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The mutual restraint effect between the expansion of *Alternanthera philoxeroides* (Mart.) Griseb and cadmium mobility in aquatic environment

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ABSTRACT

Alternanthera philoxeroides (Mart.) Griseb is one of the most malignant weeds in its invision habitats. While in the cadmium-contaminated aquatic environment, does *A. philoxeroides* possess good tolerance and adaptability? To demonstrate the effects of cadmium on *A. philoxeroides* in the polluted water bodies, a hydroponic stress experiment was conducted over a gradient of Cd concentrations (0, 2.5 and 5 mg/l) in triplicate. The seedlings were cultured in a greenhouse and harvested on days 0, 10, 20, 30 and 40, respectively. The results showed the effects of mutual restraint between Cd and *A. philoxeroides*. The *A. philoxeroides* seedlings were enriched with large amounts of Cd, and the toxicity of Cd inhibited the rapid growth of *A. philoxeroides* and induced the rapid degradation of chlorophylls in its tissues. Furthermore, the use of iron plaque effectively immobilized Cd of 1123–2883 mg/kg·DW on the root surface, thus it decreased the transferability of Cd in the aquatic environment. Due to its extensive adaptability, good Cd tolerance and the immobilization of Cd predominantly in the roots (the highest Cd concentration enriched was 7588.65 ± 628.90 mg/kg·DW in roots). *A. philoxeroides* effectively restrained the translocation of Cd and partitioned Cd in the roots within water bodies.

Capsule: The antagonistic effect exists between the invasion of *A. philoxeroides* and cadmium mobility in aquatic environments.

1. Introduction

Heavy metal contamination is a major problem in aquatic ecosystems within human-dominated regions (William, 2007). In hydrophytes, heavy metals induce oxidative stress and inhibit photosynthesis and growth, leading to cell membrane alteration and malondialdehyde (MDA) content increase via lipoperoxidation (Ding et al., 2007; Krayem et al., 2016). Heavy metals are enriched from primary producers to consumers, and organisms achieve high Cd levels in vivo through food chains or food webs. As a result, heavy metal contamination in water bodies not only threatens the development of aquatic ecosystems but is also a human health hazard (Krayem et al., 2016).

Heavy metals in the aquatic environment can be removed using a variety of approaches, such as chemical or physical adsorption, physical precipitation and phytoremediation (Liu et al., 2007; Li et al., 2016). Due to its low cost and low environmental impact, the use of phytoremediation to remove heavy metals has been a focus of researchers and environmental managers (Jasrotia et al., 2015; Rezania et al., 2016). Previous studies indicate that a good metal-accumulating wetland plant species can absorb more than 0.5% of its dry weight (DW) of a given element and bio-concentrate the element in its tissue to up to 1,000-fold the initial elemental concentration in the water body (Zayed et al., 1998). In metal-scavenging hydrophytes, heavy metal uptake mechanism includes accumulation, exclusion, translocation, osmoregulation and distribution (Rezania et al., 2016). As a result, heavy metal-scavenging hydrophytes play a critical role in cleaning up heavy metal pollution in wetlands. For example, *Alternanthera philoxeroides* (Mart.) Griseb, an amphibious species, is abundant worldwide and has a high tolerance for heavy metal-polluted environments. This plant can act as a pioneer species and can thrive in mine tailings and polluted lands or aquatic environments (typically along banks) (Naqvi and Rizvi, 2000; Minchinton et al., 2006). A record Mn concentration of 19,300 mg/kg was reported for *A. philoxeroides*, demonstrating its ability as a hyperaccumulator of Mn (Xue et al., 2003).

