways at 8 h vs. LCC 8 h were flavonoid biosynthesis (14), linoleic acid biosynthesis (6), and flavonoid and flavonol biosynthesis pathways (6). When considering time as a variable, at 2 h after FS, the top three clustered pathways were the flavonoid (15), purine (7), and phenylalanine metabolism pathways (6). At 8 h after caterpillar stress, the top three clustered pathways were the flavonoid (10), phenylpropanoid (7), and phenylalanine pathways (5). Plant hormone signal transduction and ATP-binding cassette (ABC) transporters in environmental information processing and aminoacyl-tRNA biosynthesis in genetic information processing were contained in all four comparison groups.

Log₂FC of DAMs were involved in four shared pathways: plant hormone signal transduction, ABC transporters, flavonoid biosynthesis, and aminoacyl-tRNA biosynthesis. As shown in Fig. 3, (-)-jasmonoyl-L-isoleucine, 2'-deoxyinosine, dihydromyricetin (ampelopsin), dihydroquercetin (taxifolin), JA, and trans-5-O-(p-coumaroyl) shikimate exhibited significant upregulation in the 0 h vs. 2 h and 0 h vs. 8 h comparison groups. LCC led to the downregulation of naringenin

(5,7,4'-trihydroxyflavanone), pinocembrin (dihydrochrysin), and pinostrobin.

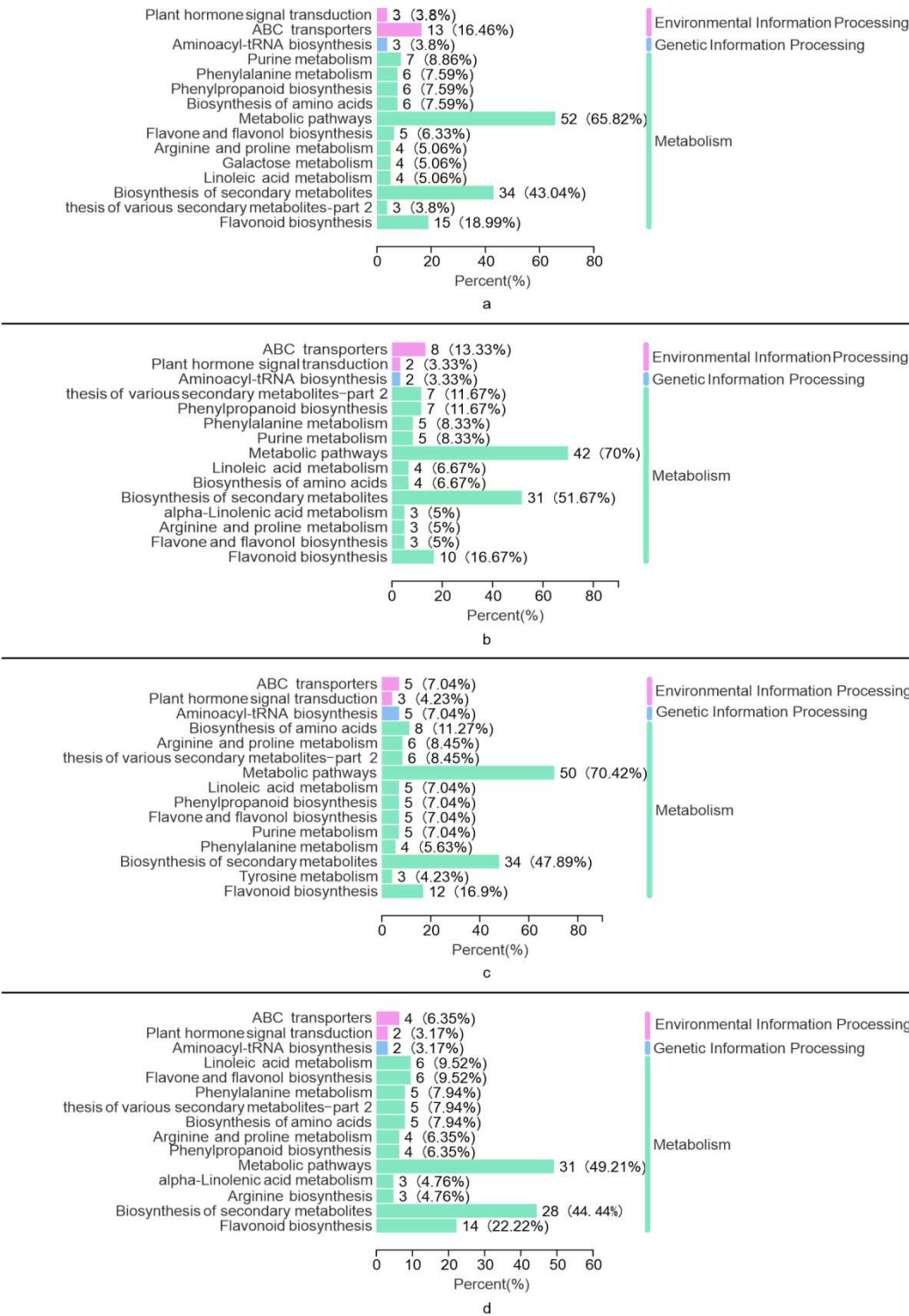


Fig 2. Bar plot of the top 15 clustered KEGG pathways of the DAMs. (a) Comparison group of 0 h vs. 2 h. (b) Comparison group of 0 h vs. 8 h. (c) Comparison group of 2 h and LCC 2 h. (d) Comparison group of 8 h and LCC at 8 h. The horizontal coordinate is the percentage of DAMs annotated to the pathway, and the vertical coordinate is the pathway name. Different colors represent different biological processes.

