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Screening the wetland plant species *Alisma plantago-aquatica*, *Carex rostrata* and *Phalaris arundinacea* for innate tolerance to zinc and comparison with *Eriophorum angustifolium* and *Festuca rubra* Merlin

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Some wetland angiosperms are tolerant to high concentrations of metals, regardless of conditions in the plants' natural habitat.

Abstract

Several wetland plant species appear to have constitutive metal tolerance. In previous studies, populations from contaminated and non-contaminated sites of the wetland plants *Typha latifolia*, *Phragmites australis*, *Glyceria fluitans* and *Eriophorum angustifolium* were found to be tolerant to high concentrations of metals. This study screened three other species of wetland plants: *Alisma plantago-aquatica*, *Carex rostrata* and *Phalaris arundinacea* for innate tolerance to zinc. The degree of tolerance was compared to known zinc-tolerant *E. angustifolium* and *Festuca rubra* Merlin. It was found that *A. plantago-aquatica* and *P. arundinacea* did not posses innate tolerance to zinc, but that *C. rostrata* was able to tolerate elevated levels of zinc, at levels comparable to those tolerated by *E. angustifolium* and *F. rubra* Merlin. The findings support the theory that some wetland angiosperm species tend to be tolerant to exposure to high levels of metals, regardless of their origin.

Keywords: Wetland; Innate metal tolerance; Zinc; *Alisma plantago-aquatica*; *Carex rostrata*; *Phalaris arundinacea*

1. Introduction

Some populations of dryland plants such as *Festuca rubra*, *Agrostis stolonifera* and *Silene vulgaris* can grow in the presence of metal levels that are toxic to non-adapted plants (Antonovics et al., 1971; Ernst, 1974; Smith and Bradshaw, 1979). Such plants do not possess innate or constitutional tolerance to metals, but tolerant plants were selected and gave rise to metal tolerant populations (Antonovics et al., 1971). Metal tolerant populations are genetically distinct from non-tolerant populations (Antonovics et al., 1971). However, the basis of metal tolerance in wetland plants seems to be different to that of dryland plants. Comparisons of populations of the wetland plant species *Typha latifolia* and *Phragmites australis* from metal enriched sites with those from non-polluted sites have shown that they were equally tolerant of metals (McNaughton et al., 1974; Taylor and Crowder, 1983, 1984; Ye et al., 1997a,b, 1998). *Glyceria fluitans* (McCabe and Otte, 2000; Moran and Otte, 2004; Matthews et al., 2004b) and *Eriophorum angustifolium* (Matthews et al., 2004a) have also been found to possess innate zinc tolerance. The information

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available supports the assumption that wetland plants, regardless of their origin, are able to grow in high metal concentrations and this has led to the theory that wetland plants have an innate tolerance to heavy metals (McCabe et al., 2001).

In this study, innate zinc tolerance in the wetland species; *Alisma plantago-aquatica*, *Carex rostrata* and *Phalaris arundinacea* was investigated. It was hypothesized that these three species would show innate tolerance to elevated levels of zinc based on observations for other wetland plant species. All three species were taken from clean sites across Ireland that had not been contaminated with heavy metals. The three species were compared with two other known zinc-tolerant species. The first was *E. angustifolium*, a wetland species taken from a non-contaminated site. It has been found to possess innate zinc tolerance (Matthews et al., 2004a). The second species was *F. rubra* Merlin, a known zinc-tolerant cultivar of *F. rubra* that has an evolved tolerance to zinc (Smith and Bradshaw, 1979). The experiment was carried out hydroponically using different concentrations of zinc-amended nutrient solutions.

