Physiological and transcriptomic analyses to reveal underlying phenolic acid action in consecutive monoculture problem of Polygonatum odoratum

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Abstract

Background: The root rot of fragrant solomonseal (Polygonatum odoratum) has occurred frequently in the traditional P. odoratum cultivating areas in recent years, causing a heavy loss in yield and quality. The phenolic acids in soil, which are the exudates from the P. odoratum root, act as allelochemicals that contribute to the consecutive monoculture problem (CMP) of the medicinal plant. The aim of this study was to get a better understanding of P. odoratum CMP.

Results: The phenolic acid contents, the nutrient chemical contents, and the enzyme activities related to the soil nutrient metabolism in the first cropping (FC) soil and continuous cropping (CC) soil were determined, and the differentially expressed genes (DEGs) related to the regulation of the phenolic acids in roots were analyzed. The results showed that five low-molecule-weight phenolic acids were detected both in the CC soil and FC soil, but the phenolic acid contents in the CC soil were significantly higher than those in the FC soil except vanillic acid. The contents of the available nitrogen, available phosphorus, and available potassium in the CC soil were significantly decreased, and the activities of urease and sucrase in the CC soil were significantly decreased. The genomic analysis showed that the phenolic acid anabolism in P. odoratum in the CC soil was promoted. These results indicated that the phenolic acids were accumulated in the CC soil, the nutrient condition in the CC soil deteriorated, and the nitrogen metabolism and sugar catabolism of the CC soil were lowered. Meantime, the anabolism of phenolic acids was increased in the CC plant.

Conclusions: The CC system promoted the phenolic acid anabolism in P. odoratum and made phenolic acids accumulate in the soil.

Keywords: Fragrant solomonseal, Root rot, Continuous cropping soil, First cropping soil, Low-molecule-weight phenolic acids, Genomic analysis
Background

Polygonatum odoratum (Mill.) Druce, popularly known as fragrant solomonseal, is a traditional Chinese perennial medicinal plant mainly cultivated in the southern parts of China and the other Southeast Asian countries such as Thailand and Vietnam. As a medicinal and edible plant, its rhizome has the functions of removing dryness, promoting secretion, and quenching thirst [1, 2], and it is widely welcomed especially in the Southeast Asian markets. However, the root rot of P. odoratum has occurred frequently in the planting areas in recent years due to the long-term continuous cropping (CC), causing the serious consecutive monoculture problem (CMP) with a sharp decline both in yield and quality. For example, in Shaodong County of Hunan Province, which was the largest traditional high-quality P. odoratum planting area in China, the medicinal peasants have almost given up the cultivation of P. odoratum due to this serious replant disease.

Researches have revealed that CMP is mainly caused by three factors covering the imbalance of soil nutrients, the shift in microorganisms towards pathogenic and allelopathic autotoxicity, and the root exudates of secondary metabolites, among which the root exudates are likely to be considered as the main factor to induce the root rot by influencing rhizosphere microbes and soil nutrients [3]. The root exudates include low-molecule-weight substances such as polysaccharides, vitamins, nucleotides, and phenolic acids. Among them, phenolic acids are most likely to be considered as the allelochemicals that can change membrane permeability, inhibit nutrient uptake, and inactivate plant endogenous hormones to influence the normal physiological process [4].

Phenolic acids are a kind of substance containing active organic acids on their aromatic rings that are mainly synthesized by the shikimic acid pathway which provides phenylpropanoids or synthesized by the polyketide pathway in plants which provides simple phenolic acids directly. Shikimic acid is synthesized from 4-phosphate erythritole via the phosphoenolpyruvic acid and pentose phosphate pathway, and then the aromatic amino acids are subsequently used as precursors for the synthesis of phenolic acids [5]. Phenolic acids in plants are products responding to environmental stresses, and they may be involved in the intracellular and intercellular signaling processes as signal transporting substances [6]. When plant roots are infected, the phenolic acid levels in the root cells are promoted to act as antioxidants and inhibit infectious microorganisms directly [7].

