Higher Novel L-Cys Degradation Activity Results in Lower Organic-S and Biomass in *Sarcocornia* than the Related Saltwort, *Salicornia*[1][OPEN]

Assylay Kurmanbayeva,[a] Aizat Bekturova,[a] Sudhakar Srivastava,[a] Aigerim Soltabayeva,[a] Armine Asatryan,[a] Yvonne Ventura,[a] Mohammad Suhail Khan,[b] Octavio Salazar,[b] Nina Fedoroff,[b,c] and Moshe Sagi[a,2]

[a]Plant Stress Laboratory, French Associates Institute for Agriculture and Biotechnology of Drylands, Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, 84990, Israel
[b]King Abdullah University of Science and Technology, Biological and Environmental Science and Engineering Division (BESE), Thuwal, 23955-6900, Saudi Arabia
[c]Evan Pugh Professor Emerita, Penn State University, State College, Pennsylvania

ORCID IDs: 0000-0001-8439-3910 (S.S.); 0000-0002-9962-810X (M.S.K.); 0000-0003-3307-9504 (M.S.).

Salicornia and *Sarcocornia* are almost identical halophytes whose edible succulent shoots hold promise for commercial production in saline water. Enhanced sulfur nutrition may be beneficial to crops naturally grown on high sulfate. However, little is known about sulfate nutrition in halophytes. Here we show that *Salicornia europaea* (ecotype RN) exhibits a significant increase in biomass and organic-S accumulation in response to supplemental sulfate, whereas *Sarcocornia fruticosa* (ecotype VM) does not, instead exhibiting increased sulfate accumulation. We investigated the role of two pathways on organic-S and biomass accumulation in *Salicornia* and *Sarcoconia*: the sulfate reductive pathway that generates Cys and L-Cys desulfhydrase that degrades Cys to H_2S, NH_3, and pyruvate. The major function of *O*-acetyl-Ser-(thiol) lyase (OAS-TL; EC 2.5.1.47) is the formation of L-Cys, but our study shows that the OAS-TL A and OAS-TL B of both halophytes are enzymes that also degrade L-Cys to H_2S. This activity was significantly higher in *Sarcocornia* than in *Salicornia*, especially upon sulfate supplementation. The activity of the sulfate reductive pathway key enzyme, adenosine 5′-phosphosulfate reductase (APR, EC 1.8.99.2), was significantly higher in *Salicornia* than in *Sarcocornia*. These results suggest that the low organic-S level in *Sarcocornia* is the result of high L-Cys degradation rate by OAS-TLs, whereas the greater organic-S and biomass accumulation in *Salicornia* is the result of higher APR activity and low L-Cys degradation rate, resulting in higher net Cys biosynthesis. These results present an initial road map for halophyte growers to attain better growth rates and nutritional value of *Salicornia* and *Sarcocornia*.

Soil salinity is one of the oldest and most important abiotic stresses affecting agricultural productivity globally. According to the Food and Agricultural Organization of the United Nations, roughly 800 million hectares of land are affected by salt. It has further been predicted that approximately 50% of arable land will be affected by salt stress by the year 2050 (Wang et al., 2003). Therefore, there is an urgent need to develop techniques to confront the adverse effects of salinity stress and develop strategies to enhance crop production under saline conditions. To do so, it is necessary to understand the physiological processes and molecular mechanisms that have evolved in plants to tolerate salt resistance, and exploit them for sustainable crop production (Fatma et al., 2013; Iqbal et al., 2013; Khan et al., 2013).

Most crop plants are glycophytes that grow in nonsaline soils and bodies of fresh water. Glycophytes are able to adapt to moderate levels of salinity, albeit with decreased productivity. Halophytic plants, on the other hand, grow and thrive in highly saline waters and soils. Among the most promising candidates for the development of novel halophytic crops are species of the

[1] This research was supported by the Israel Science Foundation grant no. 212/13, by the I-CORE Program of The Israel Science Foundation grant no. 152/11 and by the Chief Scientist, Ministry of Agriculture and Rural Development, Israel grant no. 857-0691-13. We also thank COST Action FA0901 "Putting Halophytes to Work—From Genes to Ecosystems" for their support.

[2] Address correspondence to gizi@bgu.ac.il.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is Moshe Sagi (gizi@bgu.ac.il).

A.K. participated in designing the research plans and performed the experiments and analyses; A.B. participated in sulfate extraction; S.S. read and commented on the manuscript and participated in the immunoprecipitation assay; A.S. participated in qRT-PCR; A.A. performed preliminary experiments with *Salicornia* and *Sarcocornia* grown on perlite; Y.V. compared plants grown on 100 mM sodium sulfate with those grown on 100 mM or 200 mM sodium chloride; M.S.K. and O.S. performed RNA sequencing and transcriptome de novo assembly; N.F. provided *Salicornia*'s cDNA and protein sequences and participated in manuscript editing; M.S. conceived the original idea, designed the research plan, and supervised the research work; the manuscript was jointly written by A.K. and M.S.

[OPEN] Articles can be viewed without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.17.00780

Salicornia and *Sarcocornia*. Both genera are phenotypically and ecologically very similar and occur naturally throughout the world, along coastal salt marshes, edges of saline lakes, and in areas where the vegetation is often subjected to daily tides that contain high sulfate concentrations (5 mM to 30 mM in interstitial water; Howarth and Giblin, 1983; Davy et al., 2001, 2006; de la Fuente et al., 2013; Steffen et al., 2015). The species of both genera are often referred to as pioneer plants on the sea coasts (Davy et al., 2001, 2006) and several *Salicornia* species are already used as a both fodder and a vegetable crop and can be irrigated with highly saline water, even with full seawater (Ventura et al., 2011a). The *Sarcocornia* genus differs from the annual *Salicornia* genus by its distinct perennial growth habit (Davy et al., 2006) and by differences in floral morphology (Kadereit et al., 2007). Both genera produce succulent shoots suitable for leafy vegetable production, but they differ in terms of yield and nutritional value (Ventura et al., 2011b).

Mineral nutrient levels are a major determinant of crop yield and quality; and saline environments complicate mineral nutrition and affect crop sustainability (Nazar et al., 2011a). The supply of optimal sulfur nutrition to plants is important because sulfur is an integral part of several important plant compounds, such as iron-sulfur clusters, polysaccharides, and sulfolipids, as well as a broad variety of biomolecules including vitamins such as biotin and thiamine, cofactors such as Coenzyme A and S-adenosyl-Met, peptides such as glutathione (GSH) and phytochelatins, secondary metabolites such as allyl Cys sulfoxides and glucosinolates, and the sulfur-containing amino acids Cys and Met (Kopriva 2006; Nocito et al., 2011). Cys residues (thiols) have the capacity to react with a broad spectrum of agents, ranging from free radicals, reactive oxygen species (ROSs), and cytotoxic electrophilic and organic xenobiotics, to affect the redox state of tissues and serve as signals in plant responses to stress (Mullineaux and Rausch, 2005; Koprivova et al., 2008a).

The main source of sulfur, sulfate, can either be taken up from the environment or generated within the plants from other S-containing compounds, such as sulfite (Brychkova et al., 2013, 2015). The sulfate reduction pathway (Fig. 1) is initiated in plastids (Leustek et al., 2000) and/or in the cytosol (Leustek, 2002) by the adenylation of transported sulfate by ATP sulfurylase (ATPS, EC 2.7.7.4) to generate adenosine 5′-phosphosulfate (APS). APS is then reduced to sulfite by the plastidic APS reductase (APR, EC 1.8.99.2). Further, the toxic sulfite can be oxidized to sulfate by peroxisomal sulfite oxidase (SO, E.C. 1.8.3.1.) or reduced to sulfide by the chloroplastic sulfite reductase (SiR, EC 1.8.7.1). Sulfide, together with *O*-acetyl-Ser (OAS) whose biosynthesis is catalyzed by Ser acetyltransferase (SAT, EC 2.3.1.30), is then incorporated into Cys in a reaction catalyzed by the *O*-acetyl-Ser-(thiol) lyase (OAS-TL, EC 2.5.1.47; Wirtz et al., 2004). The generated L-cys is a precursor of thiols containing metabolites (Kopriva, 2006). Cys homeostasis is controlled by the cytosolic L-Cys desulfhydrase 1 (DES1, EC 4.4.1.1), which catalyzes the breakdown of Cys to sulfide, ammonia, and pyruvate (Fig. 1; Álvarez et al., 2010).

The sulfur reduction pathway in glycophyte plants is modified in response to salinity stress (López-Berenguer et al., 2007; Koprivova and Kopriva, 2008b). ATPS, the first rate-limiting enzyme of the S assimilation pathway, is up-regulated in the glycophyte *Brassica napus* upon exposure to 150 mM NaCl (Ruiz and Blumwald, 2002). Exposure to this NaCl concentration also affects the expression of key enzymes of the sulfate reduction pathway, enhancing APR activity and increasing the abundance of the 3 *APR* isoforms 3-fold. Interestingly, an increase in APR activity was correlated

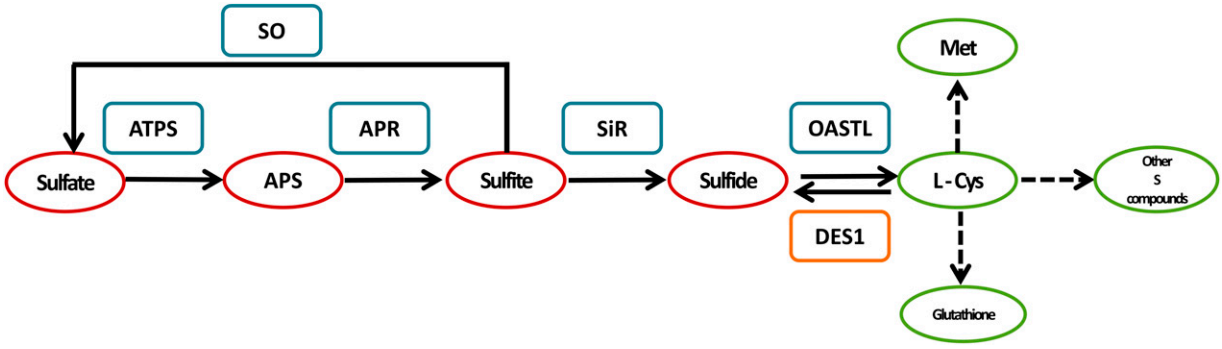

Figure 1. Schematic representation of the Sulfate reduction and Cys degradation pathways in Arabidopsis plants. ATPS catalyzes the adenylation of sulfate to APS using ATP as an electron donor. Then, APS is reduced by the plastidic enzyme APR to sulfite in the presence of two molecules of reduced GSH, which acts as an electron donor. The generated sulfite can be oxidized to sulfate by SO with the formation of H_2O_2 as a byproduct or further be reduced to sulfide by the SiR employing three molecules of reduced ferredoxin. The sulfide together with O-acetyl-L-Ser is the substrate for Cys biosynthesis catalyzed by OAS-TL. Cys homeostasis is controlled by L-Cys desulfhydrase (DES1, EC 4.4.1.1), which catalyzes the breakdown of Cys to sulfide, ammonia, and pyruvate. Red circle, inorganic S compounds; green circle, organic S compounds; blue rectangle, sulfate reduction pathway enzymes; orange rectangle, Cys degradation pathway enzyme.

with a higher rate of Cys biosynthesis to regulate the increased demand for GSH in response to the salinity stress as a defense response to ROSs (Koprivova and Kopriva, 2008b). Additionally, it has been shown that both the rate of S assimilation and the biosynthesis of thiols were greatly increased in *B. napus* (Ruiz and Blumwald, 2002) and barley (*Hordeum vulgare* L.; Astolfi and Zuchi, 2013) exposed to saline conditions.

The limited investigation of sulfur metabolism in halophytes has mainly focused on the role of S-containing metabolites such as reduced GSH and dimethylsulfoniopropionate (Nguyen et al., 2014; Colmer et al., 1996; Mulholland and Otte, 2000). Thus, it has been reported that increasing the sulfate concentration in the growth medium of 2% seawater-grown marsh cordgrass *Spartina alterniflora* resulted in a positive growth response, but no such growth response was seen in *Spartina cynosuroides* and in *Spartina anglica* grown with 0 mM to 1.6 mM sulfate supply (Stribling, 1997; Mulholland and Otte, 2000). Interestingly, *Salicornia europaea* has been determined to be extremely tolerant to sulfide ion accumulation (Ingold and Havill, 1984; Havill et al., 1985), although the tolerance mechanism is not understood. In contrast, Martin and Maricle (2015) examined 17 estuarine species, reporting that those with higher levels of cytochrome *c* oxidase activity were more sulfide-tolerant than those with lower levels.

Sulfate assimilation in glycophytes such as Arabidopsis (*Arabidopsis thaliana*), *Brassica*, and tobacco (*Nicotiana tabacum*) has been studied mainly from the perspective of S deprivation (Lappartient and Touraine, 1996; Lappartient et al., 1999; Maruyama-Nakashita et al., 2003; Nikiforova et al., 2003; Lewandowska and Sirko, 2008; Rouached et al., 2011; Király et al., 2012; Lee et al., 2012; Wipf et al., 2014). There is little information on sulfate assimilation in the presence of excessive S in glycophytes and even less in halophytes (Nazar et al., 2011b).

