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Growth and survival of *Halimione portulacoides* stem cuttings in heavy metal contaminated soils

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ABSTRACT

The halophytic shrub *Halimione portulacoides* demonstrates a high tolerance to heavy metal contamination and a capacity for accumulating metals within its tissues. On the Iberian Peninsula, this species has colonized habitats with high levels of metal pollution. The aim of this study is to analyze the response of *H. portulacoides* stem cuttings to this pollution. Growth, photosynthesis and metal uptake were examined in *H. portulacoides* through an experiment in which stem cuttings were replanted in metal-contaminated soil. This condition decreased growth and lowered both photosynthetic rate and stomatal conductance. Reduced photosynthetic performance was largely due to the reduced concentration of photosynthetic pigments. Despite these responses, there was some important evidence suggesting the phytoremediatory potential of *Halimione* stem cuttings. The results of our study indicate that this salt-marsh shrub may represent a biotool of value in the restoration of polluted areas.

1. Introduction

Planting of cuttings has specific advantages over seed sowing for restoration programmes. Cuttings may theoretically be taken at any time of the year from natural stocks while seeds are only available for relatively short periods and for some species a sufficient supply of seeds, and thus seedlings, is not always available (Huiskes, 1979; Woodhouse, 1982; Gomes Neto et al., 2006). Although potentially efficient, this system is limited in practice by the capability of cuttings to develop a well-structured root system under stressful conditions. The ability to regenerate from stem cuttings should be considered an important characteristic of metal-tolerant plants as it can enhance fast and continuous biomass production, which is an ideal property for phytoremediation. In this way, the selection of species capable of rapid establishment from cuttings in highly polluted soils could contribute to the improved success of restoration projects in heavy metal-contaminated areas.

Halimione portulacoides (L.) Aellen is a halophytic shrub frequently found on sandy and muddy coastlines and salt marshes around the coasts of Europe, North Africa and South–West Asia. It is frequently the physiognomically dominant species on well-drained and upper marshes, and often fringes channels and pools that are flooded at high tide (Chapman, 1950). Several recent studies have demonstrated that *H. portulacoides* is highly tolerant to elevated concentrations of heavy metals (Almeida et al., 2009; Duarte et al., 2007; Sousa et al., 2008; Cambrollé et al., 2012a,b). Although stem cuttings of *H. portulacoides* have been shown to regenerate both roots and aerial plant tissues in a sterile distilled water medium, there have been no studies exploring the ability of cuttings of this species to establish in metal polluted soils. The aim of the present study was to evaluate the regeneration potential of stem cuttings of *H. portulacoides* in the presence of high concentrations of heavy metals, by analyzing the survival, growth and photosynthetic response following replantation in highly metal-contaminated soils.

2. Materials and methods

2.1. Plant material and soil sample collection

Seeds of *H. portulacoides* were collected in the salt marshes of "La Mata-Torrevieja" (Alicante, SE Spain). This salt marsh area does not present elevated levels of heavy metals. The collected seeds were subsequently germinated in perlite moistened with distilled water and maintained at 25 °C for 30 days. The resulting seedlings

Abbreviations: A, net photosynthetic rate; Chl *a*, chlorophyll a; Chl *b*, chlorophyll b; C_i, intercellular CO_2 concentration; C*x*+*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; ΦPSII, quantum efficiency of PSII; Gs, stomatal conductance; NPQ, non-photochemical quenching; RGR, relative growth rate.

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were transplanted in individual plastic pots (diameter 11 cm) filled with perlite, and placed in a glasshouse with minimum–maximum temperatures of 21–25 °C, 40–60% relative humidity and natural daylight (minimum and maximum light flux: 200 and 1000 μmol m^{-2} s^{-1}, respectively). Pots were carefully irrigated with 20% Hoagland's solution (Hoagland and Arnon, 1938), as required. In addition, samples were taken from the first 15 cm depth of sediment in the Tinto marshes. All of these sediment samples were placed into plastic bags and subsequently transported to the laboratory. The area was chosen on the basis of published levels of heavy metal contamination (Sáinz and Ruiz, 2006). Tinto marsh, where the study species normally grows, presents elevated concentrations of trace metals and low pH values in both the water and sediment.

2.2. Soil measurements

Soil samples were dried at 80 °C for 48 h. The dried samples were then ground and homogenized by sieving through 2 mm nylon mesh in order to remove large stones and dead material. Measurements of soil conductivity, pH, redox potential and organic matter content ($n = 5$) were taken in the laboratory. Conductivity of the soil water was measured using a conductivity meter (Crison-522, Spain), following dilution with distilled water (1:1). Redox potential and pH of the soil were determined with a portable meter and calibrated electrode system (Crison pH/mV p-506, Spain). Organic matter content was calculated by measuring the loss on ignition of sediment samples; the furnace temperature was raised slowly over 6 h to 550 °C and maintained for a further 8 h. The proportions of sand, silt and clay in the sediment were determined by means of the Bouyoucos hydrometer method (Bouyoucos, 1936). The physicochemical properties of the soil are presented in Table 1. Soil metal concentrations were determined using the method employed by Cambrollé et al. (2008) and are presented in Table 4.