Although phenolic acids are of vital importance for the plant's interaction with the environment, few documents have been published on the relationship between the P. odoratum phenolic acids and the root rot disease. An early study showed that the low-molecule-weight substances extracted from the P. odoratum rhizosphere soil solutions exhibited an obvious self-poison effect on P. odoratum seedling growth and allelopathic effect on the rhizome spore germination rate [8]. Our recent studies have revealed that the Polygonatum CMP could be alleviated or eradicated under the rotation regimes or with an aeroponic system [9, 10], and the miRNAs-regulated genes participating in the sugar and phenylpropanoid metabolism have been enriched through the microRNA sequencing on the CC and the first cropping (FC) P. odoratum [11]. All these results prompted that the root rot in P. odoratum was likely related to the phenolic acid metabolism. Other researchers revealed that the root exudates including phenolic acids may not only involve in the signal transduction and hormone synthesis but also modify the rhizosphere soil environment to regulate the microbial community in the rhizosphere in some other medicinal plants such as Rehmannia glutinosa [5, 12]. Besides, recent researches have provided more information on the complex correlation among the root rot, phenolic acids, and CMP. Although phenolic acids in the CC soils stimulate the propagation of Fusarium which directly results in root rot, single phenolic acid also plays a role as a double-edged sword to decrease the Fusarium growth and production at a lower level, and the CC system can strengthen the phenolic acid accumulation in the rhizosphere soils of peanut, Panax notoginseng, and Chrysanthemum morifolium [13–15].

In this study, the low-molecule-weight phenolic acids from the rhizosphere soil of FC and CC P. odoratum were identified, and the relative chemical and physiological indices for evaluating the influences of the phenolic acids on the soil were also determined. Furthermore, the transcriptome profiles from the FC and CC rhizomes of P. odoratum were compared through high-throughput sequencing, and then the differentially expressed genes (DEGs) were analyzed. Then the pathway related to the phenolic acid metabolism was analyzed using the Kyoto Encyclopedia of Genes and Genomes (KEGG). This research might provide more information to understand the regulatory function of the phenolic acids in P. odoratum under the CC system.

Results

Rhizosphere soil phenolic acids

The rhizosphere soil phenolic acids were determined using the high-performance liquid chromatography (HPLC) method as compared to the standard samples of p-hydroxybenzoic acid, vanillic acid, syringic acid, cumaric acid, and ferulic acid with the retention time of 15.42, 19.29, 21.84, 27.16, and 28.94 min, respectively (Fig. S1A), and the above five phenolic acids were detected both in the FC and CC rhizosphere soil (Fig. S1B, Fig. S1C). $R^2$ of the five regression curves was 0.999 (Table 1). The good linearity indicated that the determination method was reliable. The phenolic acid
contents in the FC and CC rhizosphere soil were calculated according to the equations in Table 1. As shown in Table 2, p-hydroxybenzoic, syringic acid, cumaric acid, and ferulic acid contents in the CC soil were significantly higher than those in the FC soil, whereas vanillic acid in the CC and FC soil had no significant difference, although vanillic acid contents in the CC and FC soil exhibited much higher level than those of the other phenolic acids. The results showed that the phenolic acid contents in the CC soil were higher than those in the FC soil.

### Rhizosphere soil chemical properties and enzyme activities

The rhizosphere soil elements of the CC and FC system reflecting the soil nutritional status were determined in this experiment. As shown in Table 3, the contents of total phosphorus (TP), total potassium (TK), available nitrogen (AN), available phosphorus (AP), available potassium (AK) in the CC soil were significantly lower than those in the FC soil, whereas the total nitrogen (TN) contents between the CC soil and FC soil had no significant change. Although TN in the CC soil showed no significant difference as compared to that in the FC soil, AN, which is the form of nitrogen that can be absorbed directly by plants, decreased significantly, and the other nutrient chemicals also decreased significantly. These results insisted that the CC soil nutritional status had deteriorated.

