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Gas exchange, photosystem II photochemistry, and the antioxidant system of longan plant (*Dimocarpus longan* Lour.) leaves in response to lead (Pb) stress

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Abstract

Longan is one of the most important subtropical fruit trees and a famous special product in south China. Increased fruit demand brings longan cultivation to Pb-affected regions. Seedlings of longan (cv. Wulongling) in pots with sands were irrigated daily for 30 d with a freshly prepared nutrient solution containing different concentrations of $Pb(NO_3)_2$ (0, 100 , 200 , 400 , 600 , 800 and 1000 mg L^{-1}) to determine physiological and biochemical responses of longan seedlings to various levels of lead (Pb). The results indicated that Pb stress substantially inhibited the growth of longan plants and markedly declined in their dry biomass. However, when the plants were grown at 100 mg L^{-1} Pb, the growth and dry biomass of the plants showed no significant difference from control. In addition, the chlorophyll a fluorescence and gas exchange parameters were correlated with the growth and yield response. Pb treatments increased the minimum fluorescence (Fo) and caused a decrease in maximum fluorescence (Fm), variable fluorescence (Fv), the maximum quantum efficiency of PSII photochemistry (Fv/Fm), trapped energy flux per cross section (CS) at t=0 (TRo/CSo), electron transport flux per CS at t=0 (ETo/CSo), dissipated energy flux per CS at t=0(DIo/CSo), and the amount of active PSII reaction centers (RCs) per CS at t = 0 (RC/CSo). Furthermore, Pb stress led to decreases in the protein contents, the activity of peroxidase (POD, EC 1.11.1.7) and the accumulation of proline and malondialdehyde (MDA), and enhanced superoxide dismutase activity (SOD, EC 1.15.1.1), whereas catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX,EC 1.11.1.11) were enhanced at low Pb levels and decreased under high Pb stress. Nonetheless, these changes were closely related to the severity of the Pb stress.

Keywords: *Dimocarpus longan* Lour.; Gas exchange; Chlorophyll a fluorescence; Chlorophyll content; Antioxidant enzymes; Pb stress.

Abbreviations: APX_ascorbate peroxidase; Car_carotenoid; CAT_catalase; Chl a_chlorophyll a; Chl b_chlorophyll b; Chl a+b_total chlorophyll; Gs_stomatal conductance; DIo/CSo_dissipated energy flux per cross section (CS) at t=0;ETo/CSo_electron transport flux per CS at t=0; ETR_electron transport rate of PSII; Fo_minimum fluorescence; Fm_maximum fluorescence; Fv_variable fluorescence; Fv/Fm_maximum quantum efficiency of PSII photochemistry; H_2O_2_hydrogen peroxide; MDA_malondialdehyde; $^{O_2\cdot^-}$_superoxide anion radical; Pb_lead; Pn_net photosynthesis; POD_peroxidase; PSII_photosystem II; RC/CSo_amount of active PSII RCs per CS at t = 0; ROS_reactive oxygen species; SOD_superoxide dismutase; Tr_transpiration rate; TRo/CSo_trapped energy flux per CS at t=0.

Introduction

Heavy metal contamination is a primary ecological issue in terms of human health, and lead (Pb) has become a major contaminant that readily accumulates in soils and sediments. Lead toxicity to plants has been widely investigated (Jiang et al., 2014; Cha-Um et al., 2009; Sharma et al., 2005). Pb is not an essential element for plants, but it can be absorbed by the roots of crop and transported to shoots, and enter the food chain. Pb toxicity in plants is associated with negative effects on nutrient uptake, photosynthesis and anti-oxidant enzymes (Bharwana et al., 2014a; 2014b; Wang et al., 2011; Pourrut et al., 2011; Sengar et al., 2008). Photosynthetic efficiency, specifically for PSII behavior, has been widely assessed by using chlorophyll a fluorescence, which improved our understanding of the photosynthetic process. This technique is direct, non-destructive, highly sensitive, and reliable (Cuchiara et al., 2013; Yang et al., 2012; Buonasera et al., 2011; Mehta et al., 2010; Rohácek et al., 2002) and makes it possible to exploit the energy absorption of the pigments, the capture of an exciton, and the subsequent electron transport (Cuchiara et al., 2013; Yusuf et al., 2010; Krause and Weis, 1991). In observing the measured fluorescence transient (OJIP) by using a JIP-Test (Strasser et al., 1995; Strasser et al., 1998), it becomes possible to quantify the flux of energy through the photosystems, which is used to evaluate PSII operation during the photosynthetic performance of plants (Strasser et al., 2004; Tsimilli-Michael et al., 2008).