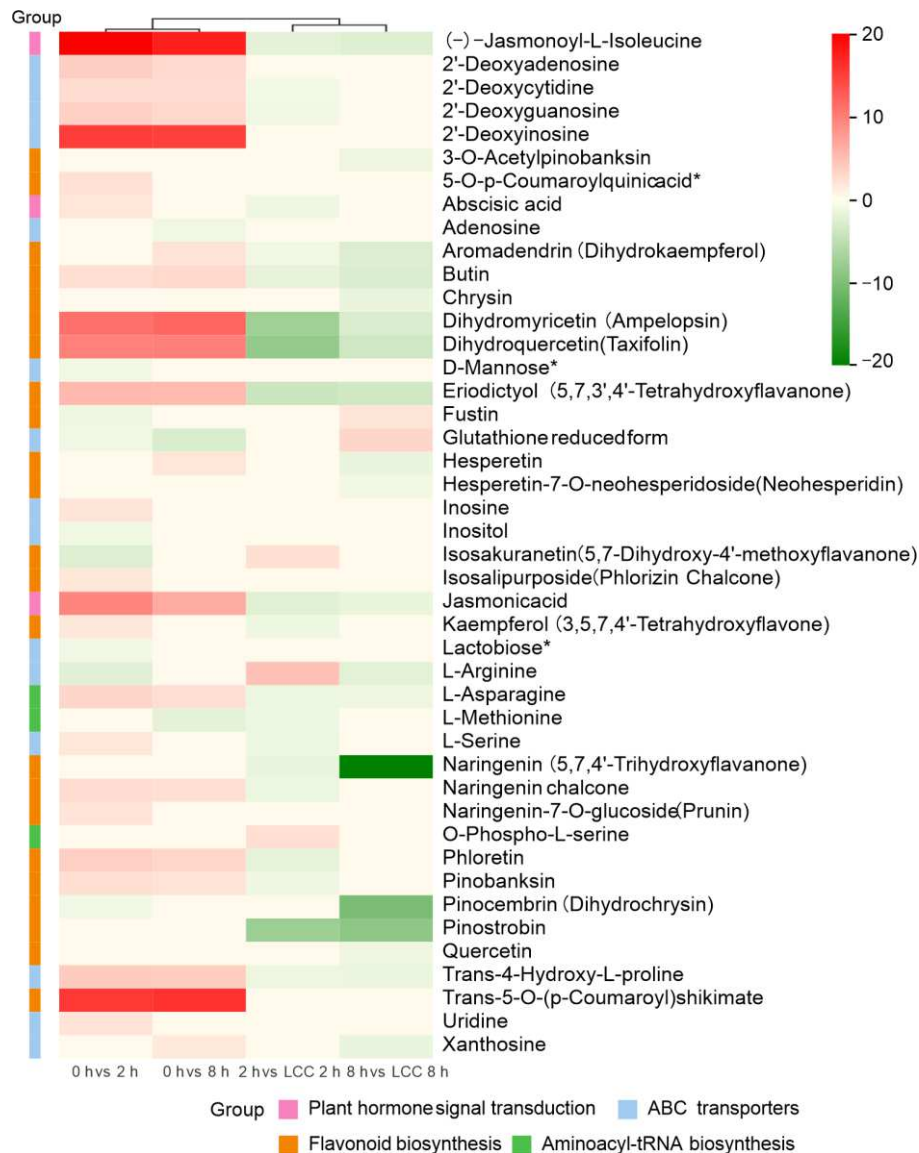


Fig 3. Log₂FC for each differential metabolite, shown as a heat map, reflecting the expression between the comparison groups.

Content and Correlation Analysis of Plant Hormones

To characterize the changes in plant hormone content during insect resistance processes in conifers, plant endogenous hormones JA, SA, IAA, and ABA were selected for the expression assay.

The concentrations of the four plant hormones in the samples were calculated using standard curves, as shown in Fig 4. The JA content in FS first increased, then decreased, and increased again over time. The peak of FS occurred at 1.5 h with 185.16 pmol·L⁻¹, which was 59.8% higher than LCC at 1.5 h. The peak of LCC occurred at 1 h with 157.43 pmol·L⁻¹, which was 19.2% higher than FS 1 h. All time points showed a higher JA content in FS than in LCC, except for 1 and 6 h. This showed that JA upregulation was induced in a short time (2 h) after FS compared to LCC.

FS led to a sharp decrease in SA at 1 and 4 h, with contents of 25.86 and 29.21 pmol·L⁻¹, respectively, which were 70.0% and 66.1% lower, respectively, than at 0 h. The minimum for LCC occurred only at 4 h after treatment and the maximum at 2 h after treatment at 115.22 pmol·L⁻¹. After 2 h, the SA content for LCC was higher than that for FS at all time points. The content of each treatment showed a significant increase over time, but no significant differences were observed between the two

treatments.

IAA content reached a peak at $12.22 \mu\text{g}\cdot\text{L}^{-1}$ in 1 h. IAA was significantly downregulated in LCC at 2 h and was significantly different from FS. At 4 and 8 h, the content of LCC was lower than that of FS, indicating that IAA was significantly induced by FS in a short time (2 h), but LCC did not cause remarkable changes over time.

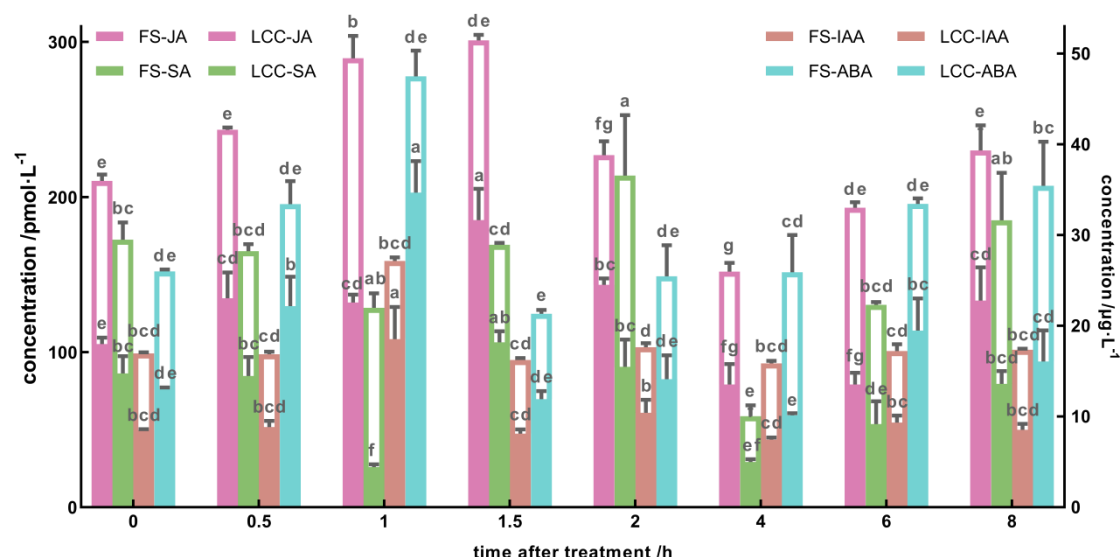


Fig 4. Concentration of JA, SA, IAA, and ABA at different times after treatment. Same letters indicate no significant difference at $P < 0.05$ level.