In contrast, sulfate was widely studied as a source of salinity and the response of halophytes to sodium sulfate was compared to that of sodium chlorides in various halophytes. Employing sodium sulfate at levels of 38 mM to 500 mM in comparison to sodium chloride resulted in toxic effects and a significant decrease in biomass accumulation in halophytes such as *Prosopis strombulifera* (Reginato et al., 2012, 2014; Llanes et al., 2013, 2014). Interestingly, *Salicornia* and *Sarcocornia* followed this inhibitory notion, exhibiting a significant decrease in biomass accumulation when grown with 100 mM sodium sulfate compared to 100 mM or even 200 mM sodium chloride (Supplementary Fig. S1).

Previously we showed the feasibility of cultivating *Salicornia* and *Sarcocornia* by applying a multiple harvest regime and irrigating with 100% seawater, generating economic yields with high nutritional value (Ventura et al., 2011a). The essentiality of supplementing artificial lighting to the natural day-length in *Salicornia* and successive harvesting regime in *Sarcocornia* for all-year-round cultivation was demonstrated, as well as the importance of molybdenum for improving total biomass accumulation in *Salicornia* grown in seawater (Ventura et al., 2010, 2011b, 2015; Ventura and Sagi, 2013).

Employing RNA and protein sequences of *Salicornia* (being highly similar to *Sarcocornia*) allows us to explore new avenues for enhancing yield and quality of this crop. Here we show that biomass and organic-S accumulation were significantly increased in *S. europaea* (ecotype RN) in response to sulfate supplementation, whereas *S. fruticosa* (ecotype VM) accumulated higher sulfate, but showed no increase in biomass. The sulfate-reductive pathway and the L-Cys desulfhydrase (DES) activities were explored for factors affecting sulfate and organic-S levels in the two halophytes. The major function of OAS-TLs is known to be the formation of L-Cys, but we found that OAS-TL also functions as a DES, degrading L-Cys to H_2S. We attribute the higher organic-S and greater biomass accumulation in *Salicornia* to the significantly lower L-Cys DES activity of OAS-TL A and OAS-TL B, especially in the presence of sulfate supplementation, as well as to the higher APR activity, both of which should lead to higher net L-Cys. By contrast, *Sarcocornia* exhibited significantly higher DES and lower APR activity levels and did not accumulate biomass in response to sulfate supplementation. These results will hold great promise for sustainable agriculture, and will help halophyte growers to improve the nutritional value and productivity of edible halophytes, such as *Salicornia* and *Sarcocornia*.

RESULTS

High Sulfate Increased Biomass in *Salicornia* But Not in *Sarcocornia*, whereas Salinity Enhancement Increased Biomass Accumulation in Both Genera Grown in Low and High Sulfate

Enhanced demand for sulfur nutrition may be expected among halophyte plants such as *Salicornia* and *Sarcocornia* that are adapted to growth in saltmarshes and sea shores exposed to frequent seawater tides containing high concentrations of sulfate, ranging between 5 mM and 30 mM (Howarth and Giblin, 1983). Interestingly, irrigation with a solution containing 50% seawater improved biomass accumulation in both genera, as compared with the absence of seawater (Ventura et al., 2011a). These results led us to examine the effect of supplementation of high sulfate levels such as 10 mM, because optimization of sulfur nutrition may not only affect biomass but also the organic sulfur accumulation in plants. Because both *Salicornia* and *Sarcocornia* exhibit poor growth in the absence of NaCl in the growth medium (Ventura et al., 2011a), treatment conditions without NaCl were not compared to those with NaCl. Assessment of the effect of the 10 mM Na_2SO_4 supplementation to the 1/2 MS (containing 0.87 mM sulfate) growth medium was carried out in the presence of either 30 mM or 180 mM NaCl, so that both

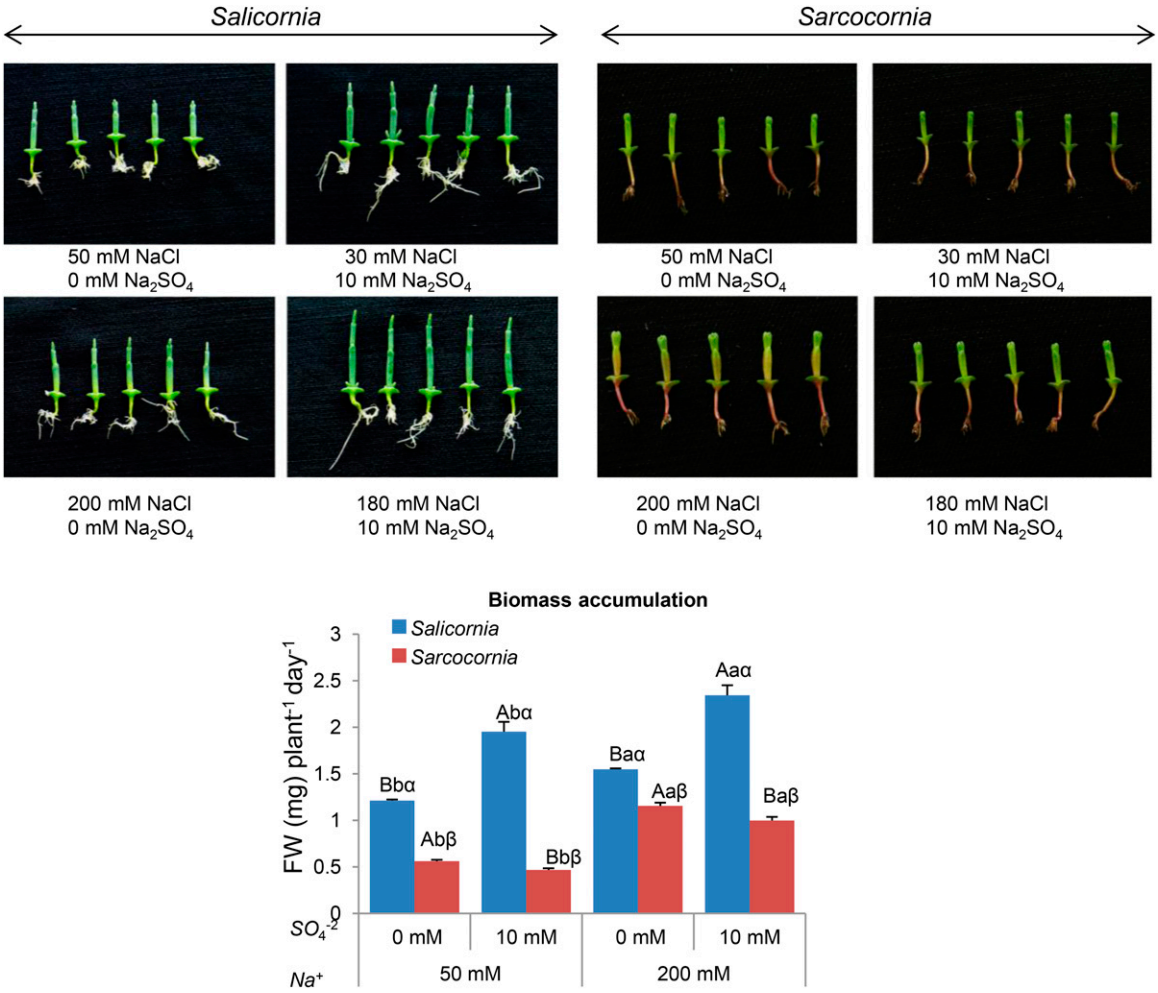

Figure 2. Effect of sodium (50, 200 mM) and sulfate (0, 10 mM) concentrations on biomass accumulation of *Salicornia* (left) and *Sarcocornia* (right). Sulfate is applied as sodium sulfate and the remainder sodium as sodium chloride. The lower and higher salinity treatments are shown in the top and bottom photos, respectively. The values are means ± SE ($n = 30$). Growth of the plants was measured as increase in biomass per day. Values denoted with different letters are significantly different according to the Tukey-Kramer HSD test, $P < 0.05$ (JMP 8.0 software). Different upper-case letters indicate significant differences between sulfate treatments in *Salicornia* or *Sarcocornia*. Different lower-case letters indicate significant differences between salinity treatments in *Salicornia* or *Sarcocornia*. Different Greek letters indicate significant difference between *Salicornia* and *Sarcocornia* subjected to the same treatment. The data are representative of one of 15 different experiments that yielded similar results.

levels include 50 mM and 200 mM total Na^+. As expected for halophytes, the biomass accumulation of both *Salicornia* and *Sarcocornia* was greater at the higher than at the lower salt concentration (Fig. 2). Although both *Salicornia* and *Sarcocornia* belong to the *Amaranthaceae* family, they differed in the effect on biomass accumulation of enhanced sulfate in the growth medium. *Salicornia* responded positively to the 10-mM sulfate supplementation, exhibiting a significant increase in the rate of biomass accumulation even when grown without NaCl supplementation (Fig. 2, left; Supplemental Fig. S2). In contrast to *Salicornia*, *Sarcocornia* exhibited a reduction in biomass accumulation in response to the addition of 10 mM sulfate to the growth mediums (Supplemental Fig. S2; Fig. 2, right). In summary, these results indicate that high sulfate is essential for optimal growth of *Salicornia*, but has a negative effect in *Sarcocornia*.

Interestingly, a similar response to sulfate was evident with *Salicornia* (RN) and *Sarcocornia* (VM) grown in pots filled with the highly air-permeable and high-water-capacity perlite, irrigated with 100 mM and 200 mM NaCl solution supplemented with 1/2 Hoagland nutrient solution, containing 0 mM and 10 mM sulfate (compare Fig. 2 to Supplemental Fig. S3), i.e. biomass accumulation was improved in *Salicornia* in response to excess sulfate, but not in *Sarcocornia*. The results indicate that growth conditions, either in plates containing 1% plant agar mixed with 1/2 MS localized in a growth-room or grown in perlite supplied with 1/2 Hoagland nutrient solution and localized in a controlled greenhouse (see the "Materials and Methods"),

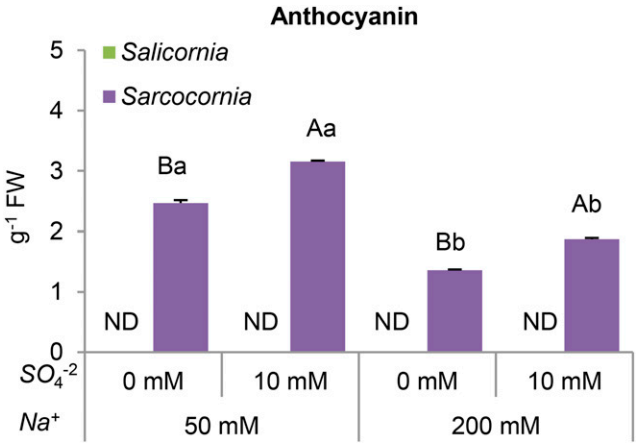

Figure 3. Effect of sodium (50, 200 mM) and sulfate (0, 10 mM) concentrations on the anthocyanin content of *Salicornia* and *Sarcocornia*. Sulfate is applied as sodium sulfate and the remainder sodium as sodium chloride. The values are means ± SE ($n = 5$). Values denoted with different letters are significantly different according to the Tukey-Kramer HSD test, $P < 0.05$ (JMP 8.0 software). Different upper-case letters indicate significant differences between sulfate treatments in *Salicornia* or *Sarcocornia*. Different lower-case letters indicate significant differences between salinity treatments in *Salicornia* or *Sarcocornia*. ND, not detectable.

did not affect the biomass accumulation response to high sulfate in *Salicornia* and *Sarcocornia*.

Effect of Sulfate and Salinity on Anthocyanin, Hydrogen Peroxide, and Superoxide Levels in *Salicornia* and *Sarcocornia*

Enhanced anthocyanin biosynthesis is a characteristic response of flowering plants to unfavorable environmental conditions (Chalker-Scott, 1999). Anthocyanin was not detectable in *Salicornia* plants (Fig. 3), whereas anthocyanin was produced in *Sarcocornia* and its level was enhanced by the addition of 10 mM sulfate. The increase in anthocyanin content was greater in plants grown in the lower salinity medium (Fig. 3), suggesting that both low salinity conditions and sulfate supplementation are stressful for *Sarcocornia*.

The generation of ROS, such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), are well-known components of the oxidative stress response and constitute one of the earliest responses of plant cells to nutrient imbalances. Increased ROS levels may result in accelerated catabolism leading to premature senescence (Yarmolinsky et al., 2014; Brychkova et al., 2015) and reduction in plant growth and loss of crop yield (You and Chan, 2015). In light of the high anthocyanin levels detected in *Sarcocornia* shoots, we investigated the effect of supplemental sulfate on ROS production in *Salicornia* and *Sarcocornia* shoots.

Superoxide levels in *Salicornia* were significantly higher in plants grown in the low salinity medium without sulfate supplementation, when compared to the other treatments (Fig. 4A). By contrast, superoxide production was at a similar level in all the treatments, being significantly higher in *Sarcocornia* than in *Salicornia* (Fig. 4A).

Lower hydrogen peroxide levels were detected in both halophytic plants at the higher salinity, suggesting that high salinity conditions are preferable for both halophytes (Fig. 4B). The sulfate supplementation increased H_2O_2 in *Sarcocornia* at both salinities (Fig. 4B), whereas in *Salicornia*, the enhancement was evident only at the lower salinity (Fig. 4B). These results indicate that sulfate supplementation is more stressful for *Sarcocornia* than for *Salicornia*.

