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Ultramorphological and physiological modifications induced by high zinc levels in *Paulownia tomentosa*

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ABSTRACT

The efficacy of *Paulownia tomentosa* in the absorption and accumulation of Zn from contaminated soils has been recently described. However, no data are available regarding the modifications induced by high levels of Zn on the anatomy and physiology of this tree species. *P. tomentosa* were grown hydroponically at different Zn concentrations (100, 500, 1000, 2000, 3000, and 5000 μM). The plant growth and leaf gas exchange parameters (net CO_2 assimilation and stomatal conductance) were significantly reduced at high Zn concentrations. Electron and confocal microscopy analysis showed differences in the cellular ultrastructure between control and treated (above 2000 μM) plants, which exhibited an accumulation of electron-dense materials. The major toxic effects of high Zn concentrations were related to damages to the cell functionality, *i.e.*, the chloroplast ultrastructure, which negatively affected the photosynthetic performance, thus leading to a significant growth inhibition. *P. tomentosa* plants are able to limit Zn-induced damages by activating effective mechanisms of Zn sequestration and accumulation of excess Zn in dedicated structures, such as petiole cell walls and root hairs, or by excluding part of the Zn in exudates located on the petiole surface.

1. Introduction

Among heavy metals, zinc (Zn) is present in biological systems at relatively high concentrations compared with other micronutrients (Wilson, 1988). Under natural conditions, zinc is present in soils at low concentrations, ranging between 30 and 150 ppm (Mulligan et al., 2001), with the available fraction being lower than 10 ppm (Ebbs and Kochian, 1997). At these concentrations, Zn is an essential element for plant metabolism and growth, but human activities, such as mining operations, have enhanced the Zn levels in numerous contaminated sites to concentrations that are potentially harmful to the environment and to human health. The range of Zn levels in polluted soils varies widely. For example, in paddy soils in central Anhui Province (China), a zinc range between 30 and 420 ppm was found (Wang et al., 2009). In topsoil collected near an abandoned Zn/Pb smelter in Austria, the zinc concentration varied between 4 and 955 ppm (Wieshammer et al., 2007), whereas Zn concentrations reached the range 800–2000 ppm in contaminated sites in Poland (Sitko et al., 2004). Furthermore, the available fraction of the zinc and its mobility in the soil are normally related to the pH, clay and hydrous oxide content, organic matter and redox conditions (Reichman, 2002), which strongly influence the zinc bioavailability for plant absorption.

Plants have developed a complex network of homeostatic mechanisms to modulate the internal concentrations of free Zn in the cytosol to maintain its toxicity below a certain threshold (Clemens, 2001). These tolerance strategies mainly include detoxification processes, complexation by organic chelators, and the accumulation and compartmentalisation of Zn ions in the vacuoles, as zinc plays a fundamental role in several critical cellular functions (Marschner, 1995). The interaction of Zn with various biochemical and metabolic processes in plants is well reported in the literature, whereas the structural and ultrastructural cell modifications induced by high levels of Zn have hardly been described (Heumann, 2002; Di Baccio et al., 2009).

Recently, *Paulownia tomentosa* (Thunb.) Steud and other tree species belonging to the genus *Paulownia* (*Paulownia fortunei* Hems., *Paulownia elongata* S.Y.Hu) have been effectively used for phytoremediation purposes (Doumett et al., 2008; Stankovic et al., 2009; Wang et al., 2009; Doumett et al., 2011) due to their tolerance to heavy metals in combination with an outstanding growth rate and leaf area expansion, *i.e.*, under appropriate conditions, a 5–7-year-old tree can grow up to 15–20 m of height, with an annual

Abbreviations: *A*, CO_2 net assimilation; ESEM, environmental scanning electron microscopy; *g*, stomatal conductance; ICP-OES, inductively coupled plasma-optical emission spectrometer; TEM, transmission electron microscopy.

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biomass production of 150 t ha^{-1} (Caparròs et al., 2008). In *P. tomentosa*, this massive production of biomass within a short time renders a significant removal of contaminants from polluted soils possible despite the rather low rate of metal absorption (Doumett et al., 2008). In addition, the extensive development of the root system of this species allows a deep soil exploration, and the high transpiration rate of the canopy makes *Paulownia* an effective natural 'pump' adapted to absorb large quantities of water from the ground. However, the physiological mechanisms related to the accumulation and the compartmentalisation of heavy metals in *P. tomentosa* are still unclear.

In the present study, we conducted an in-depth investigation of the effects of increasing Zn concentrations on the cellular ultrastructure and the photosynthetic parameters of *P. tomentosa* grown in hydroponic conditions, with particular attention given to the main strategies for Zn detoxification used by this promising phytoremediating tree species.

