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Effects of irrigation on the physiological characteristics of *Panax ginseng* molecular mechanisms of ginsenoside biosynthesis

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ABSTRACT

In 3-years-old ginseng plants grown in pot culture, we determined the changes in ginsenoside content, resistance physiological indicators, key enzyme genes activities in the biosynthetic pathways under 3-irrigation levels (I-40, I-60, I-80 field capacity). The correlation analysis showed that soil relative water content of I-60 has short-term stimulatory effects on the accumulation of potopanaxadiol (PPD) and protopanaxatriol (PPT) -type ginsenosides. The I-60, I-80 field capacity water content had significant effects on the growth of ginseng. Squalene synthetase (*SS*), β-amyrin synthase (*AS*), peroxidase (POD), malondialdehyde (MDA) contents were significantly correlated with ginsenosides biosynthesis. The secondary metabolism of ginseng includes the accumulation of ginsenosides, changes in physiological indicators of resistance gene expression of key enzymes in the synthesis pathway of ginsenosides. The secondary metabolism of ginseng may be a compensation mechanism for ginseng plants to adapt to soil water content. Therefore, for higher yield and better quality of ginseng, it should be cultivated in areas where relative soil water content is I-60.

Keywords: Enzymes, gene expression, ginsenoside, irrigation, *Panax ginseng*, physiological processes, quality, water regime, yield.

INTRODUCTION

Panax ginseng C. A. Meyer (family Araliaceae) is a perennial herbaceous medicinal plant (27). It is cultivated in large areas, its dry roots, rhizomes, stems and leaves are used as medicine (10) mainly in 3-Chinese north-eastern provinces (Jilin, Liaoning, Heilongjiang). The quality of ginseng from the Changbai Mountain production area is best. The main medicinal component of ginseng is triterpene saponins (31), which has various pharmacological effects such as improving immunity, the cardiovascular system, anti-aging tumor (5,7,17,18,24,25). Ginsenosides are classified into potopanaxadiol (PPD), protopanaxatriol (PPT), oleanane type saponins (OTS) based on different aglycones (15,26).

Plant physiological characteristics changes under biotic and abiotic stresses (33-35). Malondialdehyde (MDA) is indicator of abiotic stress and is the end product of membrane lipid peroxidation after oxidative damage (32). Under cold stress, MDA content increases with increasing membrane lipid peroxidation (20). Plants primarily respond to the damage induced by these oxides by controlling the antioxidant system (3) and producing antioxidant enzymes [superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)] and

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*Correspondence author, Changchun University of Chinese Medicine, Changchun, 130117, China antioxidant compounds. When plants face changes in the external environment, their cell membranes receive signals to induce the accumulation of plant osmotic adjustment substances. Their accumulation improves the antioxidant enzymes, which transmits the endogenous signals for the synthesis of secondary metabolites (30). Ginsenosides are the main active ingredients in *P. ginseng* and water supply is important for their biosynthesis. The biosynthesis of ginsenosides is regulated by the key enzyme genes in the complex synthesis pathways of plants. The key genes in these synthesis pathways are: *HMGR, FPS* (famesyl pryophosphate synthase), *SS* (squalene synthetase), *AS* (β-amyrin synthase) and *CYP* (Cytochrome P450) (9,28,30).

Environmental changes affects the plant gene expression, physiological and biochemical processes in plants, plant growth and the synthesis of secondary metabolites (39). Among them, signal molecules generation depends on the soil moisture content, which promotes the synthesis and accumulation of secondary metabolites. The soil water content regulates the accumulation of secondary metabolites in medicinal plants like ginseng. However, few reports showed the effects of soil moisture content on regulation mechanism, physiological and biochemical changes. The ginsenosides, physiological changes and expression of key synthesis enzyme genes in ginseng are important for its allelopathic effects. Various soils moisture regimes were maintained to study their effects on contents of ginsenosides, physiological changes and expression of key synthesis enzyme genes. This study aimed to find the effects of various soil moisture regimes on the secondary metabolites accumulation in ginseng for allelopathic researches. To improve the ginseng cultivation technology and improve the quality of ginseng.

MATERIALS AND METHODS

Materials

Three-year-old healthy and pest free ginseng (*Panax ginseng*) variety "Erma Ya" plants were purchased from Fusong County, Jilin Province, China for the pot experiment. The pots (16 cm deep, upper dia 23 cm and base dia 13 cm) were buried in the soil to maintain the temperature similar to soil. The soil water content was maintained at 60 % (I-60) field capacity. To prepare pot soil, in the 2200 Kg soil 17.5 Kg perlite and 75 Kg vermiculite was mixed. There were 3-plants per pot, which were regularly watered, weeded, fertilized and sprayed with pesticides.

Experimental treatments

The study consisted of two factors : (a). 3- soil moisture regimes/levels (i). I-40 (40 % Field capacity), (ii). I-60 (60 % Field capacity), (iii). I-80 (80 % Field capacity) and (b) 7-sampling Dates (July 30, August 10, 20, 30, September 10, 20, 30) (Table 1).

Table 1. Irrigation regimes

Treatment	Soil Moisture regime/level
I-40	40 % Field capacity
I-60	60 % Field capacity
I-80	80 % Field capacity

Experimental design

The pot experiments were conducted in the Herbal garden, Changchun University of Traditional Chinese Medicine (N43°8', E125°4') from July to September, 2019. Healthy and uniform ginseng plants were selected for the study. Each treatment cultivated 30 pots of ginseng, a total of 90 pots, and planted 3 ginseng plants in each pot. Three pots were taken from each treatment group to ensure that three biological replicates were used for subsequent analysis. In this study, three relative soil moisture conditions (I-40, I-60 and I-80 of field capacity) were maintained and watered every 5-days. The plant samples were collected at 10-days intervals on 7-Dates (July 30, August 10, 20, 30, September 10, 20, 30). Three pots were sampled per Date for each treatment i.e. total of 9 ginseng plants were collected as sample. The collected ginseng plants were placed in an ice box and brought to laboratory. The roots were immediately washed clean with water, frozen with liquid nitrogen and stored at -80 °C. The remaining parts of plants were dried in oven at 60 °C until the constant weight. It was then ground into a fine powder with high-speed mill and passed through 60-mesh sieve to determine the ginsenosides contents.

Root Fresh and dry weight

The roots of *Panax ginseng* were washed by tap water, rinsed thrice with distilled water and the surface moisture was wiped with filter paper. The fresh weight (FW) was recorded, thereafter, roots were oven dried at 45 °C in oven till constant weight and the dry weight (DW) was recorded.

