EX VITRO GROWTH AND ANTIOXIDATIVE RESPONSES OF TWO PAULOWNIA CLONES TO ZINC EXCESS

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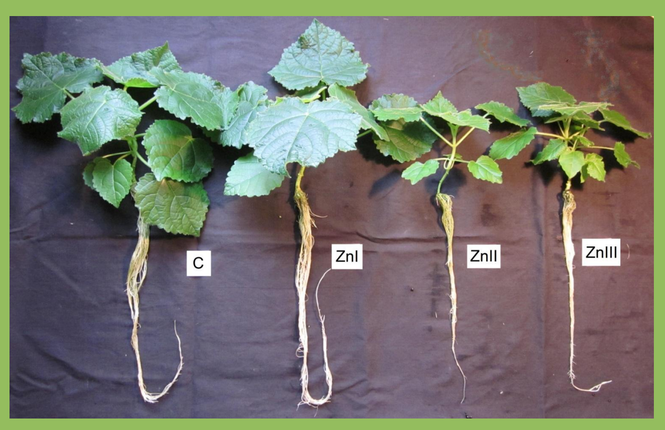
ABSTRACT

In present study, we investigated the effects of different concentrations of Zn (10, 20, 30 mg/l) on growth parameters, lipid peroxidation and accumulation of biologically active compounds, such as phenols, flavonoids and anthocyanins in *Paulownia tomentosa x fortunei* and *Paulownia elongata x fortunei*, grown in hidroponic after transferring the explants from in vitro micropropagation. Zn induced a decrease in root, stem lenght, leaf number and total leaf area of both clones. An enhanced levels of lipid peroxidation in leaf tissues with increasing the concentration of Zn indicated oxidative stres. The maximum activity of phenylalanine ammonia – lyase (PAL), a key enzyme of phenylpropanoid metabolism, was observed after treatment with 10 mg/l Zn in both clones.

Paulownia tomentosa x fortunei

Paulownia elongata x fortunei



Treatments	Root lenght [sm]	Stem lenght [sm]	Leaf number	Leaf area [sm^2]	BC
P. tomentosa x fortunei					
Control	28.25±2.12b	8.57±0.91b	12±1.7b	429±16b	
10 mg/l Zn	24.50±1.22a	6.72±0.83a	9±0.9a	198±38a	80.7
20 mg/l Zn	26.20±3.47a	7.12±0.94a	11±1.4a	227±44a	65.9
30 mg/l Zn	25.75±3.23a	7.12±1.01a	10±0.5a	218±61a	67.6
P. elongata x fortunei					
Control	37.51±2.12b	10.51±0.51b	10±0.8b	502±39d	
10 mg/l Zn	37.18±1.59b	10.20±1.21b	9±1.2b	534±61bc	43.8
20 mg/l Zn	24.02±1.31a	9.10±0.50a	9±0.9a	225±79ab	39.3
30 mg/l Zn	24.10±1.80a	8.80±0.90a	12±0.6a	171±47ab	34.8

Table 1. Mean values ± SD (n=5-6) of root and stem lenght, leaf number and total leaf area of *P. tomentosa x fortunei* and *P. elongata x fortunei*, grown in hydroponic in response to Zn stress. The bioaccumulation coefficient (BC) was obtained as the heavy metal content in plant divided by heavy metal concentration in the solution. Values with the same letter are not significantly different when means are separated by Fisher's LSD test (P<0.05).

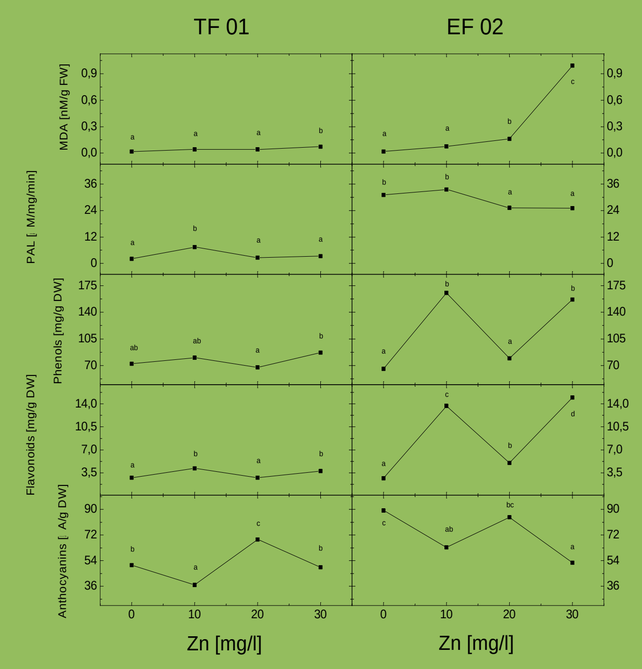


Fig. 1. Changes in MDA level,PAL activity, phenol, flavonoid and anthocyanin contents in leaves of *P. tomentosa x fortunei* and *P. elongata x fortunei*, grown hydroponically at different concentration of Zn. Values with the same letter are not significantly different when means are separated by Fisher's LSD test (P<0.05).

