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The effects of phenolic acids on the photosynthetic characteristics and growth of *Populus* × *euramericana* cv. 'Neva' seedlings

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Abstract

Populus × *euramericana* cv. 'Neva' is an important tree species in northern China. In the study, we used its potted one-year-old seedlings as experimental material and established three treatments (CK, 0.5X, and 1.0X) according to the concentrations of phenolic acids in order to examine the effects of different concentrations on the photosynthetic characteristics and growth of poplar. With increasing concentrations of phenolic acids, the net photosynthetic rate, stomatal limitation, transpiration rate, apparent quantum yield, photochemical quenching coefficient, electron transport rate, chlorophyll content, and total biomass decreased significantly. The intercellular CO_2 concentration, light-compensation point, nonphotochemical quenching, malondialdehyde content, and root/shoot ratio increased significantly. Peroxidase and superoxide dismutase activities initially decreased and then increased. We concluded that phenolic acids significantly inhibited poplar's photosynthesis and the higher phenolic acid concentration, the greater inhibition of photosynthesis occurred. This inhibition effect was mainly caused by nonstomatal factors. Phenolic acids induced noticeable photoinhibition, resulted in the irreversible damage of membrane structure, and then changed intracellular metabolic processes. To cope with phenolic acid stress, poplar seedlings increased dissipation of excess light energy and distributed relatively more biomass to underground parts within carbon allocation.

Additional key words: allelochemical; chlorophyll fluorescence; CO_2 assimilative capacity; light response; light-use efficiency; reactive oxygen species.

Introduction

Poplars, especially black poplars, are the fastest-growing and high-yield industrial material tree species in China and have been planted at a large scale in Northwest China, Northern China, the Yellow River Basin, and some areas of the Yangtze River Basin (Zhang *et al.* 2008). However, because of afforested land being limited, continuous cropping and short rotations are often used as management practices in poplar plantations, which result in serious degradation of forestland productivity (Liu *et al.* 2005). Phenolic acid accumulation in continuously cropped soil and its allelopathic effect are important contributors to soil degradation in poplar plantations (Tan *et al.* 2008, Wang *et al.* 2016). These allelochemicals, which are exuded by plant roots, accumulate gradually at the rhizosphere (Wang and Wang 2010) and result in serious successive cropping obstacles by enhancing soil sickness (Ye *et al.* 2004,

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Abbreviations: AQY – apparent quantum yield; C_a – air CO_2 concentration; C_i – intercellular CO_2 concentration; Chl – chlorophyll; E – transpiration rate; DM – dry mass; ETR – electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; LCP – light-compensation point; L_s – stomatal limitation; LSP – light-saturation point; MDA – malondialdehyde; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; POD – peroxidase; q_P– photochemical quenching coefficient; R_D – dark respiration rate; ROS – reactive oxygen species; SOD – superoxide dismutase; WUE – water-use efficiency; Φ_{PSII} – effective quantum yield of PSII photochemistry.

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Zhang *et al.* 2016), antagonizing microbial activity (Kang *et al.* 2016, Lou *et al.* 2016), inhibiting nutrition uptake (Yu and Matsui 1997), and decreasing crop yield (Li *et al.* 2010).

Previous studies on successive cropping obstacles concern all kinds of crops, trees, and medical plants, such as buckwheat (Gao *et al.* 2014), cucumber (Gu *et al.* 2016), tomato (Kang *et al.* 2016), *Sorghum bicolor* (Sène *et al.* 2000), *Gossypium hirsutum* L. (Zhang *et al.* 2016), *Cunninghamia lanceolata* (Zhang 1993), *Rehmannia glutinosa* (Zhang *et al.* 2010), and *Pogostemon cablin* (Xu *et al.* 2015). Recently, a number of studies on successive cropping obstacles at poplar plantations is increasing because poplar wood demand exceeds its supply. Most studies examine phenolic acid accumulation in the soil and its effects on soil properties and roots growth (Liu *et al.* 2005, Tan *et al.* 2008; Wang *et al.* 2011, 2013, 2016; Zhu *et al.* 2015). Tan *et al.* (2008) analyzed the contents of five phenolic acids (*p*-hydroxybenzoic acid, vanillin, ferulic acid, benzoic acid, and cinnamic acid) in continuously cropped soil at a poplar plantation by high performance liquid chromatography. Zhu *et al.* (2015) established three treatments according to the concentrations of exogenous phenolic acids to examine the influence of phenolic acids and nitrogen on branch orders of poplar fine roots. However, studies regarding the effects of phenolic acids on the photosynthetic characteristics of poplar are scarce. The response of the growth, photosynthetic light-response processes, and chlorophyll (Chl) fluorescence of poplar to phenolic acid remains unclear. In this study, we investigated the influence of different concentrations of phenolic acid on the photosynthetic characteristics (*e.g.*, light response and Chl fluorescence parameters) and growth traits in one-year-old black poplar, cultivar I-107 (*Populus* × *euramericana* cv. 'Neva') clone. This study aims to examine the stress mechanism of phenolic acids on the growth of poplar and provide a scientific basis for overcoming the continuous cropping obstacles experienced during poplar farming.