Solutions preparation

The ginseng powder (1.0 *g*) was placed in an Erlenmeyer flask and extracted as per the pharmacopeia method with slight modification (12). Thereafter, 30 mL methanol was added and extracted by ultrasonication at 90 Hz for 30 min. The supernatant was filtered and the procedure was repeated thrice. The filtrates were combined and evaporated in dish on water bath at 60 °C. The volume was made to 5 mL in a volumetric flask with a 0.22 μm pinhole filter for HPLC analysis (6). Measurements were done in triplicate.

To establish the calibration curves of 11 ginsenoside standards (Ro, Rg1, Re, Rf, Rb1, Rc, Rb2, Rb3, Rd, Rh2 and Rg3). We weighed 11 kinds of ginsenoside monomer standard substances, added methanol to prepare a solution of reference substance containing 0.5 mg per Ml of Ro, Rg1, Re, Rf, Rb1, Rc, Rb2, Rd, Rh2, Rb3 and Rg3. It was diluted to 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml, 0.03125 mg/ml for each reference substance. The solutions were filtered by 0.22 μm membrane and stored at 4 °C.

HPLC analysis

The prepared samples were analyzed using an Agilent 1260 HPLC system (Agilent, USA) and separated in an Elite Hypersil (250 mm × 4.6 mm, 5 μm) column. The mobile phase was composed of A (water) and B (acetonitrile). The gradient elution was performed as follows: 0-18.0 min, 19-23 % B; 18.1-28.0 min, 23 %-28 % B; 28.1-30.0 min, 28 %-32 % B; 30.1-50.0 min, 32 %-34 % B; 50.1-70.0 min and 34 %-80 % B. The injection volume was 10 μL, the column temperature was 25 °C, UV measurements were obtained at 203 nm and the flow rate was 1.0 mL/min (38).

Osmoregulatory substances and malondialdehyde content

In an ice bath, 0.5 *g* fresh root tissues were homogenized by grinding in 5 mL of phosphate-buffered saline (0.05 mol·L^{-1}, pH 7.8). The homogenates of fresh root tissues were assayed with SP, Pro, SS and MDA kits (NJBI). The absorbance of reaction solutions was measured at 595, 520, 620 and 530 nm using an enzyme-labeling instrument (SpectraMax 190, Molecular Devices, USA); the values obtained were used to calculate the concentrations of SP, Pro, SS and MDA, respectively (36).

Antioxidant enzymes extraction and their activity

The method of crude enzyme extract preparation used in this study was modified from a previously published method (1). The samples were ground into homogenates and transferred into centrifuge tubes; afterwards, phosphate-buffered saline (0.05 mol·L^{-}1, pH 7.8) was added up to final volumes of 5 mL. The samples were then centrifuged at 4 °C and 10000 r·min^{-1} for 10 min. SOD, POD and CAT activities were assayed using kits (NJBI) and their activities were expressed as units per milligram of protein.

Extraction of RNA and gene expression of key enzymes

Total ginseng RNA was extracted using a TaKaRa MiniBEST Universal RNA Extraction Kit (TaKaRa, CA, Japan) and 1.0 µg RNA was used for reverse transcription with a PrimeScript^{TM} RT Master Mix kit (TaKaRa, CA, Japan) in a 20 µL reaction volume. The product was stored at −20 °C. *GAPDH* was used as an internal control and the relative gene expression levels of *HMGR, SS, AS, CYP716A47, CYP716A52v2* and *CYP716A53v2* were determined (Table 2). Reverse transcriptase quantitative PCR (RT-qPCR) was done in 96-well plates in a Stratagene Mx3000P thermocycler (Agilent, Palo Alto, CA, USA) with an SYBR Green-based PCR assay. The final volume for each reaction was 20 µL with the following components: 1 µL diluted cDNA template (1 mg/mL), 10 µL SYBR Green Mix (TaKaRa, DaLian, CA, Japan), 1 µL forward primer (1 mM), 1 µL reverse primer (1 mM) and 7 µL ddH$_2$O. The reaction was performed under the following conditions: 95 °C for

Table 2. Real-time fluorescence quantitative PCR primers.

Gene	Accession No.	Primer sequence 5′-3′	Product length (bp)
GAPDH	KY400031	F: ATGGACCATCAGCAAAGGAC R: GGTAGCACTTTCCCAACAGC	117
HMGR	JX648390	F: TCTTCAAAGCCTCTGATGC R: TTTTGGGGATTGGATTTGTCA	126
SS	AB115496	F: GGACTTGTTGGATTAGGGTTG R: ACTGCCTTGGCTGAGTTTTC	107
AS	AB009030	F: GCGGAAGGGAATAAGATGAC R: CTCAGCTCTCCGGACAGC	108
CYP716A53v2	JX036031	F: ATCGGACAACGAGGCAGCAC R: GCCAACAGGCCAACTCAA	102

3 min, followed by 40 cycles of denaturation at 95 °C for 5 s, annealing at 55 °C for 32 s and extension at 72 °C for 20 s. The melting curve was obtained by heating the amplicon from 55 °C to 95 °C with increments of 0.5 °C per 5 s. Each RT-qPCR analysis was performed with three replicates. The relative gene expression levels were computed using the $2^{-\Delta\Delta Ct}$ method (36).

Statistical analysis

MS Excel 2016 (Microsoft Corp., Redmond, WA, US) was used to sort the original data and IBM SPSS Statistics 19.0 (IBM Corp., Armonk, NY, US) was used for single-factor variance analysis. GraphPad prism 6.0 (GraphPad Software Inc., San Diego, CA, US) and Origin 9.0 (OriginLab, Northampton, MA, US) were used for graphic illustration.

RESULTS AND DISCUSSION

Root fresh and dry weight

The effects of soil water regimes on ginseng root fresh weight were illustrated in Figure 1. The results showed that the fresh weight of plants increased slowly with treatments and significantly differed between the treatments during the water control period. During the moisture control period, the relative water content of soil was determined every morning in I-40, I-60, I-80 treatments. The treatments in which the water content was found less, measured quantity of water was added to maintain the moisture content. The fresh weight during September 10-30 showed significant differences. The ginseng root tissue in I-40 soil water regimes increased slowly during the entire water control period, which might be due to the dried soil, leading to slower metabolism in the plant. However, each treatment fresh

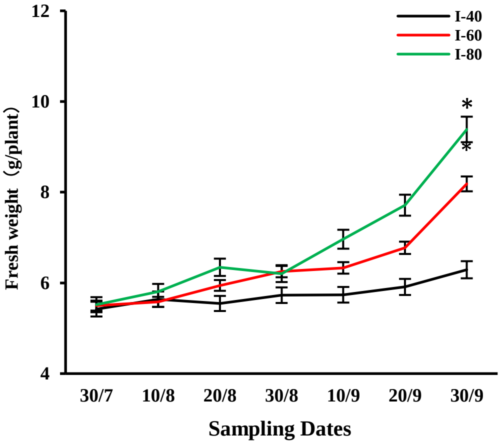


Figure 1. Effects of irrigation regimes on fresh weight of ginseng. *$P < 0.05$. **$P < 0.01$, compared with control. Vertical bars indicate the mean value ± standard deviation from 3- experiments. Note: 30/7: July 30, 10/8: August 10, 20/8: August 20, 30/8: August 30, 10/9: September 10, 20/9: September 20, 30/9: September 30.

weight reached its maximum value on September 29 and the fresh weight of various soil moisture regimes differed significantly. Among them, the minimum fresh weight (6.29 g) was in I-40 soil moisture regime. The fresh weight of I-60 and I-80 soil moisture regimes were 8.18 g and 9.38 g, respectively, which were 1.32 times and 1.49 times higher than I-40 regime. The changes in fresh weight indicated that I-60 and I-80 water levels were beneficial to the root growth of ginseng than I-40 soil water regime.

