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Diurnal changes in photosynthesis and antioxidants of *Angelica sinensis* as influenced by cropping systems

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Abstract

Diurnal dynamics of photosynthetic character of *Angelica sinensis*, as well as effect of continuous cropping on leaf photosynthetic character, antioxidant enzyme activity and growth of *A. sinensis* were investigated under field condition. The results showed that the diurnal net photosynthetic rate of *A. sinensis* in sunny day exhibited a double-peak pattern, and the peaks occurred at 9:45 and 16:45 h, respectively. There was a significant midday depression with *A. sinensis*, which was caused principally by stomatal factors such as stomatal conductance. The results also showed that net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and chlorophyll content (Chl) of *A. sinensis* plants under continuous cropping were significantly lower than those of the control. The activity of total superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), and growth parameters of *A. sinensis* plants were significantly decreased under continuous cropping condition. This study provides evidence of continuous cropping obstacle effect on photosynthesis, antioxidant enzyme activity, and growth parameters of *A. sinensis* in a field experiment, which partly explained the yield reduction of *A. sinensis* in the field, when it was cultivated continuously on the same soil.

Additional key words: *Angelica sinensis;* antioxidant enzyme activity; continuous cropping; growth parameters; photosynthesis.

Introduction

Farmers growing medicinal plants often encounter problems such as reduced production and quality when the same crop or cultivar is cultivated continuously on the same soil (Guo *et al*. 2006). This problem, described as the continuous cropping obstacle in this paper, includes plant stunting and leaf yellowing in an uneven pattern across a field. While the problem occurs worldwide at varying severity, there are locations and regions, where it does not occur.

Angelica sinensis (Oliv.) Diels (family Apiaceae) is a well known oriental herb used to treat the gynecological diseases in Traditional Chinese Medicine since ancient times (Zhang and Cheng 1997). It has been also widely used as an ingredient in cosmetic, health beverage, and drinks (Chen 2004) due to its multiple pharmacological effects. In order to meet its market demand, areas of continuous cropping with or without short rotation have increased dramatically for *A. sinensis* in the last decade. Because of this, growers face serious problems including stunting, plant mortality and *Ditylenchus destructor* infestation under continuous cropping. These difficulties reduce not only the yield, but also the quality of *A. sinensis*. Therefore, the Chinese medicinal plant, *A. sinensis*, has a significant continuous cropping obstacle effect, and it has become the main problem reducing *A. sinensis* production (Zhang *et al*. 2010a).

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Abbreviations: A – *Angelica sinensis*; C_a – CO_2 concentration in the measure chamber; C_i – intercellular CO_2 concentration; Chl – chlorophyll; E – transpiration rate; g_s – stomatal conductance; PAR – photosynthetically active radiation; P_N – net photosynthetic rate; RH – relative humidity; T_a – air temperature; T_L – leaf temperature; CAT – catalase; EDTA – ethylene diamine tetraacetic acid; FM – fresh mass; MDA – malondialdehyde; NBT – nitroblue tetrazolium; P – potato; POD – peroxidase; PVP – polyvinyl pyrrolidone; ROS – reactive oxygen species; SOD – superoxide dismutase; TBA – thiobarbituric acid; W – wheat.
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Plants are exposed to a multitude of natural biotic and abiotic stressors (Lichtenthaler 1996), and most of these stressors affect the photosynthetic performance of plant leaves (Lichtenthaler and Babani 2000). The photosynthetic performance of plants could be measured through the photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i). The photosynthetic capacity of leaves depends on the characteristics and amounts of components of the photosynthetic machinery, the production of which depends on the availability of nutrients.

The main objective of this study was to show diurnal dynamics of photosynthesis of *A. sinensis* and its relation to environmental factors, as well as the effect of continuous cropping on leaf photosynthetic characteristics, antioxidant enzymes and the growth of *A. sinensis* during the field experiment. This study focused on (*1*) the diurnal dynamics of P_N, E, g_s, and C_i of *A. sinensis* leaves and (*2*) the effect of continuous cropping on P_N, E, g_s, and C_i, chlorophyll (Chl) content, antioxidant enzyme activity, and growth parameters of *A. sinensis*. The study will help explore the continuous cropping problem, improving the cultivation and maximization of *A. sinensis* growth and production in the traditional producing areas.