Materials and methods

Study area: Our experiments were conducted at the Forestry Experimental Station of Shandong Agricultural University, Tai'an City, Shandong Province, China (36°11′ N and 117°08′E). The area belongs to a warm temperate zone with a semihumid continental monsoon climate. The frostless season lasts approximately 202 d. The average annual temperature is approximately 12.9°C, and the annual accumulated temperature over 10°C is 4,213°C. The average altitude is 150 m. The average annual precipitation is 741.8 mm, with more than 74% of the precipitation falling from June to September. The average annual relative humidity is 65%. The soil is classified as brown soil with a sandy loam texture. The pH value is approximately 8.4. The average soil bulk density is 1.29 g cm^{-3}.

Plant materials: In the middle of March 2014, one-year-old black poplar cv. I-107 (*Populus* × *euramericana* cv. 'Neva') clone seedlings with the diameter of 1 cm were derived from the National Black Poplar Germplasm Resource Base (Gaoqiao Forest Farm, Ningyang County, Tai'an City, Shandong Province, China) and cut into cuttings of 20 cm in length. The cuttings were placed in a hydroponic culture under natural light in an improved Hoagland nutrient solution (Wu 2005), which was changed once every two days. The cuttings with equal diameters were selected, and these cuttings generated root primordium in early April. Every cutting was planted in a pot (28 cm deep with an inner diameter of 30 cm) with vermiculite (approximately 8 L per pot). The improved Hoagland nutrient solution was changed once every five days.

Experimental design: In the middle of May 2014, the experiment was carried out using a randomized block design with six blocks (six replications) and three groups (three treatments) for each block. We established the following three treatments (Zhu *et al.* 2015): 0X (CK), 0.5X, and 1.0X (see the text table below) based on the actual concentration of phenolic acids in the soil of a second-generation continuously cropping poplar plantation as the reference concentration (X). The improved Hoagland nutrient solutions were added to the exogenous phenolic acids of three concentrations (0X, 0.5X, and 1.0X). Their pH values increased up to the continuously cropped soil pH value of 8.26 (Tan *et al.* 2008) by sodium carbonate (Na_2CO_3). Then, the experimental materials of tree treatments were watered with the corresponding improved Hoagland nutrient solutions in 2.0 L per pot once every ten days, respectively. The vermiculite medium was rinsed with distilled water on the fourth day before adding the nutrient solution to avoid phenolic acid accumulation.

Treatment	*p*-hydroxybenzoic acid [μg ml^{-1}]	Vanillin [μg ml^{-1}]	Ferulic acid [μg ml^{-1}]	Benzoic acid [μg ml^{-1}]	Cinnamic acid [μg ml^{-1}]
CK	0	0	0	0	0
0.5X	123	6	4	27	1
1.0X	247	11	7	54	2

Gas-exchange parameters: The gas-exchange parameters of poplar photosynthesis were measured by a portable photosynthesis system (*CIRAS-2*, *PP Systems*, Amesbury, MA, USA) in the middle of July 2014. Three strong and healthy seedlings with the same growth potential (equal seedling height and ground diameter) were selected from each treatment, and then, three fully developed mature leaves from the middle part of each seedling canopy were carefully selected and marked. The gas-exchange parameters were measured three times for each selected leaf; thus, 27 measurements were made for each treatment. All measurements were carried out between 08:00–11:00 h on sunny days. During the periods of the measurements, the leaf chamber temperature, relative humidity, and CO_2 concentration were maintained at approximately 27 ± 1.0°C, 58 ± 4.0%, and 380 ± 5.0 μmol mol^{-1}, respectively. The PAR was set at 2,000; 1,600; 1,200; 1,000; 800, 600, 300, 200, 150, 120, 100, 50, 20, and 0 μmol(photon) m^{-2} s^{-1} by a cold light-emitting diode (LED) irradiation source [range of 0–2,000 μmol(photon) m^{-2} s^{-1}] by a *CIRAS-2* LED irradiation source. At each PAR level, the tested leaf was kept in the leaf chamber for at least 120 s to reach a steady photosynthetic state before the measurements were taken. The transpiration rate (E), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), air CO_2 concentration (C_a), air temperature (T_a), relative humidity of atmosphere (RH), PAR, and P_N were recorded automatically by the portable photosynthesis system. The parameters, such as apparent quantum yield (AQY), dark respiration rate (R_D, P_N when PAR = 0), and light-compensation point (LCP, PAR when P_N = 0), were obtained by drawing the light-response curves (P_N–PAR curves) using the linear regression method under PAR ≤ 200 μmol(photon) m^{-2} s^{-1}, and the light-saturated net photosynthetic rate ($P_{N\,max}$) was estimated according to the trends of the light-response curves (Xu 2002, Lang *et al.* 2013). The rest of the gas-exchange parameters were calculated according to the following equations: water-use efficiency: $\text{WUE} = P_N/E$; and stomatal limitation: $L_s = 1 - C_i/C_a$ (Berry and Downton 1982).

Chl fluorescence parameters: Chl fluorescence is widely used as a nondestructive tool in photosynthesis research (Roháček 2002, Li *et al.* 2016). Three fully developed mature leaves from the middle part of each seedling were carefully selected and marked (different from the leaves of the photosynthetic light-response parameter measurements). The Chl fluorescence parameters, such as minimal fluorescence yield of the dark-adapted state (F_0), maximal fluorescence yield of the dark-adapted state (F_m), minimal fluorescence yield of the light-adapted state (F_0'), maximal fluorescence yield of the light-adapted state (F_m'), steady-state fluorescence yield (F_s), and electron transport rate (ETR) were measured simultaneously with the photosynthetic light-response parameter measurements using a portable Chl fluorescence monitoring system (*FMS2.02*, *Hansatech*, Norfolk, UK). The Chl fluorescence parameters were measured three times for each selected leaf. The rest of the Chl fluorescence parameters were calculated according to the following equations: maximal quantum yield of PSII photochemistry: $F_v/F_m = (F_m - F_0)/F_m$; effective quantum yield of PSII photochemistry: $\Phi_{PSII} = (F_m' - F_s)/F_m'$; photochemical quenching coefficient: $q_P = (F_m' - F_s)/(F_m' - F_0')$; and nonphotochemical quenching: $NPQ = (F_m - F_m')/F_m'$ (Roháček 2002, Li *et al.* 2015).

