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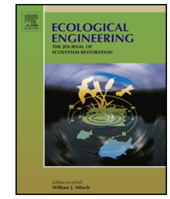

Short communication

Comparative study of Cd tolerance and accumulation potential between *Cakile maritima* L. (halophyte) and *Brassica juncea* L.

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ABSTRACT

In this work we evaluated Cd-phytoextraction ability of the halophyte *Cakile maritima* comparatively to the glycophyte *Brassica juncea* commonly recommended for phytoextraction. Seedlings were grown in nutrient solution added with 0–100 μM Cd for 21 days. Cd impaired growth in *B. juncea* but had no significant impact on *C. maritima*. The halophyte *C. maritima* maintained also higher photosynthetic activity than the glycophyte *B. juncea*. Cd decreased leaf chlorophyll (Chl) and carotenoids concentrations as well as PSII efficiency (F_v/F_m, F_v/F_0 and Φ_{PSII}) in *B. juncea* while it increased intercellular CO_2 concentration in this species. Shoot Cd content was higher in the halophyte *C. maritima* reaching 1365 μg g^{-1}dw at 100 μM while it was 548 μg g^{-1}dw in *B. juncea* at the same dose. The translocation factor (TF) was higher for *C. maritima* than for *B. juncea* at all external Cd doses. It is concluded that the halophyte *C. maritima* could be considered as a promising plant material for Cd-phytoextraction.

1. Introduction

Cd is placed as seventh hazardous substances list as provided by the American Agency for Toxic Substance and Disease Registry (Kamnev and van der Lelie, 2000). This metal cannot be biodegraded and must be extracted from contaminated soils. Phytoextracion is less expensive and more environmental friendly than conventional remediation techniques (Zhang et al., 2013). However, identification of suitable plants for this process is the most important and difficult task.

Plants for this purpose need to combine high Cd tolerance and high Cd accumulation in shoots. *Noccaea cerulescens* a Cd-hyperaccumulator gathers both requisites (Lombi et al., 2000) but slow growth and low biomass limit its application in phytoextraction. The fast growing *Brassica juncea*, although not a hyperaccumualtor, has been found to tolerate considerable shoot metal concentrations (Sanità di Toppi et al., 2001; Zaier et al., 2010) and this species is considered as a reference species for Cd tolerance and accumulation (Mohamed et al., 2012; Sharma et al., 2010; Singh et al., 2007). More recently, it has been suggested that salt-tolerant plant species would be more efficient to cope with heavy metals (Ghnaya et al., 2005; Jordan et al., 2002; López-Chuken and Young, 2005; Zaier et al., 2010), than salt-sensitive (glycophytic) crop plants commonly chosen for phytoextraction. Halophytes are able to sequester Cl^- and Na^+ in tissues without expressing toxicity. Several studies demonstrated that some tolerance mechanisms operating at the whole-plant level are not always specific to sodium and could be applied to metals (Lutts et al., 2004; Sousa et al., 2008). *C. maritma* is fast growing halophyte tolerates NaCl up to 500 mM (Debez et al., 2004). This species colonizes also heavy metals contaminated saline soils suggesting its tolerance to these elements.

In this work, we conducted to a comparative physiological study to evaluate the potential of Cd tolerance and accumulation in the halophyte *Cakile maritima* (Brassicaceae) compared with that of *Brassica juncea*. It reports the impact of increasing Cd concentrations (0–100 μM) on growth, nutrition, photosynthesis and Cd accumulation in both species. The applied objective of this

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study will be the use of this halophyte to rehabilitate saline Cd-contaminated soils.

2. Materials and methods

2.1. Plant material and culture conditions

Seeds of *Cakile maritima* were harvested from the beach of Raoued (suburb of Tunis). Seeds of Indian mustard (*Brassica juncea*, accession no. 426308) were kindly provided by the North Central Regional Plant Introduction Station of the US Department of Agriculture. The experiments were carried out under glass house conditions (16 h photoperiod, day/night temperatures of 25/20 °C and 55/75% relative humidity). After seed germination, seedlings were transferred to plastic pots filled with 5 L Hoagland's nutrient solution (pH = 5.8). Two weeks later, plants were randomly assigned to four different Cd treatments: 0–100 μM $CdCl_2$ during 21 days. At harvest, plants were divided into shoots and roots. Roots were immediately dipped in a cold solution of HCl (0.01 M) during 5 min to eliminate elements adsorbed at the root surface and then gently blotted with filter-paper (Ghnaya et al., 2007). Fresh weight of organs was immediately measured and the dry one after the desiccation of shoots and roots at 60 °C. The relative growth rate (RGR) was calculated according to Hunt (1990).

