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Comparison of manganese tolerance and accumulation among 24 *Salix* clones in a hydroponic experiment: Application for phytoremediation

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ABSTRACT

Willows (*Salix* spp.) are excellent candidates for phytoremediation owing to their large biomass, high metal tolerance and accumulation capacity. In this study, manganese tolerance and accumulation capability in 24 *Salix* clones were evaluated exposed to 1 mM Mn by a hydroponic system for 21 days. Results suggested that there were wide variations in Mn tolerance and accumulation capability among the clones. Clonal variation in biomass production ranged from growth reduction to growth stimulation. The clonal differences in Mn concentrations (μg g^{-1}, dry weight, DW) ranged from 3183.10 to 5827.7 in leaves, from 1840.48 to 4572.17 in stems, and from 2733.33 to 10,253.88 in roots exposed to excess Mn. The total Mn contents in shoots (including leaves and stems) varied 5.8-fold among clones under Mn treatment. Five clones exhibited high Mn tolerance and accumulation capacity, and clone J333 (*Salix babylonica* × *Salix matsudana*) had a relatively high Mn tolerance index and the highest Mn content in aboveground tissues. Consequently, further evaluation of the *Salix* clones for Mn tolerance and phytoremediation potential is recommended in field experiments.
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1. Introduction

Recently, soil and water contaminated with Mn have aroused considerable attention worldwide (Liu et al., 2010). Manganese contamination mainly originates from a variety of anthropogenic activities, including acid mine drainage, battery industry, catalysts, dyes, gas additives, wood preservatives, glass and ceramic articles, fertilizers, and sewage-sludge applications, which have raised Mn concentrations in many soils and waters, especially in aquatic systems (Liu et al., 2010; Paschke et al., 2005). In addition, soil acidification and flooding increase the Mn bioavailability, resulting in increasing Mn concentrations for plant uptake (Moroni et al., 2003; Najeeb et al., 2009).

Manganese is an essential element for plant growth and development; it plays an important role in enzyme activation, biological redox processes of various metabolic pathways associated with photosynthesis, respiration, and synthesis of proteins, carbohydrates, etc. (Zornoza et al., 2010). However, high concentrations of Mn may be toxic to plants. In general, excess Mn retards growth and causes chlorosis and necrosis (Dučić et al., 2006; Mou et al., 2011; Najeeb et al., 2009); excess Mn interrupts essential metabolic and reproductive processes, such as absorption, translocation, and utilization of essential elements in plants (Moroni et al., 2003; Shanahan et al., 2007). Moreover, manganese toxicity is one of the most important limiting factors for crop production in many acid soils (De la Luz Mora et al., 2009).

Conventional soil remediation for Mn toxicity comprises mainly physical–chemical methods, including air oxidation, chlorine oxidation, and contact oxidation filter, and these remediation methods are usually expensive and easily generate secondary pollution (Najeeb et al., 2009; Xu et al., 2009, 2006). Recently, phytoremediation approaches have been widely used for Mn removal (Miao et al., 2007; Najeeb et al., 2009; Xu et al., 2006). Phytoremediation, as a cost-effective and environmentally friendly technique, uses plants to remove/stabilize contaminants from the environment (Liu et al., 2010; Najeeb et al., 2009; Xu et al., 2009). Sub-categories of phytoremediation have been developed and employed for remediating environments, e.g., phytoextraction, phytofiltration, and phytostabilization (Ali et al., 2013).

The efficiency of phytoextraction is dependent upon the selection of suitable species, which requires screening and breeding of plants with high tolerance and high accumulation capability (Liu et al., 2010). Previous studies of phytoextraction mainly involved metal hyperaccumulator plants, because these plants may offer a real potential to extract heavy metals. A Mn hyperaccumulator plant is defined as a plant

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that consistently accumulates a minimum dry weight Mn concentration of 10,000 mg kg^{-1} in its aboveground parts (Liu et al., 2010; Mizuno et al., 2008). To date, 19 species have been designated as Mn hyperaccumulators (Fernando et al., 2013). Plant species known to hyperaccumulate Mn are mainly woody plants that are typically distributed in subtropical areas, belonging to the Apocynaceae, Celastraceae, Clusiaceae, Myrtaceae, and Proteaceae families (Xu et al., 2006).

Despite the abilities of these hyperaccumulators to accumulate high amounts of Mn, their use for remediation of contaminated sites is limited. Considering the feasibility of phytoremediation, hyperaccumulators have a shallow root system, grow very slowly, and produce litter biomass; hence, they do not remove large quantities of pollutants in a short time. Consequently, Mn hyperaccumulators known may not be suitable for large-scale phytoremediation application.

Recently, the genus *Salix* has emerged as efficient plant germplasm for phytoextraction. Willows (*Salix* spp.) have several characteristics that make them ideal plant species for phytoremediation application, including easy propagation and cultivation, large biomass, fast-growing, deep root system, high transpiration rate, tolerance to hypoxic conditions, and high metal accumulation capability (Dimitriou and Aronsson, 2010). Despite smaller metal concentrations with respect to hyperaccumulators, metal-tolerant willow clones hold promise for phytoremediation as they produce large biomass and metal contents in their aerial parts (Pulford and Watson, 2003). Cultural management of willows by means of short rotation coppice cultures shows large potential for phytoremediation of contaminated sites, which provide a significant opportunity for remediation applicant and simultaneous renewable energy production (Holm and Heinsoo, 2013).

