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Research article

Responses of *Hydrilla verticillata* (L.f.) Royle to zinc: In situ localization, subcellular distribution and physiological and ultrastructural modifications

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ABSTRACT

Hydrilla verticillata (L.f.) Royle exposed to 15–150 μM Zn for 7 days were analyzed with reference to the ultrastructural localization, subcellular distribution of metal and its influence on photosynthetic efficiency, malondialdehyde (MDA), adenosine triphosphate (ATP) and ultrastructure. Zn grains were found in the cell walls and within nuclei and chloroplasts using the autometallographic technique. Subcellular fractionation of Zn-containing tissues indicated 43–54% of the element was located in the cell wall fraction, followed by cell organelles (24–31%) and the soluble fraction (21–29%). A significant reduction in photosynthetic efficiency was observed in a concentration dependent manner, as indicated by the reduced efficiency of the PS II photochemical system (*Fv*/*Fm*). MDA content showed a sharp increase at all Zn concentrations, which indicated oxidative stress. Zn-exposed plants displayed a significant decrease in ATP content. Zn exposure also caused the chloroplasts and nuclei to disintegrate and the vacuolization of mitochondria, all of which suggested that Zn hastened plant senescence.

1. Introduction

As one of the essential micronutrients in plants, zinc (Zn) has multiple roles in numerous metabolic pathways at low concentrations. However, it is also a common pollutant in aquatic environments and is highly phytotoxic at higher concentrations. Accumulated data has indicated that excess Zn can strongly alter leaf morphology and ultrastructure [1,2] and profoundly affect a variety of physiological and biochemical processes, such as growth inhibition, reductions in photosynthesis and transpiration, nutrient imbalance, alteration of gene expression, and the disappearance or *de novo* synthesis of proteins [3–7]. Although Zn is not a redox-active metal, it has been shown that Zn toxicity can cause oxidative damage to major biomolecules (DNA, lipids, and proteins), as well as induce antioxidant defense mechanisms [8].

A number of studies have investigated Zn uptake, accumulation, distribution and detoxification in a wide range of hyperaccumulating or tolerant species. To counteract Zn toxicity, *Populus* × *euramericana* clone I-214 has adopted a number of defense/tolerance mechanisms involving complex structural, physiological and biochemical processes that can be attributed to both Zn excluders and accumulators [9]. One of the possible mechanisms used by *Avicennia marina* in response to excess Zn is to remove it via glandular excretion [10]. The cell walls of *Pohlia drummondii*, together with the plasma membrane, form a functional barrier that protects the cytoplasm from harmful doses of Zn [11]. The sequestration of Zn in the vacuoles of leaves plays a major role in the strong tolerance and hyperaccumulation of Zn in *Potentilla griffithii* [12]. Both Zn subcellular compartments and Zn-inducing proteins are all involved in the acclimation mechanism of *Phragmites australis* to Zn pollution [5]. Glutathione biosynthesis, increased levels of ascorbic acid and the induction of antioxidant enzyme activity may account for the higher Zn tolerance and uptake shown by a hyperaccumulating ecotype of *Sedum alfredii* [1]. However, the mechanisms involved are only partially understood [12] and there are very little toxicological data dealing with non-tolerant plants, including hydrophytes [7,10,13,14].

Hydrilla verticillata is a submerged macrophyte that is widely distributed across the world and has been shown to accumulate significant amounts of Zn [7,14] due to its fast growth rate and large biomass. Although there have been several studies on the physiological and morphogenic effects of Zn stress in *H. verticillata* [7,14,15], the precise cellular mechanisms responsible for Zn accumulation and

Abbreviations: ATP, adenosine triphosphate; DTT, dithioerythritol; FW, fresh weight; MDA, malondialdehyde; PS II, photosystem II; PVPP, polyvinylpolypyrrolidone; ROS, reactive oxygen species; TBA, thiobarbituric acid; TCA, trichloroacetic acid; TEM, transmission electron microscope.

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Table 1
Subcellular distribution of Zn in leaves of *H. verticillata*. Data are means ± S.D., $n = 3$. Different letters in the same column indicate a significant difference at the 5% level.

Zn concentration (μM)	Zn in subcellular fractions (mg kg^{-1} FW)			
	Cell wall (% of total)	Cell organelles (% of total)	Soluble fraction (% of total)	Total Zn content
Control	$121.4^{a} \pm 0.7$ (54)	$56.3^{a} \pm 1.9$ (25)	$48.7^{a} \pm 0.6$ (21)	$226.4^{a} \pm 3.2$ (100)
15	$254.5^{b} \pm 1.3$ (47)	$130.1^{b} \pm 1.6$ (24)	$158.2^{b} \pm 1.3$ (29)	$537.8^{b} \pm 4.2$ (100)
75	$474.6^{c} \pm 1.1$ (44)	$309.6^{c} \pm 3.6$ (28)	$304.9^{c} \pm 1.1$ (28)	$1089.1^{c} \pm 5.8$ (100)
150	$546.8^{d} \pm 2.3$ (43)	$390.1^{d} \pm 4.9$ (31)	$319.9^{d} \pm 2.4$ (26)	$1256.3^{d} \pm 9.6$ (100)

its consequent toxicity in this macrophyte remains unexplored. In this study, Zn subcellular distribution was evaluated using tissue fractionation and the ultrastructural localization of Zn was investigated by autometallography. To evaluate the Zn toxicity effects on *H. verticillata*, the changes in photosynthetic activity, lipid peroxidation, ATP and ultrastructure were also studied through a series of physiological and biochemical assays. The results should increase understanding of the main tissues and cellular targets involved in the accumulation/distribution of Zn and further elucidate the possible phytotoxic effects of Zn on this freshwater macrophyte.

