**Effects of arsenic on growth, photosynthesis and some antioxidant parameters of *Panax notoginseng* growing in shaded conditions**

Y. Q. Zu¹, J. J. Sun¹, Y. M. He¹, J. Wu¹, G. Q. Feng² and Y. Li²*

¹College of Resources and Environment, Yunnan Agricultural University, Kunming 650201, Yunnan Province, China.
²Wenshan Sanqi Institute, Wenshan College, Wenshan 663000, Yunnan Province, China.

**ABSTRACT**

This study assessed the effect of soil Arsenic (As) on growth - height, leaf area, biomass, relative growth rate (RGR), photosynthetic activities [net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), inter-cellular CO₂ concentration (Ci)] and selected antioxidant parameters [superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD)] of *Panax notoginseng* (Burk.) F. H. Chen. Two-years old plants in part-shade were subjected to As treatments, which were applied by supplementing soil with As to a final concentration of 20, 80, 140, 200 and 260 mg kg⁻¹ with supplementary Na₃AsO₄•12H₂O. The results obtained shows that total biomass, contents of total chlorophyll (Chl), Chl A and Chl B increased at low (20 mg kg⁻¹) As concentration; but declined at higher (80-260 mg kg⁻¹) As concentrations. The Chl A/B ratio, carotenoid content and ratio of root biomass to shoot biomass decreased in all As treatments. Pn had a 1.40-1.49 fold increase with As concentrations from 0 to 140 mg·kg⁻¹. Tr, Gs and stomatal limitation value increased significantly, whereas the inter-cellular CO₂ concentration and water use efficiency declined under As stress. Leaf SP content, relative electrical conductivity and activities of SOD, CAT and POD increased under As stress. These results indicate that stomatal limitation value should be the most important factor responsible for Pn decline under As stress. The sensitive markers of *P. notoginseng* under As stress include growth characteristics (leaf area, RGR, biomass) and antioxidant parameters (relative electrical conductivity, SOD activity).

©2016 BluePen Journals Ltd. All rights reserved

**INTRODUCTION**

Arsenic (As) is a highly toxic metal found widely in nature. Plants growing in soil with high As content may display abnormal growth, and they are also at risk of genetic mutation. Arsenic is at the top of the world's toxic top 10 hazardous substances on the 'Agency for Toxic Substances and Disease Registry' (ATSDR) (Li et al., 2008; Jedynak et al., 2010). As can be found in various industrial gas and water, and it is discharged into the environment in those waste materials. With the extensive use of As-containing pesticides, herbicides and fertilizers in agriculture, each year a large amount of As, in excess of 52000-112000 tonnes, is deposited into soil world-wide. Thus, soil As contamination has become a problem threatening the global environment as a whole (Lee et al., 2008; Yang et al., 2009; Gusman et al., 2013; Shen et al., 2014). In China, As mines are located in Hunan, Yunnan, Guangxi, and Guangdong provinces. In Yunnan, As content in red soil is in the range of...
16.4-19.20 mg/kg with a mean of 17.8 mg·kg⁻¹ (Hu and Ran, 2006). So far, extensive studies have been performed to understand how soil As pollution affects plant development in wheat, tobacco and peppers, as well as absorption, transportation, enrichment, and detoxification of the toxic metal by these plants (Mkandawire et al., 2004; Yan et al., 2011; Liu et al., 2012). It was found that micro-molar concentrations of As can actually enhance plant cellular oxidases activity and promote plant growth and development. However, excessive As is definitely harmful to leaf and root growth, resulting in low yield (Liet al., 2008; Zu et al., 2016).

As damages plant photosynthetic systems, causing symptoms including leaf necrosis, chlorophyll degradation, disturbance in chloroplast function, loss of photosynthetic pigments, decreased enzymatic activities in photosynthetic metabolism, and disruption of transportation of photosynthetic products and stomatal closure (Stoeva et al., 2004; Milivojevic et al., 2006; Rahman et al., 2007; Li et al., 2008). Arsenic induced the limitation of both stomat and non-stomat to influence growth of wheat (Liu et al., 2009). Net photosynthetic rates of some rice cultivars were increased and intercellular CO₂ concentrations, conductance to H₂O, and transpiration rate were decreased under As stress (Li et al., 2014). Most of these studies on As toxicity were conducted under natural light conditions. However, the effect of As on plant photosynthesis under shady conditions has not been fully investigated, except the few preliminary studies on Panax ginseng, Aquilaria sinensis, Pinellia ternate, and Panax notoginseng (Li et al., 2004; Xue et al., 2008; Li et al., 2009; Yuan et al., 2012).