2. Materials and methods

2.1. Plant material and growth conditions

Cuttings of *P. tomentosa* (Thunb.) Steud were rooted in a greenhouse located at the University of Florence, Italy. In spring 2008, thirty-five homogeneously rooted cuttings of *P. tomentosa*, uniform in size, were transferred into 3 L polyethylene pots filled with perlite and placed in a hydroponic cultivation system inside the greenhouse (*T* 25 °C/17 °C day/night, relative humidity max 60%). All of the plants were supplied with half-strength Hoagland's nutrient solution (pH 5.5, EC 1.3 mS cm^{-1}), continuously aerated and renewed every week. After a one-week acclimation to the hydroponic conditions, the plants were divided into seven groups that received different treatments: control (1 μM of Zn), 100, 500, 1000, 2000, 3000, 5000 μM of Zn added to the nutrient solution as sulphate salts ($ZnSO_4 \cdot 7H_2O$, purity for Zn ≥ 58%, Sigma–Aldrich).

2.2. Growth analysis

Plant material was collected 90 days after the beginning of the experiment. Before harvesting, the internode length was measured. At the time of harvest, five plants per treatment ($n = 5$) were selected, and each plant was separated into leaves, petioles, stems and roots. The dry weight of each organ was determined after drying the samples in an oven at 70 °C for 48 h. The leaf area was calculated using the Image Tool® software (freeware at www.ddsdx.uthscsa.edu/dig/itdesc.html) on leaf images (300 dpi) obtained by a CanoScan D660U scanner (Canon Europe, The Netherlands).

2.3. Zinc content determination

The zinc content was determined after digesting the plant tissues in a mixture of concentrated HNO_3 and $HClO_4$ (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 analytical standard (AA/ICP calibration/check standards for environmental analysis, 1 g L^{-1}) for Zn was supplied by Sigma–Aldrich (Italy).

2.4. Leaf gas exchange analysis

The net CO_2 assimilation (A, μmol m^{-2} s^{-1}) and stomatal conductance (g, mmol m^{-2} s^{-1}) measurements were performed on the central sector of the youngest fully expanded leaf 60 days after the beginning of the experiment. The measurements were performed between 11 am and 1 pm in a Parkinson automatic leaf chamber provided with a halogen light unit (PLC-B, PP Systems, England) and connected to a CIRAS-1 portable system (differential CO_2/H_2O infrared gas analyser, PP Systems, England). The measurements were performed at a saturating photosynthetic photon flux density (PPFD, 1500 μmol m^{-2} s^{-1}), a constant temperature (27 °C) and a relative humidity between 60 and 70%.

2.5. Transmission electron microscopy (TEM)

Electron microscopy was performed on the middle sections of leaves obtained from control plants and plants treated with 1000 μM Zn. The samples were fixed with 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 1 h and then washed twice with the same buffer, prior to a post-fixing procedure with 2% osmium tetroxide in phosphate buffer (pH 7.2). The specimens were dehydrated in a graded ethanol series (25, 50, 75, 90 and 100%); finally, the ethanol was replaced by propylene oxide. The samples were gradually embedded in Spurr resin (Spurr, 1969) and polymerised at 70 °C for 24 h. Ultrathin sections (70–90 nm in thickness) were obtained using an LKB IV ultramicrotome (Rankin Biomedical, USA), collected on Formvar-coated copper grids, stained with uranyl acetate and lead citrate and finally examined with a Philips CM12 transmission electron microscope (Philips, The Netherlands) operating at 80 kV.

2.6. Environment scanning electron microscopy (ESEM)

The leaves and petioles were monitored using an FEI Quanta 200 environment scanning electron microscope (ESEM, FEI Corporation, The Netherlands), operating in low-vacuum mode (the chamber pressure was kept at 1 Torr) at 25 kV, without pretreatment of the samples. The chemical composition of the tissue was determined by energy-dispersive X-ray spectroscopy (EDX). Each leaf and petiole area analysed by EDX in our experimental conditions corresponds to a spot of approximately 50 nm in diameter.

2.7. Confocal microscopy

Confocal imaging was performed using an upright Leica Laser Scanning Confocal Microscope SP5 (Leica Microsystems, Germany) equipped with a 63× oil immersion objective. To analyse the intracellular localisation of the zinc ions, FluoZin-3-AM (acetoxymethyl) cell permeant (Molecular Probes, USA) was used. FluoZin-3-AM was chosen because it is considered to be a very specific indicator for intracellular Zn^{2+} localisation and concentration (Gee et al., 2002). The plant samples were incubated for 30 min in a solution of 15 μM FluoZin-3-AM. After incubation, the samples were mounted in a water solution on a slide and observed. The excitation wavelength was set at 488 nm, and the emission was detected at 530 ± 20 nm.

