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Short Communication

Synergic effect of salinity and light-chilling on photosystem II photochemistry of the halophyte, *Sarcocornia fruticosa*

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ABSTRACT

Laboratory experiments were conduced to assess the synergic effect of chilling and light on photosystem II photochemistry of the halophyte, *Sarcocornia fruticosa*, grown at different salinity concentrations (0, 170, 340, 510 and 1030 mM). Chlorophyll fluorescence was measured after chilling (at 5 °C in darkness) and light-chilling (at 5 °C and 700 μmol m^{-2} s^{-1}) treatments, and after 24 h of recovery (at 20 °C and 75 μmol m^{-2} s^{-1}). At 5 °C and 700 μmol m^{-2} s^{-1}, plants grown with 0 and 170 mM NaCl showed the lowest F_v/F_m values, whereas quantum efficiency of PSII (Φ_{PSII}) was higher for plants grown at 170 and 340 mM NaCl, these results being consistent after two exposures to chilling treatments. Susceptibility to photoinhibition decreases when low temperature and high light are combined with high salinity. Therefore, populations of *S. fruticosa* that occur in arid environments with salinities c. 340 mM could show a higher tolerance to light-chilling.

Salinity is major environmental problem in arid and semi-arid regions of the world, and the use of native halophytic plants to reclaim saline areas has been proposed as a solution which is both economically and ecologically relevant (Khan et al., 2000). In this regard, *Sarcocornia fruticosa* (L.) A.J. Scott is a succulent halophyte characterized by articular stems with carnose segments, reduced and stem-united leaves, whose growth has been reported to be stimulated by salinity (Redondo-Gómez et al., 2006). This species occurs in arid and highly saline environments (Castroviejo, 1990), such as the marshes of southern Spain. Large variations in soil salinity have been measured in this zone, ranging from lower than 17 mM NaCl to extreme concentrations of more than 940 mM NaCl in the salt pans (Redondo-Gómez et al., 2006). This is an area which receives sporadic rainfalls, a proportion of the salts thus temporarily leaching from the soil, although this does not occur at any particular time of year (Pujol et al., 2000). Consequently, the level of salinity can stay high during the winters.

On the other hand, low temperatures increase sensitivity towards photoinhibition, since photon utilization capacities in plants decrease (Hirotsu et al., 2004). Likewise, susceptibility to photoinhibition significantly increases when high light is combined with low temperatures (Powles, 1984). However, the synergic effect of chilling and light on halophytes grown at different salinity concentrations is not known. Hence, the present investigation was performed to attain a wider knowledge of the synergic effect of salinity and light-chilling on photosystem II photochemistry of *S. fruticosa*.

Seeds of *S. fruticosa* were collected in December 2002 from Piedras salt marshes (37° 13′ N, 7° 9′ W; S.W. Iberian Peninsula), where minimum temperatures c. 5 °C are reached approximately five times a year, between January and March (Climatic values were obtained from Punta Umbria meteorological station, n° 555). Seeds were placed in a germinator (ASL Aparatos Científicos M-92004, Spain), and subjected to an alternating diurnal regime of 10 h of light (photon flux rate, 400–700 nm, 35 μmol m^{-2} s^{-1}) at 25 °C and 14 h of darkness at 5 °C, for 30 days (Redondo-Gómez et al., 2006). Afterwards, seedlings were planted in plastic pots in substrate designed for salt tolerant plants (Floraska, Germany: K_2O 210–290 mg/l; N 190–250 mg/l; P_2O_5 130–170 mg/l, pH ($CaCl_2$) 5.4–5.9; conductivity 400–500 μSiemens), and grown in a glasshouse at 20 °C with 40–60% relative humidity and natural daylight. Pots were gently irrigated with tap water as necessary.

When, after eleven months, seedlings had reached a height of between 10 and 15 cm, the pots were allocated to five NaCl treatments in shallow trays (ten pots per tray, with one tray per salinity treatment): 0, 170, 340, 510 and 1030 mM, in the same glasshouse.

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The salt concentration was increased in two phases to minimize shock.

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. In addition, conductivity (conductivity meter Crison-522, Spain) was monitored as well to ensure that salinity did not vary significantly during the experiment. The highest salinities in the Mediterranean salt marshes that *S. fruticosa* inhabits are maintained for no longer than 12 weeks during the summer. Therefore, measurements on the youngest fully expanded leaves were made 12 weeks after the final treatment concentrations had been reached, when the plants appeared to have achieved stable growth rates (Redondo-Gómez et al., 2006).