P. notoginseng (Burk.) F. H. Chen is an herbaceous, perennial medicinal plant in the genus Panax of the Araliaceae family. The medicinal functions of the herb include dissipating blood stasis, stopping bleeding, relieving swollen tissue and pain-killing. Being a shade-loving plant, P. notoginseng grows well under 7-17% of full light (Cui and Liao, 2001).

Several studies have investigated leaf photosynthesis of P. notoginseng when provided with various levels of shade. It was found that intercellular CO₂ concentration is the main factor affecting the net photosynthesis rate (Pn) (Zhu et al., 2013). Constant and strong light irradiation induced stomatal closure (Li et al., 2009). But it is not known how soil As content levels affect leaf photosynthesis when P. notoginseng plants are grown in shaded conditions.

P. notoginseng is a native plant in Wenshan Prefecture in Yunnan Province. Wenshan Prefecture is also the main producer of the herb. In this region, soil has a very high As content due to the properties of earth's crust paleo-chemical reactions (Yan et al., 2011). The content of As in Wenshan is in the range 6.9-242.0 mg kg⁻¹, with a mean of 65.6 mgkg⁻¹ (Zhu et al., 2013). Such a high soil As content can create food safety issues for human use of the herb.

The aim of this study was to determine the toxicity of soil As to growth characteristics, photosynthesis, membrane permeability and activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) of P. notoginseng plants in shady conditions, and use the information to develop management options for producing safe products.

MATERIALS AND METHODS

Experimental materials and design

The plot experiment was conducted using two-year old P. notoginseng plants, on a research experimental station in Wenshan of Jiaozhi village in the suburb of Yanshan County, Yunnan Province, China (104°19'46" E, 23°34'59" N; elevation 1547 m). The pH value of soil for plot experiment was 6.69, with organic matter content 16.93 g kg⁻¹, total Nitrogen content 0.60 g kg⁻¹, total Phosphorus content 1.21 g kg⁻¹, available Nitrogen content 131.20 mg kg⁻¹, available Phosphorus content 54.74 mg kg⁻¹ and total As content 24.03 mg kg⁻¹. The soil for plot experiment was upland red soil with clay loam texture.

There were six As treatment levels (0, 20, 80, 140, 200 and 260 mg kg⁻¹) with three replicates each consisting of 132 plants grown on 3 m² plots. As treatments was supplemented with Na₂AsO₄·12H₂O, which was diluted in distilled water and added to the plot soils for two weeks before one-year old P. notoginseng was transplanted on March 1, 2013.

The experiment used a randomized block design. The plots were under the shaded condition in greenhouses with double black overshadow screens made by high density polyethylene (HDPE) to control to 7-17% of full light.

Data were collected on August 23, 2013 at the flowering stage. Growth parameters, leaf chlorophyll content, leaf net photosynthetic (Pn), transpiration rate (Tr), stomatal conductance (Gs) and inter-cellular CO₂ concentration (Ci), leaf soluble protein contents, antioxidant enzyme activities, lipid peroxidation and As content in leaf were measured, as described below.

Growth parameters collection

Height was measured from the stem base to the leaf base, which was on the top of plant. Dry biomass was measured by drying shoots and roots at 65°C to constant mass. The total biomass was shoots biomass plus root biomass. The leaf area was measured with a leaf area meter WDY-500A (Hangzhou Huier Instrument Limited
Company, China). The relative growth rate was calculated using the equation proposed by Stoeva et al. (2005):

\[ \text{RGR} = \frac{(\ln W_f - \ln W_i)}{t} \]

Where is RGR (relative growth rate); \( W_f \) and \( W_i \) (dry weight natural logarithm at the end and at the beginning of experiment, respectively) and \( t \) (experiment duration in days).

**Photosynthetic pigment content determination**

To determine the photosynthetic pigment content, freshly produced leaves were harvested from plants. After removal of mid-vein, leaves were sectioned into small pieces, mixed thoroughly, and then divided into aliquots of 0.1 g each. Tissue was homogenized under liquid nitrogen (N) and chlorophyll was extracted in acetone (80%). Absorbance at 663, 646 and 470 nm was recorded for chlorophyll a (Chl A), chlorophyll b (Chl B) and carotenoids (Caro) using a 755B model ultraviolet spectrometer (Shanghai Accurate Instruments Ltd). Total Chlorophyll a (total Chl), and ratio of Chl A/B were calculated according to Li et al. (2000). Pigments content was given at mg·g\(^{-1}\)FW.

