Plant Stress 8 (2023) 100162

Contents lists available at ScienceDirect

Plant Stress

journal homepage: www.sciencedirect.com/journal/plant-stress

# *Sarcocornia fruticosa* recovery capacity after exposure to co-existed water and salinity stress

Jesús Alberto Pérez-Romero [a,*], Jose-Maria Barcia-Piedras [b], Susana Redondo-Gómez [c], Enrique Mateos-Naranjo [c]

[a] Departamento de Biología, Instituto Universitario de Investigación Marina (INMAR), Universidad de Cádiz, Puerto Real 11510, Spain
[b] Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA), Centro Las Torres, Carretera Sevilla-Cazalla de la Sierra Km 12.2, Alcalá del Río 41200, Spain
[c] Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, 1095, Sevilla 41080, Spain

ARTICLE INFO

*Keywords:*
Drought
Abiotic stress
Stress recovery
Halophyte
Photosynthesis

ABSTRACT

The capacity of halophytes species to resist abiotic stress has been tested on multiple occasions. The ability of these species such as *Sarcocornia fruticosa* to cope with severe stress conditions has been shown, as well as their utility as a phytoremediation tool or even as potential crop species. However, there is a lack of literature on the effect that these abiotic factors have on their physiological response after a recovery period. In a greenhouse experiment, *S. fruticosa* plants were subjected to a combination of water regimen (water stress/field capacity) and salinity concentration (171/510 mM NaCl) grown conditions for 30 days. After these stress periods, plants were left 15 days in recovery conditions (field capacity and 171 mM NaCl). To study the effect of stress during both periods, osmotic potential, net photosynthetic rate, stomatal conductance, intercellular $CO_2$ concentration, quantum efficiency of PS II, OJIP-derived parameters and photosynthetic pigment concentrations were measured. Our results show the already known resistance of this species to drought and salinity stress. However, the combination of both factors did affect the ability of *S. fruticosa* to maintain its level of carbon assimilation due to a decrease in stomatal conductance. In addition, the recovery period helped us to describe a synergic effect of both abiotic factors showing that plants subjected to both stresses received a better response during the recovery period than those only affected by salinity stress.

## Introduction

The rise in atmospheric $CO_2$ concentration and the increase in mean temperature worldwide are the main aspect of climate change predicted by models (IPCC, 2014). These two factors will have numerous consequences in our ecosystems, such as soil degradation due to an increase in soil salinity (IPCC, 2014). According to the International Climate Change Panel (ICCP), the Mediterranean region is one of the most vulnerable with temperature increase, decrease in rainfall and increase of seawater level (Cuttelod et al., 2009). All of these problems would constrain productivity in crops and would aggravate the intrusion of seawater into aquifers near the coast. The reduction of soils available for agriculture, in addition with the increase in world population, will leave food security in a dangerous situation (Calone et al., 2022). Furthermore, due to the COVD-19 pandemic, the number of people malnourished has increased by millions (Bongaarts, 2020). Therefore, modern agriculture needs facing the problem of maintaining its productivity in a degraded system, decreasing the impact it has on the system to ensure food security to an increasing population.

To cope with these problems, the FAO Strategic Framework 2022–2031 encourages following the concept of climate-smart agriculture (CSA) proposed by the World Bank in 2009 to improve productivity and reduce the carbon footprint and cost of agriculture (World Bank Group, 2016). This CSA is sustained by three priority lines which are: develop sustainable agriculture, increase the adaptative capacity of agroecosystems and increase carbon sinking while decreasing carbon emissions (Hussain et al., 2022). With these main objectives, CSA tolerate different technologies and methods adapted to the environment where the concept is being implemented (Campbell et al., 2014). One of these techniques is the introduction of drought or salt tolerant plants as crops species (Calone et al., 2022).

The Mediterranean region represents a biodiversity hotspot with a

* Corresponding author.
*E-mail address:* jesusalberto.perez@uca.es (J.A. Pérez-Romero).

https://doi.org/10.1016/j.stress.2023.100162
Received 7 February 2023; Received in revised form 13 April 2023; Accepted 25 April 2023
Available online 25 April 2023

large number of species adapted to extreme conditions (Calone et al., 2022). Between these species, halophytes are extremophile plants that can grow at salinity level that are toxic for most plant species and resistant to multiple harsh conditions (Calone et al., 2022). These plants are currently being cultivated for food production as a gourmet dish (Calone et al., 2022). Furthermore, the utility of these species has been stated as a biomass crop or as a source of medicinal compounds (Ventura et al., 2015; Ventura and Sagi 2013). Furthermore, they have been proven as tools for soil phytoremediation (Pérez-Romero et al., 2016), carbon sequestration (Calone et al., 2022), and recovery of saline soils (Barcia-Piedras et al., 2019).

Among them, *Sarcocornia fruticosa* has been tested in previous studies as a drought, salt, and heat waves resistant plant (Redondo-Gómez et al. 2006; Pérez-Romero et al., 2020a; Calone et al., 2022). *S. fruticosa* A.J. Scott is present in the south and west coast of Europe (Redondo-Gómez et al. 2006). It grows in the middle and high marshes from southwest Spain, where it is subjected to seasonal variation soil salinity from 17 mM NaCl to 940 mM NaCl (Redondo-Gómez et al. 2006). Furthermore, *S. fruticosa* cope with stational drought and flood periods (Redondo-Gómez et al. 2006). It belongs to the subfamily *Salicornioideae*, which includes more than 100 succulent halophile species (Calone et al., 2022). These species have recently been collected as edible crops in gourmet cuisine due to the crunchy texture and the salty taste of their fresh tips (Calone et al., 2022). In addition, they have been shown to be nutritionally valuable as they contain antioxidant, anti-inflammatory, and polyphenolic compounds (Costa et al. 2018; Calone et al., 2022). Moreover, their seeds have been used as a source of oil with health value and their biomass is used for bioethanol (Calone et al., 2022). These species have also been suggested in many previous works as phytoremediation tools of saline and heavy metals (Moreira et al. 2015; Pérez-Romero et al., 2016; Said et al., 2018). However, there is scarce information in the literature on the recovery capacity of this species after a stress period. Calone et al. (2022) have shown that *S. fruticosa* was able to fully recover from water stress after 15 days of treatment. Nevertheless, there is a lack of information about the recovery capacity for salinity stress or the permanent effect that these stress periods could have on the halophyte photosystem.

Taking all this into account, the main objective of this study is to investigate the ability of *S. fruticosa* photosynthesis system to cope with water and salt stress and to verify if the responses shown during stress period where effective in order to recover its photosynthesis system after these stressful conditions. To achieve this objective, plant physiology and water status have been measured in *S. fruticosa* plants subjected to two different regimes of water in combination (field capacity and water stress) and with two different salinities (171 and 510 mM NaCl) before and after a recovery period in which all treatments were taken at field capacity and grown at 171 mM NaCl.

## Materials and methods

### *Plant material*

*S. fruticosa* seeds collected in September 2015 from Odiel marshes (37°15′N–6°58′O; SW Spain) were stripped for each plant and stored in the dark at 4 °C until the beginning of the experiment.

