IDENTIFICATION OF GINSENG (*PANAX GINSENG* C. A. MAYER) CONTINUOUS CROPPING OBSTACLE RESPONSIVE MIRNAS AND THEIR TARGET GENES

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Abstract. The problem of continuous cropping obstacle has seriously restricted the development of the ginseng industry. In view of the little research on the mechanism of ginseng crop disorder, this study was conducted to investigate the ginseng crop disorder-related miRNAs and their target genes for the sustainability of the ginseng industry. A total of 376 miRNAs were obtained from all the tissue samples of the Continuous Cropping obstacles (CCO) and Control Check (CK). 121 miRNAs were found to be differentially expressed in the ginseng. Then, the target genes of differentially expressed miRNAs were enriched and found to be involved in phytohormone-related regulation, MAPK signaling pathway - plant, amino and nucleotide sugar metabolism, and monoterpene biosynthesis, suggesting that the alteration of these related pathways may play a key role in causing ginseng crop disorder. Finally, the correlations between miRNAs and mRNAs were integrated to construct the corresponding reciprocal networks and discover the miRNAs that might play a key role in causing ginseng crop disorder. osa-MIR818d-p3_1ss1TA and pgi-MIR6135i-p3_1ss11TG and pgi-MIR6140c-p5_1ss12CT were found to be at the core of the network, and it was hypothesized that these miRNAs play an important role in the formation of ginseng continuous cropping obstacle.
Keywords: *ginseng, non-coding RNA, RNA sequencing, environmental stress*

Introduction

Ginseng (*Panax ginseng* C. A. Meyer) is an important medicinal plant in northeastern China (Proctor et al., 1987). Ginseng has a long history of cultivation in China, Korea and Japan (Coon and Ernst, 2002). Due to the high benefit of ginseng cultivation and the limitation of cultivation soil, ginseng production areas are relatively concentrated, and the black land of Changbai Mountain is the best ginseng cultivation area. The traditional method of planting ginseng after cutting down the forest, transplanting in 3 years and harvesting in 6 years, cannot be rotated and continued continuously, since the plants are severely damaged due to chemosensitivity and return to forest after harvesting, which will also cause ecological problems such as imbalance of forest ecosystem, loss of biodiversity and decrease of soil fertility in forest land. Continuing to plant ginseng by cutting down the forest for a long time, that is, cutting down all the forests and then planting ginseng, will seriously damage the natural ecological environment, which is not in line with the national ecological environmental protection policy and planning, and in the long run, it will create the situation of having no land to plant. With the extension of ginseng cultivation, the specific gravity and capacity of the soil increase, the total porosity decreases, the physical viscous particles increase, resulting in soil caking and poor air permeability, while the organic matter in the soil is consumed in large quantities, the nutrient supply is imbalanced and the soil

tends to acidify, which affects the normal growth of ginseng. In addition, in recent years, the scale of ginseng cultivation in harvested forests has been shrinking; it is increasingly important to produce high quality ginseng. One of the important factors affecting the quality of ginseng is the various diseases that occur during the growth of ginseng; continuous cultivation of ginseng can lead to a decrease in yield as well as an increase in diseases. According to the statistics, about 70% of the production of root and stem medicinal plants are affected by successive crop disorders. Soil diseases in ginseng reduce seed germination, seedling growth, ginseng root quality, and also cause serious disease infestation (Li et al., 2011). Soil diseases reduce the yield of ginseng and also cause major problems in plant recovery on old arable land (Wu et al., 2008), and autotoxic effects can be found in plants of the *American Ginseng* (Kraus et al., 1995), *Yakobia* (Möhler et al., 2018), and *Cucumber* (Bu et al., 2016; Zhou et al., 2019).

miRNAs are a class of sRNA molecules with an average length of about 22 nucleotides, which play important roles in plant growth and development, stress resistance and secondary metabolism through post-transcriptional regulation, repressing or even silencing the expression of the target genes they act on. Studies have shown that miRNAs are important regulators of plant response to environmental stress (Ghosh et al., 2017; Singh et al., 2017; Muhammad et al., 2018). Under adversity stress, the expression of most miRNAs is significantly altered during plant growth and development (Sunkar et al., 2012), negatively regulating adversity-related genes and initiating defense systems against adverse factors (Jung et al., 2018). 280 miRNAs were found to be specifically expressed under high temperature stress by Jung et al. Zhang et al. (2016) identified five miRNAs and seven target genes in response to continuous crop disorders in succession salvia (*Salvia miltiorrhiza* Bge) roots; Li et al. (2013) identified 85 miRNAs and their target genes specifically in response to heavy crop Rehmannia glutinosa (*Rehmannia glutinosa* (Gaetn.) Libosch. ex Fisch. et Mey.), and these miRNAs may be involved in various biological processes such as plant growth and development, signaling, organ formation and response to adversity stress.

In recent years, an increasing number of studies have used the Illumina RNA-seq platform based on transcriptome analysis to explore plant responses to abiotic or biotic stresses and to understand their associated molecular mechanisms. In organisms, in addition to mRNA, there is usually an ncRNA, which does not encode proteins but has important regulatory functions. Based on size, ncRNA can be subdivided into small ncRNA of less than 200 nucleotides, including miRNA, lncRNA and cyclic RNA of length > 200 bp, consisting of a continuous closed loop (Jones-Rhoades et al., 2006; Zhang et al., 2013; Barrett et al., 2016). Studies have shown that miRNAs are closely associated with plant resistance and endostasis regulation (Zhao et al., 2016; Zhu et al., 2012). Although miRNAs have been studied in ginseng, the role of miRNAs in continuous cropping has not been reported (Wang et al., 2015; Wu et al., 2012; Li et al., 2020). In addition, with the in-depth study of miRNA functions, the regulatory mechanisms between miRNAs and genes have been gradually improved. The construction of gene regulatory networks has become an important strategy to reveal disease regulatory mechanisms and important plant traits (Durand et al., 2014; Mäkinen et al., 2017).

