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# Accumulation of allelopathic endogenous hormones and ginsenosides in *Panax ginseng* in different Models

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## ABSTRACT

In 2-years old ginseng seedlings, growth models of multiple allelopathic pathways were studied in 5-soils in pot culture for 5-months. The endogenous hormones GA, ABA and IAA levels were measured using ELISA assay and 12 ginsenosides were quantified by UPLC-QQQ-MS. We compared the accumulation and correlation between hormone and ginsenosides of PPD (Protopanaxadiol ginsenosides), PPT (Protopanaxatriol ginsenosides) and OLE (Oleanolic acid), types in ginseng under certain allelopathic effects. Compared to the healthy growth model, the leaching, decomposition, exudation and multiple allelopathic pathways combined growth models significantly differed ($P < 0.01$). Among the leaching, decomposition and exudation, the exudation had the greatest influence on the growth of ginseng. Accumulation of ginsenosides ($Rb_1$, $Rb_2$, Rc, Rd, Re, Rf, $Rg_1$, $Rg_2$) in ginseng were positively correlated to ABA content and negatively correlated to IAA concentration. The changes in hormone and ginsenosides were highly consistent and showed the correlations between the plant hormones level and ginsenosides accumulation in ginseng. In continuous cropping problem growth model, the combination of multiple allelopathic pathways enhanced the allelopathic effects on the secondary metabolism of ginseng. There were synergistic effects of different allelopathic pathways in continuous cropping problem model to increase the growth inhibition of ginseng.

**Key words:** Accumulation, allelopathic pathway, endogenous hormone, ginsenosides, *Panax ginseng*, soil sickness problem.

## 1. INTRODUCTION

*Panax ginseng* C. A. Mey. (ginseng) (Araliaceae family) is the famous species of *Panax* used in medicine and food. Ginsenosides in ginseng protects the cardiovascular system, antioxidation, antihyperlipidemia and used in treating diabetes and anticancer, preventing infectious diseases etc. (2,3,4,10,19,26). In recent years, due to the people's awareness of health care, the demand for Chinese herbal medicine in China and overseas increased and the market of ginseng and its products become prosperous. Meanwhile, wild ginseng resources could not meet the demand for ginseng, hence, the industry mainly depends on the cultivated ginseng. In cultivated *Panax*, the continuous cropping problem is the key urgent issue need to be solved (6). The soil sickness of ginseng is serious, with high seedling mortality, increases diseases, decreases the material quality and reduces the yields, which strongly restrict the clinical uses and industrial development (17). The allelopathy is one of the important causes of continuous cropping problem in medicinal plants of ginseng (15,17,25,33). The allelopathic substances released in to environment by donor plants (through volatilization, leaching, root secretion and stubble degradation), then acted on themselves or other surrounding receptor plants (28-30). In ginseng, root secretions were

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the main allelopathic pathway which adversely affected its growth, resulting in continuous cropping problems (7,15,18,22,27).

Endogenous phytohormones regulates the important physiological processes (growth, development) and stress resistance (1,8,20). One hormone could regulate the multiple stages of plant growth and development, while a specific growth and development process might be regulated by synergy of various hormones. Indole-3-acetic acid (IAA), gibberellic acid (GA) and abscisic acid (ABA) affects the resistance, nutrients transport, tuberous roots formation and division and differentiation of cells in plants (11,12,13,14,24). The regulation effect of hormones are closely dependent on their concentration.

Ginsenosides are the active substances and are the main allelochemicals in ginseng (3,16,23). The secondary metabolic processes of ginsenosides were regulated by various external environment factors and plant itself (9,32). Under allelopathic stress, the balance between primary and secondary metabolisms is disturbed (34). The concentrations of some hormones and contents of secondary metabolites are changed to make the plants adapted to the environment (1,8). In addition, there may be dependence on the concentrations of hormones and contents of secondary metabolites. In this paper, a systematic analysis model for multiple allelopathic pathways of ginseng was established. We investigated the content changes of endogenous hormone in ginseng under different allelopathic pathways. A method was developed to determine the ginsenosides by ultrahigh performance liquid chromatography triple quadrupole mass spectrometry (UPLC-QQQ-MS) in multiple-reaction monitoring (MRM) mode. The pairs of quantitative and qualitative ions in MRM mode were selected for accurate quantification of ginsenosides. We compared the accumulation and correlation of hormone and ginsenosides in ginseng under allelopathic stress.