2.8. Statistical analysis

The data from the growth analysis and the leaf gas exchange measurements were analysed by one-way ANOVA, and the means ($n = 5$) were separated using Tukey's test ($P \leq 0.05$). The statistical analysis was performed using GraphPad Prism 4.0 (GraphPad software).

Table 1
The total dry weight (g) and the leaf area (cm^2) measured in *P. tomentosa* plants grown at different Zn concentrations 90 days after the beginning of the experiment. The means were separated by Tukey's multiple comparison test ($n = 5$). The different letters indicate statistically significant differences for $P < 0.05$ ($n = 5$).

Treatments	Dry weight	Leaf area
Control	81.25a	7751.71a
100 μM	70.68a	7312.12a
500 μM	74.84a	7279.85a
1000 μM	68.09a	6741.93a
2000 μM	39.74b	4366.16b
3000 μM	27.22bc	2097.48c
5000 μM	14.92c	691.25d

3. Results

3.1. Growth parameters

The plant growth remained unaffected by Zn concentrations up to 1000 μM (Table 1). In contrast, the dry weight, leaf area and internode length dramatically decreased at Zn concentrations higher than 2000 μM (Table 1 and Fig. 1).

3.2. Leaf gas exchange parameters

No significant differences in the net CO_2 assimilation (A) were observed at Zn concentrations below 1000 μM 60 days after the beginning of the experiment (Fig. 2A). Starting from 1000 μM, the increase in the Zn concentration in the nutrient solution led to a progressive and intense decrease in A, until slight negative values were reached between 3000 and 5000 μM Zn. Similarly, the stomatal conductance (g) was negatively influenced by Zn starting from 1000 μM (Fig. 2B).

3.3. Zinc content in the plant organs

The Zn content in the different organs was measured at the end of the experiment. The results are shown in Fig. 3. Zinc was mostly stored in the root system, where the accumulation was almost linearly related with the Zn concentration in the nutrient solution. At 5000 μM, the Zn content in the roots was threefold higher than that reported at 1000 μM (Fig. 3A). A clear increase in the Zn content was also detected in the shoots (Fig. 3B). In the petioles (Fig. 3C) and leaves (Fig. 3D), the behaviour was completely different, as a linear increase in the Zn content was detected until 2000 μM, with a subsequent decrease in the plants grown with 3000 or 5000 μM of Zn in the nutrient solution.

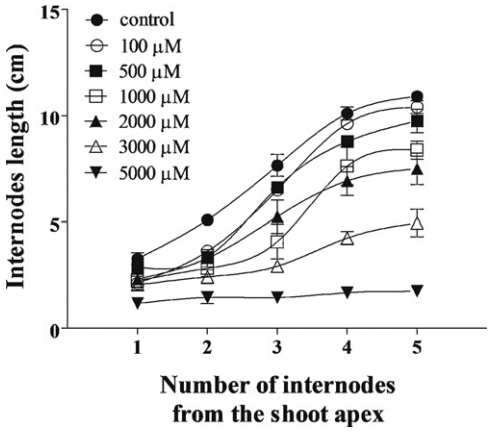

Fig. 1. The lengths (cm) of the last five internodes in plants grown at different Zn concentrations, measured 60 days after the beginning of the experiment. Internode #1 refers to the first internode below the shoot apex. The bars indicate ±SD ($n = 5$).

Table 2
The total Zn content (mg $plant^{-1}$) calculated in plants grown with increasing Zn concentrations in the NS. The means were separated using Tukey's multiple comparison test. Different letters indicate statistically significant differences for $P < 0.05$ ($n = 5$).

Treatments	Total Zn content
Control	5.66d
100 μM	16.23c
500 μM	28.35b
1000 μM	44.34a
2000 μM	36.10a
3000 μM	36.06a
5000 μM	25.72b

To estimate the real Zn absorption capacity of *P. tomentosa*, the total quantity of Zn in the entire plant was calculated (Table 2). A linear increase of metal accumulation was detected in the plants until 1000 μM; the following decrease was clearly linked to the reduced biomass production at the highest zinc concentrations (2000, 3000 and 5000 μM).

3.4. Microscopic analysis

No structural distortion was observed by TEM in the mesophyll or in the bundle sheath cells of the control plants (Fig. 4A and B), whereas the plant tissues exposed to 1000 μM Zn showed slight symptoms of toxicity. Membrane vesiculation (mv) was observed in all of the tissues (Fig. 4C), together with the withdrawal of the plasma membrane from the cell walls (Fig. 4C, cw). Moreover, a specific number of mitochondria (mt) appeared to be very enlarged, with the cristae arranged in a disorderly fashion, lower in number and vacuolated (Fig. 4D). Numerous small starch grains (sg) were observed in the control plants (Fig. 4A and B). A reduced amount of thylakoids (ts) was observed in almost all of the chloroplasts (ch, Fig. 4E). Other toxic effects observed in the 1000 μM-treated plants included an unusual shape in the cells belonging to the spongy parenchyma (sp) (Fig. 4F) and an increased number of peroxisomes (p) (Fig. 4C).

