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Full Length Article

Integrated Analysis of Metabolic Profiling of Root Exudates Revealed Potential Allelochemicals from *Pseudostellaria heterophylla*

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Abstract

Numerous studies have shown that the rhizosphere allelochemicals closely related to the formation of replant problems in *Pseudostellaria heterophylla*. In this study, ultra-high performance liquid chromatography-quadrupole time-of-flight mass technology was employed to screen the specific compounds in rhizosphere soil induced the formation of replant disease. Simultaneously, the compounds in cultured seedling of *P. heterophylla* and corresponding medium were identified using the identical methods to obtain compounds that had not been transformed or degraded by soil microbes. As a result, 176 specific molecules were identified in the rhizosphere soil, 1,011 in the rhizosphere tissue culture medium and 1,447 in plant tissue cultures were obtained by matching to the Traditional Chinese Medicine database. Further analysis found that 21 potential allelochemicals might be directly secreted from *P. heterophylla* plants, which had not been transformed or degraded in theory. Of which, 13 compounds possessed the allelopathic characteristics based on structural analysis that have been identified in other plants. Simultaneously, eight compounds were only present in the rhizosphere tissue culture medium and plant tissue cultures also were found to possess the allelopathic properties. This study provides an important reference for further screening and identification of specific allelochemicals that related closely to the formation of replant problems in *P. heterophylla*. © 2022 Friends Science Publishers

Keywords: *Pseudostellaria heterophylla*; Replant problems; Allelochemicals; Secondary metabolites; UHPLC-QTOF-MS

Introduction

Pseudostellaria heterophylla from the family of *Caryophyllaceae*, a famous genuine herb in China has been historically used as herbal medicine (Surhone *et al*. 2010; Wu *et al*. 2019a). However, *P. heterophylla* were severely affected by replant problems, which also known as the consecutive monoculture problems in production. Consecutive monoculture practice usually resulted in *P. heterophylla* plants with lower root weight and fewer numbers of roots, leading to sharp reducing of yield and medicinal value (Feng *et al*. 2010). Therefore, methods of alleviating and reducing replant problems in *P. heterophylla* have become an important research topic.

Previous studies have shown that replant problems affecting the production of traditional Chinese medicine on herbs involve obstruction of nutrient absorption, changes in soil physiochemical properties, the structural imbalance in rhizosphere microbial community and allelopathy in plants (Einhellig 1996; Lin *et al*. 2011; Li *et al*. 2012; Chen *et al*. 2021). Recent advances have found that the allelotoxic substances in rhizosphere soil are one of the critical factors triggered the generation of replant problems (Marschner and Timonen 2005; Mccully 2007). Identifying and screening of specific allelotoxic substances in plants have thus become the necessary works for deeply revealing the formation mechanism of replant problems.

Secondary metabolites from plants are important sources of plant allelochemicals (Lovett and Hoult 1998). Currently, the main method for identification of allelochemicals is gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (HPLC-MS) (Fu *et al*. 2017). In *Tagetes patula* L, there were 25 and 27 potential allelochemicals were confirmed in roots and rhizosphere soil extracts by GC-MS, respectively (Kumar *et al*. 2017). Although GC-MS technology was widely used in plant metabolomics, it could only analyze about 20% of organic matter that was thermally stable or gasified, making its application in the separation and analysis of allelochemicals greatly limited (Kong and Xu 2003). Recently, multi-stage combined techniques could widely identify complex metabolic processes in plants and

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intermediate products, thereby providing an important analytical platform for global metabolomics studies (Sangwan *et al*. 2015). Using high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) and other high-throughput metabolomics methods, a large number of plant-specific allelochemicals were identified from plants. The allelopathic potential of plant residue and root exudates of *S. oleraceus* on flavonoid composition and nodulation on two legumes were studied by HPLC-MS/MS (Gomaa *et al*. 2015). Tandem quadrupole-time-of-flight mass spectrometry (QTOF-MS) and ultrahigh-performance liquid chromatography (UHPLC) is of great advantage and is an important tool for the analysis and identification of the chemical composition of complex samples (Nguyen *et al*. 2006; Eugster *et al*. 2011).