Materials and methods

Climate and soil characteristics: The experiment was conducted in a field at Qingshui village, Min county (103°34'E, 34°27'N; 2,300 m a.s.l.), Gansu Province, China, during the growing season from March to October of 2007. The climate is alpine continental climate with the average temperatures for day and night being 25 and 14°C, respectively, and the light and dark periods being 15 and 9 h per day, respectively. The annual precipitation is approximately 596 mm, of which 79% occurs between May and September. The average frost-free season is 110 d. The annual accumulated temperature with equal to or more than 10°C is about 2,000°C. The soil of the site is sandy loam.

Experimental design: The experiment consisted of three completely randomized blocks. The three blocks were in a similar condition with each of the three *A. sinensis* blocks having different preceding crops. The block with wheat as the preceding crop (with potato as the crop preceding wheat) was the control (P-W-A). The block with *A. sinensis* as the preceding crop (with wheat as the preceding crop of *A. sinensis*) was used as the treatment (W-A-A) for continuous cropping.

Field management: The selection of *A. sinensis* varieties, fertilization interval, and planting densities were based on common practices used by local farmers. Manual weeding was used to control the weeds. *A. sinensis* plant was harvested manually on 25 October.

Gas-exchange measurements: Diurnal fluctuations in the P_N, E, g_s, and C_i of *A. sinensis* leaves were measured on three consecutive days (20–22 August) under conditions presented in Fig. 1 using a portable photosynthesis system (*CI-310, CID Bio-Science, Inc*., USA). Six similar, healthy, recently mature leaves on the top of the stem in a treatment were measured and readings were repeated 5 times per leaf (Chen *et al*. 2005).

Photosynthetic pigments: After the photosynthetic activity was determined, all leaves were harvested and transported to the laboratory in ice-coolers. We collected 0.1 g of fresh leaf mass to determine Chl content. Leaves were then ground in 80% acetone to extract both Chl *a* and Chl *b*. Pigment quantity was measured according to Lichtenthaler (1987).

Antioxidant enzyme activity and lipid peroxidation determination: At rhizome thickening stage (August 23, 2007), similar, healthy and mature leaves were collected on the middle of the stem of *A. sinensis* in each replicate treatment, transported to the laboratory in ice-coolers, and analyzed immediately to determine the activity of SOD, POD, and CAT, and content of MDA, proline, and soluble sugar.

Antioxidant enzyme extraction and activity determination were carried out following the method of Nakama and Asada (1981). Generally, each 0.5 g of leaf material was homogenized with the extraction buffer containing 50 mM phosphate buffer (pH 7.4), 1 mM EDTA, 1 g PVP, and 0.5% (v/v) Triton X-100. The homogenate was centrifuged for 20 min at 12, 000 × *g* and the supernatant obtained was used for enzyme analysis. All operations were carried out at 0–4°C. SOD activity was measured by its ability to inhibit the photochemical reaction of nitroblue tetrazolium (NBT) at 560 nm (Becana *et al*. 1986). One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of the reduction of NBT. SOD activity was expressed as the activity per g of fresh mass (FM) of fresh leaves. POD activity was measured by monitoring the increase in absorbance at 470 nm due to guaiacol oxidation at 25°C (Nakama and Asada 1981). One unit of POD activity was defined as the increase in absorbance at 470 nm for 1 min due to guaiacol oxidation. CAT activity was assayed by monitoring the disappearance of H_2O_2 at 240 nm at 25°C (Chance *et al*.1979). One unit of CAT activity was defined as the decrease at 240 nm for 1 min due to H_2O_2 consumption.

For the measurement of lipid peroxidation in leaves, the TBA test was used; it determines MDA as an end product of lipid peroxidation (Zhou *et al*. 2004). Proline was quantified according to the method described by Bates *et al*. (1973). Fresh plant samples were homogenized with 2 mL of 40% ethanol in a cold mortar containing a small amount of washed sterile sand. After agitation for 10 min, the extract was filtered through *Whatman No. 2* filter paper and a 2-mL aliquot was used. Absorbance was measured in spectrophotometer (*SP-752PC, Shanghai Spectrum Instrument, Inc.*, China) at 528 nm. Results were expressed as μg proline per g(FM) according to proline standard curve.

