Plant Physiology and Biochemistry 48 (2010) 873–878

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Antioxidative responses of *Elodea nuttallii* (Planch.) H. St. John to short-term iron exposure

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ARTICLE INFO

Article history:
Received 3 September 2009
Accepted 13 August 2010
Available online 20 August 2010

Keywords:
Iron
Physiological characteristics
Antioxidative response
Elodea nuttallii (Planch.) H. St. John

ABSTRACT

Antioxidative responses of *Elodea nuttallii* (Planch.) H. St. John to short-term iron exposure were investigated in the study. Results showed that iron accumulation in *E. nuttallii* was concentration dependent. Growth of *E. nuttallii* was promoted by low iron concentration (1–10 mg L^{-1} [Fe^{3+}]), but growth inhibition was observed when iron concentration beyond 10 mg L^{-1}. The synthesis of protein and pigments increased within 1–10 mg L^{-1} [Fe^{3+}] range. The activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione-S-transferase (GST) were up to maximal values at 10 mg L^{-1} [Fe^{3+}]. High iron concentration inhibited the synthesis of protein and pigments as well as activities of antioxidative enzymes, and accelerated degradation of pigment and production of ROS. Low iron concentration had no significant influences on PSII maximal quantum yield, activity of PSII and relative electron transport rate though PSII. Malondialdehyde (MDA) and proline concentrations were highest at 100 and 1 mg L^{-1} [Fe^{3+}], respectively.

1. Introduction

Heavy metal contamination in aquatic ecosystem is a serious problem due to their toxicology and accumulation behaviour. Heavy metals, such as Fe, Cu, Cd, Cr and Hg, are released into environment by the application of pesticides in agriculture, discharge of untreated industrial wastes, and mining operations [16,40].

Iron is an essential trace element for plants, being involved in chlorophyll biosynthesis, in many components of photosynthetic and electron transport systems, and in nitrate assimilation as an enzyme co-factor [7,12]. Excess iron, however, can result in toxicity, especially in altering chromatin structure, protein synthesis, enzyme activity, photosynthesis and respiration [8,28]. Furthermore, excess iron can stimulate the formation of free radials and reactive oxygen species (ROS) in plants [32,35].

To minimize functional and structural damages, plants have developed different mechanisms enabling them to counteract negative effects caused by metals abundance in their tissue [18]. Among them, antioxidative defense systems, including antioxidative enzymes and antioxidative compounds, are very efficient to scavenge ROS in plants.

Use of aquatic plants to remediate the contaminated aquatic environment is of considerable interest as a branch of the exciting low-cost and eco-friendly technology, phytoremediation [37]. *Elodea nuttallii* (Planch.) H. St. John is a widely used plant resistant to heavy metal, as it shows characteristics of fast growth and strong accumulation. The objective of the present research was to study the physiological and antioxidative responses of *E. nuttallii* to short-term iron exposure, and provide useful information for remediation of iron contaminated aquatic environments.

2. Materials and methods

2.1. Plant material and treatment conditions

Plants of *E. nuttallii* (Planch.) H. St. John were obtained from an uncontaminated pond at Wuhan Botanical Garden, the Chinese Academy of Sciences, China. Before metal treatment, plants were acclimatized for 5 d in laboratory conditions (115 μmol $m^{-2}s^{-1}$ light with 16 h photoperiod at 25 ± 1 °C) in 10% Hoagland's solution [13,37]. At the end of this period, uniform and healthy plants were

Abbreviations: ROS, reactive oxygen species; DW, dry weight; FW, fresh weight; Chl a, chlorophyll a; Chl b, chlorophyll b; Car, carotenoid; PEA, plant efficiency analyzer; PSII, photosystem II; Fv/Fm, maximum photochemical efficiency of PSII; Fv/Fo, the activity of PSII; ETR, electron transport rate; Fm/Fo, ETR through PSII; SOD, superoxide dismutase; NBT, nitrobluetetrazolium; CAT, catalase; POD, peroxidase; GST, glutathione-S-transferase; CDNB, 1-chloro-2,4-dinitrobenzene; MDA, malondialdehyde.

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doi:10.1016/j.plaphy.2010.08.006

collected and put into 10% Hoagland's solution without iron nutrient. Then iron was added to the containers in the form of $FeCl_3 \cdot 6H_2O$ in appropriate quantities to give the following treatments: 0.1, 1, 10, 100, 500 and 1000 mg L^{-1} [Fe^{3+}]. 0.1 mg L^{-1} iron solution was designed as control because it was commonly environmental concentration in some lakes (*e.g.* Lake Dianchi, China) [40]. After 36 h exposure, plants were harvested, washed with double distilled water, blotted and used for the study of various parameters. All chemicals were purchased from Sigma, unless stated otherwise.

2.2. Iron accumulation

After 36 h exposure to iron solution, harvested plants were washed thoroughly with demineralized water, blotted and oven dried at 80 °C for 3 d. The resulting material was placed in a desiccator and cooled to room temperature before being weighed. A subsample of known weight of dried material was then digested in 5 mL of 30% HNO_3 at 90 °C for a minimum of 8 h. The concentration of iron in the diluted digests was determined by atomic absorption spectrophotometer (AA-6800, Shimadzu Corporation, Japan) [4].

2.3. Plant growth

The growth parameter was represented as protein concentration which was estimated following the method of Bradford [6] using serum albumin as standard protein.