The effects of soil water regimes on ginseng root fresh and dry weight were given in Figure 2. The changing trend of dry weight in ginseng was similar to fresh weight. From the initial stage of regulation to August 30, the dry weight of ginseng roots in I-80 soil moisture regimes was higher than other regimes. After August 30, the ginseng root dry weight in I-80 moisture regime was higher than I-40 and I-60 regime and reached maximum (1.84 g) on September 30. While the dry weights of I-60 and I-80 regimes were 1.27 and 1.43 times more than I-40 regime, respectively. The dry weight of ginseng plants in I-40 moisture regime gradually decreased after August 30 due to the lack of soil moisture that slowed the plant metabolism and consumed secondary metabolites to overcome drought conditions. Both I-60 and I-80 moisture regimes promoted the dry weight than I-40 moisture regime.

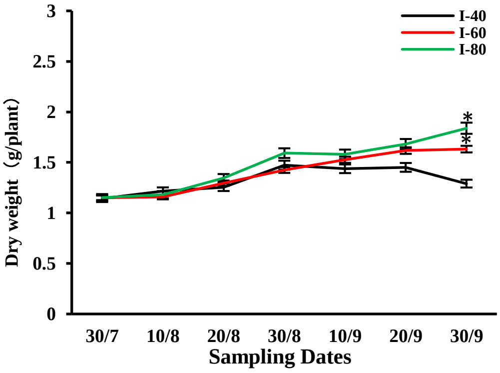


Figure 2. Effects of irrigation regimes on dry weight of ginseng. *$P < 0.05$. **$P < 0.01$, compared with control. Vertical bars indicate the mean value ± standard deviation from 3- experiments. Note: 30/7: July 30, 10/8:August 10, 20/8:August 20, 30/8:August 30, 10/9:September 10, 20/9:September 20, 30/9:September 30.

Ginsenoside content under water regimes

(i). PPD-type ginsenosides: The influence of water regimes on the content of ginsenoside (Fig. 3). The Figure 3-A showed that in the initial stage (July 30-August 30) of water regulation, PPD-type ginsenosides first increased, then decreased and then increased again. On August 30 maximum ginsenosides contents were 8.32 mg·g^{-1}, 8.92 mg·g^{-1} and 8.57 mg·g^{-1} on August 30, were in I-40, I-60, I-80 soil moisture regimes, respectively. From August 30 to September 30 only I-80 soil moisture regimes had stimulatory effects, while the other regimes were inhibitory. On September 20, the ginsenosides content in I-80 soil moisture regimes was 1.26 times higher than I-40 regime.

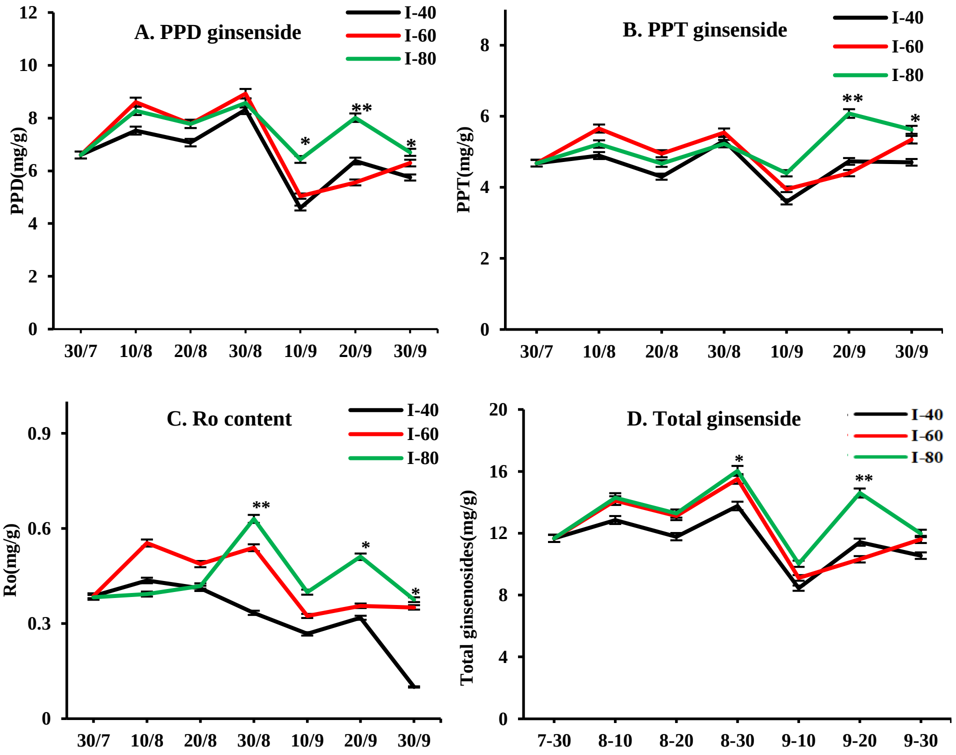


Figure 3. Effects of irrigation regimes on ginsenosides.

A: Ginsensides of PPD. B: Ginsenside of PPT. C: Ginsenside of Ro. D: Total ginsensides. *$P < 0.05$. **$P < 0.01$, compared with control. Vertical bars indicate the mean value ± standard deviation from 3- experiments. Note: 30/7: July 30, 10/8:August 10, 20/8:August 20, 30/8:August 30, 10/9:September 10, 20/9:September 20, 30/9:September 30.

(ii). PPT-type ginsenoside: The change in content of PPT-type ginsenoside was shown in Figure 3-B. The PPT-type ginsenosides did not change significantly at the initial stage of water regulation. On August 30, the I-40 and I-60 soil moisture regimes PPT-type ginsenoside reached their maximum values of 5.31 $mg \cdot g^{-1}$ and 5.54 $mg \cdot g^{-1}$, respectively. On September 20, in I-80 moisture regimes PPT-type ginsenoside contents reached the maximum value of 6.08 $mg \cdot g^{-1}$.