The rhizosphere soil enzymes related to the soil nutrient metabolism level were also determined in this experiment. As shown in Table 4, the activities of polyphenol oxidase (PPO), catalase (CAT), and acid phosphatase (ACP) had no significant differences between the CC soil and FC soil, whereas the activities of urease (UE) and sucrase (SC) in the CC soil were significantly lower than those in the FC soil. The results indicated that the soil oxidative metabolism and phosphorus utilization had not been changed between the CC soil and FC soil, but the levels of the nitrogen metabolism and sugar catabolism of the CC soil were lowered as compared to the FC soil.

### RNA-seq analysis and qRT-PCR verification

After filtering out the low-quality sequences, 299,730,812 clean reads, which were accounted for 95.38% of the raw reads, were obtained from the samples of the CC and FC P. odoratum roots, and Q20 and Q30 values and GC contents were all in the reliable ranges (Table S1). Owing to the absence of the reference genome, the total clean reads were spliced to obtain 842,213 transcripts and 510,970 unigenes, and the N50 lengths of the transcripts and unigenes were 931 and 1167 bp, respectively, and 298,939 unigenes were annotated in the databases including NR, NT, KO, SwissProt, PFAM, GO, and KOG, whereas 176,156 unigenes were annotated in the GO database. Then a total number of 15,788 DEGs were screened out in the CC vs FC root tissues including 4843 up-regulated DEGs and 10,945 down-regulated DEGs (padj < 0.05).

These DEGs were significantly enriched in 307 GO functional items. The biological processes included “oxidation-reduction process”, “carbohydrate metabolic process”, “polysaccharide metabolic process”, etc.; the molecular functions included “catalytic activity”, “oxido-reductase activity”, “hydrolase activity”, etc.; the cell components included “cell wall”, “cell periphery”, “cytoskeletal part”, etc. (Fig. 1A). The up-regulated DEGs were mainly enriched in the metabolic process, the single-organism metabolic process, the catalytic activity, the oxidoreductase activity, etc. (Fig. 1B-D); the down-regulated DEGs were mainly enriched in the oxidation-reduction process, the carbohydrate metabolic process, the hydrolase activity (acting on glycosyl bonds), the

### Table 1 Phenolic acid standard samples Regression equation using external standard method

| Phenolic acid standard sample | Regression equation | R²   | Retention time (min) |
|------------------------------|---------------------|------|----------------------|
| p-hydroxybenzoic acid        | y = 19.394x + 0.5019 | 0.999| 15.42                |
| Vanillic acid                | y = 19.040x + 0.0441 | 0.999| 19.29                |
| Syringic acid                | y = 29.678x - 0.0005 | 0.999| 21.84                |
| Cumaric acid                | y = 42.687x + 0.3221 | 0.999| 27.16                |
| Ferulic acid                | y = 27.663x - 0.0114 | 0.999| 28.94                |

Note: * was marked for the significant difference within the same column with the T-Student's method (P < 0.05, n = 3). FC stands for first cropping, and CC stands for continuous cropping.
Table 3 Chemical properties in FC and CC rhizosphere soil

| Rhizosphere soil sample | TN (g/kg) | TP (g/kg) | TK (g/kg) | AN (mg/kg) | AP (g/kg) | AK (mg/kg) |
|-------------------------|-----------|-----------|-----------|------------|-----------|------------|
| FC                      | 1.953 ± 0.027 | 0.355 ± 0.015⁹ | 1.362 ± 0.009⁹ | 83.650 ± 3.031⁹ | 0.259 ± 0.027⁹ | 34.740 ± 1.119⁹ |
| CC                      | 2.038 ± 0.045 | 0.293 ± 0.015 | 1.177 ± 0.228 | 69.650 ± 3.031 | 0.226 ± 0.052 | 25.087 ± 1.132 |

Note: values were expressed as mean ± standard error, and a was marked for the significant difference within the same column with the T-Student’s method (P < 0.05, n = 3). FC stands for first cropping, and CC stands for continuous cropping. TN stands for total nitrogen, TP stands for total phosphorus, TK stands for total potassium, AN stands for available nitrogen, AP stands for available phosphorus, and AK stands for available potassium.