2.4. Photosynthetic pigment assay

Photosynthetic pigments were extracted in 95% ethanol in dark for 24 h. Afterwards the sample was centrifuged for 10 min at 8000×*g*. The supernatant was collected and determined spectrophotometrically at 665, 649 and 470 nm (Ultrospec 3000, Pharmacia Biotech, England). The contents of chlorophylls and carotenoid were calculated using the equations given by Lichtenthaler and Buschmann [21].

2.5. Chlorophyll fluorescence measurements

Leaf chlorophyll fluorescence was measured using a Plant Efficiency Analyzer (PEA, Hansatech Instruments Ltd., England), by which the maximum photochemical efficiency of PSII (Fv/Fm), the activity of PSII (Fv/Fo) and electron transport rate (ETR) through PSII (Fm/Fo) were obtained [39].

2.6. Assay of antioxidant enzymes

The activity of SOD (EC 1.15.1.1) was assayed by inhibiting the photochemical reduction of nitrobluetetrazolium (NBT) with the method of Beauchamp and Fridovich [5]. The activity of SOD was expressed as units mg^{-1} FW. One unit of activity was the amount of protein required to inhibit 50% initial reduction of NBT under light.

The activity of CAT (EC 1.11.1.6) was determined by measuring the consumption of H_2O_2 at 240 nm [31]. Enzyme activity was expressed as units of CAT activity per milligram of fresh weight. One unit CAT activity was defined as an absorbance change of 0.01 units per minute at 240 nm (SpectraMax M2, Molecular Devices, USA).

The activity of POD (EC 1.11.1.7) was determined by using the guaiacol method [22]. Enzyme activity was expressed as units of POD activity per milligram of fresh weight. One unit of POD activity was defined as an absorbance change of 0.01 units per minute at 470 nm (SpectraMax M2, Molecular Devices, USA).

The activity of thiol antioxidant-glutathione-S-transferase (GST, EC 2.5.1.18) was measured by the method of Mannervik and Guthenberg [25] using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The activity of GST was expressed as units mg^{-1} FW. One unit of activity was defined as an absorbance change of 0.01 units per minute at 340 nm (SpectraMax M2, Molecular Devices, USA).

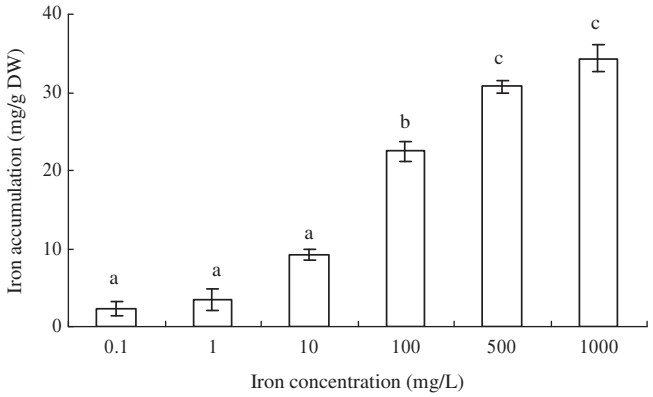

Fig. 1. Iron accumulation in *E. nuttallii* after 36 h of treatment. All the values are mean of triplicates ± SD. One-way ANOVA followed by Duncan's multiple range test was used to determined the significance of difference between treatments ($P < 0.05$).

2.7. Lipid peroxidation and proline

Lipid peroxidation was determined by estimation of malondialdehyde (MDA) concentration. Plant material was homogenized with 3 ml of 0.5% thiobarbituric acid in 20% trichloroacetic acid (w/v). The homogenate was incubated at 100 °C for 30 min and reaction was stopped in ice or cooled water. The samples were centrifuged at 10,000×*g* for 10 min and absorbance was measured spectrophotometrically at 450, 532 and 600 nm (Ultrospec 3000, Pharmacia Biotech, England). The MDA concentration was determined by the following formula: C_{MDA} (μmol L^{-1}) = $6.45(A_{532} - A_{600}) - 0.56A_{450}$, from which the absolute concentration (μmol g^{-1} FW) of MDA was calculated [19].

Proline concentration was measured according to the method described by Bates et al. [3]. Plant material was homogenized with 10 ml of 3% (v/v) sulfosalicylic acid. The homogenate was centrifuged at 800×*g* for 15 min. Free proline present in the supernatant was treated with acid-ninhydrin at 80 °C for 1 h. The reaction was terminated in an ice bath and the colored complex was extracted in toluene. Its absorbance was measured spectrophotometrically at 520 nm (Ultrospec 3000, Pharmacia Biotech, England). The standard curve for proline was prepared by dissolving proline in 3% (v/v) sulfosalicylic acid covering the concentration range 0.1–5.0 μg mL^{-1}.

Table 1
Visual appearance of *E. nuttallii* and culture medium after 36 h iron exposure.

Iron concentration (mg L^{-1})	Symptoms	Culture medium
0.1	No symptoms, leaves were green	Colorless, clear
1	No symptoms, leaves were green	Colorless, clear
10	No obvious symptoms, leaves were green	Slight orange, a small amount of precipitation
100	Chlorosis occurred	Orange, a large amount of precipitation
500	Leaves were dark brown and necrotic	Orange, no precipitation
1000	Leaves were dark brown and necrotic	Deep orange, no precipitation