In May 2017 seeds were planted on 10% agar and transported to a germinator (ASL Aparatos Cientficos M-92,004, Madrid, Spain). The germinator conditions were a day-night regime of 16 h of light (photon flux rate, 400 to 700 nm, 35 μmol $m^{-2}$ $s^{-1}$) at 25 °C and 8 h of darkness at 12 °C, for 15 days. When the seedlings germinated they were carefully transplanted into plastic pots (9 cm high x 11 cm diameter) filled with perlite. These pots were placed in a greenhouse with controlled conditions (temperature between 21 and 25 °C, 40–60% relative humidity and natural daylight of 250 μmol $m^{-2}$ $s^{-1}$ as minimum and 1000 μmol $m^{-2}$ $s^{-1}$ as maximum light flux). The pots were placed on shallow plates and watered with 20% Hoagland solution (Hoagland and Arnon, 1938) and 171 mM NaCl. This salinity concentration was chosen due to the halophytic behavior of *S. fruticosa,* which allows this species to show better performance with this salinity level (Pérez-Romero et al., 2019).

### *Experimental design*

When the plants had a mean height of 10 cm, they were separated in four different trays and transplanted to individual pots with a mix of organic commercial substrate (Gramoflor GmbH & Co. KG., Vechta, Germany) and sand (3:1). Each tray contained twenty randomly selected individual pots. Then two of the trays were irrigated until they reached field capacity (WW) and the other two were watered with 25% of the supply needed to achieve the WW (WS). Furthermore, one tray in WW and one tray in WS water regime were subjected to 171 mM or 1% NaCl of the solution volume (1%) and the in the other tray for both water regimes the NaCl concentration was raised to 510 mM or 3% NaCl of the solution volume (3%). Therefore, we obtained four different treatments during the stress phase (WW 1%, WW 3%, WS 1%, and WS 3%). These trays were maintained under the same conditions previously described.

After 30 days of experiment, we carried out the recovery phase of the experiment. Firstly, we watered with tap water to clean any excess of NaCl in the grown substrate. Then, 10 plants belonging to treatment WS 1% (WS 1% R-WW) and 10 plants from treatment WW 3% (WW 3% R-NaCl) were well watered with 171 mM NaCl solution. Lastly, 10 plants of WS 3% were well watered with 510 mM NaCl solution (WS 3% R-WW) and another set of 10 plants was well watered with 171 mM NaCl solution (WS 3% R-WW-NaCl). Recovery conditions were maintained for 10 days.

### *Osmotic potential*

After 30 days of experiment and after 10 days of recovery treatment, the osmotic potential ($\Psi_0$) of the primary branches ($n$ = 10) was determined, using a psychrometric technique with a vacuum pressure osmometer (5600 Vapro, Wescor, Logan, USA).

### *Gas exchange measurements*

After 30 days of stress conditions and after 10 days of recovery conditions, instantaneous gas exchange measurements were taken in 10 branches randomly selected between each treatment implemented. Measurements were made with an open infrared gas analyzer system (LI-6400XT, LI-COR Inc., Neb., USA) equipped with a light leaf chamber (Li-6400–02B, Li-Cor Inc.). The net photosynthetic rate ($A_N$), the stomatal conductance ($g_s$) and the intercellular $CO_2$ concentration ($C_i$) were determined. These parameters were obtained at a light photon flux density of 1500 μmol $m^{-2} s^{-1}$, leaf temperature of 25 °C, 50 ± 2% relative humidity of 50 2% and a $CO_2$ concentration surrounding leaf ($C_a$) 400 μmol $mol^{-1}$ air. The intra-water use efficiency ($_i$WUE) was calculated as the ratio between $A_N$ and $g_s$. The photosynthetic area was approximated as the area of a cylinder.

### *Chlorophyll fluorescence measurements*

Modulated chlorophyll fluorescence measurements were made in the same branches where gas exchange measurements were performed ($n$ = 10), using a FluorPen FP100 PAM (Photo System Instruments, Czech Republic) on light and 30 min dark-adapted leaves. Light energy yields of the Photosystem II (PSII) reaction centers were determined with a saturation pulse method as described by Schreiber et al. (1986), using a 0.8 s saturating light pulse with an intensity of 8000 μmol $m^{-2}$ $s^{-1}$. The quantum yield of PS II (QY) and the relative quantum yield of PS II (Q'Y) were calculated as $F_v/F_m$ and $\Phi_{PSII}$ respectively after a comparison of the minimum fluorescence values ($F'_0$), the maximum fluorescence ($F'_m$) and the operational photochemical efficiency with the values of the light and dark-adapted branches.

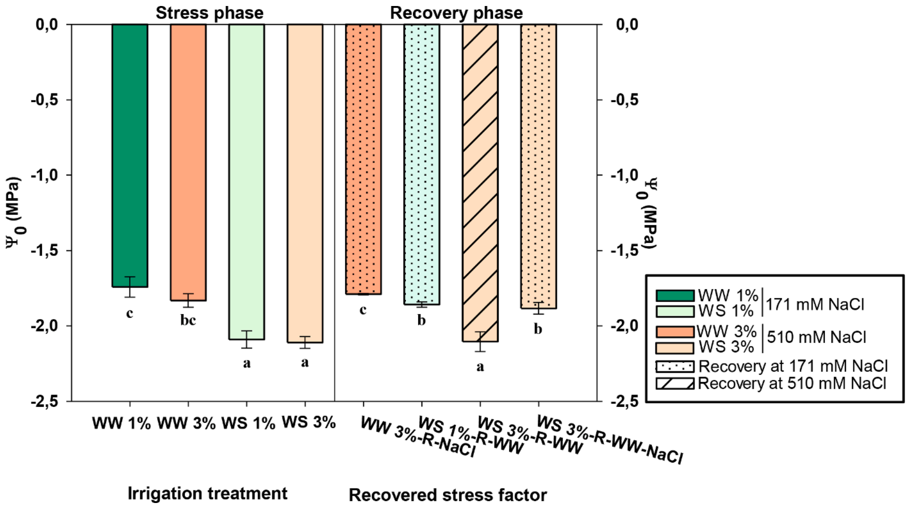


**Fig. 1.** Osmotic potential, Ψo, in randomly selected primary branches of *Sarcocornia fruticosa* after 30 days of treatment with two salinity concentrations (171 and 510 mM NaCl) and two irrigation conditions (field capacity, WW and water stress, WS) and its combinations (Stress phase) and its recovery response after 15 days of stress factor or factors offset (Recovery Phase). Legend indicates the treatments in stress period and the origin of the recovery period' treatments. Values shown mean ± SE ($n$ = 10), different letters indicate that there is significant difference between them.

Furthermore, fast kinetics of chlorophyll, or JIP test (or Kautsky curves), was also measured in dark-adapted leaves ($n$ = 5 for each one) according to Duarte et al. (2017), using preprogrammed OJIP protocols from FluorPen. All derived parameters for both RLC and OJIP were calculated according to Marshall et al. (2000) and Strasser et al. (2004).

