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Tolerance to and accumulation of arsenic in the cordgrass *Spartina densiflora* Brongn

Enrique Mateos-Naranjo *, Luis Andrades-Moreno, Susana Redondo-Gómez

Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, Apartado 1095, 41080 Sevilla, Spain

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ABSTRACT

A glasshouse study concerning the halophyte *Spartina densiflora* was carried out to determine its tolerance and capacity to accumulate As. *S. densiflora* presented a high tolerance to As-induced stress, since all plants were able to survive at concentrations higher than 6.7 mmol l^{-1} As. However, As increment caused a reduction in *S. densiflora* growth, owing to a decrease in net photosynthetic rate. This reduction was prompted by the adverse effect on the photochemical apparatus and the reduction in the absorption of essential nutrients, which was linked with the reduction in G_s, caused by the alteration of the K/Ca ratio, and with the reduction of photosynthetic pigment and Rubisco carboxilation. Arsenic tolerance was associated with the capacity to accumulate As in its roots (with values up to 2 mg g^{-1}) and largely avoid its transport to the leaves, this fact indicating that this species could be useful for arsenic phytostabilization purposes.

1. Introduction

Arsenic (As) is one of the most toxic elements in nature. Moreover, it is ubiquitous, being present in a wide range of environments, where it is highly noxious to all forms of life (Tripathi et al., 2007). It is considered as carcinogenic and its toxicity has become a global concern owing to the increasing contamination of water, soil and crops in many regions in the world (Rahman et al., 2007). Tripathi et al. (2007) explained that two strategies might help to counter the detrimental effects of As: (1) removal of As from the environment using different techniques. Actually, phytoaccumulation has recently gained importance because of its cost-effectiveness, long-term applicability and ecological aspects (Weis and Weis, 2004). This technology is based upon the ability of plants to absorb and accumulate metal contaminants in their tissues and eliminate a large amount of these elements from water or groundwater. On the other hand, (2) the development of safe crops that can be grown in presence of As contamination, minimizing the uptake and translocation of As to edible parts, by means of the transplant into tissues of a crop of genes from plants with this characteristic (Wei et al., 2005). Therefore, the research on species with a high capacity for accumulation and for growth under metal-polluted conditions can be of paramount importance for this purpose (Zhang et al., 2010).

In the salt marshes of the joint estuary of Tinto and Odiel rivers (SW Spain), one of the most polluted areas by heavy metals in the world (Sáinz and Ruiz, 2006) on account of the intense mining activity and waste from industrial zone, *Spartina densiflora* grows over sediments with between 37 and 697 mg kg^{-1} As (Mateos-Naranjo et al., 2011; Sáinz et al., 2002). This species has also demonstrated a high capacity for tolerance and accumulation of essential elements for plant development, such as copper and zinc (Mateos-Naranjo et al., 2008a,b). However, little is known about the effects of non-essential mineral elements such as As on *S. densiflora* and on plants in general. Overall, different authors have concluded that a high concentration of arsenic is toxic to most plants, since it interferes with metabolic processes and inhibits plant growth and development (Rahman et al., 2007). However, the information is very scarce in relation to the effect of arsenic on photosynthesis, the basis of plant bio-chemical system. This analysis could provide new insight into the tolerance of *S. densiflora* to arsenic stress.

The aim of this study was to evaluate the tolerance of *S. densiflora* to elevated concentrations of arsenic and quantify the capacity of this species for accumulating this element. The specific objectives were to: (1) analyze the growth of *S. densiflora* in experimental arsenic treatments ranging between 0 and 13.4 mmol l^{-1} As; (2) ascertain the extent to which the effects on the photosynthetic

Abbreviations: A, net photosynthetic rate; Chl *a*, chlorophyll a; Chl *b*, chlorophyll b; C_i, intercellular CO_2 concentration; *Cx+c*, carotenoids; F_0, minimal fluorescence level in the dark-adapted state; F_m, maximal fluorescence level in the dark-adapted state; F_v, variable fluorescence level in the dark-adapted state; F_v/F_m, maximum quantum efficiency of PSII photochemistry; Φ_{PSII}, quantum efficiency of PSII; G_s, stomatal conductance; LER, leaf elongation rate; LWC, leaf water content; NPQ, non-photochemical quenching; RGR, relative growth rate; WUE, water use efficiency.

* Corresponding author. Address: Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, Av. Reina Mercedes s/n, 41012 Sevilla, Spain. Tel.: +34 95 4557165; fax: +34 95 4615780.

E-mail address: emana@us.es (E. Mateos-Naranjo).

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apparatus (PSII chemistry), gas exchange characteristics and photosynthetic pigments determine plant performance with increasing arsenic; and (3) examine the response of accumulated calcium, copper, iron, potassium, magnesium, sodium, phosphorus and nitrogen to increasing external As and how this response affects growth.

