

## Allelopathic effects of ginsenoside Rg1 on seed germination and seedling growth of *Panax ginseng*

Qiong Li<sup>1,2</sup>, Lianxue Zhang<sup>1</sup>, Tianzhu Guan<sup>1</sup>, Yonghua Xu<sup>1\*</sup> and Changbao Chen<sup>2\*</sup>  
State -Local Joint Engineering Research Center of Ginseng Breeding and Application (Jilin), Jilin Agricultural University, Changchun, 130118, China.  
E. Mail: xyhginseng@163.com , thirteenli520@gmail.com, ccb2021@126.com

(Received in revised form: February 8, 2020)

### ABSTRACT

Soil sickness of ginseng (*Panax ginseng* C. A. Mey.) has become a major limiting factor in ginseng cultivation. We found that the in rhizosphere soil of 5-year-old ginseng mainly contained ginsenosides viz., Rb1 (2.69 mg L<sup>-1</sup>), Rg1 (1.94 mg L<sup>-1</sup>), Re (1.84 mg L<sup>-1</sup>), Rb2 (1.56 mg L<sup>-1</sup>) and Rc (1.12 mg L<sup>-1</sup>). High concentration of Rg1 (2.00 mg L<sup>-1</sup>) inhibited the seed germination and seedling growth, but was stimulatory at low concentrations (0.25 mg L<sup>-1</sup>). The Rg1 at 1.00 mg. L<sup>-1</sup> concentration stimulated the superoxide dismutase, peroxidase and ascorbic peroxidase activities of seedlings but the 2.00 mg L<sup>-1</sup> concentration decreased their activities. The Rg1 at low conc (0.25 mg L<sup>-1</sup>) increased but high concentration (2.00 mg L<sup>-1</sup>) reduced the contents of gibberellic acid and indole acetic acid. However, the content of abscisic acid was increased by all concentrations of Rg1.

**Keywords :** Allelopathic effects, antioxidant enzyme, autotoxicity, enzyme activities, gibberellic acid, ginseng, ginsenoside Rg1, indole acetic acid, *Panax ginseng*, phytohormones, seed germination, seedling growth, soil sickness.

### INTRODUCTION

Ginseng has ancient cultivation history in China, Korea and Japan (9). However, soil sickness of Ginseng reduces the seed germination, seedling growth, ginseng root quality and also causes serious diseases infestation (20). Soil sickness reduces the ginseng yields, besides causes the major problems of restoring the plants in old cultivated land. This problem is due to many factors viz., (i). deterioration of soil physical and chemical properties, (ii). nutrients imbalance, (iii). soil-borne diseases and (iv). autotoxicity (31). Autotoxicity refers to condition where plant releases certain compounds (autotoxins) into the environment, which inhibits or suppresses the growth of their own plants (4,10,36). The autotoxicity has been observed in *Jacobaea vulgaris* (21), *Cucumis sativus* L. (5,42), *Radix, Panax quinquefolii* (17). The secondary metabolites of herbaceous perennial plants, particularly the glycitein (11), 3,4-dihydroxy-acetophenon (35), veratric acid (41) have been identified in sick soil. Among these ginsenoside Rg1 has been consistently found in the root exudates of ginseng or in the rhizosphere soil growing ginseng (23). Ginsenoside is the main bioactive compound produced by ginseng plants (3,16). There are at least 20 different ginsenosides in ginseng, accounting for > 6 % of plant dry biomass. These compounds are released into the rhizosphere soil by root exudates, or in the environment by leaching and volatilization (33). The autotoxin Rg1 of notoginseng induces the excessive accumulation of reactive oxygen species (ROS) leading to the death of root cells

\*Correspondence author, <sup>1</sup>State -Local Joint Engineering Research Center of Ginseng Breeding and Application (Jilin), Jilin Agricultural University. Changchun, 130118, China; <sup>2</sup>Jilin Ginseng Academy, Changchun University of Chinese Medicine, Changchun, 130117, China.

(35).

The pharmacological properties of ginsenosides have been studied, but very little is known about the ginsenosides allelopathy in soil after release from ginseng plants. Ginsenoside may play role in soil sickness in ginseng (23,36). Due to the serious threat of autotoxic effects on the ginseng plant, we studied the allelochemicals of ginseng and their allelopathic potential. We identified the Rg1 from the ginseng rhizosphere soil and its autotoxic activity was evaluated on the metabolism of root cells, antioxidant enzymes activities and content of ginseng phytohormones.

## MATERIALS AND METHODS

Ginseng seeds were collected from Jilin Agricultural University, Changchun (236.8 m above sea level, 43°81'0639"N, 125°41'0545"E) in October, 2016. All seeds were collected from the un-infected healthy plants and stored at 4°C. All chemicals and solvents (polyvinyl pyrrolidone, dithane stainless, ascorbate, Ethylenediaminetetraacetic acid, nitro benzyltetrazole, Triton X-100, Nitrotetrazolium Blue chloride, methionine, riboflavin, 2-Methoxyphenol, nicotinamide adenine dinucleotide phosphate purchased were of analytical grade).

### Analysis of ginsenoside content

The soil used in this study was taken from the experimental site of Jilin Agricultural University, Changchun, China (43 °81"N, 125 ° 42"E). After the ginseng harvest in August 2017, soil samples were collected from the Rhizosphere of five-years-old ginseng plants. The soil was sieved (2 mm) to remove plant debris and stones (15) and dried at 45 °C. The soil samples were extracted using 80 % methanol as solvent in 1:10 ratio (v/v). It was extracted on 180 r/min constant temperature oscillator for 24 h. After concentrating, the volume was maintained at 10 mL by using Chromatographic pure mobile phase (acetonitrile: water, 20:80) and filtered by 0.45 µm filter membranes. The Waters 2489 UV/Vis Detector HPLC (High Performance Liquid Chromatography) system was used for the chromatographic analysis of C18 reversed-phase column (4.6 × 250 mm, 5µm). The sample injection volume was 10 µL. Elution with solvent (acetonitrile/water, 20:80) in step gradient manner at flow rate of 1.0 mL.min<sup>-1</sup> was done as follows: 0-24 min: 18 %-22 %; 24-26 min: 22 %-26 %, 26-30 min: 26 %-32 %, 30-50 min: 32 %-33.5 %, 50-55 min: 33.5 %-38 %, 55-65 min: 38 %, the sampling volume was 20 µL (14).

