Ecotoxicology and Environmental Safety 165 (2018) 654–661

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Morphophysiological responses and tolerance mechanisms of *Xanthium strumarium* to manganese stress

Gao Pan[a,b,1], Wensheng Liu[a,1], Heping Zhang[a,*], Peng Liu[a,b]

[a] College of Life Science and Technology, Central South University of Forestry and Technology, Changsha 410004, PR China
[b] National Engineering Laboratory for Applied Technology of Forestry & Ecology in South China, Changsha 410004, PR China

ARTICLE INFO

Keywords:
Xanthium strumarium
Phytoremediation
Morphological traits
Antioxidants
Manganese stress
Photosynthetic rate

ABSTRACT

Effective phytoremediation of manganese (Mn) requires the careful selection of a species that has a relatively high manganese tolerance. Exploring the physiological mechanisms related to Mn stress responses is crucial for identifying and employing species for Mn phytoremediation. *Xanthium strumarium* is a species that can accumulate high levels of Mn, thus it is a candidate species for Mn-phytoremediation. To reveal the tolerance mechanisms of this species to manage Mn stress, the morphological, physiological, and biochemical responses of seedlings grown in water cultures under six different Mn concentrations were analyzed. The results showed that *X. strumarium* can accumulate high levels of Mn, even as plant growth was inhibited by rising Mn concentrations. Malondialdehyde (MDA) content increased and catalase (CAT) activity decreased along with the increased Mn concentrations, while soluble protein and proline content, as well as the superoxide dismutase (SOD) and peroxidase (POD) enzymes, all increased initially and then declined. The highest value of POD, SOD, soluble protein and proline all occurred at 5000 μM of Mn stress, which means that *X. strumarium* can adapt to low concentration of Mn stress. The net photosynthetic rate (*Pn*), stomatal conductance (*Gs*), intercellular CO_2 concentration (*Ci*) and transpiration rate (*Tr*) decreased, and the stomatal limitation (*Ls*) increased in response to Mn stress. Furthermore, water use efficiency (WUE) and intrinsic water use efficiency (WUEi) increased first under low concentration of Mn, and then reduced as the concentration of Mn increased. The maximum quantum efficiency of PSII photochemistry (Fv/Fm), efficiency of excitation capture by open PSII reaction centers (Fv′/Fm′), electron transport rate (ETR) declined as Mn concentration increased. In conclusion, the above results showed that *X. strumarium* can be effectively used for phytoremediation of Mn-contaminated soils.

1. Introduction

Manganese (Mn) is a trace element that is essential for plant growth and development (Doncheva et al., 2009). Although a low concentration of Mn is a basic requirement for plant growth, an excess of Mn may inhibit plant growth and development, and even can harm human beings and livestock as it moves up the food chain. Two types of habitats are more likely to have soil with high concentrations of Mn, including those near Mn mines or tailing ponds (Yang et al., 2008) and those with especially acidic soil (i.e. pH < 5.5), which can be found throughout the tropics and subtropics (Foy, 1984; Huang et al., 2016). Some reports had estimated that approximately 40% of arable soils are acidic (Von Uexkull and Mutert, 1995), and that about 1500 t of Mn are released into the environment every year, much of which may enter the soil in the form of soluble Mn (Liang et al., 2011), and thereby cause large areas to have a toxic overabundance of Mn. As of the present, Mn toxicity has become one of the predominant growth-limiting factors for plants in acidic soils (Mou et al., 2011). In addition, the areas in which tailings from Mn mines accumulate to toxic levels have increased rapidly. Therefore, controlling Mn soil pollution has become a more urgent problem over time.