ROS such as $O_2^{\cdot -}$, H_2O_2 and HO^- were overproduced as a consequence of the phytotoxicity of Pb (Bharwana et al.,2014a; 2014b; Reddy et al.,2005). MDA is widely used to evaluate the severity and degree of plant sensitivity to ROS (Shakoor et al., 2014). High proline contents may protect plants from environmental stress. Higher activities of antioxidant enzymes, such as SOD, POD, CAT and APX, and higher proline contents are associated with a higher tolerance to abiotic stress in most plants (Cha-Um et al., 2009; Lamhamdi et al., 2011; Verma et al., 2003; Huang et al., 2014). Longan is a famous and delicious fruit in subtropical areas, and it is primarily grown in South China (Qiu, 2014). Longan leaf has been traditionally used as a folklore medicine. It is also one of the major foreign exchange-earning crops in south China because of its high

Table 1. The distribution of lead in different parts of longan seedlings. Values are means ± SD (n=3). Values followed by different letters indicating significant difference between treatments ($p \leq 0.05$).

Pb levels (mg L^{-1})	Pb concentration in roots (mg kg^{-1})	Pb concentration in stem (mg kg^{-1})	Pb concentration in leaves (mg kg^{-1})
0	10.51±1.33 e	4.28±0.31 c	1.12±0.19 d
100	82.87±9.25 e	11.50±1.28 c	1.89±0.16 c
200	265.12±26.58 d	15. 67±1.12 c	2.35±0.23 c
400	589.60±46.53 c	27.23±3.07 c	4.33±0.20 b
600	683.19±37.47 bc	103.80±11.44 b	4.76±0.27 ab
800	757.38±58.77 b	168.50±17.79 a	4.90±0.35 ab
1000	859.13±35.62 a	195.40±15.34 a	5.12±0.24 a

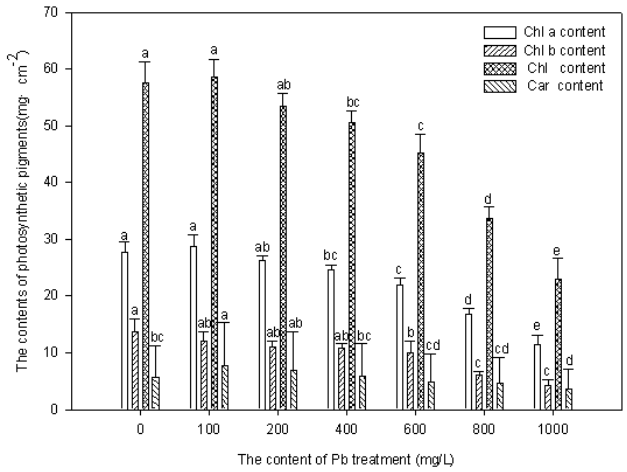

Fig 1. The effect of different Pb concentrations on mean ± SD (n = 5) photosynthetic pigments in *D. longan* leaves. Different letters indicate significant differences between treatments ($p \leq 0.05$).

Table 2. The dry biomass in leaf, stem and root of longan seedlings under Pb stress. Values are means ± SD (n=3). Values followed by different letters indicating significant difference between treatments ($p \leq 0.05$).

Pb levels (mg L^{-1})	Root dry weight $Plant^{-1}$ (g)	Stem dry weight $Plant^{-1}$ (g)	Leaf dry weight $Plant^{-1}$ (g)	Plant dry weight $Plant^{-1}$ (g)
0	6.98±0.37cd	3.27±0.23 a	3.36±0.24 a	13.60±0.75 bc
100	7.60±0.33 c	3.20±0.19 a	2.88±0.23 ab	13.68±0.28 bc
200	9.64±0.43 a	3.41±0.25 a	2.80±0.20 b	15.85±0.53 a
400	8.96±0.37 ab	3.26±0.19 a	2.60±0.25 b	14.81±0.46 ab
600	8.64±0.28 b	2.65±0.19 b	1.92±0.22 c	13.21±0.62 c
800	6.47±0.34 d	2.52±0.23b	1.64±0.16 c	10.63±0.61 d
1000	5.40±0.37 e	2.45±0.23 b	1.48±0.21 c	9.33±0.43 e