The leaves of the plants grown at 5000 μM Zn exhibited electron-dense globules (red arrow) in the bundle sheath cells (Fig. 4H), in the palisade and in the spongy parenchyma (Fig. 4G, I and J); an increase in the peroxisomes and mitochondria with dilated cristae and a dense matrix (Fig. 4K) were also observed. Moreover, the cytoplasm was vacuolated, and the chloroplasts contained large starch grains, especially in the adult leaves (Fig. 4I and J). In a few cells, the chloroplasts also showed an irregular shape, starch grains (sg) with unusual forms and many plastoglobules (pl) (Fig. 4J).

In the root cortical cells isolated from plants grown at 1000 μM Zn, an unusual deposition of electron-dense material in the form of clumps (Fig. 5A, red arrow) or globules (Fig. 5B, red arrow) was observed. Confocal microscopy also revealed a significant accumulation of Zn in the root hairs of *P. tomentosa*, with an evident compartmentalisation into megavesicles (Fig. 5C, white arrow).

In the petioles, stress due to the heavy metals was clearly indicated by the complete withdrawal of the plasma membrane from the cell walls (Fig. 5D, red arrowhead), together with the formation of vesicle-like structures and electron-dense globules (Fig. 5D, red arrow). Using confocal microscopy, Zn was detected in the chloroplast lumen and envelope (Fig. 5F, white arrows). Several punctate structures containing Zn were also clearly detectable around the chloroplasts and spread into the cytosol (Fig. 5F, white arrowhead).

ESEM micrographs and X-ray SEM microanalysis of the leaves and petioles of plants grown at 1000 μM and 5000 μM Zn are shown in Fig. 6A–C, respectively. Microanalysis performed on the trichomes located on the leaf surface did not show any Zn peak (Fig. 6A). The collenchyma collected from the petioles grown at

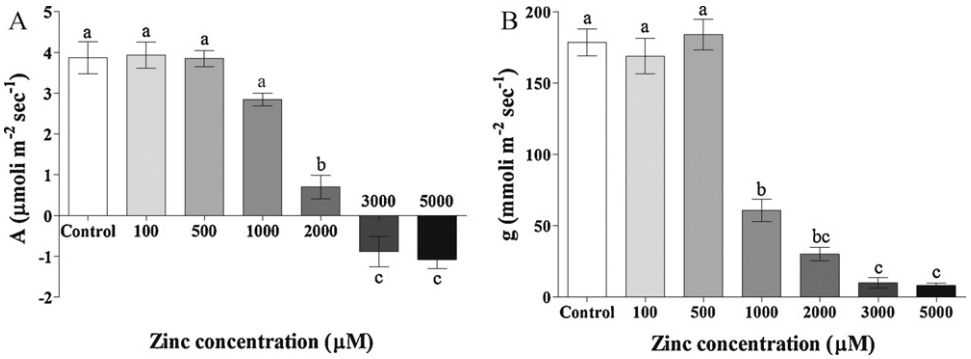

Fig. 2. The net CO_2 assimilation (A) and the stomatal conductance (B) in plants grown at increasing Zn concentrations. The means were separated using Tukey's multiple comparison test. The bars indicate ±SD. The different letters indicate statistically significant differences for $P < 0.05$ ($n = 5$).

5000 μM Zn was also analysed (Fig. 6B). The EDX microanalysis showed a high Zn accumulation on the cell walls of the collenchyma cells. Finally, an exudate deposition on the external surface of a petiole characterised by elevated Zn content was clearly detected (Fig. 6C).

4. Discussion

In recent years, the genus *Paulownia* has been investigated for phytoremediation purposes (Doumett et al., 2008; Stankovic et al., 2009; Doumett et al., 2011). In fact, *Paulownia* spp. shares with few other tree species, such as willow and poplar, the optimal characteristics of growth and adaptability and also has a higher biomass production.

In this study, we report the typical symptoms of plants under stress in *P. tomentosa* (growth inhibition, leaf area decrease) occurring at rather high Zn concentrations (above 2000 μM). This result confirms the efficacy of *P. tomentosa* as a phytoremediating species, as such concentrations are usually well tolerated primarily by herbaceous Zn hyperaccumulators (*Arabidopsis halleri*, Kashem et al., 2010; *Sedum alfredii*, Tian et al., 2009; *Thlapsi caerulescens*, Saison et al., 2004) but rarely by tree species.

