Morphological effects of zinc and its accumulation in micropropagated plants of *Paulownia tomentosa* (Thunb.) Steud.

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Abstract: *In vitro* essay of *Paulownia tomentosa* (Thunb.) Steud. was undertaken to determine the capacity of this medicinal plant to accumulate heavy metal in its tissues. In this case, the nodal explants were placed on Murashige and Skoog’s medium (1962), added with 1.0 mg l⁻¹ indole-3-butyric acid (IBA). The ZnSO₄ was supplemented to the culture medium at various concentrations 200, 400, 600, 800 and 1000 µM. The MS medium free of Zn was served as control. The height of plantlets, the number of nodes, the number and length of roots and the biomass of the *in vitro* plants were determined. The Zn content in plant tissues was measured by an inductively coupled plasma-optical emission spectrometer (ICP-OES, OPTIMA 2000 DV, PerkinElmer, USA). Results showed that highest Zn level (1000 µM) induced inhibition of tumor cell proliferation, but also decreased the fresh biomass by 22.8% and 50.5%, respectively compared to the control. Furthermore, a high Zn accumulation in the plantlet tissues which varied from 121 g/g dry matter (DM) in the control to 2580 µg/g (DM) in the presence of 1000 µM Zn was noticed. Consequently, *Paulownia tomentosa* could be considered as a bio-indicator and Zn accumulator medicinal plant.

Keywords: Micropropagation, *Paulownia tomentosa* (Thunb.) Steud., medicinal plant, Zn accumulator.

Abbreviations: IBA: indole-3-butyric acid; MS: Murashige and Skoog medium; Zn: Zinc; DM: Dry Matter.

Introduction

*Paulownia tomentosa* (Thunb.) Steud. is a perennial tree species which belonging to the Scrophulariaceae family. It is native to central and western China but it’s widely distributed in Korea, Japan, and China (Jiang et al. 2004).

Parts of the plant *P. tomentosa* (leaves, wood, and fruits) have been used in traditional Chinese herbal medicine for the treatment of tonsillitis, bronchitis, asthmatic attack, and bacterial infections such as enteritis or dysentery. In fact, the flower is the most important material used in folk medicine herbs (Kang et al. 1999, Jiang et al. 2004, Šmejkal et al. 2007). However, extracts of *P. tomentosa* contained many bioactive compounds such as flavonoids and particularly Apigenin. This last has been found to show a variety of pharmacological activities, including hypotensive (Loizzo et al 2007), anti-inflammatory (Gerritsen et al. 1995, Ko et al. 2004), antispasmodic (Capasso et al 1991), antioxidant (Cos et al 1998) and vasorelaxant (Zhang et al 2000). Besides, Apigenin may exert its anti-tumorigenic effect *in vivo* not only via the inhibition of tumor cell proliferation, but also via the impairment of the invasive potential of tumor cells (Czyz et al. 2005).

Recently, *P. tomentosa* has received an increased attention due to its marketable value for wood and biofuel production thanks to its rapid growth, high biomass production, and high stress tolerance. In fact, this species has exhibited strong transpiration rates and real tolerance to high metal concentrations in both hydroponic and field studies (Liu et al. 2007, Doumett et al. 2010). Therefore, the aim of the present study was to determine for the first time the effects of increasing Zinc concentrations on the *Paulownia tomentosa* performance, grown *in vitro* conditions and the Zn accumulation by plantlets.

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The results will be important to indicate the potential application of *Paulownia tomentosa* in phytoremediation of contaminated sites.

### Material and methods

#### Plants material

Nodal explants were collected from old plants of *Paulownia tomentosa*. These explants were grown and maintained in the greenhouse of the Department of Agronomy and Vegetal Biotechnology at the National Agronomic Institute of Tunisia.