## 2. MATERIALS AND METHODS

**Chemicals and reagents**

Acetonitrile and methanol (HPLC grade, Tedia) and formic acid (HPLC grade, Sigma-Aldrich). Plant Gibberellic Acid (GA), Plant hormone abscisic acid (ABA), Plant Indole-3-acetic acid (IAA) ELISA assay kits (Kete were purchased from Bio Co., Ltd. The concentrations of GA, ABA and IAA are defined as the number of tested substances contained in each g of fresh plant weight). Reference ginsenosides Noto $R_1$, $Rb_1$, $Rb_2$, Rc, Rd, Re, Rf, $Rg_1$, $Rg_2$, $F_2$, $F_3$, Ro ( ≥ 98 %, were purchased from Baoman Bio Ltd.).

**Preparation of multiple allelopathic pathway models in ginseng growth**

The studies were done in our shaded greenhouse, Changchun Agricultural Exposition Park, Jilin Province (43º8177" N, 125º4408" E) on November 11, 2019. Two-years-old healthy and uniform ginseng seedlings of cultivar ‘Da maya’ were collected from Fusong, Jilin Province, China. The surfaces of seedlings were sterilized with 5 % NaClO and then were buried in the soil. Pot culture experiment was done in greenhouse with shading net. The experiment was done in plastic pots (length 37 cm × width 37 cm × height 21 cm). When collecting each kind of soil sample, top 2 cm layer of soil and biomass was removed. The depth of collected soil was 15-20 cm. Each plastic pot contained 15 kg soil. The soil

was sieved through 2 mm size to remove rocks and other impurities. Each model had 3 pots replicates and each pot was planted with 9 ginseng seedlings. The pots were replicated thrice and irrigated every 3-days. We designed and compared the following 5-growth models.

**(A). Healthy growth model** : It used healthy fresh forest soil as potting substrate.

**(B). Leaching growth model**: It used healthy fresh forest soil as potting substrate. The leaves and stem of 6-years old green house grown ginseng plants were washed in water to simulate rain leachates. One liter of leachate was added every 3 days.

**(C). Decomposition growth model :** Soil was collected from the same site of healthy soil site. The whole plants (Leaf + stem + roots) of 6-year-old ginseng were buried for 90 days. The decayed residue were mixed in soil.

**(D). Exudation growth model** : The selected rhizosphere soil shaken off from 6-years old ginseng roots was used as potting substrate.

(**E). Continuous cropping barrier growth model :** It used 6-years old ginseng grown soil as potting substrate.

It is known that the interactions between the microbial population, soil physical and chemical properties and allelopathic autotoxic substances are the main causes of continuous cropping problem of ginseng (31). Therefore, model group E was set up to simulate the growth and development of ginseng under the influence of continuous cropping problem. After 5-months growth, ginseng roots samples were collected at harvest time.

**Endogenous hormone in ginseng**

Homogeneous fresh ginseng sample of 1 *g* were homogenated with 1 mL PBS buffer solution. And then the extracts were centrifuged for 20 min at 3000 r/min and 4 °C to get the supernatant for further analysis. GA, ABA and IAA levels were measured using commercial ELISA assay kit.

**Reference and sample solution preparation**

Reference solutions for 12 ginsenosides were dissolved in 80 % methanol-water (HPLC grade) to final concentration of 1 mg/mL individual. The individual reference was combined and diluted with 80 % methanol-water (HPLC grade) to make desired concentrations for UPLC-QQQ-MS quantification analysis.