Under continuous crop stress, miRNAs may also exist in ginseng in response to continuous crop stress, but it is not clear which miRNAs may be specifically expressed in the body. Therefore, in this study, we identified and screened miRNAs in crop-linked ginseng and healthy ginseng based on data obtained by RNA-seq. The target genes of

differentially expressed miRNAs were classified and functionally analyzed using GO annotation, and the biological pathways of the target genes of these differential miRNAs were revealed by KEGG pathway analysis. Finally, the correlation between miRNAs and mRNA was integrated to establish the interaction network between mRNAs and miRNAs, which laid the theoretical foundation for exploring the molecular mechanism of ginseng response to linkage disorder.

Materials and methods

Materials

Potted two-year old ginseng (*Panax ginseng* C.A. Meyer) seedlings were purchased from Wanliang Ginseng Market, Fusong County, Jilin Province; new woodland soil was collected from Pumping Township, Fusong County, Baishan City, Jilin Province; old ginseng land soil was collected from heavy crops of ginseng cultivated continuously for 6 years near the new woodland.

The experiments were conducted on April 30, 2021 in the Bacchus Garden of Changchun University of Traditional Chinese Medicine with a shade net (40 m long × 3.5 m wide × 3 m high). Healthy 2-year-old ginseng seedlings of the same species and in uniform morphological size condition were selected for the experiment using the pot method.

Control Check (CK): Healthy new forest soil was selected as the potting substrate, and experimental ginseng seedlings were moved in on the same day, each pot was filled with 9 kg of soil, 6 plants were cultivated, each treatment was repeated 3 times, total 15 pots, water was replenished once every 7 d, other than the field management.

Continuous Cropping obstacles (CCO): 6-year-old ginseng soil was selected as the substrate for potting, and experimental ginseng seedlings were transferred on the same day, each pot was filled with 9 kg of soil and 6 plants were cultivated, each treatment was repeated 3 times, totaling 15 pots, water was replenished once every 7 d, and other management was the same as that in the field.

Three plants were randomly selected and marked for each treatment during the harvesting period. The RNA samples were taken within one month after sample collection.

Constructing cDNA libraries

Construction of small RNA sequencing libraries

In this section, 6 cDNA libraries were also constructed, 3 cDNA libraries for cohort ginseng and 3 cDNA libraries for healthy ginseng. For each sample, 3 µg of RNA was used as small RNA library material. Sequencing libraries were prepared using the TruSeq Small RNA Sample Prep Kits (Illumina, San Diego, USA) kit. Libraries were constructed using the TruSeq Small RNA Sample Prep Kits (Illumina, San Diego, USA) kit according to the kit instructions. The procedure was as follows: 3′ splice sequence ligation, 5′ splice sequence ligation, reverse transcription to generate cDNA strand, PCR amplification, electrophoresis to purify the target fragment, and sequencing. The Hiseq2500 sequencer (Illumina, San Diego, USA) was used for sequencing, and the read length was 1 × 50 bp.

Data analysis process

The miRNA data analysis software provided by Lianchuan is our self-developed ACGT101-miR (LC Sciences, Houston, Texas, USA), and the analysis process of this

software is as follows: (1) remove 3' junction and garbage sequences: get clean data; (2) length screening: plant (2) length screening: sequences with retained base lengths of 18-25 nt in plants and 18-26 nt in animals; (3) various RNA database comparison analysis: the remaining sequences are compared to (without miRNA) mRNA, RFam and Repbase databases and filtered; (4) miRNA identification: valid data are obtained and compared to precursors and genomes for miRNA identification; (5) miRNA differential analysis, (6) differential miRNA target gene prediction analysis.

MiRNA identification and target gene prediction

The miRNA data analysis software provided by LianChuan is our own ACGT101-miR (LC Sciences, Houston, Texas, USA). The analysis process of the software is as follows: clean reads are obtained from the raw data after quality control processing, clean reads are removed from 3' The clean reads were removed from the 3' splice, and the sequences with a base length of 18-25 nt were retained by length screening. The remaining sequences are then compared with various RNA database sequences (excluding miRNA), such as mRNA database, RFam database (containing rRNA, tRNA, snRNA, snoRNA, etc.) and Repbase database (repetitive sequence database), and filtered, and the final data obtained is the valid data, which can be used for subsequent small RNA data analysis. The valid data were compared with the mature and precursor sequences of ginseng in the miRBase 22.0 database, as well as the genomic sequences of this species to identify known miRNAs and new predicted miRNAs.

Target gene prediction analysis was performed by PsRobot 1.2 for differential miRNAs, and GO (http://www.geneontology.org/) and KEGG (http://www.genome.jp/kegg/) functional enrichment analysis was performed for target genes of differential miRNAs.

Quantitative real-time qPCR

The total RNA isolation and cDNA preparation of ginseng and control ginseng tissue samples were performed as described in "Construction of small RNA sequencing libraries" above. To verify the reliability of the obtained miRNA data, experiments were performed on eight differentially expressed miRNAs randomly selected from the transcriptome data. miRNA primers were designed according to the Biobio Plus Tail Method kit.

Transcriptome-wide association analysis

To reveal the roles and interactions of miRNAs and mRNAs in ginseng cohorting, association analysis was performed on the obtained data. Firstly, we searched for mRNAs with targeting relationship with the differential miRNAs, and then took the intersection of these mRNAs with the corresponding combination of differential mRNAs to obtain the differential mRNAs targeted by the differential miRNAs. finally, we constructed the interaction network using Cytoscape software and visualized it.

Statistical analysis

SPSS (v.20.0) software was used for statistical analysis, and all data were expressed as _x ± SEM. RT-PCR results were performed using Student's t-test. for all analyses, $P < 0.05$ was considered statistically significant.

