

WILL HALOPHYTES IN MEDITERRANEAN SALT MARSHES BE ABLE TO ADAPT TO CLIMATE CHANGE?

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Abstract

Mediterranean salt marshes are fragile ecosystems, highly susceptible to the effects of climate change, inhabited by a characteristic halophytic flora, which includes abundant and widely distributed 'structural' halophytes, together with rare species of high ecological value. These plants are distributed along salinity gradients, primarily based on their relative salt tolerance; they are also adapted to the high temperatures and drought characteristic of Mediterranean summers. With periods of drought and heatwaves increasingly frequent and intense, global warming will also cause a rise in soil salinity. These changes could affect the distribution of some species, reducing their populations or even leading to the local disappearance of some taxa. Over the last years, we have investigated the salt and water stress responses of halophytes of several genera, in field studies and under greenhouse conditions. In this communication, we will present results showing that at least some of the species investigated possess mechanisms that can allow them to rapidly adapt and survive the climate change-induced alterations of the environment in their natural habitats

Key words: climate change adaptation, drought, heatwaves, salinity, stress tolerance.

INTRODUCTION

Climate change represents a major concern for the boost of crop yields and food production that will be required in the next decades to feed a growing human population. Global warming-induced alteration of normal seasonal weather patterns will increase the frequency, extent and intensity of meteorological phenomena, such as floods, drought periods or heatwaves, apart from the general rise of average temperatures worldwide (IPCC, 2014). In arid and semi-arid regions, and to a lesser extent in other areas, secondary salinisation of farmland cultivated under irrigation will also spread; good-quality fresh water is becoming an increasingly scarce resource for agriculture, and it will be more and more necessary to use low-quality, brackish water for irrigation.

Abiotic environmental stress conditions, especially drought and soil salinity, are the major causes for the global reduction of crop yields (Buchanan et al., 2000; Boscaiu et al., 2018). The sudden increase in temperature during a heatwave could also be devastating for a crop if its coincides with its pollination period

since pollen development and viability are extremely sensitive to heat stress (Giorno et al., 2013). Therefore, a lot of effort is being invested - with relatively little success, until now, but with promising results obtained in the last years - on enhancing the tolerance of our major crops to abiotic stress, especially to water deficit, salt stress and high temperatures. For this, all available methods are being tried, including traditional breeding (with the help of modern molecular tools), genetic engineering or even the domestication of naturally tolerant wild species (Fita et al., 2015).

Much less attention has been paid to the harmful effects of climate change on the natural habitats of wild plants, many of them extremely interesting considering ecological, conservationist and biodiversity aspects, even if not with direct economic relevance. They include, for example, different types of saline ecosystems inhabited by halophytes, 'salt-loving' plants that are able to survive and complete their life cycle in habitats with soil salinities equivalent to, at least, 200 mM NaCl - according to a generally accepted, operational definition (Flowers and Colmer 2008) -

although some of them can withstand much higher salt concentrations, even higher than that of seawater.

Over the last years, we have performed field studies to assess the responses to seasonal changes in environmental stress conditions of a large number of halophytes of several genera, growing in different salt marshes of the Valencian Community (SE Spain). This work has been complemented by the analysis of their responses to water deficit and salt stress under controlled greenhouse conditions. The general aim of this ongoing project is to try and establish the physiological, biochemical and molecular mechanisms of stress tolerance of these naturally tolerant species; we believe that they are more useful for this kind of studies than more commonly used, but non-tolerant models such as *Arabidopsis thaliana* or some crop species. Moreover, the knowledge gained from this research may contribute to the design and implementation of efficient management, conservation and regeneration programmes of these threatened habitats.

In this paper, we will present a summary of part of the work published by our group during the last years. As a general conclusion, our results suggest that at least some of the investigated species possess mechanisms that will most likely allow them to rapidly adapt and survive the climate change-induced exacerbation of the (already quite harsh) stressful environmental conditions in their natural habitats.