In previous studies, the imbalance in rhizosphere microorganisms induced by allelochemicals has found relate to replant disease formation in *P. heterophylla*. However, until now potential allelochemicals (group) in *P. heterophylla* have still not been clearly identified. To further understand the induction and formation mechanisms for replant problems in *P. heterophylla*, in this study, UHPLC-QTOF-MS were used for a detailed analysis of differences in the unplanted control soil and the rhizosphere soil of *P. heterophylla*. Simultaneously, a detailed detection of the secondary metabolite spectrum of plant tissue cultures and tissue culture rhizosphere in *P. heterophylla* without interference from environmental factors was carried out. The combination of the two sets of experiments was used to provide a detailed understanding of the release and accumulation process of secondary metabolites in the rhizosphere by *P. heterophylla*. Further, the metabolite groups that may be intimately associated with replant problems in *P. heterophylla* were identified. This study provides a foundational dataset for screening and examination of allelochemicals that probably cause replant problems of *P. heterophylla*.

Materials and Methods

Materials

The rhizosphere soil of the species "Zherong No. 2" of *P. heterophylla* (RS) was collected in Zherong County, Ningde City, Fujian Province (119.9288E, 27.1418N) in July 2016. The *P. heterophylla* was planted from November 2015 to July 2016 (The material was identified as *Pseudostellaria* of *Caryophyllaceae* by Linkun Wu, College of Life Sciences, Fujian Agriculture and Forestry University). Sampling method: there was removed 0~5 cm deep surface soil and then removed root soil by shaking the root method. The rhizosphere soil close to the root of *P. heterophylla* was collected and the obtained rhizosphere soil was mixed thoroughly. In addition, the adjacent unplanted control soil sample (UCS) was collected as a control. The samples were randomly collected from 6 points as independent replicates.

The species "Zherong No. 2" of *P. heterophylla* was cultivated using plant tissue cultures method in MS medium for 65 days at 22°C under 3000 Lux light intensity for the collection of plant tissue cultures (PTC) and rhizosphere tissue culture medium (RTCM) (Fig. 1**)**.

Extraction of compounds in the UCS and RS

The UCS and RS were naturally air-dried and filtered through 100 mesh sieve. A 30 g soil samples in sextuplicate were weighed and extracted in 150 mL of methanol, ethyl acetate and *n*-hexane using a shaker at 120 r/min for 24 h, respectively and filtered. The filtered liquid supernatant was evaporated to 10 mL using a rotary evaporator under reduced pressure at 40°C. Subsequently, the extractions in methanol, ethyl acetate and *n*-hexane were mixed and dried under nitrogen gas and then redissolved in 10 mL solution of acetonitrile: water (1: 1 ratio). The supernatant was filtered through 0.22 μm nylon filters and stored under 20°C. When injecting, taking 2 μL from each sample and pooling as QC samples, and then taking 2 μL supernatant for the UHPLC-QTOF-MS analysis. The extraction method of two soil samples was the same as described above.

Extraction of RTCM and PTC

The RTCM and PTC were completely separated. The PTC were then ground with plant tissue grinder (Shanghai Jingxin, Tissuelyser-24). Five g of PTC and 20 g of RTCM were weighted and extracted in 150 mL methanol, ethyl acetate and *n*-hexane using the same method for soil extraction.

UHPLC-QTOF-MS analysis

The compounds in extractions were separated using a Waters UHPLC BEH Amide column (1.7 μm, 2.1 × 100 mm) under Agilent 1290 Ultra High-Performance Liquid System. Water (containing 25 m*M* ammonium acetate and 25 m*M* ammonia) (A) and acetonitrile (B) were used as mobile phase following the optimal elution procedure: 0 ~ 1 min, 15% A; 12 ~ 12.1 min, 35 ~ 60% A； 15 ~ 15.1 min, 60 ~ 15% A； 20 min, 15% A. Mass spectral data were recorded on AB Sciex Triple TOF 6600 high-resolution Mass Spectrometer equipped with an ESI source operating in negative ion mode. Bombardment energy: 35 eV, 15 secondary spectra every 50ms. The ESI ion source parameter was set to as following: atomization pressure (GS1): 60 Pa, auxiliary pressure: 60 Pa, air curtain pressure: 30 Pa, temperature: 550°C, spray voltage: -4500 V. 2 μL extractions were injected for UHPLC-QTOF-MS analysis.