*Pigment analysis*

At the end of the stress period and at the end of the recovery period, photosynthetic pigments were measured in randomly collected branch samples ($n$ = 5) following the analysis of Gauss peak spectra pigment. Samples were flash frozen in liquid $N_2$ and freeze dried for 48 h in the

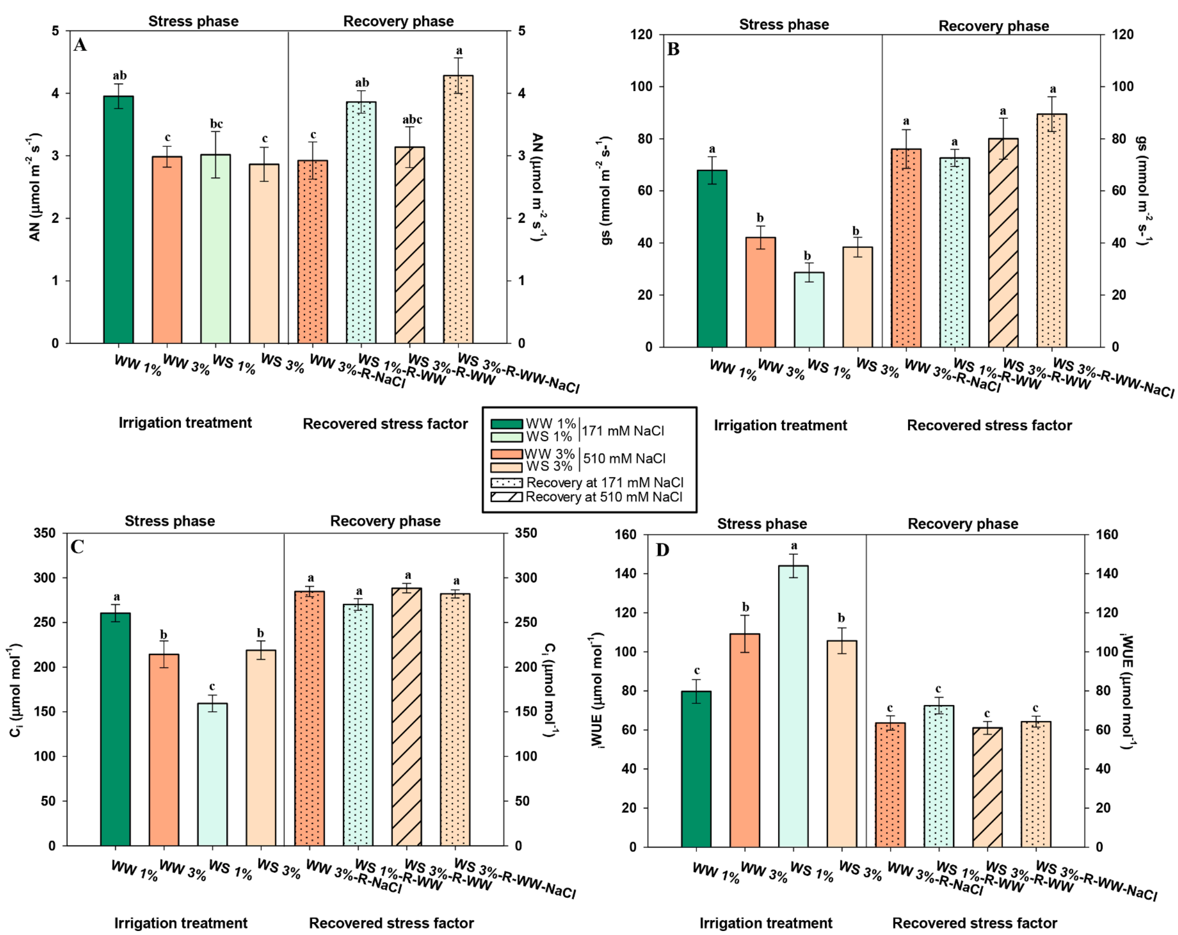


**Fig. 2.** Net photosynthetic rate, $A_N$, (A), stomatal conductance, $g_s$ (B), intercellular $CO_2$ concentration, $C_i$ (C), and intrinsic water use efficiency, $_i$WUE, (D) in randomly selected primary branches of *Sarcocornia fruticosa* after 30 days of treatment with two salinity concentrations (171 and 510 mM NaCl) and two irrigation conditions (field capacity, WW and water stress, WS) and its combinations (Stress phase) and its recovery response after 15 days of stress factor or factors offset (Recovery Phase). Legend indicates the treatments in Stress phase and the origin of the Recovery Phase' treatments. Values shown mean ± SE ($n$ = 10), different letters indicate that there is significant difference between them.

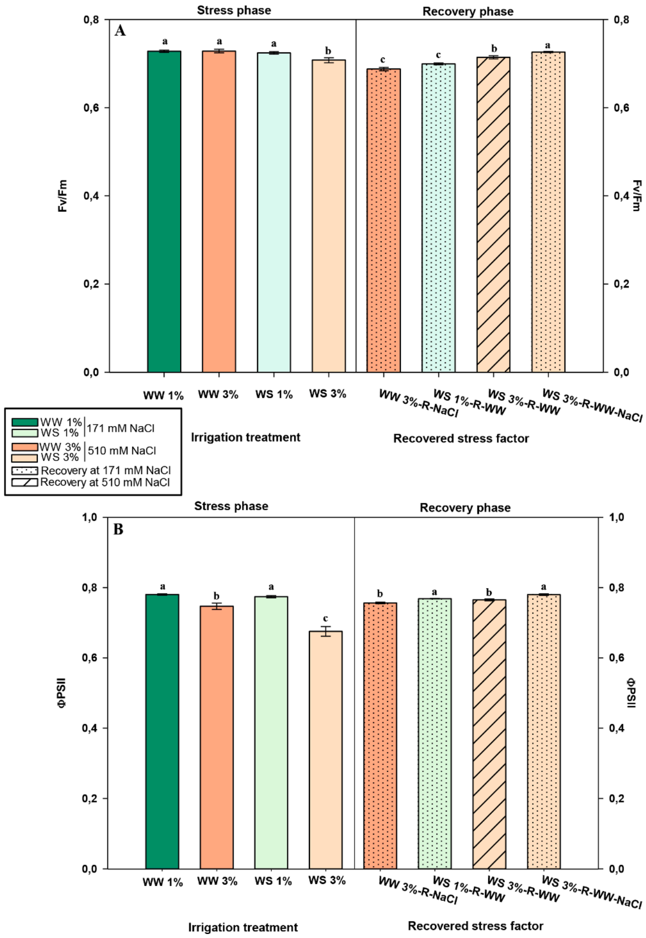


**Fig. 3.** Relative quantum yield of PS II, $\Phi_{PSII}$ (A) and maximum quantum efficiency of PS II photochemistry, $F_v/F_m$ (B) in randomly selected primary branches of *Sarcocornia fruticosa* after 30 days of treatment with two salinity concentrations (171 and 510 mM NaCl) and two irrigation conditions (field capacity, WW and water stress, WS) and its combinations (Stress phase) and its recovery response after 15 days of stress factor or factors offset (Recovery Phase). Legend indicates the treatments in stress period and the origin of the recovery period' treatments. Values shown mean ± SE ($n = 10$), different letters indicate that there is significant difference between them.