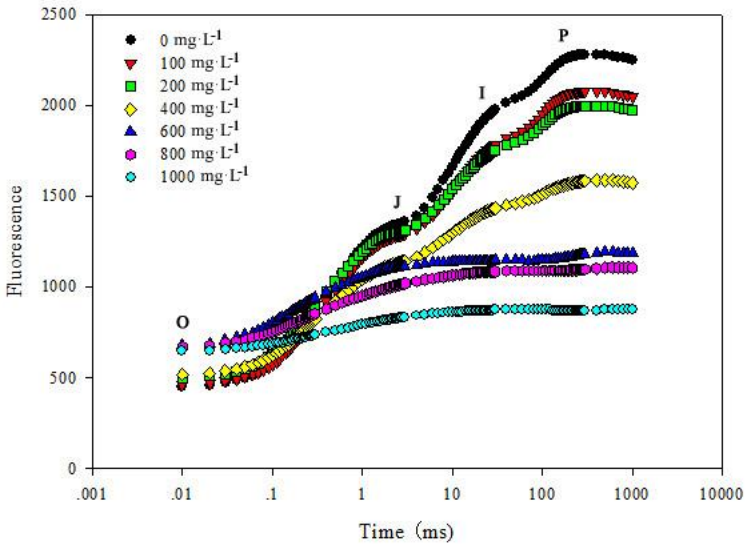

Fig 2. Effects of lead (Pb) treatments on the high irradiance actinic-light-induced chlorophyll a fluorescence (OJIP) transient of dark-adapted *D. longan* leaves plotted on a logarithmic time scale (0.01 ms to 1 s).

quality. Increased fruit demand can be achieved by increasing production areas. However, this could lead the production of *D. longan* in Pb-affected regions. To our knowledge, little is known about the Pb tolerance mechanism of longan plants. Therefore, the specific objective of this study was to explore *D. longan* plant tolerance to Pb stress, special changes in chlorophyll a fluorescence, gas exchange traits, pigment contents, soluble protein, anti-oxidant enzymes, MDA and non-enzymatic antioxidants.

Results

Pb distribution in plants and the plant growth

The roots of the longan seedlings had the highest Pb levels followed by the stems, and the leaves showed the lowest amounts of Pb. Compared with control. irrespective of the concentration of Pb applied, Pb-treated plants exhibited increased Pb concentrations in the leaves, stems and roots (Table 1) and decreased leaf dry mass (Table 2). The leaf dry weights were significantly less at 200 mg. L^{-1} Pb compared with control (Table 2), but the root dry mass with 200 mg.L^{-1} Pb treatment was significantly higher than that of the control plants (Table 2). However, the dry mass of root decreased when the plants were treated with Pb concentrations over 600 mg L^{-1} (Table 2).

Chlorophyll contents

The photosynthetic pigment (Chl a, Chl b, Chl a+b and Car) contents of longan leaves were noticeably decreased with the increasing Pb concentrations (Fig. 1). The Chl a, Chl b and Chl a+b and Car contents were not significantly affected by the presence of Pb until reaching 400 mg L^{-1} Pb, whereas their contents in the 1000 mg.L^{-1} Pb treatment were 59%, 70%, 60% and 37% lower compared with control, respectively. Pb treatment at 800 mg L^{-1} showed significant increase in the Chl a/b ratio.

Chlorophyll a fluorescence

Both Pb-treated leaves and control showed a typical polyphasic rise of OJIP transient, including O, J, I, and P phases. Pb treated leaves resulted in a decrease at the J, I and P-step. However, Pb treatments over 600 mg.L^{-1} showed dramatic increases at the O-step (Fig.2).The Fo for leaves receiving 600 mgL^{-1} Pb treatments increased significantly, whereas the Fm, Fv, Fv/Fm, TRo/CSo, ETo/CSo, DIo/CSo, and RC/CSo significantly decreased in comparison with control. However, these parameters had no significant differences in treatments of 0-200 mg L^{-1} Pb (Table 3).