### Seed germination

Twenty ginseng seeds were placed equidistant in plastic boxes (15×15 cm) lined with two layers of filter paper. As per treatments, 10 mL Rg1 of 0, 0.25, 0.50, 1.00 and 2.00 mg L<sup>-1</sup> concentrations was added into each box. The treatments were replicated 5-times. These boxes were kept in an incubator [17/7 h (day/night), photoperiod photon flux density: 40 µmol.m<sup>-2</sup>s<sup>-1</sup>, day/night temperature: 15/10 °C]. The seeds were considered germinated, when 1 cm long radicle emerged from the seed. Germination was recorded after 10 days and germination vigour at 20 days after Rg1 treatment (34). Seeds un-germinated for 5 days, were used to determine the antioxidant enzymes activities and the content of plant endogenous hormones.

### Seeding growth

Ginseng seeds were pre-germinated in petriplates (90 mm) lined with filter paper until radicle emergence. The 20 seeds were soaked in diluted Mancozeb for 3 h and then placed equidistant in petri dishes. As per treatments, 10 mL Rg1 (0, 0.25, 0.50, 1.00 and 2.00 mg L<sup>-1</sup> concentrations) was added into each petri dish. The treatments were replicated 5-times. The petri dishes were kept in Incubator (17/7 h day/night) photoperiod, photon flux density : 40  $\mu\text{mol.m}^{-2}\text{s}^{-1}$ , day/night temperature (25/20 °C). After 5,10,15 and 20-days, the radicle length of 5-randomly sampled ginseng seedlings from each Petri dish, were measured with ruler and their fresh weight was recorded. Thereafter the antioxidant enzymes activities and the content of plant endogenous hormones were determined in the seedlings.

### Antioxidant enzyme activity

Tissues (0.1 g) were weighed and ground with chilled mortar and pestle in 1 mL phosphate buffer solution (50 mM, pH 7.0), 1 mM EDTA and 2 % (w/v) polyvinyl pyrrolidone (PVP). The homogenate was filtered twice and centrifuged at 4 °C at 12000 g for 30 min. Except for Ascorbic peroxidase (APX) activity, all antioxidant enzymes activity were determined in the clear supernatant. For the activity of APX, the tissue was homogenized in phosphate buffer solution (50 mM, pH 7.0), adding 2 mM ascorbate, 1mM EDTA and 2 % (w/v) PVP. In control, distilled H<sub>2</sub>O was used instead of the enzyme extract (7,29). The activity of superoxide dismutase (SOD) was determined by observing the inhibitory effects of photo reduction of Nitrotetrazolium Blue chloride (NBT) (10). Peroxidase (POD) activity was determined by guaiacol method (24,39). Catalase (CAT) activity was measured from the rate of H<sub>2</sub>O<sub>2</sub> decomposition as measured by the decrease in absorbance at 240 nm, as per Lee *et al.* (1). APX activity was determined according to Nakano and Asada (22). Guaiacol peroxidase (GPX) activity was determined by the improved method of Cakmak and Marschner (7). Glutathione reductase (GR) Activity was determined by GSSG-dependent oxidation of NADPH (12).

### Phytohormones content

The sample (0.1 g) was frozen in liquid nitrogen and immediately ground to powder. then 200  $\mu\text{L}$  cold 80 % methanol (containing 1 mg L<sup>-1</sup> Butylated hydroxytoluene (BHT) as an antioxidant) was added and the homogenate was incubated in dark for 12 h at 4 °C. The Waters 2489 UV/Vis Detector HPLC (High Performance Liquid Chromatography) system was used for the chromatographic analysis of C18 reversed-phase column (4.6  $\times$  250 mm, 5  $\mu\text{m}$ ). Detection wavelength: gibberellin (GA) was 206 nm, heteroauxin (IAA) was 225 nm, Abscisic Acid (ABA) was 254 nm. Dissolved in 2 mL mobile phases and filtered by 0.22  $\mu\text{m}$  membrane for analysis. A diode array detector was monitored at 254 nm. Elution with solvent A (methanol/acetonitrile, 5:95) and solvent B (water/acetonitrile, 5:95) in a step gradient manner at flow rate of 0.5  $\text{ML min}^{-1}$  was done as under : 0-1 min, 25 % A; 1-4 min, 25 %-45 % A; 4-8min, 45 % A; The sample injection volume was 10  $\mu\text{L}$  (34). (Authors: please define the full names of these abbreviations here, at the first appearance in the main body of the manuscript).

### Statistical analyses

All results were presented as means  $\pm$  standard deviation (S.D.) of five replications. All data were statistically analyzed by SPSS software (ver. 18.0; IBM, New York, USA). In statistical analysis, the results were considered significant when  $p < 0.05$ . If the results of One-way ANOVA showed the significant difference at the 0.05, we used HSD (Tukey Honest Significant Differences) to compare the different treatments.

## RESULTS AND DISCUSSION

### Ginsenoside content in rhizosphere soil of Ginseng

Ginsenosides Rg1, Re, Rb1, Rc, Rb2 were mainly found in rhizosphere soil of 5-year-old ginseng plants (Fig 1). The detected concentration was: Rb1 ( $2.69 \text{ mg L}^{-1}$ ), Rg1 ( $1.94 \text{ mg L}^{-1}$ ), Re ( $1.84 \text{ mg L}^{-1}$ ), Rb2 ( $1.56 \text{ mg L}^{-1}$ ), Rc ( $1.12 \text{ mg L}^{-1}$ ).

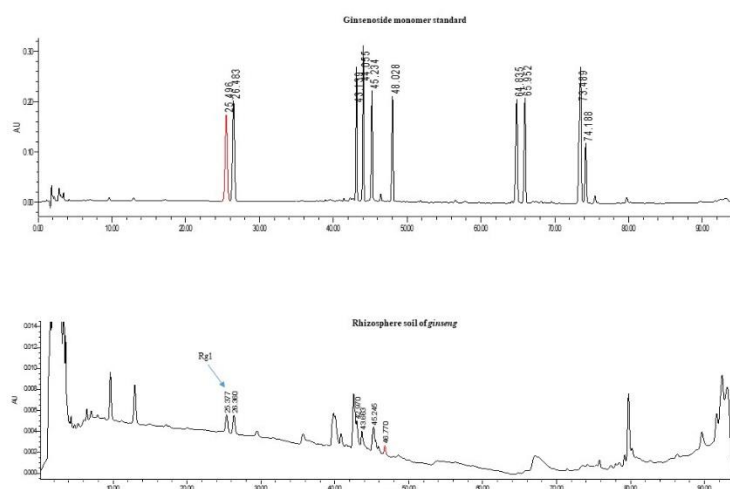


Figure 1. Determination of ginsenoside in rhizosphere soil of five-year-old *P. ginseng* by HPLC.