Fresh ginseng sample of 1 *g* extracted with 20 mL 80 % methanol-water by grinding followed by ultrasonic extraction for 60 min twice at room temperature. The extracts were centrifuged for 10 min at 10000 r/min and 4 °C to get the supernatant, which was combined and concentrated on water bath at 50 °C. The extracts were re-dissolved to make 1 mL 80 % methanol-water (HPLC grade) and filtered through 0.22 μm membrane filtration before UPLC-QQQ-MS analysis.

**Determination of ginsenosides**

Ginsenosides were separated on a Thermo Scientific Syncronis $C_{18}$ column (50 mm×3 mm, 1.7 μm). The mobile phase consisted of solvent A is 0.1 % formic acid aqueous solution and solvent B was acetonitrile. The gradient elution programme was set as under: 0-5 min at 19 % B, 5-29 min at 19-25 % B, 29-72 min at 25-40 % B, 72-77 min at 40-90 % B with a flow rate of 200 μL/min. The injection volume was 5 μL and column temperature was set at 35 ºC. The sampler temperature was set at 4 ºC.

UPLC system (Ultimate 3000, Dionex) was online coupled with TSQ Endura triple quadrupole mass spectrometer (Thermo Fisher Scientific Inc.) equipped with an electrospray ionization (ESI) ion source. Under negative ion mode, the data were acquired in multiple-reaction-monitoring (MRM) scan mode with scan range at *m/z* 100-1500. The ion transfer temperature and vaporizer temperature were set to 350 ºC and 300 ºC, respectively. The spray voltage -2.5 kV and capillary voltage was at -20 V. The sheath gas flow rate and auxiliary gas flow rate were 40 and 10 arbs, respectively. For the MRM analyses, the isolation width was 1.0 Da and the mass scale was calibrated as standard procedure. The ginsenosides references solutions were infused directly to mass spectrometer at 5 μL/min using syringe pump to optimize the best ion signals.

**Statistical analysis**

UPLC-QQQ-MS data were processed by Xcalibur software (Thermo Fisher Scientific Inc.). The processed data were imported into SPSS software 13.0 for statistical analysis.

## 3. RESULTS AND DISCUSSION

**Changes of endogenous hormones in fresh ginseng**

The levels of GA, ABA and IAA in fresh ginseng samples under different growth models were measured using ELISA assay kits and the influence of multiple allelopathic pathways on changes in them were discussed.

(i). **ABA :** The contents of ABA in ginseng from model B, C, D and E were significantly higher than in ginseng from healthy growth model (Figure 1). The content of ABA in ginseng model E was the highest and model B was the lowest among the four multiple allelopathic pathways. The ABA content variations in ginseng from different models indicated that ABA in roots of ginseng increased rapidly under stress conditions. As a growth inhibitor, ABA increased the stress resistance under multiple allelopathy regulations and the combined allelopathic influence of continuous cropping problem growth model. In leaching, decomposition and exudation growth models, ABA concentration increased gradually. It demonstrated that the growth of ginseng might be greatly affected by exudation pathway. In continuous cropping barrier growth model, the increase in ABA content was more than leaching growth model, decomposition growth model and exudation growth model. The combined multiple allelopathic effects synergistically regulated the ABA concentration. It might inhibit the growth of roots, affect the differentiation and hasten the abscission of plant organs, therefore, resulted in continuous cropping barrier.

(ii). **IAA :** The contents of IAA in ginseng from four growth models (B,C,D,E) were lower than in ginseng from healthy growth model A (Figure 1). After multiple allelopathy treatments, the contents of IAA significantly decreased. It showed that IAA was sensitive to multiple allelopathic effects of different pathways. With the increase of allelopathy, the content of IAA decreased gradually, which inhibits the division and growth of root tip, stem apex and leaflet. Comparing the different individual growth models, exudation might be the main pathway of allelopathy in ginseng and the leaching showed lesser

influence. For continuous cropping barrier growth model, the lowest content of IAA indicated the cumulative and synergistic allelopathic effects. It was consistent with the result of ABA.

