Differential Analysis of Root Exudates from *Przewalskia tangutica* Induced by Methyl Jasmonate: Postprint

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Abstract

Przewalskia tangutica is an endangered alpine plant endemic to the Qinghai-Tibet Plateau, with tropane alkaloids as its primary active constituents. The roots, seeds, and whole herb of *P. tangutica* are used medicinally, holding significant pharmaceutical value. To explore the alterations in *P. tangutica* root exudates under methyl jasmonate induction, this study utilized LC-MS/MS untargeted metabolomics to analyze root exudates from plants treated with 0 and 150 mol·L-1 methyl jasmonate for 3 d and 7 d. The results demonstrated: (1) Significant differences in the content of *P. tangutica* root exudates following treatment with 0 and 150 mol·L-1 methyl jasmonate for 3 d and 7 d. (2) Compared with the blank control, the number of root exudates increased markedly after 150 mol·L-1 methyl jasmonate treatment. (3) The KEGG pathways predominantly involved in *P. tangutica* root exudates included the α-linolenic acid metabolism pathway, plant hormone signal transduction pathway, and lysine biosynthesis pathway. In conclusion, methyl jasmonate induction influenced the metabolism of *P. tangutica* roots and modified both the content and quantity of root exudates. Through untargeted metabolomics analysis, this study preliminarily identified key metabolites participating in the secretory response of *P. tangutica* roots to methyl jasmonate induction, providing a theoretical foundation for further elucidating the changes and metabolic mechanisms of root exudates in alpine plants under methyl jasmonate induction, and offering novel perspectives for the conservation of *P. tangutica* resources.

Full Text

Differential Content and Analysis of Methyl Jasmonate-Induced Root Exudates in *Przewalskia tangutica*

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Abstract: *Przewalskia tangutica* is an endangered alpine plant endemic to the Qinghai-Tibet Plateau, with tropane alkaloids as its main active ingredients. The roots, seeds, and whole herb of *P. tangutica* have important medicinal value. To investigate changes in root exudates of *P. tangutica* under methyl jasmonate induction, this study employed LC-MS/MS non-targeted metabolomics to analyze root exudates following treatment with 0 and 150 $\text{mol} \cdot \text{L}^{-1}$ methyl jasmonate for 3 and 7 days. The results showed: (1) Significant differences in root exudate content were observed after treatment with 0 and 150 $\text{mol} \cdot \text{L}^{-1}$ methyl jasmonate for 3 and 7 days. (2) Compared with the control, the number of root exudates increased significantly after 150 $\text{mol} \cdot \text{L}^{-1}$ methyl jasmonate treatment. (3) The main KEGG pathways involved in *P. tangutica* root exudates were α-linolenic acid metabolism, plant hormone signal transduction, and lysine biosynthesis. In conclusion, methyl jasmonate induction affected root metabolism and altered both the content and quantity of root exudates in *P. tangutica*. Through non-targeted metabolomics analysis, this study preliminarily revealed key metabolites involved in the root exudate response to methyl jasmonate induction, providing a theoretical basis for further understanding changes in root exudates and metabolic mechanisms in alpine plants under methyl jasmonate treatment, and offering new insights for the conservation of *P. tangutica* resources.

Keywords: *Przewalskia tangutica*, root exudates, methyl jasmonate, non-targeted metabolomics, metabolic pathway

The Qinghai-Tibet Plateau is the highest plateau in the world, known as the "Roof of the World" (Zhang et al., 2014). In recent decades, increased extinction rates of endangered plants on the plateau have resulted from population decline, increased pests and diseases, habitat degradation, and rising soil heavy metal content (Zhong et al., 2019; Yang et al., 2024). Research has shown that low temperature, drought, and heavy metals on the Qinghai-Tibet Plateau inhibit root growth in alpine plants and reduce root exudate synthesis (Liang et al., 2024; Ran et al., 2024). For example, drought stress inhibits root activity in *Caragana sinica*, decreasing root exudate content (Qiu et al., 2013). Ba et al. (2024) found that arsenic downregulated the exudation capacity of high-

land barley (*Hordeum vulgare* var. *coeleste*) roots, negatively affecting seedling growth. Additionally, alpine plants are vulnerable to biotic stresses such as herbivory, pests, parasites, and microbial pathogens, which cause instability in root exudate composition (Xie et al., 2023; Tang et al., 2024). Chen et al. (2016) reported that heavy grazing reduced root exudation rates in *Elymus nutans*, affecting plant growth. Thus, root exudates influence plant growth by responding to environmental changes.

