BRIEF COMMUNICATION

Effects of exogenous hormones on leaf photosynthesis of *Panax ginseng*

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Abstract

Ginseng (*Panax ginseng*) is a typical perennial shade plant. Aim of this study was to investigate the effects of exogenous hormones on photosynthesis of *P. ginseng*. At different growth stages, the aerial parts of *P. ginseng* plants were cut at the stem base and they were inserted into the nutrient solutions containing different exogenous hormones. Then the leaf photosynthesis and water absorbing capacity (absorbing water mass) of the excised plants were measured. The results showed that exogenous abscisic acid (ABA) decreased significantly net photosynthetic rate (P_N), stomatal conductance, transpiration rate, and absorbed water mass of excised *P. ginseng* at all growth stages, while both cytokinin (CTK) and indole-3-acetic acid (IAA) enhanced those parameters. Comparing different growth stages, ABA caused more severe inhibition of leaf photosynthesis at the early growth stage, while CTK and IAA showed significant enhancement of leaf photosynthesis at later growth stage. ABA reduced highly intercellular CO_2 concentration of *P. ginseng* at the flowering and green fruit stages, but it had only a small effect at red fruit early and red fruit stages. During the early growth stage, the inhibitory effect of ABA on leaf P_N might be caused mainly due to the stomatal limitation. However, the reason for this reduction was complex at the later growth stage and it included stomatal and other factors.

Additional key words: absorbed water mass; ginseng; photosynthesis.

Ginseng is a highly valued herb and belongs to the most popular herbal remedies. It has been used as the medicinal plant in China for thousands of years and is widely cultivated in northeast China. The major active components of ginseng are ginsenosides, a diverse group of steroidal saponins, which demonstrate the ability to target a myriad of tissues, producing an array of pharmacological responses. In western countries, current use has been diverse with the research being focused on cancer therapeutics. Ginseng can mitigate cancer through anti-inflammatory, antioxidant, and apoptotic mechanisms to influence gene expression (Helms 2004). Several studies had focused on photosynthesis of *P. ginseng* (Zhang *et al*. 2006, Yu *et al*. 2009, Chen *et al*. 2006). Xu *et al*. (2006) had reported that P_N of *P. ginseng* was related not only to light and temperature, but also to the age of plants. Plant growth and development is finely tuned by hormonal signals (Krouk *et al*. 2011). Plant hormones play also a central role in the ability of plants to adapt to changing environments by mediating growth, photosynthesis, development, epigenetic modification, and nutrient allocation (Perales *et al*. 2005, Li *et al*. 2011, Peleg and Blumwald 2011, Latzel *et al*. 2012, Zhu *et al*. 2012). Many studies have showed that plant hormones play important roles in the regulation of plant chlorophyll (Chl) formation (Kulaeva *et al*. 2002), mineral elements (Mills *et al*. 2001), photosynthesis (Jia and Lu 2003, Khan *et al*. 2002), and stomatal conductance (Liu *et al*. 2005, Mori *et al*. 2006). To date, the effects of exogenous hormones on the photosynthesis of *P. ginseng* have not been reported. The aim of this study was to investigate the effects of different exogenous hormones on leaf photosynthesis of *P. ginseng* at different growth stages.

The research was conducted in 2010 and 2011. Seeds of ginseng (*Panax ginseng* CA Mey) were provided by Jilin Chinese Medicine College. The plants were grown in Ginseng Garden of Jilin Agricultural University under 30% of full light. The temperatures were 22–27°C

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Abbreviations: A_m − apparent mesophyll conductance; ABA − abscisic acid; AWM − absorbed water mass; C_i − intercellular CO_2 concentration; CK1− whole plants grown in nutrient solution; CK2 − excised plants grown in nutrient solution; CTK − cytokinin; E − transpiration rate; FL − flowering; g_s − stomatal conductance; GF – green fruit; IAA − indole-3-acetic acid; P_N − net photosynthetic rate; RF – red fruit; RFE – red fruit early.
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during the day and 17–22°C at night during the experiment. The 4-year-old ginseng plants were used in this study. The aerial parts of the plants were cut at the stem base, and then the excised plants were inserted into the nutrient solutions containing different exogenous hormones for 2 h. Excission was conducted at four different growth stages and five treatments were used:

Development stage			
Early		Later	
Flowering	FL	Red fruit early	RFE
Green fruit	GF	Red fruit	RF

Treatment	
CK1	whole plants in nutrient solution
CK2	excised plants in nutrient solution
ABA	excised plants in nutrient solution + 0.5 mg L^{-1} ABA
CTK	excised plants in nutrient solution + 1 mg L^{-1} 6-benzylaminopurine
IAA	excised plants in nutrition solution + 1 mg L^{-1} IAA

The hormone concentrations used were approximately equal to the actual concentration in leaves (Pan 2010). The excission treatment excluded the effects of *P. ginseng* roots on photosynthesis, and it could immediately test the effect of exogenous hormones on photosynthesis and examine the roles of hormones in photosynthetic regulation.

