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Zinc tolerance and accumulation in the halophytic species *Juncus acutus*

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ABSTRACT

The research on species with capacity to tolerate and accumulate zinc is of paramount importance for phytoremediation purposes. An experiment was designed to investigate the effect of Zn from 0 to 100 mmol l^{-1} on the growth, photosynthetic apparatus and nutrient uptake of the halophytic species *Juncus acutus*. Gas exchange, chlorophyll fluorescence and photosynthetic pigments concentration were measured. We also determined total zinc, magnesium, potassium, phosphorus and sodium concentrations, as well as C/N ratio. *J. acutus* showed high tolerance to Zn-induced stress, since all plants survived and none of them showed any toxicity symptoms, such as chlorosis, necrosis or growth reduction at concentrations up to 100 mmol l^{-1} Zn. The integrity and functionality of the photosynthetic apparatus were unaffected even at zinc concentrations greater than 500 mg kg^{-1} on tillers. Likewise, nutrient absorption was relatively unaffected. Zn tolerance was associated with the capacity to accumulate Zn in roots (with values up to 2500 mg kg^{-1}) and largely avoid its transport to tillers. These characteristics, along with its ability to establish in a wide variety of ecosystems, render this species a useful phytostabilizer for revegetation of Zn-contaminated lands.

1. Introduction

Environmental pollution by heavy metals is a serious problem worldwide, increasing in parallel with the development of human technology. Government, the industry and the public now recognize the potential dangers that metals pose to human health (Duruibe et al., 2007) through the food chain and the health of terrestrial and aquatic communities and ecosystems (Kabata-Pendias and Pendias, 2001). The danger of toxic metals is aggravated by their immutable nature and indefinite persistence in the environment (Garbisu and Alkorta, 2001; Aycicek et al., 2008). Among heavy metals, Zn is considered the main industrial pollutant of both terrestrial and aquatic environments (Barak and Helmke, 1993) and has the greatest mobility and bioavailability of all elements (Morillo et al., 2004). Although Zn is an essential microelement with many roles in plant metabolism (Kabata-Pendias and Pendias, 2001), its excess can lead to toxic effects in plants (Chaney, 1993), with specific effects on the Calvin cycle and photosystem activity (Van Assche and Clijsters, 1986).

Many remediation strategies have been considered to counter the detrimental effects of Zn excess, including physical, chemical and biological methods that immobilize or remove metals from the environment (Marques et al., 2011). Phytoremediation has recently gained importance on account of its cost-effective, long-term applicability and because it is an ecofriendly, promising clean-up solution for a wide variety of contaminated sites (Weis and Weis, 2004). This methodology depends on the use of plants to act upon the contaminants, by extracting, degrading or immobilizing them (Marques et al., 2011). The research on species which can be useful in metal phytoremediation has become a major issue (Zhang et al., 2010) and these species should be chosen on the basis of their capacity to tolerate and accumulate particular contaminants (Marques et al., 2011).

There exists a wide variation in sensitivity to metal exposure. However, exists a lack of knowledge about metal toxicity thresholds for native plant species (Ross and Kaye, 1994) and for species used to restore sites contaminated by heavy metals, such as salt marshes. Species of genus *Juncus* have been employed in wetland restoration projects around the world (Sparks et al., 2013; Marques et al., 2011), but the information on the tolerance and accumulation patterns of heavy metals in these species is really scarce. The present study is focused on the species *Juncus acutus* L., a halophytic densely caespitose plant with subcosmopolitan distribution that is common in Spanish coastal marsh communities and can be found growing in sediments containing 100–4800 ppm Zn in several estuaries of the Iberian Peninsula (Sáinz and Ruiz, 2006).

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Moreover, this species has a wide ecological range, tolerating soils with high levels of sulphates and chlorides (Fernández-Carvajal, 1982) and soils with a sandy texture and hydric stress during the dry summer season. Our hypothesis is that all these circumstances highlight the potential of *J. acutus* to be used for metal remediation in polluted areas. However, no studies have analyzed its growth and physiological responses to zinc excess.

The aim of this study was to evaluate the tolerance of *J. acutus* to elevated concentration of zinc in relation of its survival, growth and photosynthetic response, and quantify the capacity of this species for accumulating this element.

2. Materials and methods

2.1. Plant material

Seeds of *J. acutus* were collected in December 2011 from the natural marshes of Doñana National Park (37°15′N–6°58′W; SW Spain) and stored at 4 °C (in darkness) for three months. After that, seeds were placed into a germinator for a month (ASL Aparatos Científicos M-92004, Madrid, Spain) and subjected to an alternating diurnal regime of 16 h of light (photon flux rate, 400–700 nm, 35 μmol m^{-2} s^{-1}) at 25 °C and 8 h of darkness at 12 °C. Seedlings were then planted in individual plastic pots (11 cm of diameter) filled with perlite and placed in a glasshouse with controlled temperature of 21–25 °C, 40–60% relative humidity and natural daylight (minimum and maximum light flux: 250 and 1000 μmol m^{-2} s^{-1}, respectively). Pots were carefully irrigated with 20% Hoagland's solution (Hoagland and Arnon, 1938) as necessary. All the pots received the same irrigation.