Growth parameters: At rhizome thickening stage (August 23, 2007), six plants were collected at each replicated treatment and used for a determination of the plant height, main root length, shoot biomass and root biomass.

Yield and quality parameters: At the harvest stage (25 October), all plants in the area of 1 m^2 were taken out of the soil and separated into shoots (leaves and stems were combined) and roots. Roots were first cleaned using distilled water. Then the sample was oven-dried at 60°C for 48 h and weighted. Contents of essential oils and an alcohol-soluble extract were measured according to Zhang *et al*. (2010b).

Statistical analysis: All experimental data were analyzed by *ANOVA* using the *SPSS 13.0* software (*SPSS, Inc*., USA) and the significance differences were identified using the LSD method at the 0.05 and 0.01 probability level. The means and calculated standard error (SE) were reported.

Results

Plant growth parameters: As shown in Table 1, continuous cropping significantly decreased the main root length, plant height, and the biomass of *A. sinensis* plant. Compared with the control, the main root length, plant height, root biomass, and shoot biomass decreased by 20.50%, 25.85%, 61.50%, and 35.94%, respectively, under continuous cropping condition. Continuous cropping also decreased the ratio of belowground biomass to aboveground biomass of *A. sinensis* (Table 1). The increase in root growth under the control condition could enhance the root's absorptive capacity and nutrient uptake in natural and agricultural ecosystems.

Yield and quality: High yield and quality is a primary goal for medicinal plants growers. This study indicated that continuous cropping significantly decreased the yield, content of essential oils, and alcohol-soluble extract by 49.00%, 25.26%, and 12.58% (Table 1), as compared with control.

Antioxidant enzyme activity and lipid peroxidation in leaves of *A. sinensis*: Continuous cropping significantly decreased the activity of SOD, POD, and CAT in leaves of *A. sinensis*, while increased the content of MDA, proline, and soluble sugar, as compared with the control (Table 2).

Diurnal variations in leaf gas exchange of *A. sinensis* and environmental factors

Environmental factors: Diurnal fluctuations of PAR, RH, T_a, T_L, and C_a between 20 and 22 August 2007 in Qingshui village are shown in Fig. 1. The sun rose around 07:00 h of the local time and the radiation increased rapidly, so that at 08:00 h the PAR incident on the leaf

Table 1. Effect of continuous cropping on growth parameters, yield, and quality of *A. sinensis*. Lines in columns denoted by *different capital and small letters* are significantly different at *P*<0.01 and *P*<0.05, respectively. Values represent means ± SE (*n* = 6). P – potato; W – wheat; A – *Angelica sinensis*.

Rotation	Main root length [cm]	Root biomass [g plant^{-1}]	Plant height [cm]	Shoot biomass [g plant^{-1}]	Root/shoot	Yield [kg m^{-2}]	Essential oil content [%]	Alcohol-soluble extract content [%]
P-W-A	20.20 ± 2.86^{aA}	8.52 ± 1.00^{aA}	59.00 ± 1.8^{aA}	17.14 ± 1.26^{aA}	0.50 ± 0.02^{aA}	1.00 ± 0.13^{aA}	0.95 ± 0.07^{aA}	56.45 ± 2.23^{aA}
W-A-A	15.25 ± 3.96^{bB}	3.28 ± 0.70^{bB}	43.75 ± 0.8^{bB}	10.98 ± 0.43^{bB}	0.30 ± 0.01^{bB}	0.51 ± 0.07^{bB}	0.71 ± 0.08^{bB}	49.35 ± 2.53^{bA}

Table 2. Effect of continuous cropping on antioxidant enzyme activity and lipid peroxidation in leaves of *A. sinensis*. Lines in columns denoted by *different capital and small letters* are significantly different at *P*<0.01 and *P*<0.05, respectively. Values represent means ± SE (*n* = 6). SOD – superoxide dismutase; POD – peroxidase; CAT – catalase; MDA – malondialdehyde; FM – fresh mass; P – potato; W – wheat; A – *Angelica sinensis*.

