Available online at www.sciencedirect.com
ScienceDirect
Environmental and Experimental Botany 62 (2008) 69–77
Environmental and Experimental Botany
www.elsevier.com/locate/envexpbot

Efficiency of antioxidant response in *Spartina densiflora*: An adaptative success in a polluted environment☆

D. Martínez-Domínguez, M.A. de las Heras, F. Navarro, R. Torronteras, F. Córdoba*

Department of Environmental Biology and Public Health, Faculty of Experimental Sciences, University of Huelva, E-21071 Huelva, Spain

Received 10 July 2007; accepted 25 July 2007

Abstract

The effects of different culture conditions, unpolluted and polluted substrates, on an antioxidative system – antioxidant enzymes, such as catalase, ascorbate peroxidase and guaiacol peroxidase, and ascorbic acid – were investigated to establish its relationship with the acclimatization success of *Spartina densiflora*. Plants of this species growing in the polluted Odiel marshes (Huelva, Spain) showed high levels of catalase, ascorbate and guaiacol peroxidase activities and ascorbate concentration (reduced and oxidized ascorbate). In addition, we found significant oxidation of the ascorbate pool, since only 40% of ascorbate was reduced, and low levels of photosynthetic pigments, suggesting that an oxidative stress was impairing *S. densiflora*. Transplantation to an unpolluted substrate in the laboratory led to a gradual change in all tested parameters: antioxidative activities and total ascorbate concentration decreased while the percentage of reduced ascorbate and pigment concentrations increased; these data agreed with the hypothesis that oxidative stress conditions in *S. densiflora* habitat were due to a polluted substrate. After 28 days, the plants were transplanted for a second time to polluted conditions, equivalent to those in their habitats, and a rapid alteration of the antioxidative system was observed. In the first 24 h, catalase and guaiacol peroxidase activities and ascorbate concentration increased greatly and the percentage of reduced ascorbate fell drastically. Regardless of this fact, ascorbate peroxidase activity did not change until the end of the first week, while photosynthetic pigments declined at a constant rate during the whole culture period. Subsequently, we found that the antioxidative system improved its reductive capacity gradually and slowly – over weeks – but this reductive power was rapidly lost within days or even hours. It may be concluded that *S. densiflora* undergoes oxidative stress in its natural environment and is able to modulate its antioxidative system, based on the degree of pollution, in order to acclimatize successfully to its fluctuating environment.

Keywords: *Spartina densiflora*; Metal pollution; Ascorbate; Oxidative stress; Environmental adaptation

1. Introduction

High levels of metal pollution occur in the Tinto-Odiel estuary (Huelva, SW Spain), due to the wastes from nearby petrochemical industries and natural biolixiviation, together with effluents from the ancient mining activity in the Iberian Pyrite Belt located in the river basin. Metals such as Fe, Ni, Cu, Pb, Zn, As, Cd, etc. are abundant in the water and sediments (e.g. Luque et al., 1998; Santos-Bermejo et al., 2003; Nieto et al., 2007; personal data). At present, the Tinto-Odiel estuary is considered as one of the most contaminated estuaries in the world (Environmental Agency of Andalusia, 1994). However, a very high biodiversity is present in the estuary, mainly at the Odiel marshes, thus the varied marsh organisms are exposed to toxic levels of contamination. Moreover, the marsh plants are exposed to changeable salinity levels, under the influence of the sea. This environment is unstable due to the discontinuous contribution of metals, variable salinity and fluctuations in fresh water availability, depending on the tide regime, sunshine hours and industrial activities. Under these conditions, marsh plants must be able to respond rapidly to unpredictable changes in the substrate, which probably leads to a variable degree of oxidative stress.

It is well-known that reactive oxygen species (ROS) are produced in normal metabolic processes in all aerobic organisms

☆ This work was supported by Grants AGL2003-06555 (Ministerio Educación y Ciencia) and CVI 282 (Plan Andaluz de Investigación, Junta de Andalucía, Spain).
* Corresponding author at: Department of Environmental Biology and Public Health, Faculty of Experimental Sciences, Bulevar de las Artes y las Ciencias, s/n, University of Huelva, 21071 Huelva, Spain. Tel.: +34 959219875; fax: +34 959219876.
E-mail address: fcordoba@uhu.es (F. Córdoba).