Gas exchange

Pb stress led to a continuous decline in Pn, Tr, and Gs compared with control. Higher Pb stress (800 and 1000 mg.L^{-1}) led to the dramatic reduction in Pn, Tr, and Gs. The plants that were grown under control conditions exhibited the maximum values for Pn, Tr, and Gs compared with other treatments (Table 4).

Superoxide anion radical, hydrogen peroxide, MDA and free proline content

Pb treatment continuously stimulated the superoxide anion radical, hydrogen peroxide, MDA accumulation and free proline content in Pb-treated longan leaves, with a higher increase in more Pb-stressed plants. The 400 mg.L^{-1} Pb treatment showed a significant increase in the superoxide anion radical, hydrogen peroxide, MDA level and free proline content, whereas nearly 3.7, 3.2, 1.6 and 14.5 times enhancement in the superoxide anion radical, hydrogen peroxide, MDA level and free proline content were observed, respectively, in the leaves of seedlings receiving a 1000 mg.L^{-1} Pb treatment compared with control (Table 5).

Activity of antioxidant enzymes

The antioxidant enzyme (SOD, POD, CAT, and APX) activities in the leaves responded variably to different Pb treatments (Table 6). The SOD activity was consistently increased with increased Pb concentrations. The higher the concentration was, the greater the SOD activity. The CAT and APX activities were initially enhanced and then decreased with increasing Pb concentrations. The activities reached a peak at 600 mg L^{-1} and then subsequently dropped. However, the POD activity was reduced with increasing Pb levels and remained significantly lower than that of the control plants under any Pb level. The total soluble protein contents of leaves were significantly inhibited by increasing Pb concentrations.

Pearson correlation coefficients among qualitative and quantitative traits in D. longan plants

Longan plant growth parameters such as the leaf dry mass and Pn varied markedly and were positively correlated with the Chl content, but they were negatively correlated with the SOD activity, soluble protein and free proline contents (Table 7). A significantly negative correlation between Pb uptake and Chl content, Chl a fluorescence parameters and gas exchange parameters was observed. MDA, $O_2^{\cdot -}$, and H_2O_2 had significantly negative correlations with the Chl content, Chl a fluorescence parameters, and net photosynthesis.

Discussion

A common effect of Pb stress on plants is the reduction of growth and yield, but the effects are more pronounced at higher Pb levels (Sharma et al., 2005; Bharwana et al., 2014a; 2014b; Sengar et al., 2008). Our data showed that increasing the dose of Pb treatments (0-1000 mg L^{-1}) markedly affected the growth of *D. longan* plants in terms of leaf, stem and root dry weights. This finding matched the Pb-induced alterations in soluble proteins, chlorophyll content, Pn, and Tr. However, no significant difference was observed between the control plants and those grown in the presence of 100 mg L^{-1} Pb, suggesting that *D. longan* plants are tolerant to mild stress of Pb

Plant growth relies on photosynthesis, and therefore, environmental perturbations that affect growth will also affect photosynthesis (Sharma et al., 2005; Bharwana et al., 2014a; 2014b; Sengar et al., 2008). In the present study, the decline of photosynthesis in plants at low Pb levels might be related to stomatal factors such as stomatal closure and depression in carbon uptake (Table 4). At high Pb level, the decline might be related to non-stomatal factors, namely the reduction in the pigment content (Fig. 1), the reduced Pb-induced oxidative stress (Table 5) and the inhibition of photochemical processes (Table 3). In response to the decline in leaf turgor pressure, stomatal closure minimized water loss through transpiration, resulting in lower transpiration rates (Table 4). Hence, under mild stress, a small decline in stomatal conductance (Gs) may

Table 3. Effects of different concentrations of Pb on parameters of Fo,Fm,Fv ,Fv/Fm,TRo/CSo,ETo/CSo,DIo/CSo and RC/CSo of *Dimocarpus longan* Lour.Values are means ±SD (n = 6–14). Values followed by different letters indicating significant difference between treatments ($p \leq 0.05$).