dark to avoid photodegratation processes (Duarte et al., 2014). The branches were then ground in pure acetone and pigments extracted at −20 °C for 24 h in the dark, centrifuged at 4000 rpm for 15 min at 4 °C, and the resulting supernatant was scanned on a dual beam spectrophotometer (Hitachi Ltd., Japan) from 350 to 750 nm at 0.5 nm step. The resulting absorbance spectrum was used to determine all the target pigments, after application of the using Gauss-Peak Spectra (GPS) algorithm according to Kupper et al. (2007). A GPS fitting library for this Sigma Plot Software was employed. From the resulting pigment concentrations, it was also possible to calculate the De-Epoxidation State (DES) as follows (Duarte et al., 2014):

$$DES = [\text{Antheraxantin}] + [\text{Zeaxanhin}]/[\text{Violaxanthin}] + [\text{Antheraxantin}] + [\text{Zeaxanthin}]$$

*Statistical analysis*

Statistic tests were performed using a statistical software package Statistica v. 6.0 (Statsoft Inc.). The differential effect of different irrigation conditions treatments was determined by two-way analysis of variance. Multiple comparisons were analyzed using an LSD test. Before statistical analysis, Kolmogorov-Smirnov and Levene tests were used to verify the assumptions of normality and homogeneity of variances, respectively.

## Results

*Osmotic potential*

Plants grown in WW treatments showed overall lower values for $\Psi_0$ than those grown in WS. However, there were no significant differences between salinity treatments in plants grown under the same water conditions (Fig. 1).

In the recovery phase, those treatments that were in WS showed a decrease in their $\Psi_0$. Although these values were still higher than plants at WW 1% showed. This recovery was not observed for plants grown at WS 3% R-WW (Fig. 1).

*Gas exchange*

At the first phase of the experiment, all four parameters assessed have shown a similar pattern. $A_N$, $g_s$, and $C_i$ decreased significantly for all treatments with respect to the control conditions treatment (WW 1%). $_i$WUE showed the inverse pattern and was significantly lower in WW 1% treatment compared to the other three tested conditions (Fig. 2).

When recovery was applied, all four treatments values for $C_i$, $g_s$ and $_i$WUE were significantly similar to WW 1% even WS 3% R-WW. However, $A_N$ did not follow this trend. For this parameter, WW 3% R-NaCl and WS 3% R-WW did not increase significantly compared with WW 3% and WS 3% and did not reach the WW 1% values for $A_N$.

*Chlorophyll fluorescence*

Both $F_v/F_m$ and $\Phi_{PSII}$ decreased when water stress and 510 mM NaCl were applied simultaneously to *S. fruticosa*. However, $\Phi_{PSII}$ also decreased for plants belonging to the WW 3% treatment compared to WW 1%. In the recovery analysis, both parameters did not show a clear trend. $F_v/F_m$ showed some reduction in its values for WW 3% R-WW andWS 1% R-WW treatments. For $\Phi_{PSII}$, only WS 3% R-WW and R-WW-NaCl showed an improvement with respect to WS 3%.

The OJIP-derived parameters ABS/CS, TR/CS and ET/CS followed a similar trend between them. Only plants grown at WS 1% treatment showed significantly lower values. While DI/CS values were significantly higher than control conditions for both WW 3% and WS 3% treatments (Fig. 3).

When recovery treatments were applied, ABS/CS, TR/CS, and ET/CS showed the same pattern for all treatments. There was only an increase in plants grown at WS 1% R-WW which surpass the values obtained even at WW 1%. For DI/CS the difference was also only between WS 1% and WS 1% R-WW, which was significantly higher when plants recovered from water stress (Fig. 4).

*Pigment concentration*

There were no significant differences between the four treatments during stress conditions for Chl a and Chl b concentrations. It seemed that there was a higher β-carotenes and a lower DES in plants grown under WS compared to plants treated with WW independently of salinity concentration. However, the differences were not significant (Fig. 5).

Recovery treatments did not appear to have a significant effect in any treatment for Chl a, Chl b, and β-carotenes. However, DES increased significantly for WS 1% and WS 3% in both recovery treatments.

## Discussion

Halophytes have the potential to be used as new crops in an innovative agriculture threshold due to their high biomass production, their