Due to their complex composition, difficulty in collection, and susceptibility to biotic and abiotic interference, accurate identification of effective root exudate components remains challenging (Ahlawat et al., 2024). Researchers have found that collecting root exudates using hydroponic culture reduces the impact of biological and extreme environmental factors (Paterson et al., 2005), overcoming soil collection difficulties and facilitating component identification while increasing resource availability (Wang et al., 2010; Baetz & Martinoia et al., 2023). Li et al. (2024) used hydroponic collection to analyze organic acids in root exudates of three alpine meadow plants, clarifying their content and quantity. Therefore, solution-based collection methods provide better control of sterile conditions and enable clear identification of root exudate components (Ma et al., 2022; Li et al., 2023).

Methyl jasmonate, a natural plant hormone, is widely used in agricultural production and plant protection due to its environmental friendliness, non-toxicity, and broad-spectrum effects (Ramachandra & Ravishankar, 2002; Chen et al., 2023). It functions as an inducer in plant cells, participating in hormonal regulation, stimulating secondary metabolite biosynthesis, and enhancing plant defense, making it an effective elicitor for plant resource protection (Yu, 2019; Vaezi et al., 2022; Faroza et al., 2023). Wang et al. (2024) found that methyl jasmonate concentration and treatment time have critical thresholds, showing positive effects within these limits but diminishing effects beyond them. Bing and Pan (1998) reported that 100 $\text{mol} \cdot \text{L}^{-1}$ methyl jasmonate inhibited amylase activity in peanut (*Arachis hypogaea*). Zhang (2017) found that methyl jasmonate treatment for 7 days significantly increased metabolite content in *Atropa belladonna* compared to 14- and 28-day treatments. Wang et al. (2022) observed that 150 $\text{mol} \cdot \text{L}^{-1}$ methyl jasmonate significantly increased soluble sugar content in sweet potato (*Dioscorea esculenta*), while 0 and 225 $\text{mol} \cdot \text{L}^{-1}$ treatments decreased it. Yang (2019) found that soluble sugar content was highest after 3 days of methyl jasmonate treatment compared to other durations. Rasi et al. (2024) reported that 150 $\text{mol} \cdot \text{L}^{-1}$ methyl jasmonate increased scopolamine and atropine production in *Datura stramonium* roots, while 300 $\text{mol} \cdot \text{L}^{-1}$ had adverse effects. Thus, appropriate concentrations and treatment times can alter plant compound accumulation, reduce production costs for medicinal plants, and help address natural resource shortages (Wen et al., 2023). While many studies have reported on plant physiology under methyl jasmonate induction, research on the molecular mechanisms affecting root exudates remains scarce despite their fundamental role in root function. Therefore, understanding changes in effective root exudate components under methyl jasmonate treatment

is crucial for plant resource protection.

Przewalskia tangutica, a Solanaceae species representative of alpine gravel beach ecosystems, is a traditional Tibetan medicine first documented in the classic *Four Medical Tantras*. It is mainly distributed in Gansu, Qinghai, Xinjiang, Sichuan, Tibet, and the Altyn Tagh sandy areas, typically growing in alpine gravel and arid grasslands at 3,200–5,000 m elevation on the Qinghai-Tibet Plateau. Its main active components are tropane alkaloids with analgesic, antispasmodic, and anti-inflammatory effects, used clinically to treat spasmodic pain, sores, tumors, and skin diseases (Gong & Feng, 2019). As tropane alkaloids are exclusively obtained from natural plants, *P. tangutica* has been over-harvested for its high alkaloid content and was listed as a second-class endangered Tibetan medicinal plant in 2009 (Wu, 2023). Due to its narrow distribution, small wild populations, harsh habitat conditions, weak regeneration capacity, and dwindling resources, it is now being considered for inclusion as a first-class endangered Tibetan medicine (Wang & Xie, 2004; Wu, 2023). Research on *P. tangutica* has focused on tissue culture, chemical composition, and pharmacological activity, with virtually no studies on its root exudates. Given the scarcity of *P. tangutica* resources, investigating root exudate components and key secretory changes under methyl jasmonate induction is essential for resource conservation. This study examined sterile hydroponic *P. tangutica* roots using non-targeted LC-MS/MS metabolomics to analyze changes in root exudates after 0 and 150 $\text{mol} \cdot \text{L}^{-1}$ methyl jasmonate treatment for 3 and 7 days, addressing: (1) the functions of main components in *P. tangutica* root exudates, and (2) the effects of methyl jasmonate on root exudate metabolic mechanisms.