Pb levels (mgL^{-1})	Fo	Fm	Fv	Fv/Fm	TRo/CSo	ETo/CSo	DIo/CSo	RC/CSo
0	414.00±34.02b	2279.40±78.33a	1865.40±54.19a	0.819±0.011a	338.58±23.89a	171.90±6.11a	75.42±10.31c	172.35±6.45a
100	430.67±41.07b	2078.00±339.42ab	1647.33±376.92ab	0.783±0.070ab	335.06±12.42a	162.02±16.59a	95.61±42.46bc	171.71±2.86a
200	468.14±82.03b	2001.64±413.38ab	1533.50±456.95ab	0.746±0.119ab	342.32±46.51a	154.07±34.30a	125.82±84.11bc	163.44±23.20a
400	497.58±94.33b	1592.50±563.32bc	1094.92±630.94b	0.620±0.210b	295.78±77.16a	116.67±49.13b	201.8±134.44b	141.44±32.97ab
600	680.75±111.69a	1194.0±423.070cd	513.25±318.67c	0.402±0.104c	280.89±116.09a	54.58c±6.88	399.86±48.47a	118.76±31.70bc
800	669.89±100.81a	1114.33±319.62cd	444.44±280.22c	0.361±0.157c	245.09±108.43ab	64.64±23.08c	424.80±102.82a	114.84±38.00bc
1000	624.14±123.73a	882.25±173.29d	235.00±104.23c	0.259±0.080c	167.13±60.53b	44.37±16.44c	480.12±101.66a	84.37±24.73c

Table 4. Changes in gas exchange under varying Pb conditions. Values in the table are mean ±SD (n=6). Values followed by the different letter indicate significant difference between treatments ($p \leq 0.05$).

Pb levels ($mg\ L^{-1}$)	Pn ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	Tr ($mmol\ H_2O_2\ m^{-2}\ s^{-1}$)	Gs ($mmoL\ H_2O_2\ m^{-2}\ s^{-1}$)
0	2.61 ± 0.17a	1.02 ±0.05a	30.05 ±1.44a
100	2.48 ± 0.18a	1.08 ±0.04a	28.35 ±1.33b
200	2.11 ± 0.15b	1.03 ±0.06a	22.45 ±1.26c
400	2.00 ± 0.14b	0.92 ±0.07b	22.73 ±1.40c
600	1.79 ± 0.11c	0.66 ±0.05c	19.48 ±1.37d
800	0.34 ± 0.13d	0.26 ±0.03d	6.18 ±0.83e
1000	0.32 ± 0.13d	0.20 ±0.02e	4.78 ±0.80e

Table 5. The effect of different Pb concentration on contents of superoxide anion radical, hydrogen peroxide, MDA and free proline. Values in the table are mean ±SD (n=3). Values followed by the different letter indicate significant difference between treatments ($p \leq 0.05$).

Pb levels ($mg\ L^{-1}$)	$O_2 \cdot^-$ ($\Delta OD580\ m^{-2}\ s^{-1}$)	H_2O_2 ($nmol\ mg^{-1}$ protein)	MDA content ($mmol\ g^{-1}$ FW)	Free proline content ($mg\ g^{-1}$ FW)
0	0.447 ±0.069c	1.374 ±0.225d	21.011 ±1.275e	0.075 ±0.029d
100	0.568 ±0.072c	1.420 ±0.201d	21.804e ±0.925	0.076 ±0.016d
200	0.725 ±0.089c	1.895 ±0.313cd	22.482 ±0.824e	0.145 ±0044d
400	1.119 ±0.106b	2.273 ±0.371cd	27.846 ±1.178d	0.510 ±0.046c
600	1.292 ±0.121b	2.536 ±0.301c	31.441 ±1.135c	0.747b ±0.044
800	2.067 ±0.106a	4.489 ±0.344b	35.856 ±1.012b	0.701 ±0.084b
1000	2.117 ±0.162a	5.702 ±0.495a	55.458 ±1.527a	1.160 ±0.084a

Table 6. The effect of different Pb concentrations on the activities of SOD, POD, CAT and APX, and the content of soluble protein. Values are means ± SD (n = 3–6). Values followed by different letters indicating significant difference between treatments ($p \leq 0.05$).