2. Methods

2.1. Plant material

Seeds of *S. densiflora* were collected in December 2009 from Odiel Marshes, and stored at 4 °C (in darkness) for 3 months. After the storing period, seeds were placed in a germinator (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, for a month. Seedlings were then planted in individual plastic pots (11 cm of diameter) filled with pearlite 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). Immediately afterwards, the pots were allocated in shallow trays with 86 mM NaCl, since the growth of *S. densiflora* has an optimum at this external salinity concentration. The salinity treatment was established by combining 20% Hoagland's solution and NaCl of the appropriate concentration (Hoagland and Arnon, 1938). Thus, 3 l of the solution were placed in each of the trays (to a depth of 1 cm). The levels in the trays were monitored and they were topped up to the marked level with 20% Hoaglands solution (without NaCl) whenever necessary to maintain the salt concentration.

2.2. Stress treatments

In April 2010, after a month of seedling cultures, the pots were allocated to 5 As treatments in shallow trays (six pots per tray, with one tray per As treatment): 0, 0.7, 2.7, 6.7 and 13.4 mmol l^{-1} As, in the same glasshouse. Arsenic treatments were established by combining 20% Hoagland's solution and $Na_2HAsO_4{\cdot}7H_2O$ of the appropriate concentration. As concentrations were chosen to cover variations recorded by Sáinz et al. (2002) in the salt marshes of the joined estuary of Tinto and Odiel rivers.

At the beginning of the experiment, 3 l of the appropriate solution were placed in each of the trays to a depth of 1 cm. During the experiment, the levels in the trays were monitored and they were topped up to the marked level with 20% Hoagland's solution (without additional $Na_2HAsO_4{\cdot}7H_2O$) as a way to limit the change of As concentration due to water evaporation of the nutritive solution. In addition, the entire solution (including $Na_2HAsO_4{\cdot}7H_2O$) was changed weekly.

2.3. Growth and survival analysis

At the beginning and at the end of the experiment (after 30 days of treatment), four and six entire plants (roots and leaves), respectively, from each treatment were dried at 80 °C for 48 h and weighed. Also, before and after the As treatment, the number of all fully developed tillers and the percentages of alive and dead tillers were recorded; a tiller was considered dead when no green leaves remained.

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

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

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

Leaf elongation rate (LER) was measured in random leaves at the end of the experiment (n = 12, per treatment; two measurements per plant) by placing a marker of inert sealant at the base of the youngest accessible leaf. The distance between the marker and the leaf base was measured after 24 h (Mateos-Naranjo et al., 2008b).

2.4. Gas exchange

Gas exchange measurements were taken on random, fully expanded leaves (n = 10, a measurement per plant and four extra taken randomly) using an infrared gas analyzer in an open system (Li-6400, Li-COR Inc., Neb., USA) after 30 days of treatment. Net photosynthetic rate (A), intercellular CO_2 concentration (C_i), stomatal conductance to CO_2 (G_s) and transpiration rate were determined at ambient CO_2 concentration of 380 μmol mol^{-1}, temperature of 20/25 °C, 50 ± 5% relative humidity and a photon flux density of 1000 μmol m^{-2} s^{-1}. A, C_i and G_s were calculated using standard formulae of Von Caemmerer and Farquhar (1981). Photosynthetic area was approximated as the area of a trapezium. Water use efficiency (WUE) was calculated as the ratio between A and transpiration rate [mmol (CO_2 assimilated) mol^{-1} (H_2O transpired)].

2.5. Leaf water content

Leaf water content (WC) was calculated after 30 days of treatment as:

$$\mathrm{WC} = (FW - DW)/FW \times 100$$

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

2.6. Photosynthetic pigments

At the end of the experiment period, photosynthetic pigments in fully expanded penultimate leaves (n = 10, a measurement per plant and four extra taken randomly) 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 ($Cx + c$) 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 by calculation, using the method of Lichtenthaler (1987).

2.7. Measurement of chlorophyll fluorescence

Chlorophyll fluorescence was measured in random, fully developed penultimate leaves (n = 12, two measurements per plant) using a portable modulated fluorimeter (FMS-2, Hansatech Instrument Ltd., England) after 30 days of treatment. Light- and dark-adapted fluorescence parameters were measured at dawn (stable, 50 μmol m^{-2} s^{-1} ambient light) and at midday (1600 μmol m^{-2} s^{-1}) to investigate whether As concentration affected the sensitivity of plants to photoinhibition.

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. The data stored were an average taken over a 1.6 s period. Maximal fluorescence in this state (F_m) was measured after applying a saturating actinic light pulse of 15,000 μmol m^{-2} s^{-1} for 0.7 s. The value of F_m was recorded as the highest average of two consecutive points. Values of the