2.2. Pigment content

Pigments were extracted by placing 50 mg of fresh leaf in 2 mL of 100% acetone. The samples were incubated in darkness until complete chlorophyll extraction. Chlorophyll and carotenoids contents in supernatants were analyzed spectrophotometrically at 644.8, 661.6 and 470 nm.

2.3. Photosynthetic parameter measurements

2.3.1. Leaf gas exchanges

The net photosynthetic rate (Pn), stomatal conductance (gs), intercellular CO_2 concentration (Ci), transpiration rate (E) and water use efficiency (WUE; defined as the ratio Pn/E) were measured in the third fully expanded leaf from the top shoot at the end of the treatment. All measurements have been made between 11:00 a.m. and 13:00 p.m., at light saturation intensity. Six plants per treatment were assessed using a portable photosynthesis system (LCpro32471, ADC BioScientific).

2.3.2. Chlorophyll fluorescence

Parameters were recorded in parallel to gas exchange measurements on the same leaf, using a direct portable fluorometer. Leaves were acclimated to dark for 20 min before measurements were taken. After measuring the initial fluorescence (F_0), maximal fluoroscence (F_m) was determined at the beginning of each measurement using a saturating pulse of 9000 μ mol m^{-2} s^{-1} for 0.7 s. The variable fluorescence (F_v) was calculated as $F_v = F_m - F_0$. The maximum PSII quantum yield ($F_v/F_m = (F_m - F_0)/F_m$) and the effective quantum efficiency of PSII ($\Phi_{PSII} = (F'_m - F_s)/F'_m$) and the non-photochemical quenching of chlorophyll fluorescence, NPQ = $(F_m - F'_m)/F'_m$.

2.4. Determination of Cd and mineral elements

Ca, Mg, Fe, Zn and Cd concentrations were measured, after complete mineralization of tissues in 4/1 (v/v) $HNO_3/HClO_4$ mixture at 100 °C (Ghnaya et al., 2007), by atomic absorption spectrophotometry (Spectra AA 220 FS, Varian). K concentrations were determined in the same homogenate by flame spectrometry and the total nitrogen content in shoots was determined according to Kjeldahl method**.**

2.5. Statistical analysis

Analyses of variance (ANOVA) with orthogonal contrasts and mean comparison procedures were used to detect differences between treatments. Mean separation procedures were conducted using the multiple range tests with Fisher's least significant difference (LSD) ($P < 0.05$).

3. Results

3.1. Effect of Cd on plant morphology and growth

During the first week of treatment, cadmium up to 100 μM did not cause any visible toxicity symptoms in *C. maritima* while it induced chlorosis in *B. juncea*. After two weeks exposure to 100 μM Cd, severe chlorosis and leaf abscission was observed in *B. juncea*. In *C. maritima*, leaves howed chlorosis but no abscission occurred even at 100 μM Cd^{2+}.

For all treatments, *B. juncea* produced more dry matter than *C. martima*. Cd significantly reduced biomass in *B. juncea* (Fig. 1A). Contrastingly, Cd had no significant impact on biomass production in the halophyte (Fig. 1A). The variation of growth activity (RGR) in response to shoot Cd accumulation, (Fig. 1B), we demonstrated that in *B. juncea* Cd sequestered in the shoots reduced RGR from high 0.14 to low 0.11 irrespective to variation of external Cd concentration. Nevertheless, despite the higher Cd-shoot concentration, growth activity was slightly and insignificantly decreased in *C. maritima* (Fig. 1B).

3.2. Cadmium accumulation and translocation

Shoot Cd concentrations in both species increased with increasing external Cd (Table 1). For all Cd doses, the halophyte *C. martima* accumulated much more Cd in the shoots than *B. juncea*. At 100 μM, shoot Cd concentration in *C. maritima* was three times higher than in *B. juncea* (Table 1). The translocation factor (TF), were higher in *C. maritima* than in *B. juncea* at all Cd doses, (Table 1).

3.3. Cd effect on nutriment concentrations

Cd induced a drastic decrease in Ca shoot accumulation in *B. juncea* which accentuated with increasing Cd supply (Table 1). Ca shoot concentrations in *C. maritima* were less affected by Cd and showed significant decrease only at 100 μM. Mg accumulation was less affected than Ca and showed a reduction in *B. juncea* leaves when Cd supply exceeded 50 μM. K concentration decreased in the shoots *of B. juncea* subjected to Cd but no significant effect was recorded in the halophyte *C. maritima*. Total nitrogen concentrations in the shoots of plant were not affected by Cd in any of the species.

Even at the lowest concentration supplied, Cd caused a significant decrease in shoot Fe and Zn concentrations in both species (Table 1).

3.4. Cadmium effect on chlorophyll and carotenoid contents

Cadmium supply had a strong negative effect on the concentrations of chlorophylls and carotenoids in *B. juncea* but not in *C. maritima.* (Fig. 1C).