Pb levels ($mg\ L^{-1}$)	SOD activity （units g^{-1} FW s^{-1}）	POD activity （△OD470 g^{-1} FW）	CAT activity （$nmol \cdot g^{-1}$ FW$\cdot s^{-1}$）	APX activity （$nmol \cdot g^{-1}$ FW$\cdot s^{-1}$）	Soluble protein content （$mg \cdot g^{-1}$ FW）
0	17.99 ±1.23c	51.84 ±1.47a	11.17 ±1.21d	421.16 ±28.46c	17.05 ±0.53a
100	16.50 ±1.22c	44.48 ±1.69b	13.95 ±0.96c	425.40 ±32.61c	16.28 ±0.33a
200	21.89 ±1.50b	40.64 ±1.15bc	14.06 ±1.12c	555.56 ±32.22b	10.51±0.56b
400	23.87 ±1.32b	38.48 ±1.84cd	19.89 ±0.96b	681.48 ±33.95a	6.23 ±0.43c
600	24.12 ±0.93b	34.99 ±1.12d	23.31 ±1.58a	746.83 ±33.23a	5.59 ±0.34cd
800	27.48 ±0.78a	26.88 ±1.60e	5.11 ±1.32e	445.50 ±38.14c	4.83 ±0.32de
1000	27.71 ±0.85a	23.89 ±1.76e	1.50 ±0.73f	329.10 ±29.50d	4.06 ±0.34e

Table 7. Pearson correlation among different photosynthetic parameters and antioxidant enzyme activities in *D.longan* leaves under Pb stress

Parameter	Protein	SOD	POD	CAT	APX	H_2O_2	$O_2 \cdot^-$	MDA	Proline	Fo	Fm	Fv	Fv/Fm	TRo/CSo	ETo/CSo	DIo/CSo	RC/CSo	Chl	Pn	Leaf dry mass
Pb_{leaf}	-.973**	.923**	-.912**	-.123	.233	.789*	.900**	.767*	.919**	.897**	-.967**	-.963**	-.933**	-.819*	-.949**	.921**	-.918**	-.822*	-.811*	-.927**
Protein	1	-.962**	.900**	.117	-.277	-.770*	-.871*	-.733	-.879**	-.860*	.926**	.922**	.889**	.770*	.903**	-.877**	.884**	.805*	.797*	.886**
SOD		1	-.927**	-.354	.057	.876**	.937**	.802*	.888**	.848*	-.921**	-.917**	-.907**	-.838*	-.891**	.899**	-.911**	-.897**	-.903**	-.897**
POD			1	0.456	0.113	-.934**	-.970**	-.876**	-.906**	-.855*	.954**	.948**	.943**	.901**	.910**	-.932**	.936**	.941**	.952**	.976**
CAT				1	.906**	-.693	-.532	-.601	-.346	-.148	.299	.282	.358	.586	.226	-.348	.402	.630	.662	.371
APX					1	-.387	-.184	-.350	-.039	.187	-.037	-.056	.030	.315	-.101	-.019	.081	.314	.325	.045
H_2O_2						1	.963**	.956**	.902**	.744	-.889**	-.877**	-.907**	-.970**	-.841*	.893**	-.932**	-.994**	-.977**	-.902**
$O_2 \cdot^-$							1	.888**	.919**	.862*	-.954**	-.948**	-.957**	-.933**	-.917**	.949**	-.949**	-.967**	-.982**	-.959**
MDA								1	.940**	.690	-.875**	-.857*	-.891**	-.989**	-.837*	.870*	-.944**	-.965**	-.878**	-.861*
Proline									1	.852*	-.971**	-.963**	-.971**	-.958**	-.960**	.957**	-.994**	-.936**	-.856*	-.930**
Fo										1	-.931**	-.950**	-.940**	-.744	-.962**	.956**	-.877**	-.793*	-.798*	-.935**
Fm											1	.998**	.993**	.913**	.989**	-.986**	.981**	.920**	.892**	.983**
Fv												1	.994**	.897**	.994**	-.991**	.975**	.911**	.886**	.985**
Fv/Fm													1	.926**	.990**	-.998**	.986**	.938**	.906**	.983**
TRo/CSo														1	.876**	-.906**	.964**	.977**	.912**	.894**
ETo/CSo															1	-.991**	.968**	.884**	.845*	.968**
DIo/CSo																1	-.976**	-.927**	-.899**	-.981**
RC/CSo																	1	.961**	.901**	.956**
Chl																		1	.967**	.924**
Pn																			1	.921**

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

have protective effects against stress. This finding was confirmed in *Jatropha curcas* L. plants in response to Pb stress (Shu et al., 2012). At higher Pb levels, greater reductions in Pn, Tr and Gs were observed (Table 4). This decrease in the Pn, Tr and Gs at higher Pb was primarily caused by a reduction in the carboxylation rate that was out of proportion,arising from non-stomatal factors such as decreasing chlorophyll contents. Similar observation has been recorded in *Populus cathayana* plants that were exposed to lead and drought (Han et al., 2013).