In March 2004, two months after the start of NaCl treatments, pots (ten pots per tray, with one tray per salinity treatment) were firstly treated for 3 h at 5 °C in darkness (chilling treatment). Then, they were illuminated for 1 h with a photon flux density of 700 μmol m^{-2} s^{-1} at 5 °C (light-chilling treatment). Thereafter, all plants were transferred in the glasshouse at 20 °C for 24 h to allow recovery of chilled plants. On the following day, the same treatments (chilling, light-chilling and recovery) were repeated.

Chlorophyll fluorescence was measured using a portable modulated fluorimeter (FMS-2, Hansatech Instruments Ltd., England). Measurements were made on each plant in the five salinity treatments ($n = 10$). Light and dark-adapted fluorescence parameters were measured after chilling (only dark-adapted fluorescence parameters) and light-chilling treatments and the following days at dawn (recoveries; in the glasshouse at 20 °C and stable 75 μmol m^{-2} s^{-1} ambient light) to investigate whether salt concentration and chilling episodes affected the sensitivity of plants to photoinhibition (Redondo-Gómez et al., 2007). Also, control measurements at 20 °C and 75 and 700 μmol m^{-2} s^{-1} were made before the chilling experiment.

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 (<0.05 μmol m^{-2} s^{-1} for 1.8 μs) which was too small to induce significant physiological changes in the plant (Schreiber et al., 1986). The data stored were an average taken over a period of 1.6 s. 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. The value of F_m was recorded as the highest average of two consecutive points. 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 centers 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 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 ($qP = (F_m' - F_s)/(F_m' - F_0')$, where F_0' corresponds to open reaction center traps in the light-acclimated state), and non-photochemical quenching ($NPQ = (F_m - F_m')/F_m'$).

Statistical analysis was carried out using Statistica v. 6.0 (Statsoft Inc.). Data were analyzed using GLM repeated measures model. 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.

Maximum quantum efficiency of PSII photochemistry (F_v/F_m) of *S. fruticosa* varied depending on the regime (salinity, chilling and light regimes) and their interaction on the first and second days of chilling and light-chilling treatments ($p < 0.05$; Fig. 1a and b). At 5 °C and 700 μmol m^{-2} s^{-1}, plants grown with 0 and 170 mM NaCl showed lower F_v/F_m values than plants grown between 340 and 1030 mM NaCl ($p < 0.01$; as a result of lower values of photochemical quenching, qP; data not presented), and similar values to plants measured at 20 °C and 700 μmol m^{-2} s^{-1} ($p > 0.05$). Barták et al. (1998) also found similar F_v/F_m values for plants of *Lolium perenne* measured at 5 and 20 °C and 500 μmol m^{-2} s^{-1} and grown with water. Plants treated from 340 to 1030 mM NaCl and measured at 5 °C and 700 μmol m^{-2} s^{-1} showed similar F_v/F_m values, which were similar too to those in plants measured at 5 °C

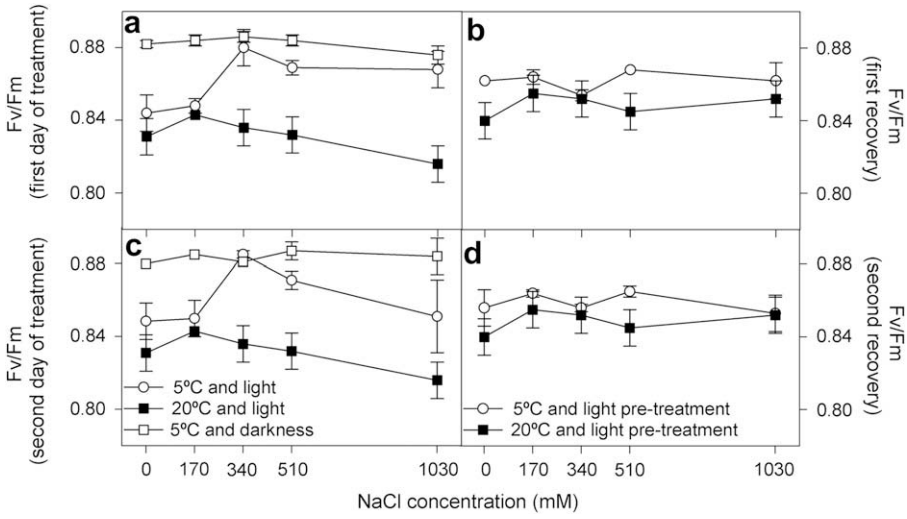

Fig. 1. Maximum quantum efficiency of PSII photochemistry (F_v/F_m) on the first (a) and second (b) days of chilling (at 5 °C in darkness) and light-chilling treatments (at 5 °C and 700 μmol m^{-2} s^{-1}), and recovering after the first (c) and second (d) days of treatments in *Sarcocornia fruticosa* plants grown with a range of salt concentrations for three months. Control was measured at 20 °C and 700 mmol m^{-2} s^{-1}. Values represent mean ±SE of ten replicates.