The Effect of Sulfate and Salinity on S-related Metabolites in *Salicornia* and *Sarcocornia*

The Effect on Sulfate, Sulfite, and Sulfide Levels

Sulfate is taken up and reduced via the sulfate-reductive pathway (Fig. 1), hence S is mostly available to plants in its fully oxidized form, the sulfate anion (Brychkova et al., 2013). As shown in Figure 5A, sulfate supplementation significantly increased sulfate levels in both plants. The effect was greater in *Sarcocornia*, especially when grown under the lower salinity (Fig. 5A), indicating that *Sarcocornia* accumulates more sulfate than *Salicornia*. Additionally, the results indicate that increasing salinity reduces sulfate accumulation. The higher sulfate accumulation in *Sarcocornia* as compared with *Salicornia* grown on plates, as shown here (Fig. 5A), is in agreement with the significantly enhanced sulfate shown in *Sarcocornia* when both halophytes were grown in pots filled with perlites and irrigated with 50% to 100% seawater supplemented with 200 ppm commercial N–P–K fertilizer (20–20–20 + microelements; Haifa Chemicals; Ventura et al., 2011a). The results further indicate that growth conditions in the plates did not affect the response of *Salicornia* and *Sarcocornia* to high sulfate.

Sulfite is generated in the chloroplast by the GSH-dependent APR, but excess accumulation of sulfite is toxic to plants. To maintain sulfite homeostasis in the chloroplast, sulfite is further reduced to sulfide by the ferredoxin-dependent SiR in the sulfate-reductive pathway. Sulfite can also be detoxified to the less toxic thiosulfate by the sulfurtransferases or be oxidized to sulfate by the molybdenum cofactor enzyme, the peroxisomal SO, or can enter the sulfolipid reductive pathway in the chloroplast to generate sulfolipid (Nakamura et al., 2000; Papenbrock and Schmidt, 2000; Tsakraklides et al., 2002).

Salinity had no effect on the sulfite level in either plant (Fig. 5B). Sulfite levels in *Salicornia* were negatively affected by sulfate supplementation (Fig. 5B), whereas in *Sarcocornia*, sulfate supplementation had no significant effect (Fig. 5B).

Sulfide, the substrate for Cys biosynthesis, is the product of sulfite reduction by sulfite reductase, and/or a product of sulfur-containing metabolite degradation

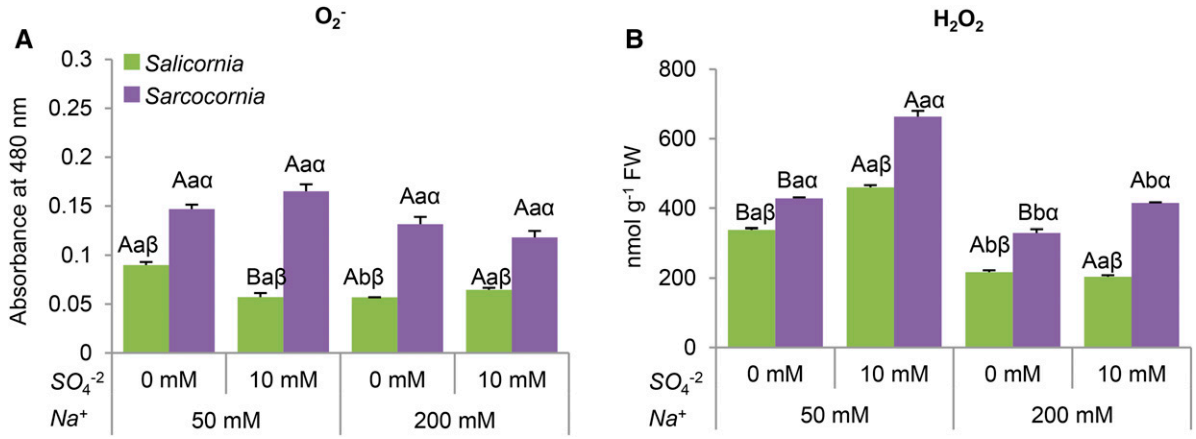

Figure 4. Effect of sodium (50, 200 mM) and sulfate (0, 10 mM) concentrations on superoxide and hydrogen peroxide content of *Salicornia* and *Sarcocornia*. Sulfate is applied as sodium sulfate and the remainder sodium as sodium chloride. A, Superoxide (O_2^-) content in *Salicornia* and *Sarcocornia*. The values are means ± SE ($n = 4$). B, Hydrogen peroxide (H_2O_2) content in *Salicornia* and *Sarcocornia*. The values are means ± SE ($n = 4$). Values denoted with different letters are significantly different according to the Tukey-Kramer HSD test, $P < 0.05$ (JMP 8.0 software). Different upper-case letters indicate significant differences between sulfate treatments in *Salicornia* or *Sarcocornia*. Different lower-case letters indicate significant differences between salinity treatments in *Salicornia* or *Sarcocornia*. Different Greek letters indicate significant difference between *Salicornia* and *Sarcocornia* subjected to the same treatment.

(Yarmolinsky et al., 2014; Brychkova et al., 2015). Sulfide levels were unaffected by salinity increase in both plants (Fig. 5C). A significant reduction in H_2S content was seen in *Salicornia*, but not in *Sarcocornia* plants supplemented with 10 mM sulfate at the low salinity (Fig. 5C).

The Effect on Cys and GSH

Cys is the final product of the S assimilation pathway, and is the rate-limiting factor for GSH and Met biosynthesis. GSH is a storage form of reduced S in plants, playing an important role in controlling the redox status of plant tissue, protection against biotic and abiotic stresses, precursor of phytochelatins, detoxification of xenobiotics, and more (Rao and Reddy, 2008; Zechmann et al., 2008).

The level of free Cys in *Salicornia* was unaffected by salinity and sulfate treatments, whereas in *Sarcocornia*, Cys level decreased with salinity, being significantly lower than in *Salicornia*. Sulfate supplementation resulted in Cys enhancement at the lowest salinity level tested (Fig. 6A). Sulfate supplementation increased total GSH in both *Salicornia* and *Sarcocornia*, but was lower at the higher salt concentration in both plants (Fig. 6B).

Total Sulfur and Organic Sulfur

The effect of salinity and sulfate supplementation on total and organic-S was measured. Remarkably, except for plants grown at the highest salinity without supplementation of sulfate, the total sulfur level in *Sarcocornia* was significantly higher than in *Salicornia* plants (Fig. 7A). In both halophytes, the total sulfur level increased with increasing sulfate and decreased with increasing salinity in the growth medium (Fig. 7A).

Importantly, the organic sulfur level followed biomass accumulation in *Salicornia* but not in *Sarcocornia* when both halophytes were supplemented with high sulfate (compare Fig. 2, lowest insets, to Fig. 7B). Salinity negatively affected organic sulfur level in *Salicornia*, and in the higher salinity treatment without supplementation of sulfate in *Sarcocornia* (Fig. 7B). Impressively, at the lower salinity treatments without sulfate supplementation, the organic sulfur level in *Sarcocornia* was higher as compared to *Salicornia*, yet in the other treatments, organic sulfur was significantly higher in *Salicornia* (Fig. 7A).

Effect of Sulfate and Salinity Levels on Sulfate-Reduction Pathway Components in *Salicornia* and *Sarcocornia*

The differences in organic sulfur between *Sarcocornia* and *Salicornia* can be the result of differences in the sulfate assimilation pathways and/or in organic-S catabolism expressed as Cys degradation. These possibilities were further examined to uncover the factor/s responsible for these differences.

APR Expression

Chloroplast-localized APR is known to be a key regulatory point in the sulfate assimilation pathway (Vauclare et al., 2002; Kopriva, 2006; Khan et al., 2010) and may shed light on the cause/s for the different response of *Salicornia* as compared to *Sarcocornia* under sulfate supplementation at different salinity levels described above (Figs. 2, 3, and 4).

A reduction in *APR* transcripts was evident in response to 10 mM sulfate treatment in both *Salicornia* and *Sarcocornia*, whereas *APR* transcript abundance was

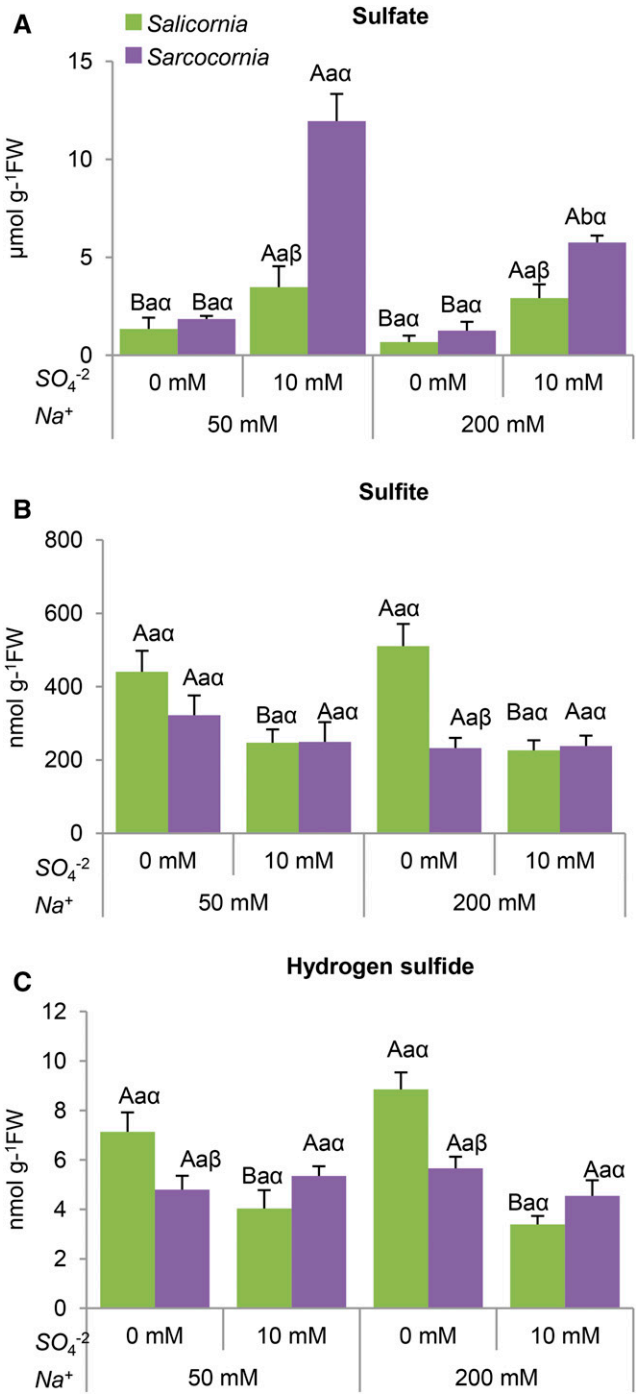

Figure 5. Effect of sodium (50, 200 mM) and sulfate (0, 10 mM) concentrations on sulfate, sulfite, and hydrogen sulfide contents in *Salicornia* and *Sarcocornia*. Sulfate is applied as sodium sulfate and the remainder sodium as sodium chloride. A, Sulfate levels in *Salicornia* and *Sarcocornia*. The values are means ± SE ($n = 3$). B, Sulfite levels in *Salicornia* and in *Sarcocornia*. The values are means ± SE ($n = 3$). The data are from one of three different experiments that yielded similar results. C, Hydrogen sulfide levels in *Salicornia* and in *Sarcocornia*. The values are means ± SE ($n = 4$). Values denoted with different letters are significantly different according to the Tukey-Kramer HSD test, $P < 0.05$ (JMP 8.0 software). Different upper-case letters indicate significant differences between sulfate treatments in *Salicornia* or *Sarcocornia*. Different lower-case letters indicate significant differences between salinity treatments in *Salicornia* or *Sarcocornia*. Different Greek letters indicate significant difference between *Salicornia* and *Sarcocornia* subjected to the same treatment.

significantly higher in *Salicornia* grown in 200 mM NaCl without sulfate supplementation than in either halophyte exposed to other treatments (Supplemental Fig. S4A).

Salinity positively affected APR activity in *Salicornia*. In *Sarcocornia* a positive response was evident only when high sulfate was supplemented. A significant decline in APR activity was evident in both types of plants when supplemented with 10 mM sulfate at either salinity level. Importantly, APR activity was higher in *Salicornia* than *Sarcocornia* under all growth conditions, being insignificantly higher only in plants grown with the low salinity medium without sulfate supplementation (Fig. 8A).

These results indicate that in the halophytes *Salicornia* and *Sarcocornia*, *APR* expression is reduced by sulfate supplementation. The results also show that APR activity is higher in *Salicornia*, especially in sulfate-supplemented plants.

SiR Expression

Chloroplast-localized SiR catalyzes the reduction of sulfite to sulfide in the sulfate-reductive pathway (Yarmolinsky et al., 2014; Brychkova et al., 2015).

SiR transcript abundance was not affected by salinity and sulfate treatments in *Salicornia*. In contrast, sulfate supplementation to *Sarcocornia* caused a decrease in *SiR* transcript levels at low salinity, whereas in the absence of sulfate supply, the enhanced salinity resulted in decreased *SiR* transcript. (Supplemental Fig. S4B).