During plant photosynthesis, the shift of energy through the PSII at the reaction center level and the excited cross-section level can be assessed by using the OJIP test (Strasser et al., 1995). The present study showed that Fm, Fv, Fv/Fm, TRo/CSo, ETo/CSo and RC/CSo were significantly decreased with the increased Pb treatments; by contrast, the Fo and DIo/CSo increased (Table 3). Lead decreased the Fv/Fm and altered the OJIP transient (Figure 2), indicating that photo-inhibition occurs in leaves that were exposed to Pb. The reduction in the Fv/Fm was caused by both the decline of Fm and the increase in Fv (Table 3). Because Pb treatment decreased the RC/CSo (Table 3), the increase in the Fo may have resulted from the inactivation of RC in PSII (Jiang et al., 2008; Yamane et al., 1997) and the accumulation of reduced QA (Jiang et al., 2008; Bukhov et al., 1990). Moreover, ETo/CSo decreased to a greater extent in 400 mg.L^{-1} Pb-treated leaves, indicating the re-oxidation of reduced QA via electron transport from PS II to PS I, which was likely caused by an over-reduction of the photo-system in response to Pb stress. The decline in the ETo/CSo suggested that the damage to the PS II acceptor side occurred in Pb-treated leaves. The present investigation showed that the Pn was significantly and positively correlated with PS II photochemistry and chlorophyll levels. However, ETo/CSo, TRo/CSo, RC/CSo, Fm, Fv, and Fv/Fm were significantly and negatively correlated with the SOD activity and free proline content (Table 7). Similar result was observed in cotton that was stressed by lead (Bharwana et al., 2014a; 2014b).

Lead stress leads to ROS overproduction, leading to oxidative destruction in plant cells (Shamsi et al., 2008; Asada, 1996; Pinho and Ladeiro, 2012; Apel and Hirt, 2004). Increases in MDA and H_2O_2 contents under Pb stress accounted for lipid peroxidation or plasma membrane damage, which in turn inhibited plant growth (Zhang et al., 2009). In our experiment, the MDA, $O_2^{\cdot -}$ and H_2O_2 contents showed continuous accumulation in leaves with increasing Pb stress (Table 5), suggesting that Pb stress actually induced the decline in PSII activity. Similar findings for Pb-induced membrane lipid peroxidation by means of ROS were recorded by Shakoor et al. (2014) and Kanwal et al. (2014).

In our study, SOD activity was continuously enhanced with increased Pb stress, whereas APX and CAT activity were initially enhanced and subsequently decreased with increased Pb concentrations. This finding indicates that CAT and APX activity at lower Pb stress (100-600 mg L^{-1} Pb) could quench H_2O_2 and protect the *D. longan* plants from oxidative injury, and the scavenging functions of CAT and APX were impaired under higher Pb stress (800-1000 mg L^{-1} Pb). Nonetheless, cooperation from antioxidant enzymes is essential for the scavenging of ROS in plant cells. This finding indicated that the $O_2 \cdot^-$ scavenging was performed properly by the SOD and the combined action of CAT, APX, and POD converted the H_2O_2 to water. Obviously, lead influences the activity of antioxidative enzymes, which vary with the Pb levels.

The protein contents of leaves were significantly reduced at a stress level over 200 mg L^{-1}Pb, which may result from the oxidative damage. Similar Pb effects have been documented in association with metal toxicity (Bharwana et al., 2014a; Gupta et al., 2009]). In this study, a progressive enhancement in proline was recorded with increasing Pb stress (Table 5), suggesting that elevated proline protected *D. longan* plants from oxidative stress, lipid peroxidation and cell damage, and the tolerance improved. A similar finding was recorded in *Boehmeria nivea* L. in response to salt stress (Lamhamdi et al., 2011). Therefore, the free proline content combined with the SOD may play a key role in *D. longan* tolerance to Pb stress.