Results

Effect of continuous crop on the phenological traits of ginseng

The above-ground part of the stems and leaves had a lot of yellow spot disease, and the roots showed serious fibrous root abscission, accompanied by root rot, rust rot and other typical problems of crop failure in old ginseng fields. Compared with CK, the mean plant height, mean primary root length, and harvesting period of CCO all reached significant differences. The mean fibrous root number even reached significant differences at the fruiting and harvesting stages. This indicates that continuous crop has a significant impact on the growth of ginseng, mainly in terms of shorter plants, reduced number of leaves, fewer fibrous roots and shorter main roots, which in turn affects the yield and quality of ginseng (*Fig. 1*).
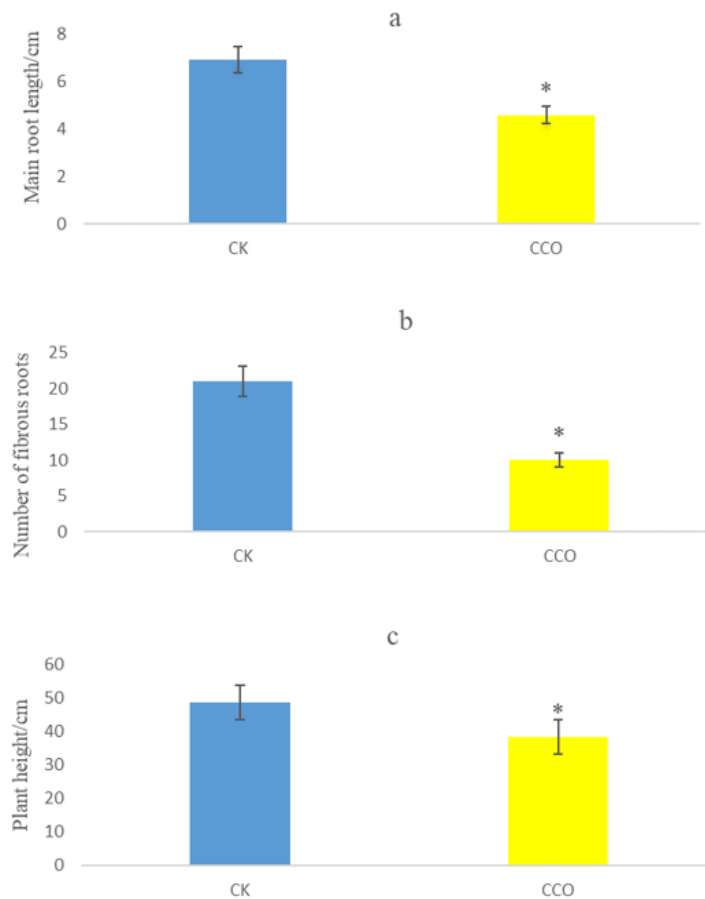

Figure 1. *(a) Effect of continuous soil crop of ginseng on the main root length of ginseng. (b) Effect of continuous soil crop on the number of fibrous roots of ginseng. (c) Effect of continuous soil crop of ginseng on the height of ginseng plants (*P <0.05, vc CK)*

Quality control of RNA-seq reads

A total of 86,060,736 raw reads were obtained from the sequencing of 6 sRNA cDNA libraries, and 84,523,614 clean reads were generated after removing splice-related reads and low-quality reads, with $Q20 > 99\%$ and $Q30 > 97\%$ for each sample (*Tables 1* and *2*).

Table 1. *Overview of miRNA sequencing data*

lib	CK1				CK2				CK3			
	Total	% of Total	uniq	% of uniq	Total	% of Total	uniq	% of uniq	Total	% of Total	uniq	% of uniq
Raw reads	13555394	100.00	4925689	100.00	15635919	100.00	5359722	100.00	10989035	100.00	3987821	100.00
3ADT&length filter	2320908	17.12	653477	13.27	4455550	28.50	1105344	20.62	3682123	33.51	867817	21.76
Junk reads	50889	0.38	35006	0.71	50667	0.32	35186	0.66	36837	0.34	26979	0.68
Rfam	662070	4.88	14255	0.29	673281	4.31	14132	0.26	419133	3.81	11081	0.28
Repeats	2549	0.02	85	0.00	2958	0.02	90	0.00	1487	0.01	54	0.00
Valid reads	10519376	77.60	4222888	85.73	10454100	66.86	4204990	78.46	6849742	62.33	3081905	77.28
rRNA	513059	3.78	10843	0.22	520368	3.33	10775	0.20	324781	2.96	8689	0.22
tRNA	99989	0.74	1695	0.03	102251	0.65	1661	0.03	64541	0.59	1122	0.03
snoRNA	5804	0.04	309	0.01	5821	0.04	326	0.01	3147	0.03	187	0.00
snRNA	1265	0.01	116	0.00	1069	0.01	101	0.00	609	0.01	62	0.00
Other Rfam RNA	41953	0.31	1292	0.03	43772	0.28	1269	0.02	26055	0.24	1021	0.03

Table 2. *Overview of miRNA sequencing data*

lib	CCO1				CCO2				CCO3			
	Total	% of Total	uniq	% of uniq	Total	% of Total	uniq	% of uniq	Total	% of Total	uniq	% of uniq
Raw reads	17866326	100.00	6614761	100.00	29977664	100.00	10380242	100.00	18544217	100.00	6922396	100.00
3ADT&length filter	6459393	36.15	1644609	24.86	4431911	14.78	1740193	16.76	5186211	27.97	1507444	21.78
Junk reads	58738	0.33	41287	0.62	117214	0.39	67826	0.65	62404	0.34	42923	0.62
Rfam	674747	3.78	17143	0.26	1498833	5.00	30041	0.29	836318	4.51	19710	0.28
Repeats	3456	0.02	81	0.00	8115	0.03	188	0.00	5333	0.03	117	0.00
valid reads	10670373	59.72	4911667	74.25	23922319	79.80	8542045	82.29	12454677	67.16	5352246	77.32
rRNA	505066	2.83	12582	0.19	1120824	3.74	21044	0.20	627918	3.39	14239	0.21
tRNA	123332	0.69	2980	0.05	282027	0.94	6092	0.06	147099	0.79	3574	0.05
snoRNA	4108	0.02	228	0.00	8242	0.03	471	0.00	5922	0.03	314	0.00
snRNA	1505	0.01	141	0.00	5030	0.02	415	0.00	2243	0.01	200	0.00
other Rfam RNA	40736	0.23	1212	0.02	82710	0.28	2019	0.02	53136	0.29	1383	0.02