Biochemical parameters and growth indexes: The peroxidase (POD, EC1.11.1.1) and superoxide dismutase (SOD, EC1.15.1.1) activities, Chl content, and malondialdehyde (MDA) content were measured by the spectrophotometric method (Cai 2013). The POD activity was expressed as 0.1 times of the change of A_{470} (the absorbance of poplar leaves at the wavelength of 470 nm) per min and per fresh mass. The SOD activity was expressed as the quantity of enzyme required to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) to half of the control (the normal photochemical reduction of NBT without SOD) per fresh mass. At the end of the experiment, the biomass was determined. The roots, stems (branches), and leaves of the experimental seedlings (six plants per treatment) were cut off, cleaned, and dried to a constant mass under 85°C in a drying oven. The dry mass of the roots (DM_r), stems (branches) (DM_s), and leaves (DM_l) were weighed, respectively. The root/shoot ratio (R/S) was calculated as follows: $R/S = DM_r/(DM_s + DM_l)$.

Statistical analysis: The *Excel 2007* for *Windows* (*EXCEL 2007*, *MS*, Redmond, USA) and *Statistical Program for the Social Sciences* (*SPSS 19.0*, *IBM*, Chicago, USA) were used to analyze the parameters, perform statistical evaluations, and make charts. The results of the different treatments were examined at the 5% level by one-way analysis of variance (*ANOVA*) and the least significant difference method (LSD). The data shown in charts are the average values ± standard error. The different lowercase letters indicate significant differences (P<0.05).

Results

Gas-exchange parameters: With increasing phenolic acid concentrations (0X, 0.5X, and 1.0 X), the P_N, L_s, E, and WUE significantly decreased, and C_i significantly increased under the same PAR [1,200 μmol(photon) m^{-2} s^{-1}] (Table 1). According to the criterion of the photosynthesis limitations (Farquhar and Sharkey 1982), the main reason that the photosynthesis of poplar seedlings declined was the limitation from nonstomatal factors. Compared with CK, the 0.5X and 1.0X treatments reduced P_N by 9.9 and 18.2%, the L_s by 15.8 and 47.8%, the E by 1.3 and 3.6%, the WUE by 8.8 and 15.1%, and the C_i increased by 19.3 and 58.5%, respectively. The L_s and C_i changed fast indicating that they were both sensitive to phenolic acid. The gas-exchange parameters showed significant differences between CK and the 0.5X and 1.0X treatments, except the E between CK and the 0.5X treatment.

Light-response parameters: The light-response curves of the poplar in all treatments showed similar trends (Fig. 1). Under PAR ≤ 200 μmol(photon) m^{-2} s^{-1}, increasing PAR enhanced P_N linearly, indicating that in this PAR range, PAR was the main factor influencing photosynthesis. Beyond this range, with PAR increasing until the light-saturation point [LSP, approximately 1,600 μmol(photon) m^{-2} s^{-1}], P_N rose slowly to P_{Nmax}. In this PAR range, the increase in P_N slowed because of the effects of the other factors (*e.g.*, C_i, C_a, T_a, and RH). As PAR increased over LSP, the light-response curves of the different treatments showed a greater difference. Under strong light, the P_N of CK stabilized, while in the other treatments, especially after the 1.0X treatment, it was significantly reduced, indicating that phenolic acids led to the photoinhibition of poplar leaves.

Table 1. The gas-exchange parameters of poplar seedlings under different concentrations of phenolic acids and the same PAR [1,200 μmol(photon) m^{-2} s^{-1}] (Means ± SE). The different *lowercase letters* indicate significant differences (LSD test, $p<0.05$), and $n = 27$. P_N – net photosynthetic rate; E – transpiration rate; WUE – water-use efficiency; C_i – intercellular CO_2 concentration; L_s – stomatal limitation.

Treatment	P_N [μmol m^{-2} s^{-1}]	E [μmol m^{-2} s^{-1}]	WUE [μmol $mmol^{-1}$]	C_i [μmol mol^{-1}]	L_s
CK	20.7 ± 0.6^a	4.49 ± 0.04^a	4.7 ± 0.2^a	171 ± 5^a	0.64 ± 0.01^a
0.5X	18.6 ± 0.8^b	4.41 ± 0.03^a	4.2 ± 0.3^b	204 ± 8^b	0.42 ± 0.01^b
1.0X	16.9 ± 0.9^c	4.02 ± 0.04^b	3.9 ± 0.2^c	271 ± 10^c	0.21 ± 0.01^c