FS caused a continuous increase in ABA content to $34.68 \mu\text{g}\cdot\text{L}^{-1}$ for 1 h and then decreased; subsequently, there was an upregulation at 6 and 8 h, with a 49.8 and 23.6% increase, respectively, over that at 0 h, respectively. The maximum ABA content with LCC was $19.36 \mu\text{g}\cdot\text{L}^{-1}$, which occurred at 8 h, and a significant difference in expression from 0 h was only observed at 8 h. The minimum value was $9.40 \mu\text{g}\cdot\text{L}^{-1}$, which occurred at 1.5 h. Although there was a significant difference between the two treatments at 4 and 6 h, the content at 4 h for LCC was higher than that in FS, while at 6 h, the two treatments led to opposite results.

In conclusion, resistance-related hormones JA and SA were greatly affected by feeding stimulation, while growth-related hormones IAA and ABA were less influenced by the different treatments at all time points, except for a significant increase at FS 1 h. Correlation analysis of the four plant hormones at different times showed significant positive correlations between JA and SA, as well as between IAA and ABA, with correlation coefficients of 0.78 and 0.83, respectively, at the $P < 0.5$ level; SA showed negative correlations with IAA and ABA.

Content of Total Flavonoid

To validate the metabolomics results, the total flavonoid content, the top class of substances with the greatest accumulation differences in the metabolome, was determined. As shown in Fig 5, both treatments induced flavonoid accumulation over time. The total flavonoid content was 0.24 and 0.12 $\text{mg}\cdot\text{ml}^{-1}$ for 8 h and LCC 8 h, respectively, which were significantly different from 0.08 $\text{mg}\cdot\text{ml}^{-1}$ at 0 h. FS treatment induced a highly significant difference in accumulation. There was no significant difference between 2 h and LCC 2 h, but a significant difference was observed between 8 h and LCC 8 h. There was a significant difference between 2 h and 8 h for FS, while in LCC, they were not significantly different.

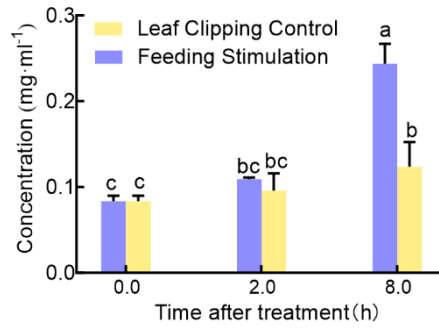


Fig 5. Total flavonoid concentration at different times after treatment. Same letters indicate no significant difference at $P < 0.05$ level.

A two-way analysis of variance (ANOVA) of total flavonoid content with different treatments and different times after treatment showed a significant main effect of treatment ($F(1,10) = 26.16$, $P < 0.001$) and a significant main effect of different times after treatment ($F(1, 10) = 39.43$, $P < 0.001$). Post hoc tests with the LSD method between the main effects of different treatments, as shown in Tab 2, showed a significant difference in the flavonoid content in needles between FS and the control, as well as an equivalent significant difference between FS and LCC, but no significant difference was observed between the control and LCC.

Tab 2. Post-hoc multiple comparison tests of different modes of treatment

(i) treatment modes	(j) treatment modes	M(I-J)	SE	Sig. ^c
0h	LCC	-0.02 ^{a,b}	0.01	0.11
	FS	-0.06 ^{a,b,*}	0.01	0.00
LCC	0h	0.02 ^{a,b}	0.01	0.11
	FS	-0.04 ^{a,b,*}	0.01	0.00
FS	0h	0.06 ^{a,b,*}	0.01	0.00
	LCC	0.04 ^{a,b,*}	0.01	0.00

Based on estimated marginal means. a. Modified estimate of overall marginal mean (I). b. Modified estimate of overall marginal mean (J). c. Adjustment for multiple comparisons: Least significant difference (equivalent to no adjustment). *. Differences in means are significant at the 0.05 level.

Discussion

Differential Accumulated Flavonoids in Chinese Pine after FS and LCC

Stress on plants induces a large number of ROS. Flavonoids in response to stress in plants are mainly involved in the antioxidant scavenging of free radicals (Ahammed and Yang 2022; Liu, *et al.* 2021b; Ma, *et al.* 2021). In this study, a significant amount of DAMs were enriched in the flavonoid pathway in both the FS and LCC comparison groups, as shown in Fig 2. According to the KEGG pathway, the C4H cleavage of cinnamic acid to 4-coumaric acid occurs in the phenylalanine pathway (Shi, *et al.* 2022), which in turn generates p-coumaroyl CoA, followed by naringenin chalcone synthesis catalyzed by chalcone synthase (Liu, *et al.* 2021c), the precursor of most different accumulated flavonoids in this study. This process was upregulated by caterpillars, but there was no difference in expression between treatments at 8 h. However, as shown in Fig 6, naringenin, a downstream substance of chalcone, was not induced by FS but was significantly higher in FS than in LCC at 8 h. Therefore, mechanism damage can promote naringin depletion and become more intense over time. The downstream substance of naringenin, eriodictyol, was induced by FS but not by LCC.

The longer the feeding stimulation by pine caterpillars, the weaker the reaction of naringenin flow to isosakuranin, causing eriodictyol and others to form flavanonol, dihydrokaempferol, dihydroquercetin, and dihydromyricetin catalyzed by flavanone 3-hydroxylase (F3H)(Liu, *et al.* 2021c). There were more significant differences in dihydromyricetin and dihydroquercetin at 2 h than at 8 h for different treatment modes, but the opposite trend was observed in aromadendrin (dihydrokaempferol) accumulation. Furthermore, no differences were observed in its downstream substance, kaempferol, between the FS and LCC comparison groups at 8 h. This indicates that anthocyanin may play a key role in resistance. In alfalfa, graphene induces the upregulation of isoquercitrin and proline, which are involved in the plant scavenging of ROS to protect them from oxidative damage(Chen, *et al.* 2021). At 8 h after treatment, pine caterpillars induced the dihydrokaempferol–dihydroquercetin–quercetin reaction, and at the same time, kaempferol was involved in flavonoid and flavonol biosynthesis (Tab S1). These results are consistent with metabolite changes in sugarcane after stickleback feeding and in tea trees after looper feeding (Wang Weiwei 2018; Zhang Jiasong 2020).