Table 4 Soil enzyme activities in FC and CC rhizosphere soil

| Rhizosphere soil sample | PPO (mg/d/g) | CAT (μmol/d/g) | UE (μg/d/g) | ACP (μmol/d/g) | SC (mg/d/g) |
|-------------------------|--------------|----------------|-------------|----------------|-------------|
| FC                      | 49.269 ± 0.438 | 17.867 ± 1.022 | 392.069 ± 32.173⁸ | 22.871 ± 0.830 | 34.800 ± 0.188⁹ |
| CC                      | 47.727 ± 0.688 | 17.284 ± 1.026 | 298.174 ± 14.843 | 22.004 ± 0.321 | 20.801 ± 0.177 |

Note: values were expressed as mean ± standard error, and a was marked for the significant difference within the same column with the T-Student’s method (P < 0.05, n = 3). FC stands for first cropping, and CC stands for continuous cropping. PPO stands for polyphenol oxidase, CAT stands for catalase, UE stands for urease, ACP stands for acid phosphatase, and SC stands for sucrase.

Discussion

The soil phenolic acids are usually released by plant leaching, root exudation, or decomposition of plant residues [16, 17]. For the low-molecule-weight phenolic acids, there are two groups including benzoic acid derivatives and cinnamic acid derivatives, which play an important role in plant growth and interactions with the environment. These molecules reduce water utilization, hydraulic conductivity, and nutrient uptake to influence the plant’s physiological state [18]. In this study, five low-molecule-weight phenolic acids were identified from the P. odoratum rhizosphere soil, i.e., p-hydroxybenzoic acid, vanillic acid, syringic acid, cinnamic acid, and ferulic acid, where p-hydroxybenzoic acid, vanillic acid, and syringic acid were from benzoic acid derivatives, and cinnamic acid and ferulic acid were from cinnamic acid derivatives. Although their total contents were very low, the following discussion will focus on the high-molecule-weight phenolic acids identified from the P. odoratum rhizosphere soil, including chlorogenic acid, ferulic acid, sinapic acid, and diferulic acid.

Hydrolysis of phenolic compounds was observed in the P. odoratum rhizosphere soil, indicating an increase in fermentative activities. The soil phenolic acids are usually released by plant leaching, root exudation, or decomposition of plant residues [16, 17]. For the low-molecule-weight phenolic acids, there are two groups including benzoic acid derivatives and cinnamic acid derivatives, which play an important role in plant growth and interactions with the environment. These molecules reduce water utilization, hydraulic conductivity, and nutrient uptake to influence the plant’s physiological state [18]. In this study, five low-molecule-weight phenolic acids were identified from the P. odoratum rhizosphere soil, i.e., p-hydroxybenzoic acid, vanillic acid, syringic acid, cinnamic acid, and ferulic acid, where p-hydroxybenzoic acid, vanillic acid, and syringic acid were from benzoic acid derivatives, and cinnamic acid and ferulic acid were from cinnamic acid derivatives. Although their total contents were very low, the following discussion will focus on the high-molecule-weight phenolic acids identified from the P. odoratum rhizosphere soil, including chlorogenic acid, ferulic acid, sinapic acid, and diferulic acid.
Fig. 1 (See legend on next page.)
subtle in the soil, the phenolic acid contents in the CC soil were significantly higher than those in the FC soil. These results supported that the CC system might result in an accumulation of phenolic acids secreted by the medicinal plant.

The content level and balance of nitrogen, phosphorus, and potassium in soil are essential for plant growth and development [19]. It was observed that TP, TK, AN, AP, and AK were significantly reduced in the CC soil. Subsequently, this phenomenon might lead to nutrient deterioration in the CC soil for medicinal plant growth. Soil AN is the nitrogen form which is easy to be absorbed and used directly by plants, mainly including NH$_4^+$, NO$_3^-$, amino acid, amide, and hydrolysable protein nitrogen [20]. The early research had proved that nitrogen in soil inhibits the formation of phenolic acids in plants, and there was a negative correlation between the total phenolic acids and the soil nitrogen content according to the “carbon/nutrient balance hypothesis” [21]. It was also observed this correlation in our experiment. The result showed that the AN in the CC soil was significantly lower than that in the FC soil, whereas the phenolic acids in the CC soil were at a higher level.