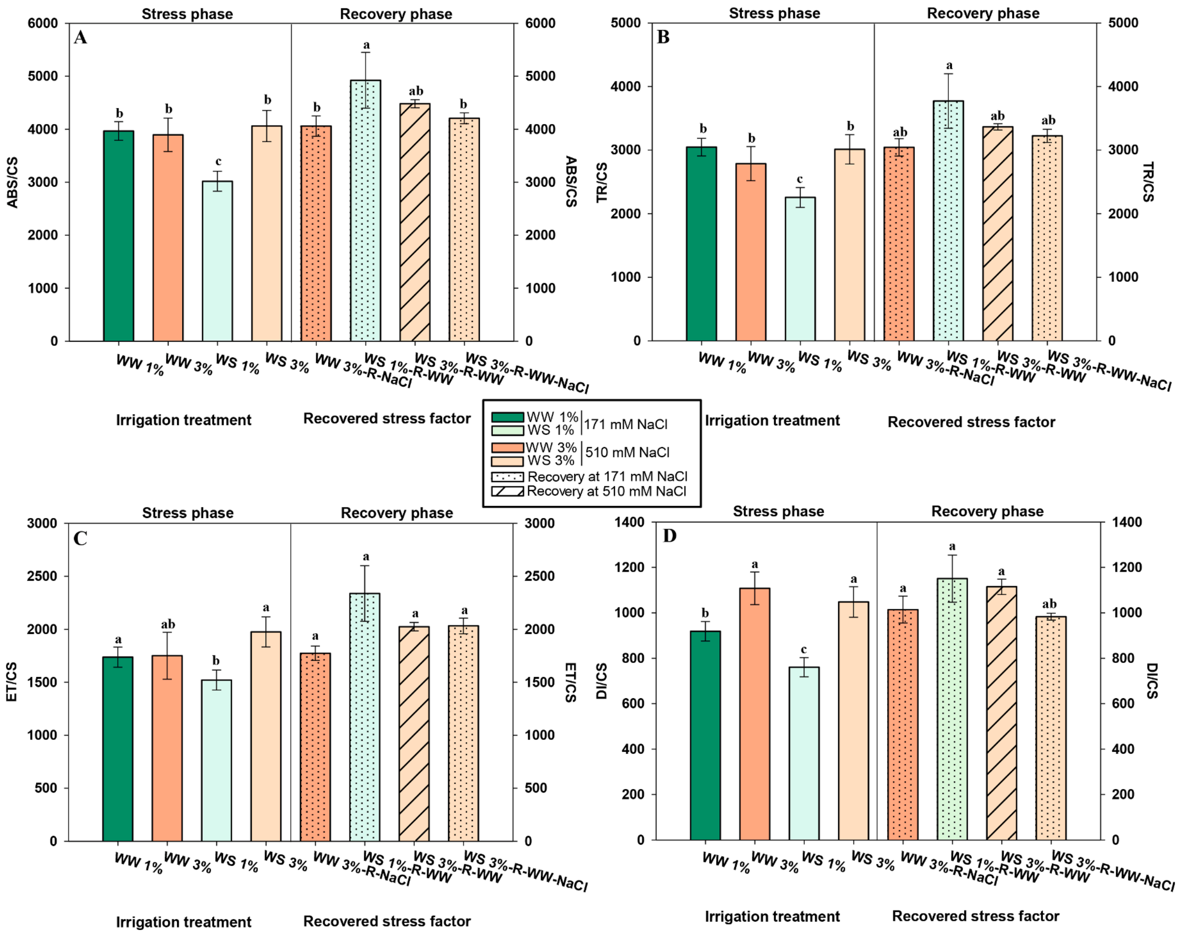


**Fig. 4.** Absorbed energy flux, ABS/CS (**A**), trapped energy flux, TR/CS (**B**), transport energy flux ET/CS (**C**) and dissipated energy fluxes, DI/CS (**D**) per cross section in randomly selected primary branches of *Sarcocornia fruticosa* after 30 days of treatment with two salinity concentrations (171 and 510 mM NaCl) and two irrigation conditions (field capacity, WW and water stress, WS) and its combinations (Stress phase) and its recovery response after 15 days of stress factor or factors offset (Recovery Phase). Legend indicates the treatments in stress period and the origin of the recovery period' treatments. Values shown mean ± SE (*n* = 10), different letters indicate that there is significant difference between them.

nutritional values, and their ability to grow in extreme conditions (Ventura and Sagi 2013; Ventura et al., 2015; Pérez-Romero et al., 2018; Calone et al., 2022). There is abundant literature on the ability of halophytes to cope with stress (Redondo-Gómez et al. 2006; Carreiras et al., 2020; Pérez-Romero et al., 2020a) or their phytoremediation potential (Pérez-Romero et al., 2016; 2022; Mateos-Naranjo et al., 2018; Barcia-Piedras et al., 2019). However, few studies can be found on recovery from abiotic stresses in halophytes species (Calone et al., 2022). This study showed how a perennial halophyte such as *S. fruticosa* responded to water and salt stress and if the negative effects of such a stress persist after a period of recovery.

The water state of *S. fruticosa* has been proven to be resistant to salinity presence in the growth solution (Redondo-Gómez et al. 2006; Pérez-Romero et al., 2020a; Calone et al., 2022). Our results agreed with these previous studies showing a stable $\Psi_0$ independently of NaCl concentration. However, this parameter increased for plants grown at WS conditions. This increment has been observed as a mechanism of response to balance water state together with a decrease in $g_s$ (Hormaetxe et al., 2006). *S. fruticosa* plants that were under WS conditions showed this decrease in $g_s$ and also an increment in $_i$WUE compared with control conditions showing concordance with what Hormaetxe et al. (2006) found. What was more interesting was the ability displayed by *S. fruticosa* to recover. Calone et al. (2022) obtained similar results after the recovery period shown values similar to plants grown in control conditions for three halophyte species. Nevertheless, our results showed one single exception, plants grown in the WS 3% R-WW treatment showed higher $\Psi_0$ values than the other recovery treatments. Even when salinity did not affect $\Psi_0$, the exposition to 510 mM NaCl seemed to interfere with the recovery process and did not allow these plants to restore its $\Psi_0$ to non-stressed values.

This recovery pattern can also be seen on the gas-exchange parameters. The negative effect of NaCl concentration is usually due to accumulation of $Na^+$ and $Cl^-$ in plant tissue that could have a direct negative effect on the photosynthetic apparatus (Brugnoli and Lauteri, 1991). Furthermore, the presence of high NaCl in soil reduces the availability of water for plants (Belkheiri and Mulas 2013). Therefore, salinity and drought stress are generally related (Belhkeri and Mulas, 2013). Our results supported this relationship a similar decrease in $A_N$, $C_i$ and $g_s$ when plants were exposed to water and/or salt stress.. In addition, the presence of high salinity and WS at the same time did not aggravate the negative effect on gas exchange parameters highlighting the relationship between these two abiotic factors as stress motors. As a consequence of salt and water stress, *S. fruticosa* plants showed higher values of $_i$WUE due to a greater decrease in $g_s$ than in $A_N$ compared to plants grown under conditions of WW 1%. The enhancement of $_i$WUE did not avoid the negative effect that salinity or water stress had on $A_N$. As previously hypothesized (Flexas et al., 2004; Verena et al., 2016) this decrease on $A_N$ could be related to less availability of $CO_2$ due to stomatal closure. The values obtained for $C_i$ and $g_s$ were in concordance with this hypothesis. Restrictions in $CO_2$ availability could possibly cause increased susceptibility to photodamage (Colom and Vazzana, 2001).