1.1 Experimental Materials

Przewalskia tangutica plants were collected in 2023 from Maqin County, Guoluo Prefecture, Qinghai Province (98°00 –100°56 E, 33°43 –35°16 N, average elevation 4,100 m). Plump, uniform-sized seeds with intact morphological characteristics and free from pests and diseases were selected from *P. tangutica* capsules and stored at 4°C for later use (specimen number: X23051136) at the Northwest Institute of Plateau Biology.

1.2 Sterile Seedling Culture of *P. tangutica*

Following the method of Lei et al. (2015), seeds were soaked in 250 $\text{mg} \cdot \text{L}^{-1}$ gibberellin for 24 h, then sterilized with 75% ethanol and 2.5% sodium hypochlorite. They were spot-inoculated in tissue culture bottles containing 100 mL of MS solid medium, with 6 seeds per bottle, totaling 720 seedlings (cultured in darkness for 14 days at 25°C, then under 3,000 lx light for 4 months at 25°C with 24 h illumination).

1.3 Root Exudate Collection and Processing

Four-month-old sterile *P. tangutica* seedlings were transferred to 100 mL Hoagland solution, with 5 plants per bottle. Methyl jasmonate stock solution (10 L) was dissolved with 456 L anhydrous ethanol to prepare a 150 $mol \cdot L^{-1}$ treatment solution, which was applied to plants using a 1 mL sterile syringe twice daily at 1-day intervals (4 sample groups, with 5 bottles per group and 6 replicates per sample group, totaling 180 plants per group). Collected root exudates were concentrated to approximately 1 mL using a rotary evaporator (45°C, 66 $r \cdot min^{-1}$) and filtered through a 0.22 m aqueous membrane. A 100 L liquid sample was transferred to a 1.5 mL centrifuge tube, mixed with 400 L acetonitrile:methanol (1:1) extraction solution containing 0.02 $mg \cdot mL^{-1}$ L-2-chlorophenylalanine internal standard, vortexed for 30 s, and ultrasonically extracted at low temperature for 30 min (5°C, 40 kHz). Samples were then held at -20°C for 30 min, centrifuged at 13,000 g for 15 min at 4°C, and the supernatant was collected. The supernatant was dried with nitrogen gas and redissolved in 100 L acetonitrile:water (1:1) reconstitution solution, ultrasonically extracted at low temperature for 5 min (5°C, 40 kHz), centrifuged at 4°C, and the final supernatant was filtered through a 0.22 m membrane and transferred to sample vials for analysis.

1.4 LC-MS/MS Analysis

Chromatographic conditions: HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm); mobile phase A: 95% water + 5% acetonitrile (0.1% formic acid); mobile phase B: 47.5% acetonitrile + 47.5% isopropanol + 5% water (0.1% formic acid); flow rate: 0.40 $mL \cdot min^{-1}$; column temperature: 40°C.

Mass spectrometry conditions: Mass scan range: 70–1,050 $m \cdot z^{-1}$; sheath gas flow rate: 50 $L \cdot h^{-1}$; auxiliary gas flow rate: 13 $L \cdot h^{-1}$; auxiliary gas heater temperature: 425°C; positive mode spray voltage: 3,500 V; negative mode spray voltage: -3,500 V; ion transfer tube temperature: 325°C; normalized collision energy: 20 V–40 V–60 V; primary resolution: 60,000; secondary resolution: 7,500; data acquisition in DDA mode.

1.5 Metabolite Identification

After instrumental analysis, raw LC-MS data were imported into the metabolomics processing software Progenesis QI (Waters Corporation, Milford, USA) for baseline filtering, peak identification, integration, retention time correction, and peak alignment, yielding a data matrix of retention time, mass-to-charge ratio, and peak intensity. MS and MS/MS spectral information were matched against public metabolite databases including HMDB (http://www.hmdb.ca/) and Metlin (https://metlin.scripps.edu/), as well as a self-built database from Shanghai Majorbio Bio-Pharm Technology Co., Ltd., to obtain metabolite information.