2. Results

2.1. Subcellular distribution of Zn

Compared to the control, a significant increase of Zn content in cell wall, cell organelles and soluble fraction was detected as Zn concentration increased in the nutrient solution (Table 1). Meanwhile, the proportion of Zn in different subcellular fractions remained fairly constant for all treatments in leaves, namely, roughly 50% of Zn is in the cell wall and roughly the other 50% is equally distributed between organelles and soluble fraction.

2.2. Photosynthetic efficiency

In comparison to the control plants, Zn stress induced significant decreases in *Fv*/*Fm* ($P < 0.01$) as a function of Zn concentration (Fig. 1). The maximum reduction of 66% were seen at the highest Zn concentration, which indicated that severe damage to the PS II reaction centers had occurred due to Zn toxicity.

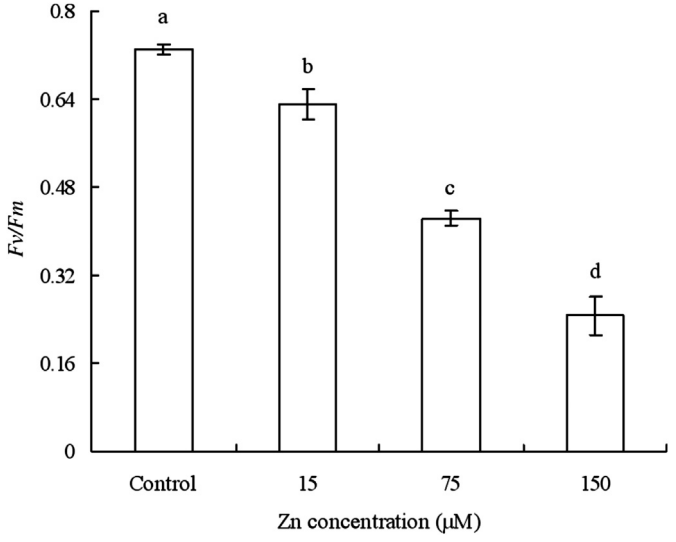

Fig. 1. *Fv*/*Fm* in leaves of *H. verticillata* exposed to different concentrations of Zn. Data are means ± S.D. ($n = 6$). Values designated over the bars in different letters are significantly different at $P < 0.05$ between means.

2.3. ATP content

After 7 days of growth, a significant loss in ATP content was observed as Zn concentrations increased (Fig. 2). A maximum of 105.84×10^{-10} M g^{-1} FW was recorded in the culture medium of the control plants and a minimum of 1.43×10^{-10} M g^{-1} FW was found in plants exposed to 150 μM Zn.

2.4. Lipid peroxidation

In order to assess the membrane damage caused by Zn, MDA content was measured in order to analyze lipid peroxidation. It can be seen from Fig. 3 that MDA increased gradually and was significantly positively correlated with the Zn concentration in the solution ($P < 0.05$). The maximum increase in MDA on day 7 at 150 μM was 40% higher than the control.

2.5. Leaf distribution of Zn at the cellular level

Zn distributions in plant tissues, after staining using the sulfide–silver method and examination by TEM, are seen as electron dense depositions [12,16,17]. In this study, under the control conditions, no dark deposits were found in the leaf tissues (Fig. 4A). In contrast, dark grains of various sizes were clearly observed in the leaf cells of *H. verticillata* when exposed to Zn and the cell walls contained densely packed particles (Fig. 4B–D). A number of small deposits were also seen in the nuclei (Fig. 4C), the chloroplasts (Fig. 4D) and in the cytoplasm (Fig. 4B–D).

2.6. Ultrastructural changes

The leaf cells of the control plants had a typical ultramorphology (Fig. 5A). Elliptical-shaped chloroplasts had orderly-arranged grana

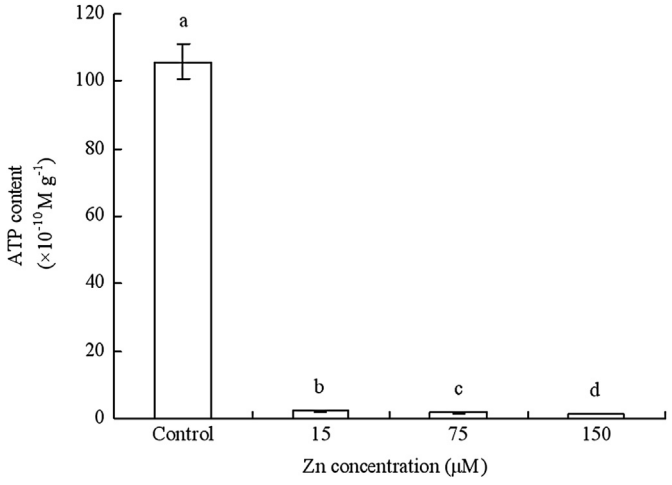

Fig. 2. ATP contents in leaves of *H. verticillata* in response to various levels of Zn stress. Data are means ± S.D. ($n = 3$), values designated over the bars in different letters are significantly different at $P < 0.05$ after one-way ANOVA.