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BRIEF COMMUNICATION

Evaluation of zinc accumulation potential of *Hydrilla verticillata*

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Abstract

Biofortification of foods with essential micronutrients and phytoremediation of the contaminated sites are the two sides of the same coin for metals like zinc. In the present study, Zn accumulation potential, growth and antioxidant status of *Hydrilla verticillata* (L.f.) Royle plants were studied upon supplementation of Zn (0 - 5 000 μM) for 2 and 7 d. At 5000 μM Zn, plants accumulated about 7.60 and 18.07 mg(Zn) g^{-1}(d.m.) after 2 and 7 d, respectively. Plants exposed to Zn concentrations up to 500 μM showed significantly increased contents of low molecular mass antioxidants and activities of antioxidant enzymes in comparison with controls. Only upon exposure of plants to 5 000 μM Zn, toxicity was observed after 7 d. Therefore, owing to their high Zn accumulation capacity, *Hydrilla* plants may be used both as a Zn source (*via* culturing in *ca*. 100 μM Zn supplemented nutrient medium) or as a phytoremediator.

Additional key words: antioxidants, food supplement, phytoremediation.

Biofortification of mineral micronutrients in food crops for the benefit of human nutrition and phytoremediation of metal/metalloid contaminated sites are the two sides of the same coin and represent two potential applications of our current knowledge of metal homeostasis and detoxification in plants. Both ideas have appeared during last few decades (Guerinot and Salt 2001, Palmgren *et al.* 2008, Zhao and McGrath 2009). Zinc is an essential element for both plants and animals (Marschner 1995) and an adequate supply of Zn is critical for normal cell function (Cakmak 2000). Zinc is found in most food, however, often in too low concentration. Further, a number of factors are known to affect the bioavailability of Zn, such as calcium decreases Zn bioavailability while sulfur-containing amino acids enhance its bioavailability (House *et al.* 1996). Identification of a suitable edible plant that may supply required quantity of bioavailable Zn may be a good solution to the problem in some world areas.

On the other hand, Zn is also a major industrial pollutant of the terrestrial and aquatic environment. Hence, there is a need to remediate the contaminated sites in a cost-effective manner for which the technique of phytoremediation may be a good option (Gatti 2008). The hyperaccumulation threshold for Zn is 10000 μg g^{-1}(d.m.) (Sarret *et al.* 2002). There are only a few known Zn hyperaccumulators like *Thlaspi caerulescens* (Brown *et al.* 1995) and *Sedum alfredii* (Yang *et al.* 2002). However, to the best of our knowledge, there are no reports of any aquatic plant showing Zn hyperaccumulation.

Hydrilla verticillata (L.f.) Royle, a fresh water weed plant, containing vital nutrients, amino acids and a other beneficial food compounds. This plant has also been demonstrated to accumulate significant amounts of various essential heavy metals, such as Fe, Ni and Cu (Srivastava *et al.* 2006) as well as toxic metals and metalloids (Pb, Hg, Cd and As; Gupta *et al.* 1998, Srivastava *et al.* 2007). With a view of using *Hydrilla* plants as a food source of Zn and also as a phytoreme-

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Abbreviations: APX - ascorbate peroxidase; Car - carotenoids; CAT - catalase; Chl - chlorophyll; GPX - guaiacol peroxidase; MDA - malondialdehyde; NP-SH - non-protein thiols; SOD - superoxide dismutase.
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diator, Zn accumulation potential, growth and antioxidant status of the plants was studied in the present study.

Plants (about 7 cm tip portion) were grown in 10 % Hoagland's solution (Hoagland and Arnon 1950) and acclimatized for 5 d in laboratory conditions (an irradiance of 115 μmol m^{-2} s^{-1}, a 14-h photoperiod, temperature of 25 ± 2 °C). Experiments were set up in triplicate and each replicate contained 10 plants of equal size (approximately 4 g fresh mass in total). Plants were exposed to different concentrations of Zn (100, 500, 1000 and 5000 μM; prepared using $ZnCl_2$; *Sigma*) supplemented in 10 % Hoagland's solution under the above-mentioned conditions for a period of 2 d or 7 d. Flasks containing 10 % Hoagland solution without Zn supplementation served as control (0.08 μM Zn). After harvesting, plants were washed with double-distilled water, blotted and used to determine various parameters.