2.2. Stress treatments

In October 2012, after five months of seedling culture, the pots (with between 5 and 6 tillers) were randomly allocated still inside the glasshouse to five Zn treatments (six pots per tray, one tray per Zn treatment): 0, 10, 30, 60 and 100 mmol l^{-1} Zn. The treatment with 0 mmol l^{-1} Zn was considered the control treatment. Zinc treatments were established by combining 20% Hoagland' solution and $ZnSO_4·7H_2O$ of the appropriate concentration. The control, 0 mmol l^{-1} Zn treatment, had exactly 0.002 mmol l^{-1} Zn, as Hoagland' solution contains a small amount of Zn as an essential trace nutrient. Zn concentrations were chosen to cover variations recorded by Sáinz and Ruiz (2006) in the salt marshes of the joint estuary of the Tinto and Odiel Rivers.

At the beginning of the experiment, 1 l of appropriate solution was placed in each tray (Hoagland al 20% + $ZnSO_4·7H_2O$) to a depth of 1 cm. During the experiment, the levels of trays were monitored and topped up to the marked level with 20% Hoagland' solution (without additional $ZnSO_4·7H_2O$) to limit the change of Zn concentration caused by water evaporation from the nutrient solution. Also, the entire solution (including $ZnSO_4·7H_2O$) was changed every three days.

2.3. Growth analysis

At the beginning and the end of the experiment (after 50 days of treatment) three and five entire plants, respectively, from each treatment were dried at 80 °C for 48 h and weighed. Also, before and after the Zn treatment, the number and height of all fully developed tillers were measured.

The relative growth rate (RGR) in ash-free dry mass of whole plants was calculated using the formula:

$$\mathrm{RGR} = \left(\ln B_f - \ln B_i\right) \times D^{-1} \left(\mathrm{g} \quad \mathrm{g}^{-1} \quad \mathrm{day}^{-1}\right)$$

where B_f = final dry mass, B_i = initial dry mass (an average of the three plants from each treatment dried at the beginning of the experiment) and D = duration of experiment (days).

2.4. Gas exchange

Measurements were taken on random, fully developed photosynthetic tillers (n = 10, two measurements per plant) using an infrared gas analyser in an open system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) after 50 days of treatment. Maximum net photosynthetic rate (A), intercellular CO_2 concentration (C_i) and stomatal conductance to CO_2 (G_s) were determined at CO_2 concentration of 400 μmol CO_2 mol^{-1} air, temperature of 25–30 °C, 42.4 ± 0.4% relative humidity and a photon flux density of 1000 μmol m^{-2} s^{-1} once a steady-state was reached. A, C_i and G_s were calculated using standard formulas of Von Caemmerer and Farquhar (1981). Photosynthetic area was approximated as the area of a cylinder. Intrinsic water use efficiency (WUE_i) was calculated as the ratio between A and G_s.

2.5. Tiller water content

Tiller water content (TWC) was calculated after 50 days of treatment as (n = 5, one measurement per plant):

$$\mathrm{TWC} = \frac{(\mathrm{FW} - \mathrm{DW})}{\mathrm{FW}} \times 100$$

where FW is the fresh mass of the tillers and DW is the dry mass after oven-drying at 80 °C for 48 h.

2.6. Photosynthetic pigments

At the end of the experimental period, photosynthetic pigments in fully developed, photosynthetic tillers (n = 5) from each treatment were extracted using 0.05 g of fresh material in 10 ml of 80% aqueous acetone. After filtering, 1 ml of the suspension was diluted with a further 2 ml of acetone and chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoid ($C_x + c$) contents were determined with a Hitachi U-2001 spectrophotometer (Hitachi Ltd., Japan) using three wavelengths (663.2, 646.8 and 470.0 nm). Concentrations of pigments (μg $gfwt^{-1}$) were obtained through calculation following Lichtenthaler (1987).

2.7. Measurement of chlorophyll fluorescence

Chlorophyll fluorescence was measured using a portable modulated fluorimeter (Mini-PAM, Heinz Walz, Germany) after 50 days of treatment, in tillers similar to those used previously. Measurements were made on each plant in the five zinc treatments (n = 10, two measurements per plant). Light and dark-adapted fluorescence parameters were measured at dawn (stable 75 μmol m^{-2} s^{-1} ambient light) and at midday (1500 μmol m^{-2} s^{-1}) to investigate whether zinc concentration affected the sensitivity of plants to photoinhibition (Qiu et al., 2003).

Plants were dark-adapted for 30 min using leaf-clips designed for this purpose. The minimal fluorescence level in the dark-adapted state (F_0) was measured using a modulated pulse (<0.05 μmol m^{-2} s^{-1} for 1.8 μs) too small to induce significant physiological changes in the plant (Schreiber et al., 1986). The data stored were an average taken over a 1.6 s period. Maximal fluorescence level in this state (F_m) was measured after applying a saturating actinic light pulse of 10,000 μmol m^{-2} s^{-1} for 0.8 s (Bolhàr-Nordenkampf and Öquist, 1993). The value of F_m was recorded as the highest average of two consecutive points. Values of the variable fluorescence ($F_v = F_m - F_0$) and maximum quantum efficiency of PSII photochemistry (F_v/F_m) were calculated from F_0