1.6 Data Analysis

Analyses were performed on the Majorbio Cloud Platform (cloud.majorbio.com). The ropls package (Version 1.6.2) in R was used for principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA), with 7-fold cross-validation to assess model stability. Significantly differential metabolites were selected based on variable importance in projection (VIP) values from the PLS-DA model and Student's t-test P-values. Differential metabolites were annotated to metabolic pathways through the KEGG database (https://www.kegg.jp/kegg/pathway.html). Pathway enrichment analysis was conducted using the Python package scipy.stats, with Fisher's exact test used to identify biological pathways most relevant to the experimental treatments.

2.1 Identification of *P. tangutica* Root Exudates

Differential root exudate metabolites from various treatments were annotated across 11 categories based on their characteristics, showing high overall diversity (Table 1). Lipids and organic acid compounds showed identical counts after 0 $\mathrm{mol \cdot L^{-1}}$ methyl jasmonate treatment for 3 days, decreasing to 213 and 211 types (22.30% and 22.09%, respectively) after 7 days. In contrast, organic oxygen compounds and benzenoids increased over time, while alkaloids, organic oxygen compounds, heterocycles, and phenylpropanoids showed minimal change. After 150 $\mathrm{mol \cdot L^{-1}}$ methyl jasmonate treatment for 3 days, lipids and organic acids numbered 201 and 249 types (20.16% and 24.97%, respectively), changing to 243 and 211 types (23.91% and 20.77%) after 7 days. Lipids, phenylpropanoids, polyketides, and benzenoids increased with treatment duration, while organic acids and derivatives, alkaloids, heterocycles, nucleosides, and nucleotides decreased; organic nitrogen compounds remained unchanged. These results indicate that methyl jasmonate concentration and treatment time significantly impact metabolite accumulation.

2.2 PCA Analysis of Metabolomic Data

Principal component analysis is a multivariate method for revealing inter-group differences, summarizing data variance, and measuring sample variability (Marukatat, 2023). PCA was performed on root exudates from *P. tangutica* treated with 150 $\mathrm{mol \cdot L^{-1}}$ methyl jasmonate for 3 and 7 days (EG3, EG7) and 0 $\mathrm{mol \cdot L^{-1}}$ methyl jasmonate for 3 and 7 days (CK3, CK7) (Figure 1 [Figure 1: see original paper]). QC sample clustering indicated stable and reliable results, with six samples within each group clustering together, demonstrating high similarity of secondary metabolites within groups. Conversely, the four differently treated samples were distributed in distinct regions defined by PC1 and PC2, indicating significant differences in metabolite types or relative content among treatments.

2.3 Analysis of Overall Differential Metabolites

To understand differences in root exudate metabolites under various treatments, comparative analysis was performed among the four sample groups EG3, EG7, CK3, and CK7 (VIP>1, P<0.05). As shown in Figure 2 [Figure 2: see original paper], the EG3 vs CK3 comparison revealed 468 upregulated and 333 downregulated root exudates; EG7 vs CK7 showed 356 upregulated and 311 downregulated; and EG7 vs EG3 exhibited 293 upregulated and 391 downregulated. These results demonstrate that methyl jasmonate both promotes and inhibits root exudate synthesis in *P. tangutica*, though more metabolites were promoted than inhibited compared to the control.

2.4 Clustering Analysis of Differential Metabolites

To identify differential components in *P. tangutica* root exudates under different treatments, hierarchical clustering trees were constructed for differentially abundant metabolites, revealing which metabolites changed at each experimental stage. As shown in Figure 3 [Figure 3: see original paper], clustering of the top 20 metabolites in root exudates is presented with a metabolite dendrogram on the left, metabolite names on the right, a sample dendrogram at the top, and sample names below. Closer branches indicate more similar content, with different colors representing relative metabolite abundance. Compared with control samples, six metabolites showed higher content after 150 $\text{mol} \cdot \text{L}^{-1}$ methyl jasmonate treatment for 3 days, including sucrose, trehalose, and inulin disaccharide; eight metabolites were more abundant after 7 days, including cimifugin, digitoxin, and scopolamine. This indicates that metabolic functions of differential metabolites were more active after 150 $\text{mol} \cdot \text{L}^{-1}$ methyl jasmonate treatment for 7 days.