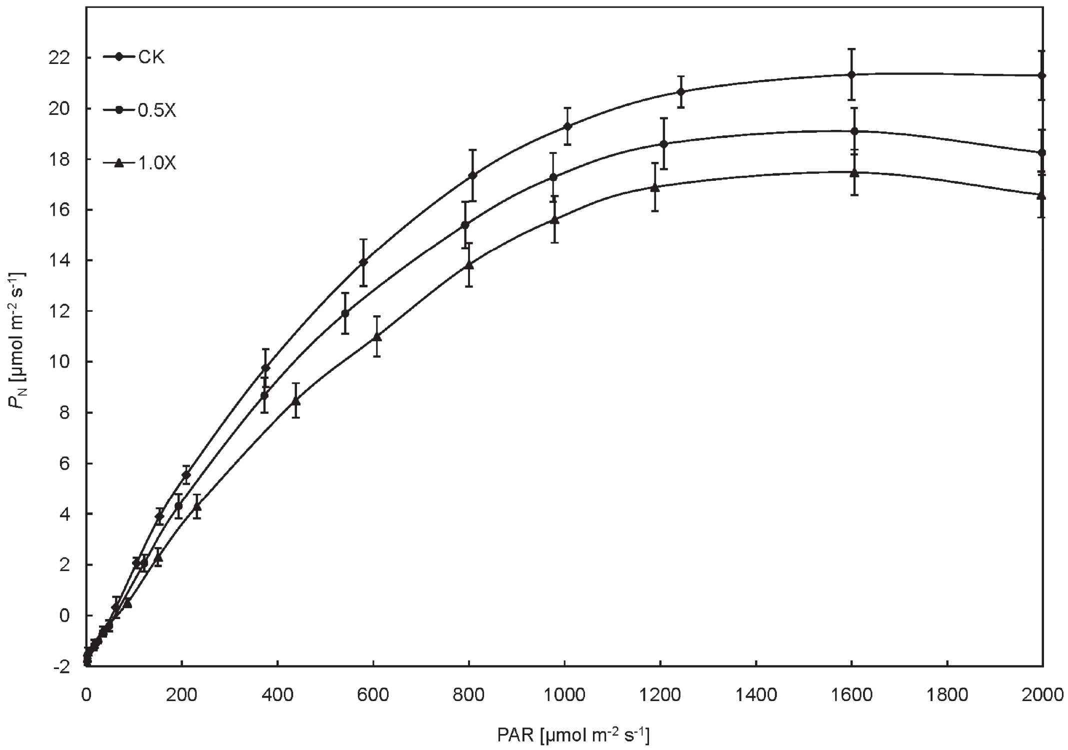

Fig. 1. Light-response curves of poplar under different phenolic acid concentrations. Values are the means ± SE; $n = 27$. P_N – net photosynthetic rate; PAR – photosynthetically active radiation.

With increasing phenolic acid concentrations, the P_{Nmax}, AQY, and R_D all significantly decreased, and LCP significantly increased (Table 2). Compared with CK, the 0.5X and 1.0X treatments reduced P_{Nmax} by 10.3 and 17.8%, the AQY by 13.0 and 30.9%, the R_D by 3.1 and 17.9%, and the LCP increased by 11.4 and 18.8%, respectively. The light-response parameters all showed significant differences between the diverse treatments.

Chl fluorescence parameters: With increasing phenolic acid concentrations, the F_v/F_m, Φ_{PSII}, q_P, and ETR all significantly decreased, and the NPQ significantly increased (Table 3). Compared with CK, 0.5X and 1.0X treatments reduced F_v/F_m by 1.0 and 1.6%, the Φ_{PSII} by 3.3 and 4.0%, the q_P by 3.0 and 5.0%, the ETR by 12.0 and 27.5%, and the NPQ increased by 27.5 and 48.4%, respectively. The NPQ increased fast indicating that it was sensitive to phenolic acids. The Chl fluorescence parameters between CK and the 1.0X treatment showed significant differences.

Biochemical parameters and growth indexes: With increasing phenolic acid concentrations, the POD and SOD activities initially decreased and then increased, the Chl content and total biomass significantly decreased, and the MDA content and R/S significantly increased (Table 4). Compared with CK, the POD and SOD activity was reduced by 41.2 and 45.1% in the 0.5X treatment, respectively, while the 1.0X treatment increased the POD and SOD activity by 0.1% and 19.6%, respectively. The 0.5X and 1.0X treatments lowered the Chl content by 9.2 and 18.0%, the total biomass by 13.3 and 32.3%, the R/S increased by 9.2 and 10.9%, and the MDA content by 31.3 and 146.7%, respectively. The MDA content increased fast indicating that it was sensitive to phenolic acid. The POD and SOD activities between CK and the 1.0X treatments showed no significant differences, but they were significantly higher than those in the 0.5X treatment.

Table 2. The light-response parameters of poplar seedlings under different concentrations of phenolic acids (means ± SE). The *different lowercase letters* indicate significant differences (LSD test, $p<0.05$), and $n = 27$. P_{Nmax} – light-saturated net photosynthetic rate; AQY – apparent quantum yield; LCP – light-compensation point; R_D – dark respiration rate.

