BRIEF COMMUNICATION

Modular response to salinity in the annual halophyte, *Salicornia ramosissima*

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Abstract

Chlorophyll fluorescence measurements showed that plasticity to salinity in stems of *Salicornia ramosissima* is expressed at a modular level, so intraplant variation should be considered in further studies.

Additional key words: chlorophyll fluorescence; halophyte; modular response; NaCl.

Phenotypic plasticity in plants is expressed at a modular level within the individual, *i.e*. individual meristems, leaves, branches and roots respond to changes and differences in local environmental conditions (Weiner 2004, de Kroon *et al.* 2005). Likewise, the response of modules may significantly be altered, both quantitatively and qualitatively, by interactive effects with other connected modules that experience different conditions (de Kroon *et al.* 2005). However, the physiological response of different modules of plants grown at different conditions (*e.g*. salinity) is not yet known. Hence, the present investigation was performed to ascertain whether photosystem II photochemistry of modular subunits (internodes) of *S. ramosissima* J. Woods responds uniformly to salinity. This species is a succulent halophyte characterized by articular stems with carnose segments (internodes), reduced and stem-united leaves, which can tolerate high salinity levels (Silva *et al.* 2007). We hypothesize that internodes of this species may respond as more-or-less independent units or modules.

In May 2004 adult plants of *Salicornia ramosissima* were obtained from a low-marsh population at Odiel marshes, in the joint estuary of Odiel and Tinto rivers (37°14'N-6°57'O, Huelva, South-west Spain). Plants were planted in individual plastic pots (10 cm in diameter and 12 cm in height) filled with soil from the marsh, and grown in a glasshouse at 20ºC with 40–60% relative humidity and natural daylight. Pots were gently irrigated with tap water as necessary.

In June 2004 the pots were allocated to two NaCl treatments in shallow trays (ten pots per tray, with one tray per salinity treatment): 0 and 510 mM, in the same glasshouse. At the beginning of the experiment, 5 l of the appropriate solution were placed in each of the trays and the level was marked. During the experiment, the levels in the trays were monitored and topped up to the marked level whenever necessary to maintain constant salt concentration with fresh water.

Chlorophyll fluorescence was measured using a portable modulated fluorimeter (*FMS-2, Hansatech Instruments Ltd*., King′s Lynn, England) after two weeks of treatment. Measurements were made on the five fully developed internodes nearest to distal ends of the branches in the two salinity treatments (n = 10, per internode and salinity treatment; *see* Fig. 1). Light- and dark-adapted fluorescence parameters were measured at dawn (stable 75 μmol m^{-2} s^{-1} ambient light) and at midday (1,400 μmol m^{-2} s^{-1}) to investigate whether salt concentration affected the sensitivity of plants to photoinhibition (Redondo-Gómez *et al.* 2007). Plants were dark-adapted for 30 min, using leaf clips designed for this purpose. The minimal fluorescence level in the dark-adapted state (F_0) was measured using a modulated pulse over 0.7 s (<0.05 μmol m^{-2} s^{-1} for 1.8 μs) which was too

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Abbreviations: 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; g_s – stomatal conductance; NPQ – non-photochemical quenching; Φ_{PSII} – quantum efficiency of PSII.
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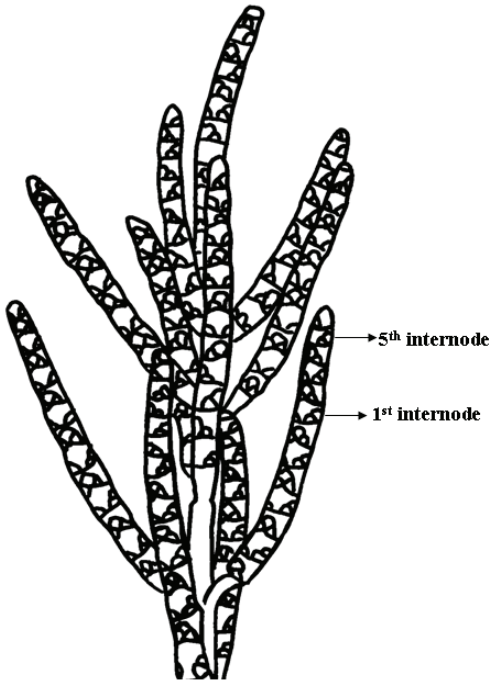

Fig. 1. Drawing of *Salicornia ramosissima* indicating the position of internodes measured, being 1 the most basal internode and 5 the most apical.

small to induce significant physiological changes in the plant. 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. Values of the 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 (Maxwell and Johnson 2000).