After *S. fruticosa* was subjected to a recovery period, all treatments

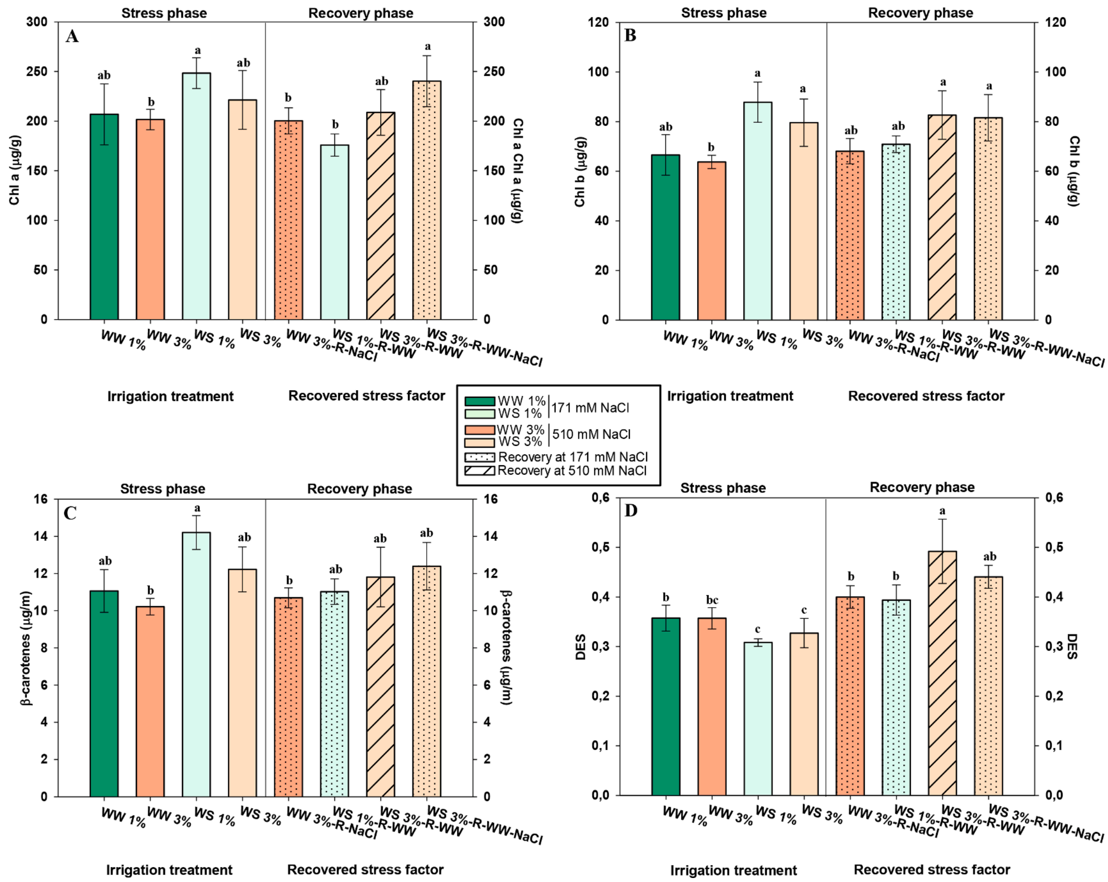


**Fig. 5.** Chlorophyll a, Chl a (A), chlorophyll *b*, Chl b (B), beta carotenes, β-carotenes (C) concentration and depoxidation index, DES (D) in randomly selected primary branches of *Sarcocornia fruticosa* after 30 days of treatment with two salinity concentrations (171 and 510 mM NaCl) and two irrigation conditions (field capacity, WW and water stress, WS) and its combinations (Stress phase) and its recovery response after 15 days of stress factor or factors offset (Recovery Phase). Legend indicates the treatments in stress period and the origin of the recovery period' treatments. Values shown mean ± SE ($n$ = 10), different letters indicate that there is significant difference between them.

showed $g_s$, $C_i$, and $_i$WUE values similar to plants grown under optimal conditions (WW 1%). However, $A_N$ did not recovered control values for plants previously grown at 510 mM NaCl independently of water conditions. Other studies had shown that $A_N$ in halophytes was not affected by a decrease in $C_i$ but by a decrease in $g_m$ or by inhibition of RuBisCO activity (Mateos-Naranjo et al., 2015; Pérez-Romero et al., 2018) this could explain the reduction in $A_N$ even after $C_i$ has been recovered. Nevertheless, plants grown at WW 3% R-WW-NaCl showed an improvement on $A_N$ and values similar to those grown at WW 1%. This could be related to a synergic effect of both types of stress, Pérez-Romero et al., 2018; 2019; 2020a) demonstrated the importance of considering the synergic effect between abiotic factors in order to better understand the response of plant species in their natural habitat. This study reflected these complex relationships established between plants and their environment and showed how exposure to water stress in the presence of 510 mM NaCl may affect the ability of *S. fruticosa* to recover after the stress period. The $A_N$ value did not show an improvement when WW 3% was subjected to conditions R-NaCl. The lack of recovery of $A_N$ along with the improvement in $g_s$ and $C_i$ could indicate that there was salinity-driven damage caused at the photosystem or pigment level (Carreiras et al., 2020) that did not recover after 15 days. Thus, $A_N$ did not increase even when plants had enough $CO_2$ available in their stroma. Exposure to water and salinity stress mitigated this effect in plants grown first at WS 3% and then recovered for both stress factors (R-WW-NaCl). However, *S. fruticosa* treated with WS 3% and recovered only of WS (R-WW) did not show the same trend, highlighting the important role of NaCl excess in the grown solution in the recovery ability of this species.

Nevertheless, the decrease observed in the gas exchange parameters was not related to the destruction of photosynthetic pigments as the pigment concentration for chlorophylls and carotenoids obtained shown. Calone et al. (2022) found similar results in *S. fruticosa,* the concentration of chlorophyll a, b and beta-carotenes being stable in salinity and water stress presence. As well as our results shown, Calone et al. (2022) found that the recovery period did not affect pigment concentration. However, recovery treatment affected DES index values, being this parameter lower for plants in WS treatments compared to their recovered counterparts. This decrement could indicate a xanthophyll cycle malfunction (Duarte et al., 2013). On the other hand, it could be a mechanism to reduce damage due to excess energy dissipation through conversion of violaxanthin to zeaxanthin (Duarte et al., 2013). There has been evidence that halophytes that exhibit an increase in DES index values were able to respond better to abiotic stress (Pérez-Romero et al., 2018; 2020a). Therefore, the increase in this parameter in *S. fruticosa* plants recovering from the stress period could indicate that these plants responded to water and salinity stress by dissipating excess energy, allowing them to restore their physiological status.

Furthermore, when studying the fluorescence-derived parameters, there is no evidence of permanent damage in the *S. fruticosa* PS II imposed by the stress conditions. In fact, the OJIP derived parameters showed that only the plants grown under WS 1% appeared to be negatively affected by the conditions imposed and after the recovery period these plants were completely recovered with values higher than those of the control conditions. PS II of halophytes has demonstrated great levels of tolerance to salinity and water stress (Mateos-Naranjo et al., 2015; Pérez-Romero et al., 2018; 2020b). This characteristic resistance is usually related to its ability to activate some energy dissipation strategies (Mateos-Naranjo et al., 2015; Pérez-Romero et al., 2018; 2019; 2020a). As the dissipated energy flux (DI/CS) showed, in the recovery periods plants that were subjected to water stress had higher values than when they were in the stress period. This is in accordance with our results of the DES index, DI/CS is the amount of energy that it is not being fixed by the PS II and is related with energy dissipation mechanisms such as xanthophyll cycle or photorespiration that alleviate stress decreasing the pressure in PS II during abiotic stress exposition (Pérez-Romero et al., 2020a). Therefore, as the DES index pointed out, *S. fruticosa* at the recovery period responded to the negative effects indicating that the stress consequences could be strong enough to had a long-term effect. However, as the physiology parameters indicated this response to stress at the recovery period allowed this halophyte to restore its photosynthetic parameters.

The lack of recovery for $A_N$ at WW 3% R-NaCl could be due to a reduction in $F_v/F_m$ in the recovery period. This was the only treatment that showed this trend for this parameter, *S. fruticosa* has demonstrated its PS II tolerance to abiotic stress such as heat waves and salinity levels higher than 510 mM NaCl (Pérez-Romero et al., 2020a). Our results are in accordance with this tolerance, as OJIP derived parameters and $\Phi_{PSII}$ indicated for the treatments tested. Nonetheless, the decrease observed at WW 3% R-NaCl could be related to the DES index and the DI/CS values. Neither of those parameters increases for this treatment in the recovery period. Therefore, the xanthophyll cycle and the excess energy dissipation exhibited by the other plants grown under the recovery conditions did not appear in these plants at WW 3% R-NaCl. This could have led to a damage in its PS II efficiency, as $F_v/F_m$ and $A_N$ indicated, even when the concentrations of $g_s$, $C_i$, and photosynthetic pigments showed values similar to those under control conditions.