In heavy metal-contaminated areas, *A. philoxeroides* has evolved into a cumulative ecotype under heavy metal stress (Hu et al., 2013). Baker et al. (1989) proposed some criteria for classifying a plant as a hyperaccumulator: it should be capable of accumulating more than 10,000 mg/kg for Mn and Zn and more than 100 mg/kg for Cd in their shoots, and the TF should be more than 1 (Baker et al., 1989). Based on

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data from Hu et al. (2013), *A. philoxeroides* meets these criteria and can be considered a potential species for the phytoremediation of soils, sediments and aquatic environments polluted with Cd, Mn and Zn. They showed that the Cd and Zn concentrations in *A. philoxeroides* were 21 and 43,154 mg/kg in the roots, respectively, and 155 and 13,784 mg/kg in the stems, respectively. In addition, the bioaccumulation factors (BCFs) of Cd and Zn were 36.78 and 49.23, respectively, and the translocation factors (TFs) were 7.38 and 3.19, respectively. However, the Cd accumulation and extraction capabilities of *A. philoxeroides* are debated. Liu et al. (2007) investigated the uptake and distribution of Cd, Pb and Zn in 19 wetland plant species from constructed wetlands, and they reported that the Cd concentrations in *A. philoxeroides* were 20.56 and 96.66 mg/kg in the aboveground and underground parts, respectively. The TF was less than 1, although the Cd accumulated mass was 28.17 mg/kg in the entire plant, representing the highest value among all of the studied species. These different results might be due to study differences in the growth media and the Cd concentration that was bioavailable to *A. philoxeroides*.

Cd can be absorbed through symplast and non-symplast transport in the roots. During symplast transport, Cd is transported through selective or non-selective ionophores and channel proteins (e.g., Ca^{2+}, Mg^{2+} and Fe^{2+} channel proteins) into the roots (Welch et al., 1999; Zhang et al., 2015). Plants absorb Cd through Mg, Ca or Fe channels because of their high biological activities and the bioavailability of Cd. Fe is important to plant metabolism and growth. Absorbed Cd competes with Fe in the plant metabolism and can interrupt the Fe balance directly or indirectly, causing iron deficiency (Gao et al., 2011). Fe deficiency causes a reduction in enzyme activities, chlorophyll synthesis and plant growth (Li et al., 2017a). As a result, Cd is one of the primary heavy metal pollutants, and it has received extensive attention in the field of phytoremediation.

Here, we report a hydroponics experiment conducted in a controlled laboratory environment to understand the interactive effects of Cd concentration and stress time on *A. philoxeroides*. The aims of this study were to 1) reveal the tolerance of *A. philoxeroides* to Cd, 2) explore the tolerance mechanisms of *A. philoxeroides* to Cd and 3) assess the potential of *A. philoxeroides* as a phytoremediation species for Cd.

2. Materials and methods

2.1. Experimental design and sample culture

Fresh *A. philoxeroides* shoots with two nodes were collected from the Yangtze River Valley in Zhenjiang City, Jiangsu Province, China. The shoots were cultured in a greenhouse at a temperature of 25–28 °C (60–80% relative humidity, and 12 h light/dark) until the roots and leaves were fully developed. One week before the Cd stress treatment, the plants were transferred to 1/10 modified Hoagland culture solution to undergo an adaptation period (Li et al., 2015). The seedlings were then treated with Cd ($CdCl_2$) at 0, 2.5 or 5.0 mg/l in triplicate, and defined as the control, medium-dose treatment and high-dose treatment, respectively. Five seedlings were inserted into each pot containing 5 l of culture solution with or without Cd, with a total of 45 pots. The culture solution was renewed every 3 days. The plants were harvested on days 0, 10, 20, 30 and 40 in each tretment, and their physiological and biochemical indexes were determined immediately.