Of all pollution control measures used to deal with metal contamination of the soil, phytoremediation has emerged as a promising, cost-effective, and environmentally friendly technology that has notable advantages, including ecological safety, reliability, adaptability, and positive aesthetic qualities (Pulford and Wasson, 2003). The first step in the phytoremediation process requires selecting hyperaccumulator or tolerant plant species that possess a high capacity to extract metals from contaminated soil and translocate them to the affected area (Fernando et al., 2009). Although some preliminary work

* Corresponding author.
E-mail address: hepzhang@sina.com (H. Zhang).
[1] Both authors contributed equally to this work.

https://doi.org/10.1016/j.ecoenv.2018.08.107
Received 12 March 2018; Received in revised form 23 August 2018; Accepted 30 August 2018

regarding selecting tolerant plant species as candidates for Mn-phytoremediation has been performed (Xue et al., 2004; Yang et al., 2008; Liu et al., 2010, 2015), the selected suitable species remains limited. It is also important to explore the physiological and biochemical mechanisms of phytoremediation, while much less is known about the mechanism in species suitable for Mn-phytoremediation.

In general, excess of Mn has adverse effects on plants both early in development and at crucial periods during the plant's life history, and an excess may influence the morphology, physiology, and cellular biochemistry of plants. For example, Mn stress influences plant growth, alterations in biomass and root system architecture, and retardation of plant reproduction (Ducic et al., 2006).

Mn stress also interferes with metabolic processes and thereby leads to cellular energy deficiency, which may induce oxidative stress (Boojar and Goodarzi, 2008) and as a consequence the formation of reactive oxygen species (ROS). ROS include singlet oxygen ($^1O_2$), the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH·); all are highly toxic and can lead to lipid peroxidation, protein oxidation, and nucleic acid damage. Chemical damage from ROS exposure can in turn ultimately result in the death of plant cells (Gill and Tuteja, 2010). To prevent damage from ROS exposure, plants possess antioxidant defense mechanisms that scavenge for ROS, such as accumulation of low-molecular weight antioxidants, and thereby alleviate the destructive potential of oxidation. Superoxide dismutases (SODs), peroxidases (PODs) and catalases (CATs) are the most important antioxidative defense enzymes.

Mn participates in formation of the structure of photosynthetic proteins and enzymes. Besides, it affects the water-splitting system of photosystem II (PSII). Suppression of photosynthesis under the stress of excess of Mn is generally due to the limitation of stomatal conductance, the absorption of carbon dioxide (CO_2), the creation of water imbalances, diminution in chlorophyll contents, and weakness of photosynthetic capacity which, as a result, stunts plant growth (Hauck et al., 2003; Lambers et al., 2008; Koyro et al., 2013). In addition, the essential processes of energy absorption, utilization, and dissipation are more susceptible to Mn stress, and a lower rate of photosynthetic electron transport can result in the over-reduction of reaction centers as well as inhibition or damage in PSII (Maxwell and Johnson, 2000).

Xanthium strumarium (synonym: *Xanthium sibiricum* Patrin ex Widder) is an annual herb belonging to Asteraceae. It is an important candidate species for phytoremediation of Mn-contaminated soils, since it grows rapidly, accumulates substantial biomass, covers large areas, and persists in a wide range of environments (Pan et al., 2017). *X. strumarium* can grow in Mn mine area and normally set flowers and seeds. Furthermore, this species is an important herb in Traditional Chinese Medicine (TCM), and therefore is suitable for both ecological and economic utilization. To date, several studies had been performed using this species, including those assessing its chemical constituents and mechanisms of pharmacological action (Bui et al., 2012; Jiang et al., 2017), while data regarding its potential use in heavy metal phytoremediation is scarce. In this study, we examined the response of *X. strumarium* to Mn stress, and explored the intracellular mechanisms of physiological and biochemical processes that may be affected by Mn accumulation. To do so, we (1) determined the effect of different concentrations of Mn metal ions on the growth of *X. strumarium*; (2) calculated the Mn concentration after translocation of *X. strumarium*; (3) investigated the physiological and biochemical responses of *X. strumarium* to Mn stress, including antioxidant enzyme activity, and the concentrations of soluble protein, proline, malondialdehyde (MDA); and (4) evaluated the extent to which the impacts of Mn stress on photosynthetic characteristics and chlorophyll fluorescence. The results of the present work may assist in the employment of this plant for phytoremediation.