Data processing

The statistical analyses were performed using SIMCA software (V14.0, MKS Data Analytics Solutions, Umea, Sweden). Unsupervised principal component analysis

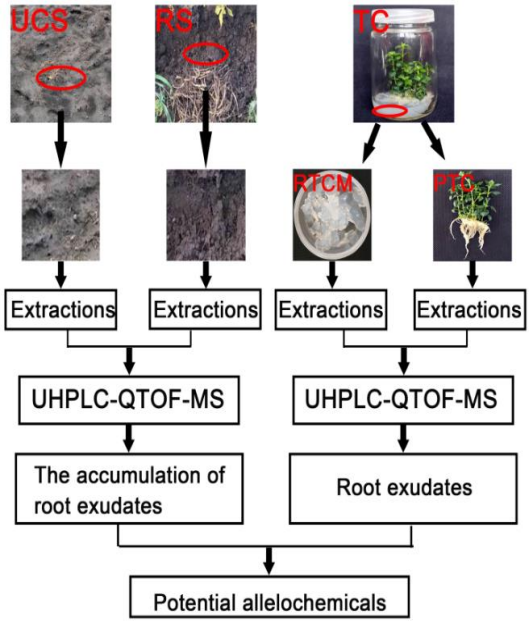

Fig. 1: Flow chart of experimental design. UCS (unplanted control soil); RS (rhizosphere soil of *P. heterophylla*). TC (tissue culture). RTCM (rhizosphere tissue culture medium). PTC (plant tissue cultures)

(PCA), partial least squares discriminant analysis (PLS-DA) and supervised (orthogonal) partial least squares (OPLS-DA) were used to observe the overall distribution of samples and the stability of the whole analysis process, to distinguish the overall differences of metabolic profiles among groups and to identify the different metabolites among groups. In OPLS-DA analysis, variables with VIP greater than 1 were considered as differential variables. The identification of compounds of interest was conducted by searching against the Traditional Chinese Medicine database (TCM, AB SCIEX).

Results

Identification of critical metabolites in rhizosphere of *P. heterophylla* in field conditions

To develop a preliminary understanding of the set of compounds in the rhizosphere of *P. heterophylla*, the molecules in the RS and UCS were profiled and totally 878 entities were obtained. By searching against TCM databases, 636 compounds were identified of which 176 compounds which were significantly accumulate in the RS (set A) while some compounds that were decreased. Through classification analysis of the set A, we found that these compounds are mainly long-chain fatty acids, organic acids, terpenoids and steroids while decreased compounds are mainly amino acids and their derivatives, alkaloids and sugars (Fig. 2a, b and c). The set A probably secreted by the root system or products of microbial action on root exudates, while the decrease of the compounds probably resulted by the utilization of plant.

Identification of critical metabolites released from *P. heterophylla* under tissue culture conditions

The compounds in the RTCM (set B) and PTC (set C) were analyzed. 1,011 and 1,447 compounds were identified in the set B and C by searching against TCM databases, respectively. Of which, 177 compounds were simultaneously identified in the set B and C. Classification analysis showed that these common substances were mainly organic acids, long-chain fatty acids, amino acids and their derivatives, which are probably released into the tissue culture medium during plant growth (Fig. 3a, b and c).

Integrated analysis of rhizosphere exudates in field and tissue condition reveals candidate allelochemicals set of *P. heterophylla*

Venn diagram was used to analyze the relationship between the release and accumulation of secretions (Fig. 4a, b and c) and 21 compounds were detected both in the set A, B and C. These substances are mainly organic acids, long-chain fatty acids and alkaloids. Moreover, there were 31 compounds were detected both in the set A and set B. These compounds are mainly organic acids, long-chain fatty acids and flavonoids. Furthermore, 153 compounds were detected both in the set B and set C. Most of these substances are organic acids, long-chain fatty acids, amino acids and their derivatives. These results showed that the compounds that were secreted from *P. heterophylla* plants into the rhizosphere are main esters, long-chain fatty acids, organic acids, terpenoids and alkaloids.