In field/greenhouse experiments, most willow species/clones have been investigated for their phytoremediation potential (Dos Santos Utmazian et al., 2007; Rosselli et al., 2003; Zhivotovsky et al., 2010). From growth chamber experiments, pot experiments, and field experiments, numerous authors have reported that variation in the phytoremediation capacity depends on the willow clones used (Dos Santos Utmazian et al., 2007; Zacchini et al., 2009; Zhivotovsky et al., 2010).

Hydroponic screening has been widely applied to evaluate genotypic variation for Mn tolerance and accumulation ability (Khabaz-Saberi et al., 2010; Moroni et al., 2003; Rout et al., 2001; Stoyanova et al., 2009; Wang et al., 2002). In addition, hydroponic screening has also been considered as optimum method to speed up clone selection in *Salix* (Dos Santos Utmazian et al., 2007; Zhivotovsky et al., 2010). Watson et al. (2003) have pointed out that results obtained in hydroponic and field experiments are well correlated.

Early phytoremediation studies focused on identifying Mn hyperaccumulator species (Liu et al., 2010; Mizuno et al., 2008; Xue et al., 2004). Genotypic variation in Mn tolerance and accumulation has been observed in crops such as wheat (Khabaz-Saberi et al., 2010), rice (Wang et al., 2002), rapeseed (Moroni et al., 2003), and other woody plants (Dučić et al., 2006; Kitao et al., 2001; Yao et al., 2012). Toxicity thresholds of Mn have been recently established for *Salix geyeriana* and *Salix monticola* (Shanahan et al., 2007). However, to date, and to our knowledge, manganese tolerance and accumulation in different willow clones are poorly understood.

The genus *Salix* comprises more than 450 species, including trees, shrubs, and creeping shrub, which are widespread in both northern and southern hemispheres, and the center of diversity is believed to be in Asia, with around 275 species in China (189 endemics) (Karp et al., 2011). Owing to the wide genetic variability, the genus *Salix* provides a tremendous opportunity to select “super clones” improving heavy metal tolerance and accumulation capacity. The aims of this study were to evaluate the response to Mn tolerance and accumulation in different *Salix* clones by a hydroponic system. This information will be useful to propose the most suitable *Salix* species or clones for phytoremediation projects in Mn-contaminated soils and waters.

2. Materials and methods

2.1. Plant material and growth condition

The 24 willow clones tested (listed in Table 1) in this study were chosen from the National Willow Germplasm Resource in the Jiangsu Academy of Forestry, Nanjing (33° 31′ N, 118° 47′ E), China. They are widely planted in many regions in China, which exhibit good growth performance due to high biomass and fast growth rate. Ten-centimeter cuttings of each clone were prepared from 1-year-old stems; three cuttings of the same clone were inserted in Styrofoam and transferred to a plastic bucket. Before treatment, the plants were supplied weekly with an aerated nutrient solution. The nutrient solution consisted of 1 mM $Ca(NO_3)_2$, 1.25 mM KNO_3, 0.5 mM $MgSO_4$, and 0.5 mM $NH_4H_2PO_4$ and contained the following micronutrients: 25 μM Fe-EDTA, 23.1 μM H_3BO_3, 0.4 μM $ZnCl_2$, 0.18 μM $CuCl_2$, 4.57 μM $MnCl_2$, and 0.06 μM Na_2MoO_4 (as described by Watson et al., 2003). After 30 days of plant growth under hydroponic culture conditions, plants were treated for 21 days with nutrient solution containing 1 mM Mn (added as $MnSO_4$); background nutrient solution contained 4.57 μM Mn as control.

Each treatment was carried out in three replicates. The pH of the nutrient solution was maintained at 5.5–6.0 by using H_2SO_4/NaOH. Nutrient solution was replaced once a week, and aeration was supplied by using a pump during the experiment. The experiments were conducted in a greenhouse, provided with 16/8-h light/dark period, temperature of 25/18 °C, and relative humidity of 70/80%.

2.2. Sample preparation and analysis

After 21 days of Mn treatment, plants were harvested and separated into leaves, stems, and roots. The roots were washed carefully in 10 mM EDTA-Na_2 for 15 min to remove adsorbed metals on the surface of the root, and then rinsed in double-deionized water. Plant materials were dried at 70 °C for 48 h in a forced-air oven. The dry weight of the plant materials was recorded. The dried material was milled to a fine powder, and approximately 0.2 g of plant sample was wet-digested with HNO_3:$HClO_4$ (4:1 v/v). The obtained extracts were analyzed for Mn by using atomic absorption spectrometry (PE AAnalyst 800, PerkinElmer Inc., USA).

Table 1
Salix species/clones tested in the experiment.

Clone	Specie/hybrid
SB7	*Salix babylonica*
SB9	*S. babylonica*
SB13	*S. babylonica*
SM24	*S. matsudana*
SM30	*S. matsudana*
SM31	*S. matsudana*
SM33	*S. matsudana*
SS61	*S. suchowensis*
SI63	*S. integra*
J9-6	*S. integra* × *S. suchowensis*
J194	(*S. matsudana* × *S. chosenia arbutifolia*) × *S. matsudana*
J333	*S. babylonica* × *S. matsudana*
SI336	*S. integra*
SV681	*S. viminalis*
SV683	*S. viminalis*
SS708	*S. suchowensis*
J795	*S. matsudana* × *S. alba*
J8-26	*S. integra* × *S. suchowensis*
J842	*S. babylonica* × *S. alba*
J844	*S. babylonica* × *S. alba*
J903	(*S. matsudana* × *S. chosenia arbutifolia*) × *S. matsudana*
J1011	*S. babylonica* × *S. alba*
J1052	*S. suchowensis* × *S. leucopithecia*
SI102-2	*S. integra*

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