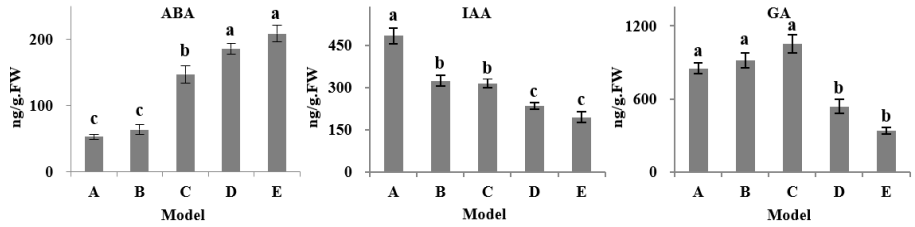



Figure 1. The levels of GA, ABA and IAA in ginseng in different growth models. A : Healthy growth model; B : Leaching growth model; C : Decomposition growth model; D : Exudation growth model; E : Continuous cropping barrier growth model.
Means with similar letters are not significantly different at $P < 0.05$.

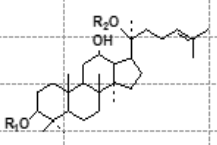
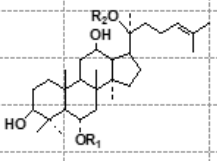
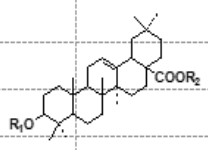
(iii). **GA :** The levels of GA in ginseng decreased in model D and E compared to healthy growth model A. In model B and C, the concentrations of GA increased slightly and without significant difference from the healthy growth model A. It indicated that the allelopathic effects of leaching and decomposition were weaker than exudation and continuous cropping barrier, which stimulated the increase in GA content. During the growth of ginseng, GA was stimulatory in low concentration and inhibitory in high concentration. Because of interactions of various hormones, GA might adapt to environmental changes by balancing the hormonal homeostasis in conjunction with other hormones.

**Accumulation of ginsenosides in fresh ginseng**

The experimental parameters of UPLC-QQQ-MS were optimized automatically and manually. The appropriate chromatographic conditions were obtained on the basis of flow rate, column temperature and gradient elution, which could improve separation and peak shape. The optimization of MS was conducted under direct infusion to achieve the highest signal intensity. UPLC was coupled to the mass spectrometer to monitor ion pairs of 12 ginsenosides simultaneously. The ion pairs of quantitation in MRM mode and corresponding collision energies selected were shown in Table 1.

The methodology of UPLC-QQQ-MS was validated using linearity, repeatability, accuracy, recovery and limits of detection (LOD) and quantification (LOQ), which were presented in Table 2. The coefficient of correlation ($R^2$) and linear range were obtained from analytical curves. Each ginsenosides showed good linearity in the range of studied concentrations. Intraday repeatability was studied in a single day with measurements of every 4 h and interday repeatability was measured by the same procedure in three separate days with high, medium and low concentrations of mixed references. The relative standard deviation (RSD) of intraday and interday repeatability ranged between 1.07 % and 4.46 %.

Table 1. Quantification parameters of ginsenosides detection by UPLC-QQQ-MS in MRM mode.

