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Mn accumulation and tolerance in *Celosia argentea* Linn.: A new Mn-hyperaccumulating plant species

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HIGHLIGHTS

- This is the first report of *Celosia argentea* Linn. as Mn hyperaccumulator.
- *C. argentea* shows a great tolerance to manganese.
- *C. argentea* has relatively high biomass and growth rates.
- *C. argentea* is a suitable species for Mn phytoremediation.

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ABSTRACT

Identifying a hyperaccumulator is an important groundwork for the phytoextraction of heavy metal-contaminated soil. *Celosia argentea* Linn., which grew on a Mn tailing wasteland, was found to hyperaccumulate Mn (14 362 mg kg^{-1} in leaf dry matter) in this study. To investigate Mn tolerance and accumulation in *C. argentea*, a hydroponic culture experiment was conducted in a greenhouse. Results showed that the biomass and the relative growth rate of *C. argentea* were insignificantly different ($p > 0.05$) at the Mn supply level ranging from 2.5 mg L^{-1} (control) to 400 mg L^{-1}. Manganese concentrations in leaves, stems, and roots reached maxima of 20 228, 8872, and 2823 mg kg^{-1} at 600 mg Mn L^{-1}, respectively. The relative rate of Mn accumulation increased by 91.2% at 400 mg Mn L^{-1}. Over 95% of the total Mn taken up by *C. argentea* was translocated to shoots. Thus, *C. argentea* exhibits the basic characteristics of a Mn-hyperaccumulator. This species has great potential to remediate Mn-contaminated soil cheaply and can also aid the studies of Mn uptake, translocation, speciation, distribution and detoxification in plants.

1. Introduction

Manganese is an essential trace element for life tissues; however, it may become toxic in excessive amounts [1,2]. Manganese toxicity causes brown spots on mature leaves, interveinal chlorosis and necrosis, deformation of young leaves, and growth retardation [3,4]. Exposure to excessively high concentrations of Mn can lead to numerous health problems, such as neurodegenerative disorders, abnormalities of the reproductive system, and malfunction of the immune system [5]. Manganese is also a common soil contaminant through inputs from natural processes and anthropogenic activities. High concentrations of Mn in soil are a widespread environmental problem in southeastern Australia, northeastern USA, and southern China [6–8]. Thus, developing efficient techniques to remove Mn from soil is urgently and imperatively needed.

Phytoremediation, which mainly uses hyperaccumulators to remove excess heavy metals from contaminated soil, is regarded as a promising cost-effective method without major secondary environmental issues, particularly for remediating large areas of soil with a relatively low level of heavy-metal contamination [9]. The success of this technique highly depends on the hyperaccumulators, which can extremely accumulate and tolerate heavy metals. Although more than 400 species were found to be hyperaccumulators, only approximately 17 species target Mn. These Mn hyperaccumulators include *Gossia bidwillii* and *Austromyrtus bidwillii* from eastern Australia [10,11], *Phytolacca acinosa* Roxb. [12], *Phytolacca americana* L. [13], *Polygonum hydropiper* L. [14], *Polygonum pubescens* Bl. [15], and *Polygonum perfoliatum* L. [16] from China, nine species listed by Reeves and Baker [17], and an unidentified *Eugenia* species [18]. Given that some hyperaccumulators are small and grow slowly, only a limited selection of known hyperaccumulators may be suitable for large-scale phytoremediation [19]. Therefore, identifying new feasible Mn hyperaccumulators is essential for the successful phytoremediation of Mn-contaminated soil.

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Celosia argentea Linn., an annual herb, is distributed all over China and Southeast Asia. This species exhibits rapid growth, strong adaptability, and high propagation rate. A field survey showed that *C. argentea* grew well on a droughty Mn-mine-tailing dump contaminated by multiple heavy metals [20]. Therefore, it was hypothesized that *C. argentea* could be used for phytoremediation. In the present work, field surveys on Mn accumulation in *C. argentea* were conducted in Daxin Mn Mine, Guangxi Province, China. A hydroponic experiment was also performed to examine the accumulation and tolerance abilities of this species for Mn. The objectives of this study were as follows: (1) to determine whether *C. argentea* is a Mn hyperaccumulator, (2) to assess the tolerance of *C. argentea* to Mn stress, and (3) to evaluate the potential of using this species to remediate Mn-contaminated sites.