The phenolic acids are detrimental to the rhizosphere soil environment and can change the soil microbial community [9]. Phenolic acids have a hydroxyl group and a carboxyl group. For this reason, they can hydrogen-bond with the soil enzymes and change the soil pH value to worsen the soil’s physiological status. The activities of soil enzymes can reflect the soil health status [22]. Soil SC hydrolyzes sucrose into monosaccharides, which are closely related to soil nutrition, whereas soil UE hydrolyzes urea to produce ammonia, which is the source of AN. In this experiment, it was found that the activities of UE and SC in the CC soil were decreased as compared to those in the FC soil. Under this situation, the utilization of sugar and the production of AN were weakened in the CC soil, and then the lack of nitrogen could lead to a comparatively higher content of phenolic acids in the CC plant as compared to that in the FC plant. Consequently, more phenolic acids were secreted and finally accumulated in the CC soil.

The high-throughput sequencing and relative qRT-PCR analysis also revealed that the gene expressions of the enzymes that catalyzed the key steps in the synthesis of the phenolic acids were regulated in the P. odoratum root tissues. The pentose pathway and relative pathways are the major source for metabolic intermediates in plants [23] and can provide erythrose-4-phosphate as a precursor for shikimic acid. In the study, 12 kinds of DEGs that were closely relative to the shikimic acid and

![Graph A](image1.png)

**Fig. 1** GO enrichments of DEGs in CC vs FC root tissues of *Polygonatum odoratum*. DEGs stands for differentially expressed genes. FC stands for first cropping, and CC stands for continuous cropping. **A**: DEGs in CC vs FC root tissues of *Polygonatum odoratum*; **B**–**D**: up-regulated DEGs in biological process, molecular function, and cellular component, respectively; **E–G**: down-regulated DEGs in biological process, molecular function, and cellular component, respectively. In Fig. 1A, BP stands for biological process, CC stands for cellular component, and MF stands for molecular function.

![Graph B](image2.png)

**Fig. 2** KEGG pathway enrichment of DEGs of CC vs FC root tissues of *Polygonatum odoratum*. DEGs stands for differentially expressed genes. FC stands for first cropping, and CC stands for continuous cropping. **A**: Up-regulated DEGs of CC vs FC root tissues of KEGG pathway enrichment, **B**: down-regulated DEGs of CC vs FC root tissues of KEGG pathway enrichment.
chorismicate metabolisms were enriched, where eight of them were up-regulated, and four of them were down-regulated (Fig. 5).

Among the up-regulated genes, PDG, rpe, and SORD encoded 6-phosphoglucuronate dehydrogenase, ribulose-phosphate 3-epimerase, and L-iditol 2-dehydrogenase, respectively, and they were the enzymes for synthesis of ribulose-5-phosphate, xylulose-5-phosphate, and xylusose, respectively [24–26]. The up-regulation of these genes was beneficial to the xylusoe-5-phosphate biosynthesis. FBP and mαZ encoded fructose-1, 6-biphosphatase and α-glucosidase, respectively. The up-regulation of them was favorable for the synthesis of fructose-6-phosphate, which was material for the synthesis of erthrose-4-phosphate, a precursor of shikmic acid [27,
aroK and aroC encoded shikimate kinase and chorismate synthase, respectively [29, 30], and the up-regulation of them was beneficial to chorismate biosynthesis, which was the precursor of the low-molecule-weight phenolic acids. On the contrary, the down-regulation of glgC, E3.2.1.4, and GPI, which encoded glucose-1-phosphate adenylyltransferase, endoglucanase, and glucose-6-phosphate isomerase, respectively, could inhibit the cellodextrin and amylose metabolism [31–33]. This process in turn could provide more materials for the erythrose-4-phosphate biosynthesis. Early researches have proved that the phenolic acid biosynthesis in plants is triggered by biotic and abiotic stresses [34, 35]. Likewise, the results in our study also supported that the biosynthesis of phenolic acids in the CC plant proceeded through the activation of the primary pathway (shikimic acid) and the suppression of the relative branch pathway (cellodextrin and amylose).