MEDITERRANEAN SALT MARSHES

Salt marshes are some of the most fragile and threatened ecosystems worldwide, much affected by anthropogenic actions. In the Mediterranean coast of Spain, as in many other regions, these habitats have been largely reduced or eliminated, because they were considered as insalubrious in the past, due to the expansion of agriculture, urbanism and industry, or by suffering an intense touristic pressure. Today, the remaining littoral salt marshes of the Valencian Community are protected habitats; protected, that is, from harmful human interventions, but still highly susceptible to the effects of climate change. Salt marshes are inhabited by a characteristic halophytic flora, which includes abundant and widely distributed ‘structural’ halophytes,

along with rare and endemic species of high ecological value. Although all these species are ‘halophytes’, as defined above, their degree of tolerance is variable, so that they are distributed along salinity gradients in the salt marsh, primarily based on their relative salt tolerance. However, soil salinity is not the only stress factor for the halophytes growing in Mediterranean salt marshes. They must also be adapted to the high temperatures and intense droughts of Mediterranean summers, often following heavy rains in spring. Therefore, in some years the plants may switch in a few months from waterlogging in spring to extremely dry conditions in summer, when the soil surface is covered by a salt crust, due to the intense evapotranspiration (Figure 1).



Figure 1. Salt marsh in ‘La Albufera’ Natural Park, near the city of Valencia, in spring (left) and summer (right)

In the Mediterranean Spanish coast, climate change has already caused, in the last years, out-of-season heavy rains, cold spells and high temperatures, and the periods of drought and heatwaves are becoming longer, more frequent and more intense. Therefore, the salt marsh vegetation will be affected shortly not only by higher soil salinity but also by an increase in other stressful environmental factors. As indicated in the title, the question arises as to whether the halophytic species present in these salt marshes will be able to quickly adapt to these changes and survive.

RESPONSES OF HALOPHYTES TO ENVIRONMENTAL STRESS: FIELD STUDIES

Our initial studies were carried out in a marked-off experimental plot within a littoral salt marsh in ‘La Albufera’ Natural Park, near the city of Valencia. Several (over ten) salt-tolerant wild species present in this plot were selected for this project, including three succulent dicotyledonous halophytes, two of them highly salt-tolerant (*Sarcocornia fruticosa*

and *Inula crithmoides*) and the third one (*Plantago crassifolia*) resistant to somewhat lower salinity levels. The study started in 2008 and lasted several years, although only data from summer 2009 (July 1st) to autumn 2010 (November 23rd) were initially published and are included in this report. During this period, in different seasons, we collected climate data and soil samples, to estimate the type and intensity of the environmental stress affecting the plants in their natural habitat and their seasonal variations. Simultaneously with soil samples, leaf material was collected from the plants, to determine the levels of several stress biomarkers (ions, compatible solutes, markers of oxidative stress, antioxidants). The same individual plants, labelled in the field, were used for all samplings (all selected species are perennial).

Seasonal variations in atmospheric and soil parameters in the experimental zone

During the study period, meteorological data [rainfall, evapotranspiration (ETP), and mean, maximum, and minimum temperatures], registered by a nearby (~10 km away) agroclimatological station, were collected in a weekly basis. From these data, the mean temperature and ETP were calculated for two months before every sampling date. According to the general patterns of Mediterranean climate, this analysis revealed substantial seasonal variations in precipitations (abundant in autumn and spring and very low in summer) and ETP (highest in summer); changes in average temperatures, however, were relatively small as the salt marsh is close to the sea (data not shown). These seasonal oscillations in atmospheric conditions were reflected in parallel changes in soil properties. Some data of the soil analyses are shown in Figure 2 and Table 1.

Soil salinity, measured as electric conductivity in soil/water (1:1) extracts ($EC_{1:1}$), reached the highest values in summer of both, 2009 and 2010, almost 10-fold larger than in spring 2010, when the lowest EC was measured. Intermediate soil salinities were determined in autumn, relatively smaller in 2009 when rainfall was more intense than in 2010 (Figure 2). Soil water content followed the opposite pattern, with the highest humidity measured in spring and the lowest in summer, especially in

2009 since sampling was preceded by an extremely dry period (Figure 2).

