Estuarine, Coastal and Shelf Science 175 (2016) 10–14

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Short communication

Advances in the use of *Halimione portulacoides* stem cuttings for phytoremediation of Zn-polluted soils

Jesús Cambrollé*, Juan M. Mancilla-Leytón, Sara Muñoz-Vallés, Antonio Cambrón-Sena, M. Enrique Figueroa

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

ARTICLE INFO

Article history:
Received 23 July 2015
Received in revised form
28 January 2016
Accepted 26 March 2016
Available online 28 March 2016

Keywords:
Halimione portulacoides
Heavy metals
Phytoremediation
Pollution tolerance
Wetlands

ABSTRACT

The salt-marsh shrub *Halimione portulacoides* can grow in soils containing extremely high concentrations of Zn and stem cuttings have recently been shown to aid the recovery of polluted soils although further work on this methodology have is required. A greenhouse experiment was carried out to analyze the effects of a range of Zn concentrations (0–130 mmol l^{-1}) on the establishment, growth and photosynthetic performance of stem cuttings of different sizes of *Halimione portulacoides*, with the aim of determine the phytotoxicity thresholds and the optimal initial size of the cuttings to be used in phytoremediation strategies. Stem cuttings were able to survive and grow at external Zn concentrations of 130 mmol l^{-1}. Plants from smaller cuttings showed greater growth inhibition, which could have been related to the inability to establish a root system sufficiently developed so as to avoid the translocation of Zn to aerial parts. The present study demonstrates that stem cuttings of *H. portulacoides* can establish and develop a root system under concentrations as high as 130 mmol l^{-1} Zn (approximately 9000 mg kg^{-1}). In highly polluted soils, with concentrations from 50 mmol l^{-1}, it would be advisable to use stem cuttings with a minimum size of approximately 10 cm in length and a minimum biomass of 100 mg dry weight. This study indicate that the use of *H. portulacoides* stem cuttings could play an important role in the restoration of coastal ecosystems contaminated with heavy metals.

1. Introduction

Soil contamination by trace metals became a major environmental issue worldwide in the last century as a consequence of the intensification of mining and mineral processing, which dispersed toxic trace metals and led to widely contaminated areas. Heavy metals are deemed serious pollutants because of their toxicity, persistence and non-degradability in the environment. Despite this, certain plant species have evolved tolerance to heavy metals and remain unaffected under exposure to extremely high concentrations of these elements (Ernst et al., 2000). Some of these metal-tolerant plants can be used to decontaminate environments with high metal concentrations.

Phytoremediation, the use of plants to extract, sequester, and/or detoxify pollutants, is widely viewed as the ecologically responsible alternative to the environmentally destructive physical remediation methods currently practiced (Meagher, 2000; Garbisu et al., 2002). This technology requires knowledge of some biological aspects of the species to be used. In this sense, when implementing this type of technologies, it is essential to know the phytotoxicity limits, the accumulation capacity and the mechanisms of tolerance of the plant species, amongst other aspects. Knowing the strategy of metal uptake and tolerance of a certain species, it is possible to evaluate its potential for its use in the revegetation of contaminated soils or even for the phytoextraction of metals in highly polluted areas (Dahmani-Muller et al., 2000).

Halimione portulacoides (L.) Aellen is a halophytic shrub frequently found on sandy and muddy sea-shores and salt marshes around the coasts of Europe, North Africa and South-West Asia. The species is frequently the physiognomic dominant on well-drained and upper marshes, often fringing channels and pools that are flooded at high tide (Chapman, 1950). Although there are studies

Abbreviations: A, net photosynthetic rate; F_0, minimal fluorescence level in the dark-adapted state; F_m, maximal fluorescence level in the dark-adapted state; F_s, steady state fluorescence yield; F_v, variable fluorescence level in the dark-adapted state; F_v/F_m, maximum quantum efficiency of PSII photochemistry; RGR, relative growth rate.

* Corresponding author.
E-mail address: cambrolle@us.es (J. Cambrollé).

http://dx.doi.org/10.1016/j.ecss.2016.03.019

focused on evaluating the phytoremediation capacity of this species, and its tolerance mechanisms and phytotoxicity limits have been recently investigated (Cambrollé et al., 2012a, b), some basic aspects, which are essential to understand in order to put decontamination strategies successfully into practice, are still completely unknown.

Recently, Andrades-Moreno et al. (2013) explored for the first time the ability of *H. portulacoides* to develop from stem cuttings under high concentrations of heavy metals, highlighting the possible applicability of cuttings of this species for the recovery of polluted soils. The use of stem cuttings in phytoremediation can have important advantages over the use of adult plants. 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 (Woodhouse, 1982; Gomes Neto et al., 2006). In addition to this, in the case of species with high regeneration potential such as *H. portulacoides*, the use of stem cuttings can reduce time and costs in restoration projects. However, some determining aspects for the use of this methodology, such as the phytotoxicity limits and the optimal size of stem cuttings for transplantation, have not yet been studied.