**Photosynthesis parameters determination**

Photosynthesis was measured between 0900-1100 when the air temperature was 20-25°C, using a LI-6400 portable photometer (LI-COR) with setting of LED light source, leaf chamber size of 6 cm\(^2\) and photon flux density (PFD) of 800 μmol·m\(^{-2}\)·s\(^{-1}\). Leaflets on the largest fully expanded composite leaves were selected to record \( Pn, \) Tr, Gs and Ci. Each measurement was repeated five times. Water use efficiency (WUE) was derived as WUE = \( Pn/Tr \) and stomatal density (PFD) of 800 μmol·m\(^{-2}\)·s\(^{-1}\) were selected to record \( Pn, \) Tr, Gs and Ci. Each measurement was repeated five times. Water use efficiency (WUE) was derived as WUE = \( Pn/Tr \) and stomatal limitation value was calculated as \( Ls = 1-(Ci/Ca) \). \( C_0 \) (CO₂ concentration in the gas chamber) was preset at 395 μmol mol\(^{-1}\).

**Antioxidant parameters determination**

Fresh leaves (1.0 g) were homogenized with a mortar pestle using liquid N in 10 mL of an extraction buffer (20 mMTris-HCl in 1% polyvinylpyrrolidone, pH 7.4). After filtration through two layers of gauze to remove any debris, the homogenate was centrifuged at 10,000 g for 20 min. The supernatant was used to analyse enzyme activity. SOD was determined on the basis of its ability to inhibit the photochemical reduction of nitro blue tetrazolium. The reaction solution contained 50 mM phosphate buffer (pH 7.8), 13 mMethionine, 75 μM nitroblue tetrazolium, 2 μM riboflavin, 100 nM Ethylene diaminetetraacetic acid (EDTA) and H\(_2\)O. The riboflavin was added last. The reaction mixture was read at 560 nm. One unit of SOD activity was designated as the amount of enzyme that caused 50% inhibition of initial reaction rate. Based on H\(_2\)O₂ hydrolysis, the decreasing absorbance was measured at 240 nm (A240). Reduction of 0.1 at A240 in 1 min was designated as one unit of enzyme activity. POD activity was measured by guaiacol spectrophotometry. When exposed to H\(_2\)O₂, POD catalyzed the guaiacol to tetraguaiacol, which had an optical density (OD) of 470 nm. The reaction solution contained 100 mM phosphate buffer (pH 6.0), 33 mMguaiacol and 0.3 mM H\(_2\)O₂. Specific activity of POD was calculated from the increase in OD470 for 30 s. Total SOD, CAT and POD activity was expressed as U g\(^{-1}\)(FM) min\(^{-1}\).

Lipid peroxidation, the thiobarbituric acid (TBA) test, which determined malondialdehyde (MDA) content, was applied. The amount of MDA-TBA complex (red pigment) was measured by means of its specific absorbance at 532 nm. Non-specific absorbance at 600 nm was also subtracted. The data were calculated using the coefficient of absorbance of 155 mM\(^{-1}\)·cm\(^{-1}\).

Fresh leaf tissues (1.0 g) were homogenized in 5 ml of a prechilled 0.05 mol L\(^{-1}\)phosphate buffer supplemented with 1% polyvinyl pyrrolidone for the extraction of MDA. After bringing the volume to 25 mL, 10 mL of the mixture was taken to centrifugation at 4000×g for 10 min. MDA in the supernatant (200 μL) was assayed using the thiobarbituric acid method and the value was expressed as nmol mg\(^{-1}\) protein.

**Arsenic content determination**

Measurements of As content in leaves: Dried leaves (0.5 g) was digested in the mixture of 5 ml water, 5 ml of concentrated HNO₃ and 1.5 ml of H₂O₂ by using the microwave oven. Decomposition temperature was 140°C, ramp time 15 min and hold time 13 min. After digestion the solution was diluted to 25 ml with deionized water and filtered through an acid-resistant cellulose filter. Blank samples were prepared in a similar way. The As content was determined by atomic absorption spectroscopy (AA-6300C, Shimadzu, Japan).
### Table 1. Effects of Arsenic on growth parameters of *P. notoginseng*.