For Zn estimation, all plant samples were oven dried at 80 °C, digested in $HClO_4$:HNO_3 (1:3 v/v) at 100°C and then diluted with double-distilled water. For desorbing the adsorbed Zn, one set of plant samples were kept in ice-cold 10 mM $CaCl_2$ for half an hour and then washed in double-distilled water. Zinc concentrations were determined on an atomic absorption spectrophotometer (*GBC Avanta Σ*, Victoria, Australia). Protein content was measured following the method of Lowry *et al.* (1951). The contents of chlorophylls and carotenoids were estimated following the method of Arnon (1949) and Duxbury and Yentsch (1956), respectively. Lipid peroxidation was determined by estimation of the malondialdehyde (MDA) content according to Heath and Packer (1968). The homogenization and assay of the activities of superoxide dismutase (SOD; EC 1.15.1.1), guaiacol peroxidase (GPX; EC 1.11.1.7), ascorbate peroxidase (APX; EC 1.11.1.11) and catalase (CAT; EC 1.11.1.6) were performed following the methods of Beauchamp and Fridovich (1971), Hemeda and Klein (1990), Nakano and Asada (1981) and Aebi (1974), respectively, as described previously (Srivastava *et al.* 2006). The content of total non-protein thiols (NP-SH) and cysteine were measured following the method of Ellman (1959) and Gaitonde (1967).

The experiments were performed in a randomized block design. All data were subjected to an analysis of variance (*ANOVA*). Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments (Gomez and Gomez 1984).

The accumulation of Zn by the plants was found to be a concentration and duration dependent so the maximum content of total Zn was achieved after 7 d at 5000 μM Zn (Table 1). Out of the total Zn, a significant proportion was absorbed (about 70 % at each concentration) while the rest (*ca*. 30 %) was adsorbed on the surface as revealed by desorption of adsorbed Zn using $CaCl_2$. Thus plants showed hyperaccumulation of Zn (1.8 % Zn of their d.m.; Sarret *et al.* 2002). Significant Zn accumulation has also been reported by other aquatic plants like *Potamogeton pectinatus* (Tripathi *et al.* 2003) and *Ceratophyllum demersum* (Umebese and Motajo 2008). However, to the best of our knowledge, this is the first report of an aquatic weed and edible plant showing Zn hyperaccumulation. This plant, therefore, may find two-sided application: firstly as a Zn source and secondly as a phytoremediator. Despite such a high Zn accumulation (Chaney 1989), no toxicity symptoms as chlorosis or necrosis of the plant tissues (except some decline in various parameters) were observed in the present study. Hence, plants might be efficiently employing one or two of the various known mechanisms of Zn homeostasis and detoxification, *e.g.*, binding Zn with organic acids and phosphates (Sarret *et al.* 2002), polypeptides like glutathione (Sun *et al.* 2005) and phytochelatins (Tsuji *et al.* 2002) or through transport to vacuoles (Kobae *et al.* 2004).

Plant growth and the contents of total soluble proteins and photosynthetic pigments were slightly enhanced upon Zn supplementation (up to 500 μM) as compared with control treatment (Table 1). Thus, being an essential micronutrient, Zn enhanced the growth of plants probably through an increase in photosynthesis and protein metabolism as reported previously (*e.g.* Omar 2002). Positive effects of Zn application on photosynthesis and growth were also recorded in maize plants under sufficient water supply (Wang *et al.* 2009). Similarly, in Cd-treated regenerants of *Bacopa monniera*, a decline in the adverse effects of Cd on photosynthesis and growth was observed upon Zn supplementation (Ali *et al.* 2000). However, at maximum concentration of 5000 μM Zn, biomass of the plants and the contents of pigments and proteins declined significantly particularly after 7 d. This toxic effect may be attributed to disturbances to the nutrient composition of the plant (Stoyanova and Doncheva 2002).