(iii). Ro content: Ro contents were shown in Figure 3-C. The Ro contents in ginseng Plants grown at I-60 and I-80 soil moisture regimes were higher than I-40 regime i.e. higher soil moisture promoted the synthesis of ginsenoside Ro. On August 30, the Ro contents in I-80 soil moisture reached the maximum content of 0.63 $mg \cdot g^{-1}$ which was 1.91 times higher than I-40 moisture regime ($P < 0.01$).

(iv). Total saponins: Their contents changes were shown in Figure 3-D. The changes in total saponins contents were similar in all treatments. The moisture status of I-60 and I-80 promotes the synthesis of ginsenosides. On August 30, the total saponins content in ginseng plants I-60 and I-80 were maximum: 15.51 mg·g-1 and 16.03 mg·g-1, respectively and were 1.17 times and 1.13 times higher than I-40 respectively. Around September 10, due to continuous rainfall, high air humidity and high soil water content and ginseng was close to maturity (Harvest period end September to mid-October), these were not conducive to the accumulation of ginsenosides, hence significantly decreased the ginsenoside content. The metabolites in the plant were transformed into energy storage substances, which might decrease the content of secondary metabolites (23). Therefore, the soil moisture content of I-80 is suitable for the growth of ginseng and the accumulation of medicinal ingredients.

Environmental conditions seriously affects the synthesis and accumulation of saponins in ginseng (37). The content of PPD-type ginsenoside and Ro in the I-40 soil moisture at the initial stage was significantly low ($P < 0.01$) than other moisture regimes, indicating that the relative soil water content of I-40 inhibited the accumulation of ginsenosides. At the initial stage, the relative soil water content of I-60, promoted the synthesis of PPD, PPT-type ginsenosides and Ro, but inhibited the synthesis of saponins in the later stage of experiment, which was similar to the results of Bao *et al.* (2). The short-term water deficit significantly affected the accumulation of ginsenosides, indicating that the secondary metabolism of ginseng was more sensitive to the water availability at initial stage. The saponin content suddenly decreased on September 10, it might be due to rainy days in early September, high moisture content in the soil and high air humidity and the overwinter buds were growing on main roots of ginseng. The substances synthesized in the plants might be converted into energy storage materials. In overwintering buds, the effective ingredients were reduced. In summary, the accumulation of ginsenosides by regulating the soil moisture levels have short-term effects.

Malondialdehyde and osmoregulatory substances content

Enzymes :

(i). SOD: The change in SOD activity with soil moisture content was shown in Figure 4-A. The I-80 soil moisture content reached the maximum value of 27.31 U·mg·prot^{-1} on September 10, which was 1.06 and 1.02 times higher than I-40 and I-60 soil moisture regimes. With the extension of treatment time, the SOD activity decreased due to the long-term water regulation that reduced the SOD activity in the roots and could not remove the excess oxygen free radicals produced in the plant.

(ii). POD: The influence of water regulation on POD activity was shown in Figure 4-B. The overall trend was increasing-decreasing-increasing. From August 30 to September 10, POD activity decreased significantly. At this time, the trend of POD activity was opposite to SOD activity. SOD is the first line of defence against oxidation in plant cells and its higher activity removes the excess superoxide ions. After the increase in SOD enzyme activity, the POD enzyme activity was enhanced. The POD and SOD enzyme converts the superoxide H_2O_2 present in plant cells into O_2 and H_2O, which are less harmful to cells.

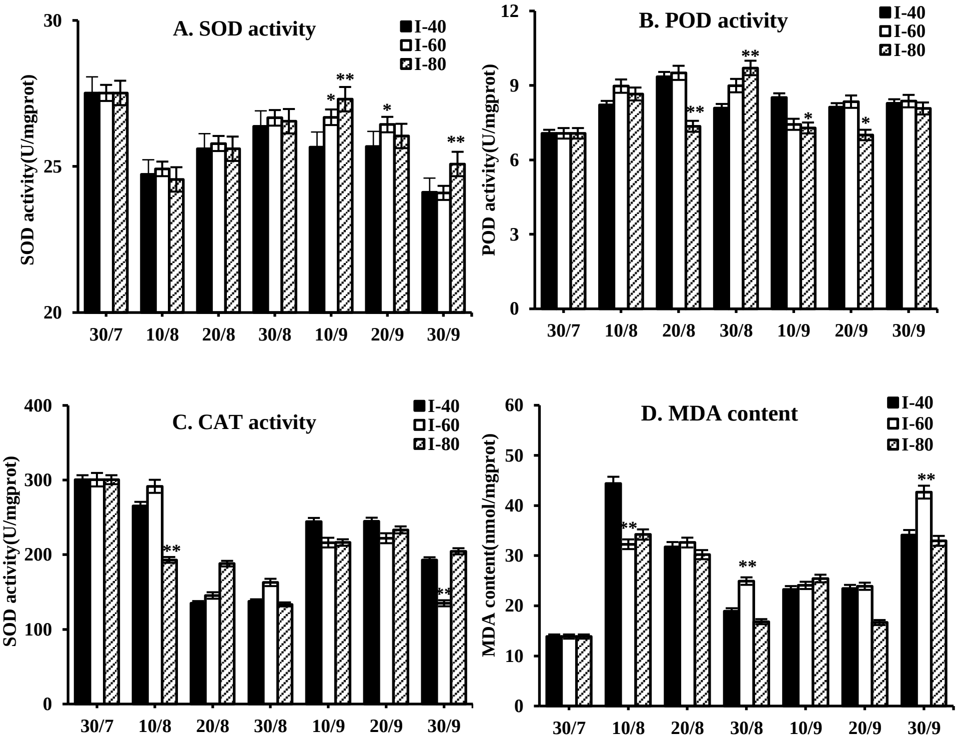


Figure 4. Effects of irrigation regimes on osmoregulatory substance and malondialdehyde.

A: SOD activity, B: POD activity, C: CAT activity. D: MDA content. $*P < 0.05$. $**P < 0.01$, compared with control. Vertical bars indicate the mean value ± standard deviation from 3- experiments. Note: 30/7: July 30, 10/8:August 10, 20/8:August 20, 30/8:August 30, 10/9:September 10, 20/9:September 20, 30/9:September 30.

(iii). CAT: The influence of water regulation on CAT activity was shown in Figure 4-C. The trends of CAT activity and SOD activity were identical. On September 10, the I-40 soil moisture content had the highest activity of 244.38 U·mgprot^{-1}, which was 1.12 times and 1.13 times higher, respectively than of I-60 and I-80 soil moisture. In scavenging oxygen free radicals in plant cells, antioxidant enzymes acted synergistically.