Previous reports and classifies of allelochemicals were compared with these results to determine the potential allelochemicals secreted by *P. heterophylla* into the rhizosphere. Twenty one compounds indicated the potential allelopathic characteristics based on structural analysis, while 13 compounds such as pyruvaldehyde, succinic acid and benzoic acid were simultaneously present in the set A, set B and set C. A total of 8 compounds such as indole, vanillic acid and salicylic acid, were presented in both the set B and set C. Majority of these potential allelochemicals belong to benzoic acid and its derivatives, water-soluble organic acids, alkaloids, flavonoids and so on (Fig. 5).

Discussion

Currently, researchers believe that allelopathy is a main causes of replant problems and mediated rhizosphere microbial ecology imbalance by allelopathy (Wu *et al*. 2016; Zhang *et al*. 2021). Therefore, the isolation and identification of allelochemicals is the prerequisite for the accurate and comprehensive understanding of allelopathy and is the key to understanding the cause of replant problems. In order to exclude complex conditions in the field and accurately analyze allelochemicals from *P. heterophylla*, UHPLC-QTOF-MS were employed to combined analyze the

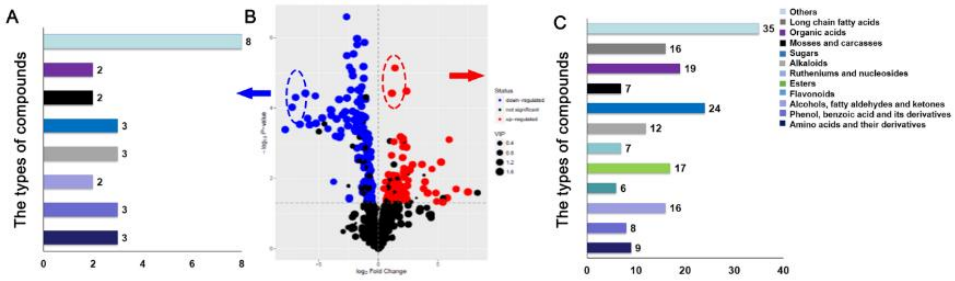

Fig. 2: Comparison and classification of compounds in the RS and UCS. (A) The types of compounds that are decreased in the rhizosphere soil after the planting of *P. heterophylla*. (B) Black: the non-significantly different compounds; Blue: the decreased compounds. Red: the significant accumulated compounds. (C) The types of compounds that are significant accumulated in the rhizosphere soil after the planting of *P. heterophylla*

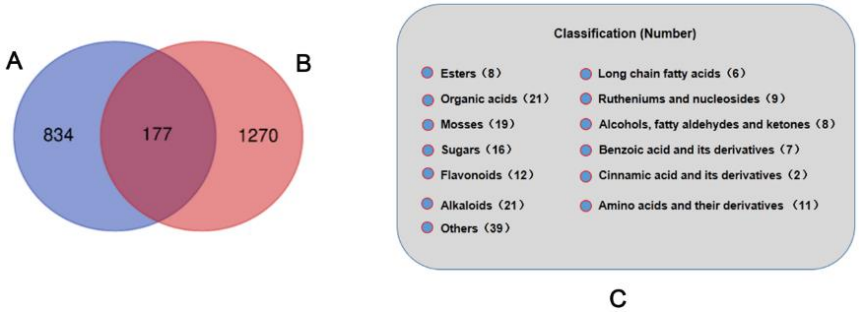

Fig. 3: Metabolic profiling of compounds in *P. heterophylla* plant tissue cultures and rhizosphere tissue. (A) A set of compounds in the rhizosphere tissue culture medium of *P. heterophylla* (set B). (B) A set of compounds in the plant tissue cultures of *P. heterophylla* (set C). (C) The types of common compounds