The same internode section of each plant was used to measure light-adapted parameters. Steady state fluorescence yield (F_s) was recorded after adapting plants to ambient light conditions (1,400 μmol m^{-2} s^{-1}) 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 PSII photochemistry. Using fluorescence parameters determined in both light- and dark-adapted states, the following were calculated: quantum efficiency of PSII [$\Phi_{PSII} = (F_m' - F_s)/ F_m'$]; photochemical quenching [$q_P = (F_m' - F_s)/(F_m' - F_0'$], where F_0' corresponds to open reaction center traps in the light-acclimated state, and it was estimated following Baker and Rosenqvist (2004], and nonphotochemical quenching [$NPQ = (F_m - F_m']/F_m'$].

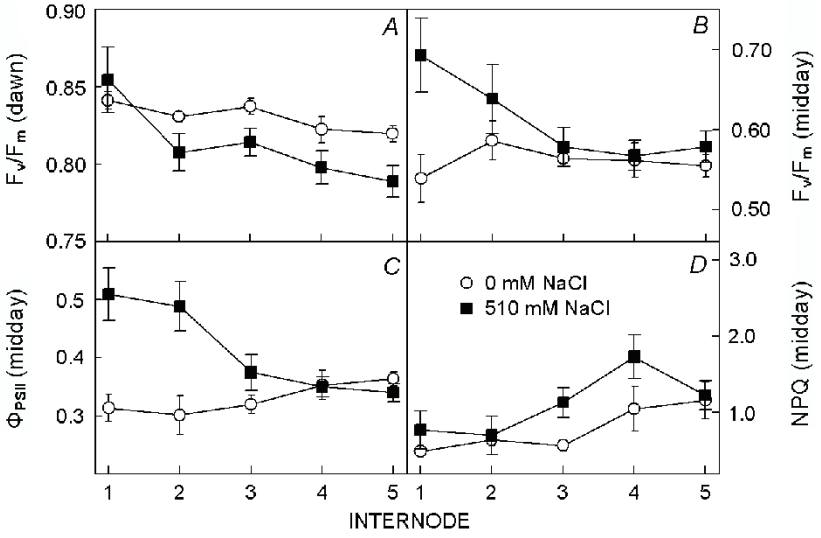

Fig. 2. *A*: Maximum quantum efficiency of PSII photochemistry (F_v/F_m) at dawn, *B*: F_v/F_m at midday, *C*: quantum efficiency of PSII (Φ_{PSII}), and *D*: non-photochemical quenching (NPQ) at midday in *Salicornia ramosissima* in response to treatment with 0 and 510 mM NaCl for two weeks. Values represent mean ±SE of ten replicates.

Statistical analysis was carried out using *Statistica v. 6.0* (*Statsoft*, Tulsa, USA). Data were analyzed using one- and two-way analysis of variance (*F*-test). Data were first tested for normality with the Kolmogorov-Smirnov test and for homogeneity of variance with the Brown-Forsythe test. Significant test results were followed by *Tukey* test for identification of important contrasts. Differences between measurements of fluorescence at dawn and midday were compared by the Student test (*t*-test).