The heavy metal uptake by the roots and the successive translocation to the above-ground organs are primarily driven by transpiration. Therefore, the measurement of the gas exchange parameters provides indirect but useful insights to be applied for selecting suitable plant species for phytoremediation (Angle et al., 2003). It is well known that the photosynthetic parameters in higher plants are negatively affected by high levels of Zn (Di Baccio et al., 2003; Krämer, 2005). In fact, chloroplasts are particularly sensitive to oxidative stress, and the presence of Zn at high concentrations (above 1000 μM) inhibits the photosynthetic electron transport *via* the production of toxic oxygen species, such as O_2^- or H_2O_2 (Baishnab et al., 1980; Kappus, 1985), thus leading to a disassembly of the thylakoids and a consequent significant decrease in the net CO_2 assimilation. Accordingly, we detected the presence of

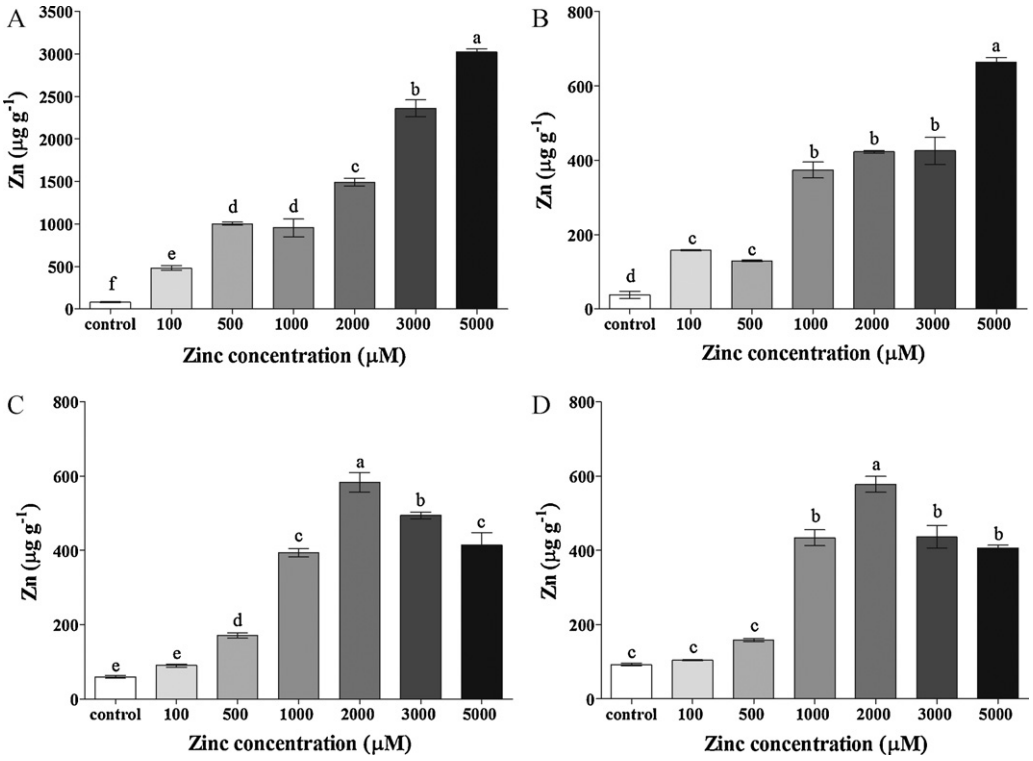

Fig. 3. The zinc concentrations in different plant organs after 90 days from the beginning of the experiment: (A) roots, (B) shoots, (C) petioles and (D) leaves. The means were separated using Tukey's multiple comparison test. The bars indicate ±SD. The different letters indicate statistically significant differences ($P < 0.05$; $n = 5$).