(iv). MDA: The influence of moisture regimes on the MDA content was shown in Figure 4-D. The trend of MDA content, first declined and then increased. MDA is an indicator of lipid membrane peroxidation and its content directly reflects the degree of damage to plant cell membranes. In the initial stage of water regulation, the MDA content in I-40 soil moisture was significantly higher than other moisture regimes, which were 1.38 and 1.30 times higher than I-60 and I-80 regimes, respectively. During the water regulation period, the MDA content in I-80 soil moisture was lower than other groups, indicating that its cell membrane was less damaged.

In the initial stage of water regulation, as the soil water content decreased, the MDA content increased and the activities of POD and CAT decreased. This is similar to the results of Zhang *et al.* (37). The SOD activity decreased at the beginning, increased shortly after the treatment and then decreased, which is same as the results of Liu *et al.* (16). This might due to the fact that the content of reactive oxygen species (ROS) under water stress exceeded the cell's ability to withstand it. The peroxidative damage of cell lipid membrane was severe, which weakened the synthesis and accumulation of intracellular proteins and other compounds, decreasing the antioxidant enzyme activities (4,13).

Key enzymes response to water regimes

The expression results of key enzyme genes in the ginsenoside biosynthesis pathway were shown in Figure 5. In the first sample taken on July 30 as a control (By default, the first point of the real-time fluorescent quantitative PCR test is the control blank, and the basic value is 1.), the real-time fluorescent quantitative PCR results showed that the response of key enzyme genes in ginsenoside synthesis differed with water regimes.

(i). *HMGR* gene: Its expression level during the whole treatment period was shown in Figure 5-A. On August 20, the gene expression level at I-40 and I-60 moisture levels was significantly higher than I-80 moisture level. It might be due to short-term water stress that stimulated the gene expression. On September 10, the gene expression in all soil moisture regimes decreased than other sampling periods. The gene expression at I-40 moisture regime was 1.54 times higher than I-80 moisture regimes.

(ii). *SS* gene: The changes in its expression in response to soil moisture regimes were shown in Figure 5-B. On August 30, the gene expression in I-40 moisture regimes was 3.38 and 1.89 times higher than I-60 and I-80 moisture levels, respectively. The I-80 moisture level expression reached its maximum on September 20.

(iii). *AS* gene : The changes in its expression under water regimes were shown in Figure 5-C. From September 20 to September 30, this gene expression in I-80 moisture level was 2.41 and 2.32 times higher than I-40 moisture level. In the early stage of water regulation, the gene expression in I-40 soil moisture level was active but gradually decreased with time. While the expression level in I-80 moisture level showed an upward trend with increased treatment time and reached the maximum on September 20.

(iv). *CYP716A53v2* gene : During the entire water regulation period, its expression was shown in Figure 5-D. This gene in I-60 and I-80 moisture levels was more active. The I-60 moisture regime reached the maximum value of 1.13 in the initial stage of water regulation and the I-80 moisture regime reached the maximum value of 1.15 at the end. The gene in I-40 soil moisture level reached the maximum value on August 10.

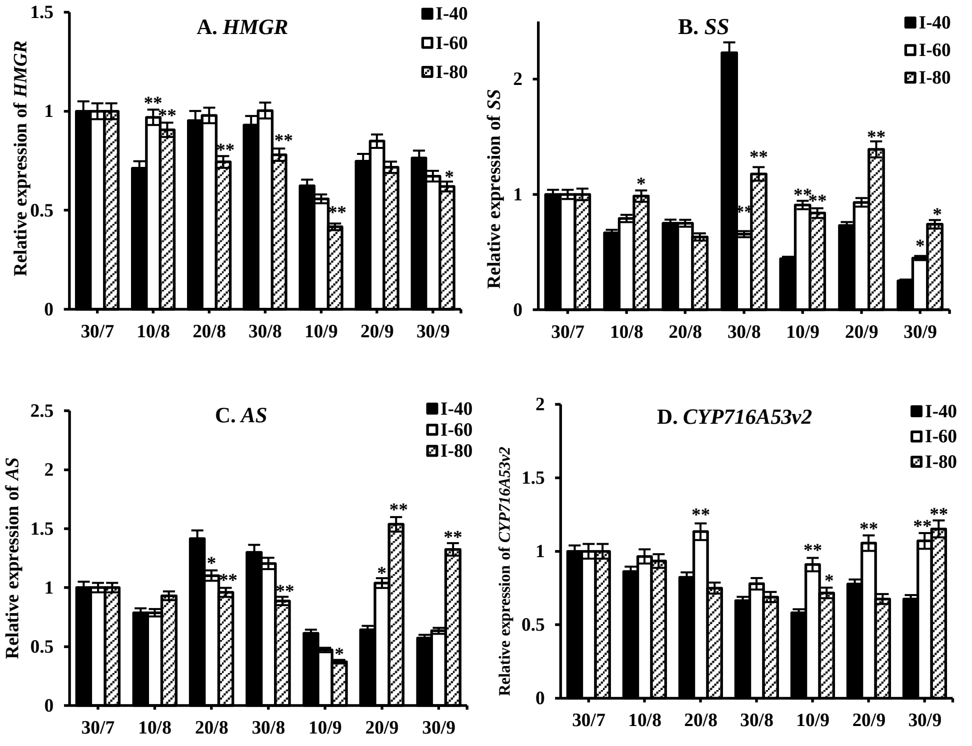


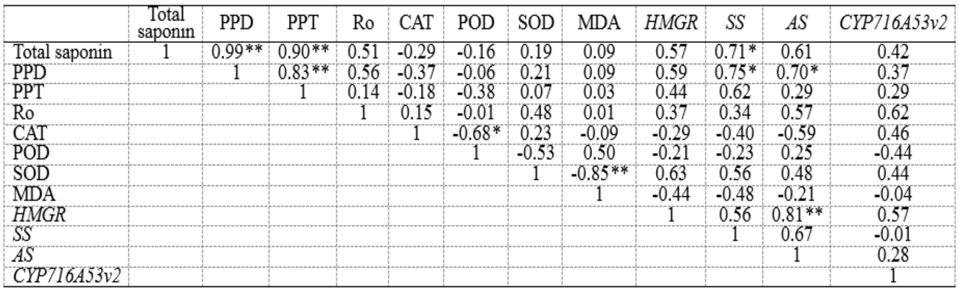
Figure 5. Effects of irrigation regimes on gene expression of key enzyme.

A: *HMGR* expression, B:*SS* expression, C: *AS* expression. D: *CYP716A53v2* expression. *$P < 0.05$. **$P < 0.01$, compared with control. Vertical bars indicate the mean value ± standard deviation from 3- experiments. Note: 30/7: July 30, 10/8: August 10, 20/8:August 20, 30/8:August 30, 10/9:September 10, 20/9:September 20, 30/9:September 30.