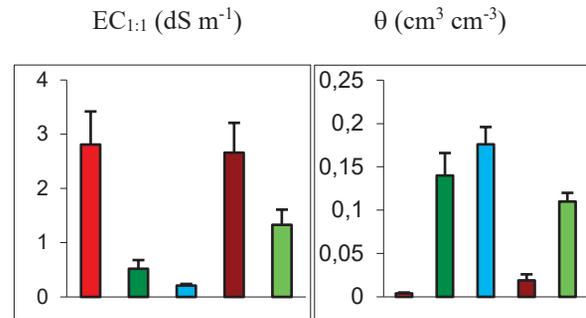


Figure 2. Soil electric conductivity ($EC_{1:1}$) and soil humidity (θ) in the experimental zone, in samples collected in (from left to right) summer 2009 (red), autumn 2009 (green), spring 2010 (blue), summer 2010 (dark red) and autumn 2010 (light green) (Adapted from Gil et al., 2014)

The seasonal oscillations in soil electric conductivity were paralleled by changes in Na^+ and Cl^- contents in the soil, again with the highest concentrations measured in summer 2009 and the lowest in spring 2010. The observed differences between these extreme values were more than 8-fold for Na^+ , and more than 30-fold for Cl^- . On the other hand, soil K^+ contents were very low and did not change significantly during the whole study period (Table 1).

Table 1. Ions contents in the soil samples of Figure 2 (Adapted from Gil et al., 2014)

Sampling	Na^+ ($\mu\text{mol g}^{-1}$)	Cl^- ($\mu\text{mol g}^{-1}$)	K^+ ($\mu\text{mol g}^{-1}$)
Summer09	26.7 ± 6.0c	30.5 ± 7.2d	0.6 ± 0.4a
Autumn09	3.3 ± 0.9a	4.2 ± 1.8b	0.5 ± 0.1a
Spring10	3.2 ± 0.7a	0.9 ± 0.3a	0.5 ± 0.1a
Summer10	20.0 ± 10.4c	22.8 ± 9.3d	0.7 ± 0.4a
Autumn10	8.3 ± 3.1b	9.2 ± 4.3c	0.5 ± 0.1a

We concluded from these measurements that the plants in the salt marsh are subjected to big seasonal changes in several stressful environmental parameters. Therefore, it is clear that they are already adapted to strong salinity and drought conditions, to a greater or lesser extent depending on the species, as these conditions are prevalent at least in summer. Elucidation of the mechanisms underlying the stress tolerance of a particular species should help to determine whether or not such mechanisms will be still effective if the

intensity of the stress conditions increases as a consequence of climate change.

Constitutive mechanisms of stress tolerance in highly tolerant, dicotyledonous halophytes

One common mechanism of salt tolerance in dicotyledonous halophytes is based on the active transport of Na^+ and Cl^- ions to the aerial part of the plants. Then, they are predominantly stored in the vacuoles of the leaf cells, to avoid reaching toxic levels in their cytoplasm. Succulent plants, such as *S. fruticosa*, *I. crithmoides* and, to a lesser extent, *P. crassifolia*, with huge vacuole volume, can store the ions to high concentrations in the leaves. Indeed, this is what we observed when we measured leaf Na^+ contents in the plants (Figure 3).

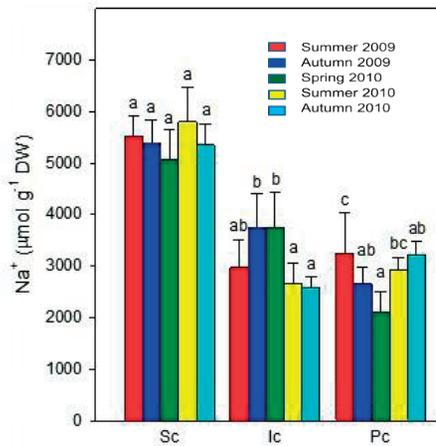


Figure 3. Sodium contents in leaves of *Sarcocornia fruticosa* (Sc), *Inula crithmoides* (Ic) and *Plantago crassifolia* (Pc). Leaf material was collected in the indicated seasons from the same individual plants. Means \pm SD (n = 5) (Adapted from Gil et al., 2014)