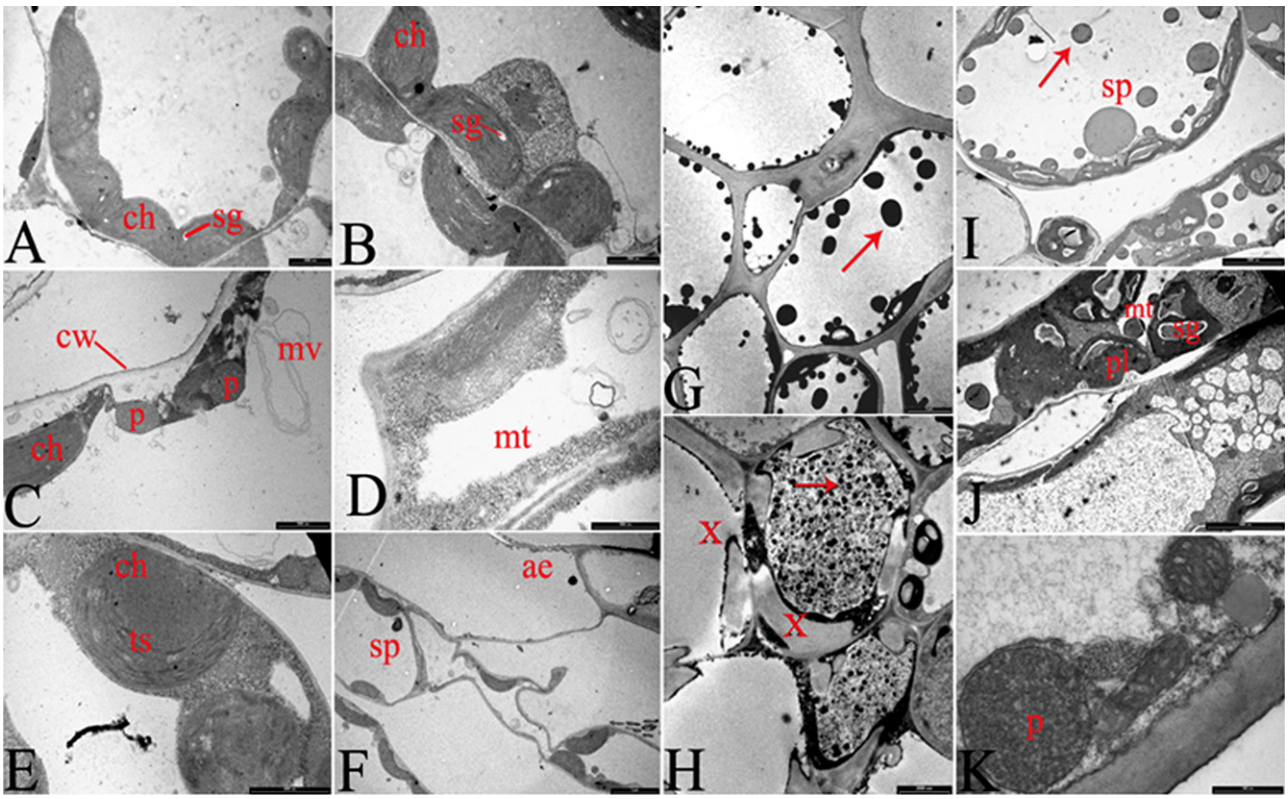

Fig. 4. Electron micrographs of leaf mesophyll cells of *P. tomentosa* plants. (A and B) TEM micrographs of control leaves with well-developed chloroplasts (ch) and small, numerous starch grains (sg). (C–F) TEM micrographs of leaf cells of the plants grown at 1000 μM Zn showing membrane vesiculation (mv), withdrawal of the plasma membrane from the cell wall (cw) and an increase in the number of peroxisomes (p, C); enlarged mitochondria (mt) (D); a reduced portion of thylakoids (ts, E) and unusually shaped spongy parenchyma (sp) cells (F). (G–K) TEM micrographs of leaf cells of plants grown at 5000 μM Zn showing electron-dense globules (arrow) in the spongy parenchyma (G, I, J) and in the xylem elements (x) in the bundle sheath cells (H); vacuolated cytoplasm and chloroplasts (ch) with large starch grains (sg, I, J); chloroplasts with irregular shape and many plastoglobules (pl, J); and an increase in the number of peroxisomes (p, K). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Zn in the chloroplast lumen and the envelope in plants treated with high levels of Zn (above 1000 μM), with several punctate structures containing Zn also visible around the chloroplasts (Fig. 6E and F). We also found a large increase in the number of peroxisomes, which are known to be a main site for the production of ROS (Romero-Puertas et al., 1999; Baker and Graham, 2002; Mittova et al., 2004; del Río et al., 2006), supporting the hypothesis of a ROS-mediated impairment of the photosynthetic activity.

In addition, ultramorphological changes were detected by TEM. Changes in the cellular organisation at high Zn levels were evident. A clear alteration in the leaf cellular ultrastructure was detected in the plants grown at 1000 μM of Zn, even if no symptoms of damage were detectable by visual assessment. In particular, an increase in membrane vesiculation, a different development of the vacuoles and the withdrawal of the plasma membrane from the cell walls were evident (Fig. 4C). The ultrastructural analysis revealed a large increase in the size of the mitochondria and dramatic changes in the chloroplasts' morphology, which showed few starch grains and many plastoglobules, as was previously reported in plants grown at high levels of heavy metals, such as Cd or Mn (McCarthy et al., 2008; Najeeb et al., 2009).