Treatment	P_{Nmax} [μmol m^{-2} s^{-1}]	AQY [mol mol^{-1}]	LCP [μmol m^{-2} s^{-1}]	R_D [μmol m^{-2} s^{-1}]
CK	21.3 ± 0.3^a	0.036 ± 0.001^a	51.0 ± 2.6^c	1.85 ± 0.41^a
0.5X	19.1 ± 0.5^b	0.032 ± 0.002^b	56.8 ± 3.1^b	1.80 ± 0.17^b
1.0X	17.5 ± 0.2^c	0.025 ± 0.002^c	60.6 ± 2.1^a	1.52 ± 0.46^c

Table 3. The chlorophyll (Chl) fluorescence parameters of poplar seedlings under different concentrations of phenolic acids (means ± SE). The *different lowercase letters* indicate significant differences (LSD test, $p<0.05$), and $n = 27$. F_v/F_m – maximal quantum yield of PSII photochemistry; Φ_{PSII} – effective quantum yield of PSII photochemistry; q_P – photochemical quenching coefficient; NPQ – nonphotochemical quenching; ETR – electron transport rate.

	F_v/F_m	Φ_{PSII}	q_P	NPQ	ETR
CK	0.828 ± 0.005^a	0.58 ± 0.01^a	0.85 ± 0.01^a	0.65 ± 0.05^c	4.2 ± 0.2^a
0.5X	0.821 ± 0.006^a	0.55 ± 0.02^b	0.82 ± 0.01^b	0.84 ± 0.10^b	3.6 ± 0.1^b
1.0X	0.803 ± 0.006^b	0.54 ± 0.01^b	0.79 ± 0.01^c	0.98 ± 0.09^a	3.0 ± 0.1^c

Table 4. The biochemistry and growth parameters of poplar seedlings under different concentrations of phenolic acids (means ± SE). The *different lowercase letters* indicate significant differences (LSD test, $p<0.05$), and $n = 27$. POD – peroxidase; SOD – superoxide dismutase; MDA – malondialdehyde; Chl – chlorophyll.

Treatment	POD activities [U g^{-1}]	SOD activities [U g^{-1}]	MDA content [μmol g^{-1}]	Chl content [mol g^{-1}]	total biomass [g]	root/shoot ratio
CK	494 ± 40^a	222 ± 10^a	20.1 ± 2.4^c	6.60 ± 0.11^a	233 ± 9^a	0.496 ± 0.004^b
0.5X	290 ± 20^b	121 ± 8^b	27.4 ± 1.9^b	5.84 ± 0.07^b	200 ± 7^b	0.574 ± 0.007^a
1.0X	495 ± 41^a	265 ± 6^a	50.9 ± 3.0^a	4.99 ± 0.07^c	157 ± 8^c	0.580 ± 0.004^a

Discussion

The phenolic acids secreted from plant roots had strong allelopathic activity, which not only inhibit other plants and rhizosphere microflora, but it could also restrain the growth of the plants themselves and result in continuous cropping obstacles (Wang and Wang 2010). Continuous cropping obstacles were not only derived from the land productivity degradation but also from the toxic effect of the phenolic acids on plants. Allelochemicals stimulated the cell membrane of plant roots by first affecting its membrane potential, membrane permeability, and membrane activity. The signal was then conveyed to the ground part of the plants, thereby affecting plant enzyme activity and photosynthesis process by various mechanisms (Einhelling 1995). In this study, with increasing phenolic acid concentrations, the P_N, L_s, E, WUE, P_{Nmax}, AQY, R_D, F_v/F_m, Φ_{PSII}, q_P, ETR, Chl content, and total biomass decreased significantly, whereas the C_i, LCP, NPQ, MDA content, and R/S increased significantly. Furthermore, POD and SOD activities initially decreased and then increased.

P_N and AQY are important indexes of photosynthesis and light-use efficiency (Xu 2002, Lang *et al.* 2013, Xia *et al.* 2014b) and directly reflect the operating states of the photosynthetic apparatus (Liang *et al.* 2006). The higher the P_N, the greater the photosynthetic efficiency of plants under high light, and the higher the AQY, the greater the photosynthetic efficiency of plants under low light (Xu 2002). In this study, with increasing phenolic acid concentrations, the decline of the P_N, E, and L_s and the increasing C_i under the same PAR [1200 μmol(photon) m^{-2} s^{-1}] suggest that phenolic acids significantly reduced photosynthesis and transpiration of poplar seedlings; the main reason for the P_N decline was the limitation from nonstomatal factors (Farquhar and Sharkey 1982), that is to say, phenolic acids reduced the photosynthetic ability of poplar and could do harm to poplar photosynthesis organ. Previous studies found that allelochemicals could reduce the photosynthetic activity of the key enzyme – Rubisco (Amarjeet *et al.* 2005). However, further study is needed to determine whether phenolic acids inhibit the Rubisco activity of poplar.

The LCP, LSP, and P_{Nmax} of plant leaves are the main indicators of the photosynthetic response to adverse conditions (Xia *et al.* 2014a, Gao *et al.* 2017). In this study, with increasing phenolic acid concentrations, the decline of P_{Nmax} and AQY, and the increase of LCP suggested that phenolic acids reduced photosynthetic and light-use efficiency under both strong and weak light (Li *et al.* 2015) and inhibited poplar's photosynthetic apparatus. The decline of the photosynthetic efficiency is the most significant feature of photoinhibition (Xu 2002). Thus, exposure to phenolic acids resulted in the photoinhibition of poplar photosynthesis and the decline of the adaptive capacity of poplar photosynthetic apparatus under strong light. With increasing phenolic acid concentrations, the decline of R_D indicates that phenolic acids inhibited the mitochondrial respiration and physiological activities of poplar, and the higher the phenolic acid concentration, the greater the inhibition of the poplar's physiological activities.