Figs. 2*A* and 2*B* show F_v/F_m measured at midday, which indicate plants response to light stress, and the recovery of this parameter on the following day at dawn. There was a difference between values of F_v/F_m at midday and dawn (the values varied around 0.63 and 0.82, respectively) as a result of lower values of q_P at midday (*t*-test, $P<0.0001$; data not presented). At midday, the reduction in F_v/F_m values revealed that *S. ramosissima* plants experienced photoinhibition at the highest light flux. Photoinhibition is caused by damage to photosynthetic components, and this effect can be short-term and reversible (dynamic photoinhibition) or long-term and irreversible (chronic photoinhibition; Werner

et al. 2002). In the case of *S. ramosissima,* photoinhibition was mainly dynamic, since low midday F_v/F_m values at 0 and 510 mM NaCl recovered by dawn to optimal values for unstressed plants (Björkman and Demming 1987). Also quantum efficiency of PSII (Φ_{PSII}) and non-photochemical quenching (NPQ) demonstrated the difference between sampling times (*t*-test, $P<0.01$). Φ_{PSII} was lower at midday as a consequence of the decrease in q_P and the increase in NPQ, as a defense system, producing an increase in thermal dissipation in the PSII antennae to ameliorate the effects of excess light energy (Müller *et al.* 2001). In addition, an increase in NPQ may be related to the contribution of different relaxation-time components (Megdiche *et al.* 2008).

At midday, F_v/F_m values of the most basal internode of the branch (internode 1) were higher at 510 mM NaCl (*t*-test, $P<0.0001$) than at 0 mM NaCl. It is possible that a stable quencher was formed in presence of NaCl and that it did not decay during the dark adaptation period before measurement. Alternatively, it could be concluded as well that under low NaCl conditions plants are more prone to photoinhibition, through damage to PSII reaction centres (Redondo-Gómez *et al.* 2010). Redondo-Gómez *et al.* (2007 and 2008) found that absence of salt represented an environmental stress factor for *Atriplex portulacoides* and *Suaeda splendens*, respectively, since there was a marked midday reduction in F_v/F_m at 0 mM NaCl. In contrast, interestingly enough, there were not differences between salinity treatments for all the rest of internodes measured ($P>0.05$). Thus, if fluorescence parameters had been only measured in apical internodes of the branches, no difference would have been found between salinity treatments because modular organization of plants would not have been taken into account. De Kroon *et al.* (2005) explained that plotting the response of genetic individuals (whole plants) to different environmental conditions is an illustrative and useful method if: (*1*) the relevant environmental variation occurs at the scale of the individual; and (*2*) if all modular subunits of a functional individual perceive and respond uniformly to environmental variation. The second condition is not met by *S. ramosissima*, as argued previously.

Overall, at both, dawn and midday, F_v/F_m of plants grown with salt were affected by the internode position (*ANOVA*, $P<0.05$). Therefore, apical internodes of the branches showed lower F_v/F_m values than basal internodes (experiencing higher photoinhibition; Fig. 2). It is well established that environmental stress factors such as high radiation may damage plant cells through production of reactive oxygen species, including superoxide (Scandalios 1997). In this regard, Yamane *et al.* (2009) suggested that the capacity to scavenge reactive oxygen species in rice decreases with age, and thus the apical region of the leaf blade (young tissue) suffered severer damage by Na^+ than the basal region (old tissue). Also, Φ_{PSII} and NPQ of *S. ramosissima* plants at 510 mM NaCl varied with internode position (*ANOVA*, $P<0.01$ and $P<0.05$, respectively; Fig. 2). The lower Φ_{PSII} values of apical internodes (Fig. 2*C*; as a result of lower values of photochemical quenching, q_P; data not presented), which showed the higher NPQ values (Fig. 2*D*), indicate that salinity treatment enhances photoinhibition induced by light stress for these internodes. This is that plasticity to salinity is expressed at a modular level, so we should aim at measuring intraplant variation rather than viewing this variation as undesirable noise blurring our estimates of elusive whole-plant plasticities (de Kroon *et al.* 2005). In addition, modular response should be considered in further ecophysiological studies of other halophytic genera which have stems composed of succulent assimilating internodes (*e.g.* species of genera *Arthrocnemum* and *Sarcocornia*; Redondo-Gómez *et al.* 2005).

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