In the petioles, several punctate structures or vesicles were observed by microscopic analysis (Fig. 5D and F), together with an accumulation of Zn in the chloroplast lumen and the envelope that likely caused the observed alterations in the chloroplast morphology and the envelope disgregration. The clearly detectable structures containing Zn around the chloroplasts and spread into the cytosol are similar to the so-called 'zincosomes' identified in yeast and mammalian cells grown in the presence of Zn (Haase and Beyersmann, 1999; Devirgiliis et al., 2004). Zincosomes are cytosolic vesicles that store Zn in case of metabolic needs. The presence of zincosomes in plant tissues is still controversial, although recent evidence of Zn-accumulating vesicles was detected in *Arabidopsis thaliana* seedlings (Kawachi et al., 2009). Based on our results, *P. tomentosa* appears to possess similar structures. In many species tolerant to heavy metals, the root hairs also play a crucial role as a defence barrier to avoid the traslocation of toxic substances to the aerial parts (Olko et al., 2008). The presence of zincosomes in *P. tomentosa*, together with the compartimentalisation in the vesicles or the vacuoles observed in the root hairs (Fig. 5C), can therefore be interpreted as a strategy adopted by the tree to detoxify the cytosol from heavy metals (Martinoia et al., 2007; Kawachi et al., 2009) and to prevent the translocation of Zn to the entire plant.

Together with the translocation to the above-ground organs, the distribution and the accumulation of heavy metals in the different organs of a plant also represent key factors for a successful phytoextraction. Unfortunately, the zinc accumulation in *P. tomentosa* appeared to be mostly located in the root system. Moreover, the Zn content in the roots increased with the increasing Zn concentration of the substrate. This behaviour could be a consequence of Zn binding to negatively charged sites in the cell walls of the roots or as an improvement of Zn storage in the vacuole of the root cells, thus leading to a reduced Zn translocation to the shoots at higher Zn concentrations in the nutrient solution or in the soil (Pulford and Watson, 2003; Greger, 2004). However, a linear increase in the Zn accumulation was observed in the shoots, petioles and leaves when the plants were grown with low Zn concentrations (<2000 μM). By comparing the values of Zn measured in *P. tomentosa* with values