FS also induced a significant upregulation of dihydromyricetin and dihydroquercetin, which are involved in anthocyanin biosynthesis (Fig 3). The expression of JA and anthocyanin-related genes in maize (*Zea mays* L.) was highly upregulated by whitefly (*Bemisia tabaci* (Genn.)) attack(Park, *et al.* 2015). Anthocyanin in corn can affect the growth and development of tobacco hornworm (*Manduca sexta* L.) caterpillars(Tayal, *et al.* 2020). In *Arabidopsis thaliana*, JA releases WD-repeat/bHLH/MYB complexes to accumulate anthocyanin biosynthesis(Qi, *et al.* 2011). Therefore, anthocyanins, as flavonoids, react to stress in plants with JA. In this study, the JA concentration reached its peak 1 h after FS, confirming this statement.

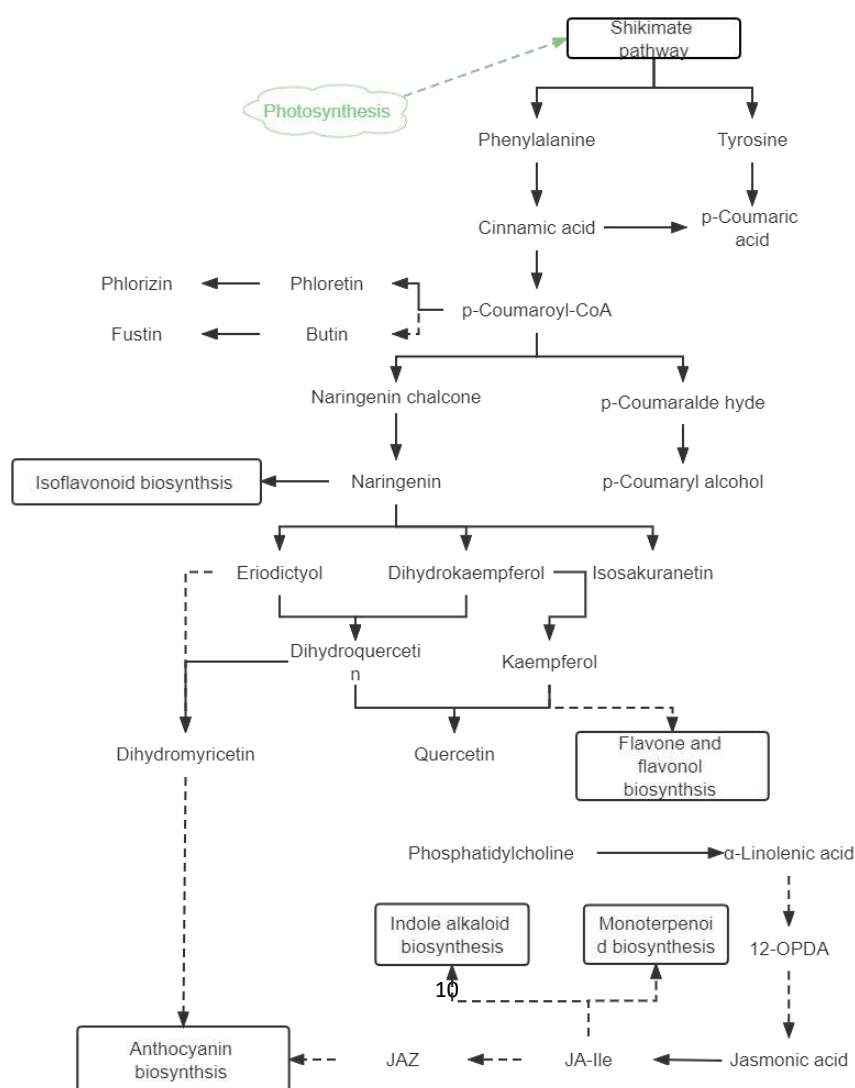


Fig 6. Pathway and expression of flavonoids, phenylpropanoids, and JA with different accumulations. Solid lines show the reaction with no intermediate steps, and dashed lines show the reactions requiring an intermediate process.

In this study, the total flavonoid concentration was determined to validate the metabolomic results. A two-way ANOVA on total flavonoid content revealed changes in flavonoid content due to different treatments and the time after treatment, with a significant difference between FS and LCC treatments at 0 and 8 h, indicating significant differences due to feeding stimulation. This result can not only validate metabolomic results but also indicate that other behaviors or substances of insects, in addition to mechanical damage from feeding, could trigger a defense response.

Differential Accumulated Phenylpropanoid-derived Compounds in Chinese Pine after FS and LCC

Flavonoids are derived from phenylalanine, and their expression can be induced by insect herbivory. In plants, phenylalanine deaminates to cinnamic acid using phenylalanine ammonia-lyase (PAL), which is the first step in generating other phenylpropanoid-derived compounds (Barros and Dixon 2020), considered a bridge between primary metabolism and the synthesis of lignin, flavonoids, or other resistance substances during defense (Guo, *et al.* 2018). Phenylpropanoid-derived compounds widely participate in the response to biotic stress. Genes in the phenylpropanoid pathway are upregulated in *Ulmus minor* by an egg treatment of the elm leaf beetle (*Xanthogaleruca luteola*) (Schott, *et al.* 2021). PAL can increase SA content to regulate the plant–cereal cyst nematode (CCN) interaction (Zhang, *et al.* 2021b). As shown in Fig 2, more DAMs were enriched in the phenylalanine metabolism and phenylpropanoid biosynthesis pathways in the 0 h vs. 2 h and 0 h vs. 8 h comparison groups. Coniferyl-aldehyde was upregulated after FS. P-coumaric acid, caffeic acid, p-coumaraldehyde, and conifer-alcohol were depressed at LCC 2 h, while cinnamic acid and cinnamaldehyde were depressed at LCC 8 h. Precursors of guaiacyl lignin, coniferyl-aldehyde, and conifer-alcohol also accumulated differently after FS (Table S1). P-coumaric CoA is related to the phenylalanine metabolism and flavonoid metabolism pathways, as shown in Figure 6, and accumulated under FS, although the change was not significant. As mentioned previously, anthocyanin is regulated by JA, and changes in phenylalanine may occur earlier than 2 h after stress.