SiR activity in both plants decreased with increasing salinity, being significant only without sulfate supply in *Salicornia* (Fig. 8B). The supplementation with 10 mM sulfate of the low salinity medium resulted in a 2-fold decrease in SiR activity in *Salicornia*, but there was no difference in SiR activity in *Sarcocornia* at either salinity level (Fig. 8B). SiR activity in *Salicornia* plants grown in high salinity was unaffected by supplementation with 10 mM sulfate.

SO Expression

The internal sulfite generated during the sulfate reduction pathway or as a result of S amino acids catabolism (Brychkova et al., 2013) can be oxidized to sulfate by the molybdenum cofactor-containing peroxisomal SO. The abundance of *SO* transcripts decreased with sulfate addition under low salinity conditions in *Salicornia*, but not in *Sarcocornia*. At the higher salinity, sulfate supplementation had little effect on *SO* transcript abundance in *Salicornia*, but increased it in *Sarcoconia* (Supplemental Fig. S4C). Importantly, salinity level had little effect on SO activity, whereas sulfate addition decreased SO activity in either plant (Fig. 8C).

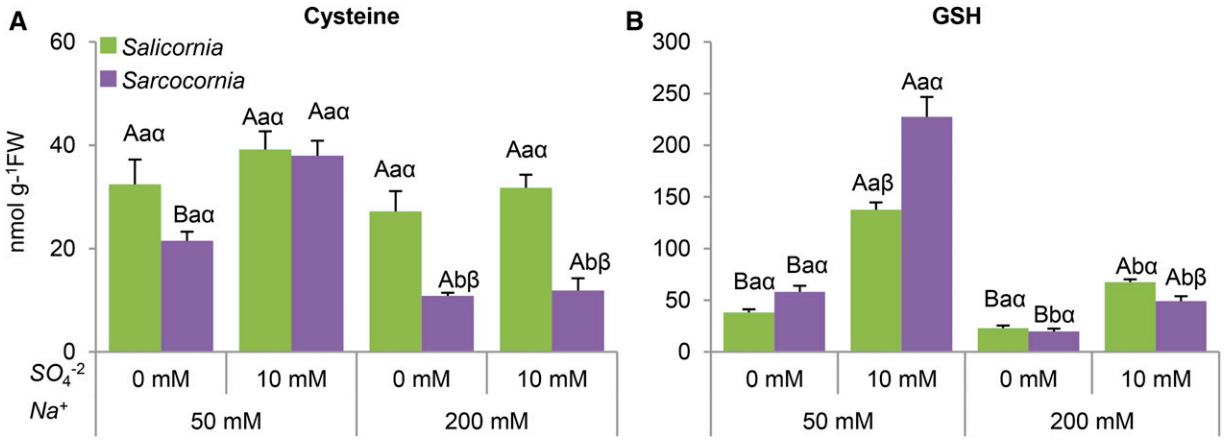

Figure 6. The effect of sodium (50, 200 mM) and sulfate (0, 10 mM) concentrations on Cys and GSH content in the shoots of *Salicornia and Sarcocornia*. Sulfate is applied as sodium sulfate and the remainder sodium as sodium chloride. A, Cys levels in *Salicornia* and *Sarcocornia*. Error bars indicate SE ($n = 4$). The data are from one of four different experiments that yielded similar results. B, Glutathione levels in *Salicornia* and *Sarcocornia*. The values are means ± SE ($n = 4$). The data are from one of four different experiments that yielded similar results. Values denoted with different letters are significantly different according to the Tukey-Kramer HSD test, $P < 0.05$ (JMP 8.0 software). Different upper-case letters indicate significant differences between sulfate treatments in *Salicornia* or *Sarcocornia*. Different lower-case letters indicate significant differences between salinity treatments in *Salicornia* or *Sarcocornia*. Different Greek letters indicate significant difference between *Salicornia* and *Sarcocornia* subjected to the same treatment.

Impressively, more than 2-fold higher SO activity was noticed in *Sarcocornia* in any of the treatments applied, suggesting a requirement for high SO activity to oxidize excess sulfite to sulfate.

OAS-TL Expression

OAS-TL catalyzes the biosynthesis of Cys using OAS and sulfide generated by the sulfate-reduction pathway and/or as a result of the degradation of thiol-containing metabolites (Álvarez et al., 2010, 2012).

The genes encoding OAS-TL A and OAS-TL B, which are localized to the cytosol and chloroplast, respectively, were examined for changes in transcript abundance in response to sulfate supplementation. At the lower salinity, we observed a decrease in the abundance of *Salicornia OAS-TL A*, but not *OAS-TL B* transcripts in response to sulfate supplementation. By

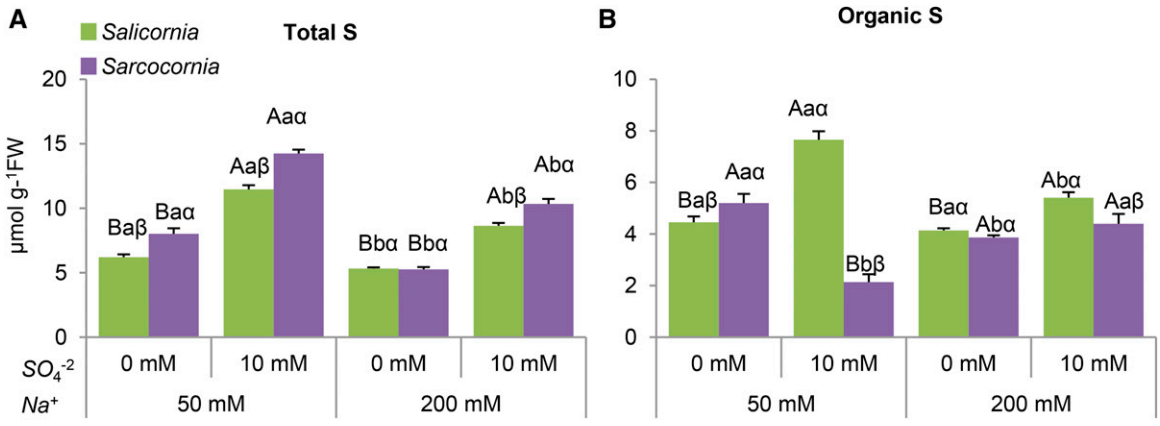

Figure 7. The effect of sodium (50, 200 mM) and sulfate (0, 10 mM) concentrations on total and organic sulfur content in the shoots of *Salicornia* and *Sarcocornia*. Sulfate is applied as sodium sulfate and the remainder sodium as sodium chloride. A, Total S content in *Salicornia* and *Sarcocornia*. The values are means ± SE ($n = 3$). B, Total organic S-compounds were calculated by the subtraction of total sulfur content from the known inorganic S-metabolites content in *Salicornia* and *Sarcocornia*. The values are means ± SE ($n = 3$). Values denoted with different letters are significantly different according to the Tukey-Kramer HSD test, $P < 0.05$ (JMP 8.0 software). Different upper-case letters indicate significant differences between sulfate treatments in *Salicornia* or *Sarcocornia*. Different lower-case letters indicate significant differences between salinity treatments in *Salicornia* or *Sarcocornia*. Different Greek letters indicate significant difference between *Salicornia* and *Sarcocornia* subjected to the same treatment.

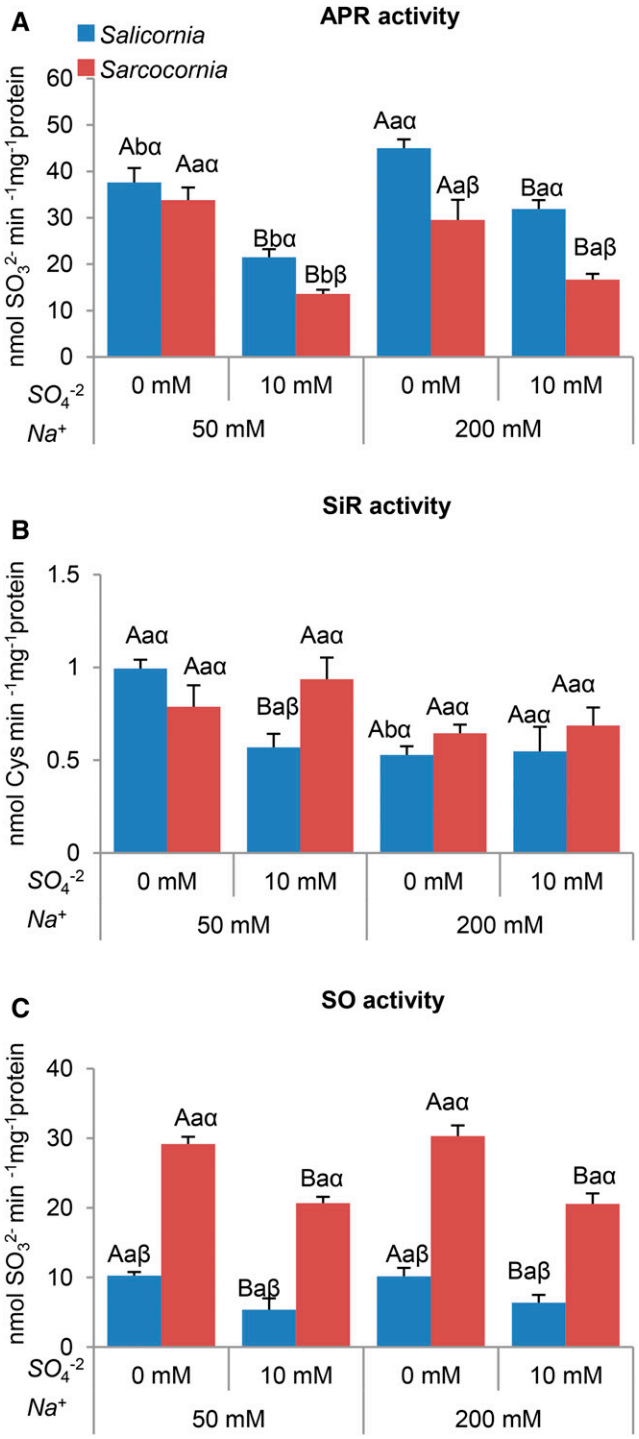

Figure 8. Effect of sodium (50, 200 mM) and sulfate (0, 10 mM) concentrations on APR, SiR, and SO activities in *Salicornia* and *Sarcocornia*. Sulfate is applied as sodium sulfate and the remainder sodium as sodium chloride. A, APR activity was detected by sulfite appearance in *Salicornia* and *Sarcoconia*. The values are means ± SE ($n = 4$). B, SiR activity was detected by Cys appearance in *Salicornia* and *Sarcocornia*. The values are means ± SE ($n = 3$). C, SO activity was detected as sulfite disappearance in *Salicornia* and *Sarcoconia*. The values are means ± SE ($n = 4$). Values denoted with different letters are significantly different according to the Tukey-Kramer HSD test, $P < 0.05$ (JMP 8.0 software). Different upper-case letters indicate significant differences between sulfate treatments in *Salicornia* or *Sarcocornia*. Different lower-case letters indicate significant differences between salinity treatments in *Salicornia* or *Sarcocornia*. Different Greek letters indicate significant difference between *Salicornia* and *Sarcocornia* subjected to the same treatment.

contrast, *Sarcocornia* showed a significant increase in the abundance of both *OAS-TL A* and *OAS-TL B* transcripts in response to sulfate supplementation. We observed an increase in both *OAS-TL A* and *OAS-TL B* transcript abundance in *Sarcocornia* at the higher salinity level, whereas in *Salicornia*, the increase was observed mainly in the abundance of *OAS-TL B* transcripts (Supplemental Fig.S4, D and E). Interestingly, whereas salinity levels in the tested range had no effect on OAS-TL activity in either halophyte, the OAS-TL activities detected as Cys generation followed transcript expression, exhibiting enhanced activity in *Sarcocornia* supplemented with high sulfate. The results were significant at the lowest salinity. In contrast, a higher OAS-TL activity rate was noticed in *Salicornia* at the low compared to high sulfate supplementation (Fig. 9A) conditions. This may indicate a response to sulfur starvation (Barroso et al., 1998; Ravina et al., 1999; Carfagna et al., 2011, 2016), as the organic-S and biomass accumulation in *Salicornia* were indeed significantly increased at the highest level of supplemental sulfate (Figs. 7B and 2). The results indicate also a higher capacity of Cys biosynthesis by OAS-TL activity in *Sarcocornia* as compared to *Salicornia* supplied with the highest sulfate concentration. Yet, considering the organic-S content, one would expect the opposite, unless higher organic-S degradation activities exist in *Sarcocornia*.

A Higher L-Cys DES Activity Was Evident in *Sarcocornia* as Compared with *Salicornia*

The differences in organic-S levels between the halophytes might result from either anabolic or catabolic processes. We therefore investigated the levels of molecular and biochemical factors playing a role in the catabolism of sulfur-containing compounds. L-Cys degradation activity was monitored by sulfide production using both in gel and kinetic assays (Fig. 9, B and C, respectively). Significantly, *Sarcocornia* exhibited a higher L-Cys-degrading activity level (L-Cys desulfhydrase) than did *Salicornia* and exhibited a significant increase when supplemented with high sulfate, whereas in *Salicornia* L-Cys desulfhydrase activity was decreased with the low salinity and did not change much with high salinity when supplemented with enhanced sulfate (Fig. 9, B and C). The results of both in-gel and kinetic assays that detected sulfide production show that the L-Cys-degrading activity is higher in *Sarcocornia* than in *Salicornia* and that it shows a more marked increase in response to sulfate supplementation in *Sarcocornia*.