The tube mouths were plugged with cotton wool (Fig. 1). After 2 h of the treatment (at 9:00 h), the absorbed water mass (AWM) and photosynthesis of excised plants were measured. AWM was calculated as $(M_1 - M_2)/2$, where M_1 is the combined mass of the nutrient solution, cotton wool, and test tubes, and M_2 is the combined mass of the nutrient solution, cotton wool, and test tubes after 2 h. AWM was expressed as mg(H_2O) s^{-1}. P_N, g_s, transpiration (E) and intercellular CO_2 concentration (C_i) of leaves were measured with a portable photosynthesis system (*Model LI-6400*, *LI-COR Inc*., Lincoln, NE, USA) at 9:00–11:00 h. The fixed light source was red and blue light with intensity of 300 μmol m^{-2} s^{-1}. The ambient CO_2 concentration was set at 380 μmol mol^{-1}. Apparent mesophyll conductance (A_m) was calculated as follows: $A_m = P_N/C_i$. The experiment was set in a randomized complete block design with five replicates. Statistical analysis of the data was performed using the statistical program *SPSS 13.0* (*SPSS company*, USA). The data in figures and tables are the means of 2010 and 2011 experiments. The treatment mean values at the same growth stage were compared by post-hoc least significant difference (LSD) test.

The excission and both the CTK and IAA treatments increased P_N of *P. ginseng*, while it was reduced by the exogenous ABA treatment (Fig. 1). This indicated that ABA inhibited photosynthesis at all growth stages, while CTK and IAA greatly elevated leaf P_N. After the excission, g_s of CK2 was higher than that of CK1 (Fig. 1*B*). ABA had the significant inhibitory effect on g_s at all growth stages, while CTK and IAA increased g_s. The impacts of different treatments on E were similar to g_s (Fig. 1*C*). Fig. 1*D* showed that the excission treatment had a small effect on leaf C_i. Exogenous ABA reduced C_i at the flowering (FL) and green fruit (GF) stages, but did not affect C_i at the red fruit early (RFE) and red fruit (RF) stages, while the impacts of CTK and IAA on C_i were insignificant. ABA reduced P_N/C_i at GF later, but CTK and IAA stimulated greatly P_N/C_i at all growth stages (Fig. 1*E*). Exogenous ABA decreased AWM at all growth stages, whereas CTK and IAA enhanced it significantly (Fig. 1*F*).

ABA had the severer inhibitory effect on P_N, g_s, E, and C_i at the early growth stages than at the late ones, while CTK and IAA caused more significant enhancement of P_N, g_s, and E at the later growth stages and they had small effects on C_i (Table 1). The percentage change of each treatment relative to CK2 was calculated (Table 1).

Many studies reported that ABA, CTK, and IAA can regulate leaf photosynthesis (Khan *et al*. 2002, Ahmed *et al*. 2006, Shao *et al*. 2011, Prokopová *et al*. 2010). Guinn *et al*. (1993) showed that ABA inhibited the photosynthesis of the cotton, whereas both CTK and IAA facilitated it. In this study, the treatments with the exogenous hormones also showed that ABA had the inhibitory effect on P_N of *P. ginseng*, but it was stimulated by both CTK and IAA; and that the effects was growth stage–dependent (Table 1, Fig. 1). ABA inhibited more severely leaf photosynthesis at the early growth stages, while CTK and IAA caused more significant enhancement of leaf photosynthesis at the later growth stages (Table 1, Fig. 1).

Many studies reported that CTK and IAA can affect g_s (Zhang *et al*. 2008, Tanaka *et al*. 2006). Our results showed that both CTK and IAA significantly elevated g_s and E and increased P_N/C_i and AWM at all growth stages. However, CTK and IAA had only a small effect on the C_i. The stomata opening caused by exogenous CTK and IAA treatments might be the main reason for the increase of P_N. Many studies have indicated that ABA plays the important role in regulating g_s. ABA can induce stomata closure by increasing NO and H_2O_2 concentrations in guard cells (Neill *et al*. 2002, Desikan *et al*. 2004, Zhu *et al*. 2010). Our results showed that ABA inhibited greatly g_s at all growth stages. ABA reduced significantly C_i at FL and GF stages, but it had a small effect on C_i at RFE and RF stages. This indicated that the inhibition effect of ABA on leaf P_N might be mainly due to the stomatal limitation at FL and GF stages, while at and RF stages it might result from the combined effects of stomatal and