Although Zn is not a redox-active metal, it has been shown that Zn toxicity can lead to oxidative damage as well as induce antioxidant defense mechanisms (Prasad *et al.* 1999). In the present study, a significant increase in oxidative stress, measured as lipid peroxidation of membranes, was observed only at 7 d beyond 1000 μM Zn (Table 1). The activities of SOD and APX exhibited a concentration and duration dependent increase (Table 1). However, the activities of GPX and CAT (Table 1) increased only at lower concentrations. Thus, a positive response of antioxidant enzymes presumably allowed plants to effectively control oxidative stress. Similarly, in Zn-hyperaccumulating ecotype of *Sedum alfredii*, the activities of SOD, CAT, GPX and APX were enhanced at Zn concentration of 500 μM (Jin *et al.* 2008).

In addition, the content of low molecular mass antioxidant cysteine also increased significantly up to 1000 μM. The bioavailability of Zn is known to be enhanced by the presence of high content of sulfur-containing amino acids (House *et al.* 1996). Therefore, a significant increase in the content of cysteine might also lead to an increased bioavailability of Zn. Total NP-SH also showed significant increase at all concentrations in comparison to

Table 1. Zinc accumulation by *Hydrilla verticillata* plants exposed to different concentrations of zinc for 2 d and 7 d and effects on biomass, total soluble proteins, photosynthetic pigments, antioxidant enzymes, cysteine and non-protein thiols. All values are mean of triplicates ± SD. Different letters indicate significantly different values at a particular duration (DMRT, $P \leq 0.05$). Zinc accumulation was measured without and with $CaCl_2$ washing.