Rotation	SOD [U g^{-1}(FM)]	POD [U min^{-1} g^{-1}(FM)]	CAT [U min^{-1} g^{-1}(FM)]	MDA [μmol g^{-1}(FM)]	Proline [μg g^{-1}(FM)]	Soluble sugar [%]
P-W-A	290.52 ± 1.83^{aA}	16.46 ± 0.27^{aA}	26.08 ± 0.91^{aA}	2.36 ± 0.07^{bB}	37.15 ± 0.73^{bB}	6.10 ± 0.04^{bB}
W-A-A	200.36 ± 8.65^{bB}	14.67 ± 0.64^{bB}	14.65 ± 0.64^{bB}	2.73 ± 0.04^{aA}	82.46 ± 1.80^{aA}	7.88 ± 0.11^{aA}

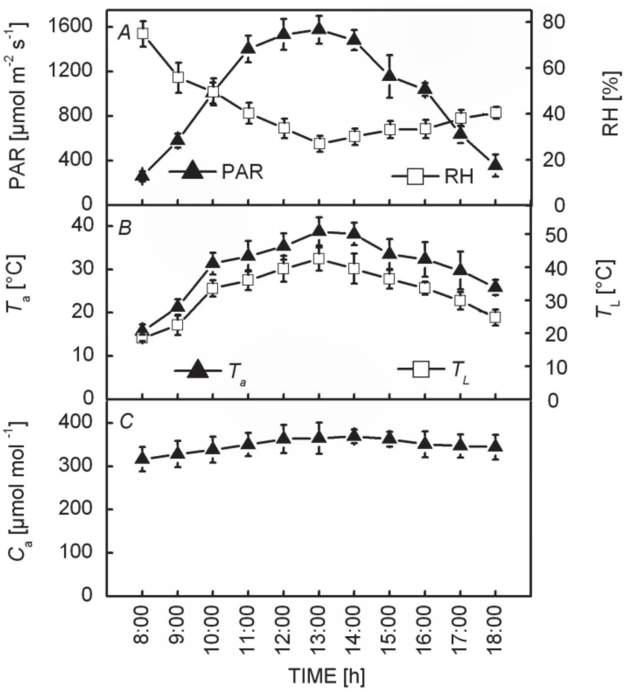

Fig.1. Diurnal fluctuations of photosynthetically active radiation (PAR) and relative humidity (RH) (*A*), air temperature (T_a) and leaf temperature (T_L) (*B*), and CO_2 concentration in the measuring chamber(C_a) (*C*) in full sunlight between 20 and 22 August 2007 in Qingshui. Values are means ± SE (*n* = 6).

epidermis of plants exceeded 200 μmol m^{-2} s^{-1}. At 09:00 h, PAR was 600 μmol m^{-2} s^{-1}, peaking at above 1,400 μmol m^{-2} s^{-1} between 11:00 and 14:00 h. The PAR decreased thereafter dropping below 600 μmol m^{-2} s^{-1} after 17:00 h (Fig. 1*A*). The diurnal fluctuations of RH was contrary to PAR, in the morning around 07:00 h, the RH was about 75%, then decreased rapidly and dropped to about 30% at midday, and then slowly increased and reached about 40% at 18:00 h. At equivalent irradiance, RH was significantly higher in the morning than in the afternoon (Fig. 1*A*).

T_L increased along with the increase in PAR and T_a from about 18°C just after sunrise to about 34°C at 10:00 h and 40°C at midday. At 15:00 h, T_L dropped to 35°C, reaching 25°C in the late afternoon (Fig. 1*B*). At equivalent irradiance, T_L was cooler in the morning than in the afternoon. At sunset, leaves were 6–8°C warmer than at dawn. The diurnal fluctuations of T_a was similar to T_L, but T_a was slightly lower than T_L all day.

C_a was about 310 μmol mol^{-1} in the early morning, increasing to about 370 μmol mol^{-1} at 14:00 h, and at 16:00 h it decreased below 350 μmol mol^{-1}, further decreasing to about 340 μmol mol^{-1} at sunset (Fig. 1*C*). C_a was slightly higher in the afternoon than in the morning.