#### Surface disinfection, sterilization and culture media

Disinfection of nodal explants was carried out in the laminar airflow chamber by using ethanol (few seconds) and soaked in 0.1% (w/v) HgCl2 for 5 min. Then, the shoot tips were rinsed with sterile distilled water and surface sterilized with 10% (w/v) sodium hypochlorite for 5 min. Thus, sterilized nodal explants were cultured on Murashige and Skoog’s medium (1962) supplemented with 1 mg l⁻¹ IBA and 3% (w/v) sucrose. The medium was gelled with 0.6% (w/v) agar (Sigma) and the pH was adjusted to 5.8 with 0.1 N NaOH or HCl before autoclaving at 120°C for 20 min under a pressure of 1.1 kg cm⁻².

### Zn treatments and experimental design

The metal salt Zinc Sulphate 7-hydrate (ZnSO₄·7H₂O) was added to the culture medium at various concentrations: 200, 400, 600, 800 and 1000 µM. The MS medium without adding of Zn was served as control. The experimental design was conducted as a factorial randomized block with three replications.

### Growth conditions

The cultures were incubated at 23 ± 1°C under 16/8h (light/dark cycle) photoperiod and irradiance (36 µmol m⁻² s⁻¹) provided by cool-white fluorescent lamps.

Observations were made after four weeks of culture and focused on plantlet heights, number of nodes, root number and average length, biomass accumulation and the content of dry matter Zn.

### Zinc analysis

Vitro plants were carefully washed with distilled water, oven dried at 65°C for about 48 hours to stop enzymatic reactions and to obtain a constant weight, then ground to a fine powder before analysis.

The zinc content was determined after digesting the plant tissues in a mixture of concentrated HNO₃ and H₂O₂ (2:1 v/v., Sigma-Aldrich, Italy) using a digester (VELP Scientifica, Italy). After digestion, the Zn content was measured by an Inductively Coupled Plasma-optical emission spectrometer (ICP-OES, OPTIMA 2000 DV, PerkinElmer, USA). The ICP-OES analytical standard (AA/ICP calibration/check standards for environmental analysis, 1 g l⁻¹) for Zn was supplied by Sigma-Aldrich (Italy).

### Statistical analysis

Fifteen explants were used per treatment in triplicates. Data were subjected to statistical analysis using the program package SAS (SAS 1999). The one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test at the significance level of 5% was used to compare means.

### Results and Discussion

#### Zn effect on shoot height and nodes number of plants

The *in vitro* shoot height of *Paulownia tomentosa* in the culture media supplemented with Zn at different concentrations showed a significantly difference between the treatments (Table 1).

The highest shoots were obtained on MS culture medium free of Zn (control) and no significant difference was observed in media with 200 M of Zn. However, the plant height was significantly reduced by 18.86%, compared to the control, at 1000 µM of Zn.
When the Zn concentration was up to 400 µM, the number of nodes decreased slightly but without a significant difference between treatments. However, no difference was observed between shoots grown on the control or on media supplemented with 200 and 400 µM of Zn. The number of nodes was significantly reduced by 20.63% compared to the control at the highest level of Zn (1000 µM) in the culture medium (Table 1). Indeed, the presence of Zn in the culture media caused a decrease in the aerial parts growth of plantlets. These findings corroborate those of Gerard et al. (2000) who observed a decrease of the biomass produced in cultures of Lolium perenne in soils contaminated by Zn. In the same context, Wang et al. (2010) showed that the germination rate and shoot length of Paulownia fortunei decreased when Zn concentrations are higher at mining sites rich in this metal. On the other hand, Ruscinska et al. (1999) showed that the presence of metals can cause a decrease in biomass, an inhibition of photosynthesis, respiration disturbances and degeneration of main cell organelles. In Brassica napus, Ben Ghnaya (2008) noted that the treatment in the greenhouse at 2000 µM Zn caused a decrease reaching 75% in shoot and root biomass which is depending on the genotype.

Table 1. Effects of ZnSO₄ on vegetative growth and rooting of in vitro plantlets Paulownia tomentosa (Thunb) Steud.