Identification and characterization of miRNAs

Based on the analysis and statistics of the raw sequencing data, we further conducted the length distribution statistics of the Total and Unique of the Valid data filtered by Rfam, Repeats and other databases. The length distribution of sRNAs obtained by filtering is shown in *Figures 2* and *3*. All six samples were mainly concentrated between 21nt and 24nt, and 21nt and 24nt were the most abundant.
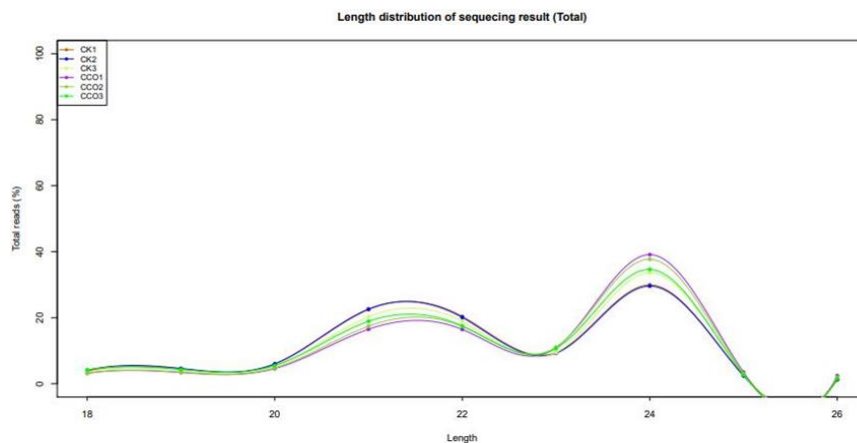

Figure 2. *Length distribution of total valid reads*
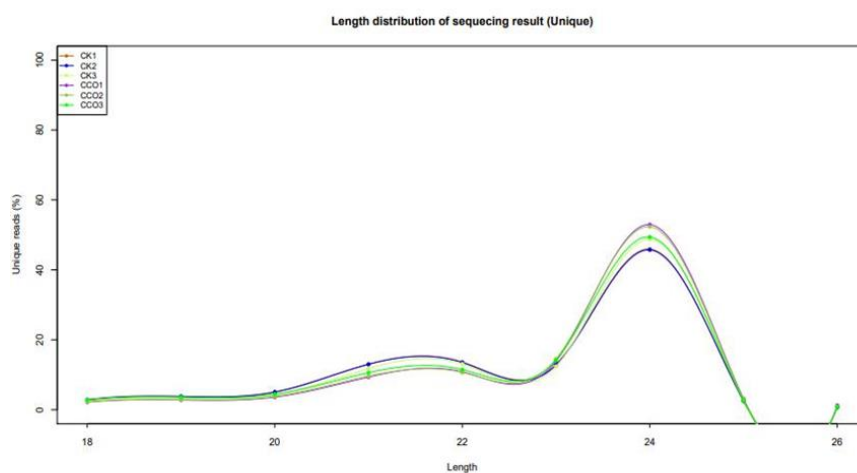
Figure 3. *Length distribution of unique valid reads*

Considering that miRNAs are highly evolutionary conserved, the presence of miRNAs identified in ginseng tissues was counted in other species. As shown in *Figure 4,* many miRNAs identified in ginseng tissues showed high conservation and high homology with soybean (*Glycine max*), apple (*Malus domestica*), poplar (*Populus species*), Arabidopsis (*Arabidopsis thaliana* (L.) Heynh.

In addition, the obtained miRNAs were analyzed by family analysis, and the miRNA families were counted. As shown in *Figure 5,* among these miRNA families, MIR6135, MIR6140, and MIR6136 were the three larger families, containing 45, 39, and 37 miRNAs, respectively.
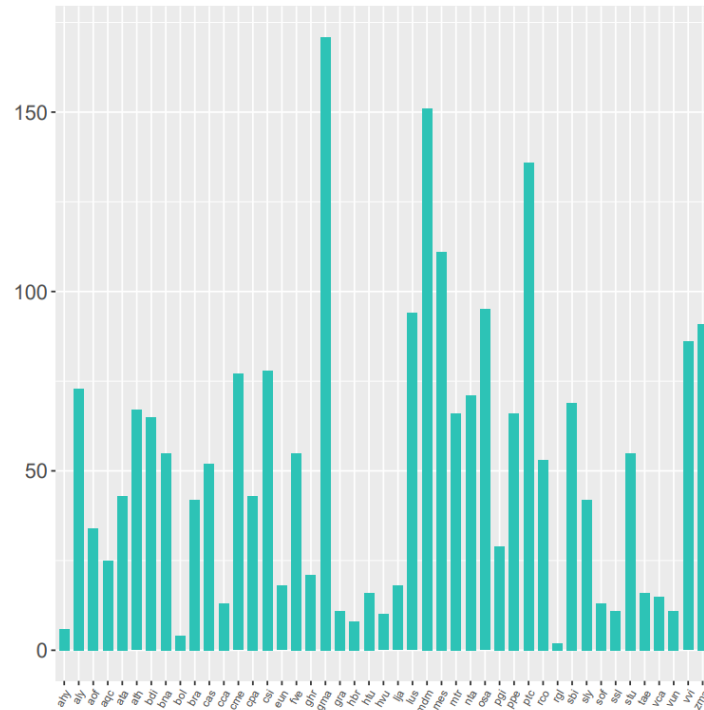
Figure 4. *Statistics on the frequency of miRNA of Panax ginseng in other species*
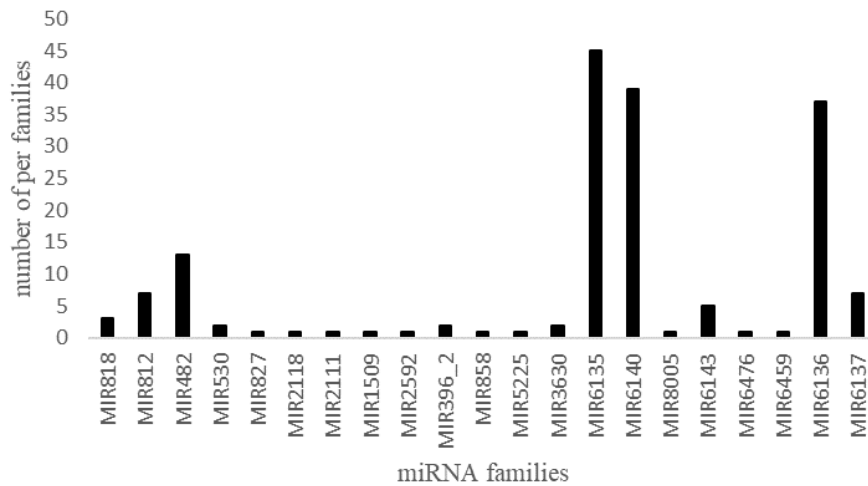

Figure 5. *miRNA family statistics of miRNAs in ginseng*

Expression analysis of miRNAs

As shown in *Figure 6,* the Wayne diagram shows that 282 miRNAs were co-expressed by the samples in one group, 14 were specifically expressed in CK and 80 were specifically expressed in CCO.