Photosynthesis and transpiration: Diurnal variation of P_N in *A. sinensis* leaves exhibited a double-peak pattern

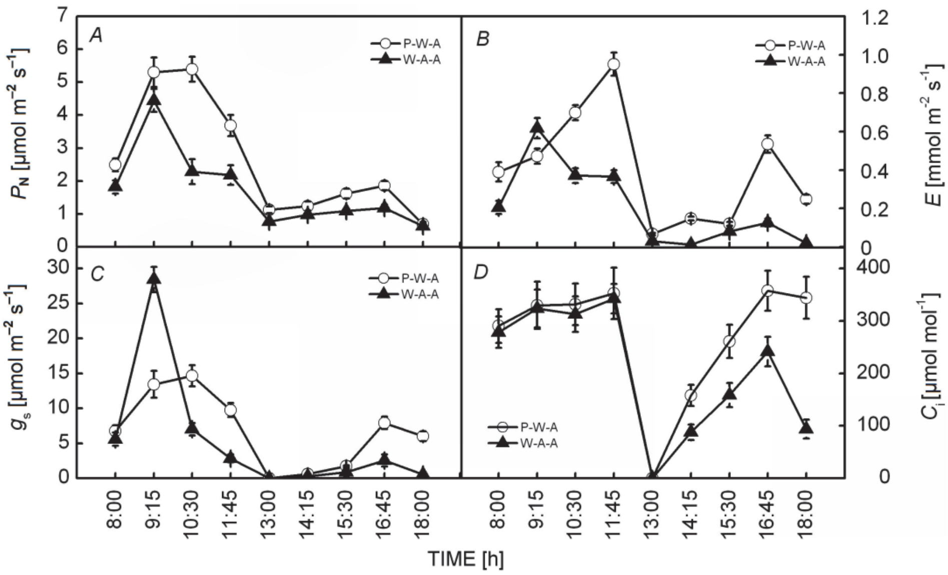

Fig. 2. Diurnal changes in net photosynthetic rate, P_N (*A*), transpiration rate, *E* (*B*), stomatal conductance, g_s (*C*), intercellular CO_2 concentration, C_i (*D*) in *A. sinensis* leaves at different treatment. Values are means ± SE (*n* = 6).

with obvious midday depression (13:00 h), and the peaks occurred at 09:15 and 16:45 h, respectively.The daily mean of P_N was significantly lower in continuous cropping than in control (Table 3). The average P_N for the 3 d of measurement for the different rotation systems (Fig. 2*A*) increased rapidly from zero at dawn (data not shown) to a maximum approximately at 09:15 h. This was followed by a progressive decrease, which was smaller and delayed in control compared with continuous cropping, and at 13:00 h, P_N dropped to the lowest value that is the 'midday depression' phenomenon. P_N gradually increased and reached the second peak in the late

Table 3. Effect of continuous cropping on photosynthetic parameters in leaves of *A. sinensis*. Lines in columns denoted by *different capital and small letters* are significantly different at $P<0.01$ and $P<0.05$, respectively. Values represent means ± SE ($n = 6$). P_N – net photosynthetic rate; E – transpiration rate, g_s – stomatal conductance; C_i – intercellular CO_2 concentration; Chl *a*(*b*) – chlorophyll *a*(*b*); FM – fresh mass.

Rotation	P_N [μmol m^{-2} s^{-1}]	E [mmol m^{-2} s^{-1}]	g_s [mmol m^{-2} s^{-1}]	C_i [μmol mol^{-1}]	Chl (*a*+*b*) [mg g^{-1}(FM)]	Chl *a* [mg g^{-1} FM]	Chl *b* [mg g^{-1} FM]	Chl *a*/Chl *b*
P-W-A	2.60±0.78^{aA}	0.39±0.03^{aA}	6.77±0.50^{aA}	269.57±11.91^{aA}	1.070 ± 0.009^{aA}	0.812 ± 0.007^{aA}	0.258 ± 0.003^{aA}	3.144 ± 0.032^{bB}
W-A-A	1.71±0.19^{bB}	0.19±0.02^{bA}	5.34±0.26^{bB}	204.47±12.34^{bB}	0.626 ± 0.011^{bB}	0.489 ± 0.014^{bB}	0.137 ± 0.006^{bB}	3.575 ± 0.236^{aA}