<table>
<thead>
<tr>
<th>Arsenic content (mg kg⁻¹)</th>
<th>Arsenic content in leaf (mg kg⁻¹)</th>
<th>Shoot height (cm)</th>
<th>Leaf area (cm²)</th>
<th>Shoot biomass (g plant⁻¹)</th>
<th>Root biomass (g plant⁻¹)</th>
<th>Total biomass (g plant⁻¹)</th>
<th>Ratio of root biomass to shoot biomass</th>
<th>Relative growth rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.12±0.01f</td>
<td>12.04±0.12a</td>
<td>32.3±2.02a</td>
<td>1.39±0.04a</td>
<td>2.58±0.05a</td>
<td>3.97±0.05a</td>
<td>1.86±0.07a</td>
<td>1.3±0.01a</td>
</tr>
<tr>
<td>20</td>
<td>0.18±0.02e</td>
<td>12.79±0.15a</td>
<td>33.6±2.12a</td>
<td>1.53±0.02a</td>
<td>2.72±0.02b</td>
<td>3.85±0.04b</td>
<td>0.78±0.06c</td>
<td>1.4±0.02a</td>
</tr>
<tr>
<td>80</td>
<td>0.26±0.02d</td>
<td>13.01±0.10a</td>
<td>32.3±3.01a</td>
<td>1.63±0.10a</td>
<td>1.19±0.02b</td>
<td>2.60±0.07b</td>
<td>0.97±0.01b</td>
<td>1.3±0.03a</td>
</tr>
<tr>
<td>140</td>
<td>0.30±0.01c</td>
<td>11.92±0.17b</td>
<td>27.28±1.04b</td>
<td>1.21±0.02b</td>
<td>1.02±0.09bc</td>
<td>2.23±0.04bc</td>
<td>0.99±0.02b</td>
<td>1.2±0.02b</td>
</tr>
<tr>
<td>200</td>
<td>0.34±0.02b</td>
<td>11.82±0.14bc</td>
<td>24.65±1.02c</td>
<td>1.18±0.07b</td>
<td>1.00±0.02c</td>
<td>2.18±0.04c</td>
<td>0.85±0.03c</td>
<td>1.2±0.01b</td>
</tr>
<tr>
<td>260</td>
<td>0.44±0.02a</td>
<td>11.58±0.09c</td>
<td>24.04±1.12c</td>
<td>1.10±0.04b</td>
<td>0.95±0.01c</td>
<td>2.05±0.02c</td>
<td>0.86±0.02c</td>
<td>1.1±0.03c</td>
</tr>
</tbody>
</table>

Note: Data are means ± SD. The different small letters indicated significant difference at *P*<0.05.

### Statistical analyses

All data presented here are the mean value ± standard deviation (SD) calculated from three replicates. Statistically significant differences among treatments were determined by using the least significant difference (LSD) test at *P*<0.05. Linear correlation analysis was conducted using statistical procedures in SPSS 11.5 for Windows.

### RESULTS

#### Effects of Arsenic on growth parameters of *P. notoginseng*

As contents in leaves of *P. notoginseng* increased with increased As concentrations. There was a significant positive correlation between As treatment concentration and leaf As content (*r*=0.985, *n*=6, *P*<0.01). Shoot height under 20-80 mg kg⁻¹ As treatments was not significantly different from the control (Table 1). Shoot height under 140-260 mg kg⁻¹ As treatments was inhibited with increasing As concentrations. Shoot height decreased by 4.1% under 260 mg kg⁻¹ As treatments compared with the control. Leaf area under 20-80 mg kg⁻¹ As treatments was not significantly different from the control (Table 1). Leaf area under 140-260 mg kg⁻¹ As treatments were inhibited with increasing As concentrations. Leaf area decreased by 23.7 and 25.6% under 200 and 260 mg kg⁻¹ As treatments compared with the control. Significant negative correlations were found between As treatment concentration and leaf As content (*r*=-0.955, *n*=6, *P*<0.01), and leaf area (*r*=-0.887, *n*=6, *P*<0.05), respectively.

Shoot biomass, root biomass and total biomass decreased with increased As concentrations, except for 20 mg kg⁻¹ As treatments increased by 10.1% compared with the control. Shoot biomass, root biomass and total biomass was inhibited by 20.9, 63.2 and 48.4% under 260 mg kg⁻¹ As treatments compared with the control. Significant negative correlations were observed between As treatment concentration and total biomass (*r*=-0.831, *n*=6, *P*<0.05) and shoot biomass (*r*=-0.914, *n*=6, *P*<0.05), respectively. Ratio of root biomass to shoot biomass decreased with increased As concentrations. Ratio of root biomass to shoot biomass decreased by 54.3 and 54.8%, respectively, under 200 mg kg⁻¹ and 260 mg kg⁻¹ As treatments compared with the control. A significant negative correlation between As treatment concentration and ratio of root biomass to shoot biomass was observed (*r*=-0.885, *n*=6, *P*<0.05).