<table>
<thead>
<tr>
<th>Zn (µM)</th>
<th>0</th>
<th>200</th>
<th>400</th>
<th>600</th>
<th>800</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoots length (cm)</td>
<td>4.766 ± 0.563 –</td>
<td>4.367 ± 0.550 –</td>
<td>4.133 ± 0.550 –</td>
<td>4.067 ± 0.563 –</td>
<td>4.033 ± 0.611 –</td>
<td>3.867 ± 0.550 –</td>
</tr>
<tr>
<td>No. of nodes</td>
<td>5.333 ± 0.724 a</td>
<td>5.267 ± 0.704 a</td>
<td>5.067 ± 0.594 a</td>
<td>4.533 ± 0.743 b</td>
<td>4.467 ± 0.640 c</td>
<td>4.200 ± 0.676 b</td>
</tr>
<tr>
<td>Roots length (cm)</td>
<td>2.606 ± 0.426 a</td>
<td>1.749 ± 0.347 b</td>
<td>1.401 ± 0.257 c</td>
<td>1.339 ± 0.277 d</td>
<td>0.977 ± 0.208 e</td>
<td>0.891 ± 0.149 f</td>
</tr>
<tr>
<td>No. of roots</td>
<td>7.067 ± 0.884 a</td>
<td>6.267 ± 0.884 a</td>
<td>3.933 ± 0.594 a</td>
<td>3.067 ± 0.458 a</td>
<td>2.667 ± 0.488 a</td>
<td>1.067 ± 0.258 a</td>
</tr>
</tbody>
</table>

Mean values within the column followed by the same letter in superscript are not significantly different at P<0.5% (Duncan test).

Effect of Zinc on in vitro rooting

In vitro regenered shoot from nodal explants were cultured on MS medium supplemented with 1 mg l⁻¹ IBA in combination with different concentrations (0, 200, 400, 600, 800, 1000 µM) of Zn (Figure 1).

Figure 1: In vitro plantlets Paulownia tomentosa (Thunb.) Steud. cultured in presence of ZnSO₄. A: Control; B: 800 µM ZnSO₄; and C: 1000 µM ZnSO₄.

The results showed that high levels of Zn in MS medium didn’t inhibited root formation. However, number and root length average varied significantly and is related to Zn concentration in MS medium (Table 1). The addition of Zn to the culture medium caused a significant reduction in the average number of roots from 7 per vitroplant in the control to a single root in
plants grown on a culture medium supplemented with 1000 µM of Zn.

Furthermore, root length average varied significantly in all the treatments (Table 1). Indeed, the length of roots was significantly reduced by 65.8% compared to the control at 1000 µM of Zn in the culture medium.

The effect of Zn on root quality was studied in other plants. Schwartz et al. (1999) reported that in Thlapsi caerulescens, short roots arranged in aggregates in presence of high concentrations of this metal in soil and in uncontaminated soil the roots of the same plant are very thin and long.

In addition, Shi et al. (2011) showed that root length, root surface and root volume of Glochidion puberum grown in lead/zinc (Pb/Zn) mine tailings areas were reduced by 70, 80, and 86%, respectively, compared to that grown in uncontaminated soil.

Effect of Zinc on fresh and dry biomass

As shown in Figure 2, the addition of Zn to the culture medium at different concentrations affected severely the fresh and dry biomass of in vitro plantlets and this effect is more pronounced with increasing levels of Zn.

Indeed, the fresh biomass decreased from 9.62 g to 7.42 g in the control and with 1000 µM Zn concentration, respectively. However, the fresh biomass of the plantlets treated with 200 and 400 µM of Zn is relatively stable (9 g) (Figure 2).