2. Materials and methods

2.1. Pot and hydroponic experiments

Seeds of *X. strumarium* were picked from more than 100 randomly selected individual plants at a sample plot in the Xiang River scenic belt of Changsha, Hunan, China (28°15′ N, 112°94′ E). After kept in the air temperature for 35 days, healthy and plump seeds were selected and sown in humid sand dishes, which were placed in an artificial climate incubator with a light (150 μmol/m^2 s)/dark photoperiod of 14/10 h, a day/night temperature of 25 °C/20 °C, and a relative humidity of 75–80%. After 40 days, healthy seedlings were selected and transplanted to pots (20 cm in diameter and 14 cm in height) filled with a 3-kg sand-perlite mixture (1:1), and were placed in a glasshouse with a temperature range of 20–30 °C, and a relative humidity range of 45–85%. Then the sand-perlite mixture of each pot was treated by Hoagland's nutrient solution and incubated for two months. Pots were watered with Hoagland's nutrient solution as necessary. The Hoagland's nutrient solution contained (in mg/L) 607 K_2SO_4, 115 $NH_4H_2PO_4$, 493 $MgSO_4$, 20 $C_{10}H_{12}FeN_2NaO_8{\cdot}3H_2O$, 15 $FeSO_4{\cdot}7H_2O$, 2.86 H_3BO_3, 4.5 $Na_2B_4O_7{\cdot}10H_2O$, 2.13 $MnSO_4$, 0.05 $CuSO_4$, 0.22 $ZnSO_4$, and 0.02 $(NH_4)_2SO_4$. Solution pH was adjusted to 5.5 ± 0.2 with NaOH as required. After 40 days of growth, healthy and uniform seedlings were selected and transplanted to solution culture containers (71 cm in length, 46 cm in width, and 18 cm in height). Containers were placed in the same glasshouse as pots. Each container contained 25 L of Hoagland's nutrient solution and was covered with a white foam plastic plate (65 cm in length, 40 cm in width, and 2 cm in height). Seedlings were grown in Hoagland's nutrient solution for a week before Mn treatment. The Mn treatments were assigned as follows: Hoagland's nutrient solution containing 0 (control), 1000, 5000, 10,000, 15,000 and 20,000 μM Mn supplied as $MnCl_2{\cdot}4H_2O$. Three hundred and seventy-five seedlings (fifteen containers) of *X. strumarium* were treated with Mn^{2+} solution and seventy-five seedlings (three containers) of *X. strumarium* were treated with Hoagland's nutrient solution only. Each treatment had three replicates (three containers, 75 seedlings). The solution in each container was replaced every three days. After four weeks, plant materials were obtained for photosynthesis assays and morphological measurements, and were then harvested for element content analysis.

2.2. Morphological measurements

After cultivation under different Mn concentrations for 28 days, plant height was measured by Vernier caliper. The fresh weights of leaves, stems, and roots were determined using an electronic balance, and were then dried in a vacuum oven at 105 °C for 30 min and at 70 °C for 72 h. Then the dry weights were determined using an electronic balance. The root images of *X. strumarium* were obtained by Image Scan using an EPSON Expression 11000XL scanner. Total length, surface area, volume, and average root diameter of individual plants were measured and analyzed by using the WinRHIZO image analysis system (2013e, Regent Instruments Canada Inc.).

2.3. Assays of antioxidant enzyme activity

Superoxide dismutase (SOD) activity analysis was carried out as Gao (2006) by using the nitro blue tetrazolium (NBT) method. A 0.5 g fresh leaf sample was homogenized and then was centrifuged. The absorbance of the reaction mixture was recorded at a wavelength of 560 nm using a UV/visible spectrophotometer (UV-5100B, METASH, China) to calculate the value. Peroxidase (POD) and catalase (CAT) activity were also assayed by the method described in Gao (2006). The absorbance of the reaction mixture was recorded at a wavelength of 470 nm and 240 nm using a UV/visible spectrophotometer.