Aquatic Botany 113 (2014) 90–99

Contents lists available at ScienceDirect

Aquatic Botany

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

Desiccation stress in intertidal seaweeds: Effects on morphology, antioxidant responses and photosynthetic performance

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ARTICLE INFO

Article history:
Received 6 June 2013
Received in revised form 5 November 2013
Accepted 17 November 2013
Available online 7 December 2013

Keywords:
Desiccation stress
Zonation pattern
Seaweeds

ABSTRACT

Seaweeds are differentially distributed between the upper and lower limits of the intertidal zone of rocky coasts around the world. Daily changes in tide height cause water loss, triggering desiccation stress as a consequence. How this stress affected some of the morphological characteristics and physiological responses in representative intertidal seaweeds with contrasting vertical distributions was explored in the present work. The selected species were *Mazzaella laminarioides* (upper-middle distribution), *Scytosiphon lomentaria* and *Ulva compressa* (middle distribution), and *Lessonia spicata* and *Gelidium rex* (lower distribution). To assess tolerance response to desiccation, cellular and morphological alterations, ROS production, enzymatic activity of catalase (CAT) and ascorbate peroxidase (AP) and photosynthesis performance were measured after a simulated emersion stress experiment. Results show different tolerance responses to desiccation, with seaweeds having higher intertidal distributions displaying greater antioxidant enzymatic activity, suggesting a higher capacity to buffer ROS excess induced during desiccation. Contrarily, this capacity seems to be absent or deficient in low intertidal species (i.e. *L. spicata* and *G. rex*), where AP and CAT activities were below detection limits, ROS were higher than normal and caused an over-oxidation of bio-molecules and photosynthetic disarray, explaining from a functional stand point their low distribution in the intertidal zone.

1. Introduction

Marine macroalgae are pre-eminent producers occupying a basal position in aquatic food webs (Lobban and Harrison, 1994). Rocky intertidal zones are dynamic and stressful habitats for seaweeds as a result of the rapid changes in physical conditions associated with tides, in addition to the changes brought by seasonal variations (Kumar and Reddy, 2012). Seaweeds are distributed in bands parallel along the rocky intertidal zone, where distribution and relative abundance along the lower limits is mainly controlled by biotic factors such as predation and intra and interspecific competition, while the upper limit distribution is mostly determined by abiotic factors such as UV radiation, light, salinity, temperature, nutrient availability and air exposure (Zaneveld, 1969). Davison and Pearson (1996) and Ji and Tanaka (2002), while studying the photosynthetic and respiratory performance of various seaweeds collected from different intertidal regimes following 2 h of desiccation, suggested that the ability to withstand desiccation stress (fast recovery during rehydration) and not that to avoid desiccation (water retaining ability), is the key factor determining their vertical distribution.

Exposure of seaweeds during low tidal emersions demands the alga to prepare not only for desiccation but also for subsequent rehydration and eventual cellular damage (Burritt et al., 2002). Extended desiccation may cause a decline in photosynthesis rate while interrupting the electron flow between photosystem (PS) I and II (Heber et al., 2010; Gao et al., 2011). Additionally, fluctuating and dynamic environmental conditions in the intertidal zone trigger the accumulation of reactive oxygen species (ROS) (Collén and Davison, 1999a,b; Contreras et al., 2005, 2009; Kumar et al., 2010, 2011) that, if not buffered, result in an oxidative stress condition. Further, cellular dehydration resulting from desiccation increases

Abbreviations: AP, ascorbate peroxidase; CAT, catalase; DHAR, dehydroascorbate reductase; GR, glutathione reductase; PRX, peroxiredoxin; ROS, reactive oxygen species; RWC, relative water content.

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http://dx.doi.org/10.1016/j.aquabot.2013.11.004

electrolyte concentration in the cell and brings changes to membrane structures, including thylakoids (Kim and Garbary, 2007). Acclimation to an adverse environment involves complex enzymatic and non-enzymatic antioxidant mechanisms functioning in an orchestrated manner to mitigate changes in cellular osmolarity, ion disequilibrium and ROS excess (Sampath-Wiley et al., 2008; Contreras et al., 2009; Kumar et al., 2011).

The effects of desiccation on the vertical distribution of macroalgae are poorly understood. Only few reports highlight the induction of ROS and activation of the antioxidant system in seaweeds in response to desiccation (Collén and Davison, 1999a,b; Burritt et al., 2002; Ross and Van Alstyne, 2007; Contreras-Porcia et al., 2011, 2013; Kumar et al., 2011; López-Cristoffanini et al., 2013). Independent studies investigating algal responses to fluctuating environmental conditions show alterations in photosynthetic performance (*Fv*/*Fm*), cellular morphology and ontogenetic development (e.g. Abe et al., 2001; Varela et al., 2006; Kumar et al., 2011; Contreras-Porcia et al., 2012; Gao and Wang, 2012). However, integrative studies addressing cellular responses to desiccation are lacking.