RGR under 20-80 mg kg⁻¹ As treatments was not significantly different from the control (Table 1). RGR under 140-260 mg kg⁻¹ As treatments were inhibited with increasing As levels. Relative growth rate decreased by 22.2% under 260 mg kg⁻¹ As treatments compared with the control. A significant negative correlation between As treatment concentration and RGR was observed (*r*=-0.863, *n*=6, *P*<0.05).

#### Effect of Arsenic on leaf photosynthetic pigment contents of *P. notoginseng*

Contents of Chl A, Chl B and carotenoids from each of the five As treatments were significantly different from the untreated control (Table 2). Contents of Chl A, Chl B and total chlorophyll increased first and then decreased with increasing As concentrations. In the 20 mg kg⁻¹ As treatment, there was an increase of 6.4% in Chl A, 5.7% in Chl B and 6.2% in total chlorophyll, respectively. However, treatments with higher As concentrations resulted in decreased photosynthetic pigments, and the most serious effect was found in the 260 mg kg⁻¹ As treatment. Compared to the control, Chl A content significantly decreased by 8.4%, and total chlorophyll by 7.7%.

Carotenoid contents declined gradually when soil As content level was raised from 0 to 260 mg kg⁻¹.
Compared to the untreated control, the carotenoid content was a significant 15.7% lower in the highest As level treatment (260 mg kg\(^{-1}\)). Thus, it can be concluded that carotenoid content was repressed by As treatments. When compared between the five As treatment levels, the two high As concentrations (200 and 260 mg kg\(^{-1}\)) led to significantly lower carotenoid contents.

As treatment induced a significantly low Chl A/B ratio. When exposed to 260 mg kg\(^{-1}\) As, Chl A/B ratio decreased by 3.8%. The ratio of total chlorophyll to total carotenoid contents increased proportionally in accordance with As concentrations (Figure 1).

**Effects of Arsenic treatments on leaf photosynthetic properties of **\(P.\) **notoginseng**

Pn increased 1.48-1.40-fold in the 20 mg kg\(^{-1}\) and 80 mg kg\(^{-1}\)As treatments, respectively. But when As was raised to >140 mg kg\(^{-1}\), Pn declined rapidly until it was significantly lower than the control (Figure 2).

Respiration was induced by As treatments. The 200
and 260 mg kg$^{-1}$As treatments resulted in a 1.67-1.33-fold increase compared to the control. A similar trend of responses to As treatments was found in stomatal conductance. As induced a significantly higher level of stomatal conductance. A 1.64-1.46-fold increase was induced in the 200 and 260 mg kg$^{-1}$ As treatments, respectively, compared to the control.

When exposed to As treatments, intercellular CO$_2$ concentration was significantly lower than in the untreated control group. Furthermore, the degree of As-induced changes was proportional to As concentration. Intracellular CO$_2$ concentration was 26.6-37.5% of the control.

**Effect of Arsenic on water use efficiency and stomatal limitation level of *P. notoginseng***

Water use efficiency of *P. notoginseng* decreased proportionally in response to increasing As concentrations. The As treatment of 260 mg kg$^{-1}$ led to 61.0% of the control and the As treatment of 200 mg kg$^{-1}$ As treatment led to 67.8% of the control (Figure 3). Stomatal limitation value was significantly higher in As treated than untreated groups. In addition, the degree of As-induced changes was proportional to As concentration. The scale of increase in stomatal limitation value was 1.26-1.38-fold.
relative electrical conductivity increased gradually when soil As content level was raised from 0-260 mg kg\(^{-1}\). Compared to the control, relative electrical conductivity was a significant 57.1% higher in the treatment with the highest As concentration (260 mg kg\(^{-1}\)). When compared between the five As treatments, the two high As concentrations (200 and 260 mg kg\(^{-1}\)) led to significantly high relative electrical conductivity. Significantly positive correlations were observed between As treatment concentration and leaf As content \((r=0.886, n=6, P<0.05)\), and relative electrical conductivity \((r=0.924, n=6, P<0.01)\), respectively (Table 3).

Antioxidant enzyme, SOD, CAT and POD activities increased with 20-260 mg kg\(^{-1}\) As concentrations compared with the control. Compared to the control, SOD activity was a significant 42.9% higher in the 260 mg kg\(^{-1}\) As treatment. Significant positive correlations were
observed between As treatment concentration leaf As content (r=0.875, n=6, P<0.05) and SOD activity (r=0.880, n=6, P<0.05), respectively. CAT activity increased from As 0 to 80 mg·kg⁻¹ concentrations and then decreased from As 140 to 260 mg·kg⁻¹. Compared to the control, POD activity was a significant 57.3% higher in the 140 mg·kg⁻¹ As treatment. Thus, it can be concluded that antioxidant enzyme activities were stimulated by As treatments. When compared between the five As treatment levels, the antioxidant enzyme activities increased and then decreased at the highest As level treatments (260 mg·kg⁻¹), except for SOD activity (Table 3).