2. Materials and methods

2.1. Field survey and sampling

The field surveys were conducted in Daxin Mn Mine, which is located in Daxin County, Guangxi Province, southern China (106°40′ E to 106°44′ E, 22°54′ N to 22°55′ N). This area has a subtropical monsoon climate, with an annual average temperature of 21.3 °C and an average rainfall of 1424 mm. The proven Mn reserves in this area were 131 million tons, accounting for a quarter of the total Mn reserves in China. Daxin Mn Mine started to operate (opencast mining) in 1958. The long-term mining activities had resulted in heavy-metal contamination of the soil in this area [21]. A preliminary survey was conducted in April 2012 to investigate plant species and phytoaccumulation of heavy metals in Daxin Mn Mine. It was found that *C. argentea* was the dominant species in the tailing wasteland and contained high levels of Mn in shoots. A detailed survey on *C. argentea* was conducted in August 2012. Five individual plants were collected randomly within the sampling area. Corresponding soil samples (0–30 cm) were also collected from the plant sampling locations.

2.2. Hydroponic culture

C. argentea seeds were collected from a tailing wasteland in Daxin Mn Mine. The seeds were germinated and cultivated in sand for two weeks. The seedlings were then transplanted into a 15 cm diameter round plastic pot containing half-strength Hoagland's solution. The plants were acclimated in the solution for 15 days prior to the addition of high levels of Mn supplied as $MnCl_2$. The plants were exposed to the following Mn concentrations (in half-Hoagland's solution): 2.5 (control), 25, 50, 100, 200, 400, and 600 mg Mn L^{-1} (the control treatment contained sufficient Mn to prevent Mn deficiency). Each treatment was replicated thrice, with three plants per pot per replicate. The plants were aerated and maintained in a greenhouse with a controlled environment (25 °C day/18 °C night, 70–75% relative humidity, and 14 h photoperiod) for 30 days. The solutions were adjusted daily to pH 5.5 with 0.1 M NaOH or 0.1 M HCl and renewed every 4 days, with the volume being restored to its original level.

2.3. Plant and soil sample analyses

During harvest, the plants were rinsed thrice in deionized water and separated into leaves, stems, and roots. The tissues were initially dried at 105 °C for 30 min, and then at 70 °C for 48 h to constant weight. Tissue biomass (dry weight, DW) was determined. The dried tissues were ground into powder using an agate mortar and digested by HNO_3/H_2O_2 microwave digestion. The Mn concentrations in each tissue sample were quantified using a flame atomic absorption spectrophotometer (FAAS, PE-AA700). All soil samples were air-dried and sifted through a 2 mm sieve. The sieved soil samples were digested with $HCl + HNO_3 + HClO_4$ (3:1:1, v/v). The Mn concentrations of the soil samples were also determined by using the FAAS. Reagent blanks, a standard reference soil sample (GBW07403) and standard plant samples (GBW10010), were employed in the analysis to ensure accuracy and precision. The results were found within ±5% of the certified value.

Fig. 1. *C. argentea* growing on a Mn tailing wasteland.

2.4. Growth and Mn accumulation rates

Five plants were harvested at the beginning of the experiment. The dry weight and Mn concentrations of the plants were determined as described in the previous section. The relative growth rate (RGR) of whole plants was calculated by using the following formula:

$$RGR = \frac{\ln Bf - \ln Bi}{D}$$

where Bf = final dry mass, Bi = initial dry mass (an average of the five plants dried at the beginning of the experiment), and D = duration of experiment (days).

The relative rate of Mn accumulation (RRMn) in the plants could be measured in the same manner as RGR and was calculated by using the following equation:

$$RRMn = \frac{\ln Mf - \ln Mi}{D}$$

where Mf = Mn accumulation per plant at the end of the experiment, and Mi = Mn accumulation per plant at the beginning of the experiment.

2.5. Statistical analyses

All data were analyzed through the software PASW Statistics 18.0. One-way ANOVA was conducted to determine the influences of the various factors. All tests were conducted at a 95% confidence interval ($\alpha = 0.05$).

3. Results

3.1. Mn accumulation in C. argentea growing on contaminated sites

In the field surveys, few plants were found surviving on the Mn-tailing wasteland. However, *C. argentea* successfully colonized this harsh and stressed habitat and occupied most of the area (Fig. 1).