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 biochemically active compounds, such as phenols, flavonoids and anthocyanins in Paulownia tomentosa x fortunei and Paulownia elongata x fortunei, grown in hydroponic after transferring the explants from in vitro micropropagation. Zn induced a decrease in root, stem length, 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 stress. 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.

RESULTS AND DISCUSSION

Effect of heavy metal stress on plants growth. With increasing heavy metal levels, the root and stem length, leaf number and 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 (Hermie 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 area leaf of both clones after treatment are not corresponding 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 Dibacco 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 activity followed the same trend as the changes of 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 anthocyanins 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.

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 M2 medium, 2 % sucrose, 6 % agar and vitamins supplemented with 4,92 µM BAP 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/m²/s and 24/18°C 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 Proplan solution.

Hydroponic experiment. Ex vitro seedlings were transplanted to Hellriegel (1898) nutrient solution with an addition of A-E 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(NO₃)₂·6H₂O. 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 assays. 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 determined 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).

Table 1. Mean values. 0.30 (n=6) of root and stem length, 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 estimated 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).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root length (cm)</th>
<th>Stem length (cm)</th>
<th>Leaf number</th>
<th>Total leaf area (cm²)</th>
<th>BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.86±0.46</td>
<td>19.6±0.96</td>
<td>7.12±0.7</td>
<td>26.0±2.5</td>
<td></td>
</tr>
<tr>
<td>10 mg/l</td>
<td>7.12±0.55</td>
<td>17.5±0.58</td>
<td>10.8±0.58</td>
<td>30.8±6.5</td>
<td></td>
</tr>
<tr>
<td>20 mg/l</td>
<td>7.06±1.03</td>
<td>17.1±0.76</td>
<td>10.5±0.5</td>
<td>29.5±1.6</td>
<td></td>
</tr>
<tr>
<td>30 mg/l</td>
<td>6.96±0.46</td>
<td>17.5±0.75</td>
<td>10.1±0.5</td>
<td>29.0±1.5</td>
<td></td>
</tr>
</tbody>
</table>

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).