DISCUSSION

Responses of growth characteristics and resistance to Arsenic treatments of P. notoginseng in shaded conditions

Arsenic is toxic for plants. Growth of crops (such as rice) could be facilitated with low soil As contents (<40 mg·kg⁻¹) (Chen and Liu, 1993). Phosphonation of respiration could be disturbed strongly by As, which is one of the inhibitors to respiration and decreases consumption of photosynthetic production, resulting in increased Pn and growth improvement under low As concentrations. On the contrary, high soil As content could suppress plant growth. Shoot height, biomass and relative growth rate under 20-80 mg·kg⁻¹ As treatments were not significantly different from the control, which suggested that growth characteristics of P. notoginseng could be maintained in a stable condition.

The toxic content of As for P. notoginseng was ≥140 mg·kg⁻¹. At such concentrations shoot height, leaf area, biomass and relative growth rate decreased. Especially toxicity of excessive As to roots was higher than shoots due to the decreased ratio of root to shoot biomass with increasing As concentrations. In accordance with Liu et al. (2009) we presume that As inhibits transpiration, root activity and the absorption of water and nutrients by disorganizing the membrane structure of leaf and root cells, thus facilitating lipid peroxidation. Increased MDA accumulation and relative electrical conductivity were observed due to As stress (Liu et al., 2009). Enhanced lipid peroxidation indicates that As toxicity resulted in the increased production of reactive oxygen species (ROS), which caused membrane damage. The induction of antioxidant enzyme, including superoxide dismutase, catalase and peroxidase, are considered to play important roles in As stress. The activities of antioxidant enzymes were significantly more at As=80 mg·kg⁻¹. This suggests that activities of antioxidant enzymes were negatively correlated with shoot growth and biomass reduction and were more sensitive than growth characteristics. Since the activities of antioxidant enzymes are related to ROS formation, it is evident that As induced ROS accumulation. As compounds induce reducing reactions and thus enhance oxidase activities within cells.

Protein degradation is considered an adaptation of cells to sugar deficiency (Stoeva et al., 2005). On the contrary, the soluble protein amount of P. notoginseng increased under As stress, which suggested that soluble protein could be related to resistance and synthesized in order to respond to As stress.

Responses of photosynthetic pigments to As treatments of P. notoginseng in shaded conditions

From the curves of leaf contents in Ch A and Ch B and total chlorophyll as a function of As concentration, it can be seen that low concentrations of As promoted biosynthesis of those pigments, but it was harmful at high concentrations. A study by Liu et al. (2009) showed that As at ≤10 mg·kg⁻¹ had no inhibitory or damaging effect on wheat chlorophyll synthesis, but contents of Chl A, Chl B and carotenoids significantly decreased in As treatments ≥90 mg·kg⁻¹ (Liu et al., 2009). As also induced similar changes in photosynthetic pigments in Brasenia schreberi, maize (Zea mays), and rice (Oryza sativa L.) (Song et al., 2000; Stoeva et al., 2004, 2005). It was postulated that As can replace Mg and Fe in chlorophyll structure and interfering chlorophyll synthase, thus disrupting the biosynthetic pathways of chlorophyll. On the other hand, As may activate enzymes in chlorophyll degradation pathways. Activation of the two processes is responsible for the decreased photosynthetic pigment content in As treated plants (Li et al., 2008; Chen et al., 1985).

Chlorophyll A/B ratio significantly decreased under As treated conditions, and Chl A had a greater response than Chl B. Studies on peas (Pisum sativum) and Eulatriopsis binata also identified the lowered Chl A/B ratio as an indication of As toxicity (Paivoke and Simola, 2001; Yuan et al., 2012). Chl A is a light harvesting pigment and also a central pigment in the light reaction center (PSI), whereas Chl B only has a role in light harvesting. A low Chl A/B ratio indicates that stroma thylakoid membranes may be disrupted by As treatments. Since the integrity of the membrane system is essential for effective absorption and transmission of photons, such structural damage will decrease the efficiency in the conversion of quantum energy into chemical energy forms, and eventually low photosynthetic rates (Stoeva and Bineva, 2003; Shi et al., 2006; Chen et al., 2011).