MATERIAL AND METHODS

Plant material. Seeds and *in vivo* explants from *P. tomentosa x fortunei and P. elongata* x *P. fortunei* were used for developing of *in vitro* multiplication protocol. Shoots were cultured on Murashige and Skoog's (1962) nutrient medium supplemented with 3 % (w/v) sucrose, 0,8 % agar, vitamins, 4,439 µM BAP and 0,537 µM IAA. After multiplication the shoots were transferred to rooting medium based on half strength basal salts MS medium, 2 % sucrose, 6 % agar and vitamins suplemented with 4,92 µM IBA and 1,075 µM IAA. The pH of all media was adjusted to 5,7 before autoclaving. All cultures were incubated under controlled conditions – 16 h photoperiod, light intensity of 35 µmol/m^2/s and 24/18ºC day/night temperature. After three weeks of rooting the shoots were washed from the medium with tap water and roots were rinsed with 1,5 ml/l Proplant solution.

Hydroponic experiment. *Ex vitro* seedlings were transplanted to *Hellriegel* (1898) nutrient solution with an addition of A-Z microelements in growth chamber. The heavy metal treatment was applied on the 48-th day after transplanting and one of the following concentrations of Zn were added: 10, 20 and 30 mg/l supplied as $Zn(NO_3)_2.6H_2O$. Plants grown in nutrient solution without metals served as controls. Plants were harvested after 10 days of treatment. Fresh mass of each of the plant sample was measured gravimetrically. Dry mass of shoots and roots was determined after oven-drying (60°C) for two days until constant weight. Leaf area was calculated using SigmaScan Pro 5 software. The bioaccumulation coefficient (BC) was calculated as the heavy metal content in plant divided by heavy metal concentration in the solution (*Nanda-Kumar et al., 1995*).

Enzyme and metabolite assayes. PAL activity was measured according to the procedure of *Yuan et al.* (2002). Total protein content was determined using the method of *Lowry et al.* (1951). The level of lipid peroxidation was assayed by the method of *Heath et al.* (1968). Total phenol content was determind by the method of *Pfeffer et al.* (1998), total flavonoid content was measured by the method of *Zhishen* (1999) and total anthocyanin content was assayed by the method of *Mancinelli et al.* (1975).

RESULTS AND DISCCUSION

Effect of heavy metal stress on plants growth. With increasing heavy metal levels, the root and stem lenght, leaf number and total leaf area of both clones were reduced (table 1). Total leaf area of *P. elongata x fortunei* increased slightly at 10 mg/l Zn compared to control. Visual symptoms of leaf injury caused by excess of Zn consist of chlorosis, necrosis and red-brown coloration of leaf, and in some cases, necrotic lesions lead to the death of the entire leaf (*Hermle et al., 2007; Wang et al., 2009*). In our study, no visible symptoms of Zn toxicity were observed in the leaves of *P. tomentosa x fortunei* and *P. elongata x fortunei*. Decreased total leaf area of both clones after treatment are not coresponding to an increased in leaf number (Table 1). This means that Zn affected leaf expansion before their biomass allocation and confirmed with the results of DiBaccio et al. (2010). The leaf Zn concentration followed the same trend as the Zn content, showing that the Zn uptake is directly proportional to biomass production (data not shown). The bioaccumulation coefficient (BC) decreased with increasing Zn concentration (Table 1).

Effect of heavy metal stress on plants malondialdehyde. Our results showed that MDA concentration, indicating degree of lipid peroxidation, increased significant in the leaves of *P. elongata x fortunei* after treatment with 30 mg/l Zn (Fig. 1).

Effect of heavy metal stress on plants secondary metabolism. We observed higher concentrations of total phenols and flavonoids after Zn treatment. The values measured at the middle Zn level (20 mg/l) are lowest than that at 10 and 30 mg/l Zn. Anthocyanin content is highest at 20 mg/l Zn. PAL avtivity followed the same trend of the changes as phenol and flavonoid contents after Zn treatment.

Irrespective of enhanced levels of MDA after Zn treatment, content of lower molecular antioxidants, such as phenols, flavonoids and antocyanins in the leaves of both clones changed in a different manner. Probably the pathways for synthesis of these low molecular antioxidants are different and PAL play an insignificant role in the induction of phenolic metabolism in *Paulownia* plants as a response to Zn stress.

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