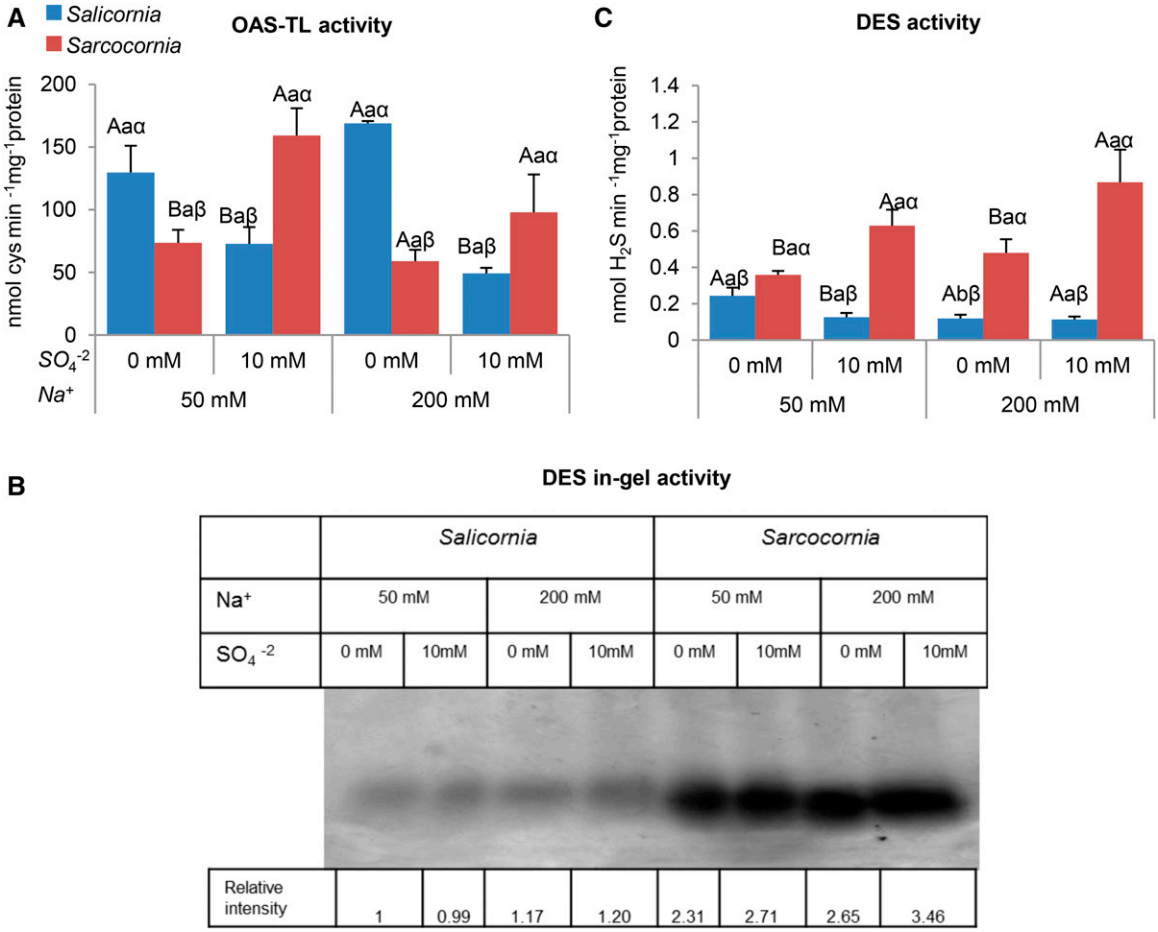

Figure 9. Effect of sodium (50, 200 mM) and sulfate (0, 10 mM) concentrations on OAS-TL and Cys degradation (DES) activities in *Salicornia* and *Sarcocornia*. Sulfate is applied as sodium sulfate and the remainder sodium as sodium chloride. A, OAS-TL activity was detected as Cys appearance in *Salicornia* and *Sarcocornia*. The values are means ± SE ($n = 3$). B, L-Cys DES was detected in the gel assay as a brown precipitate resulting from the reaction of hydrogen sulfide, generated by DES enzymatic activity, with lead acetate. The data are from three different experiments that yielded similar results. C, DES activity was also quantified as the release of sulfide from L-Cys. The values are means ± SE ($n = 4$). Values denoted with different letters are significantly different according to the Tukey-Kramer HSD test, $P < 0.05$ (JMP 8.0 software). Different upper-case letters indicate significant differences between sulfate treatments in *Salicornia* or *Sarcocornia*. Different lower-case letters indicate significant differences between salinity treatments in *Salicornia* or *Sarcocornia*. Different Greek letters indicate significant difference between *Salicornia* and *Sarcocornia* subjected to the same treatment.

Identification of the Cys Desulfhydrase Activity Source by Trypsinization of the Activity Bands in *Salicornia* and *Sarcocornia*

Specific unique peptides trypsinized from the L-Cys desulfhydrase activity bands of *Salicornia* and *Sarcocornia* (sliced from the bands shown in Figure 9B) were identified by their similarity to a OAS-TL A (Q00834) of *Spinacia oleracea*, being 93% identical to *Salicornia* sequence (Supplemental Fig. S5A). The number of identified unique trypsinized peptides were able to overlap 67% of the *Salicornia's* OAS-TL A protein full sequence and 55% of the *Sarcocornia* protein sequence (Supplemental Table S2). Further, additional unique peptides were identified by their similarity to the chloroplast-localized OAS-TL B, exhibiting 87% sequence identity when AAA16973 (*S. oleracea*) was compared to *Salicornia*'s OAS-TL B protein sequence (Supplemental Fig. S5B). The obtained number of trypsinized peptides overlapped 91% of *Salicornia*'s OAS-TL B protein whereas in *Sarcocornia* the amount of identified unique peptides overlapped 57.5% of OAS-TL B (Supplemental Table S3). Importantly, no peptide was found when the search for unique peptides was based on the similarity to Arabidopsis L-Cys desulfhydrase 1 (DES1, AT5G28030) or Pyridoxal P-dependent transferases DES1 (AT3G62130). Considering the absence of proteins with sequence similarity to L-Cys desulfhydrase 1 activity among the proteins identified in the sliced activity bands, these results indicate that OAS-TL A and OAS-TL B proteins may participate in L-Cys degradation in *Salicornia* and *Sarcocornia* (Fig. 9).

Identification of the Cys DES Activity Source by Immunodetection and Immunoprecipitation

The Western blot analysis of the DES activity bands (sliced from the band shown in Fig. 9B and fractionated by SDS-PAGE), employing antibody raised against Arabidopsis OAS-TL A, revealed two cross-reacting bands; the lower is likely OAS-TL A, as it shows identical gel mobility as shown for Arabidopsis OAS-TL A protein. Both protein bands exhibited significantly higher intensity in *Sarcocornia* as compared to *Salicornia* (Fig. 10A). Using OAS-TL B antibody, which cross reacted with Arabidopsis OAS-TL A, OAS-TL B, and OAS-TL, also cross reacted with two bands in *Sarcocornia* and *Salicornia* (Fig. 10B). The suitability of the antibodies raised against the Arabidopsis recombinant OAS-TL (kindly provided by Prof. Dr. S. Kopriva, University of Cologne, Köln) with *Salicornia*'s (and *Sarcocornia*'s) OAS-TL A and OAS-TL B proteins can be explained by their 81% and 78% sequence identity, respectively (Supplemental Fig. S6, A and B).

Immunoprecipitation analysis of *Salicornia* and *Sarcocornia* protein extract was performed employing OAS-TL A and OAS-TL B antibodies (see "Materials and Methods"). The L-Cys DES activity bands were fully, or almost fully, abrogated when proteins were extracted from *Salicornia* or *Sarcocornia*, respectively, were incubated with Arabidopsis OAS-TL A antibody as compared to the normal activity with proteins incubated without the antibody (Fig. 10C). Immunoprecipitation with OAS-TL B antibody in both plants revealed a complete disappearance of the L-Cys DES activity bands whereas the proteins that were incubated at the absence of the antibody in the pull-down assay exhibited desulfhydrase activity bands (Fig. 10D). Considering the relatively close identity of *Salicornia* OAS-TL A and OAS-TL B to the Arabidopsis proteins (Supplemental Fig. S6, A and B; GenBank/EMBL data libraries, accession nos. P47998 and P47999, respectively), as well as the absence of L-Cys DES1 protein (accession no. NP-001330588) among the proteins that generated the L-Cys desulfhydrase activity bands, these results indicate that the DES activity by the halophyte protein extracts resulted from OAS-TL A and OAS-TL B.

DISCUSSION

Salicornia Is Better Adapted to High Sulfate than *Sarcocornia*

Studies on S nutrition of *Salicornia* and *Sarcocornia* are few, and those that exist were carried out with sulfur

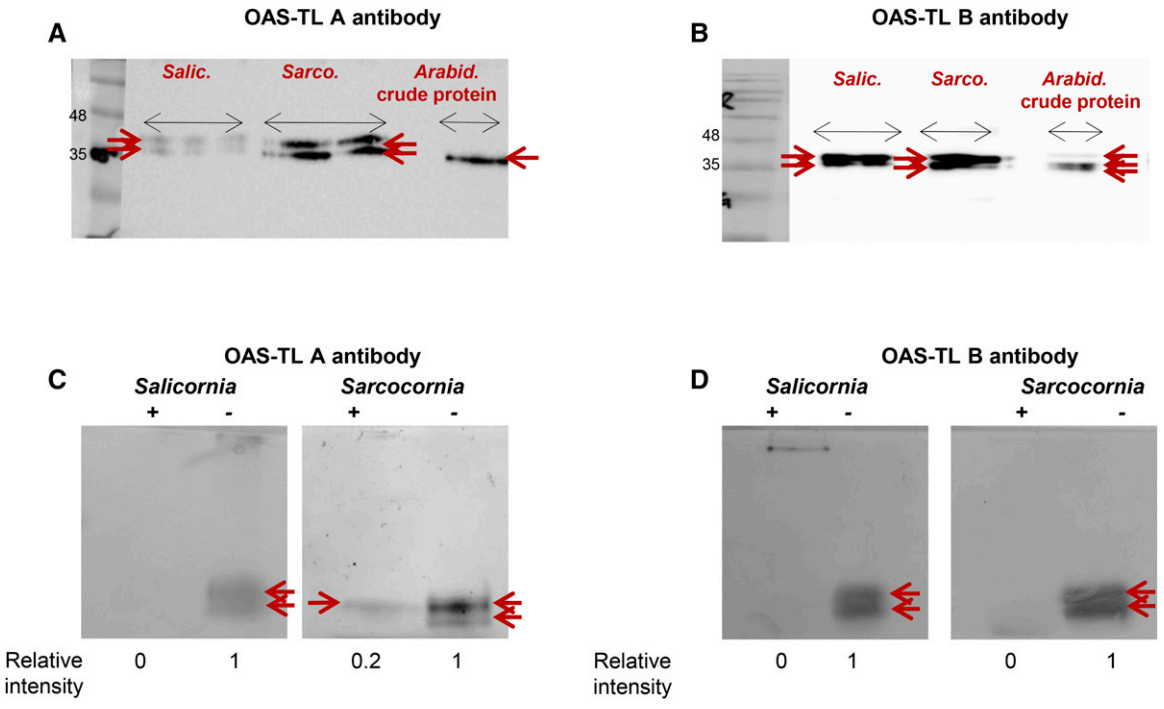

Figure 10. Immunodetection and immunoprecipitation of *Salicornia* and *Sarcocornia* OAS-TL proteins. A and B, Immunodetection of OAS-TL A and OAS-TL B, respectively. Fifty μg proteins of *Salicornia* and *Sarcocornia* were subjected to in-gel Cys desulfhydrase activity as shown in Fig. 9B. The activity bands were then sliced and were fractionated by 12.5% SDS PAGE together with Arabidopsis wild-type (Col) crude extract and analyzed by Western blot with antibodies raised against Arabidopsis OAS-TL A or OAS-TL B. C and D, Immunoprecipitation of 30 μg *Salicornia* and *Sarcocornia* desulfhydrase activity employing antibodies raised against Arabidopsis OAS-TL A and OAS-TL B, respectively. Plus sign (+) indicates "with antibody"; (−) minus sign indicates "without antibody"; red pointer indicates activity band.

sources other than sulfate. The positive response of *Salicornia europaea* to sulfide as compared with other tested halophyte species, including *Aster tripolium*, *Halimione portulacoides*, *Suaeda maritima*, and *Puccinellia maritima*, was attributed to its habitat (Ingold and Havill, 1984).