| Ginsenosides | Structures | $R_1$ | $R_2$ | Precursor ion (*m/z*) | RF Lens (V) | Quantitation ion (*m/z*) | Collision Energy (eV) |
|---|---|---|---|---|---|---|---|
| $Rb_1$ | | -$Glc^3$-Glc | -$Glc^6$-Glc | 1107.83 | 298 | 945.44 | 49.69 |
| $Rb_2$ |  | -$Glc^3$-Glc | -$Glc^6$-Ara(p) | 1077.64 | 298 | 945.48 | 49.24 |
| Rc | | -$Glc^3$-Glc | -$Glc^6$-Ara(f) | 1077.85 | 298 | 945.52 | 49.19 |
| Rd | | -$Glc^3$-Glc | -Glc | 945.51 | 298 | 783.44 | 49.49 |
| $F_2$ | Protopanaxadiol | -Glc | -Glc | 783.64 | 204 | 621.52 | 28.76 |
| | | | | | | | |
| Re | | -$Glc^3$-Rha | -Glc | 945.45 | 298 | 783.38 | 49.74 |
| Noto $R_1$ | | -$Glc^3$-Xyl | -Glc | 931.52 | 283 | 799.54 | 47.11 |
| Rf |  | -$Glc^3$-Glc | -H | 799.48 | 272 | 637.39 | 52.19 |
| $Rg_1$ | | -Glc | -Glc | 799.54 | 252 | 637.37 | 35.94 |
| $Rg_2$ | | -$Glc^3$-Rha | -H | 783.55 | 298 | 637.44 | 37.46 |
| $F_3$ | Protopanaxatriol | -H | -$Glc^6$-Ara(p) | 841.47 | 230 | 637.51 | 25.47 |
| | | | | | | | |
| |  | | | | | | |
| | | | | | | | |
| | | | | | | | |
| $R_o$ | Oleanane | -$Glc^3$-Glc | -Glc | 955.55 | 262 | 793.33 | 51.01 |

The recoveries were assessed with known amounts of references with high, medium and low concentrations spiked into samples, ranged from 97.23 to 102.45 %. The determination was done in five repetitions. The LOD and LOQ were conducted based on the response at signal-to-noise ratio (*S/N*) of 3:1 and 10:1. The validation showed that this UPLC-QQQ-MS method was sensitive and accurate to simultaneously quantify the ginsenosides.

The 12 ginsenosides in ginseng samples from different growth models were determined by the validated UPLC-QQQ-MS method. The ginsenosides detection were confirmed by the retention time and ion pairs comparison. The peak areas of quantitation ion pairs were integrated and calculated by regression equation to calculate the contents of ginsenosides. The distributions of ginsenosides in ginseng from different growth models were represented by column diagram (Figure 2).

Table 2. Validation of UPLC-QQQ-MS method for ginsenosides detection.