Correlations between ginsenoside content and physiological stress indicators

The results of correlation analysis between the ginsenoside content and the physiological indexes of resistance at the relative soil water content of I-40 were shown in Table 3. Total ginsenoside content was significantly positively correlated with PPT-type ginsenosides and PPD-type ginsenosides ($P < 0.01$), as well as *SS* ($P < 0.05$). PPD-type ginsenosides were significantly correlated with PPT-type ginsenosides ($P < 0.01$), with *SS* and *AS* ($P < 0.05$). Seo *et al.* (22) found that enhancing the SS activity of ginseng could increase the content of plant sterols and triterpene saponins, which are similar to our experimental results.

Table 3. Correlation analysis of ginsenosides with key enzyme genes and resistance physiological indicators at I-40 field capacity

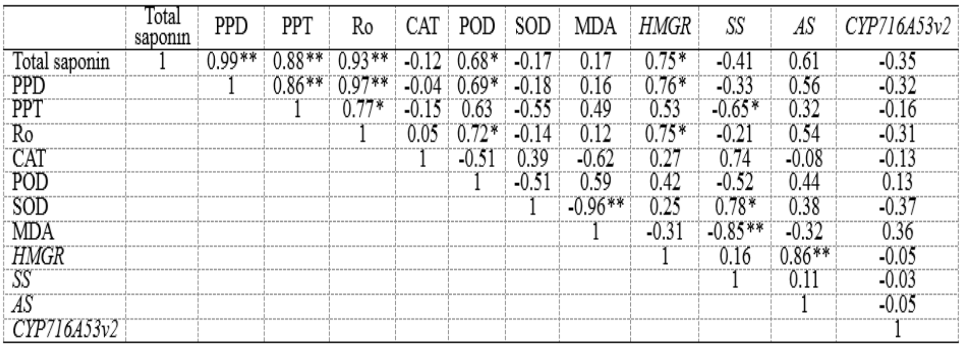


	Total saponin	PPD	PPT	Ro	CAT	POD	SOD	MDA	*HMGR*	*SS*	*AS*	*CYP716A53v2*
Total saponin	1	0.99**	0.90**	0.51	-0.29	-0.16	0.19	0.09	0.57	0.71*	0.61	0.42
PPD		1	0.83**	0.56	-0.37	-0.06	0.21	0.09	0.59	0.75*	0.70*	0.37
PPT			1	0.14	-0.18	-0.38	0.07	0.03	0.44	0.62	0.29	0.29
Ro				1	0.15	-0.01	0.48	0.01	0.37	0.34	0.57	0.62
CAT					1	-0.68*	0.23	-0.09	-0.29	-0.40	-0.59	0.46
POD						1	-0.53	0.50	-0.21	-0.23	0.25	-0.44
SOD							1	-0.85**	0.63	0.56	0.48	0.44
MDA								1	-0.44	-0.48	-0.21	-0.04
HMGR									1	0.56	0.81**	0.57
SS										1	0.67	-0.01
AS											1	0.28
CYP716A53v2												1

SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; MDA: malondialdehyde. *$p < 0.05$. ** $p < 0.01$. Same below.

When the relative soil water content was I-60, the results of the correlation analysis between the ginsenoside content and the physiological indexes of resistance were shown in Table 4. Total ginsenoside content was significantly positively correlated with PPT-type ginsenosides, PPD-type ginsenosides, Ro ($P < 0.01$), as well as POD and *HMGR* ($P < 0.05$). PPD-type ginsenosides were significantly correlated with PPT-type ginsenosides, Ro ($P < 0.01$), as well as POD and *HMGR* ($P < 0.05$). The HMGR gene is the first rate-limiting enzyme of the MVA pathway and plays an important role in the biosynthesis of ginsenosides (11,30).

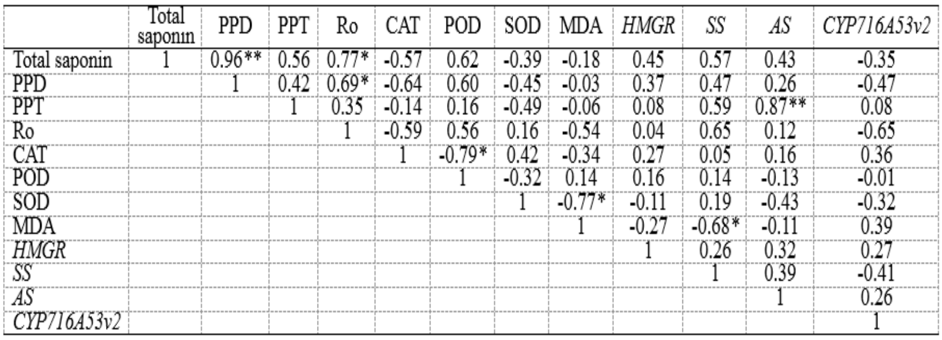
Table 4. Correlation analysis of ginsenosides with key enzyme genes and resistance physiological indicators at I-60 field capacity



	Total saponin	PPD	PPT	Ro	CAT	POD	SOD	MDA	*HMGR*	*SS*	*AS*	*CYP716A53v2*
Total saponin	1	0.99**	0.88**	0.93**	-0.12	0.68*	-0.17	0.17	0.75*	-0.41	0.61	-0.35
PPD		1	0.86**	0.97**	-0.04	0.69*	-0.18	0.16	0.76*	-0.33	0.56	-0.32
PPT			1	0.77*	-0.15	0.63	-0.55	0.49	0.53	-0.65*	0.32	-0.16
Ro				1	0.05	0.72*	-0.14	0.12	0.75*	-0.21	0.54	-0.31
CAT					1	-0.51	0.39	-0.62	0.27	0.74	-0.08	-0.13
POD						1	-0.51	0.59	0.42	-0.52	0.44	0.13
SOD							1	-0.96**	0.25	0.78*	0.38	-0.37
MDA								1	-0.31	-0.85**	-0.32	0.36
HMGR									1	0.16	0.86**	-0.05
SS										1	0.11	-0.03
AS											1	-0.05
CYP716A53v2												1

When the relative soil water content was I-80, the results of correlation analysis between the ginsenoside content and the physiological indexes of resistance were shown in Table 5. Total ginsenoside content was significantly positively correlated with PPD-type ginsenosides ($P < 0.01$) as well as with Ro ($P < 0.05$). PPD-type ginsenosides were significantly correlated with Ro ($P < 0.05$). PPT-type ginsenosides were significantly correlated with *AS* ($P < 0.01$).