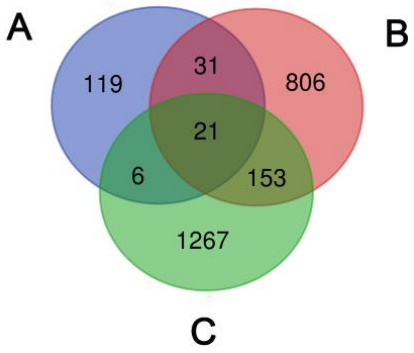

Fig. 4: Distribution analysis of compounds of interest in *P. heterophylla* tissue and rhizosphere. (A) A set of compounds that are significant accumulated in the rhizosphere soil after the planting of *P. heterophylla* (set A). (B) A set of compounds in the rhizosphere tissue culture medium of *P. heterophylla* (set B). (C) A set of compounds in the plant tissue cultures of *P. heterophylla* (set C)

compounds of the plant tissue culture (PTC), rhizosphere tissue culture medium (RTCM) and the significant accumulated compounds in the rhizosphere soil after the planting of *P. heterophylla* (set A). Compared to previous methods (Qin *et al*. 2009), our method precluded the effects of microorganisms and determined which compounds were significantly accumulated in rhizosphere soil and those directly originated from plant secretions and which ones had cumulative effects. Our study identified 21 potential allelochemicals that caused replant problems in *P. heterophylla*. Among these compounds, 13 compounds were simultaneously present in the PTC, RTCM and set A. These compounds may be secreted by the plants into the rhizosphere and are potential allelochemicals with cumulative effects on the soil. In addition, the eight compounds were present in the PTC and RTCM but not significantly accumulated in rhizosphere soil, which may be original compounds exuded by the plants. These compounds (groups) did not undergo metabolic conversion by the soil or microorganisms.

The potential allelochemicals found in this study can be divided into eight categories, which include water-soluble organic acids; long-chain fatty acids; simple phenols, benzoic acid and its derivatives; cinnamic acid and its derivatives; flavonoids; alkaloids and other compounds (Rice 1984). Currently, research on allelochemicals in replant problems in *P. heterophylla* have mainly focused on simple phenols and water-soluble organic acids (Zhao *et al*. 2015; Wu *et al*. 2017). No studies involving alkaloids and flavonoids in replant problems in *P. heterophylla* have been conducted to date, despite extensive investigations on other plants. Paszkowski and Kremer (1988) isolated six types of flavonoids from velvetleaf and found that these compounds

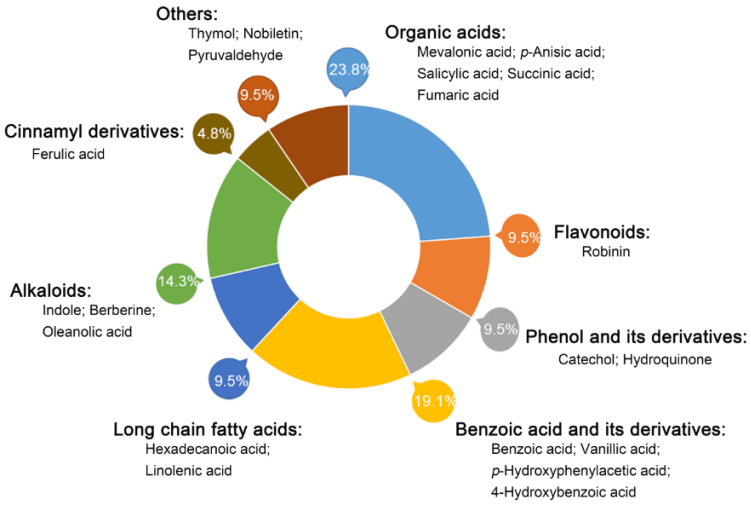

Fig. 5: Classification of candidate allelochemicals set in the rhizosphere of *P. heterophylla*

have significant inhibitory effects of the germination and radicle growth in all tested species using 1 m*M* concentrations. Ambika (2002) studied the inhibitory effects of *Chromolaena odorata* weed on crop growth and found phenols, alkaloids and amino acids were mainly responsible allelochemicals in this regard. Therefore, more attention should be paid to these compounds in future.