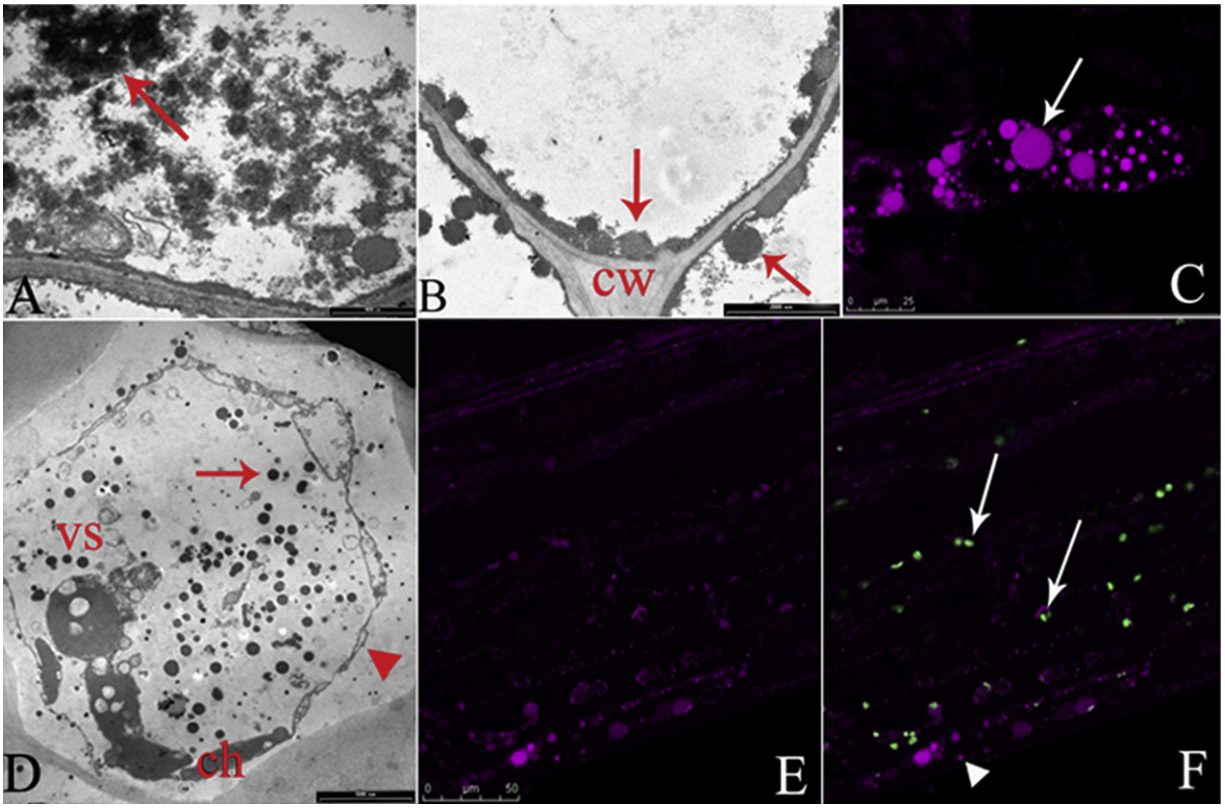

Fig. 5. Electron and confocal micrographs of the root and petiole cells of *P. tomentosa* plants. (A and B) TEM micrographs of the root cells of plants grown at 1000 μM Zn showing electron-dense material (arrow) in the form of clumps (A) or globules (B) in the vacuole. (C) Confocal micrograph of a root hair showing Zn compartmentalised in the megavesicles (white arrow). (D) TEM micrograph of a petiole cell showing the complete withdrawal of the plasma membrane from the cell wall (arrowhead), vesicles (*vs*) and electron-dense globules (red arrow). (E and F) Confocal micrograph of petiole cells showing Zn in the chloroplast lumen and envelope (white arrows) and several punctate structures containing Zn (white arrowhead). The emission of chlorophyll between 630 and 750 nm was detected to visualise the chloroplasts (F).

obtained in other tree species, we can see that *P. tomentosa* is able to store greater quantities of Zn than *Populus* (Di Baccio et al., 2003; Sebastiani et al., 2004), *Alnus incana*, *Betula pendula* and *Fraxinus excelsior* (Rosselli et al., 2003). In contrast, *Salix* species appear to be more efficient in storing Zn (Dos Santos Utmazian et al., 2007). Of course, the various conditions of growth and substrate found in the literature are often so different as to render the comparison very difficult.

ESEM has previously been successfully used to localise heavy metals in plant tissues (Fernando et al., 2006; Turnau et al., 2007; Solís-Domínguez et al., 2007) due to the opportunity to observe the sample under natural conditions (*i.e.*, without fixation, dehydration and coating). In this paper, we used ESEM and other microscopic techniques to investigate the localisation of Zn in *Paulownia* tissues. Although trichomes are normally considered to be one of the main sites for heavy metal accumulation in hyperaccumulators (see, for example, Zhao et al., 2000 on *Arabidopsis halleri*), no significant Zn content was found in the trichomes of *Paulownia* leaves using EDX microanalysis. To establish how *Paulownia* is able to compartmentalise the heavy metal, sections of the petioles were analysed. EDX microanalysis revealed a significant Zn accumulation in the cell walls of the collenchyma cells (Fig. 6B) and a strong deposition of exudates with high Zn content on the external surfaces of the petioles (Fig. 6C); this effective system utilised by *P. tomentosa* to exclude a portion of the large quantity of Zn absorbed has been previously observed by Sarret et al. (2006) in tobacco plants.

In conclusion, our results attempt to explain the ability of *P. tomentosa* plants to tolerate high levels of Zn through the use of

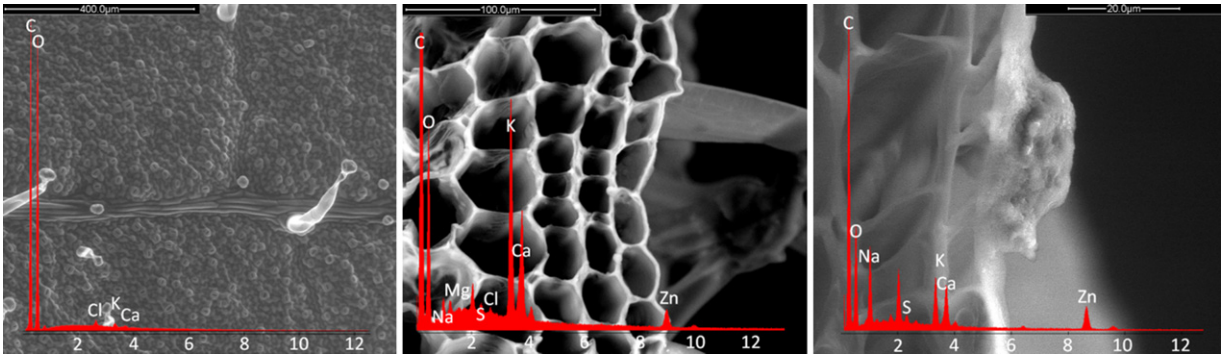

Fig. 6. ESEM micrographs and X-ray SEM microanalysis of (A) a trichome on the leaf surface of a plant grown at 1000 μM Zn; (B) collenchyma cells of a petiole section of a plant grown at 5000 μM Zn; (C) exudate on the external surface of a petiole in a plant grown at 5000 μM Zn.

sophisticated mechanisms that are able to capture the heavy metal in particular structures, such as the petiole cell walls and the vacuoles in the root hairs, or that are capable of extruding part of the Zn in exudates located on the surface of the petiole.

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