Table 5. Correlation analysis of ginsenosides with key enzyme genes and resistance physiological indicators at I-80 field capacity



	Total saponin	PPD	PPT	Ro	CAT	POD	SOD	MDA	*HMGR*	*SS*	*AS*	*CYP716A53v2*
Total saponin	1	0.96**	0.56	0.77*	-0.57	0.62	-0.39	-0.18	0.45	0.57	0.43	-0.35
PPD		1	0.42	0.69*	-0.64	0.60	-0.45	-0.03	0.37	0.47	0.26	-0.47
PPT			1	0.35	-0.14	0.16	-0.49	-0.06	0.08	0.59	0.87**	0.08
Ro				1	-0.59	0.56	0.16	-0.54	0.04	0.65	0.12	-0.65
CAT					1	-0.79*	0.42	-0.34	0.27	0.05	0.16	0.36
POD						1	-0.32	0.14	0.16	0.14	-0.13	-0.01
SOD							1	-0.77*	-0.11	0.19	-0.43	-0.32
MDA								1	-0.27	-0.68*	-0.11	0.39
HMGR									1	0.26	0.32	0.27
SS										1	0.39	-0.41
AS											1	0.26
CYP716A53v2												1

Besides, under different water regulation conditions, there was certain correlations between the physiological indexes of resistance and the expression of key enzyme genes. Ginsenosides had less correlation with physiological indicators of resistance. In contrast, there were significant correlations between the expression levels of key enzymes in the synthesis pathway, antioxidant enzyme activity and osmotic regulators. The results indicated that antioxidant enzyme activities, osmoregulatory substances and key enzyme gene expressions jointly participated in ginseng resistance to water regulation.

The relative water content of soil plays an important role in the growth and quality of ginseng. When the relative water content of soil reached I-60 or less, the fresh and dry weight of plant and the total saponins contents differed significantly, similar to the results of previous studies (14,19). When the relative soil water content was > I-80, the diseases index of root disease was high, root rot was serious and the reed head and body were short. The SS, AS, POD and MDA play an important role in ginseng's response to soil moisture regimes.

In this study, the ginsenosides content was increased in I-60 and I-80 soil moisture regime but was significantly inhibited by I-40 soil moisture level. This is consistent with the results of other studies on the accumulation of secondary metabolites in medicinal plants under water regulation (8,29,32). Water regulation affects the secondary metabolic processes. Under appropriate radix, the ginseng responds by increasing the activities of protective enzymes and the amounts of osmoprotective compounds to protect cells from oxidative stress. This promotes the biosynthesis of ginsenosides via the expression of key enzymes in the biosynthetic pathway and enhances the secondary metabolite accumulation and enzymatic activities, eventually increasing the content of ginsenosides.

CONCLUSIONS

The water supply to *Panax ginseng* activated its antioxidant enzyme system and accumulated the osmoregulatory substances. However, water regulation also influenced the yield of *P. ginseng*. Soil relative water content of I-60 promoted the accumulation of PPD and PPT-type ginsenosides for short time. At the end of water regulation, I-60 and I-80 soil moisture regime significantly affects the growth of ginseng. *SS* and *AS* genes were significantly related to ginsenosides, while, POD and MDA were significantly related to physiological indicators of resistance. They played an important role in ginseng's response to soil moisture levels. Therefore, it is recommended to grow ginseng in areas with relative soil water content of I-60, for higher yield and better quality. This study provided the basis to improve the ginseng quality and maintain the effective contents of medicinal ingredients in ginseng plants.

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CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

REFERENCES

1. Ba, Q.S., Zhang, G.S., Wang, J.S., Che, H.X., Liu H.Z., Niu, N., Ma, S.C. and Wang, J.W. (2013). Relationship between metabolism of reactive oxygen species and chemically induced male sterility in wheat. *Canadian Journal of Plant Science* **93**: 675-681.
2. Bao, J. (2008). *Effects of Different Soil and Different Soil Water Content on Photosynthetic Characterristics and Growth of Panax ginseng*. Dissertation. Jilin Agricultural University, Changchun.
3. Cheng, L., Han, M., Yang, L.M., Yang, L., Sun, Z. and Zhang, T. (2018). Changes in the physiological characteristics and baicalin biosynthesis metabolism of *Scutellaria baicalensis* Georgi under drought stress. *Industrial Crops* and *Products* **122**: 473-482.
4. Ekmekçi, Y., Tanyolaç, D. and Ayhan, B. (2008). Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars. *Journal of Plant Physiology* **165**: 600-611.
5. Fang, N.M.C.H. (2018). Qualitative and quantitative analysis of the saponins in *Panax notoginseng* leaves using ultra-performance liquid chromatography coupled with time-of-flight tandem mass spectrometry and high-performance liquid chromatography coupled with UV detector. *Journal of Ginseng Research* **42**: 149-157.
6. Guo, S., Yang, L., Zhang,Y., Yang, L. and Han, M. (2015). Analysis of microwave-assisted extraction of total saponins and ginsenosides in ginseng. *Food Science* **36**: 1-6.
7. Ji, Y., Rao, Z., Cui, J., Bao, H., Chen, C., Shu, C. and Ru, G.J. (2012). Ginsenosides extracted from nanoscale chinese white ginseng enhances anticancer effect. *Journal of Nanoscience* and *Nanotechnology* **12**: 1-5.
8. Jia, X., C. Sun, G. Li, G. Li and G. Chen. (2015). Effects of progressive drought stress on the physiology, antioxidative enzymes and secondary metabolites of *Radix astragali*. *Acta Physiologiae Plantarum* **37**:1-14.
9. Juxin, Y., Zhang, D., Zhuang, J., Huang, Y., Mu, Y. and Lv, S. (2017). Study on the correlation between gene expression and enzyme activity of seven key enzymes and ginsenoside content in ginseng in over the time in Ji'an, China. *International Journal of Molecular Sciences* **18**: 1-12.
10. Kim, J.H., Yi, Y.S., Kim, M.Y. and Cho, J.Y. (2017). Role of ginsenosides, the main active components of *Panax ginseng*, in inflammatory responses and diseases. *Journal of Ginseng Research* **41**: 435-443.