0098-8472/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2007.07.005

(Asada and Takahashi, 1987; Mittler, 2002) and also well-established is their implication as key molecules in pathogen defense, programmed cell death, abiotic stress responses and systemic signaling (Desikan et al., 2001; Mittler, 2002). However, several stress conditions (metal pollution, salt stress, chilling, UV radiation, pathogen attack, etc.) can unbalance the steady-state level of ROS production (Foyer et al., 1997). These ROS include the superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2), which are produced during electron transport activities in the cell membrane as well as by a number of metabolic pathways (Shi et al., 2006). ROS accumulation induces oxidative processes such as membrane lipid peroxidation, protein oxidation, enzyme inhibition and DNA and RNA damage, resulting in cell damage and, eventually, cell death (Dat et al., 2000; Hammond-Kosack and Jones, 1996). To avoid the deleterious effect of ROS, plant cells possess efficient antioxidant defense mechanisms, comprising both enzymatic components, such as ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD) or guaiacol peroxidase (POD); as well as non-enzymatic components, such as ascorbic acid (ASC) or glutathione (Arrigoni and De Tullio, 2002). The capacity of ascorbic acid to eliminate directly or indirectly – via APX activity – different ROS including singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radicals is widely reported (Foyer and Halliwell, 1976; Asada and Takahashi, 1987; Padh, 1990; Asada, 1992). These defensive mechanisms against oxidative damage have been specifically observed in plants subjected to saline stress (Gossett et al., 1994, 1996; Lee et al., 2001) or metal pollution, in the form of excessive iron (Kampfenkel et al., 1995), similar environmental conditions applying to the plants growing in the Odiel marshes.

Marsh plants may be suitable to investigate defensive responses against environmental stress from multiple and variable sources. In our study, we chose *Spartina densiflora* Brong, an invasive cordgrass from South America (Mobberley, 1956), because of its wide distribution in the Odiel marshes, salinity tolerance and metal accumulating properties (Luque et al., 1999). The successful adaptation of this species to stressing conditions in the Odiel marsh could be related to an efficient defensive antioxidant mechanism. If so, we can expect that *Spartina* would rapidly modulate its antioxidant system when environmental stressors, mainly metals in soils, fluctuate from low to high levels (and vice versa). In order to validate this hypothesis, we have developed a new experimental approach with *Spartina* individuals exposed to different soil conditions (polluted versus unpolluted) in a continuously controlled culture. The present study reveals that *S. densiflora* undergoes oxidative stress in its polluted location but is able to rapidly modulate its redox status when cultured under the presence or absence of polluting agents.

2. Materials and methods

2.1. Plant material and growth conditions

S. densiflora specimens were collected in the Odiel River salt marshes (Huelva, SW, Spain). Ten plants of *S. densiflora* were transplanted from their polluted habitat to individual pots in our laboratory and divided into two groups. A group of five plants, denominated *Control plants*, was cultivated using their natural, metal-polluted sandy substrate. Another group of five plants, named *Experimental plants*, was cultivated in non-stressing conditions with clean, metal-unpolluted substrate (marine sand). After 28 days of culture (*period A*), both Control and Experimental plants were again transplanted to a polluted substrate obtained from the marshes, and their growth was followed for 15 days (*period B*). A schematic drawing of the experimental design is shown in Fig. 1.

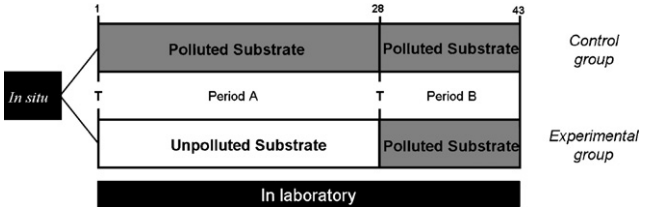

Fig. 1. Scheme of the experimental design. Ten plants of *Spartina densiflora* were collected *in situ* and transplanted (T) to individual pots in the laboratory: five pots contained polluted substrate as *in situ* (*Control plants*) and the other five pots contained unpolluted substrate (*Experimental plants*). After 28 days in these conditions (Period A), all plants were again transplanted to individual pots containing polluted substrate in both Control and Experimental groups until 43 days after collection (Period B). Periodically, leaf samples were removed to analyse metabolites and enzymes as indicated in Section 2.

From the onset of the experimental periods, all plants were cultured in individual pots in a greenhouse, with humidity, temperature, light intensity and photoperiod controlled similar to conditions in the Odiel River marshes. Plants were watered with Hoagland's nutritious solution (Hoagland and Arnon, 1950) and, since *S. densiflora* is a halophyte, this nutritious solution was enriched with 4 g/l of NaCl.

Periodically, leaf samples, similar in aspect and size, were removed and conserved at −80 °C until homogenization and biochemical analysis was performed.

2.2. Determination of metals concentrations

The determination of metals concentrations was carried out according to Chaoui et al. (1997) with minor modifications. At harvest, leaves of *S. densiflora* were washed in distilled water and then desiccated for 48 h at 70 °C. Then, oven-dried plant material was wet-ashed with an acid mixture (HNO_3:$HClO_4$, 4:1) and analysed for Fe, Cu, Ni, Pb, Zn, As, Cd by atomic absorption spectrophotometry.

Soil samples from the Odiel marshes were analysed in the same way, but without being washed in distilled water.

2.3. Photosynthetic pigments determination

For the determination of carotenes, chlorophylls *a* and *b*, and total chlorophyll, we used approximately 100 mg fresh weight of leaves. Plant material from *S. densiflora* was homogenized using 50% ice-cold acetone in a mortar, with washed sea sand, and later on, the extract was centrifuged (Arnon, 1949). Finally, the parameters previously mentioned were determined by means of spectrophotometry from the supernatant (Lichtenthaler, 1987).