In the most tolerant species, *S. fruticosa*, Na^+ reached very high levels of around 6 mmol g^{-1} DW; ion contents were somewhat lower in *I. crithmoides* (Figure 3). A very similar pattern, with somewhat higher absolute values, was obtained for Cl^- (Gil et al., 2014). What is more important, in both species leaf ion levels were not significantly different, in general, in the different samplings, despite the wide seasonal variation in their concentrations in the soil (Table 1). Regarding the relatively less tolerant *P. crassifolia*, leaf Na^+ (and also Cl^-) levels showed seasonal patterns roughly following their changes in the soil; that is, highest concentrations in summer and lowest in spring. Still, even in this season, when soil salinity is at a minimum, the absolute values of

ion contents in the leaves were quite high: about 2 mmol g^{-1} DW for Na^+ and $>3 \text{ mmol g}^{-1}$ DW for Cl^- (Figure 3, and data not shown). Accumulation of compatible solutes or osmolytes is another general response of plants to drought, salinity and other stress conditions causing cellular dehydration. These compounds contribute to cellular osmotic balance under stress and have additional roles in stress responses as low-molecular-weight chaperons, ROS scavengers and signalling molecules (Slama et al., 2015).

Glycine betaine (GB) is the major functional osmolyte in *S. fruticosa* and *I. crithmoides*, and it accumulated to high concentrations in the leaves of both species, especially in the former, during the whole period of study. In general, no significant seasonal changes in GB contents were observed, except for the substantially higher value measured in summer 2009 in *I. crithmoides* (Figure 4A). *Plantago crassifolia*, on the other hand, showed very low GB levels in all samplings, as this species is not a GB-accumulator (Figure 4A). It is well-established that species of the genus *Plantago* use sorbitol as the major functional osmolyte and, indeed, we detected the accumulation of sorbitol in *P. crassifolia* following seasonal patterns roughly parallel to those of soil salinity (Figure 4B).

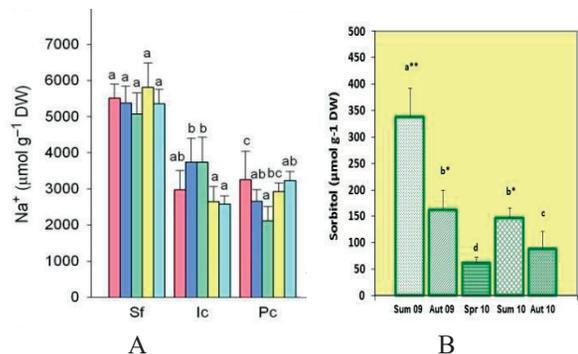


Figure 4. Glycine betaine (A) leaf contents in *Sarcocornia fruticosa* (Sc), *Inula crithmoides* (Ic) and *Plantago crassifolia* (Pc). Samplings as in Figure 3. Sorbitol (B) leaf contents in *Plantago crassifolia*. Leaf material was collected in the indicated seasons from the same individual plants. In both panels, means \pm SD (n = 5) are shown

Adapted from Gil et al., 2014 (A) and Gil et al. 2011 (B)

These data clearly indicate that halophytes, at least the most salt-tolerant ones, possess constitutive mechanisms that allow them to rapidly respond to changes in the environment,

for example, to seasonal oscillations in soil salinity and soil water content, which can be very wide in the salt marsh habitat. These mechanisms include the accumulation in the leaves of specific organic osmolytes (glycine betaine in the case of *S. fruticosa* and *I. crithmoides*), but also of inorganic osmolytes, Na⁺ and Cl⁻ ions that contribute to osmotic balance under stress conditions. In terms of energy consumption, it is much 'cheaper' maintaining large leaf concentrations of organic and inorganic osmolytes all the year-long, independently of the external conditions, than the stress-induced active transport of ions to the leaves or, especially, the *de novo* synthesis of GB or other organic osmolytes. Therefore, contrary to non-tolerant species, halophytes can invest a greater fraction of their resources in maintaining primary metabolism and growth under stress.