Carotenoids content decreased gradually in response to increasing As concentrations. Stoeva and Bineva (2003) showed that As treatment led to low carotenoid content in maize. When comparing the As treated and untreated groups for the ratios of total chlorophyll and
Figure 1 shows that significant change occurred at the flowering stage. The results indicate that As treatments have more influence on carotenoid than chlorophyll contents during the flowering stage.

Carotenoids belong to a group of stress defense compounds which play important roles in conferring resistance or tolerance to oxidative stresses (Drezkiewica and Basznzki, 2010). Decreasing carotenoid content may lead to low cellular capacity for the removal of reactive oxygen species. One of the consequences is the denaturation of the D1 protein complex in the PSII center and chlorophyll degradation (Chen et al., 2011). For plants at the flowering stage, significantly lower carotenoid content can exaggerate the harmful effects of As. In summary, for plants growing under shaded conditions, the low contents of Chl A and carotenoid represent the greatest changes in photosynthetic pigments induced by As toxicity.

Responses of photosynthetic parameters to Arsenic treatments of P. notoginseng plants in shaded conditions

At flowering stage, As treatments ≤140 mg kg⁻¹ actually induced a significant increase in leaf Pn. With increasing As concentrations >140 mg kg⁻¹, Pn significantly decreased. These results accord with observations that As treatments lead to low Pn in maize, beans, rice barley, and tea tree (Stoeva et al., 2005; Shao et al., 2012).

The metabolism of photosynthetic pigments are responsible for the As-induced low Pn. Correlation analysis confirmed a significant positive correlation (Y=-19.24+38.20X, R=0.892, F=15.62, P<0.05, n=6) between carotenoid content and Pn. Additionally, respiration rate, stomatal conductance, and intercellular CO₂ concentrations also affect responses to As treatments, as these parameters are involved in physiological properties such as cellular water status, stomatal opening and cellular CO₂ metabolism (Farquhar and Sharkey, 1982).

Translocation of photosynthetic products is affected by respiration. For plants at flowering stage, respiration rate showed a very dynamic response to As. When As was <140 mg kg⁻¹, respiration rate and photosynthetic rate both increased, which indicates that these two parameters are very sensitive and the activation of these two physiological responses may enable plants to develop tolerance to As treatments. A high respiration rate indicates higher stomatal conductance. This study found that these two parameters had similar responses to As treatments. Furthermore, correlation analysis found a significant positive correlation between leaf respiration rate and stomatal conductance (Y=9.41+18.40X, R=0.9864, F=144.19, P<0.01, n=6) at the flowering stage. When exposed to As, P. notoginseng plants enhanced respiration by increasing stomatal conductance, which may be a mechanism to improve tolerance to As stress. Plants at the flowering stage actually showed an increase in Pn when As was applied at ≤140 mg kg⁻¹, which may result from a synergistic effect with the increasing chlorophyll content, stomatal conductance and respiration rate. However, As repressed respiration rate and stomatal conductance in rice and wheat (Chen et al., 2011; Drezkiewica and Basznzki, 2010). These conflicting results may be attributed to differences in the environmental conditions under which those experiments were performed, and the inter-specific variation in terms of tolerance to As toxicity. In a study using flag leaves of wheat growing on three soil types, it was found that respiration rate and stomatal conductance increased for plants growing in loam soil supplemented with high As concentration, but these two parameters was enhanced by low As but repressed by high As concentrations for plants in sandy and clay soil (Lu et al., 2011).

The degree of stomatal closure directly influences CO₂ uptake by plants. Thus, the low intercellular CO₂ concentration may be responsible for the decline of Pn in As treated plants. Such As-induced effects were less serious for plants, which resulted in a higher intercellular CO₂ concentrations. But the role of such relationships in relation to As tolerance cannot be explained based on results from this study.