### Ginseng seed germination

The Rg1 at  $0.25 \text{ mg L}^{-1}$  content promoted the seed germination and vigour by 3.62 % and 2.90 %, respectively than distilled water control (Fig. 2). Rg1 at  $0.50\text{--}2.00 \text{ mg L}^{-1}$  concentration, inhibited the germination and vigour, especially at  $2.00 \text{ mg L}^{-1}$ . The Rg1 at  $2.00 \text{ mg L}^{-1}$  concentration, remarkably decreased the germination rate and vigour by 42.99 % and 33.82 %, respectively, showing strong inhibitory effects.

### Enzyme activities and phytohormones content in ginseng seed germination

In the range of  $0.25\text{--}1.00 \text{ mg L}^{-1}$  concentration, the activities of SOD, APX and CAT were increased, but at the highest concentration ( $2 \text{ mg L}^{-1}$ ) their activities were decreased over control (Fig. 3). However, in POD, the activity was concentration dependent, an increase was observed in response to Rg1 treatment, and the maximum

stimulation (102.57%) was at 2.00 mg L<sup>-1</sup> concentration. At 1.00 mg L<sup>-1</sup> Rg1 concentration, except the APX, the activities of all antioxidant enzymes were increased than control, indicating that ginseng had positive self-protective effects at low Rg1 concentration. However, high concentration of Rg1 (2.00 mg L<sup>-1</sup>) decreased the antioxidant enzyme activity, this reflected the negative self-inactivation effect, which decreased the seed germination.

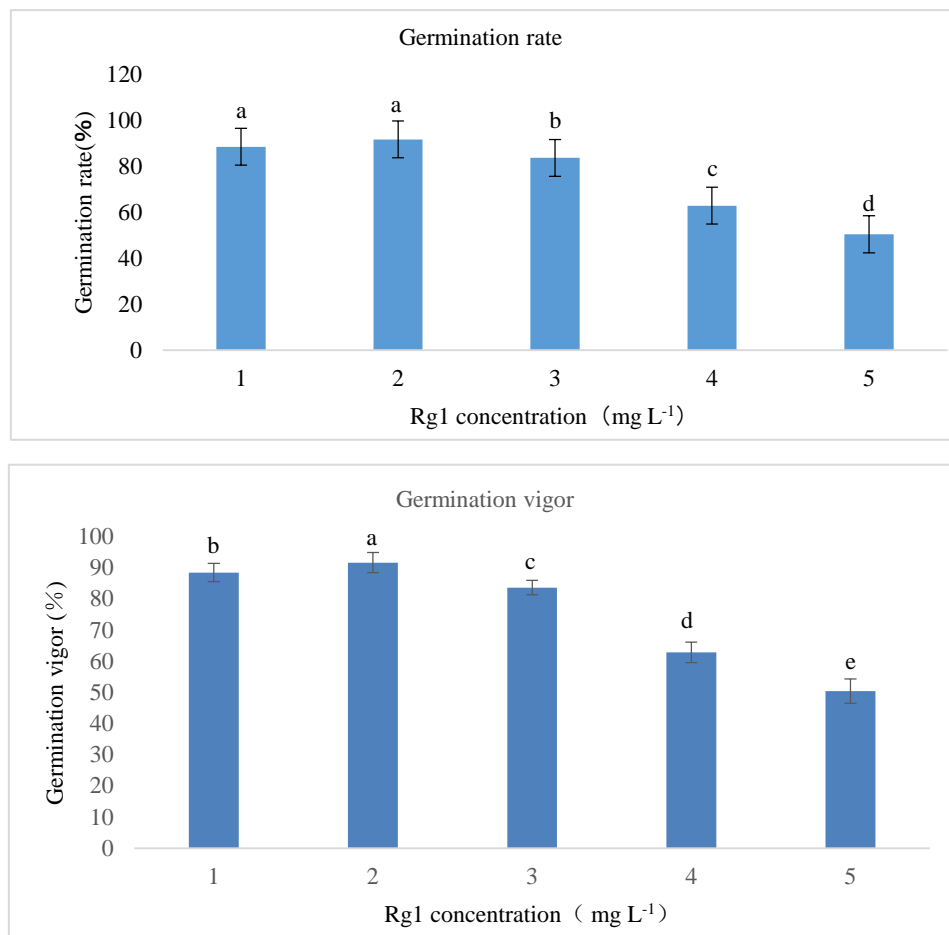


Figure 2. Effects of Rg1 on seed germination of *P. ginseng*. 1: Control, 2: 0.25 mg L<sup>-1</sup>, 3: 0.50 mg L<sup>-1</sup>, 4: 1.00 mg L<sup>-1</sup>, 5: 2.00 mg L<sup>-1</sup>. Each value of extract represents means  $\pm$ SE (standard errors) (n=5). Means with similar letters are not significantly different at  $p < 0.05$ .

Rg1 at moderate concentration (0.25–0.50 mg L<sup>-1</sup>) significantly increased the content of GA<sub>3</sub> and IAA, while its high concentrations were inhibitory. In 0.50 mg L<sup>-1</sup> Rg1 treatment, the GA<sub>3</sub> and IAA content were highest (16.72 % increase) and 34.36 % higher

than control. At 2.00 mg L<sup>-1</sup> Rg1, the contents of GA<sub>3</sub> and IAA decreased by 36.44 % and 31.49 % respectively (Fig. 4). The low concentration 0.25 mg L<sup>-1</sup> of Rg1, decreased the hormone ABA content (24 % decrease), while high Rg1 concentration significantly stimulated the ABA content.