Here we show that the addition of 10 mM sulfate to the 1/2 MS growth medium resulted in a significant increase in biomass accumulation in *Salicornia*, whereas in *Sarcocornia*, it gave the opposite response (Fig. 2). *Salicornia* therefore appears to be better adapted to high sulfate conditions, likely because *Salicornia's* natural habitat is seawater that contains relatively high sulfate levels (Howarth and Giblin, 1983). Whereas *S. europaea* is found in areas exposed to frequent seawater tidal flooding, *S. fruticosa* is normally found in environments exposed to high soil salinities, high vegetation coverage, and less frequent flooding (Rogel et al., 2000).

At typical soil sulfate concentrations, most of the sulfate enters glycophytic plants, is reduced in the leaves and is allocated to the various sinks, yet 10% to 20% of the sulfur generally accumulates as sulfate (Cram, 1990). In coastal halophytes, at high environmental sulfate levels, up to 93% of total sulfur is normally expected to appear as sulfate (Ernst, 1990). Interestingly, at the lowest sulfate concentration used in this study, both *Salicornia* and *Sarcocornia* exhibited similar sulfate accumulation ratios of approximately 25% to 15% and 22.5% to 24% of the total sulfur content, respectively. However, when supplemented with high sulfate, *Sarcocornia* behaved more like a coastal halophyte, with 85% and 55% of the total sulfur being sulfate, whereas *Salicornia* exhibited more efficient use of the applied sulfate for growth and organic-S biosynthesis, resulting in 35% to 29% sulfate to the total sulfur ratio (calculated from Figs. 5A and 7A). The results here are in agreement with the previously reported observation that *Sarcocornia* accumulated twice as much sulfate as *Salicornia* when irrigated with Red Sea water (Ventura et al., 2011a), indicating that *Salicornia* is very well adapted to its natural habitat in areas exposed to frequent seawater tidal flooding.

High Salinity Is Favorable for Both *Salicornia* and *Sarcocornia*, Whereas High Sulfate Is a Stressor for *Sarcocornia*

Above a certain threshold, specifically for glycophytes as well as halophytes, salinity may generate ionic imbalance, which results in ionic toxicity, osmotic stress, and the generation of ROS (Allakhverdiev et al., 2000; Hasegawa et al., 2000; Chaparzadeh et al., 2004; Parida and Jha, 2010; Chawla et al., 2013). *Salicornia* and *Sarcocornia* thrived with 200 mM sodium rather than with 50 mM (Fig. 2), exhibiting lower anthocyanins in *Sarcocornia* and H_2O_2 in both *Salicornia* and *Sarcocornia* treated with the highest salinity (Figs. 3 and 4B). Interestingly, the enhanced sulfate supplementation resulted in an increase in anthocyanins and H_2O_2 in *Sarcocornia* (Figs. 3 and 4B). The H_2O_2 enhancement is most likely the cause for anthocyanin production, serving as a ROS scavenger in *Sarcocornia* (Takahama, 1992; Yamasaki et al., 1997; Chalker-Scott, 1999; Gould et al., 2002; Schüssler et al., 2008). The results indicate that high salinity is favorable for both halophytes, whereas the high sulfate is a stressor for *Sarcocornia*.

The high sulfate level detected in *Sarcocornia* shoots (Fig. 5A) could be the result of massive sulfate uptake that did not enter the sulfate reduction pathway. It also could be the result of the oxidation by SO of excess endogenous sulfite generated by APR and/or the result of S-amino acids degradation as demonstrated recently (Brychkova et al., 2013; Yarmolinsky et al., 2014). Excess sulfate uptake is thought to be energetically wasteful, employed to avoid osmotic potential imbalances (Hawkesford and De Kok, 2006). Yet, the capacities for osmotic adjustment can be reduced when relatively high sulfate is present in the growth medium, resulting in toxicity symptoms, as was shown in halophytes such as *P. strombulifera* (Llanes et al., 2013). Interestingly, estimation of the osmotic potential in both halophyte plants studied revealed a significantly higher osmolality in *Sarcocornia* than in *Salicornia* extracts when sulfate was added to the growth medium (Supplemental Fig. S7). Because biomass accumulation in *Sarcocornia* is generally decreased in the presence of high sulfate, whereas in *Salicornia* biomass accumulation is enhanced (Fig. 2), the sulfate accumulation (Fig. 5A) can be seen also as a cause of energetic waste. Whether the accumulated sulfate is a result of endogenous sulfite oxidation and/or avoidance of osmotic potential imbalances, both are energetically wasteful.

OAS-TL A and OAS-TL B Exhibit Significant L-Cys DES Activity in *Sarcocornia*, Especially in the Presence of High Sulfate

The synthesis of Cys and its degradation should be well coordinated. L-Cys desulfhydrase activity results in the release of sulfide, whereas OAS-TL consumes sulfide for Cys biosynthesis. It has been claimed that the kinetic properties of the OAS-TLs A, OAS-TL B, and OAS-TL C in Arabidopsis most likely do not allow a significant reverse reaction (Wirtz et al., 2004). Yet, free H_2S was shown to be partially released by OAS-TLs (Papenbrock et al., 2007), acting not only in L-Cys de novo biosynthesis but also in its homeostasis (Riemenschneider et al., 2005). Interestingly, both OAS-TL and DES activities showed a positive correlation in response to the *Brassicaceae*-infecting pathogen, *Pyrenopeziza brassicae* (Bloem et al., 2004). Similar results have been reported in *Vitis vinifera*, consequent from chilling stress (Fu et al., 2013). These indicate the feasibility of certain conditions where L-Cys desulfhydrase activities of OAS-TL can be affected at least to a certain level.

Sulfide for Cys biosynthesis can be derived from Cys degradation, cyanide detoxification, and iron-sulfur

cluster degradation, but the bulk of sulfide is believed to be generated by the assimilatory sulfate reduction pathway (Birke et al., 2015a, 2015b). Yet, this appears to be in doubt because the rate of sulfide generation by sulfite reductase activity in Arabidopsis leaves has been reported to be 2.5 to 4 nmol mg^{-1} protein min^{-1} (Khan et al., 2010; Yarmolinsky et al., 2013), whereas the activity of Arabidopsis L-Cys DES1 that degrades Cys to sulfide, pyruvate, and ammonia has been reported to be 7 to 10 nmol mg^{-1} protein min^{-1} (Álvarez et al., 2010). This indicates that the DES activity is a possible source of sulfide, in addition to sulfite reductase activity. Intriguingly, the DES1 activity in Arabidopsis leaves accounted for only 13% to 19% of the total desulfhydrase activity (calculated from Álvarez et al., 2010), indicating the existence of additional unidentified enzymes with DES activity.

In view of the significantly lower organic-S content of *Sarcocornia* compared to *Salicornia* (Fig. 7B), it seems possible that sulfate supplementation stimulates L-Cys degradation by the *Sarcocornia* OAS-TL (Fig. 9, A and B). Several lines of evidence support this notion. First, we showed a significantly higher DES activity in *Sarcocornia* as compared to *Salicornia* in the presence of supplemental sulfate (Fig. 9B). The enhancement of DES activity in sulfate-supplemented *Sarcocornia* was also detected using the in-gel activity, as well as by employing the alternative kinetic DES assay, both, based on the detection of H_2S (Fig. 9, B and C). Finally, the DES activity was shown to be attributable directly to OAS-TL A and OAS-TL B by peptide identification in the L-Cys DES activity bands of *Salicornia* and *Sarcocornia* (Tables S2 and S3), as well as by identification of the activity bands and their abrogation by immunoprecipitation, with Arabidopsis OAS-TL A or OAS-TL B antibodies (Fig. 10). The complete pull-down of the L-Cys desulfhydrase activity bands by OAS-TL B antibody in both *Salicornia* and *Sarcocornia* (Fig. 10D), and the complete abrogation in *Salicornia* and almost full pull-down in *Sarcocornia* by OAS-TL A antibody (Fig. 10C), indicate that even if DES1 comigrated with the OAS-TLs, the vast majority of DES activity is still that of OAS-TL A and OAS-TL B.

The Higher APR Activity in *Salicornia* than in *Sarcocornia* Suggests Enhanced Sulfate Reduction Activity in *Salicornia*

The higher total-S but lower organic-S accumulated in *Sarcocornia* as compared with *Salicornia* supplemented with high sulfate, in either salinities levels (Fig. 7), indicates that the lower sulfate level in *Salicornia* (Fig. 5A) is more the result of a higher sulfate assimilation rate by the sulfate reduction pathway, than only the result of lower sulfate uptake. The key step in the pathway is the reduction of APS to sulfite catalyzed by APR (Vauclare et al., 2002) considered to be the key control point in the sulfate assimilation pathway (Vauclare et al., 2002; Kopriva, 2006; Khan et al., 2010). APR activity and expression increase upon sulfur starvation and decrease with sulfate availability (Hirai et al., 2003; Maruyama-Nakashita et al., 2003; Nikiforova et al., 2003). In accordance with the concept of demand-driven regulation of sulfate assimilation, APR activity was down-regulated when 10 mM sulfate was supplied to either *Salicornia* or *Sarcocornia* (Fig. 8A). Yet, APR activity was significantly higher in *Salicornia* than in *Sarcocornia* (Fig. 8A), which may explain the lower sulfate and higher organic-S levels in *Salicornia* (Figs. 5A and 7B). Enhanced APR activity indicates a higher need for S-containing metabolites and is followed by higher synthesis levels of thiols such as Cys (Romero et al., 2001; Koprivova et al., 2008a). Similarly, a significantly higher Cys level was detected in *Salicornia* than in *Sarcocornia* (Fig. 6A), indicating a higher demand for reduced sulfur for the synthesis of essential S-containing metabolites. Moreover, sulfur assimilation is a highly regulated process that controls responses to developmental cues. In plants, the organic sulfur (such as Cys and Met) is extremely important for growth, especially when plant organs are developing rapidly (Martin et al., 2005). Accordingly, the higher organic sulfur content appears to be important in achieving the higher growth rate observed in *Salicornia* as compared with *Sarcocornia* (Fig. 2). In summary, the higher organic-S level in *Salicornia* (as compared with *Sarcocornia*) is likely indicative of an enhanced APR response to the demand for enhanced biomass production and is likely to be the result of reduced degradation of organic-S compounds such as L-Cys.

High Sulfate Accumulation and SO Activity Rates in *Sarcocornia* But Not in *Salicornia* Are Indicative of Enhanced Organic S Degradation Activity in *Sarcocornia*

The high sulfate level detected in *Sarcocornia* (Fig. 5A) is first of all a consequence of the higher uptake of sulfate, as inferred from its generally higher total sulfur content as compared with *Salicornia* (Fig. 7A). It also could be the result of the high oxidation rate of sulfite generated as the result of plant metabolism, as part of the sulfate reduction pathway, and/or degradation of sulfur-containing metabolites. Turnover of S-containing amino acids, a massive component of plant organic S (Stulen and De Kok, 1993), has recently been reported (Brychkova et al., 2013). The absence of active plant SO resulted in the accumulation of sulfite as a result of dark-induced accelerated catabolism of protein-bound Cys and Met, whereas a significant enhancement of sulfate was evident in the presence of active SO in wild-type plants (Brychkova et al., 2013, 2015). This notion is supported by the high SO activity rate in *Sarcocornia*, because high expression of the constitutively expressed SO protein (0.1% of total crude leaf protein; Lang et al., 2007) can be explained mainly by the need to detoxify excess sulfite, most likely the result of degraded organic sulfur. Although SO activity decreased when *Sarcocornia* plants were supplied with

sulfate, the greater sulfate accumulation can be attributed to the generally high SO activity (Fig. 8C), which efficiently oxidizes excess sulfite resulting in decreased nontoxic sulfite levels (Fig. 5B). In this context, the significantly higher ROS levels in *Sarcocornia* than in *Salicornia* plants (Fig. 4) are likely indicative of the higher ROS generation by SO (Hänsch et al., 2006; Brychkova et al., 2007, 2012).

CONCLUSIONS

By exploring the sulfate reductive pathway that generates L-Cys and the L-Cys DES activities to identify factors affecting organic-S and sulfate levels, we identified a major role for OAS-TLs, enzymes known to catalyze L-Cys as the final step of the sulfate reductive pathway. We showed that OAS-TL A and OAS-TL B exhibit significant L-Cys DES activity and that this activity is significantly higher in *Sarcocornia* than in *Salicornia*, especially upon sulfate supplementation. In addition, the activity of APR, the key enzyme in sulfate assimilation, was significantly higher in *Salicornia* than in *Sarcocornia*, whereas the activity of SO, which regulates sulfite levels in the sulfate reductive pathway, was significantly higher in *Sarcocornia*. These results indicate that the low organic-S in *Sarcocornia* is the result of enhanced organic-S degradation by the Cys degrading activity of OAS-TL A and OAS-TL B, likely followed by SO oxidation of sulfite originated from protein-bound sulfur amino acid degradation. The low Cys generation by the reduced APR activity observed in *Sarcocornia* further contributed to the lower organic-S in this plant. The significantly higher APR activity rate and the very low L-Cys DES activities in *Salicornia* is suggestive for its higher net Cys generation, resulting in higher organic-S levels for biomass accumulation. The results of this study provide evidence that *Salicornia* thrives at a high sulfate concentration, indicating its adaptation not only to high salinity, but also to high sulfate that exists in its natural habitat. In contrast, *Sarcocornia* is sensitive to high sulfate, as evident not only by the lower biomass and organic-S accumulation but also by the high anthocyanin and ROS production. These results present an initial road map for halophyte growers to gain better growth and nutritional value of *Salicornia* and *Sarcocornia*.