Materials and Methods

Plant materials and growth conditions

Longan (*Dimocarpus longan* L. cv. Wulongling) seeds were provided by the Putian Institute of Pomology, Fujian Province, and they were germinated in pots filled with sand inside pots and then irrigated with nutrient solution according to Wang and Zhuang (1981). Twelve weeks after germination, uniform seedlings were selected and transplanted to pots filled with sand. Three seedlings per pot were grown in a net house in a natural photoperiod at Fujian Agriculture and Forestry University. Each pot was supplied with 250 ml of nutrient solution every alternate day.

Pb treatments

Forty weeks after transplanting, each pot was supplied daily with a freshly prepared nutrient solution containing different concentrations of $Pb(NO_3)_2$ (0, 100 , 200 , 400 , 600 , 800 and 1000 mg L^{-1}) until the interstitial spaces between sand particles was completely filled. There were 15 pots per treatment in a completely randomized design. One month later, the leaves from all the treatments were observed.

Observations

Gas exchange parameters and chlorophyll a fluorescence measurements were determined after the treatment was stopped. Plants were sampled (at the 3-5th leaf from the top) to assess different physiological and biochemical index. After washing, the leaves were frozen in liquid N_2 and stored at 80℃ for biochemical analysis. The remaining plants were kept to assess their growth and yield-related traits.

Leaf chlorophyll a fluorescence transient

An OJIP transient was measured with a Handy Plant Efficiency Analyser (Handy PEA, Hansatech Instruments, Norfolk, U.K.) according to Strasser et al (1995) and Jiang et al (2008).

Leaf gas exchange

Leaf gas exchange measurements were performed by using a portable photosynthesis system (CID-301, USA) at an ambient CO_2 concentration and with a photosynthetic photon flux (PPF) of 1000 μmol m^{-2} s^{-1} according to Qiu et al (2002). During the measurements, the leaf temperature and ambient vapor pressure were 26-30°C and 1.53 ± 0.01 kPa, respectively.

Determination of leaf pigment

Leaf pigment was extracted and Chl a,Chl b, Chl a + b and Car were determined by following the methods of Arnon (1949) and Rao et al (1996).

Determination of Pb concentration and dry mass (DM)

At the end of the experiment, 5 plants per treatment from five pots were harvested. The plants were divided into roots, stems and leaves. The plant materials were extensively washed with deionized water. They were then dried at 80 °C for 48 h and DM was measured according to Chen et al (2005). The Pb concentrations were determined according to Fang (1991).

Soluble proteins and antioxidant enzyme activity

Frozen leaf samples (0.5 g) were ground in liquid nitrogen with a mortar and pestle and homogenized in 5 ml of 50 mM sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 11000 g for 20 min at 4°C. The supernatant was collected and used for protein and antioxidant enzyme activity analyses. The soluble proteins were determined as described by Bradford (1976). The SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium as described by Stewart and Bewley (1980). POD activity was estimated by the method followed by Zhu (1990). CAT activity was estimated by using the Rao method (1996). APX activity was measured according to Nakano and Asada (1981).

Determination of MDA

Lipid peroxidation was determined by estimating the MDA content following the method described by Hodges et al (1999).

Determination of H_2O_2

The content of H_2O_2 was measured according to the method described by Orendi et al (2001).

Determination of $O_2 \cdot^-$ generation

The content of $O_2 \cdot^-$ was assayed by its ability to reduce nitroblue tetrazolium (NBT) following the method described by Doke (1983).

Determination of proline

The proline content was determined according to the method used by Bates et al (1973).

Statistical analysis

The experiments were carried out with 3–15 replicates. An analysis of variance (ANOVA) was performed with a statistical package, namely Statistical Product and Service Solutions (SPSS) version 13.0, followed by the least significant difference (LSD) test at $p \leq 0.05$. The results are presented as the means ± SD.

Conclusions

When *D. longan* plants were subjected to Pb stress, they responded through alterations in their physiological and biochemical processes, which led to growth inhibition. Pb treatments induced increases in Fo, and they decreased the Fm, Fv, Fv/Fm, TRo/CSo, ETo/CSo, DIo/CSo, and RC/CSo. The decline in these parameters at high Pb stress was related to the loss of chlorophyll contents, leading to a significant decline in Pn, Tr, and Gs. Furthermore, Pb stress led to decreases in the soluble protein content, POD activity and increases in the SOD activity, proline and MDA contents, whereas CAT and APX were enhanced at low Pb levels and decreased under high Pb stress. These changes were closely related to the level of Pb stress and the interaction of these factors.

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