afternoon (at 16:45 h), then decreased gradually until the sunset. During the midday period, the 'midday depression' phenomenon was more apparent in continuous cropping system compared with the control (Fig. 2*A*). At the equivalent irradiance in each treatment, P_N was significantly higher in the morning than in the afternoon. The diurnal variation of E responded similarly to P_N (Fig. 2*B*). However, difference existed in the time and value of diurnal variation curve peaks for P_N and E. As shown in Fig. 2*B*, the peaks of E occurred at 09:15 and 16:45 h in the continuous cropping system respectively, while the peaks occurred at 11:45 and 16:45 h in the control. The daily mean of E was significantly higher in the control than in the continuous cropping system (Table 3).

Stomatal conductance and internal CO_2 concentration: In the early morning g_s was lower, it increased rapidly and reached the maximum at 09:15 h in the control and at 10:30 h in the continuous cropping, then decreased towards midday (13:00 h) and increased, peaking at about 16:45 h and then decreased until the sunset (Fig. 2*C*). C_i increased slowly from about 280 μmol mol^{-1} at 08:00 h to a maximum approximately at 11:45 h, then decreased rapidly and dropped to the lowest at midday, and then rapidly increased, peaking at about 16:45 h and then decreased until the sunset (Fig. 2*D*). According to the day average (Table 3), g_s and C_i were significantly higher in the control than in the continuous cropping.

Contents of photosynthetic pigments in leaves of *A. sinensis*: Continuous cropping significantly decreased the contents of Chl *a*, Chl *b*, and Chl *(a+b)*, while increased the ratio of Chl *a/b*, compared with the control (Table 3).

Discussion

Growth parameters: The effect of continuous cropping on vegetative growth has been investigated for different medicinal plants. Zhang *et al*. (2005) found that the output of aboveground and belowground parts of *Salvia miltiorrhiza* was lessened, the amount of roots, diameter and the length of roots were reduced; active principle of *S. miltiorrhiza* was decreased, shape of *S. miltiorrhiza* was deformed. Those factors lead the quality of herbal drugs to lessen under continuous cropping conditions. Wang *et al*. (1998) also indicated that continuous cropping decreased the vegetative growth and a yield of *Panax notoginseng*. In addition, continuous cropping was disadvantageous to the growth of *Rehmannia glutinosa, Atractylodes lancea*, *Panax ginseng*, and *Coptis chinensis* Franch (Guo *et al*. 2006, Liu *et al*. 2006). However, continuous cropping increased the biomass and yield in *Achyranthes bidentata* Blume (Wang *et al*. 2005). This study indicated that continuous *A. sinensis* cropping significantly decreased the plant height, main root length, biomass of *A. sinensis* plants during the vegetative period, and it led to lower yields. In addition, partitioning of growth between shoots and roots showed a significantly higher decrease in roots over shoots, resulting in decline in the ratio of the root to shoot biomass in continuously cropped plants. A similar finding was obtained in *Rehmannia glutinosa* plants (Zhang *et al*. 2011).

Antioxidant enzyme activity and lipid peroxidation: It is well known that plants generate more reactive oxygen species (ROS) and stimulate resistance responses of plants when exposed to stressful conditions (Hancock *et al*. 2002, Thoma *et al*. 2003). These ROS are either toxic by-products of aerobic metabolism or key regulators of growth, development, and the defense pathways (Ding *et al*. 2007, Laloi *et al*. 2004, Mehdy *et al*. 1996, Mittler *et al*. 2004). Toxic ROS can affect membrane permeability, induce lipid peroxidation, and ultimately lead to programmed cell death. In fact, plants possess efficient systems for scavenging ROS that protect them from destructive oxidative reactions (Olmos *et al*. 1994). As part of this system, antioxidant enzymes are key elements in the defense mechanisms. Many changes have been observed in the activities of antioxidant enzymes in plants under continuous cropping. In this study, the activity of antioxidant enzymes (SOD, POD, and CAT) decreased under continuous cropping condition, which was in agreement with results obtained from cucumber (Zhang *et al*. 2007) and grape (Guo *et al*. 2010).