Coumarins are also resistant substances derived from the phenylpropanoid pathway (Kai, *et al.* 2008; Siwinska, *et al.* 2014), and scopoletin could eliminate superoxide anions in vivo (Shaw, *et al.* 2003), especially in fungus infection (Sun, *et al.* 2014). Surprisingly, scopoletin showed downregulation at 8 h compared with LCC at 8 h. Although scopoletin can reduce the larval weight of *Spodoptera frugiperda* (J. E. Smith) (Giongo, *et al.* 2016) and induce calcium overload in *T. cinnabarinus* (Zhou, *et al.* 2019), it was downregulated by feeding stimulation of pine caterpillar, and whether this process relies on jasmonate signaling should be further studied.

Changes in ABC Transporters in Response to FS and LCC

Most ABC transporters rely on the energy of ATP hydrolysis to drive defense substances' transport in plants and are located in the membrane, and many kinds of stress could induce the expression of ABC subfamilies (Mirzahossini, *et al.* 2015; Pierman, *et al.* 2017). Cd stress causes upregulation in poplar roots and *Brassica napus* (Wang, *et al.* 2019a; Zhang, *et al.* 2018). Some hormones also have to be transported through ABC transporters (Dahuja, *et al.* 2021). Glutathione (L-γ-glutamyl-L-cysteinylglycine, GSH) is imported into cells through ABC transporters. When facing stress, ROS can be scavenged by the ascorbate–glutathione pathway (Ding, *et al.* 2020). GSH has an antagonism with JA and SA (Couto, *et al.* 2016). As shown in Fig 3 and 4, the GSH-reduced form was

downregulated at both 2 and 8 h after FS, while JA and SA were upregulated. However, the interaction between phytohormones and GSH requires further study.

Changes in Plant Hormones in Response to FS and LCC

Plants immediately synthesize considerable JA once they perceive stimulation, and jasmonyl isoleucine can bind to COI1 receptors to mediate the ubiquitination and degradation of JAZs, thereby activating JA signaling pathways(Zhang, *et al.* 2021a) and regulating most steps of anthocyanin formation in the flavonoid pathway(Wasternack and Strnad 2019). In this study, JA content peaked at FS 1.5 h, while flavonoid pathway changes in the 2 h metabolome were observed; therefore, pine caterpillar feeding stimulation induced JA expression, and its regulation in the subsequent flavonoid pathway participated in oxidative stress. As shown in Tab 2, JA and jasmonyl isoleucine accumulated differentially at 2 and 8 h in both treatments and were higher for FS than for LCC, which is consistent with the changing trend of plant hormones. JA content increased rapidly in the short term during feeding stimulation and then decreased to a level with no significant difference in expression from the pretreatment (Fig 4), avoiding excessive plant defenses that hindered its growth and development due to excessive JA accumulation.

The accumulation differences of substances in the SA pathway occur after many plants are infested with pathogens(Klessig, *et al.* 2018), but insect stress also leads to the cooperative expression of the SA signaling pathway with the JA signaling pathway(Zhang 2020; Zhao 2019). Plants initiate earlier resistance to pathogens through the SA pathway(Alfonso, *et al.* 2021). In plants, SA can be synthesized through phenylalanine and isobranhich acid, both of which have mangiferic acid as a precursor(Li, *et al.* 2019; Zhong, *et al.* 2021). In this study, there was no significant difference in SA content between FS and LCC, and the expression of related substances was not detected in the comparison of both treatments; however, the long duration of feeding stimulation led to changes in SA content, with a significant positive correlation between SA and JA content.

IAA can be generated from L-tryptophan and indole in the tryptophan pathway. This study showed that in the tryptophan pathway, indole produces not only IAA but also 2-carboxamidobenzoate, but this reaction was only seen in the comparison group with short-term treatments (2 h). Most of the carbon skeleton compounds in plants are produced by the mangiferous acid pathway, and both synthesis pathways of SA and IAA, phenylalanine and tryptophan pathways, are generated from the product chorismic acids in the mangiferous acid pathway. IAA is the signal of anthocyanin accumulation at insect chewing sites and induces systematic acquired resistance(Machado, *et al.* 2016). Although there was no difference between SA and IAA accumulation in either comparison group of the metabolome, the difference between these two substances showed a negative correlation at 1 h after different treatments, as shown in Fig 4. The IAA concentration was upregulated before JA, which is consistent with a previous study(Machado, *et al.* 2016). Thus, enzymes in the heterobranched acid pathway play an important role in balancing defense and growth.

As shown in Tab S1, γ -aminobutyric acid (GABA) was upregulated 2 h after feeding stimulation. ABA signaling pathway activation can be observed by applying GABA to water-stressed apple shoots(Liu, *et al.* 2021a). Meanwhile, GABA can activate the gene expression of ACC, the precursor of ethylene synthesis, and ethylene-induced root growth requires IAA biosynthesis, transport, signaling, and response. The same positive correlation between IAA and ABA can be induced by feeding stimulation of pine caterpillars. ABA regulates stomatal opening or closing to reduce water loss in plants, which has been widely reported as a response to abiotic stress(Hu, *et al.* 2021; Li, *et al.* 2022). Downregulation of ABA signaling induced by brown planthoppers can increase SA-dependent defense

in response to salt stress in susceptible rice varieties(Quais, *et al.* 2020). Similarly, the increase in ABA and JA content in rice after silencing E3 ligase also demonstrates brown planthopper-induced plant hormone antagonism(Zhang, *et al.* 2021c). Correlation analysis indicated a correlation between SA and ABA, but the lower significance of these plant hormones induced by the feeding stimulation of pine caterpillars on Chinese pine instead of high significance may occur because of the foraging pattern, that is, the brown planthopper feeds on the phloem, where ABA transport occurs over a long distance.

Conclusion

In this study, it was found that Chinese pine can trigger the flavonoid pathway when faced with feeding stimulation by the pine caterpillar. Furthermore, pine trees upregulated JA and IAA to balance growth and defense and confer resistance. Our metabolome data could offer a resource for other research on Chinese pine and provide a reference for plant growth and resistance balance under biotic stress.

Supplementary Materials: Table S1: DAMs of the four group: 0 h vs 2 h, 0 h vs 8 h, 2 h vs LCC 2 h, and 8 h vs LCC 8 h.