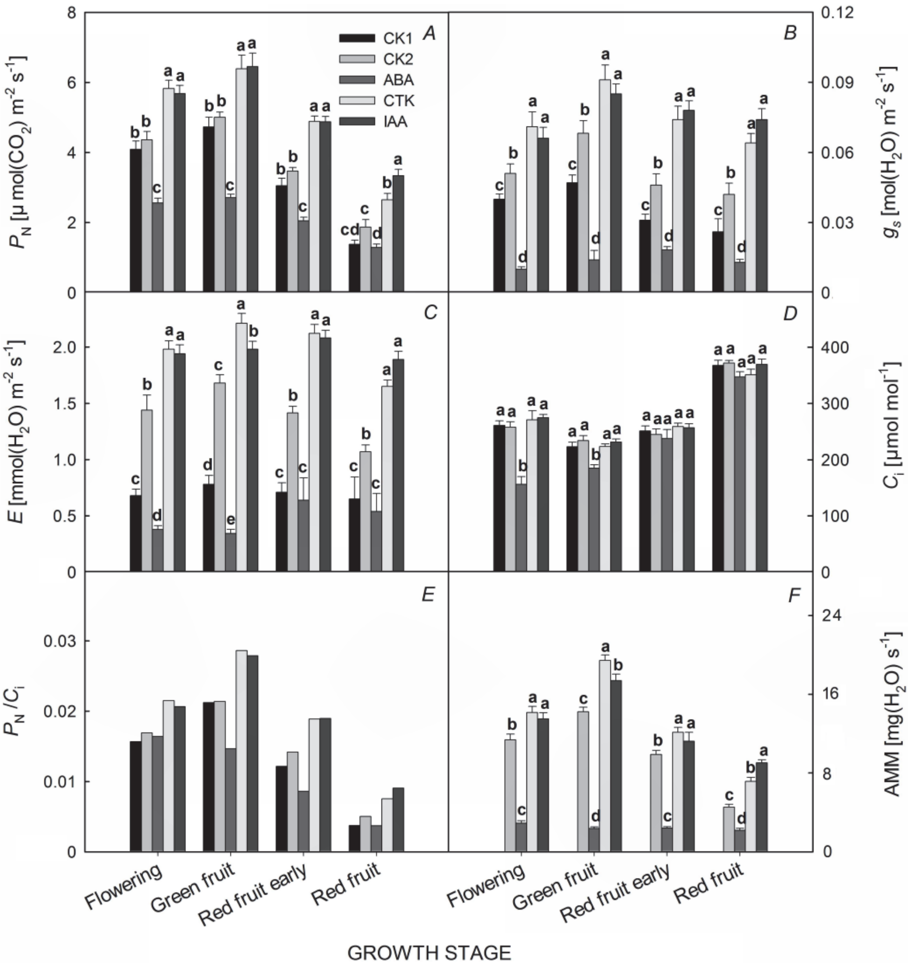

Fig. 1. Effects of exogenous hormones on photosynthesis and absorbed water mass (AWM) of *P. ginseng* at different growth stages. Means followed by different letters among treatments at the same growth stage are significantly different, according to least significant difference (LSD) test (P<0.05). P_N – net photosynthetic rate, g_s – stomatal conductance, E – transpiration rate, C_i – intercellular CO_2 concentration, CK2 – excised plants in nutrient solution; CK1 – whole plants in nutrient solution; ABA – abscisic acid; CTK – cytokinin; IAA – indole-3-acetic acid.

Table 1. Interactive effects of growth stages and exogenous hormones on leaf photosynthesis of *P. ginseng* relative to CK2. CK2 – excised plants in nutrient solution; ABA – abscisic acid; CTK – cytokinin; IAA – indole-3-acetic acid.

Growth stage	P_N [%]				g_s [%]			E [%]			C_i [%]		
	CK2	ABA	CTK	IAA	ABA	CTK	IAA	ABA	CTK	IAA	ABA	CTK	IAA
Flowering	-	–41.3	33.6	30.3	–80.4	39.2	29.4	–73.6	37.6	34.8	–39.5	5.0	6.6
Green fruit	-	–45.9	27.7	28.9	–79.5	33.5	24.7	–79.5	31.5	17.9	–20.9	–4.5	–1.1
Red fruit early	-	–40.9	40.9	40.5	–60.1	60.9	69.6	–54.7	49.9	47.1	–2.9	5.7	4.9
Red fruit	-	–31.0	41.7	78.6	–69.0	52.4	76.2	–49.5	54.2	76.6	–6.7	–5.6	–0.8

other factors. It had been reported that ABA inhibited significantly leaf ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity (Jiang *et al.* 2006, Wei and Ning 2002). Our study showed that ABA also inhibited significantly P_N/C_i. The ABA inhibition of leaf P_N might result from the combination of the stomatal factor and Rubisco inhibition at RFE and RF stages. Our results showed that the effects of plant hormones on the photosynthesis might be complex.

Application of the exogenous hormones has been widely used in the research of plant hormones (Aliyu *et al*. 2011). For example, Aliyu *et al*. (2011) reported that application of exogenous plant hormones improves flowering and fruiting in cashew (*Anacardium occidentale* L.). Latzel *et al*. (2012) reported that exogenous jasmonic acid and salicylic acid treatments induced epigenetic variation of *Arabidopsis thaliana*. Exogenous phytohormones also influenced accumulation of secondary metabolites, such as plumbagin (Gangopadhyay *et al*. 2011) and polyphenol (Quiroga *et al*. 2012). In these works**,** application of the exogenous hormones treatment was performed on leaf surfaces (foliar application). The treatment method used in the present study might be more effective than the foliar application. The excission treatment can exclude the effects of roots on photosynthesis, and immediately test the effects of exogenous hormones on photosynthesis and examine the roles of hormones in photosynthetic regulation.

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