Parameter	Time	Control	100 μM Zn	500 μM Zn	1000 μM Zn	5000 μM Zn
Zinc accumulation	2 d	0.17±0.02^{d}	0.80±0.03^{d}	2.28±0.34^{c}	6.12±0.60^{b}	11.33±1.57^{a}
[mg g^{-1}(d.m.)]	7 d	0.39±0.02^{d}	2.09±0.20^{d}	6.82±0.66^{c}	16.55±1.60^{b}	25.61±2.50^{a}
Zinc accumulation + $CaCl_2$	2 d	0.13±0.01^{d}	0.57±0.08^{d}	1.44±0.21^{c}	4.53±0.53^{b}	7.60±1.10^{a}
[mg g^{-1}(d.m.)]	7 d	0.26±0.04^{d}	1.49±0.19^{d}	4.57±0.53^{c}	12.55±1.68^{b}	18.07±2.68^{a}
Biomass	2 d	4.36±0.50^{a}	4.41±0.67^{a}	4.46±0.88^{a}	4.52±0.87^{a}	4.32±0.55^{a}
[g (f.m.)]	7 d	5.13±0.34^{a}	5.19±0.87^{a}	4.73±0.46^{a}	4.36±0.22^{a}	4.02±0.19^{b}
Total soluble protein	2 d	6.51±1.20^{d}	8.54±0.87^{c}	9.47±1.23^{b}	10.31±1.99^{a}	5.01±0.99^{a}
[mg g^{-1}(f.m.)]	7 d	6.61±0.89^{c}	9.57±1.31^{a}	9.27±1.55^{a}	7.65±1.92^{b}	2.87±0.56^{d}
Chlorophyll *a*	2 d	0.74±0.05^{a}	0.83±0.03^{a}	0.81±0.06^{a}	0.68±0.11^{a}	0.61±0.10^{a}
[mg g^{-1}(f.m.)]	7 d	0.74±0.09^{a}	0.83±0.13^{a}	0.76±0.08^{a}	0.60±0.10^{a}	0.52±0.05^{b}
Chlorophyll *b*	2 d	0.58±0.07^{a}	0.59±0.08^{a}	0.61±0.12^{a}	0.59±0.15^{a}	0.51±0.05^{a}
[mg g^{-1}(f.m.)]	7 d	0.57±0.04^{a}	0.60±0.09^{a}	0.54±0.03^{a}	0.48±0.10^{a}	0.33±0.09^{b}
Carotenoid	2 d	0.44±0.02^{a}	0.46±0.07^{a}	0.46±0.03^{a}	0.44±0.05^{a}	0.38±0.08^{a}
[mg g^{-1}(f.m.)]	7 d	0.44±0.07^{a}	0.47±0.10^{a}	0.41±0.09^{a}	0.38±0.03^{a}	0.29±0.04^{b}
MDA	2 d	4.18±0.45^{a}	4.38±0.88^{a}	4.59±0.95^{a}	4.75±0.24^{a}	5.01±0.51^{a}
[μmol g^{-1}(f.m.)]	7 d	4.21±0.54^{b}	4.60±0.22^{b}	5.04±0.14^{b}	6.00±0.74^{a}	7.00±0.92^{a}
SOD	2 d	360.00±51.0^{b}	363.00±82.0^{b}	375.00±34.0^{b}	386.00±60.0^{b}	546.00±77.0^{a}
[mkat mg^{-1}(protein)]	7 d	363.00±50.0^{c}	391.00±34.0^{c}	426.00±51.0^{c}	629.00±85.0^{b}	1110.00±102 ^{a}
APX	2 d	18.00±1.66^{d}	21.00±1.87^{d}	26.00±4.08^{c}	32.00±4.13^{b}	53.00±7.12^{a}
[mkat mg^{-1}(protein)]	7 d	19.00±1.14^{d}	23.00±2.38^{d}	29.00±3.57^{c}	40.00±3.74^{b}	92.00±13.2^{a}
GPX	2 d	147.00±20.0^{b}	174.00±16.0^{a}	165.00±17.0^{a}	147.00±14.0^{b}	90.00±9.00^{c}
[mkat mg^{-1}(protein)]	7 d	153.00±25.0^{b}	173.00±34.0^{a}	142.00±16.0^{b}	123.00±14.0^{c}	45.00±3.00^{d}
CAT	2 d	163.00±17.0^{c}	287.00±35.0^{a}	209.00±20.0^{b}	199.00±16.0^{b}	144.00±17.0^{c}
[mkat mg^{-1}(protein)]	7 d	164.00±13.0^{b}	272.00±34.0^{a}	178.00±17.0^{b}	158.00±20.0^{b}	74.00±14.0^{c}
Cysteine	2 d	76.00±9.00^{c}	80.00±7.00^{c}	98.00±13.0^{b}	113.00±21.0^{a}	74.00±11.0^{c}
[nmol g^{-1}(f.m.)]	7 d	76.00±15.0^{b}	86.00±11.0^{b}	119.00±12.0^{a}	120.00±21.0^{a}	56.00±5.00^{c}
Non-protein thiols	2 d	0.64±0.07^{c}	2.00±0.12^{b}	3.42±0.56^{a}	3.94±0.61^{a}	1.83±0.22^{b}
[μmol g^{-1}(f.m.)]	7 d	0.65±0.11^{c}	2.50±0.23^{a}	2.13±0.42^{a}	1.85±0.25^{a}	0.95±0.11^{b}

control (Table 1). Total NP-SH comprise of mainly glutathione (GSH) and the metal binding ligands, phytochelatins (PCs), as constituent thiols. In *Sedum alfredii,* significant increase in the content of GSH was suggested to be involved in conferring Zn hyper-accumulation phenotype (Sun *et al.* 2005). It is important to note that GSH also plays an important role as an antioxidant in the cells (Srivastava *et al.* 2006). Thus, the significant increase in the NP-SH content might be involved in Zn tolerance, homeostasis and detoxification in *Hydrilla* plants.

In conclusion, the present study demonstrated that *Hydrilla* plants accumulated significant amounts of Zn even upon exposure to low Zn concentrations (*ca*. 100 μM) and showed enhanced content of photosynthetic pigments, proteins and various antioxidants. Hence, these plants may be used as a source of Zn. Further, upon increasing the exposure concentration, Zn accumulation increased, reaching well beyond the hyperaccumulation limit, without showing any visible symptoms of toxicity. Hence, these plants may also find application in the phytoremediation of Zn-contaminated aquatic habitats. These two applications of the plants, however, need to be carefully controlled to avoid any toxicity to humans due to consumption of plants having very high load of Zn.

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