Oxidative stress and activation of antioxidant systems

Different abiotic stress conditions, including drought and salinity, cause as a secondary effect, oxidative stress in plants, through the increase in 'reactive oxygen species' (ROS) concentrations. The degree of oxidative stress affecting the plants is routinely assessed by measuring the stress-induced changes in the levels of biomarkers like malondialdehyde (MDA), a product of membrane lipid peroxidation. Plants respond to oxidative stress by activating a series of antioxidant systems, both enzymatic and non-enzymatic (Apel and Hirt, 2004).

In our field study, however, we did not observe any significant seasonal change in MDA concentrations, in any of the investigated halophytes. Consequently, we also did not detect changes in the specific activities of some of the major antioxidant enzymes, SOD (superoxide dismutase), CAT (catalase) or GR (glutathione reductase), or in the levels of antioxidant compounds such as total phenolics or flavonoids (Gil et al., 2014). We concluded that, under field conditions, the observed stress tolerance mechanisms based on the control of ion transport and ion homeostasis, and on the synthesis and accumulation of specific osmolytes are efficient enough to avoid the generation of oxidative stress. Therefore, it is not necessary for the plants to activate

antioxidant enzymes or to synthesise antioxidant metabolites to counteract the deleterious effects of salt or water stress.

RESPONSES OF HALOPHYTES TO CONTROLLED STRESS TREATMENTS: GREENHOUSE STUDIES

Field studies carried out with several halophytes, including those described above, indicate that these plants can rapidly adapt to changing environmental conditions in their natural habitat. To address the question of their possible tolerance to increased levels of stress in the salt marsh, which can be expected as a consequence of climate change, plants of the same and many other species have been subjected to controlled water deficit and salt stress treatments in the greenhouse.

It is essential to point out that a direct comparison of the results obtained in the field and the greenhouse is not possible. First, the developmental stage of the plants is different. Adult plants of unknown age (all selected species are perennial) were studied in the field, whereas young plants obtained by seed germination were employed in the greenhouse experiments, and it is well established that stress tolerance varies with the age of the plants (Vicente et al., 2004). Second, plants in their natural environment are simultaneously affected by different stress factors. In a Mediterranean salt marsh in summer, they will include high soil salinity, intense drought and UV radiation and possibly high temperatures, especially during a heatwave. In the experimental set-up of the greenhouse, it is intended that plants are maintained under optimal conditions, except for the specific stress treatment applied. Finally, the root system of potted plants is constrained in a reduce volume under uniform conditions of, for example, substrate salinity or moisture. These plants do not have the possibility of responding to stress by morphological or developmental adaptations; in plants in the field, on the contrary, stress can induce root growth to reach more profound and more humid soil layers or to search for soil zones of lower salinity.

Nevertheless, controlled stress treatments in the greenhouse provided relevant information on the possible limits of tolerance of the investigated species. Our results indicated that

all tested halophytes could survive, for relatively long periods, stress conditions much stronger than those they would normally encounter in their natural habitats. The stress treatments affect the plants in different degrees, according to their relative levels of tolerance. For example, some species of the genus *Limonium* survived for four weeks after complete withholding of irrigation (González-Orenga et al., 2020), and in the presence of salt concentrations as high as 800 mM NaCl - more than double the salinity of seawater - (Al Hassan et al., 2017), showing no or very slight inhibition of growth. Moreover, two halophytes of the genus *Juncus*, *J. maritimus* and *J. acutus*, also survived eight weeks of water deficit or salt stress (400 mM NaCl) treatments, although in this case a significant inhibition of growth was observed in the stressed plants, as compared to the controls (Al Hassan et al., 2016a). Similar results were obtained with two salt-tolerant *Plantago* species, *P. crassifolia* and *P. coronopus*, which withstood an 800 mM NaCl treatment for four weeks, salinity level much higher than that of their natural habitats (Al Hassan et al., 2016b).

The mechanisms underlying the tolerance to salt and water stress of these plants are diverse, although some of them are shared by different, often taxonomically unrelated species. As an example, some results from our experiments with *P. crassifolia* are described and discussed in the following sections.