11. Kim, Y.J., Lee, O.R., Oh, J.Y., Jang, M.G. and Yang, D.C. (2014). Functional analysis of 3-hydroxy-3-methylglutaryl coenzyme a reductase encoding genes in triterpene saponin-producing ginseng. *Plant Physiology* **165**: 373-87.
12. Kuang, L., Li, C., Cao, S., Li, H., Wang, Z., Wu, S. and Fan, Y. (2020). *Pharmacopoeia of People's Republic of China*. China Medical Science and Technology Press, Beijing.
13. Lee, D. H. and Lee, C. B. (2000). Chilling stress—induced changes of antioxidant enzymes in the leaves of cucumber: in gel enzyme activity assays. *Plant Science* **159**: 75-85.
14. Li, J., Zhang, J., Jia, H., Yue, Z., Lu, M., Xin, X. and Hu, J. (2018). Genome-wide characterization of the shsp gene family in *Salix suchowensis* reveals its functions under different abiotic stresses. *International Journal of Molecular Sciences* **19**: 1-16.
15. Liu, G., Li, X., Wang, N., Zhou, H., Wei, W., Gui, M., Yang, B. and Jin, Y. (2010). Three new dammarane-type triterpene saponins from the leaves of *Panax ginseng* C.A. Meyer. *Journal of Asian Natural Products Research* **10**: 865-873.
16. Liu, P., Wen-shan, G., Han-chun, P., Chao-nian, F. and Xin-kai, Z. (2006). Effects of high temperature on antioxidant enzymes and lipid peroxidation in flag leaves of wheat during grain filling period. *Agricultural Sciences in China* **5**: 425-430.
17. Liu, W., Wang, Z., Hou, J.G., Zhou, Y.D., He, Y.F., Jiang, S., Wang, Y.P., Ren, S. and Li W. (2018). The liver protection effects of maltol, a flavouring agent, on carbon tetrachloride-induced acute liver injury in mice via inhibiting apoptosis and inflammatory response. *Molecules* **23**: 1-16.
18. Lo'Ay, A.A. and Elkhateeb, A.Y. (2018). Antioxidant enzyme activities and exogenous ascorbic acid treatment of 'Williams' banana during long-term cold storage stress. *Scientia Horticulturae* **234**: 210-219.
19. Oh, J.Y., Kim, Y., Jang, M., Joo, S.C., Kwon, W., Kim, S., Jung, S. and Yang, D. (2014). Investigation of ginsenosides in different tissues after elicitor treatment in *Panax ginseng*. *Journal of Ginseng Research* **38**: 270-277.
20. Rahimi, Y., Taleei, A. and Ranjbar, M. (2018). Long-term water deficit modulates antioxidant capacity of peppermint (*Mentha piperita* L.). *Scientia Horticulturae* **237**: 36-43.
21. Seo, J.W., Jeong, J.H., Shin, C.G. and Lo, S.C. (2005). Over expression of squalene synthase in *Eleutherococcus senticosus* increases phytosterol and triterpene accumulation. *Phytochemistry* **66**: 869-877.
22. Shao, H.H., Zhang, G.S., Liu, G., Zhang, K.M. and Zhang, Z.L. (2021). Correlation analysis of soil microorganisms and saponins from different growing regions of *Panax notoginseng*. *Allelopathy Journal* **53**:189-210.
23. Sung-Dae, Kim Young-Joo, Ki Jung-Sik, Huh Sae-Woong and Kim Sohn, D. (2013). Improvement of erectile function by Korean red ginseng (*Panax ginseng*) in a male rat model of metabolic syndrome. *Asian Journal of Andrology* **15**: 395-399.
24. 24. Thiyagarajan, R., Kim, S.W., Hwan, J., Yoo, S.K. and Kim, S. (2012). Effects of fermented *Panax ginseng* extract (GINST) on oxidative stress and antioxidant activities in major organs of aged rats. *Experimental Gerontology* **47**: 77-84.
25. Tung, N.H., Song, G.Y., Nhiem, N.X. and Ding, Y. (2010). Dammarane-type saponins from the flower buds of *Panax ginseng* and their intracellular radical scavenging capacity. *Journal of Agricultural and Food Chemistry* **58**: 868-874.
26. Wang, J., Li, J., Li, J., Liu, S., Wu, X., Li J. and Gao, W. (2016). Transcriptome profiling shows gene regulation patterns in ginsenoside pathway in response to methyl jasmonate in *Panax Quinquefolium* adventitious root. *Scientific Reports* **6**:1-11.
27. Xu, Y.G., Liu, H.J., Zhang, K.M., Zhu, S.S. and Yang, M. (2020). Allelopathic Plants: 28. Genus *Panax* L. *Allelopathy Journal* **51**: 21-40.
28. Yadav, R.K., Sangwan, R.S., Sabir, F., Srivastava, A.K. and Sangwan, N.S. (2014). Effects of prolonged water stress on specialized secondary metabolites, peltate glandular trichomes and pathway gene expression in *Artemisia annua* L. *Plant Physiology* and *Biochemistry* **74**: 70-83.
29. Yang, J., Hu, Z., Zhang, T., Gu, A., Gong T. and Zhu, P. (2018). Progress on the studies of the key enzymes of ginsenoside biosynthesis. *Molecules* **589**: 1-12.
30. Yin Xiong, L.C.J.M. (2017). Chemical and bioactive comparison of *Panax notoginseng* root and rhizome in raw and steamed forms. *Journal of Ginseng Research* **43** :385-393.

31. Zahir, A., Abbasi, B.H., Adil, M., Anjum, S., Zia, M. and Ihsan-ul-Haq. (2014). Synergistic effects of drought stress and photoperiods on phenology and secondary metabolism of *Silybum marianum*. *Applied Biochemistry and Biotechnology* **174**: 693-707.
32. Zhang, K.M., Shen, Y. and Fang, Y.M. (2018). Effects of root of *Bidens pilosa* on photosynthesis and cell membrane in *Pteris multifida* gametophyte. *Fresenius Environmental Bulletin* **2**: 771-778.
33. Zhang, K.M., Shen, Y., Miu, X., Yang, J., Prasanta, B., Zhou, X.Q., Fang, Y.M. and Xing, B.S. (2019). The regulation of programmed cell death and antioxidant system working in plant cell under root exudates of invasion species. *Ecotoxicology* and *Environmental Safety* **173**: 203-213.
34. Zhang, K.M., Shen, Y., Fang, Y.M. and Prasanta B. (2017). Effects of up-ground parts of *Bidens pilosa* on chlorophyll fluorescence in *Pteris multifida* gametophyte. *Allelopathy Journal* **11**: 231-238.
35. Zhang, T., C. Chen, Y. Chen, Q. Zhang, Q. Li and W. Qi. (2021). Changes in the leaf physiological characteristics and tissue-specific distribution of ginsenosides in *Panax ginseng* during flowering stage under cold stress. *Frontiers in Bioengineering* and *Biotechnology* **9**: 1-18.
36. Zhang, T., Han, M., Yang, L., Han, Z. and Yang, L. (2018). The effects of environmental factors on ginsenoside biosynthetic enzyme gene expression and saponin abundance. *Molecules* **24**: 14-32.
37. Zhang, T., Gao, Y., Han, M. and Yang, L. (2021). Changes in the physiological characteristics of *Panax ginseng* embryogenic calli and molecular mechanism of ginsenoside biosynthesis under cold stress. *Planta* **253**: 1-23.
38. Zhang, Y.G., Han, M., Jiang, X., Zhao, S.N. and Yang, L.M. (2013). Physiological and ecological response of *Scutellaria baicalensis* Georgi to drought and rewatering. *China Journal of Chinese Materia Medica* **38**: 3845-3850.
39. Zhu, X.Z., Zhang, K.M. and Tang, S.J. (2021). Allelopathic plants 29: *Eupatorium adenophorum* Sprengel. *Allelopathy Journal* **53**: 15-22.

PUBLISHER NOTE