Halophytes resistance to water stress has been reported before (Mateos-Naranjo et al., 2015; Pérez-Romero et al., 2018; 2020b). However, in this study we can see how plants grown under WS 1% conditions showed an overall decreased in many of the parameters studied as AN, gs, Ci or OJIP derived parameters. Nevertheless, photodamage does not generally occur during water stress (Colom and Vazzana 2001) and our recovery period results seemed to support the lack of permanent photodamage. All parameters measured during recovery period for plants grown at WS 1% R-WW treatments displayed values equal or even higher than those of plants grown at control conditions. Thus, these results showed that even if drought could temporary affect photosynthesis function in *S. fruticosa* this species had the ability to overcome the negative effects when a recovery period was established.

## Conclusions

Our data highlight the well-known relationship between water and salinity stress and the great ability of *S. fruticosa* to resist salinity and water stress even when they occurred at the same time. The main achievement of this work is to study the ability of its photosystem to recover after exposure to these abiotic factors, showing that the exposition to high NaCl along with water deficit allowed this species to restore its optimal state better than those of *S. fruticosa* plants that were only subjected to salinity stress. The mechanisms that this species has to cope with water and salt stress seemed to be similar and due to that it allowed a restoration of its physiological state. Therefore, plants under both tested stresses were able to recover in 15 days as the results for gas exchange and chlorophyll fluorescence suggested.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgements

The authors thank the Research General Services of the University of Seville Greenhouse and Herbarium (SGI and SGH, CITIUS) for providing facilities and equipment. We also thank the Ministerio de Ciencia e Innovación-AEI for the financial support of Project PID2021–124750NB-I00, MCIN/ AEI /10.13039/501100011033/FEDER and Project TED2021–131605B-I00, MCIN/AEI /10.13039/501100011033, Next-GenerationEU/ PRTR. J.A. Pérez-Romero thanks the Ministerio de Ciencia y Educación for his personal financial support (FJC2020–043865-I). And J.M. Barcia-Piedras thanks the Fondo Europeo de Desarrollo Regional for his personal financial support (RSRR.RSRR1900.002).

## References

Barcia-Piedras, J.M., Pérez-Romero, J.A., Mateos-Naranjo, E., Camacho, M., Redondo-Gómez, S., 2019. Effect of prior salt experience on desalination capacity of the halophyte Arthrocnemum macrostachyum. Desalination 463, 50–54. https://doi.org/10.1016/j.desal.2019.03.006.

Belkheiri, O., Mulas, M., 2013. The effects of salt stress on growth, water relations and ion accumulation in two halophyte Atriplex species. Environ. Exp. Bot. 86, 17–28. https://doi.org/10.1016/j.envexpbot.2011.07.001.

Bongaarts, J., FAO, IFAD, UNICEF, WFP, WHO, 2020. The State of Food Security and Nutrition in the World 2020. Tranforming Food Systems For Affordable Healty Diets. FAO, Rome, Italy. ISBN 9789251329016.

Brugnoli, E., Lauteri, M., 1991. Effects of salinity on stomatal conductance, photosynthetic capacity, and carbon isotope discrimination of salt- tolerant (Gossypium hirsutum L.) and salt-sensitive (Phaseolus vulgaris L.) C3 non-halophytes. Plant Physiol. 95, 628–635. https://doi.org/10.1104/pp.95.2.628.

Calone, R., Mircea, D.M., González-Orenga, S., Boscaiu, M., Lambertini, C., Barbanti, L., Vicente, O., 2022. Recovery from Salinity and Drought Stress in the Perennial Sarcocornia fruticosa vs. the Annual Salicornia europaea and S. veneta. Plants 11 (8), 1058. https://doi.org/10.3390/plants11081058.

Campbell, B.M., Thornton, P., Zougmoré, R., van Asten, P., Lipper, L., 2014. Sustainable intensification: what is its role in climate smart agriculture? Curr. Opin. Environ. Sustain. 8, 39–43. https://doi.org/10.1016/j.cosust.2014.07.002.

Carreiras, J., Pérez-Romero, J.A., Mateos-Naranjo, E., Redondo-Gómez, S., Matos, A.R., Caçador, I., Duarte, B., 2020. The effect of heavy metal contamination pre-conditioning in the heat stress tolerance of native and invasive Mediterranean halophytes. Ecol. Indic. 111, 106045 https://doi.org/10.1016/j.ecolind.2019.106045.

Colom, M.R., Vazzana, C., 2001. Drought stress effects on three cultivars of Eragrostis curvula: photosynthesis and water relations. Plant Growth Regul. 34 (2), 195–202. https://doi.org/10.1023/A:1013392421117.

Cuttelod, A., García, N., Malak, D.A., Temple, H.J., & Katariya, V. (2009). The Mediterranean: a biodiversity hotspot under threat. Wildlife in a Changing World–an analysis of the 2008 IUCN Red List of Threatened Species, 89(2019), 9.

Duarte, B., Santos, D., Marques, J.C., Cacador, I., 2013. Ecophysiological adaptations of two halophytes to salt stress: photosynthesis, PS II photochemistry and anti-oxidant feedback–implications for resilience in climate change. Plant Physiol. Biochem. 67, 178–188. https://doi.org/10.1016/j.plaphy.2013.03.004.

Duarte, B., Silva, H., Marques, J.C., Caçador, I., Sleimi, N., 2014. Light-dark $O_2$ dynamics in submerged leaves of C3 and C4 halophytes under increased dissolved $CO_2$: clues for saltmarsh response to climate change. Ann. Bot. Plants 6, plu06. https://doi.org/10.1093/aobpla/plu067.

Duarte, B., Cabrita, M.T., Gameiro, C., Matos, A.R., Godinho, R., Marques, J.C., Cacador, I., 2017. Disentangling the photochemical salinity tolerance in Aster tripolium L.: connecting biophysical traits with changes in fatty acid composition. Plant Biol. 19 (2), 239–248. https://doi.org/10.1111/plb.12517.

Flexas, J., Bota, J., Loreto, F., Cornic, G., Sharkey, T.D., 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. Plant Biol. 6 (03), 269–279. https://doi.org/10.1055/s-2004-820867.

Hoagland, D.R., Arnon, D.I., 1938. The water culture method for growing plants without soil. Calif. Agric. Ext. Serv. Circ. 347, 32.

Hormaetxe, K., Becerril, J.M., Hernández, A., Esteban, R., García-Plazaola, J.I., 2006. Plasticity of photoprotective mechanisms of Buxus sempervirens L. leaves in response to extreme temperatures. Plant Biol. 9 (1), 59–68. https://doi.org/10.1055/s-2006-924456.

Hussain, S., Amin, A., Mubeen, M., Khaliq, T., Shahid, M., Hammad, H.M., Sultana, S.R., Awais, M., Murtaza, B., Amjad, M.;., et al., 2022. Climate smart agriculture (CSA) technologies. Building Climate Resilience in Agriculture: Theory, Practice and Future Perspective 319–338. https://doi.org/10.1007/978-3-030-79408-8_20. Jatoi, W.N., Mubeen, M., Ahmad, A., Cheema, M.A., Lin, Z., Hashmi, M.Z.ISBN 978-3-030-79408-8.