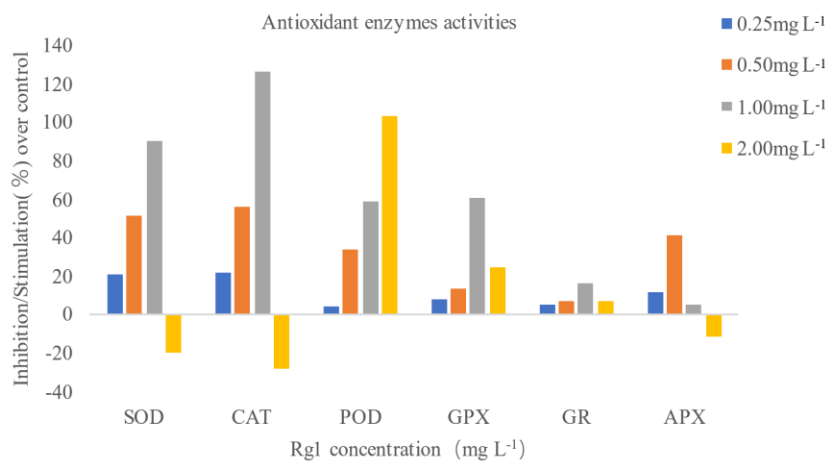


Figure 3. Effects of Rg1 application on the antioxidant enzymes activities.

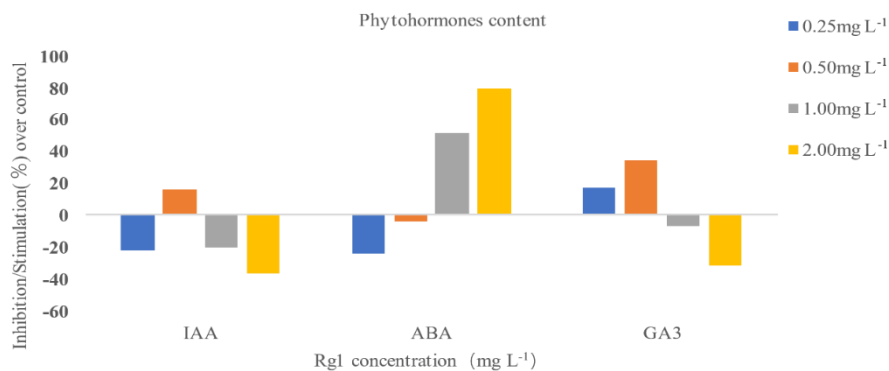


Figure 4. Effects of Rg1 application on the phytohormones contents.

#### Effects of Rg1 on early seedlings growth of *ginseng*

The length and fresh weight of radicle were maximum 20 days after Rg1 stress treatment than control (Fig 5). Rg1 treatment of 1.0 mg L<sup>-1</sup> for 20 days inhibited the

growth and development of seedlings, the roots length and fresh weight were decreased by 60 % and 58 % respectively (Fig 6). The results showed that high concentration of Rg1 ( $0.5 \text{ mg L}^{-1}$ ) inhibited the growth of ginseng seedlings but low concentration ( $0.25 \text{ mg L}^{-1}$ ) promoted the seedlings growth. The Rg1 treatment  $0.5 \text{ mg L}^{-1}$  concentration, inhibited the germination and seedling growth of ginseng. The concentration of Rg1 was  $1.94 \text{ mg L}^{-1}$  in rhizosphere soil of 5-year-old ginseng. Thus, our investigation provided evidence about the phytotoxic potential of ginseng. Coumarin and 4,8-DHT exhibits concentration-dependent stimulatory or inhibitory effects on seedling growth (27,34). In lettuce, the low concentrations of caffeic and ferulic acids promoted the elongation of hypocotyls, but higher concentrations inhibited the seedling growth and seed germination (17).

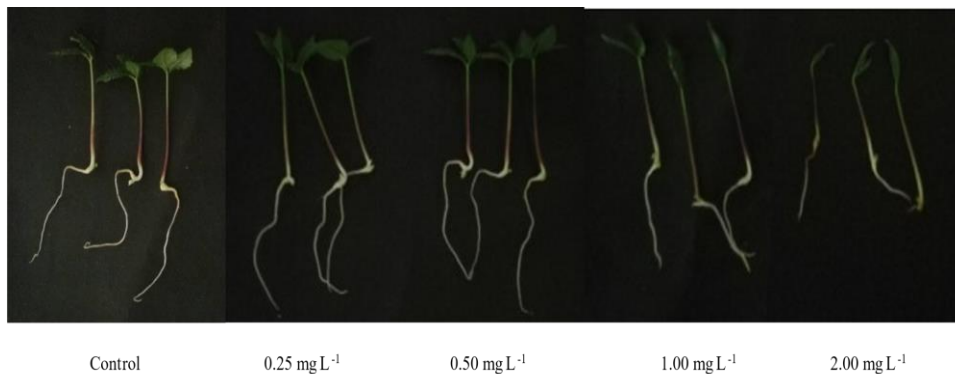


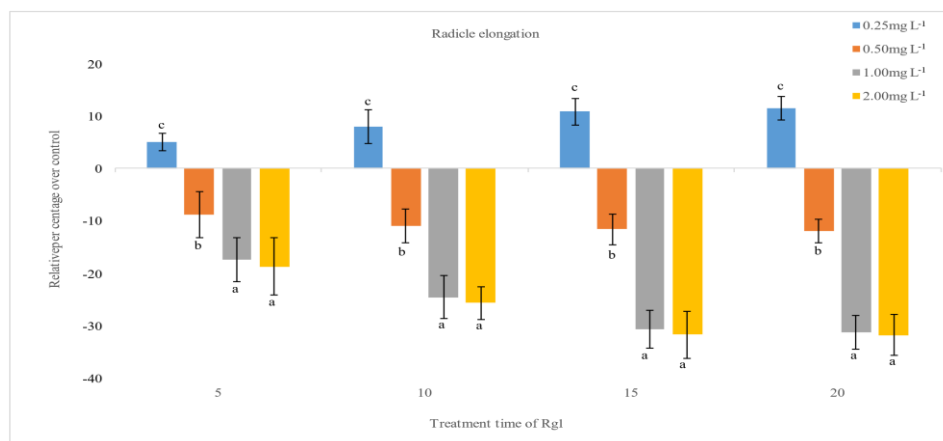
Figure 5. Effects of Rg1 on early seedlings growth of *P. ginseng*.