Tyramine is the product of tyrosine decarboxylation, and partial phenolic acids in plants can be conjugated with tyramine especially when the plant is under stress.
The tyramine-conjugated phenolic compounds act as phytoalexins to make plants adapt to the external stress, and the biosynthetic processes including the shikimate, phenylpropanoid, and arylmonoamine pathways are activated [38]. The conjugation is also favorable for more phenolic acids to accumulate. In this experiment, it was also found that the expression of tyrosine decarboxylase in the CC plant was up-regulated and polyphenol oxidase was down-regulated. It was speculated that the change might help to promote the phenolic acid anabolism in *P. odoratum* in the CC soil and made them accumulate in the soil.

The inducement of the root rot of *P. odoratum* in the CC soils was very complicated. The multiple factors to
cause CMP may mainly include soil element imbalance, soil microbial population transforming to harmful population, and autotoxicity caused by the secretions from plant roots, but the comprehensive mechanism for CMP remains unclear [3]. For *P. odoratum* in the CC soils, the nutrient composition and the soil enzymes promoted the synthesis of phenolic acids. On the other hand, there might be multiple gene families activated together to accelerate the phenolic acid synthesis process coordinately to influence the CMP formation.

Conclusions

In this study, five low-molecule-weight phenolic acids were identified from the *P. odoratum* rhizosphere soil. Among them, the phenolic acid contents in the CC soil were significantly higher than those in the FC soil except vanillic acid. The contents of AN, AP, and AK in the CC soil were significantly higher than those in the FC soil, and the nitrogen metabolism and sugar catabolism of the CC soil were lowered. The genomic analysis showed that the phenolic acid anabolism in *P. odoratum* in the CC soil was promoted. This research presented us with a preliminary understanding of the role of phenolic acids in the CMP of *P. odoratum*. In perspective, proteomics and metabolomics are needed to look into more metabolic mechanism details of phenolic acid metabolism on the root rot of *P. odoratum*.

Methods

Experiment materials and chemicals

The *P. odoratum* cultivar “Zhushiwei” was selected as the experimental material and authenticated by associate Professor Zefa Liu, a horticulturist from Loudi Agricultural Institute, Hunan Province, China. The roots and rhizosphere soil of *P. odoratum* were collected in May, 2017 from the Gutang Town Experimental Station of Loudi City, Hunan Province, China with the permission of Loudi Agricultural Institute, Hunan Province, China. For the CC system, *P. odoratum* was cultivated in the land where the same plants had been harvested; for the FC system, *P. odoratum* was cultivated on the same date in the land near CC where the cabbages had been harvested. The collection of rhizosphere soil was according to Riley and Barber’s method [39, 40]. Briefly, the soil with a complete root system was excavated in the selected plot, the large soil without root was shaken off gently, and then the soil adhered to the root circumference (0–5 mm from the root circumference) was collected with a brush as the rhizosphere soil. The rhizosphere soil was air-dried under room temperature, passed through the 2 mm sieve, and stored at 4 °C until use.

Methanol, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, cuminic acid, ferulic acid, and methanol were of chromatographic purity, and the other chemicals were of analytical purity.

Rhizosphere soil phenolic acid determination

The rhizosphere soil of 1.0 g was added with 3.0 mL 1 N NaOH, and then shaken at 4 °C overnight. After centrifuged with 8000×g at 4 °C for 10 min, the supernatant was adjusted to pH 2.5 with HCl. After the liquid was extracted two times with 10 mL ethyl acetate, it was concentrated to dryness under nitrogen, and then dissolved in methanol. The solution was filtered with a 0.45 μm membrane for the HPLC analysis.