MATERIALS AND METHODS

Plant Materials, Growth Conditions, and Biomass Determination

Salicornia europaea (ecotype RN, collected in the Dead Sea area, Israel) and *Sarcocornia fruticosa* (ecotype VM, collected in the Ramat HaNegev district, Israel) were used in the experiments (Ventura et al., 2010; Ventura and Sagi, 2013). Experiments were carried out in the growth room at Ben-Gurion University of the Negev, under the following conditions: 14-h light/10-h dark, 25°C, 75% to 85% relative humidity, and under light intensity of 150 μmol m^{-2} s^{-1}.

Seeds were germinated and grown in standard 90 mm petri dishes on 1/2 MS medium supplemented 1% plant agar and 1% Suc for 10 d. The seedlings were transferred to large petri dishes of 155-mm diameter × 30-mm height, supplied with 1/2 MS medium (Murashige and Skoog, 1962; containing 0.87 mM sulfate level) supplemented with either 50 mM NaCl or 200 mM NaCl without the addition of Na_2SO_4. The NaCl concentration was 30 mM NaCl or 180 mM NaCl when 10 mM Na_2SO_4 was supplemented. All treatments were performed in three replicates.

The weight of shoot biomass accumulation was determined 14 d after the treatment onset for *S. europaea* (RN) and 21 d for *S. fruticosa* (VM). Results were expressed as average plant growth rate in mg d^{-1} $plant^{-1}$. Samples were immediately frozen in liquid N and stored at −80°C for further use.

To estimate biomass accumulation in the preliminary experiments, *Salicornia* and *Sarcocornia* seeds were germinated in pots (0.4 L) filled with the highly air-permeable and high-water-capacity perlite (up to 2 mm particles size; Agrekal Habonim Industries). After germination, seedlings were thinned out to similar numbers of 40 seedlings per pot in four replicas and subjected to 100 mM NaCl and 200 mM NaCl in half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) supplemented with 0 mM or 10 mM sulfate. Plants were grown in controlled greenhouses at Ben Gurion University of the Negev, Israel, under approximately 13.5-h sunlight (May 2012), day temperature 25 to 31°C, night temperature approximately 20°C, relative humidity approximately 60%, and light intensity ranged between 400 and 600 μmol m^{-2} s^{-1}. The biomass accumulation data from this experiment is presented in Supplemental Fig. S3.

For RNA sequencing, *S. europaea* (RN) seeds were germinated in plastic trays on Metromix-360 garden soil in a greenhouse at King Abdullah University of Science and Technology (Biological and Environmental Science and Engineering Division) and the seedlings were maintained under natural daylight conditions until sampled.

RNA Sequencing and Transcriptome De Novo Assembly

Four-month-old *S. europaea* (RN) plants were sampled for RNA sequencing and transcriptome de novo assembly. Total RNA was extracted using Trizol and used for preparation of RNAseq libraries. Paired-end libraries were prepared from *S. europaea* RNA using a TruSeq RNA Library Prep Kit v2 (Illumina) to prepare RNASeq libraries using manufacturers low sample protocol and libraries were sequenced on a HiSeq2000 platform (Illumina). A total of approximately 550 million paired-end reads of insert size 100 bp were produced. Low-quality reads and adapter sequences were filtered using a Trimmomatic (Bolger et al., 2014), leaving approximately 500 million high quality reads for downstream analyses. Transcriptome de novo assembly was performed using the software Trinity (Grabherr et al., 2011) and annotated following the Trinotate pipeline (Haas et al., 2013). A total of 56,107 protein sequences were predicted using TransDecoder (Haas et al., 2013). Predicted protein sequences were annotated by performing BLASTp against Swiss-Prot, protein domains were searched for through Pfam, and annotated using Cluster of Orthologous Genes and Gene Ontology annotations.

RNA Extraction, cDNA Synthesis, and Quantitative Real-Time Reverse Transcription-PCR in *Salicornia* and *Sarcocornia*

Total RNA extraction, cDNA synthesis, and the quantitative analysis of transcripts of *Salicornia* and *Sarcocornia* shoots grown in 1/2 MS medium was performed as described in Brychkova et al. (2007) employing the primers shown in Supplemental Table S1 as described below. To employ at least one of the two primers as splice junction overlapping primer, *Actin* (*Act*), *SiR*, *Transcription initiation factor* (*TFIID*), *APR*, *OAS-TL A*, and *OAS-TL B* primers were designed based on similarity analysis to *Beta vulgaris*, whereas *SO* primers for *Salicornia* were designed based on similarity to *Populus trichocarpa*. All the primers designed for *Salicornia* were suitable for *Sarcocornia* transcripts analysis as well. The quantitative PCR products were separated on a 1% agarose gel, excised from the gel, and sequenced for identity verification (see alignment results with each of the related transcripts in Supplemental Fig. S8). *ACT* (88% identity to *B. vulgaris* subsp. *vulgaris* actin-related protein 7-like, Accession XR_789363.1 | PREDICTED) and *TFIID* (85% identity to *B. vulgaris* subsp. *vulgaris* transcription initiation factor *TFIID* subunit 6, Accession XM_010690649) were unaffected by the treatments and thus were employed as reference housekeeping genes. The transcript levels in *Salicornia* or *Sarcocornia* were compared to 50 mM *NaCl* and 0 mM Na_2SO_4 treatment after normalization to *TFIID* or *ACT* and presented as relative expression (means ± SE, $n = 4$). Only results normalized with *TFIID* are presented.

Protein Extraction, Determination of Soluble Protein, and Kinetic Assays for APR, SO, SiR, and OAT-TL Enzymes

Protein extraction, desalting, concentration determination, and kinetic assays for APR, SO, SiR, and OAS-TL in *Salicornia* and *Sarcocornia* shoots grown in 1/2 MS medium were carried out as previously described for tomato leaves *Solanum lycopersicum* "Rheinlands Ruhm" (Brychkova et al., 2013). 1 U of 124-fold purified Arabidopsis (*Arabidopsis thaliana*) OAS-TL was added to the reaction mix of the SiR assay as a sulfide trap. SiR and OAS-TL activities were expressed in nmol Cys mg^{-1} protein min^{-1} (Brychkova et al., 2012) and APR and SO in nmol sulfite mg^{-1} protein min^{-1}.

Protein Extraction, Determination of Soluble Protein, and Kinetic Assays for DES Activity

Protein extraction of *Salicornia* and *Sarcocornia* shoots grown in 1/2 MS medium and desalting for DES was performed as described before for sulfurtransferases (Brychkova et al., 2013). DES activity was detected based on direct detection of H_2S formation in the presence of L-Cys as described in Riemenschneider et al. (2005). The modified assay solution contained 0.1 M Tris-HCl, pH 9.0, 2.5 mM DTT, 0.8 mM L-Cys, and 10 μg of desalted protein in a total volume of 0.2 mL. After incubation at 37°C for 30 min, H_2S was detected according to Bloem et al. (2004) with 30 mM $FeCl_3$ dissolved in 1.2 N HCl and 20 mM N,N-dimethyl-p-phenylenediamine dihydrochloride dissolved in 7.2 N HCl. The formation of methylene blue was measured at 670 nm with Epoch Microplate Spectrophotometer supported by Gen5 1.10 software (BioTek). Na_2S*9H_2O was used as a standard.

Measurement of Sulfur-containing Metabolites

To determine total sulfur content, approximately 10 mg dried and powdered *Salicornia* and *Sarcocornia* shoots grown in 1/2 MS medium were placed in tin containers in an elemental analyzer (EA1108 CHNS/O; Fisons Instruments) and the amounts of total S in each sample were quantified according to a standard calibration curve prepared with reduced GSH.

Sulfate and sulfite from *Salicornia* and *Sarcocornia* shoots grown in 1/2 MS medium were determined as described in Brychkova et al. (2012). Briefly, for sulfate determination, the leaf samples were extracted in 24 mM formaldehyde in 2 mM Na_2CO_3/0.75 mM $NaHCO_3$ solution (1:100, w/v) and determined employing a DIONEX LC20 ion chromatograph with an ED 50 Electrochemical Detector and an AS9-SC Analytical IonPak (4 mm × 250 mm). Sulfite was determined by employing chicken SO using NADH-peroxidase-dependent determination of H_2O_2 generated as a byproduct of sulfite oxidation to sulfate, catalyzed by chicken SO. For sulfide detection, samples were extracted in a solution containing 50 mM citric acid, 100 mM Na_2HPO_4, and 1 mM salicylic acid, pH 5.0 in 1:40 (w:v) ratio and detected by employing the ISO-H2S-100 microsensor for H_2S according to Yarmolinsky et al. (2014). Cys and total GSH were separated and quantified by HPLC according to Ohkama-Ohtsu et al. (2007). Organic S was calculated by subtracting the sum of sulfate, sulfite, thiosulfate, and sulfide from the total sulfur content.

Protein Extraction for an In-Gel DES Assay

Shoots samples of *Salicornia* and *Sarcocornia* plants grown in 1/2 MS medium were ground using a mortar and pestle in extraction buffer (ratio 1:4) containing 0.25 M Suc, 50 mM Tris-HCl (pH 8.5), 3 mM EDTA, 1 mM sodium molybdate, 4 mM DTT, 15 mM GSH, 0.025% of Triton X-100, and a cocktail of protease inhibitors including aprotinin (10 μg mL^{-1}), leupeptin (10 μg mL^{-1}), and pepstatin (10 μg mL^{-1}). The homogenate was centrifuged at 18,000*g* for 20 min and the total soluble protein content was determined by the Bradford assay (Bradford, 1976).

DES In-Gel Activity Assay

Fifty μg of extracted protein was loaded into each lane and fractionated by 7.5% Native PAGE. DES activity was detected using a modification of an in-gel visualization protocol for H_2S (Manchenko, 2002). Lead acetate was employed to detect the generated H_2S, producing dark brown lead sulfide bands. The reaction solution contained 0.15 M Tris–HCl, pH 8.5, 1 mM DTT, 50 mM β-mercaptoethanol, 20 mM L-Cys, 0.1 mM pyridoxal 5-P, 0.4 mM lead acetate. The reaction was stopped by immersion of the gel in double-distilled water.

Protein Sequencing

To identify the proteins participating in the L-Cys desulfhydrase activity the activity bands from the in-gel activity of DES (Fig. 9B) were sliced from the native gel, and fractionated with 12% SDS-PAGE. Thereafter the proteins were stained by Coomassie Brilliant Blue, and the stained bands were excised from the gel, trypsinized, and the resulting peptides were separated by HPLC and analyzed by LC-MS/MS on Q-Exactive (Thermo Fisher Scientific) at The Smoler Protein Research Center (Technion University, Haifa, Israel).

All the identified peptides were filtered with high confidence, top rank, mass accuracy, and a minimum of two peptides. High confidence peptides passed the 1% false discovery rate (FDR) threshold (*FDR = the estimated fraction of false positives in a list of peptides). Semiquantitation was done by calculating the peak area of each peptide. The area of the protein is the average of the three most intense peptides from each protein. Analysis of peptide sequences was performed by employing the Proteome Discoverer software, ver. 1.4.1.14 (Thermo Fisher Scientific) against the *Salicornia* sequence (kindly supplied by Prof. Nina Fedoroff and performed as described in the paragraph titled "RNA Sequencing and Transcriptome De Novo Assembly").

Immunodetection and Immunoprecipitation of L-Cys DES

For immunodetection analysis, the DES activity bands (sliced from the band shown in Fig. 9B) together with Arabidopsis wild-type bands were fractionated by 12.5% SDS PAGE as described in Brychkova et al. (2013) and blotted onto polyvinylidene difluoride membranes (Immuno-Blot membranes; Bio-Rad). The blotted proteins were subjected to immuno-detection with antibodies raised against Arabidopsis cytosolic OAS-TL A (diluted 1:2000) and chloroplastic OAS-TL B. The latter (diluted 1:5000; kindly provided by Prof. Dr. S. Kopriva, University of Cologne, Köln) could identify the three OAS-TL (A, B, and C). PBS-diluted (1:5000) secondary antibodies (antirabbit IgG; Sigma-Aldrich) was followed. Protein bands were visualized with the ChemiDoc Touch Imaging system (Bio-Rad) after staining with an ECL detection system, using the SuperSignal West Pico Chemiluminescent Substrate (Pierce) according to the manufacturer's instructions. Band intensities were quantified by using ImageJ software (National Institutes of Health).

For the immunoprecipitation assay, 50 μg of protein from *Salicornia* and *Sarcocornia* were incubated with 30 μL of the OAS-TL A or OAS-TL B antibodies in Tris-buffer saline for 30 min at room temperature and then kept at 4°C for overnight. Protein extract that had not been mixed with antibody was employed as control. The mixture and the control solutions were then incubated with 50 μL of Protein G Agarose at 4°C for 2 h with continuous shaking and then centrifuged at 10,000*g* for 5 min, followed by removal of supernatant for analysis by the in-gel DES assay.