#### Enzyme activity in leaves of ginseng seedlings

The POD enzyme activities first increased, then declined with  $2.00 \text{ mg L}^{-1}$  Rg1 treatment compared with control (Fig 7). The SOD activities increased in the early stage and the maximum enzyme activities were at  $1.00 \text{ mg L}^{-1}$  Rg1 treatment after 15 days. On the 5th day after the treatment with  $2.00 \text{ mg L}^{-1}$  Rg1, the activity of CAT enzyme reached the maximum value, and declined after wards.

Rg1 ( $1.00 \text{ mg L}^{-1}$ ) treatment increased the activities of SOD, POD and CAT in ginseng, indicating that moderate Rg1 stress could improve the resistance ability. Rg1 ( $2.00 \text{ mg L}^{-1}$ ) inhibited the activities of these enzymes, due to the decreased tolerance of Rg1 stress to high concentration. APX activity of ginseng seedlings was briefly stimulated by  $0.25$  and  $0.50 \text{ mg L}^{-1}$  Rg1 than control, 5 days after treatment, but decreased with prolongation of treatment period. The activity of APX and GPX increased significantly, 5-days after treatment with high concentrations of Rg1 ( $1.00$  and  $2.00 \text{ mg L}^{-1}$ ). The maximum value of 30.82 % in APX and 30.84 % in GPX was reached 10 days after  $1.00 \text{ mg}$  treatment than control (Fig 7). The Rg1 had variable effects on the antioxidant enzymes activities in ginseng seedlings. In early seedling growth, Rg1 at  $1.00 \text{ mg L}^{-1}$  concentration increased the activities of all antioxidant enzymes except GR. The results

showed that moderate concentration of Rg1 increased the activity of antioxidant enzymes, which helped the ginseng seedlings to maintain their ROS content, below harmful level, to enhance the resistance to ginseng. These results are consistent with other studies of antioxidant enzymes described under allelochemical stress (8). The low and medium ginsenoside isolated from ginseng significantly stimulates the activities of SOD, POD and CAT in roots of American ginseng (38). Similarly, Paclobutrazol increased the antioxidant enzymes in wheat leaves (17) and glycine betaine in wheat leaves (2). This is the self-protection mechanism of plants in response to biotic and abiotic stresses. However, at  $2.00 \text{ mg L}^{-1}$ , the activity of antioxidant enzymes decreased. The decrease in enzyme activities was also observed in other studies on allelochemical modes of actions (33). Two allelochemicals (4,7-dimethyl-1-(propan-2-ylidene)-1,4,4a, 8a-tetra-hydronaphthalene-2, 6(1H, 7H)-dione and 6-hydroxyl-5-isopropyl-3, 8-dimethyl-4a, 5, 6, 7, 8, 8a-hexahydronaphthalen-2(1H)-one) isolated from the leachates of *Ageratina adenophora* at high concentration (0.94 and 0.74 mM) decreased the activities of POD and SOD in rice seedlings after 48h. It was speculated that the accumulation of ROS induced during severe Rg1 stress goes beyond the clearance ability of antioxidant enzymes. Too much ROS can induce cell damage and cause ginseng seedlings death.



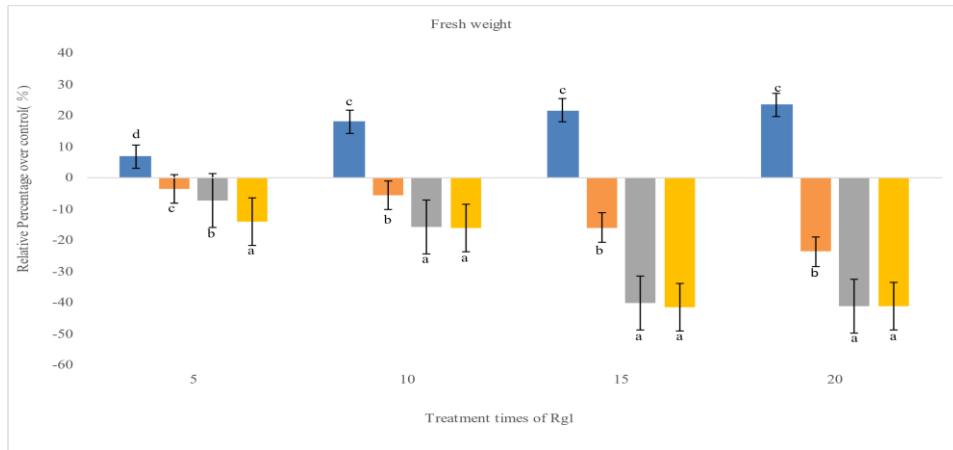
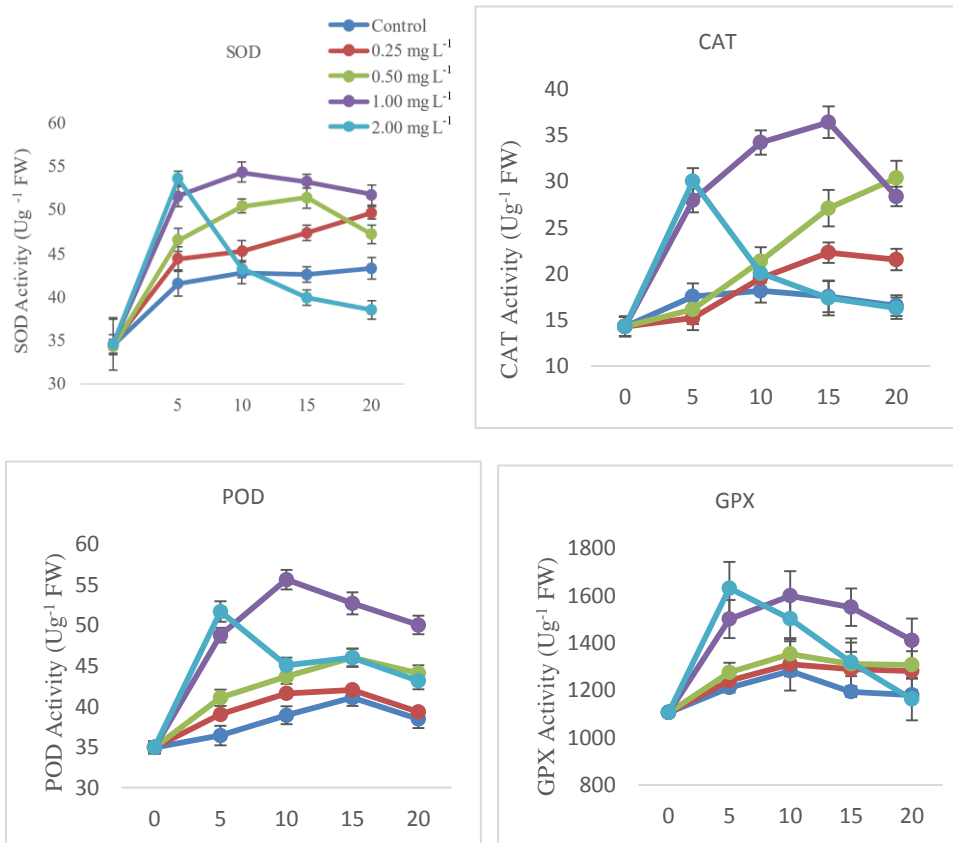