2.2. Chlorophyll assay

Fresh leaf samples weighing 0.5 g were ground in 95% alcohol. The homogenate was filtered in darkness. The extract was diluted with 95% alcohol to 25 ml. The absorbance of the supernatant at 645 and 663 nm was recorded by a UV–visible spectrophotometer. The concentrations (mg l^{-1}) of chlorophyll *a* (*chl a*), chlorophyll *b* (*chl b*) and total chlorophyll (total *chl*) in the supernatant were calculated using the following equations (Li, 2000):

$$\text{Chlorophyll } a(\text{mg/g } FW) = (12.7\,A_{663} - 2.69\,A_{645}) \times V/(1000 \times FW)$$

$$\text{Chlorophyll } b(\text{mg/g } FW) = (22.9\,A_{645} - 4.68\,A_{663}) \times V/(1000 \times FW)$$

$$\text{Total chlorophyll}(\text{mg/g } FW) = (20.2\,A_{645} + 8.02\,A_{663}) \times V/(1000 \times FW)$$

where A_{663} and A_{645} are the absorbance at 663 nm and 645 nm, respectively; V is the volume of extracting solution (ml); and FW is the fresh weight of the sample (g).

2.3. 2-thiobarbituric acid reactive substances (TBARSs) concentration

The equivalent of MDA concentration was expressed as the rate of oxidative damage, which was evaluated by analysing the concentration of 2-thiobarbituric acid reactive substances (TBARSs) in leaves and roots of *A. philoxeroides*. For the measurement of lipid peroxidation in plants, the TBA (2-thiobarbituric acid) test which determines MDA as an end product of lipid peroxidation was used (Cakmak and Horst, 2006). The TBARS concentration was estimated with the method of Cakmak and Horst (2006) with slight modifications. Samples of 0.5 g (FW) were homogenized (with silica sand added as appropriate) in 5 ml 5% (w/v) trichloroacetic acid (TCA) and then centrifuged at 8000*g* for 10 min. Next, 2 ml supernatant was added to 2 ml 0.6% (w/v) 2-thiobarbituric acid (TBA) in 5% (w/v) TCA. Samples were incubated at 100 °C for 10 min, with the reaction stopped by ice bath. Then, the samples were centrifuged at 6000*g* for 15 min. The absorbance of the supernatant was measured at 532 nm using a UV–visible spectrophotometer. The reading was corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The TBARS concentration was calculated using the following equation:

$$\left(\text{TBARS concentration}(\text{nmol}\cdot\text{g}^{-1}\text{FW}) = \frac{(A_{532} - A_{600})\text{V}}{\varepsilon \times \text{FW}}\right)$$

where A_{532} and A_{600} are the absorbance at 532 nm and 600 nm, respectively; V is the volume of crushing medium (ml); ε is the specific extinction coefficient (155 mm cm^{-1}); and FW is the fresh weight of the sample (g).

2.4. Extraction of iron plaque

The iron plaque on the fresh root surfaces of *A. philoxeroides* was extracted according to the DCB technique (Hu et al., 2007). A dithionite-citrate-bicarbonate solution was used to extract the iron plaque for 60 min at room temperature. The resulting solution was filled up to 100 ml with deionized water, defined as DCB extracts. The extractable Fe and Cd concentrations in the DCB extracts were determined by inductively coupled plasma mass spectrometry (ICP-MS, XSeries II, Thermo, USA). After DCB extraction, the roots were oven dried at 70 °C to a constant weight.

2.5. Analysis of Cd concentration in tissues of A. philoxeroides by ICP-MS

Dry plant samples (approximately 0.5 g) were digested as described by Soto-Jimenez and Paez-Osuna (2001). Reagent blanks and standard references of plant material (GBW-07603) (from the National Research Center for Standards in China) were included to verify the accuracy and precision of the analysis procedure (Li et al., 2017b). All of the reagents were Merck analytical grade or Suprapur quality, and all of the materials (bottles, beakers, glass funnels, measuring cylinders, filters and digestion tanks) were acid-cleaned (14% (v/v) nitric acid) and rinsed with deionized water prior to use. Fe and Cd concentrations in the samples were detected by ICP-MS (XSeries II, Thermo, USA).

2.6. Statistical analysis

All of the results presented and discussed here are based on the mean values and standard deviation (S.D.) of three replicates. One-way