The information obtained from this study is highly relevant to phytoremediation of metal polluted sites, for example for use in constructed wetlands for the treatment of runoff water with high metals content (Kadlec and Knight, 1996) or for phytostabilization of mine tailings (Smith and Bradshaw, 1979). In constructing a treatment wetland, it is important to select populations of plants that are pre-adapted for survival in the prevailing environment (Salt et al., 1995). So typically, plants used in phytoremediation are from metal tolerant populations. Innate tolerance in some species of wetland plants would increase the amount of suitable material available for phytoremediation, as any plants from any population could be used. This could reduce the cost involved in the construction of treatment wetlands.

2. Materials and methods

2.1. Collection of plants and soil samples

Plants were collected in March 2003. *A. plantago-aquatica* L. (water plantain) and *C. rostrata* Stokes (bottle sedge) were collected from a pond in Djouce Wood, Co. Wicklow, Ireland (grid reference O 219 112). *P. arundinacea* L. (reed canary grass) was collected from Lough Ennel, Mullingar, Co. Westmeath, Ireland (7° 25′ W, 53° 28′ N, grid reference N 240 246). *E. angustifolium* Honckeny (bog cotton) was collected from a bog in Kippure, Co. Wicklow, Ireland (grid reference T 141 143). *F. rubra* L. Merlin (creeping red fescue) was grown from seed supplied by Perryfields Ltd, Inkberrow, England. Plant and soil samples collected from the field were placed in plastic bags and transported back to the Thornfield Horticultural Unit glasshouse complex in University College Dublin, Ireland, where the growth experiments took place. Samples of soil, porewater and plants were also transferred to the laboratory at University College Dublin and analyzed for zinc content. Soil samples were randomly collected from soil around the plants. Porewater samples were taken to determine the total amount of zinc available to the plant. Ten dialysis vials, consisting of 20 ml plastic scintillation vials covered with 20 μm millipore mesh, were buried at each site at depths of between 5 and 10 cm (cf. Doyle and Otte, 1997). These were left in situ for three weeks to equilibrate, retrieved, filtered using 0.45 μm membrane filters, acidified with a drop (0.03 ml) of HNO_3 and analyzed for zinc.

2.2. Chemical analysis of soil, porewater and plant samples

All plant material was first washed in a solution of 10 mmol L^{-1} $CaCl_2$ at 0 °C for 30 min to remove any wall bound metals (Harmens et al., 1993). Samples of soil and plants were then dried in an oven at 60 °C until constant weight was obtained. Zinc concentrations were determined following digestion of 100 mg of dried homogenized soil or 50 mg of dried homogenized plant material in Teflon bombs containing 2 ml HNO_3:HCl in a (4:1) mix (cf. Otte et al., 1995). The samples were cooled, diluted and filtered using 0.45 μm membrane filters. Acid digests of soil and plant samples were analyzed for zinc using a Unicam 929 flame atomic absorption spectrometer. Porewater samples were acidified with HNO_3 before analysis. Plant standard reference samples supplied by Glen Spectra Reference Materials, England, of oriental tobacco leaves (CTA-OTL-1) were also analyzed for zinc content and deviated on average by ±2% from certified values for zinc.

2.3. Glasshouse experiment

All plants were pre-grown for two months to acclimatize them to their new environment in nutrient solution containing: KNO_3 (1.5 mmol L^{-1}), $Ca(NO_3)_2$ (1 mmol L^{-1}), $NH_4H_2PO_4$ (0.5 mmol L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.25 mmol L^{-1}), KCl (1 μmol L^{-1}), H_3BO_3 (25 μmol L^{-1}), $MnSO_4 \cdot 7H_2O$ (2 μmol L^{-1}), $CuSO_4 \cdot 5H_2O$ (0.1 μmol L^{-1}), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.1 μmol L^{-1}) and Fe(Na)EDTA (0.1 mmol L^{-1}). The pH of the solution was adjusted to 5.5 using NaOH and HCl and the solution was changed weekly. This type of nutrient solution has been found not to interfere with metal availability to plants in metal tolerance experiments (Harmens et al., 1993). The experiment commenced on 22 April 2003 and lasted until 30 July 2003. This study was carried out hydroponically in black