Author Contributions: All authors contributed to the study conception and design. Original draft preparation by Zhao Yanan; data curation by Zhao Yanan and Sun Tianhua; experiment setting by Zhao Yanan, Sun Tianhua, Wang Lifeng and Xu Qiang; review and editing by Zhou Guona; visualization by Liu Junxia; supervision by Gao Baojia; funding acquisition by Gao Baojia. All authors read and approved the final manuscript.

Funding: This research was funded by Molecular Mechanisms of Population Differentiation and Adaptation of Forest Pests with Environmental Stress(China), grant number 30771739.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank Metware Biotechnology Co., Ltd. for their technical support, Huangtuliangzi Forestry for material support and Zhang Ziheng, Li Hanxiao, and Meng Danyang for their help to set experiments. We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Declaration:

Conflicts of Interest: The authors declare no conflict of interest.

References:

- AGUDELO-ROMERO P, ERBAN A, REGO C, *et al.* (2015) Transcriptome and metabolome reprogramming in *Vitis vinifera* cv. Trincadeira berries upon infection with *Botrytis cinerea*. J Exp Bot, 66: 1769-1785.
- AHAMMED G J, YANG Y. (2022) Anthocyanin-mediated arsenic tolerance in plants. Environ Pollut, 292(Pt B):118475.
- ALFONSO E, STAHL E, GLAUSER G, *et al.* (2021) Insect eggs trigger systemic acquired resistance against a fungal and an oomycete pathogen. New Phytol, 232: 2491-2505.
- BAILEY-SERRES J, PARKER J E, AINSWORTH E A, *et al.* (2019) Genetic strategies for improving crop yields. Nature, 575: 109-118.
- BARROS J, DIXON R A.(2020) Plant Phenylalanine/Tyrosine Ammonia-lyases. Trends Plant Sci,

25: 66-79.

BERENS M L, BERRY H M, MINE A, *et al.* (2017) Evolution of Hormone Signaling Networks in Plant Defense. *Annu Rev Phytopathol*, 55: 401-425.

BHARATH P, GAHIR S, RAGHAVENDRA A S.(2021) Absciscic Acid-Induced Stomatal Closure: An Important Component of Plant Defense Against Abiotic and Biotic Stress. *Front Plant Sci*, 12:615114.

CHEN J, WANG J, WANG R, *et al.*(2020) Integrated metabolomics and transcriptome analysis on flavonoid biosynthesis in safflower (*Carthamus tinctorius* L.) under MeJA treatment. *BMC Plant Biol*, 20: 353.

CHEN Z, GUO Z, NIU J, *et al.* (2021) Phytotoxic effect and molecular mechanism induced by graphene towards alfalfa (*Medicago sativa* L.) by integrating transcriptomic and metabolomics analysis. *Chemosphere*, 290:133368.

CHOUDHURY F K, DEVIREDDY A R, AZAD R K, *et al.* (2018) Rapid Accumulation of Glutathione During Light Stress in Arabidopsis. *Plant Cell Physiol*, 59: 1817-1826.

COUTO N, WOOD J, BARBER J.(2016) The role of glutathione reductase and related enzymes on cellular redox homeostasis network. *Free Radical Bio Med*, 95:27-42.

CUI N, LU H, WANG T, *et al.* (2019) Armet, an aphid effector protein, induces pathogen resistance in plants by promoting the accumulation of salicylic acid. *Philos Trans R Soc Lond B Biol Sci*, 374: 20180314.

DAHUJA A, KUMAR R R, SAKHARE A, *et al.*(2021) Role of ATP-binding cassette transporters in maintaining plant homeostasis under abiotic and biotic stresses. *Physiol Plantarum*, 171: 785-801.

DENISON F C, PAUL A L, ZUPANSKA A K, *et al.*(2011) 14-3-3 proteins in plant physiology. *Semin Cell Dev Biol*, 22: 720-727.

DING H Y, WANG B P, HAN Y, *et al.*(2020) The pivotal function of dehydroascorbate reductase in glutathione homeostasis in plants. *J Exp Bot*, 71: 3405-16.

FARAHBAKHS F, HAMZEHZARGHANI H, MASSAH A, *et al.* (2019) Comparative metabolomics of temperature sensitive resistance to wheat streak mosaic virus (WSMV) in resistant and susceptible wheat cultivars. *J Plant Physiol*, 2019, 237:30-42.

FERRY N, EDWARDS M G, GATEHOUSE J A, *et al.* (2004) Plant-insect interactions: molecular

approaches to insect resistance. *Curr Opin Biotechnol*, 15: 155-61.

GIONGO A M, VENDRAMIM J D, FREITAS S D, *et al.*(2016) Toxicity of Secondary Metabolites from Meliaceae Against *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). *Neotrop Entomol*, 45: 725-733.

GUO L, WANG P, JAINI R, *et al.* (2018) Dynamic modeling of subcellular phenylpropanoid metabolism in *Arabidopsis* lignifying cells. *Metab Eng*, 49:36-46.

HAMANISHI E T, BARCHET G L, DAUWE R, *et al.* (2015) Poplar trees reconfigure the transcriptome and metabolome in response to drought in a genotype- and time-of-day-dependent manner. *BMC Genomics*, 16:329.

HU W, ZHANG J, YAN K, *et al.*(2021) Beneficial effects of abscisic acid and melatonin in overcoming drought stress in cotton (*Gossypium hirsutum* L.). *Physiol Plant*, 173: 2041-2054.

JEON J E, KIM J G, FISCHER C R, *et al.* (2020) A Pathogen-Responsive Gene Cluster for Highly Modified Fatty Acids in Tomato. *Cell*, 180: 176-87 e19.

KAI K, MIZUTANI M, KAWAMURA N, *et al.* (2008) Scopoletin is biosynthesized via ortho-hydroxylation of feruloyl CoA by a 2-oxoglutarate-dependent dioxygenase in *Arabidopsis thaliana*. *Plant Journal*, 55: 989-99.

KANDASAMY D, GERSHENZON J, ANDERSSON M N, *et al.* (2019) Volatile organic compounds influence the interaction of the Eurasian spruce bark beetle (*Ips typographus*) with its fungal symbionts. *ISME J*, 13: 1788-800.

KLESSIG D F, CHOI H W, DEMPSEY D A.(2018) Systemic Acquired Resistance and Salicylic Acid: Past, Present, and Future. *Mol Plant Microbe Interact*, 31: 871-88.

LI Q, FAN J, SUN J, *et al.* (2019) Anti-plant Defense Response Strategies Mediated by the Secondary Symbiont *Hamiltonella defensa* in the Wheat Aphid *Sitobion miscanthi*. *Front Microbiol*, 10:2419.