The Φ_{PSII} is the index of effective quantum yield of PSII photochemistry in the PSII reaction center, and the decline of Φ_{PSII} is regarded as the reduction of the electron transport rate and CO_2 assimilative capacity (Maxwell and Jonhson 2000). The ETR reflects the electron transport rate in the photochemical reactions. In this study, with increasing phenolic acid concentrations, the decline of Φ_{PSII} and ETR implied that phenolic acids led to the reduction of the conversion efficiency of light energy, electron transport rate, and CO_2 assimilative capacity in the PSII reaction center. The q_P is an indicator of the proportion of open PSII reaction centers and the light energy absorbed by antennae pigment used for photochemical electron transfer (van Kooten and Snel 1990). The NPQ reflects the ability of plants to release excess light energy by heat dissipation, to avoid light damage, and achieve photoprotection (Bilger *et al.* 1995). In this study, with increasing phenolic acid concentrations, the decline of q_P and the increase of NPQ indicates that phenolic acids reduced the proportion of the light energy absorbed by antennae pigment used for photochemical reactions and increased the proportion of that used for heat dissipation. This is an important means for poplar to reduce excess light energy accumulation in the PSII reaction center, avoid light damage and achieve photoprotection. The F_v/F_m is the index of quantum yield of PSII photochemistry, and the decline of F_v/F_m is regarded as the reduction of conversion efficiency of primary light energy and the photoinhibition in the PSII reaction center (Demmig-Adams *et al.* 1996). The F_v/F_m is relatively constant and generally shows the range from 0.80 to 0.85 as plants are not subjected to environmental stress and go through fully dark-adapted treatment (Xu 2002). In this study, the F_v/F_m decreased with increasing phenolic acid concentration, and the F_v/F_m in the 1.0X treatment was significantly lower than those in CK and 0.5X treatments, which implied that the higher the phenolic acid concentration, the higher photoinhibition in poplar leaves. Moreover, the F_v/F_m in the 1.0X treatment was 0.80 (Table 3), indicating that 1.0X treatment is the maximum concentration of phenolic acids which poplar seedlings can tolerate to maintain potential conversion efficiency of primary light energy in the PSII reaction center.

Under stress conditions, plants are highly sensitive to variations in environmental factors and the photosynthetic apparatus is often the primary site damaged by such an adversity. MDA is the final product of lipid peroxidation in the cell membrane as the production of free radicals exceeds the removal capacity of the protective enzyme system, and its content can reflect stress tolerance of plants (Pei *et al.* 2013). In this study, the MDA contents after the 0.5X and 1.0X treatments were significantly higher than those of CK (Table 4), which implies that poplar leaf cells were subjected to serious damage. Moreover, with increasing phenolic acid concentration, the increase of MDA content implies that the higher the phenolic acid concentration, the more injury is caused in poplar cell membranes. Allelochemical-induced lipid peroxidation resulted in free radical formation in plasma membranes and inhibition of POD and CAT activities (Baziramakenga *et al.* 1995). Antioxidant enzymes, such as POD, CAT, and SOD are the most important enzymes in the free radical scavenge system, which maintain the balance of the production and elimination of free radicals in plant cells (Pei *et al.* 2013). In this study, with increasing phenolic acid concentrations, POD and SOD activities initially decreased, then increased, POD and SOD activities showed no significant difference, and they all were significantly higher than those of the 0.5X treatment, which implied that in the 0.5X treatment, phenolic acids markedly inhibited POD and SOD activities and after the

1.0X treatment, the cell membrane of poplar leaves were severely injured and both activities were induced sharply. Zhang *et al.* (2010) found that the accumulation of reactive oxygen species (ROS) and free radicals in plant cells resulted in a damaged membrane structure, which in turn led to a decrease of the Chl content of *Rehmannia glutinosa*. Chl is the key of plant photosynthesis, which absorbs and transfers light energy to the photosynthetic apparatus. The Chl content is an important index to evaluate the intensity of photosynthesis, and photosynthetic and dry matter accumulation capacities (Zhang *et al.* 2012). Within certain limits, the P_N increases with increasing Chl content (Zhang *et al.* 2010). In this study, with increasing phenolic acid concentrations, the Chl content decreased significantly, which could be due to Chl degradation promoted by phenolic acids. Conversely, Chl synthesis was hindered by a significant decline in nitrate absorption and the availability of soil nitrogen (N) due to phenolic acids (Wang *et al.* 2011, Wang *et al.* 2013); N is an indispensable element for Chl synthesis (Xu 2002). The photosynthetic yield was significantly correlated with N supply (Uribelarrea *et al.* 2009). The decline of total biomass occurred due to phenolic acids significant injury of poplar photosynthetic apparatus, thereby resulting in the reduction of its photosynthetic capacity and CO_2 assimilate accumulation. The increase of R/S occurred because phenolic acids significantly inhibited N supply and injured poplar roots (Wang *et al.* 2011). Relatively more biomass was distributed in the below-ground plant parts to promote root growth and nutrient absorption, which is an important physiological strategy for poplar coping with phenolic acid stress.