Figure 6. Effects of Rg1 on seedlings growth of *P. ginseng*. Error bars present standard errors of five independent biological replicates. The same lower-case letter indicates a non-significant difference ( $p > 0.05$ ), different lower-case letters indicate a significant difference ( $p < 0.05$ ).



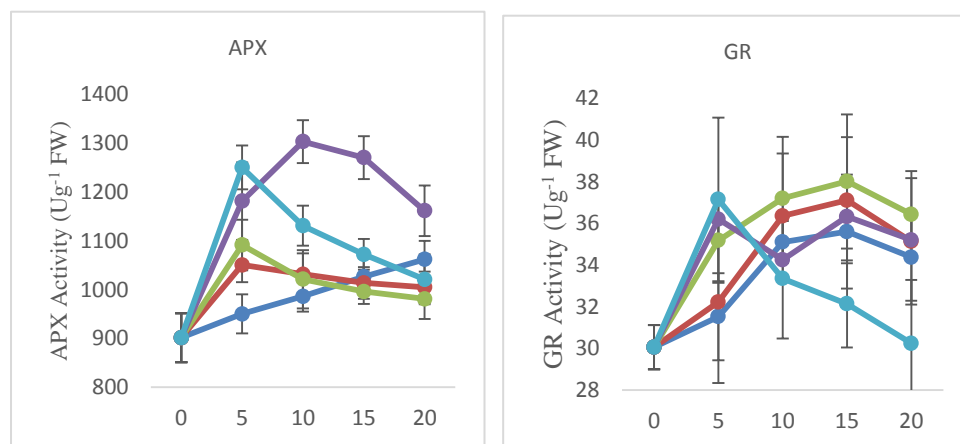
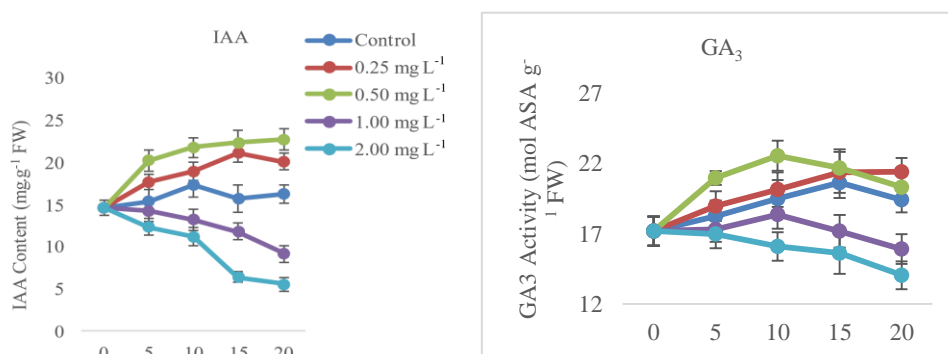


Figure 7. Effects of Rg1 on the antioxidant enzymes activities during the early seedlings growth. Error bars present standard errors of five independent biological replicates.

### Hormone content in leaves of ginseng seedlings

At 0.50 mg L<sup>-1</sup> Rg1 the content of GA<sub>3</sub> increased by 31.16 % in 10 days old ginseng seedlings (Fig 8). At 2.00 mg L<sup>-1</sup> Rg1 treatment, the contents of IAA, GA<sub>3</sub> decreased by 61.98 % and 18.31 %, respectively. Likewise, at 1.00 mg L<sup>-1</sup> Rg1 treatment, after 20 days the contents of IAA, GA<sub>3</sub> decreased by 37.13 % and 7.5 %, respectively, than untreated seedlings. Unlike the content of IAA and GA<sub>3</sub>, the ABA content increased with applied Rg1.



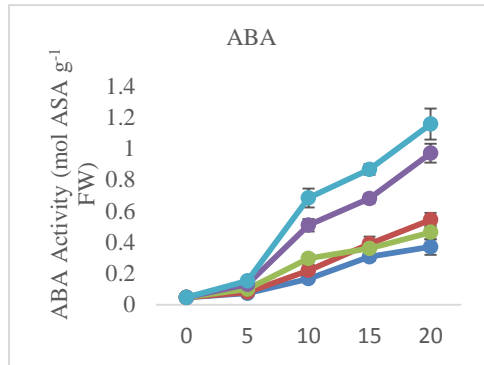


Figure 8. Effects of Rg1 on the content of endogenous plant hormones during the early seedlings growth. Error bars present standard errors of five independent biological replicates.

In addition, the ABA content of seedlings treated with 2.00 mg L<sup>-1</sup> was significantly higher than control. The increase in ABA content at 0.25 mg L<sup>-1</sup> and 0.50 mg L<sup>-1</sup> Rg1 was 3.14, 2.15 and 2.52 folds, respectively ( $p < 0.05$ ). Previous researches have shown that allelochemicals increases the plant hormones contents of crops and weeds (25,37). Evidence from physiological studies indicated that IAA, ZT (Zeatin) and GA<sub>3</sub> affected the cell enlargement and balanced the plant growth (14,19,40). Low concentration of Rg1 increased the content of IAA, ZT and GA and promoted the seedlings growth. Contrarily, the high concentration of Rg1 decreased the content of IAA, ZT and GA<sub>3</sub> and subsequently reduced the seedlings growth. Radicle length and fresh weight were related to the contents of IAA, ZT and GA<sub>3</sub> and were affected by Rg1. This is similar to the results of abiotic stress and biological stress treatment in other plants such as Faba bean (*Vicia faba* L.) and rice etc. (27,28,29). On the other hand, the content of ABA in seedlings treated with Rg1 was significantly higher than control, indicating that the increase in Rg1 stress increased the content of ABA, which was adaptation process to Rg1 stress. These results suggested that the endogenous hormones might have interactive effects on the ginseng seedlings to respond and adapt to Rg1 stress. Therefore, further research is needed to determine, how endogenous hormones regulate the growth of ginseng seedlings under Rg1 stress.