| Ginsenosi-des | Conc (μg/mL) | Regression equations | Linear range (ng/μL) | $R^2$ | LOD (pg/μL) | LOQ (pg/μL) | Intraday repeatability RSD (%) | Interday repeatability RSD (%) | Recovery (%) |
|---|---|---|---|---|---|---|---|---|---|
| $Rb_1$ | 0.1 | Y=71.01X+8.26 | 0.05-10 | 0.9990 | 0.02 | 0.06 | 1.22 | 1.37 | 99.05±1.53 |
| | 0.5 | | | | | | 2.61 | 1.83 | 100.88±1.15 |
| | 5 | | | | | | 1.71 | 1.99 | 98.59±1.23 |
| $Rb_2$ | 0.1 | Y=9.63X+6.42 | 0.01-10 | 0.9982 | 0.02 | 0.05 | 3.61 | 1.22 | 99.92±1.73 |
| | 0.5 | | | | | | 3.38 | 3.15 | 102.21±0.95 |
| | 5 | | | | | | 1.45 | 1.46 | 97.85±2.02 |
| Rc | 0.1 | Y=5.40X+1.99 | 0.05-15 | 0.9990 | 0.06 | 0.2 | 2.25 | 1.07 | 97.32±1.50 |
| | 0.5 | | | | | | 2.86 | 2.20 | 102.28±0.87 |
| | 5 | | | | | | 2.99 | 1.80 | 98.52±1.15 |
| Rd | 0.1 | Y=7.89X+8.20 | 0.01-10 | 0.9996 | 0.016 | 0.05 | 3.81 | 2.07 | 100.79±1.04 |
| | 0.5 | | | | | | 1.59 | 1.54 | 99.88±1.50 |
| | 5 | | | | | | 1.97 | 1.96 | 97.29±1.91 |
| $F_2$ | 0.02 | Y=11.71X+8.90 | 0.002-2 | 0.9988 | 0.003 | 0.01 | 2.65 | 3.98 | 100.88±0.90 |
| | 0.1 | | | | | | 2.01 | 3.40 | 98.23±1.84 |
| | 1 | | | | | | 3.72 | 2.66 | 98.34±1.63 |
| Re | 0.1 | Y=6.20X+1.03 | 0.01-20 | 0.9992 | 0.017 | 0.05 | 3.64 | 3.09 | 98.89±1.70 |
| | 0.5 | | | | | | 1.15 | 1.57 | 101.49±1.81 |
| | 5 | | | | | | 1.07 | 1.76 | 100.62±1.39 |
| Rf | 0.02 | Y=13.24X-8.47 | 0.002-2 | 0.9980 | 0.003 | 0.01 | 3.05 | 3.36 | 99.21±1.32 |
| | 0.1 | | | | | | 2.85 | 3.34 | 100.99±1.76 |
| | 1 | | | | | | 2.21 | 2.81 | 97.9±0.58 |
| $Rg_1$ | 0.01 | Y=1.23X+1.91 | 0.001-1 | 0.9990 | 0.005 | 0.02 | 4.12 | 3.33 | 102.45±1.96 |
| | 0.05 | | | | | | 2.24 | 3.17 | 98.79±1.32 |
| | 0.5 | | | | | | 1.87 | 2.97 | 98.9±1.04 |
| $Rg_2$ | 0.02 | Y=10.25X+5.72 | 0.01-2 | 0.9996 | 0.01 | 0.04 | 3.54 | 4.46 | 99.44±0.88 |
| | 0.1 | | | | | | 2.80 | 2.57 | 99.01±1.67 |
| | 1 | | | | | | 1.35 | 1.87 | 100.68±1.38 |
| $F_3$ | 0.02 | Y=136.3X-3.64 | 0.01-2 | 0.9982 | 0.03 | 0.1 | 3.66 | 3.11 | 97.23±1.26 |
| | 0.1 | | | | | | 2.74 | 3.32 | 99.9±1.53 |
| | 1 | | | | | | 1.74 | 1.92 | 102.44±1.44 |
| Noto $R_1$ | 0.02 | Y=5.91X+1.55 | 0.01-2 | 0.9984 | 0.02 | 0.06 | 3.60 | 4.38 | 99.23±1.04 |
| | 0.1 | | | | | | 1.70 | 3.18 | 97.34±1.47 |
| | 1 | | | | | | 1.52 | 3.26 | 101.88±1.50 |
| $R_o$ | 0.2 | Y=1.61X+1.04 | 0.1-20 | 0.9987 | 0.01 | 0.03 | 2.74 | 4.19 | 101.77±1.18 |
| | 1 | | | | | | 3.53 | 3.65 | 98.34±1.04 |
| | 10 | | | | | | 1.96 | 2.63 | 97.23±1.26 |

$R^2$: Correlations coefficient

The 12-Ginsenosides different aglycone structures could be divided into three types (Table 1): (i). Protopanaxadiol ginsenosides (**PPD**): $Rb_1$, $Rb_2$, Rc, Rd, $F_2$, (ii). Protopanaxatriol ginsenosides (**PPT**) Re, Noto $R_1$, Rf, $Rg_1$, $Rg_2$, $F_3$, and (iii). Oleanolic acid type ginsenosides (**OLE**): Ro.

**(i). PPD ($Rb_1$, $Rb_2$, Rc, Rd, $F_2$):** The contents of $Rb_1$, $Rb_2$ and Rc ginsenosides, were significantly decreased in ginseng plants from model B and E compared to healthy growth model A, but increased in model C and D. The content of Rd was significantly decreased in model E and was increased in other three models. However, the contents of $F_2$ were significantly increased in model B and model E and decreased slightly in model C and D. The total contents of PPD type ginsenosides in ginseng of model B and E were significantly decreased than healthy growth model and significantly increased in model C and model D.