Conclusions: The photosynthetic gas-exchange and Chl fluorescence parameters showed that phenol acid stress could thoroughly inhibit photosynthesis of poplar seedlings. This inhibition effect was mainly caused by nonstomatal factors. Phenolic acids reduced the light energy conversion efficiency, electron transport rate, and CO_2 assimilative capacity of poplar leaves led to noticeable photoinhibition in photosynthesis, and induced the arising of ROS largely in leaf cells as poplar encountered excess light energy. Moreover, phenolic acids inhibited POD and SOD activities markedly. The POD and SOD are the most important enzymes of the free radical scavenging system. So the balance of the production and elimination of free radicals was disturbed and ROS accumulated massively in poplar cells. The accumulation of ROS resulted in the lipid peroxidation of membrane systems and the arising of MDA content, which resulted in the irreversible damage of membrane structure. The damage of membrane structure of poplar cells led to the decline of the Chl content.

Thus, we concluded that phenolic acid stress significantly inhibited photosynthesis, transpiration, respiration, and organic matter accumulation in poplar seedlings. The higher the phenolic acid concentration, the greater the inhibition of the poplar's physiological activities. The 1.0X treatment was the maximum concentration of phenolic acids which poplar seedlings could tolerate in order to maintain potential conversion efficiency of light energy in the PSII. Poplar increased the dissipation of excess light energy to alleviate the damage of photosynthetic apparatus in strong light. This is its physiological strategy to cope with phenolic acid stress. Furthermore, carbon allocation results in relatively higher biomass in the below-ground plant parts to promote root growth and nutrient absorption.

References

Amarjeet B., Mohini S., Sangeeta S.: Allelopathic effects of *Parthenium hysterophorus* on the chlorophyll, nitrogen, protein and ascorbic acid content of *Lantana camara*. – Allelopathy J. **15**: 305-310, 2005.

Baziramakenga R., Leroux G.D., Simard R.R.: Effects of benzoic and cinnamic acids on membrane permeability of soybean roots. – J. Chem. Ecol. **21**: 1271-1285, 1995.

Berry J.A., Downton W.J.S.: Environmental regulation of photosynthesis. – Govindjee (ed.): Photosynthesis, Vol. II. Pp. 263-343. Academic Press, New York 1982.

Bilger W., Fisahn J., Brummet W. *et al.*: Violaxanthin cycle pigment contents in potato and tobacco plants with genetically reduced photosynthetic capacity. – Plant Physiol. **108**: 1479-1486, 1995.

Cai Q.S.: [Plant Physiology Experiment.] Pp. 178-188. China Agricultural University Press, Beijing 2013. [In Chinese]

Demmig-Adams B., Adams W.W.III., Barker D.H. *et al.*: Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. – Physiol. Plantarum **98**: 253-264, 1996.

Einhelling F.A.: Allelopathy: Current status and future goals. – In: Inderjit Dakshini K.M.M., Einhelling F.A. (ed.): Allelopathy: Organisms, Processes and Applications. Pp. 1-24. American Chemical Society, Washington DC 1995.

Farquhar G.D., Sharkey T.D.: Stomatal conductance and photosynthesis. – Annu. Rev. Plant Physio. **33**: 317-345, 1982.

Gao Y., Xia J.B., Chen Y.P. *et al.*: Effects of extreme soil water stress on photosynthetic efficiencyand water consumption characteristics of *Tamarix chinensisin* China's Yellow River Delta – J. For. Res. **28**: 491-501, 2017.

Gao Y., Gao X.L., Zhang D.Q. *et al.*: [Effects of continuous cropping on buckwheat yield, soil nutrient and enzyme activities.] – Soils **46**: 1091-1096, 2014. [In Chinese]

Gu X.L., Chen W., Cai F. *et al.*: [Effect of *Trichoderma* biofertilizer on continuous cropping cucumber cultivation with reduced rates of chemical fertilizer application.] – Acta Pedol. Sin. **53**: 1296-1305, 2016. [In Chinese]

Kang Y.l., Jing F., Sun W.Q. *et al.*: [Effects of continuous cropping of processing tomato on physical-chemical properties of and microbial biomass in the Soil.] – Acta Pedol. Sin. **53**: 533-542, 2016. [In Chinese]

Lang Y., Wang M., Zhang G.C. *et al.*: Experimental and simulated light responses of photosynthesis in leaves of three tree species under different soil water conditions. – Photosynthetica