## CONCLUSIONS

Rg1 is one of the possible factors leading to the soil sickness of ginseng. The Rg1 isolated from the ginseng plants, inhibited the seeds germination, radicle elongation and fresh weight of ginseng at high concentration (0.5-2.00 mg L<sup>-1</sup>) and had stimulatory effects at low concentration (0.25 mg L<sup>-1</sup>). Rg1 had variable effects on antioxidant enzymes activities and plant hormones content in ginseng seedlings. Proper concentration (0.25-0.50 mg L<sup>-1</sup>) of Rg1 can increase the antioxidant enzymes activity and is beneficial to maintain the balance between the production and scavenging of ROS. High Rg1 concentration (1.00-2.00 mg L<sup>-1</sup>) induced excessive ROS production and inhibited the growth of ginseng seedlings. Furthermore, Rg1 inhibited the radicle elongation and

tolerance to Rg1. However, to estimate the autotoxicity potential of ginseng, further pot and field experiments needs to be conducted.

### ACKNOWLEDGEMENTS

This research was financially supported by the Natural Science Foundation of Jilin province, China (No.20190201297JC) and the National Natural Science Foundation of China (31701354). National Key R&D Program of China (2017YFC1702101).

### REFERENCES

1. Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology* **105**:121-126.
2. Ali, S., Chaudhary, A., Rizwan, M., Anwar, H.T., Adrees, M., Farid, M., Irshad, M.K., Hayat, T. and Anjum, S.A. (2015). Alleviation of chromium toxicity by glycinebetaine is related to elevated antioxidant enzymes and suppressed chromium uptake and oxidative stress in wheat (*Triticum aestivum* L.). *Environmental Science and Pollution Research* **22**: 10669-10678.
3. Bernards, M.A., Ivanov, D.A., Neculai, M.A. and Nicol, R.W. (2011). Ginsenosides: Phytoanticipins or host recognition factors? In: *The Biological Activity of Phytochemicals* (Ed., D.R. Gang), pp. 13-32. Springer, New York.
4. Bi, X., Yang, J. and Gao, W. (2010). Autotoxicity of phenolic compounds from the soil of American ginseng (*Panax quinquefolium* L.). *Allelopathy Journal* **25**: 115-122.
5. Bu, R., Xie, J., Yu, J., Liao, W., Xiao, X., Lv, J., Wang, C., Ye, J. and Calderón-Urrea, A. (2016). Autotoxicity in cucumber (*Cucumis sativus* L.) seedlings is alleviated by silicon through an increase in the activity of antioxidant enzymes and by mitigating lipid peroxidation. *Journal of Plant Biology* **59**: 247-259.
6. Cakmak, I. and Marschner, H. (1992). Magnesium deficiency and high light intensity enhanced activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. *Plant physiology* **98**: 1222-1227.
7. Chiboub, M., Jebara, S.H., Abid, G., and Jebara, M. (2019). Co-inoculation effects of *Rhizobium sulae* and *Pseudomonas* sp. on growth, antioxidant status, and expression pattern of genes associated with heavy metal tolerance and accumulation of cadmium in *Sulla coronaria*. *Journal of Plant Growth Regulation* **1**: 1-13.
8. Coelho, É.M.P., Barbosa, M.C., Mito, M.S., Mantovanelli, G.C., Oliveira, R.S. and Ishii-Iwamoto, E.L. (2017). The activity of the antioxidant defense system of the weed species *Senna obtusifolia* L. and its resistance to allelochemical stress. *Journal of chemical ecology* **43**: 725-738.
9. Coon, J.T. and Ernst, E. (2002). *Panax ginseng*. *Drug Safety* **25**: 323-344.
10. Dorning, M. and Cipollini, D. (2006). Leaf and root extracts of the invasive shrub, *Lonicera maackii*, inhibit seed germination of three herbs with no autotoxic effects. *Plant Ecology* **184**: 287-296.
11. Guo, Z.Y., Kong, C.H., Wang, J.G. and Wang, Y.F. (2011). Rhizosphere isoflavones (Daidzein and genistein) levels and their relation to the microbial community structure of mono-cropped soybean soil in field and controlled conditions. *Soil Biology and Biochemistry* **43**: 2257-2264.
12. Halliwell, B. and Foyer, C. (1978). Properties and physiological function of a glutathione reductase purified from spinach leaves by affinity chromatography. *Planta* **139**: 9-17.
13. Huang, L.F., Song, L.X., Xia, X.J., Mao, W.H., Shi, K., Zhou, Y.H. and Yu, J.Q. (2013). Plant-soil feedbacks and soil sickness: From mechanisms to application in agriculture. *Journal of Chemical Ecology* **39**: 232-242.
14. Hui, W., Wang, Y., Yan, S., Shi, J., Huang, W., Zayed, M.Z., Peng, C., Chen, X. and Wu, G. (2018). Simultaneous analysis of endogenous plant growth substances during floral sex differentiation in *Jatropha curcas* L. using HPLC-ESI-MS/MS. *Scientia Horticulturae* **241**: 209-217.
15. Jin, X., Zhang, J., Shi, Y., Wu, F. and Zhou, X. (2019). Green manures of Indian mustard and wild rocket enhance cucumber resistance to Fusarium wilt through modulating rhizosphere bacterial community composition. *Plant and Soil* **441**: 283-300.
16. Kimura, Y., Sumiyoshi, M., Kawahira, K. and Sakanaka, M. (2006). Effects of ginseng saponins isolated from Red Ginseng roots on burn wound healing in mice. *British Journal of Pharmacology* **148**: 860-870.