2.5 Identification and Quantification of Key Alkaloid Features

To investigate alkaloid differences in *P. tangutica* root exudates under various treatments, differential analysis was performed on alkaloids collected from the four sample groups EG3, EG7, CK3, and CK7. Based on VIP>1 combined with primary and secondary mass spectral information compared with actual samples, standard compounds were used for qualitative identification and confirmation of characteristic components, screening for differential metabolites (Zhao et al., 2024). This study focused on metabolites playing important classification roles in the OPLS-DA model, screening according to VIP values and their influence intensity and explanatory power among sample groups. A total of 13 characteristic alkaloid components were selected for analysis (Table 2). Among these, tropane alkaloids were the most abundant, accounting for 38.46% of the total. Calystegine B2 and scopolamine showed the highest VIP values at 1.98 and 1.81, respectively. Scopolamine content increased with methyl jasmonate concentration and treatment time, while calystegine B2 content decreased.

2.6 KEGG Pathway Analysis of *P. tangutica* Root Exudates

Metabolic pathway analysis mapped identified root exudates onto KEGG pathways using a topological method (relative-betweenness centrality). As shown in Table 3 , plant hormone signal transduction, lysine biosynthesis, and α-linolenic acid metabolism were significantly enriched pathways in *P. tangutica* root exudates.

The plant hormone signal transduction pathway involved seven metabolites, with four upregulated metabolites in both EG3 vs CK3 and EG7 vs EG3 comparisons, and five metabolites (three upregulated, two downregulated) in EG7 vs CK7. The lysine biosynthesis pathway contained 13 annotated metabolites: nine metabolites (five upregulated, four downregulated) in EG3 vs CK3; nine metabolites (eight upregulated, one downregulated) in EG7 vs CK7; and five metabolites (four upregulated, one downregulated) in EG7 vs EG3. The α-linolenic acid metabolism pathway involved 12 metabolites: five metabolites (four upregulated, one downregulated) in EG3 vs CK3; eight metabolites (six upregulated, two downregulated) in EG7 vs CK7; and six upregulated metabolites in EG7 vs EG3. These findings indicate that 150 $\mathrm{mol \cdot L^{-1}}$ methyl jasmonate treatment for 3 and 7 days altered pathway-involved metabolites, with significantly increased participation after 7 days.

Tropane alkaloids, as effective components in Solanaceae plants, provide pharmacological benefits and pest control by inhibiting insect growth and development (Wu, 2021). Yang et al. (2018) found that methyl jasmonate induced high expression levels of TR1 and H6H genes in *Atropa belladonna* roots, increasing scopolamine and hyoscyamine content. This study similarly showed accumulation of hyoscyamine and scopolamine in *P. tangutica* root exudates after 150 $\mathrm{mol \cdot L^{-1}}$ methyl jasmonate induction for 7 days, suggesting that this treatment can regulate TR1 and H6H gene expression, promote exudate accumulation, and enhance self-defense. Zeng et al. (2024) discovered that calystegine is synthesized via tigloyl pseudotropine synthase, inhibiting glucosidase activity and showing good hypoglycemic effects. Carbohydrate compounds, as effective components in *P. tangutica* root exudates, can be transported extracellularly via SWEET proteins to maintain cellular osmotic balance and facilitate plant growth (Hu et al., 2017). In this study, calystegine B2 content decreased with increasing methyl jasmonate concentration and treatment time, while carbohydrate content peaked after 150 $\mathrm{mol \cdot L^{-1}}$ methyl jasmonate treatment for 3 days and remained elevated at 7 days. This suggests that methyl jasmonate reduces calystegine B2 content, alleviating inhibition of carbohydrate synthesis to maintain *P. tangutica* growth and development.

Furthermore, after treatment with 0 and 150 $\mathrm{mol \cdot L^{-1}}$ methyl jasmonate for 3 and 7 days, *P. tangutica* root exudates contained organic acids, alcohols, amines, and phenols—similar to the diverse compounds identified in soybean (*Glycine max*) seedling root exudates by Ma et al. (2011) and consistent with reported root exudate components in domestic literature (Zhang et al., 2014; Cai & Yu,

2022; Xia et al., 2024). However, Cao and Ou (2008) found that salicylic acid treatment reduced carbohydrate content in *Ginkgo biloba* seedlings, while Bao et al. (2020) reported that methyl jasmonate more effectively alleviated salt stress and increased amino acid content in roots compared to salicylic acid. This study showed increased carbohydrate and amino acid content after methyl jasmonate induction, indicating positive effects on *P. tangutica* root metabolites.