The main objective of this study was to evaluate Zn tolerance in stem cuttings of different sizes of *H. portulacoides*. The specific objectives were: (1) to determine the Zn phytotoxicity thresholds of stem cuttings of different initial sizes, by analyzing the establishment, survival and growth of plants in a range of external Zn concentrations, from 0 to 130 mmol l^{-1}; (2) to ascertain the extent to which Zn determines plant performance in plants grown from stem cuttings, in terms of influence on the photosynthetic apparatus (PSII chemistry) and gas exchange characteristics; and (3) to determine the optimal initial size of the stem cuttings of *H. portulacoides* to be used in phytoremediation strategies for the recovery of heavy metal-polluted soils.

2. Materials and methods

2.1. Plant material and stress treatments

Seeds of *Halimione portulacoides* were collected in the salt marshes of 'La Mata-Torrevieja' (Alicante, SE Spain). The collected seeds were subsequently germinated in perlite moistened with distilled water, and maintained at 25 °C for 30 days. The resulting seedlings were sown 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.

When seedlings were between 20 and 25 cm in height (after 4 months of growth), apical stem cuttings were taken from the seedlings. Cuts were made at different lengths with the aim of obtaining stem cuttings of three different sizes: small cuttings, with 2 nodes (including the apical node), approx. 35 mg dry weight and 5 cm long; medium cuttings, with 4 nodes (including the apical node), approx. 110 mg dry weight and 10 cm long; and large stem cuttings, with 6 nodes (including the apical node), approx. 280 mg dry weight and 15 cm long. Then, the stem cuttings were planted 4 cm deep in pots with vermiculite moistened with Hoagland's solution.

The stem cuttings of the different sizes were subjected to different Zn treatments: 0, 15, 50 and 130 mmol l^{-1}. One tray was used for each Zn treatment and cutting size, with 12 pots per tray. Zn treatments were prepared and monitored following Cambrollé et al. (2012b).

2.2. Growth

From each treatment, four complete plants (roots and shoots) were harvested at the beginning, and the remaining eight at the end of the experiment (i.e. following 70 days of treatment). These plants were dried at 80 °C for 48 h and then weighed. Relative growth rate (RGR) of whole plants and leaf area were calculated following the methods described in Cambrollé et al. (2012b). Plant height was measured from the base of the stem to the tip of the uppermost leaf.

2.3. Gas exchange

Gas exchange measurements were taken from randomly selected, fully expanded leaves (n = 12, one measurement per plant plus four extra measurements taken randomly per treatment), following 70 days of treatment, using an infrared gas analyzer in an open system (LI-6400, LI-COR Inc., Neb., USA). Net photosynthetic rate (A) was determined at an ambient CO_2 concentration of 400 μmol mol^{-1}, temperature of 20/25 °C, 50± 5% relative humidity and a photon flux density of 1000 μmol m^{-2} s^{-1}. Values of the parameters were calculated using the standard formulae of Von Caemmerer and Farquhar (1981).

2.4. Chlorophyll fluorescence

Chlorophyll fluorescence was measured in randomly selected, fully developed leaves (n = 12; one measurement per plant and four extra measurements taken randomly per treatment) using a portable modulated fluorimeter (FMS-2, Hansatech Instruments Ltd., England) at the end of the experimental period, following the methods described in Cambrollé et al. (2012a). Values of variable fluorescence ($F_v = F_m - F_0$) and 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 centres, and dark adapted values of F_v/F_m can be used to quantify photoinhibition (Krivosheeva et al., 1996).

2.5. Statistical analysis

Statistical analysis was carried out using Statistica v. 6.0 (Statsoft Inc.). Pearson coefficients were calculated to assess the correlation between different variables. Data were analyzed using one- and two-way analysis of variance (*F*-test). Data were tested for normality with the Kolmogorov-Smirnov test and for homogeneity of variance with the Brown-Forsythe test. Tukey tests were applied to significant test results for identification of important contrasts. Measured differences between fluorescence at dawn and midday were compared by the Student test (*t*-test).

3. Results

3.1. Survival

All the medium and large stem cuttings developed roots and survived during the 70 days of the experiment in the different treatments tested. 100% of the small stem cuttings survived at Zn concentrations of up to 50 mmol l^{-1}, whereas the survival rate of those treated with 130 mmol l^{-1} was 75%. At the highest Zn concentration, symptoms of chlorosis and necrosis were observed in leaf tissue; these effects were especially visible in the small stem cuttings.