The potential allelochemicals that were found in this study such as succinic acid and vanillic acid have been proven to be allelochemicals in relevant studies on *P. heterophylla* replant problems (Zhao *et al.* 2015; Wu *et al.* 2017; Wu *et al.* 2019b). Although berberine, oleanolic acid and other compounds were not reported in related studies on replant problems in *P. heterophylla*, these compounds have been proven by other studies involving other plants to be allelochemicals. Dai *et al.* (2013) found that berberine shows concentration-dependent inhibitory effects on the growth of the *Microcystis aeruginosa* 905, and inhibition rates increase with higher berberine concentrations. Wang *et al.* (2016) isolated oleanolic acid from the leaf extraction of *Alstonia scholaris* and found that it could inhibited the activity of gram-negative bacteria. Rasmussen and Einhellig (1977) found that *p*-coumaric and ferulic acids synergistically inhibited the germination and growth of sorghum seeds, resulting in greater toxicity. Therefore, the compounds identified in this study may be classical as broad-spectrum allelochemicals.

The present study has identified 21 potential allelochemicals that may be utilized as a dataset for preliminary screening and examination of allelochemicals that cause replant problems involving *P. heterophylla*. However, whether this set of allelochemicals can cause replant problems in *P. heterophylla* remains unclear. Thus, further investigation is needed to determine if a single substance or coordination by multiple substances are needed to elicit their effects.

Conclusion

Our study primarily found that 21 potential allelochemicals might be directly secreted from *P. heterophylla* plants, which had not been transformed or degraded. Although the function of these allelochemicals needs to be verified by further experiments, our study provided data sets information for identifying allelochemicals that resulted in the replant problems of *P. heterophylla.* At the same time, this study also provokes for further elucidating the formation mechanism of replant problems of *P. heterophylla* and its rhizosphere ecological process.

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Author Contributions

ZYZ designed the experiments; JF, BZ, HYL and MJL executed the experiments and wrote the manuscript; JF, BZ, HYL and MJL analyzed the data; MJL, LG, FJF and JMW discussed the results.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

References

Ambika SR (2002). Allelopathic plants. 5. *Chromolaena odorata* (L) King and Robinson. *Allelopathy J* 9:35–41

Chen J, LT Zhou, IU Din, Y Arafat, Q Li, JY Wang, TT Wu, LK Wu, HM Wu, XJ Qin, GR Pokhrel, S Lin, WX Lin (2021). Antagonistic activity of *Trichoderma* spp. against *Fusarium oxysporum* in rhizosphere of *Radix pseudostellariae* triggers the expression of host defense genes and improves its growth under long-term monoculture system. *Front Microbiol* 12:422–434

Dai W, SL Zhang, YJ Lin, XD Bi, RJ Yan, KZ Xing (2013). Allelopathic effects of berberine, a plant alkaloid, on the algae, *Microcystis aeruginosa* (FACHB-905), at different initial densities. *Isr J Aquacult* 65:906–910

Einhellig FA (1996). Interactions involving allelopathy in cropping systems. *Agron J* 88:886–893

Eugster PJ, G Davy, R Serge, V Jean-Luc, C Pierre-Alain, W Jean-Luc (2011). Ultra high pressure liquid chromatography for crude plant extract profiling. *JAOAC Intl* 94:51–70

Feng YQ, PH Xia, J Long, Q Wu, J Wei (2010). Effects of continuous cropping on yield and quality of *Radix pseudostellariae*. *Guizhou Agric Sci* 38:61–63

Fu J, B Zhang, HY Liu, MJ Li, L Gu, J Li, FQ Wang, ZY Zhang (2017). A consideration on screening of allelochemicals by using "knock-out/in" of targeting ingredients based on identification model of medicinal effect. *Chin J Chin Mater Med* 42:805–808

Gomaa NH, MO Hassan, GM Fahmy, L González, O Hammouda, AM Atteya, NH Gomaa, MO Hassan, GM Fahmy, L González (2015). Flavonoid profiling and nodulation of some legumes in response to the allelopathic stress of *Sonchus oleraceus* L. *Acta Bot Bras* 29:553–560

Kong CH, XH Xu (2003). *Separation and structure identification of organic matter*. Chemical Industry Press, Beijing, China

Kumar A, KK Chahal, D Kataria (2017). Comparison of chemical composition of root and rhizosphere soil extracts of *Tagetes patula* L.: GC-MS Analysis. *Asian J Chem* 29:797–800

Li Z, S Wang, Q Tao, J Pan, L Si, Z Gong, R Cai (2012). A putative positive feedback regulation mechanism in CsACS2 expression suggests a modified model for sex determination in cucumber (*Cucumis sativus* L.). *J Exp Bot* 63:4475–4484