2.3. Plant response to soil treatment experiment

After 12 month of growth, apical stem cuttings of 20 cm were taken from the seedlings, with 25–35 leaves per cutting. The cuttings were then allocated to individual plastic pots filled with homogenates of the Tinto soil. Other stem cuttings were allocated to individual plastic pots filled with perlite (control treatment). Pots were placed in shallow trays (26 pots per tray, one tray per treatment; $n = 52$). At the beginning of the experiment, 3 l of 20% Hoagland's solution (for the control treatment) and 3 l of distilled water (for the soil treatment) were placed in the trays to a depth of 1 cm. During the experiment, the levels of Hoagland's solution or distilled water in the trays were monitored and maintained. At the end of the experiment, 120 d after transplantation, all complete plants (leaves, stems and roots) were harvested from the soil treatment and control and dried at 80 °C for 48 h before being weighed. In addition, gas exchange, chlorophyll fluorescence and pigment concentration measurements were measured in randomly selected fully expanded leaves. Finally, the concentrations of heavy metals in the tissues were determined.

Table 1
Physicochemical properties of soil from Tinto marshes. Values represent mean ± S.E, $n = 5$.

Physico-chemical properties				
Texture (silt/clay/sand percentage)	Redox potential (mV)	Conductivity (mS cm^{-1})	pH	Organic matter (%)
71/13/16	353 ± 12	15.5 ± 0.9	4.9 ± 0.3	11.6 ± 0.4

2.4. Growth analysis

From each treatment, 6 plants (stem cuttings) were harvested at the beginning, and the remaining 20 at the end of the experiment (i.e. following 120 d of treatment). These plants were dried at 80 °C for 48 h and then weighed.

The relative growth rate (RGR) of whole plants was calculated using the formula:

$$RGR = (\ln Bf - \ln Bi) \cdot D^{-1} (g\ g^{-1} day^{-1})$$

where Bf is the final dry mass, Bi the initial dry mass (average of the six stem cuttings from each treatment dried at the beginning of the experiment) and D is the duration of experiment (days).

2.5. Gas exchange

Gas exchange measurements were taken from randomly selected, fully expanded leaves ($n = 20$, one measurement per plant), following 120 d of treatment, using an infrared gas analyzer in an open system (Li-6400, Li-COR Inc., Neb., USA). Net photosynthetic rate (A), intercellular CO_2 concentration (C_i) and stomatal conductance to CO_2 (G_s) were determined at an ambient CO_2 concentration of 360 ppm CO_2, temperature of 20 °C, 50 ± 5% relative humidity and a photon flux density of 1000 μmol m^{-2} s^{-1}. Values of the paramenters A, C_i and G_s were calculated using the standard formulae of Von Caemmerer and Farquhar (1981).

2.6. Chlorophyll fluorescence

Chlorophyll fluorescence was measured in randomly selected fully developed leaves ($n = 20$) using a portable modulated fluorimeter (FMS-2, Hansatech Instrument Ltd., England). 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}), in order to investigate whether metal pollution of the soil affected the sensitivity of plants to photoinhibition.

Plants were dark-adapted for 30 min, using purpose designed leaf clips. Minimal fluorescence level was measured in the dark-adapted state (F_0) using a modulated pulse (<0.05 μmol m^{-2} s^{-1} for 1.8 μs), which was too small to induce significant physiological changes in the plant. The data recorded represented 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 variable fluorescence ($F_v = F_m - F_0$) and the maximum quantum efficiency of PSII photochemistry (F_v/F_m) were calculated from F_0 and F_m. This ratio of variable to maximal fluorescence correlates with the number of functional PSII reaction centers, while the dark-adapted values of F_v/F_m can be used to quantify photoinhibition (Krivosheeva et al., 1996).

The same leaf section of each plant was used to measure light-adapted parameters. Steady state fluorescence yield (F_s) was recorded following adaptation of the plants to ambient light conditions for 30 min. A saturating actinic light pulse of 15,000 μmol m^{-2} s^{-1} for 0.7 s was then used to produce the maximum fluorescence yield (F'_m) by temporarily inhibiting the PSII photochemistry.

Using fluorescence parameters determined in both light- and dark adapted states, the following values were calculated: quantum efficiency of PSII ($\Phi PSII = (F'_m F_s / F'_m)$, which measures the proportion of light absorbed by the chlorophyll associated with the