Along the Chilean coast, the upper-most part of the intertidal zone is characterized by a seasonal dominance of *Porphyra* and *Pyropia* species such as *Pyropia columbina* (Montagne) WA Nelson (formerly *Porphyra columbina* Montagne) (Bangiales, Rhodophyta). The upper-middle zone is dominated, among others, by *Mazzaella laminarioides* (Gigartinales, Rhodophyta) and the middle zone by *Ulva compressa* (Ulvales, Chlorophyta), *Scytosiphon lomentaria* (Ectocarpales, Heterokontophyta), *Ceramium* spp. and *Polysiphonia* spp. (Ceramiales, Rhodophyta). The lower intertidal zone is dominated by *Codium* spp. (Bryopsidales, Chlorophyta), *Lessonia* spp. (Laminariales, Heterokontophyta), *Gelidium* spp. (Gelidiales, Rhodophyta) and also several species of crustose calcareous red algae (Hoffmann and Santelices, 1997). Experimental studies on the littoral zone have been important in unraveling the factors regulating the vertical distribution of these species (Alveal, 1970; Moreno and Jaramillo, 1983; Buschmann, 1990). Recently, Contreras-Porcia et al. (2011) demonstrated that *P. columbina* exposed to natural desiccation during low tide loose 90–95% water and displays an excess of ROS, elevated activities of antioxidant enzymes and high concentration of photosynthetic pigments. Furthermore, desiccation results in over expression of tolerance genes, as those coding for ABC (ATP binding cassette) transporter proteins, antioxidant enzymes, heat shock proteins, cytochrome P450, cell wall proteins and specific transcriptional factors, among others (López, 2012; Contreras-Porcia et al., 2013). It is highly likely that these mechanisms, present in *P. columbina*, represent part of the functional tools available to the plants to tolerate desiccation and, at the end, may help to explain its ecological dominance in the higher part of the intertidal zone. In this context, we assessed the general validity of the responses in *P. columbina* by testing the hypothesis that the presence and adequacy of functional responses to desiccation stress in a selected group of common intertidal seaweeds defines their altitudinal position in the intertidal zone.

2. Materials and methods

The presence and adequacy of functional responses to desiccation in *M. laminarioides*, *S. lomentaria*, *U. compressa*, *Lessonia spicata* and *Gelidium rex* were determined by monitoring and recording (i) cellular alteration, (ii) attenuation of ROS over-production, (iii) oxidation of biomolecules, (iv) antioxidant enzymatic activity, and (v) photosynthetic response after desiccation. These responses were recorded in plants affected by desiccation in vitro, and compared with those in plants rehydrated in fresh seawater.

2.1. Sampling

Hydrated vegetative individuals of each species were collected along 250–300 m of coastline in Maitencillo beach (32°39.5′ S, 71°26.6′ W) during low tide. Plants were kept in plastic bags with seawater and transported to the laboratory in a cooler with ice packs at 5–7 °C. Once in the laboratory, these hydrated individuals were exhaustively rinsed with 0.45 μm-filtered seawater, cleaned using an ultrasonic bath (575T, Crest, NJ, USA) and acclimated to laboratory conditions in a growth chamber for 24 h at 14 ± 2 °C, 12:12 light:dark photoperiod and 30–40 μmol photon m^{-2} s^{-1} irradiance.

2.2. In vitro experiment of desiccation and recovery

In vitro experiments were conducted according to previous work in *P. columbina* (Contreras-Porcia et al., 2011), where responses to 4 h desiccation were similar in magnitude to those recorded after 4 h of natural desiccation. In vitro desiccation experiments included an initial blot drying of the hydrated plants followed by air exposure in a growth chamber at 16 °C and 70–80 μm photon m^{-2} s^{-1} irradiance for 4 h. In addition, a subset of dehydrated fronds was immediately re-hydrated in 0.45 μm-filtered seawater, during 4 h, to characterize the recovery from oxidative stress caused by desiccation. This timing was selected because plant tissues of all species under study have reached 95–100% relative water content (RWC). Control plants were obtained after acclimation, and frozen immediately in liquid nitrogen.

2.3. Level of desiccation

The level of desiccation experienced by different algal species during the in vitro trials was expressed as relative water content (RWC%) following the formula: $RWC\% = [(Wd - Wdo) \times (Wf - Wdo)^{-1}] \times 100$, where *Wf* is the wet weight of fully hydrated fronds, *Wd* is the dehydrated weight after desiccation, and *Wdo* is the dry weight determined after drying for 48 h at 80 °C. In this context, the RWC is the complement of desiccation status and thus, a fully hydrated thallus has RWC of 100% and a fully dehydrated thallus has RWC near to 0%; decreasing RWC means increasing desiccation.

2.4. Morphological effects of desiccation

Hand-made cross sections of naturally hydrated, dehydrated and rehydrated fronds (five of each category) were used to characterize morphological changes at light microscopy level. The hydrated sections were mounted in seawater and the dehydrated section in a synthetic, water-free resin (Permount™, Electron Microscopy Sciences, PA, USA) to prevent rehydration during analysis. Cell size in each category of fronds was defined as the mean value from 100 cell measurements. Images were captured with a Nikon Microscopy Unit (Nikon Corp., Tokyo, Japan) coupled to a digital recording system (CoolSNAP-Pro*cf*, Media Cybernetics, MD, USA) and analyzed using the Image Pro Plus Version 4.5 software (Media Cybernetics, MD, USA).

Changes in ultrastructure as a result of desiccation stress were analyzed by transmission electron microscopy (TEM) using triplicate samples of hydrated, dehydrated and rehydrated fronds. Tissue samples were fixed in 0.22 μm-filtered seawater containing 3% (v/v) glutaraldehyde and 1% (v/v) *p*-formaldehyde for 3 days at 5 °C (Correa and McLachlan, 1991). Post-fixation for 2 h at 5 °C in 0.05 M sodium cacodylate buffer (pH 7.8) with the addition of 2% (w/v) OsO_4 and 1% (w/v) potassium hexacyanoferrate, was followed by dehydration in ethanol series (10–100%, v/v) and embedding