Differential expression analysis of miRNAs

The analysis of differentially expressed miRNAs is shown in *Figure 7*. After normalized data processing, the screening of differentially expressed genes with a

threshold of P < = 0.05 showed that a total of 121 miRNAs were differentially expressed, of which 63 were up-regulated and 58 were down-regulated.
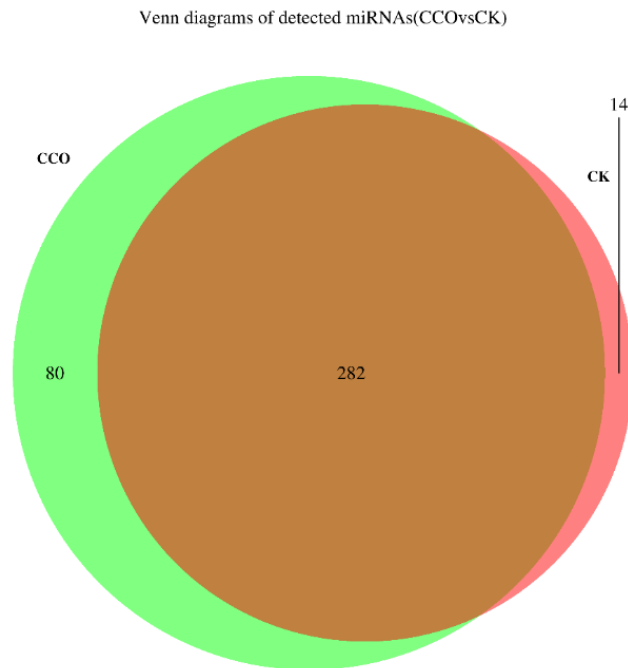
Figure 6. Venn diagram showing miRNAs differently expressed in CCO and CK

Target gene prediction and functional analysis of differentially expressed miRNAs

In order to investigate the potential regulatory roles of miRNAs in ginseng, target gene prediction was performed using PsRobot (Dai and Zhao, 2011) software for significantly different miRNAs, and GO and KEGG analyses were used to further predict miRNA target gene functions.

GO is the international standard classification system for gene functions. All of the DEGs can be divided into three categories, including biological process (BP), cellular component (CC), and molecular function (MF).

The GO enrichment analysis of miRNA target genes revealed that in the BP entry, miRNA target genes were mainly enriched in biological process and regulation of translation. CC entries were significantly enriched in nucleus, cytoplasm and plasma membrane. In addition, DNA-binding transcription factor activity and DNA binding were significantly enriched in the MF entry, and binding was enriched in the most target genes. This suggests that differentially expressed miRNAs may be involved in a variety of regulatory processes and cellular structural components in ginseng tissues (*Fig. 8*).