Regulation of ion transport in the responses to stress in *P. crassifolia*

Accumulation of Na⁺ to high concentrations (up to ~ 3 mmol g⁻¹ DW) was detected in the leaves of *P. crassifolia* plants, in parallel with the increase of salinity in the irrigation water, following the behaviour generally shown by dicotyledonous halophytes (Figure 5). It should be noticed that Na⁺ contents were also quite high (1 mmol g⁻¹ DW) in the control plants, which were grown under very low salinity in the pots. These results confirmed the field data in that *P. crassifolia*, as other halophytes, activates the transport of ions to the leaves in response to increasing soil salinity, and that this response is in part constitutive, also operating in the absence of stress.

The same pattern of leaf accumulation was observed for Cl⁻ in *P. crassifolia*, and similar

for both ions, Na⁺ and Cl⁻ in *P. coronopus*, a congener halophytic species. It is worth mentioning that these specific responses to salt stress, based on the active transport of ions to the aboveground organs of the plants and the constitutive accumulation of Na⁺ in leaves in the absence of stress, are relevant for the mechanisms of salt tolerance in *Plantago*.

In a glycophyte member of the same genus, *P. major*, the same treatments led to lower ion accumulation in the leaves, as compared with the halophytes, and very low Na⁺ and Cl⁻ concentrations were measured in the controls (Al Hassan et al., 2016b).

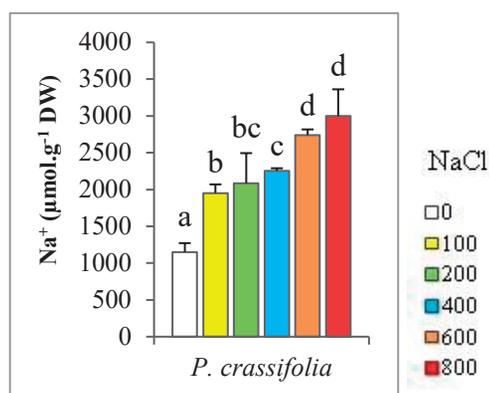


Figure 5. Leaf Na⁺ contents in *P. crassifolia* plants, after four weeks of growth in the presence of the indicated salt concentrations in the irrigation water.

(Adapted from Al Hassan et al., 2016b)

Interestingly, a significant increase in foliar Na⁺ (but not Cl⁻) contents has been observed in the halophytes *P. crassifolia* and *P. coronopus* (but not the glycophyte *P. major*) in response to the water deficit treatment (Table 2). Therefore, the halophytes can activate Na⁺ transport to the leaves as a mechanism of tolerance not only to high soil salinity but also to drought. This result highlights the role of sodium as ‘inorganic osmolyte’ in these tolerant species, contributing, together with organic compatible solutes to osmotic adjustment under salt stress and water deficit conditions.

Maintenance of relatively large leaf K⁺ concentrations is considered as an essential contributor to salt tolerance, due to the crucial role of this ‘physiological cation’ in plant metabolism. Salt treatments generally lead to a decrease in K⁺ contents, in parallel with the increase of Na⁺, as both cations compete for the same binding sites in proteins and Na⁺

interferes with K⁺ uptake into the cells by using its physiological membrane transporters (Flowers and Colmer, 2008).

We observed this general pattern also in the *Plantago* halophytes, but only at low and moderate salinities. In the presence of 600 mM and 800 mM NaCl, K⁺ contents increased again, to reach control levels (Al Hassan et al., 2016a). This result indicates the activation of K⁺ transport to the leaves at very high salinities, usually not reached in the natural habitats of the plants. This is not a mechanism specific for *P. crassifolia* and *P. coronopus*. We have observed the same response in some halophytes of other genera, such as *Juncus maritimus* and *J. acutus*, *Inula crithmoides* or some *Limonium* species, but not in typical glycophytes, such as *P. major* or *J. articulatus*.