51: 370-378, 2013.
Li H., Zhang G.C., Xie H.C. *et al.*: The effects of the phenol concentrations on photosynthetic parameters of *Salix babylonica* L. – Photosynthetica **53**: 430-435, 2015.
Li H., Zhang G.C., Xie H.C. *et al*.: [The effect of phenol concentration on photosynthetic physiological parameters of *Salix babylonica*.] – Chin. Bull. Bot. **51**: 31-39, 2016. [In Chinese]
Li Z.H., Wang Q., Ruan X. *et al*.: Phenolics and plant allelopathy. – Molecules **15**: 8933-8952, 2010.
Liang C.J., Huang X.H., Zhou Q.: [Effect of cerium on photosynthesis in soybean seedling under supplementary UV-B radiation stress: II Photosynthetic quantum yield and carboxylation efficiency.] – J. Agro-Environ. Sci. **25**: 580-583, 2006. [In Chinese]
Liu F.D., Jiang Y.Z., Wang H.T. *et al.*: [Effect of continuous cropping on poplar plantation.] – J. Soil Water Conserv. **19**: 102-105, 2005. [In Chinese]
Lou Y., Davis A.S., Yannarell A.C.: Interactions between allelochemicals and the microbial community affect weed suppression following cover crop residue incorporation into soil. – Plant Soil **399**: 357-371, 2016.
Maxwell K., Jonhson G.N.: Chlorophyll fluorescence – a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.
Pei B., Zhang G.C., Zhang S.Y. *et al.*: [Effects of soil drought stress on photosynthetic characteristics and antioxidant enzyme activities in *Hippophae rhamnoides* Linn. seedlings.] – Acta Ecol. Sin. **33**: 1386-1396, 2013. [In Chinese]
Roháček K.: Chlorophyll fluorescence parameters: The definitions, photosynthetic meaning and mutual relationships. – Photosynthetica **40**: 13-29, 2002.
Sène M., Doré T., Pellissier F.: Effect of phenolic acids in soil under and between rows of a prior sorghum (*Sorghum bicolor*) crop on germination, emergence, and seedling growth of peanut (*Arachis hypogeal*). – J. Chem. Ecol. **26**: 625-637, 2000.
Tan X.M., Wang H.T., Kong L.G. *et al.*: [Accumulation of phenolic acids in soil of a continuous cropping poplar plantation and their effects on soil microbes.] – J. Shandong Univ. **43**: 14-19, 2008. [In Chinese]
Uribelarrea M., Crafts-Brandner S.J., Below F.E.: Physiological N response of field-grown maize hybrids (*Zea mays* L.) with divergent yield potential and grain protein concentration. – Plant Soil **316**: 151-160, 2009.
van Kooten O., Snel J.F.H .: The use of chlorophyll fluorescence nomenclature in plant stress physiology. – Photosynth. Res. **25**: 147-150, 1990.
Wang H.T., Yang Y., Wang Y.P. *et al*.: [Effects of exogenous phenolic acids on nitrate absorption and utilization of hydroponic cuttings of *Populus* × *euramericana* 'Neva'.] – Chin. J. Plant Ecol. **35**: 214-222, 2011. [In Chinese]
Wang W.B., Ma X.S., Dong Y.F. *et al*.: [Community characteristics and degradation metabolism regulation of soil phenolic acid degrading bacteria in poplar plantations under continuous cropping and crop rotation.] – Chin. J. Appl. Environ. Biol. **22**: 0815-0822, 2016. [In Chinese]
Wang Y.P., Wang H.T.: [Allelochemicals from roots exudation and its environment behavior in soil.] – Chin. J. Soil Sci. **41**: 501-507, 2010. [In Chinese]
Wang Y.P., Wang H.T., Xu T. *et al.*: [Effects of exogenous phenolic acid on soil nutrient availability and enzyme activities in a poplar plantation.] – Chin. J. Appl. Ecol. **24**: 667-674, 2013. [In Chinese]
Wu X.H.: [Studies on the allelopathy of several submerged Potamogetonaceae species on planktonic algae.] – PhD. Thesis. Graduate University of Chinese Academy of Sciences, Wuhan 2005. [In Chinese]
Xia J.B., Zhang G.C., Wang R.R. *et al*.: Effect of soil water availability on photosynthesis in *Ziziphus jujuba* var. *spinosus* in a sand habitat formed from seashells: comparison of four models. – Photosynthetica **52**: 253-261, 2014a.
Xia J.B., Zhang G.C., Zhang S.Y. *et al.*: Photosynthetic and water use characteristics in three natural secondary shrubs on Shell Islands, Shandong, China. – Plant Biosyst. **148**: 109-117, 2014b.
Xu D.Q.: [Photosynthetic Efficiency.] Pp. 2-35. Shanghai Sci. Technol. Press, Shanghai 2002. [In Chinese]
Xu Y., Wu Y.G., Chen Y. *et al.*: Autotoxicity in *Pogostemon cablin* and their allelochemicals. – Rev. Bras. Farmacogn. **25**: 117-123, 2015.
Ye S.F., Yu J.Q., Peng Y.H. *et al*.: Incidence of fusarium wilt in *Cucumis sativus* L. is promoted by cinnamic acid, an autotoxin in root exudates. – Plant Soil **263**: 143-150, 2004.
Yu J.Q., Matsui Y.: Effects of root exudates of cucumber (*Cucumis sativus*) and allelochemicals on ion uptake by cucumber seedling. – J. Chem. Ecol. **23**: 817-827, 1997.
Zhang Q.S.: Potential role of allelopathy in the soil and decomposing root of Chinese-fir replant woodland. – Plant Soil **151**: 205-210, 1993.
Zhang Y.N., Niu J.M., Zhang Q. *et al*.: [A discussion on applications of vegetation index for estimating aboveground biomass of typical steppe.] – Acta Pratacul. Sin. **21**: 229-238, 2012. [In Chinese]
Zhang Y.N., Wang X.X., Li X.G. *et al*.: [Effects of continuous cropping on physiological and biochemical resistance of cotton to *Fusarium* wilt.] – Acta Ecol. Sin. **36**: 4456-4464, 2016. [In Chinese]
Zhang Z.X., Sun X.G., Mu L.Q. *et al*.: [Dendrology.] Pp. 232-235. China Forestry Press, Beijing 2008. [In Chinese]
Zhang Z.Y., Yin W.J., Li J. *et al.*: [Physio-ecological properties of continuous cropping *Rehmannia glutinosa*.] – Chin. J. Plant Ecol. **34**: 547-554. 2010. [In Chinese]
Zhu W.R., Wang Q.T., Liu M.L. *et al.*: [Interactive effects of phenolic acid and nitrogen on morphological traits of poplar (*Populus* × *euramericana* 'Neva') fine roots.] – Chin. J. Plant Ecol. **39**: 1198-1208, 2015. [In Chinese]