The GO enrichment analysis of miRNA target genes revealed that miRNA target genes were mainly enriched in "auxin-activated signaling pathway", "cell differentiation", "cellular differentiation", and "cellular differentiation". cell differentiation", "meristem initiation", "transcription, DNA-templating "(transcription, DNA-templated), suggesting that differentially expressed miRNAs may be involved in multiple regulatory processes and the composition of cellular structures in human ginseng (*Fig. 9*).
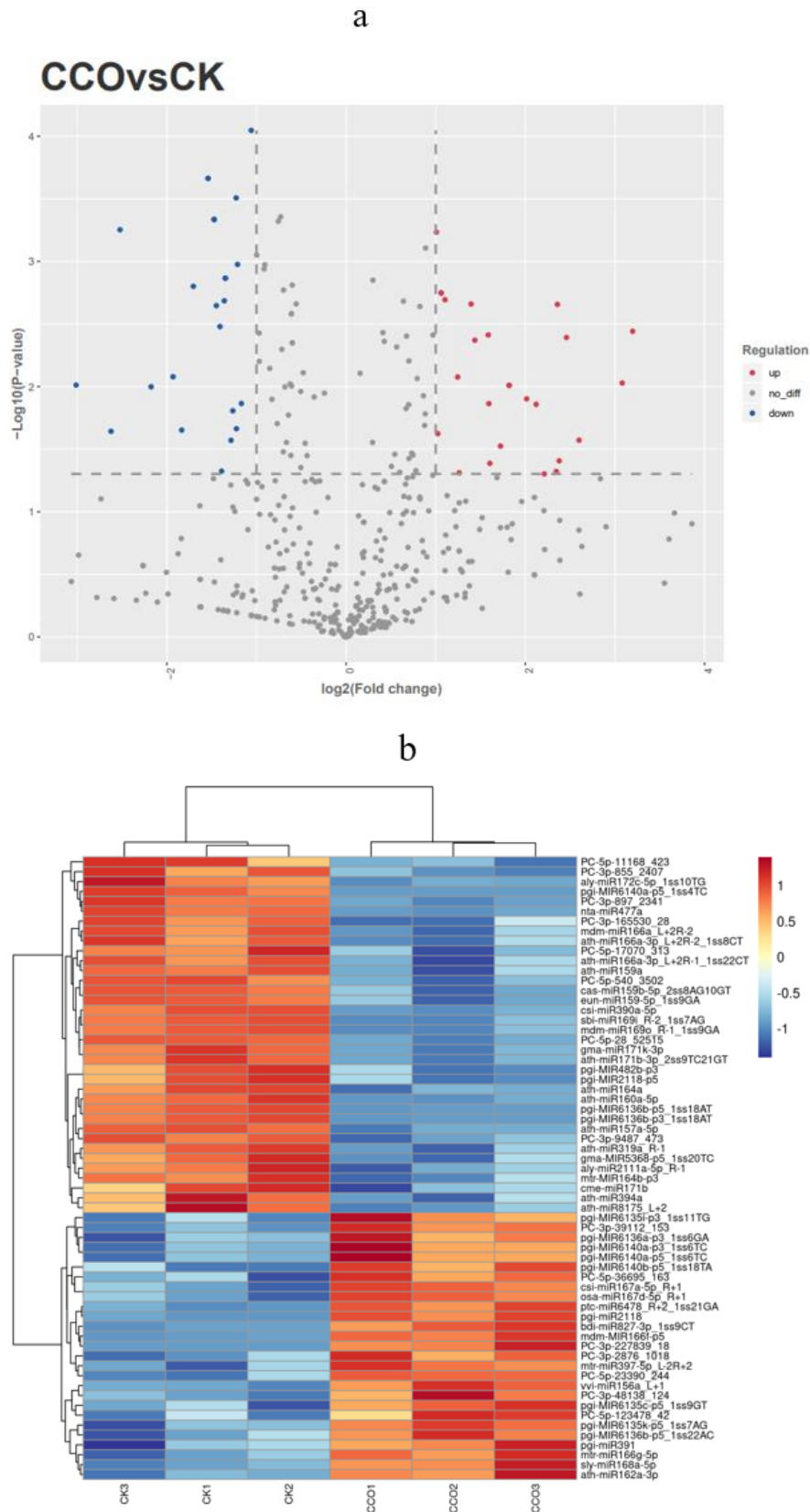
Figure 7. *(a) Expression profiling changes of miRNA. Volcano plot indicating upregulated and downregulated miRNA. (b) The heatmap of differentially expressed miRNA of Panax ginseng callus*
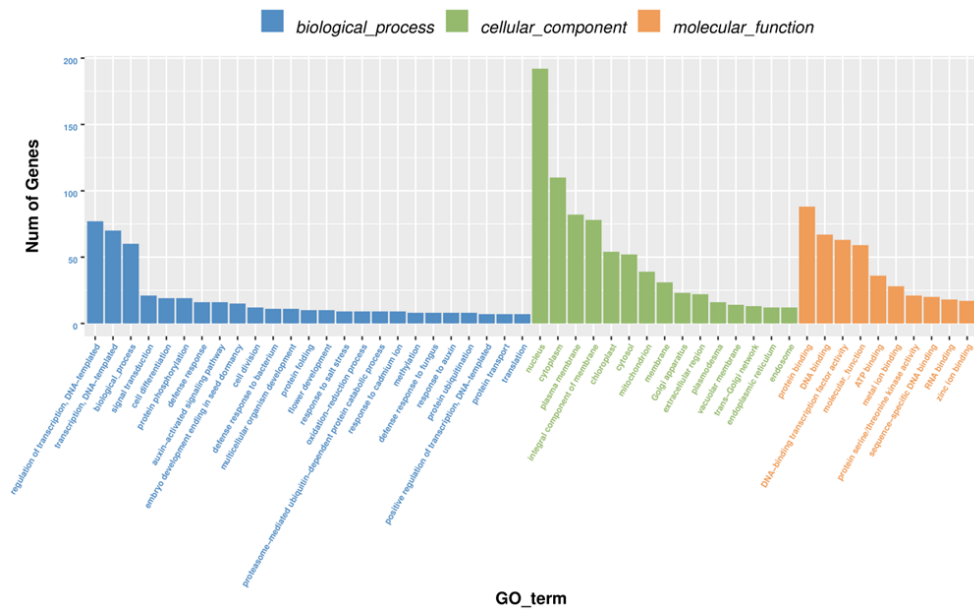
Figure 8. *Differential gene composition structure based on GO*
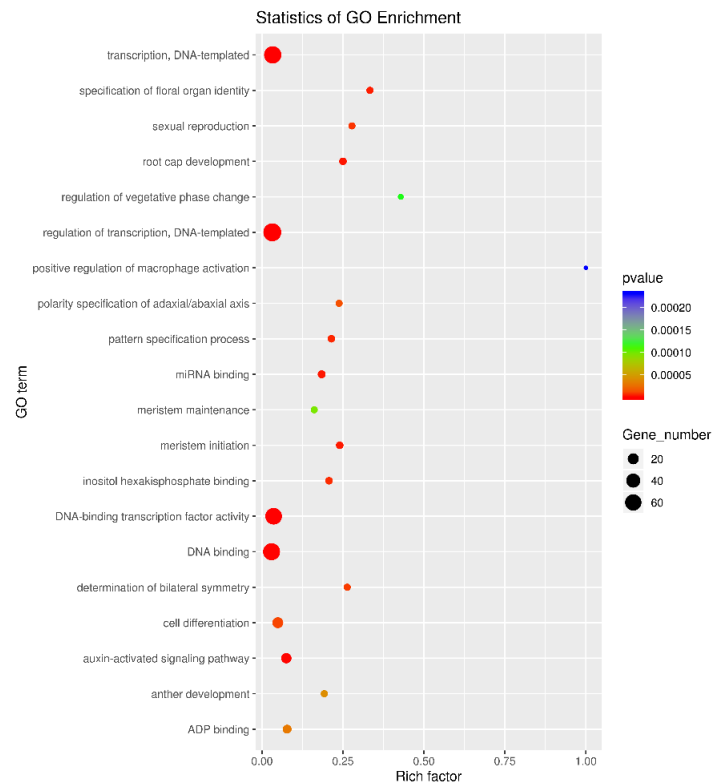

Figure 9. *Function enrichment analysis of miRNA target gene GO in ginseng under continuous cropping regulation*