Lin RY, HB Wang, XK Guo, CY Ye, HB He, Y Zhou, WX Lin (2011). Impact of applied phenolic acids on the microbes, enzymes and available nutrients in paddy soils. *Allelopathy J* 28:225–236

Lovett JV, AHC Hoult (1998). *Allelopathy in Plants*. Springer, New York, USA

Marschner P, S Timonen (2005). Interactions between plant species and mycorrhizal colonization on the bacterial community composition in the rhizosphere. *Appl Soil Ecol* 28:23–36

Mccully M (2007). Rhizosphere allelopathy. *Allelopathy J* 19:75–84

Nguyen TT, D Guillarme, S Rudaz, JL Veuthey (2006). Chromatographic behaviour and comparison of column packed with sub-2 μm stationary phases in liquid chromatography. *J Chromatogr A* 1128:105–113

Paszkowski WL, RJ Kremer (1988). Biological activity and tentative identification of flavonoid components in velvetleaf (*Abutilon theophrasti Medik.*) seed coats. *J Chem Ecol* 14:1573–1582

Qin YM, LW Nie, YQ Huang, Q Wang, X Liu (2009). Detection of *Paeonia ostii* autotoxins and their mechanism. *Acta Ecol Sin* 29:1153–1161

Rasmussen JA, FA Einhellig (1977). Synergistic inhibitory effects of *p*-coumaric and ferulic acids on germination and growth of grain sorghum. *J Chem Ecol* 3:197–205

Rice EL (1984). *Allelopathy*. 2nd edn. Academic Press, New York, USA

Sangwan NS, P Tiwari, SK Mishra, RK Yadav, S Tripathi, AK Kushwaha, RS Sangwan (2015). Plant metabolomics: an overview of technology platforms for applications in metabolism. *Plantomics*: *Omics Plant Sci* 257–298

Surhone LM, MT Tennoe, SF Henssonow (2010). *Pseudostellaria Heterophylla*. Betascript Publishing, Beau Bassin, Mauritius

Wang CM, HT Chen, ZY Wu, YL Jhan, CL Shyu, CH Chou (2016). Antibacterial and synergistic activity of pentacyclic triterpenoids isolated from *Alstonia scholaris*. *Molecules* 21:139–149

Wu LK, J Chen, HM Wu, JY Wang, YH Wu, S Lin, MU Khan, ZY Zhang, WX Lin (2016). Effects of consecutive monoculture of *Pseudostellaria heterophylla* on soil fungal community as determined by pyrosequencing. *Sci Rep* 6:26601–26610

Wu HM, LK Wu, Q Zhu, JY Wang, XJ Qin, JH Xu, LF Kong, J Chen, S Lin, MU Khan, H Amjad, WX Lin (2017). The role of organic acids on microbial deterioration in the *Radix pseudostellariae* rhizosphere under continuous monoculture regimes. *Sci Rep* 7:3497–3509

Wu HM, XJ Qin, JY Wang, LK Wu, J Chen, JK Fan, L Zheng, HP Tangtai, Y Arafat, WW Lin, XM Luo, S Lin, WX Lin (2019a). Rhizosphere responses to environmental conditions in *Radix pseudostellariae* under continuous monoculture regimes. *Agric Ecosyst Environ* 270:19–31

Wu LK, B Yang, ML Li, J Chen, ZG Xiao, HM Wu, QY Tong, XM Luo, WX Lin (2019b). Modification of rhizosphere bacterial community structure and functional potentials to control *Pseudostellaria heterophylla* replant disease. *Plant Dis* 1:104–114

Zhang MD, YS He, WX Zhou, LQ Ai, HH Liu, L Chen, Y Xie (2021). Effects of continuous cropping of *Codonopsis tangshen* on rhizospheric soil bacterial community as determined by pyrosequencing. *Diversity* 13:317–332

Zhao Y, L Wu, L Chu, Y Yang, Z Li, S Azeem, Z Zhang, C Fang, W Lin (2015). Interaction of *Pseudostellaria heterophylla* with *Fusarium oxysporum* f.sp. *heterophylla* mediated by its root exudates in a consecutive monoculture system. *Sci Rep* 5:8197–8203