The HPLC conditions were as follows: volume of 10 μL was loaded on a Kromasil C18 column (250 mm × 4.6 mm × 5 μm) (Akzo Nobel, Amsterdam, Netherlands) with a Rigol HPLC L3000 (Rigol Technologies, Beijing, China). The gradient elution was with eluent A 1% phosphoric acid and eluent B methanol. The elution gradients were 80% A + 20% B on 0–10 min, 70% A + 30% B on 10–20 min, 50% A + 50% B on 20–30 min, 50% A + 50% B on 30–40 min, 80% A + 20% B on 40–45 min, and 80% A + 20% B on 45–55 min, respectively, with the rate of 1 mL/min at 30 °C, and the signal was detected at 280 nm with an ultraviolet detector. For the standard curve, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, cuminic acid, and ferulic acid were used as standard samples and detected as above. The external standard method was used for the quantitative analysis of the phenolic acids as per Wang et al. [41].

Determination of rhizosphere soil chemical properties and enzyme activity

The TN content of the rhizosphere soil was determined with the Kjeldahl method, the TP content of the rhizosphere soil was determined with the molybdenum-blue colorimetry after the soil was extracted with the mixture of concentrated sulfuric acid and perchloric acid, the TK content of the rhizosphere soil was determined with the flame spectrophotometry after the soil was extracted with the mixture of concentrated sulfuric acid and perchloric acid, the AN content of the rhizosphere soil was determined with the diffusion method after hydrolysis with the alkaline solution, the AP content of the rhizosphere soil was determined with the molybdenum-blue colorimetry after the soil was extracted with the mixture of concentrated sulfuric acid and perchloric acid, the AK content of the rhizosphere soil was determined with the flame spectrophotometry after the soil was extracted with sodium hydroxide, and the AK content of the rhizosphere soil was determined with the catechol method [43]. The CAT activity of the rhizosphere soil was determined as per Kraus and Fletcher [44]. The UE activity of the rhizosphere soil was determined with the indophenol blue colorimetric method [45]. The ACP of the rhizosphere soil activity was
with Trizol reagent according to the manufacturer's instruction. To obtain cDNA, the total RNA (0.5 μg) was reversely transcribed using a TUREscript cDNA Synthesis Kit (Aidlab Biotechnologies Co., Ltd., Beijing, China) with oligo dT as a primer. The candidate genes and the reference gene were analyzed with a qTOWER2.2 real-time PCR (Analytik Jena AG, Jena, Thuringia, Germany) with the primers in Table S3. The protocol for qRT-PCR was as follows: 3 min at 95 °C, followed by 10 s at 95 °C and 30 s at 58 °C with 39 cycles, and then was ended after a melt curve analysis from 60 °C to 95 °C with an increment of 1 °C for 4 s at each step. Three biological replicates were used for each analysis and the data were analyzed using the $2^{ΔΔCt}$ method.

Data statistics
All the experiments were repeated three times at the biological sample level, and the differences were analyzed using IBM SPSS 19.0 (International Business Machines Corporation, Armonk, NY, USA) with the T-student’s method ($P < 0.05$).

Abbreviations
ACP: Acid phosphatase; AN: Available nitrogen; AP: Available phosphorus; AK: Available potassium; CAT: Catalase; CC: Continuous cropping; CMP: Consecutive monoculture problem; DEGs: Differentially expressed genes; FC: The first cropping; KEGG: The Kyoto Encyclopedia of Genes and Genomes; HPLC: High-performance liquid chromatography; PPO: Polyphenol oxidase; qRT-PCR: Quantitative real-time PCR; SC: Sucrase; TK: Total potassium; TP: Total phosphorus; TN: Total nitrogen; UE: Urease

Supplementary Information
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Authors’ contributions
NXZ performed most of the experiments. JCZ managed the fields and provided the materials. LAY participated in the preparation of the manuscript. CY and HYH conceived and coordinated the studies, and HYH wrote and edited the manuscript. All the authors have read and approved the final manuscript.

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Availabilty of data and materials
All data supporting the findings were contained in the manuscript and its supplementary files except the RNA-seq raw data. And all the RNA-seq raw data were uploaded in the SRA of NCBI (PRJNAS07291).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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