The results of KEGG enrichment analysis of differentially expressed miRNA prediction are shown in *Figure 10*. miRNA target genes were mainly enriched in "plant hormone signal transduction", "MAPK signaling pathway - plant", "Amino sugar and nucleotide

metabolism", and "Amino sugar and nucleotide metabolism". MAPK signaling pathway - plant", "Amino sugar and nucleotide sugar metabolism", and "MAPK signaling pathway - plant". sugar metabolism" and "monoterpenoid biosynthesis". In addition, several amino acid metabolic pathways and fatty acid-related pathways were found to be enriched.
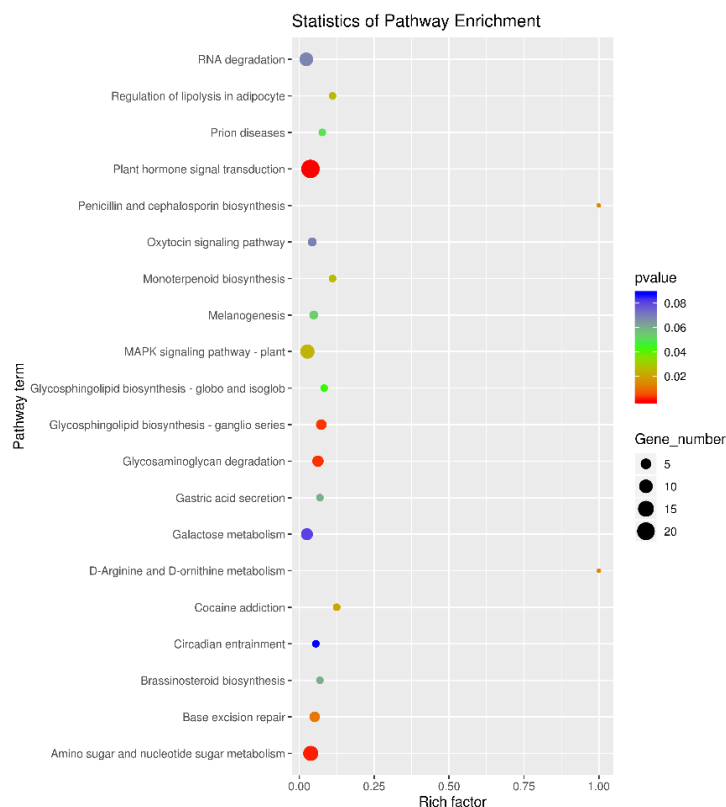

Figure 10. *Function enrichment analysis of miRNA target gene KEGG in ginseng under continuous cropping regulation*

Real-time quantitative polymerase chain reaction (qPCR) to verify the expression of miRNAs

In order to verify the accuracy of RNA-seq results and provide a basis for further study, nine differentially expressed miRNAs were randomly selected from the differentially expressed ncRNAs for qRT-PCR analysis, and the expression levels of these miRNAs were verified by qRT-PCR and transcriptome sequencing. The pgi-MIR482b-p3 , pgi-MIR6135c-p5_1ss9GT , pgi-MIR6136a-p3_1ss6GA , pgi-MIR6135k-p5_1ss7AG, pgi-MIR6136b-p5_1ss22AC, pgi-miR391, pgi-MIR6136b-p5_1ss18AT, pgi-miR2118. The corresponding ncRNA expression levels obtained by RNA-seq are shown in *Figure 11*. All validation results fully demonstrated the reliability and accuracy of the transcriptome sequencing data.

Transcriptome association analysis

The results are shown in *Figure 12,* and it was found that one miRNA was often targeted to multiple target genes in the target gene prediction process, for example, osa-MIR818d-p3_1ss1TA and pgi-MIR6135i-p3_1ss11TG. Some miRNAs may share

common target genes, such as pgi-MIR6140d-p3_1ss4AG and pgi-MIR6140c-p5_1ss12CT, pgi-miR6140a_L+1R-1, etc. This not only indicates the diversity and specificity of miRNA target genes, but also implies that miRNAs encoding the same target gene play synergistic roles in plant growth and development. plant growth and development play synergistic roles. It is hypothesized that these miRNAs may play an important regulatory role in the continuous crop response of ginseng.
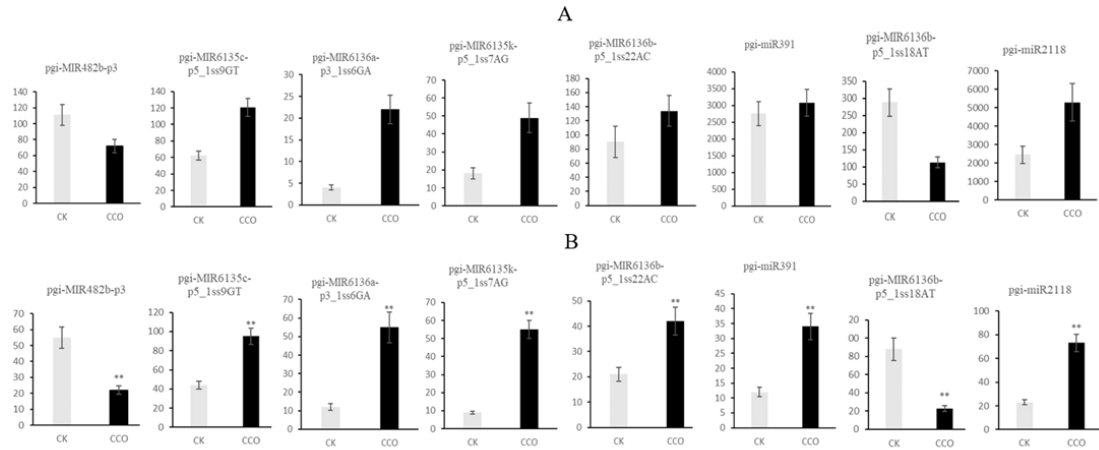

*Figure 11. qRT-PCR validation of significant differentially expressed genes. (A) RNA-seq; (B) qRT-PCR, the data were presented as the mean ± SEM (n = 3); **p < 0.05*
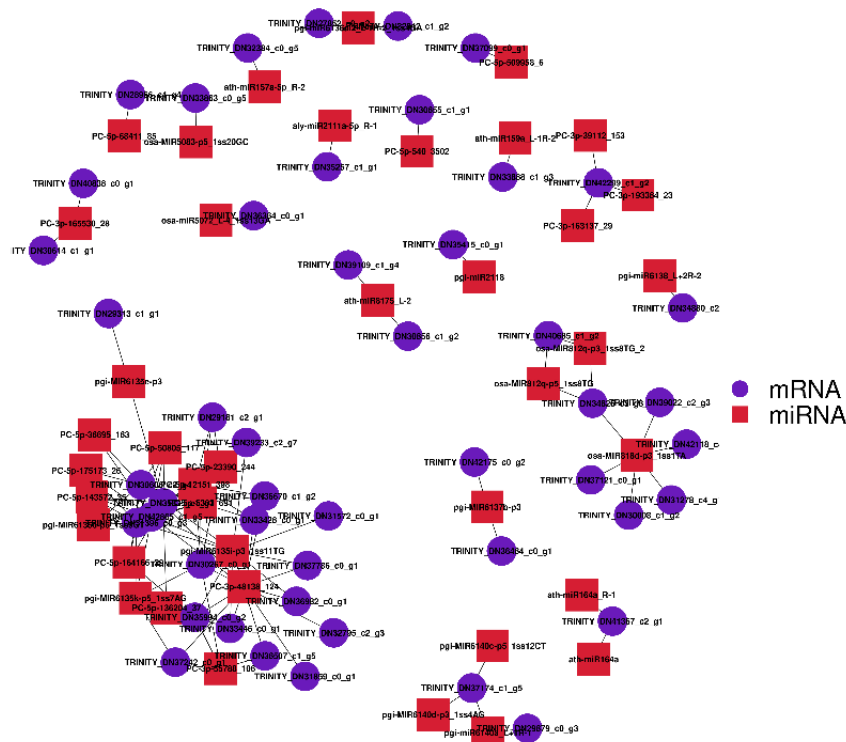