17. Kraus, T.E., McKersie, B.D. and Fletcher, R.A. (1995). Paclobutrazol-induced tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity. *Journal of Plant Physiology* **145**: 570-576.
18. Li, J., Liu, X., Wang, Q., Huangfu, J., Schuman, M.C. and Lou, Y. (2019). A group D MAPK protects plants from autotoxicity by suppressing herbivore-induced defense signaling. *Plant Physiology* **179**: 1386-1401.
19. Li, X., Wang, L., Wang, S., Yang, Q., Zhou, Q. and Huang, X. (2018). A preliminary analysis of the effects of bisphenol A on the plant root growth via changes in endogenous plant hormones. *Ecotoxicology and Environmental Safety* **150**: 152-158.
20. Li, Y., Huang, X. and Ding, W. (2011). Autotoxicity of *Panax ginseng* rhizosphere and non-rhizosphere soil extracts on early seedlings growth and identification of chemicals. *Allelopathy Journal* **28**: 95-102.
21. Möhler, H., Diekötter, T., Herrmann, J.D. and Donath, T.W. (2018). Allelopathic vs. autotoxic potential of a grassland weed-evidence from a seed germination experiment. *Plant Ecology & Diversity* **11**: 539-549.
22. Nakano, Y. and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology* **22**: 867-880.
23. Nicol, R.W., Yousef, L., Traquair, J.A. and Bernards, M.A. (2003). Ginsenosides stimulate the growth of soil borne pathogens of American ginseng. *Phytochemistry* **64**: 257-264.
24. Polle, A., Otter, T. and Seifert, F. (1994). Apoplastic peroxidases and lignification in needles of Norway spruce (*Picea abies* L.). *Plant Physiology* **106**: 53-60.
25. Ren, K., Hayat, S., Qi, X., Liu, T. and Cheng, Z. (2018). The garlic allelochemical DADS influences cucumber root growth involved in regulating hormone levels and modulating cell cycling. *Journal of Plant Physiology* **230**: 51-60.
26. Sah, S.K., Reddy, K.R. and Li, J. (2016). Abscisic acid and abiotic stress tolerance in crop plants. *Frontiers in Plant Science* **7**: 571.
27. Saleh, A.M., Madany, M.M. and González, L. (2015). The effects of coumarin application on early growth and some physiological parameters in Faba bean (*Vicia faba* L.). *Journal of Plant Growth Regulation* **34**: 233-241.
28. Shu, K., Zhou, W., Chen, F., Luo, X. and Yang, W. (2018). Abscisic acid and gibberellins antagonistically mediate plant development and abiotic stress responses. *Frontiers in Plant Science* **9**: 416.
29. Shen, S., Liu, Y., Wang, F., Yao, G., Xie, L. and Xu, B. (2019). Graphene oxide regulates root development and influences IAA concentration in rice. *Journal of Plant Growth Regulation* **38**: 241-248.
30. Tamman, A., Badr, R., Abou-Zeid, H., Hassan, Y. and Bader, A. (2019). Nickel and ozone stresses induce differential growth, antioxidant activity and mRNA transcription in *Oryza sativa* cultivars. *Journal of Plant Interactions* **14**: 87-101.
31. Wu, L., Zhao, Y., Guan, Y. and Pang, S. (2008). A review on studies of the reason and control methods of succession cropping obstacle of *Panax ginseng* CA Mey. *Special Wild Economic Animal Plant Research* **2**: 68-72.
32. Xie, J.T., Mehendale, S.R., Wang, A., Han, A.H., Wu, J.A., Osinski, J. and Yuan, C.S. (2004). American ginseng leaf: Ginsenoside analysis and hypoglycemic activity. *Pharmacological Research* **49**: 113-117.
33. Yang, G. (2006). Physiological effects of allelochemicals from leachates of *Ageratina adenophora* (Spreng.) on rice seedlings. *Allelopathy Journal* **18**: 237-246.
34. Yang, L., Ma, X.Y., Ruan, X., Jiang, D.A., Pan, C.D. and Wang, Q. (2016). Enantioselective separation of 4, 8-DHT and phytotoxicity of the enantiomers on various plant species. *Molecules* **21**:528.
35. Yang, L., Ruan, X., Jiang, D., Zhang, J., Pan, C. and Wang, Q. (2017). Physiological effects of autotoxicity due to DHAP stress on *Picea schrenkiana* regeneration. *PLoS one* **12**: e0177047.
36. Yang, M., Zhang, X., Xu, Y., Mei, X., Jiang, B., Liao, J., Yin, Z., Zheng, J., Zhao, Z. and Fan, L. (2015). Autotoxic ginsenosides in the rhizosphere contribute to the replant failure of *Panax notoginseng*. *PLoS One* **10**: e0118555.
37. Yuan, X.K. and Yang, Z.Q. (2018). The effects of endogenous hormones on plant morphology and fruit quality of tomato under difference between day and night temperature. *Horticultural Science* **45**: 131-138.
38. Zhang, A.H., Lei, F.J., Fang, S.W., Jia, M.H. and Zhang, L.X. (2011). Effects of ginsenosides on the growth and activity of antioxidant enzymes in American ginseng seedlings. *Journal of Medicinal Plants Research* **5**: 3217-3223.
39. Zhang, J. (1990). Response of cell protective enzymes in corn leaf to water stress at seedling stage. *Acta Agriculturae Boreali-Sinica* (Suppl.) **5**: 19-23. (in Chinese).

40. Zhang, W., Lu, L.Y., Hu, L.Y., Cao, W., Sun, K., Sun, Q.B., Siddique, M.A., Shi, R.H. and Dai, C.C. (2018). Evidences for the involvement of auxin, ethylene and ROS signaling during allelochemical benzoic acid-mediated primary root inhibition of *Arabidopsis*. *Plant and Cell Physiology* **59**: 1889-1904.
41. Zhou, B., Kong, C.H., Li, Y.H., Wang, P. and Xu, X.H. (2013). Crabgrass (*Digitaria sanguinalis*) allelochemicals that interfere with crop growth and the soil microbial community. *Journal of Agricultural and Food Chemistry* **61**: 5310-5317.
42. Zhou, X., Wang, J., Jin, X., Li, D., Shi, Y. and Wu, F. (2019) Effects of selected cucumber root exudates components on soil *Trichoderma* spp. communities. *Allelopathy Journal* **47**: 257-266.