Table 2. Leaf Na⁺ and Cl⁻ contents in plants of the indicated *Plantago* species, after four weeks of withholding irrigation. C, control; WS: water stress. Values are means ± SD (n = 5). For each species and ion, different letters indicate statistically significant differences between WS and control treatments

Species	Treatment	Na ⁺	Cl ⁻
		(μmol g ⁻¹ DW)	(μmol g ⁻¹ DW)
<i>P. crassifolia</i>	C	1099.1 ± 186.2 a	1183.6 ± 260.6 a
	WS	1486.4 ± 174.8 b	884.7 ± 117.6 a
<i>P. coronopus</i>	C	1343.9 ± 93.1 a	516.5 ± 98.5 a
	WS	1636.4 ± 198.8 b	554.9 ± 150.9 a
<i>P. major</i>	C	249.1 ± 38.4 a	395.9 ± 10.6 a
	WS	236.1 ± 23.7 a	443.65 ± 60.6 a

Activation of Pro synthesis at high salinities

The greenhouse experiments also confirmed the role of sorbitol as the major osmolyte in *Plantago*, present at high concentration in the leaves of control, non-stressed plants of the three investigated species. An additional, salt-induced increase in sorbitol contents was detected in all cases, especially in the most tolerant *P. crassifolia*. However, the absolute sorbitol values at all external salinities were similar in the three taxa. Therefore, the differences in tolerance cannot be explained by differential accumulation of sorbitol in the halophytes and *P. major*, even though sorbitol is probably the major contributor to osmotic adjustment under stress in all three species (Al Hassan et al., 2016a). However, we did observe a clear difference between the halophytes and

the glycophyte in the salt-induced changes of leaf proline (Pro) contents.

In the field study, very low Pro levels were measured in the selected dicotyledonous halophytes, including *P. crassifolia*, in all seasonal samplings. This was confirmed when the plants were treated in the greenhouse with NaCl solutions of up to 400 mM; however, higher salinities (600 mM and 800 mM NaCl) induced the synthesis and accumulation of Pro in *P. crassifolia* to levels about 15-fold more abundant than in the control, up to ca. 30 μmol g⁻¹ DW (Figure 6). The same response, reaching even higher Pro concentrations (~ 45 μmol g⁻¹ DW) was observed in *P. coronopus*. In *P. major*, on the contrary, Pro contents remained very low, below 3-4 μmol g⁻¹ DW, indicating that Pro accumulation is a relevant mechanism of salt tolerance in *Plantago* when the plants are subjected to very high salinity levels (Al Hassan et al., 2016a).

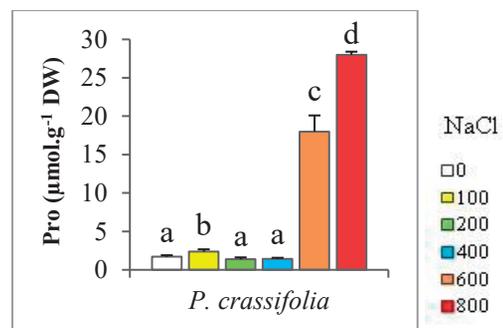


Figure 6. Leaf proline (Pro) contents in *P. crassifolia* plants, after four weeks of growth in the presence of the indicated salt concentrations in the irrigation water (Adapted from Al Hassan et al., 2016b)

Oxidative stress and antioxidant systems

As mentioned above, the studied halophytes did not seem to be affected by oxidative stress in their natural habitats. Under stronger, artificial stress conditions in the greenhouse, oxidative stress is however generated, as shown in several species by the increase in MDA concentrations; under these conditions, the plants responded by the activation of antioxidant systems.

If climate change induces an increase in the level of stress affecting the halophytic vegetation of the salt marsh, the activation of the antioxidant machinery of the halophytes will be triggered to counteract the secondary oxidative stress generated.

CONCLUSIONS

Halophytic species present in littoral salt marshes of the Mediterranean Spanish coast are well adapted to the harsh conditions of their natural habitat and the extreme seasonal oscillations in soil salinity and humidity. This adaptation is achieved mainly through the control of ion transport and maintenance of osmotic balance by the accumulation of organic and inorganic osmolytes. Besides, these species appear to possess built-in mechanisms that will allow them surviving the potential exacerbation of environmental stress factors as a consequence of climate change. These mechanisms, observed under artificial conditions in the greenhouse, include, for example, triggering the synthesis of additional organic osmolytes or K^+ transport to the leaves under very high salinities.