Figure 12. Interacted network of miRNA-mRNA in ginseng

Discussion

In this paper, we investigated the differential expression of miRNAs in CK and CCO. The functional pathways of the differentially expressed miRNAs were analyzed by GO enrichment analysis, KEGG pathway, and the regulatory networks of mRNAs and miRNAs were established. These findings suggest that miRNAs play an important regulatory role in the formation of ginseng crop disorder.

Non-coding RNA (ncRNA, non-coding RNA) is a class of RNA molecules that cannot be translated into proteins. Based on their morphology and function, ncRNAs can be classified into various types. With the continuous exploration of the function of non-coding RNAs, the regulatory mechanisms between various types of RNAs have been gradually explored (Yuan et al., 2016; Rokavec et al., 2017), and the reciprocal networks at the transcriptional level are becoming more and more negligible. Transcriptome analysis methods have provided the basis for the study of many plant diseases, and numerous studies have previously identified miRNA responses to abiotic stresses and biotic infestations in plants (Dong et al., 2018; Sun et al., 2018; Wang et al., 2017; Zhao et al., 2017; Xu et al., 2013; Zhou et al., 2012; Sunkar et al., 2012; Shan et al., 2020). At present, the transcriptional regulatory mechanisms of mRNAs and miRNAs in continuous ginseng are still unclear. Therefore, in this paper, we analyzed miRNAs in continuous ginseng and predicted the functional and regulatory roles between mRNAs and it, which is important for further in-depth and comprehensive study on the mechanism of continuous crop disorder in ginseng.

A total of 376 miRNAs were obtained in the samples through rigorous screening and prediction by various bioinformatics software and online tools, and the necessary characterization of miRNAs in the samples was carried out. Then, differential expression analysis and clustering analysis of miRNAs between crop ginseng and control ginseng showed that 121 miRNAs were significantly differentially expressed in crop ginseng.

In order to understand the biological functions and potential regulatory mechanisms of miRNAs in crop ginseng, target gene prediction was performed on the screened differentially expressed miRNAs, and then the obtained target genes were subjected to GO and KEGG functional enrichment analysis. The results of GO enrichment analysis of differentially expressed miRNAs showed that compared with healthy ginseng, miRNAs were linked to the "auxin-activated signaling pathway", "cell differentiation", "meristem initiation", "transcription, DNA-templated" The high enrichment of processes suggests that differential miRNAs may perform relevant regulatory roles in the composition of multiple regulatory processes and cellular structures in ginseng.

In the results of KEGG enrichment analysis of the target genes of differential miRNAs, it was found that the target genes of miRNAs were involved in phytohormone-related pathways. This indicates that the hormone anabolic pathway may be significantly altered in continuous-crop ginseng and miRNAs play an important regulatory role in it. These results suggest that miRNAs may be involved in the regulation of ginseng stress resistance in continuous ginseng. In addition, the differential miRNA target genes in continuous ginseng were significantly enriched in the biosynthesis of various primary and secondary metabolites such as terpenoids, glycans, and amino acids. This again demonstrates that there are significant differences in metabolic and biological processes in continuous ginseng compared to control ginseng. Meanwhile, miRNAs play a corresponding regulatory role in these metabolic processes.

In order to understand the interactions between mRNAs and miRNAs, correlations between differentially expressed mRNAs and miRNAs from CK and CCO were analyzed, and interactions networks were constructed between differentially expressed mRNAs and miRNAs obtained from CK and CCO (Seitz, 2009; Salmena et al., 2011; Lu et al., 2019; Wang et al., 2018), and a more in-depth characterization of miRNA-mRNA interactions associated with crop disorders in ginseng. The miRNA-mRNA network was constructed by comparing the target genes of differentially expressed miRNAs with mRNAs. Several miRNAs, such as osa-MIR818d-p3_1ss1TA and pgi-MIR6135i-p3_1ss11TG, were found to be at the core of the network, and it was inferred that these miRNAs might play a very important role in the formation of ginseng crop disorder.

Conclusion

In this study, miRNAs were identified in ginseng and control ginseng, and functional enrichment analysis was performed on the target genes of the screened differentially expressed miRNAs. The 376 miRNAs were obtained in CK and CCO, and the obtained miRNAs were characterized. Then, GO and KEGG analyses of the target genes of differentially expressed miRNAs revealed that differentially expressed miRNAs play important regulatory roles in phytohormone-related regulation, transcriptional-translational processes, MAPK signaling pathways - plant, amino and nucleotide sugar metabolism, and monoterpene biosynthesis in the continuous cropping ginseng. Finally, the correlation between miRNAs and mRNAs was integrated, the corresponding interaction networks were constructed and miRNAs that may play a key role in ginseng crop disorder were identified. these results provide a basis for revealing the molecular mechanism of ginseng crop disorder and also enrich the understanding of miRNAs in ginseng continuous crop. The study on the molecular mechanism of continuous cropping obstacle of ginseng can provide reference for cultivation and breeding of ginseng, and contribute to breeding more resistant varieties of ginseng. It can also provide thoughts on the causes of ginseng crop disorders in order to solve them from the perspective of soil improvement. Also provide support for the sustainability of the ginseng industry.

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