Lipid peroxidation is a well established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues. MDA is the ultimate product of membrane lipid peroxidation, its content can reflect plant antioxidation ability and the strength of the metabolism. The results of this study showed that continuous cropping significantly increased MDA content in *A. sinensis* leaves, indicating that *A. sinensis* plant experienced an increased ROS generation and accumulation in leaves, leading to increased membrane peroxidation, and finally shown as the increase in MDA content.

Photosynthesis characteristics: Photosynthesis is also limited by continuous cropping (Zhang *et al*. 2011). Continuous cropping is one of the most severe limitation to medicinal plants (He *et al*. 2009, Zhang *et al*. 2005). Continuous cropping had a strong effect on the diurnal fluctuation patterns of *A. sinensis* leaves, which are closely linked to the biological rhythm of the plant. P_N, E, g_s, and C_i were reduced in plants under continuous cropping condition (Fig. 2*A–D*), which supports the results obtained for peanut (Zheng *et al*. 2008). The authors were able to show that, in addition to reduced growth, continuous cropping led to a decline of the photosynthetic activity of peanut due to a decrease in g_s, an inhibition of chloroplast activity, and a breakdown of Chl.

An apparent P_N 'midday depression' phenomenon in relation to continuous cropping was observed at the point, when PAR reached its diurnal maximum and the temperature was high (Fig. 2*A*). The 'midday depression' phenomenon was more apparent in the continuous cropping system compared with the control. High PAR and temperature might inhibit P_N by controlling stomata closure, because a similar pattern occurred with g_s and C_i. Photosynthetic limitation has been traditionally analyzed in terms of stomatal and nonstomatal limitations (Wu *et al*. 2008). In this study, the variation trend of P_N, g_s, and C_i indicated that P_N 'midday depression' phenomenon of *A. sinensis* leaves resulted from the stomatal limitation.

Chl content and Chl *a/b* ratio of leaves are widely used to characterize the general state of the photosynthetic apparatus (Terashima and Hikosaka 1995, Kitajima and Hogan 2003). Leaf Chl content is one of the most important factors in determining the photosynthetic rate and dry matter production (Dai *et al*. 2009). The changes in the Chl *a/b* ratio are related to the balance of the irradiance absorption capacity of the two photosystems. Increasing Chl *a/b* ratio is associated with the decrease in the size of PSII light-harvesting antenna, ensuring that the supply of electrons from PSII is sufficient to keep pace with the rate of excitation of PSI (Kitajima and Hogan 2003). The current study showed that the lower photosynthetic performance of *A. sinensis* plants might be associated with the decreasing Chl content under continuous cropping (Table 3). In plastic-greenhouse cultivation systems, continuous cropping can reduce crop quality, delay marketing, and consequently decrease profitability (Yao *et al*. 2006). Continuous cropping significantly affected the growth of *A. sinensis* as indicated in this study. The low Chl content, g_s, and P_N values, combined with the lower growth parameters (plant height, biomass) and quality parameters (essential oil and alcohol-soluble extract) (Table 1) of plants grown under continuous cropping suggest that continuous cropping damaged the *A. sinensis* plants.

Conclusion: The plasma membrane lipid peroxidation of the plants led to the impaired structure and function of plant cells under continuous cropping. Also, P_N, g_s, C_i, and Chl content of the *A. sinensis* plants under continuous cropping were significantly lower than those of the control. Therefore, it was suggested that the accumulation of ROS and free radicals in plant cells, caused by the stressful condition of continuous cropping, resulted in the damaged membrane structure, which in turn led to decreased Chl content and g_s, reduced photosynthetic capacity, and consequently to the retarded growth of the plants, reduced yield, and the quality of *A. sinensis*. This study provides the evidence of continuous cropping obstacle effect on *A. sinensis*, which partly explained the yield reduction of *A. sinensis* cultivated continuously in the same soil.

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