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REFERENCES

- Al Hassan, M., Estrelles, E., Soriano, P., López-Gresa, M. P., Bellés, J. M., Boscaiu, M., Vicente, O. (2017). Unraveling salt tolerance mechanisms in halophytes: A comparative study on four Mediterranean *Limonium* species with different geographic distribution patterns. *Frontiers in Plant Science*, 8, 1438. doi: 10.3389/fpls.2017.01438.
- Al Hassan, M., López-Gresa, M.P., Boscaiu, M., Vicente, O. (2016a). Stress tolerance mechanisms in *Juncus*: responses to salinity and drought in three *Juncus* species adapted to different natural environments. *Functional Plant Biology*, 43, 949-960. doi: 10.1071/FP16007.
- Al Hassan, M., Pacurar, A., López-Gresa, M.P., Donat-Torres, M.P., Llinares, J.V., Boscaiu, M., Vicente, O. (2016b). Effects of salt stress on three ecologically distinct *Plantago* species. *PLoS ONE*, 11(8), e0160236. doi:10.1371/journal.pone.0160236.
- Apel, K., Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, 55, 373-399.
- Boscaiu, M., Naranjo, M., Vicente, O. (2018). Strategies to increase crop yields in a climate change scenario. *Scientific Bulletin. Series F. Biotechnologies*, 22, 15-20.
- Buchanan, B.B., Gruissem, W., Jones, R.L. (2000). *Biochemistry and Molecular Biology of Plants*. American Society of Plant Physiologists, Rockville, MD., USA.
- Fita, A., Rodríguez-Burruezo, A., Boscaiu, M., Prohens, J., Vicente, O. (2015). Breeding and domesticating crops adapted to drought and salinity: a new paradigm for increasing food production. *Frontiers in Plant Science*, 6, 978. doi: 10.3389/fpls.2015.00978.
- Flowers, T.J., Colmer, T.D. (2008). Salinity tolerance in halophytes. *New Phytologist*, 179, 945-963.
- Gil, R., Bautista, I., Boscaiu, M., Lidón, A., Wankhade, S., Sánchez, H., Llinares, J., Vicente, O. (2014). Responses of five Mediterranean halophytes to seasonal changes in environmental conditions. *AoB Plants*, 6, plu049. doi:10.1093/aobpla/plu049
- Gil, R., Lull, C., Boscaiu, M., Bautista, I., Lidón, A., Vicente, O. (2011). Soluble carbohydrates as osmolytes in several halophytes from a Mediterranean salt marsh. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 39, 9-17.
- Giorno, F., Wolters-Arts, M., Mariani, C., Rieu, I. (2013). Ensuring reproduction at high temperatures: The heat stress response during anther and pollen development. *Plants*, 2, 489-506. doi: 10.3390/plants2030489.
- González-Orenga, S., Llinares, J.V., Al Hassan, M., Fita, A., Collado, F., Lisón, P., Vicente, O., Boscaiu, M. (2020). Physiological and morphological characterization of *Limonium* species in their natural habitats: Insights into their abiotic stress responses. *Plant and Soil*, 449, 267-284.
- Slama, I., Abdelly, C., Bouchereau, A., Flowers, T., Savoure, A. (2015). Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Annals of Botany*, 115, 433-447.
- Vicente, O., Boscaiu, M., Naranjo, M.A., Estrelles, E., Bellés, J.M. and Soriano, P. (2004). Responses to salt stress in the halophyte *Plantago crassifolia* (Plantaginaceae). *Journal of Arid Environments*, 58, 463-481.
- ***IPCC Intergovernmental panel on climate change. In Proceedings of the 5th Assessment Report, WGII, Climate Change 2014: Impacts, Adaptation, and Vulnerability. Retrieved January, 15, 2020 from <http://www.ipcc.ch/report/ar5/wg2/>.