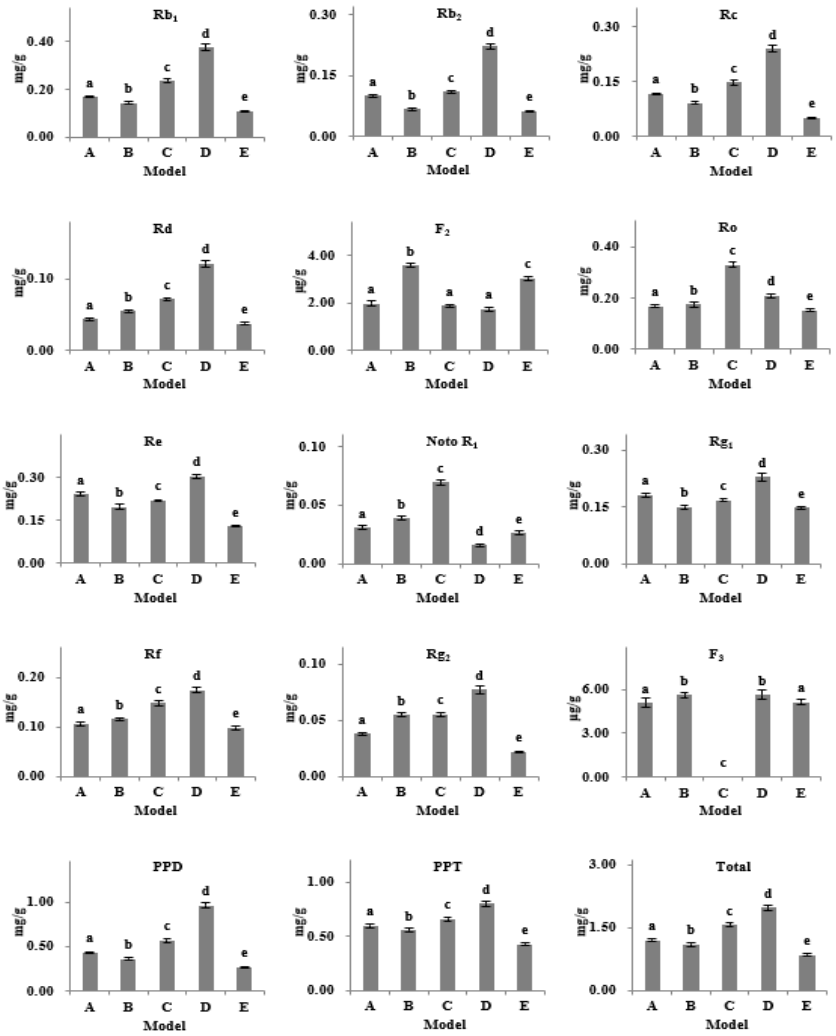



Figure 2. The ginsenosides contents in ginseng plants from different growth models.
A : Healthy growth model); B : Leaching growth model; C : Decomposition growth model; D : Exudation growth model; E : Continuous cropping barrier growth model.
Means with similar letters are not significantly different at $P < 0.05$.

**(ii). PPT (Re, Noto $R_1$, Rf, $Rg_1$, $Rg_2$, $F_3$):** The contents of Re and $Rg_1$ ginsenosides, were significantly decreased in model B, C and E than healthy growth model A, but increased in model D. The contents of Noto $R_1$ were decreased in ginseng model D and E and increased in model B and C with significant difference between models. The contents of Rf and $Rg_2$ were significantly decreased in model E and gradually increased in other three growth models. However, the contents of $F_3$ were slightly increased in model B, D and E, with no detection in model C. The total contents of PPT type

ginsenosides in model B and E were significantly decreased than healthy growth model A and significantly increased in model C and D.