Changes in intercellular CO₂ concentration and stomatal limitation value can be used to identify the key factors involved in low Pn. It is known that lowering intercellular CO₂ concentration, together with increasing stomatal conductance and the stomatal limitation value, result in low photosynthetic rate. When an increase in intercellular CO₂ concentration concurs with a decline in stomatal limitation, it would suggest a non-stomatal mechanism responsible for the low Pn (Zhang et al., 2008). In P. notoginseng, As treatments led to lower intercellular CO₂ concentrations, higher stomatal limitation and stomatal conductance, therefore, stomatal limitation is a major factor responsible for the low photosynthetic rate under As treatment conditions (Yuan et al., 2012). As treatments induced an increase in stomatal conductance and respiration rate and stomatal limitation value but a declining intercellular CO₂ concentration. These results indicate that the effect of stomatal limitation on photosynthesis should not be taken as a simple process involving only stomatal opening or closure. Changes in stomatal structure, the number and size of stomata, and the degree of stomatal opening, and intercellular CO₂ and water exchange properties and plant water use efficiency properties all contribute to the mechanisms that make plants more susceptible or tolerant to As (Liu et al., 2012). According to the stomatal optimality theory, plants would use the strategy for maximizing carbon gain by regulating stomatal opening (water loss) in the short-term. Thus, the decreased water use efficiency and higher respiration...
rate may both be used as mechanisms to develop
tolerance to As stress in *P. notoginseng* (Zhang et al.,
2008). In this study, the decline in water use efficiency
and a simultaneous greater stomatal limitation in
response to As treatment could be associated with
relieving As-induced stresses on *P. notoginseng* in
shaded conditions.

In summary, a low water use efficiency and low
intercellular CO₂ concentration together with an elevated
stomatal limitation were induced by As treatments. These
changes are responsible for the low photosynthetic rate
in *P. notoginseng* grown in shaded conditions. The higher
respiration rate and stomatal conductance may be
activated as a strategy to increase tolerance to As stress.

Conclusion

In this study, the effects of As on growth, photosynthetic
properties and selected antioxidant parameters of *P.
notoginseng* in shade were investigated. The results
obtained show that leaf As content, height, biomass
relative growth rate and leaf photosynthetic rate declined
in response to high As concentrations in soil, as well as
reduced contents of chlorophyll A and carotenoids, lowered
water use efficiency and intercellular CO₂ concentrations, and higher stomatal limitation values.
Based on these results, stomatal property is the major
factor contributing to the low photosynthetic rates under
As treatment conditions. Higher respiration rate and
stomatal conductance are proposed to enhance As
tolerance. Activities of superoxide dismutase, catalase,
peroxidase increased under As stress resulting from
increased soluble protein content, which suggests that
resistance of *P. notoginseng* to As could be increased
through antioxidant enzymes. The sensitive markers of
*P. notoginseng* to As stress are leaf area, relative growth
rate, biomass and superoxide dismutase activity. It is
recommended that the As concentration in soil for *P.
notoginseng* production should be less than 140 mg kg⁻¹.

ACKNOWLEDGEMENTS

The study was supported by the National Natural Science
Foundation of China (Grant Nos. 41261096, 31560163
and U1202236). We thank Prof. Michael A Fullen for his
valuable comments and English writing improvement.

REFERENCES

photosynthetic characteristics of Cd uptake and translocation
inseeding of two *Helianthus tuberosus* varieties. Acta Pratagculiiae
growth and development and its mechanism. Sichuan Agricultural
of trace elements in the eastern part of Yunnan Province. Yunnan
Drežkiewicz M. & Baszniki T. (2010). Interference of nickel with the
73:982-986.
Arsenate and arsinite: The toxic effects on photosynthesis and
growth of lettuce plants. Acta Physiologiae Plantarum. 35(4):1201-
1215.
Studies on the different arsenic forms and the influence of sample
pretreatment on arsenic speciation in white mustard (*Sinapis alba*).
exposure to arsenic due to rice ingestion in the vicinity of
96:231-235.
Li H. S., Sun Q. & Zhao S. J. (2000).The experimental principle and
technology of plant physiological and biochemical. Beijing: Higher
Li R. Y., Shen X. H., Zhang Y. H., Zhou Z. G. Xie J. J., Li Y. X., Xu X.
H. & Qin Z. J. (2014). Effects of inorganic Arsenic on seed
germination and photosynthetic characteristics of various rice
transmission rate on American ginseng’s photosynthesis. Chinese J
treatment on morphological structure and photosynthetic indices of
one-year-old *Panax notoginseng* plants. Journal of Yunnan
S. (2012). Effects of salt stress on growth and photosynthetic traits of
photosynthesis characteristics of wheat (*Triticum aestivum* L.) under
and physiological characteristics in *Halogeton glomeratus* with heavy
phosphorus content in different organs and chlorophyll fluorescence
of arsenic species to *Lemna gibba* L. and the influence of phosphate
on arsenic bioavailability. Environ. Toxicol. 19:26-34.
Mineral nutrients, chlorophyll content, and phytase activity.
& Tasnim A. (2007). Effects of arsenic on photosynthesis, growth
and yield of five widely cultivated rice (*Oryza sativa* L.) varieties in
Effects of arsenic stress on growth and photosynthetic characteristics
of wheat during filling stage in three textures of soil. Acta Agriculturae