The α-linolenic acid metabolism and plant hormone signal transduction pathways are crucial physiological functions in plant responses to abiotic stress, primarily involving lipid compounds (Guimaraes & Venancio et al., 2022; Zhang et al., 2024). In the α-linolenic acid metabolism pathway, linolenic acid is the direct substrate for jasmonic acid synthesis (Tang et al., 2024). Methyl jasmonate application can increase lipoxygenase activity to promote linolenic acid synthesis (Gui et al., 2005). 12-OPDA, an unsaturated fatty acid, is a jasmonic acid synthesis intermediate (Yuho et al., 2024). Jiang et al. (2014) found that ACOX and MFP2 genes regulate 12-OPDA synthesis during α-linolenic acid metabolism. Exogenous methyl jasmonate can regulate related gene expression to promote metabolite synthesis and enhance plant defense mechanisms (Benevenuto et al., 2019). Chen et al. (2022) reported that methyl jasmonate treatment upregulated PpCOI1, PpJAZ, and PpMYC2 genes, promoting jasmonic acid accumulation and conferring cold tolerance. In the plant hormone signal transduction pathway, jasmonic acid-isoleucine (JA-Ile), regulated by the ABCG16 gene, activates jasmonic acid signaling and is a key mechanism for plants to regulate environmental stress and growth (Thurow et al., 2020; Lin et al., 2024). 3-Indoleacetic acid, a natural plant factor, participates in antioxidant regulation and promotes plant growth (Zhu, 2018). This study showed increased jasmonic acid content in both α-linolenic acid metabolism and plant hormone signal transduction pathways after 150 $\mathrm{mol \cdot L^{-1}}$ methyl jasmonate treatment for 7 days. Linolenic acid content increased after 3 days, 12-OPDA content increased after 7 days, JA-Ile content increased after 3 days, and 3-indoleacetic acid content increased after 7 days. These results demonstrate that signal molecules in both pathways respond actively to methyl jasmonate induction, stimulating gene expression to promote metabolite synthesis and adapt to stress. The involvement of jasmonic acid in both pathways indicates close interaction and coordination to maintain metabolic balance (Zhang et al., 2022).

The lysine biosynthesis pathway primarily involves amino acids, which regulate physiological processes, balance cellular osmotic pressure, and promote plant growth and stress adaptation (Zeng et al., 2024; Wu et al., 2024). Research shows that 2-aminoadipic acid, an intermediate in lysine biosynthesis derived from saccharopine degradation, regulates cellular osmotic pressure, maintains cell water content, and improves drought resistance (Arruda et al., 2000; Zhang et al., 2022). Saccharopine plays a major regulatory role in plant lysine synthesis, degrading excess lysine to maintain stable lysine concentrations (Sun et al., 2013). Aspartic acid, a lysine biosynthesis precursor, can chelate heavy metals, enhance root activity, and promote endogenous hormone synthesis and secretion, thereby increasing plant biomass and yield (Yang et al., 2019; Zhao et

al., 2024). In this study, 2-aminoadipic acid content increased after 150 $mol \cdot L^{-1}$ methyl jasmonate treatment for 3 days, while saccharopine and aspartic acid content increased after 7 days, with 2-aminoadipic acid showing no change. This indicates that 150 $mol \cdot L^{-1}$ methyl jasmonate promotes amino acid synthesis in the lysine biosynthesis pathway, playing important roles in heavy metal mitigation, osmotic regulation, and plant growth.

This study collected *P. tangutica* root exudates after 0 and 150 $mol \cdot L^{-1}$ methyl jasmonate treatment for 3 and 7 days using hydroponic culture, identifying a total of 2,921 chemical components. The chemical composition and metabolic mechanisms of *P. tangutica* root exudates were preliminarily characterized, providing an important data foundation for future studies. The results demonstrated significant differences in root exudate components and content under different treatments, with maximum accumulation of carbohydrates and organic acids after 150 $mol \cdot L^{-1}$ methyl jasmonate treatment for 3 days, and significant promotion of alkaloid and lipid synthesis after 7 days. The number of metabolites after methyl jasmonate induction exceeded that of the control, indicating that methyl jasmonate induces chemical differences in root exudates. Furthermore, 150 $mol \cdot L^{-1}$ methyl jasmonate promoted root exudate synthesis and enhanced defense responses in *P. tangutica* by regulating upregulation of related gene expression, playing a positive role in resource conservation. Therefore, methyl jasmonate not only affects the chemical composition and content of root exudates but also regulates related gene expression. This study provides comprehensive baseline data for investigating *P. tangutica* root exudates, though further research is needed on the mechanisms of root metabolites under methyl jasmonate action to provide new perspectives on *P. tangutica* physiological activity and resource conservation.

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Note: Figure translations are in progress. See original paper for figures.