**(iii). OLE** : The Ro ginsenosides contents were significantly decreased in model E compared to healthy growth model, but increased in models B, C, D, with the highest content in model C.

Compared to healthy growth model, the contents of 10-ginsenosides ($Rb_1$, $Rb_2$, Rc, Rd, Ro, Re, Noto $R_1$, $Rg_1$, Rf, $Rg_2$ among the 12 ginsenosides determined were decreased significantly in ginseng continuous cropping barrier growth model. The results showed that there were some allelochemicals in continuous cropping barrier soil, which could change the secondary metabolism of ginseng root. Under the influence of complex multiple allelopathic pathways, the effects of continuous cropping barrier growth model and the contents of ginsenosides decreased greatly. For leaching, decomposition and exudation growth models, allelopathic substances were released and their contents increased gradually. With the increase of allelopathy effects, the accumulations of ginsenosides were stimulated in ginseng plants. The contents of ginsenosides in ginseng were most affected by the exudation pathway. Ginseng plants showed certain resistance and repairability under low allelopathic stress.

Compared to continuous cropping barrier growth model, the contents of 12 ginsenosides determined in leaching growth model, decomposition growth model and leaching growth model were significantly higher in ginseng continuous cropping barrier growth model. These results suggested that the combination of multiple allelopathic pathways may enhance the allelopathy of ginseng. In the single allelopathic release pathway growth model, the changes in endogenous hormones and ginsenoside accumulation in fresh ginseng were similar, indicating high correlation between them. There was no consistency between the concentrations of endogenous hormones and ginsenosides in Group E of continuous cropping obstacle compound growth model. This may be due to the multiple effects of other physical, chemical and biological factors on plant growth and development in the continuous cropping model (5,21,35).

In leaching, decomposition and exudation growth models, the ginsenosides $Rb_1$, $Rb_2$, Rc, Rd, Re, Rf, $Rg_1$, Rg accumulation trend in ginseng were positively correlated to ABA content, while negatively correlated to IAA concentration. The maximum values of ginsenosides Ro, Noto $R_1$ and hormone GA were found in the decomposition growth model. A variety of metabolic pathways could be involved in secondary metabolites synthesis and most of the pathways were cross-linked with each other in plants growth. Secondary metabolic pathways could produce many hormones precursors. GA, IAA and abscisic acid were synthesized by isoprenoid pathway. The Mevalonate pathway (MVA) related to isoprenoid pathway was recognized as the necessary synthesis of triterpene aglycones of ginsenosides. The stress caused by different release pathways of allelochemicals could disrupt the homeostasis of plant internal environment. To adapt to the environmental changes, the plant undergoes series of metabolic changes. The changes in trend of hormone and ginsenosides contents were highly consistent, demonstrated that there was a potential correlation between plant hormone level and ginsenosides accumulation in ginseng growth.

## 4. CONCLUSIONS

We studied, the influence of multiple allelopathic pathways on changes of endogenous hormone and ginsenosides accumulation in fresh ginseng. Among the three single allelopathic pathways (leaching, decomposition and exudation), the exudation pathway had greater influence on the growth of ginseng. In the multiple allelopathic pathways combined model, continuous cropping barrier growth model, there were synergistic effects of different allelochemicals releasing pathways. It was correlated with other inducements of continuous cropping barrier to aggravate the growth inhibition of ginseng. In the single allelopathic release pathway growth model, the consistent changes of endogenous hormone and ginsenosides accumulation in fresh ginseng from different models demonstrated the high correlation between them and certain concentration dependence. In view of the autotoxic effects of different pathways of allelopathy in ginseng's growth, the differences in hormone levels and ginsenosides accumulation were investigated from physiological and metabolic perspectives. This study was helpful to understand the change in endogenous substances of ginseng under different allelopathic influence. The regulation effects of hormones on the synthesis of ginsenosides in ginseng indicated accumulation mechanism of endogenous substances of ginseng under allelopathic effects.

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## DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

## CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

## ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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## PUBLISHER NOTE