As well, fresh biomass of plantlets treated with 600, 800 and 1000 µM Zn is near to 7.5 g. A regression by 22.8% and 50.5% on the fresh and dry biomass under 1000 µM Zn concentration compared to the control respectively was recorded. These results agree with those found by Ben Ghnaya (2008) which observed a significant decrease in dry matter weight and fresh tissue culture in all the variety of Brassica napus L. Drakkar by adding 500 and 1000 M of Zn to the culture medium. However, Mench et al. (1981) showed that the vegetative growth of maize seedlings is related with the Zn concentration in the solution. On the other hand, the addition of different concentrations of Zn to a culture of Solanum nigrum seeds (0, 100, 500 and 1000 mg Zn/kg of sand) affects the growth of seedlings. Plants grown at 100 mg kg⁻¹ showed significantly lower leaf, stem, root and total biomass than control ones and plants growing at 500 and 1000 mg kg⁻¹ presented significantly the lowest biomass (Marques et al. 2006).

In fact, the abiotic stress caused by heavy metals, modifies the structure of essential enzyme by altering the protein structure and causing deficiency symptoms in plants. The plasma
membrane is particularly sensitive to heavy metal toxicity since membrane permeability and thus, its functionality can be affected by altering the intrinsic membrane protein such that H⁺-ATPases (Hall 2002). This stress results in a production of reactive oxygen species (ROS). Overproduction of these ROS leads to oxidative damage in plant tissues due to lipid peroxidation leading to the formation of degradative products such as alkanes and aldehydes (Ferrat et al. 2003). Therefore, toxic symptoms such as chlorosis, growth retardation, browning of roots and a cell cycle arrest can be observed. To fight this oxidative stress, plants produce defense systems enzymatic and non enzymatic antioxidants in order to regulate the concentration of ROS and thus maintain ion homeostasis within cells (Clemens 2001, Hall 2002).

Zn levels in vitro plants

The Zn content in the *in vitro* plantlets was measured at the end of the experiment. As shown in Fig. 3, the Zn levels recorded *in vitro* plantlets vary from 121 mg g⁻¹ in the control to 2580 mg/g *in vitro* plants grown in the medium containing the highest concentration of Zn (1000 µM).

![Figure 3. Zn levels (µg g⁻¹ DW) on tissues of *in vitro* plantlets *Paulownia tomentosa* (Thunb) Steud.](image)

Indeed, Zn was mostly stored in the tissues plantlets of *Paulownia*, where the accumulation was almost linearly related with the Zn concentration in the MS medium. Similar results were reported by Azzarello et al. (2012) whose detected a linear increase of Zn accumulation in *Paulownia tomentosa* plants with the 1000 µM of Zn. At highest zinc concentrations (2000, 3000 and 5000 µM), a linear decrease of Zn accumulation was detected and was clearly linked to the reduced biomass production.

On the other hand, Marques et al. (2006) showed that *Solanum nigrum* accumulated up to 1450, 3240 and 3810 mg Zn kg⁻¹ in the leaves, stems and roots, respectively but without any visual toxicity signs. This confirmed the idea that *Paulownia tomentosa* is Zn tolerant and hyperaccumulator plant.

### Conclusion

In order to evaluate the tolerance and the ability to Zn accumulation in *Paulownia tomentosa* grown under *in vitro* conditions, different morphological parameters as well as analysis of the Zn content in tissues of plantlets were determined. Results showed that 1000 µM Zn concentration caused a reduction of about 18.86% and 50.5%, respectively on shoot height and dry biomass compared to the control. This decline was more pronounced in the root system. However, a significant reduction in the number of roots was noticed and estimated by 84.9% with 1000 µM Zn concentration compared to the control. The high levels of Zn in the culture medium limited the root formation. However, the determination of Zn levels showed good accumulation in the tissues of plantlets with ranging from 121 µg g⁻¹ DW to 2580 g g⁻¹ DW. Overall, results showed that
Paulownia tomentosa could be considered as a bio-indicator and Zn accumulator medicinal plant since it can tolerate and accumulate high Zn content.

References


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