LI R, SU X, ZHOU R, *et al.* (2022) Molecular mechanism of mulberry response to drought stress revealed by complementary transcriptomic and iTRAQ analyses. *BMC Plant Biol*, 22: 36.

LIU C, WANG H, ZHANG X, *et al.*(2021) Activation of the ABA Signal Pathway Mediated by GABA Improves the Drought Resistance of Apple Seedlings. *Int J Mol Sci*, 22: 12676.

LIU S, FANG S, LIU C, *et al.* (2021) Transcriptomics Integrated With Metabolomics Reveal the

479 Effects of Ultraviolet-B Radiation on Flavonoid Biosynthesis in Antarctic Moss. *Front Plant*
480 *Sci*, 12:788377.

481 LIU W, FENG Y, YU S, *et al.* (2021) The Flavonoid Biosynthesis Network in Plants. *Int J Mol Sci*,
482 22: 12824.

483 MA T L, LI W J, HONG Y S, *et al.* (2021) TMT based proteomic profiling of *Sophora*
484 *alopecuroides* leaves reveal flavonoid biosynthesis processes in response to salt stress. *J*
485 *Proteomics*, 253:104457.

486 MACHADO R A R, ROBERT C A M, ARCE C C M, *et al.* (2016) Auxin Is Rapidly Induced by
487 Herbivore Attack and Regulates a Subset of Systemic, Jasmonate-Dependent Defenses. *Plant*
488 *Physiol*, 172(1): 521-532.

489 MICHAEL T P, VANBUREN R. (2020) Building near-complete plant genomes. *Curr Opin Plant*
490 *Biol*, 54:26-33.

491 MIRZAHOSSINI Z, SHABANI L, SABZALIAN M R, *et al.* (2015) ABC transporter and
492 metallothionein expression affected by NI and *Epichloe* endophyte infection in tall fescue.
493 *Ecotoxicol Environ Saf*, 120:13-19.

494 NASCIMENTO F X, ROSSI M J, GLICK B R. (2018) Ethylene and
495 1-Aminocyclopropane-1-carboxylate (ACC) in Plant-Bacterial Interactions. *Front Plant Sci*,
496 9:114.

497 NIU S, LI J, BO W, *et al.* (2022) The Chinese pine genome and methylome unveil key features of
498 conifer evolution. *Cell*, 185: 204-17 e14.

499 OU X, LI T, ZHAO Y, *et al.* (2022) Calcium-dependent ABA signaling functions in stomatal
500 immunity by regulating rapid SA responses in guard cells. *J Plant Physiol*, 268:153585.

501 PARK Y S, BAE D W, RYU C M. (2015) Aboveground Whitefly Infestation Modulates
502 Transcriptional Levels of Anthocyanin Biosynthesis and Jasmonic Acid Signaling-Related
503 Genes and Augments the Cope with Drought Stress of Maize. *Plos One*, 10: e0143879.

504 PASHKOVSKIY P P, VANKOVA R, ZLOBIN I E, *et al.* (2019) Comparative analysis of abscisic
505 acid levels and expression of abscisic acid-related genes in Scots pine and Norway spruce
506 seedlings under water deficit. *Plant Physiol Biochem*, 140:105-112.

507 PIERMAN B, TOUSSAINT F, BERTIN A, *et al.* (2017) Activity of the purified plant ABC
508 transporter NtPDR1 is stimulated by diterpenes and sesquiterpenes involved in constitutive

and induced defenses. J Biol Chem, 292: 19491-19502.

PITAKRATTANANUKOOL S, KAWAKATSU T, ANUNTALABHOCHAI S, *et al.* (2012) Overexpression of *OsRab7B3*, a small GTP-binding protein gene, enhances leaf senescence in transgenic rice. Biosci Biotechnol Biochem, 76: 1296-1302.

QI T, SONG S, REN Q, *et al.* (2011) The Jasmonate-ZIM-domain proteins interact with the WD-Repeat/bHLH/MYB complexes to regulate Jasmonate-mediated anthocyanin accumulation and trichome initiation in *Arabidopsis thaliana*. Plant Cell, 23: 1795-814.

QIN S J, QI J Y, LIU R J, *et al.* (2021) Physiological Response of *Pinus tabulaeformis* Infected with *Bursaphelenchus xylophilus* in Natural State. Journal of Shenyang Agricultural University, 52: 625-632. [in Chinese] DOI:10.3969/j.issn.1000-1700.2021.05.014

QUAIS M K, MUNAWAR A, ANSARI N A, *et al.* (2020) Interactions between brown planthopper (*Nilaparvata lugens*) and salinity stressed rice (*Oryza sativa*) plant are cultivar-specific. Sci Rep-Uk, 10:8051.

RIVAS-UBACH A, BARBETA A, SARDANS J, *et al.* (2016) Topsoil depth substantially influences the responses to drought of the foliar metabolomes of Mediterranean forests. Perspect Plant Ecol, 21:41-54.

RUAN J, ZHOU Y, ZHOU M, *et al.* (2019) Jasmonic Acid Signaling Pathway in Plants. Int J Mol Sci, 20:2479.

YUAN, M., HUANG, Y., GE, W. *et al.* (2019) Involvement of jasmonic acid, ethylene and salicylic acid signaling pathways behind the systemic resistance induced by *Trichoderma longibrachiatum* H9 in cucumber. BMC Genomics. 20: 144.

SCHOTT J, FUCHS B, BOTTCHE C, *et al.* (2021) Responses to larval herbivory in the phenylpropanoid pathway of *Ulmus minor* are boosted by prior insect egg deposition. Planta, 255: 16.

SHAW C Y, CHEN C H, HSU C C, *et al.* (2003) Antioxidant properties of scopoletin isolated from *Sinomonium acutum*. Phytother Res, 17: 823-825.

SHI J, YAN X, SUN T, *et al.* (2022) Homeostatic regulation of flavonoid and lignin biosynthesis in phenylpropanoid pathway of transgenic tobacco. Gene, 809:146017.

SHI Y Y, FENG J Z, YU L H, *et al.* (2017) The inducing effect of insect feeding and leaf cutting on some defensive substance in Chinese pine (*Pinus Tabulaeformis*) needle. Journal of

539 Agricultural University of Hebei, 40: 81-86. [in Chinese] DOI:
540 10.13320/j.cnki.jauh.2017.0015

541 SIWINSKA J, KADZINSKI L, BANASIUK R, *et al.* (2014) Identification of QTLs affecting
542 scopolin and scopoletin biosynthesis in *Arabidopsis thaliana*. BMC Plant Biol, 14:280.