IPCC, 2014. Core writing team. Pachauri, R.K., Meyer, L.A. Climate Change 2014: Synthesis Report. IPCC, Geneva, Switzerland, p. 151. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel On Climate Change.
Kupper, H., Seibert, S., Parameswaran, A., 2007. A. Fast, sensitive, and inexpensive alternative to analytical pigment HPLC: quantification of chlorophylls and carotenoids in crude extracts by fitting with Gauss peak Spectra. Anal. Chem. 79 (20), 7611–7627. https://doi.org/10.1021/ac070236m.
Marshall, H.L., Geider, R.J., Flynn, K.J., 2000. A mechanistic model of photoinhibition. New Phytol. 145 (2), 347–359. https://doi.org/10.1046/j.1469-8137.2000.00575.x.
Mateos-Naranjo, E., Mesa, J., Pajuelo, E., Perez-Martin, A., Caviedes, M.A., Rodriguez-Llorente, I.D., 2015. Deciphering the role of plant growth-promoting rhizobacteria in the tolerance of the invasive cordgrass Spartina densiflora to physicochemical properties of salt-marsh soils. Plant Soil 394 (1–2), 45–55. https://doi.org/10.1007/s11104-015-2504-7.
Mateos-Naranjo, E., Pérez-Romero, J.A., Redondo-Gómez, S., Mesa-Marín, J., Castellanos, E.M., Davy, A.J., 2018. Salinity alleviates zinc toxicity in the saltmarsh zinc-accumulator Juncus acutus. Ecotoxicol. Environ. Saf. 163, 478–485. https://doi.org/10.1016/j.ecoenv.2018.07.092.
Moreira da Silva, M., Anibal, J., Duarte, B., Chicharo, L., 2015. Sarcocornia fruticosa and Spartina maritima as heavy metals remediators in an European southwestern salt marsh (Ria Formosa, Portugal). J. Environ. Prot. Ecol. 16 (4), 1468–1477. https://scibulcom.net/en/article/qVqQiZ7L7Yuaphczxwxz (accessed on 12 April 2023).
Pérez-Romero, J.A., Redondo-Gómez, S., Mateos-Naranjo, E., 2016. Growth and photosynthetic limitation analysis of the Cd-accumulator Salicornia ramosissima under excessive cadmium concentrations and optimum salinity conditions. Plant Physiol. Biochem. 109, 103–113. https://doi.org/10.1016/j.plaphy.2016.09.011.
Pérez-Romero, J.A., Idaszkin, Y.L., Barcia-Piedras, J.M., Duarte, B., Redondo-Gómez, S., Caçador, I., Mateos-Naranjo, E., 2018. Disentangling the effect of atmospheric $CO_2$ enrichment on the halophyte Salicornia ramosissima J. Woods physiological performance under optimal and suboptimal saline conditions. Plant Physiol. Biochem. 127, 617–629. https://doi.org/10.1016/j.plaphy.2018.04.041.
Pérez-Romero, J.A., Barcia-Piedras, J.M., Redondo-Gomez, S., Mateos-Naranjo, E., 2019. Impact of short-term extreme temperature events on physiological performance of Salicornia ramosissima J. Woods under optimal and sub-optimal saline conditions. Sci. Rep. 9 (1), 659. https://doi.org/10.1038/s41598-018-37346-4.
Pérez-Romero, J.A., Barcia-Piedras, J.M., Redondo-Gómez, S., Mateos-Naranjo, E., 2020a. Sarcocornia fruticosa photosynthetic response to short-term extreme temperature events in combination with optimal and sub-optimal salinity concentrations. Plant Physiol. Biochem. 148, 45–52. https://doi.org/10.1016/j.plaphy.2019.12.026.
Pérez-Romero, J.A., Mateos-Naranjo, E., López-Jurado, J., Redondo-Gómez, S., Torres-Ruiz, J.M., 2020b. Importance of physiological traits vulnerability in determine halophytes tolerance to salinity excess: a comparative assessment in Atriplex halimus. Plants 9 (6), 690. https://doi.org/10.3390/plants9060690.
Pérez-Romero, J.A., Barcia-Piedras, J.M., Redondo-Gómez, S., Caçador, I., Duarte, B., Mateos-Naranjo, E., 2022. Salinity modulates Juncus acutus L. tolerance to diesel fuel pollution. Plants 11 (6), 758. https://doi.org/10.3390/plants11060758.
Redondo-Gomez, S., Wharmby, C., Castillo, J.M., Mateos-Naranjo, E., Luque, C.J., De Cires, A., Luque, T., Davy, A.J., Enrique Figueroa, M., 2006. Growth and photosynthetic responses to salinity in an extreme halophyte, Sarcocornia fruticosa. Physiol. Plant. 128 (1), 116–124. https://doi.org/10.1111/j.1399-3054.2006.00719.x.
Said, O.B., da Silva, M.M., Hannier, F., Beyrem, &.H., Chicharo, L., 2018. Using Sarcocornia fruticosa and Saccharomyces cerevisiae to remediate metal contaminated sediments of the Ria Formosa lagoon (SE Portugal). Ecohydrol. Hydrobiol. 19, 588–597. https://doi.org/10.1016/j.ecohyd.2018.10.002.
Schreiber, U., Schliwa, W., Bilger, U., 1986. Continuous recording of pho- tochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorimeter. Photosynth. Res. 10, 51–62. https://doi.org/10.1007/BF00024185.
Strasser, R.J., Tsimilli-Michael, M., Srivastava, A., 2004. Analysis of the Chlorophyll fluorescence transient. Papageorgiou, C, George, G Chlorophyll Fluorescence: a Signature of Photosynthesis. Springer, Dordrecht, Netherlands, pp. 321–362. https://doi.org/10.1007/978-1-4020-3218-9_12.
Ventura, Y., Eshel, A., Pasternak, D., Sagi, M., 2015. The development of halophyte-based agriculture: past and present. Ann. Bot. 115 (3), 529–540. https://doi.org/10.1093/aob/mcu173.
Ventura, Y., Sagi, M., 2013. Halophyte crop cultivation: the case for Salicornia and Sarcocornia. Environ. Exp. Bot. 92, 144–153. https://doi.org/10.1016/j.envexpbot.2012.07.010.
Verena, I., Becker, A., Johannes, A., Goessling, J.W., Duarte, B., Cacador, I., 2016. Combined effects of soil salinity and high temperature on photosynthesis and growth of quinoa plants (Chenopodium quinoa). Funct. Plant Biol. 44 (7), 665–678. https://doi.org/10.1071/FP16370.
World Bank Group, 2016. Climate-Smart Agriculture Indicators. World Bank, Washington, DC, USA, p. 126. https://documents1.worldbank.org/curated/en/187151469504088937/pdf/105162-WP-P132359-PUBLIC-CSAIndicatorsReportweb.pdf (accessed on 12 April 2023).