543 SUN H H, WANG L, ZHANG B Q, *et al.* (2014) Scopoletin is a phytoalexin against *Alternaria*
544 alternata in wild tobacco dependent on jasmonate signalling. J Exp Bot, 65: 4305-4315.

545 SZYMANSKI J, BOCOBZA S, PANDA S, *et al.* (2020) Analysis of wild tomato introgression
546 lines elucidates the genetic basis of transcriptome and metabolome variation underlying fruit
547 traits and pathogen response. Nat Genet, 52: 1111-1121.

548 TAKABAYASHI J, SHIOJIRI K. (2019) Multifunctionality of herbivory-induced plant volatiles in
549 chemical communication in tritrophic interactions. Curr Opin Insect Sci, 32:110-117.

550 TAYAL M, SOMAVAT P, RODRIGUEZ I, *et al.* (2020) Polyphenol-Rich Purple Corn Pericarp Extract
551 Adversely Impacts Herbivore Growth and Development. Insects, 11(2):98.

552 VERHAGE A, VLAARDINGERBROEK I, RAAYMAKERS C, *et al.* (2011) Rewiring of the
553 Jasmonate Signaling Pathway in *Arabidopsis* during Insect Herbivory. Front Plant Sci, 2:47.

554 WANG H H, LIU Y Y, PENG Z H, *et al.* (2019) Ectopic Expression of Poplar ABC Transporter
555 PtoABCG36 Confers Cd Tolerance in *Arabidopsis thaliana*. International Journal of
556 Molecular Sciences, 20:3293.

557 WANG J, LV J, LIU Z, *et al.* (2019) Integration of Transcriptomics and Metabolomics for Pepper
558 (*Capsicum annuum* L.) in Response to Heat Stress. Int J Mol Sci, 20:5042.

559 WANG W W. (2018) Resistance Evaluation and Mechanism of *Camellia sinensis* Response to
560 *Ectropis obliqua*. Dissertation, Huazhong Agricultural University, China.

561 WANG Y C, ZHOU G N, ZHANG B, *et al.* (2016) Difference in Protein Expression of *Pinus*
562 *tabulaeformis* Induced by *Dendrolimus tabulaeformis* Feeding and Leaf-Cutting Stimulation.
563 Scientia Silvae Sinicae, 52: 68-75. [in Chinese] doi:10. 11707/j. 1001-7488. 20160809

564 WASTERNAK C, STRNAD M. (2019) Jasmonates are signals in the biosynthesis of secondary
565 metabolites - Pathways, transcription factors and applied aspects - A brief review. N
566 Biotechnol, 48:1-11.

567 XU Y H, LIU R, YAN L, *et al.* (2012) Light-harvesting chlorophyll *a/b*-binding proteins are
568 required for stomatal response to abscisic acid in *Arabidopsis*. J Exp Bot, 63: 1095-1106.

- ZHANG B, GAO B J, LIU Y. (2017) The Effects of Leaf-Cutting and Feeding Stimulation on the Activities and Dynamic of Defense Enzymes in Chinese Pine. *Ecological Science*, 36: 118-122. [in Chinese] doi:10.14108/j.cnki.1008-8873.2017.01.016
- ZHANG G, LIU W, GU Z, *et al.*(2021) Roles of the wound hormone jasmonate in plant regeneration. *J Exp Bot* published online ahead of print. Accessed 20 Nov 2021
- ZHANG H, HUANG Q, YI L, *et al.* (2021) PAL-mediated SA biosynthesis pathway contributes to nematode resistance in wheat. *Plant J*, 107: 698-712.
- ZHANG J S. (2020) The Metabolomics Analysis of Sugarcane in Response of Oriental Armwarm *Mythimna Separate* Feeding. Dissertation, Fujian Agriculture and Forestry University, China.
- ZHANG L B, MA C, CHAO H B, *et al.* (2019) Integration of metabolome and transcriptome reveals flavonoid accumulation in the intergeneric hybrid between *Brassica rapa* and *Raphanus sativus*. *Sci Rep-Uk*, 9:18368.
- ZHANG X D, ZHAO K X, YANG Z M. (2018) Identification of genomic ATP binding cassette (ABC) transporter genes and Cd-responsive ABCs in *Brassica napus*. *Gene*, 664:139-151.
- ZHANG Y B, CHEN M T, ZHOU S X, *et al.* (2021) Silencing an E3 Ubiquitin Ligase Gene *OsJMJ715* Enhances the Resistance of Rice to a Piercing-Sucking Herbivore by Activating ABA and JA Signaling Pathways. *International Journal of Molecular Sciences*, 22:13020.
- ZHANG Y, LI Y Y, CHEN X M, *et al.* (2020) Combined Metabolome and Transcriptome Analyses Reveal the Effects of Mycorrhizal Fungus *Ceratobasidium* sp. AR2 on the Flavonoid Accumulation in *Anoectochilus roxburghii* during Different Growth Stages. *International Journal of Molecular Sciences*, 21:564.
- ZHANG Z, LONG Y, YIN X, *et al.* (2021) Sulfur-Induced Resistance against *Pseudomonas syringae* pv. *actinidiae* via Triggering Salicylic Acid Signaling Pathway in Kiwifruit. *Int J Mol Sci*, 22: 12710.
- ZHAO X M. (2019) The defence response of tea plants to green leafhopper infestation: a multi-omics study. Dissertation, Fujian Agriculture and Forestry University, China.
- ZHONG Q, HU H, FAN B, *et al.* (2021) Biosynthesis and Roles of Salicylic Acid in Balancing Stress Response and Growth in Plants. *Int J Mol Sci*, 22:11672.
- ZHOU H, ZHANG Y Q, LAI T, *et al.* (2019) Acaricidal Mechanism of Scopoletin Against *Tetranychus cinnabarinus*. *Front Physiol*, 10:164.

599 ZHU H, AI H, HU Z, *et al.* (2020) Comparative transcriptome combined with metabolome
600 analyses revealed key factors involved in nitric oxide (NO)-regulated cadmium stress
601 adaptation in tall fescue. BMC Genomics, 21: 601.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.xlsx](#)