Determination of Anthocyanin Content and Osmolality

The anthocyanin content was determined based on a modification of protocols described by Laby et al. (2000) and Kant et al. (2006). Approximately 100 mg of fresh shoot tissue of *Salicornia* and *Sarcocornia* plants grown in 1/2 MS medium was crushed in 600 μL methanol acidified with 1% HCl. The extract was centrifuged for 10 s at 4000*g*. Five-hundred μL of double distilled water was added to the collected sand, mixture was gently vortexed, and then 700 μL chloroform was added and mixed for 20 s followed by centrifugation at 4000*g* for 2 min. The total anthocyanin in the aqueous phase was determined by detecting the optical density at A530 nm and A657 nm. The amount of anthocyanin was calculated by subtracting the A657 from the A530 (Laby et al., 2000).

The osmolality was measured according to Thalmann et al. (2016). The fresh shoot tissues of *Salicornia* and *Sarcocornia* grown in 1/2 MS medium were crushed with iron beads, diluted 1/3 with water, and centrifuged for 10 min at 18,000*g* at 4°C. The supernatant was used to determine the osmolality employing a Micro-Osmometer (Advance Instruments).

ROS Determination

For detecting O_2^- and H_2O_2, frozen shoots of *Salicornia* and *Sarcocornia* were extracted in 50 mM P buffer (pH 7.5) at a ratio of 1:8 (w/v) and centrifuged (Eppendorf 5417R) twice at 18,000*g* for 20 min. The reaction mixture for detecting O_2^- consisted of 4 mM epinephrine as an electron acceptor in 100 mM Tris-HCl buffer (pH 7.8) in the presence or absence of 2100 U/mL CuZn-SOD as described in Yesbergenova et al. (2005). Absorbance was measured at 480 nm

employing an Epoch Microplate Spectrophotometer supported by Gen5 1.10 software (BioTek).

The reaction mixture for detecting H_2O_2 consisted of 0.85 mm 4-aminoantipyrine, 3.4 mm 3,5-dichloro-2-hydroxobenzene sulfonate, 4.5 U mL^{-1} HRP in 2 mL of 50 mM P buffer (pH 7.5) in the presence or absence of 2 mM tungstic acid and 100 μM DPI as described in Yesbergenova et al. (2005). Absorbance was measured after 5 min at 515 nm as described above.

Accession Numbers

Protein sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers: NP_001330588 (DES1, Arabidopsis), P47998 (OAS-TL A, Arabidopsis), P47999 (OAS-TL B, Arabidopsis), Q00834 (OAS-TL A, *Spinach oleracea*), and AAA16973 (OAS-TL B, *S. oleracea*).

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Effect of NaCl (100 and 200 mM) and Na_2SO_4 (100 mM) on biomass accumulation of *Salicornia* (RN) and *Sarcocornia* (VM).

Supplemental Figure S2. Effect of sodium (0 mM) and sulfate (0 and 10 mM) levels on biomass accumulation of *Salicornia* (upper panels) and *Sarcocornia* (lower panels).

Supplemental Figure S3. Effect of sodium (100 and 200 mM) and sulfate (0 and 10 mM) levels on biomass accumulation of *Salicornia* and *Sarcocornia*.

Supplemental Figure S4. Effect of sodium (50 and 200 mM) and sulfate (0, 10 mM) application on selected enzymes' expression in *Salicornia* and *Sarcocornia*.

Supplemental Figure S5. Multiple sequence alignment of *Salicornia*'s OAS-TL A and OAS-TL B protein sequence against *S. oleracea*'s OAS-TL A and OAS-TL B protein sequence.

Supplemental Figure S6. Multiple sequence alignment of *Salicornia*'s OAS-TL A and OAS-TL B protein sequence against Arabidopsis's OAS-TL A and OAS-TL B protein sequence.

Supplemental Figure S7. Effect of sodium (50 and 200 mM) and sulfate (0 and 10 mM) application on osmolality level in *Salicornia* and *Sarcocornia*.

Supplemental Figure S8. *Salicornia* selected gene sequences and quantitative real-time PCR product verification results in multiple sequence alignment.

Supplemental Table S1. List of gene primers used for quantitative real-time PCR with *Salicornia* and *Sarcocornia*.

Supplemental Table S2. List of identified and overlapped unique peptides of OAS-TL A in *Salicornia* and *Sarcocornia*.

Supplemental Table S3. List of identified and overlapped unique peptides of OAS-TL B in *Salicornia* and *Sarcocornia*.

ACKNOWLEDGMENTS

We thank Dr. Dominic Standing and Ms. Talya Samani for their technical support and Prof. S. Kopriva (University of Cologne, Köln) for providing OAS-TL A and OAS-TL B antibodies.

Received July 6, 2017; accepted July 22, 2017; published July 25, 2017.

LITERATURE CITED

Allakhverdiev SI, Sakamoto A, Nishiyama Y, Inaba M, Murata N (2000) Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. Plant Physiol **123:** 1047–1056

Álvarez C, Bermúdez MÁ, Romero LC, Gotor C, García I (2012) Cysteine homeostasis plays an essential role in plant immunity. New Phytol **193:** 165–177

Álvarez C, Calo L, Romero LC, García I, Gotor C (2010) An *O*-acetylserine(thiol)lyase homolog with L-cysteine desulfhydrase activity regulates cysteine homeostasis in Arabidopsis. Plant Physiol **152:** 656–669

Astolfi S, Zuchi S (2013) Adequate S supply protects barley plants from adverse effects of salinity stress by increasing thiol contents. Acta Physiol Plant **35:** 175–181

Barroso C, Vega JM, Gotor C (1998) The role of roots in cysteine biosynthesis by *Arabidopsis thaliana*. J Physiol Biochem **54:** 189–194

Birke H, De Kok LJ, Wirtz M, Hell R (2015b) The role of compartment-specific cysteine synthesis for sulfur homeostasis during H2S exposure in Arabidopsis. Plant Cell Physiol **56:** 358–367

Birke H, Hildebrandt TM, Wirtz M, Hell R (2015a) Sulfide detoxification in plant mitochondria. Methods Enzymol **555:** 271–286

Bloem E, Riemenschneider A, Volker J, Papenbrock J, Schmidt A, Salac I, Haneklaus S, Schnug E (2004) Sulphur supply and infection with *Pyrenopeziza brassicae* influence L-cysteine desulphydrase activity in *Brassica napus* L. J Exp Bot **55:** 2305–2312

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics **30:** 2114–2120

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72:** 248–254

Brychkova G, Grishkevich V, Fluhr R, Sagi M (2013) An essential role for tomato sulfite oxidase and enzymes of the sulfite network in maintaining leaf sulfite homeostasis. Plant Physiol **161:** 148–164

Brychkova G, Xia Z, Yang G, Yesbergenova Z, Zhang Z, Davydov O, Fluhr R, Sagi M (2007) Sulfite oxidase protects plants against sulfur dioxide toxicity. Plant J **50:** 696–709

Brychkova G, Yarmolinsky D, Batushansky A, Grishkevich V, Khozin-Goldberg I, Fait A, Amir R, Fluhr R, Sagi M (2015) Sulfite oxidase activity is essential for normal sulfur, nitrogen and carbon metabolism in tomato leaves. Plants (Basel) **4:** 573–605

Brychkova G, Yarmolinsky D, Fluhr R, Sagi M (2012) The determination of sulfite levels and its oxidation in plant leaves. Plant Sci **190:** 123–130

Carfagna S, Bottone C, Cataletto PR, Petriccione M, Pinto G, Salbitani G, Vona V, Pollio A, Ciniglia C (2016) Impact of sulfur starvation in autotrophic and heterotrophic cultures of the extremophilic microalga *Galdieria phlegrea* (*Cyanidiophyceae*). Plant Cell Physiol **57:** 1890–1898

Carfagna S, Vona V, Di Martino V, Esposito S, Rigano C (2011) Nitrogen assimilation and cysteine biosynthesis in barley: evidence for root sulphur assimilation upon recovery from N deprivation. Environ Exp Bot **71:** 18–24

Chalker-Scott L (1999) Environmental significance of anthocyanins in plant stress responses. Photochem Photobiol **70:** 1–9

Chaparzadeh N, D'Amico ML, Khavari-Nejad R-A, Izzo R, Navari-Izzo F (2004) Antioxidative responses of *Calendula officinalis* under salinity conditions. Plant Physiol Biochem **42:** 695–701

Chawla S, Jain S, Jain V (2013) Salinity induced oxidative stress and antioxidant system in salt-tolerant and salt-sensitive cultivars of rice (*Oryza sativa* L.). J Plant Biochem Biotechnol **22:** 27–34

Colmer TD, Fan, TWM, Läuchli A, Higashi RM (1996) Interactive effects of salinity, nitrogen and sulphur on the organic solutes in *Spartina alterniflora* leaf blades. J Exp Bot **47:** 369–375

Cram W (1990) Uptake and transport of sulfate. H Rennenberg, C Brunold, LJ De Kok, I Stulen, eds. *In* Sulfur Nutrition and Sulfur Assimilation in Higher Plants: Fundamental, Environmental, and Agricultural Aspects. SPB Academic Publishing, The Hague, The Netherlands. pp 3–11

Davy AJ, Bishop GF, Costa CSB (2001) *Salicornia* L. (*Salicornia pusilla* J. Woods, *S. ramosissima* J. Woods, S. *europaea* L., *S. obscura* P.W. Ball & Tutin, *S. nitens* P.W. Ball & Tutin, *S. fragilis* P.W. Ball & Tutin and *S. dolichostachya* Moss). J Ecol **89:** 681–707

Davy AJ, Bishop GF, Mossman H, Redondo-Gómez S, Castillo JM, Castellanos EM, Luque T, Figueroa ME (2006) Biological flora of the British Isles: *Sarcocornia perennis* (Miller). A.J. Scott. Journal of Ecology **94:** 1035–1048

de la Fuente V, Oggerin M, Rufo L, Rodríguez N, Ortuñez E, Sánchez-Mata D, Amils R (2013) A micromorphological and phylogenetic study of *Sarcocornia* AJ Scott (Chenopodiaceae) on the Iberian Peninsula. Plant Biosyst **147:** 158–173

Ernst WHO (1990) Ecological aspects of sulfur metabolism. H Rennenberg, C Brunold, LJ De Kok, I Stulen, eds. *In* Sulfur Nutrition and Sulfur Assimilation in Higher Plants: Fundamental, Environmental, and

Agricultural Aspects. SPB Academic Publishing, The Hague, The Netherlands. pp 131–144

Fatma M, Khan MIR, Masood A, Khan NA (2013) Coordinate changes in assimilatory sulfate reduction are correlated to salt tolerance: involvement of phytohormones. Annu Rev Res Biol **3:** 267–295

Fu P, Wang W, Hou L, Liu X (2013) Hydrogen sulfide is involved in the chilling stress response in *Vitis vinifera* L. Acta Soc Bot Pol **82:** 295–302

Gould K, McKelvie J, Markham K (2002) Do anthocyanins function as antioxidants in leaves? Imaging of H_2O_2 in red and green leaves after mechanical injury. Plant Cell Environ **25:** 1261–1269

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, et al (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol **29:** 644–652

Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, Couger MB, Eccles D, Li B, Lieber M, MacManes MD, Ott M, et al (2013) De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat Protoc **8:** 1494–1512

Hänsch R, Lang C, Riebeseel E, Lindigkeit R, Gessler A, Rennenberg H, Mendel RR (2006) Plant sulfite oxidase as novel producer of H_2O_2: combination of enzyme catalysis with a subsequent non-enzymatic reaction step. J Biol Chem **281:** 6884–6888

Hasegawa PM, Bressan RA, Zhu J-K, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol **51:** 463–499

Havill DC, Ingold A, Pearson J (1985) 6. Sulphide tolerance in coastal halophytes. WG Beeftink, J Rozema, AHL Huiskes, eds. *In* Ecology of Coastal Vegetation. Springer, New York. pp 279–285

Hawkesford MJ, De Kok LJ (2006) Managing sulphur metabolism in plants. Plant Cell Environ **29:** 382–395

Hirai MY, Fujiwara T, Awazuhara M, Kimura T, Noji M, Saito K (2003) Global expression profiling of sulfur-starved Arabidopsis by DNA macroarray reveals the role of O-acetyl-l-serine as a general regulator of gene expression in response to sulfur nutrition. Plant J **33:** 651–663

Hoagland DR, Arnon DI (1950) The Water-Culture Method for Growing Plants without Soil. College of Agriculture, University of California, Berkeley, CA, **C347**

Howarth RW, Giblin A (1983) Sulfate reduction in the salt marshes at Sapelo Island, Georgia. Limnol Oceanogr **28:** 70–82

Ingold A, Havill D (1984) The influence of sulphide on the distribution of higher plants in salt marshes. J Ecol **72:** 1043–1054

Iqbal N, Masood A, Khan MIR, Asgher M, Fatma M, Khan NA (2013) Cross-talk between sulfur assimilation and ethylene signaling in plants. Plant Signal Behav **8:** e22478

Kadereit G, Ball P, Beer S, Mucina L, Sokoloff D, Teege P, Yaprak AE, Freitag H (2007) A taxonomic nightmare comes true: Phylogeny and biogeography of glassworts (*Salicornia* L., *Chenopodiaceae*). Taxon **56:** 1143–1170

Kant S, Kant P, Raveh E, Barak S (2006) Evidence that differential gene expression between the halophyte, *Thellungiella halophila*, and *Arabidopsis thaliana* is responsible for higher levels of the compatible osmolyte proline and tight control of Na^+ uptake in *T. halophila*. Plant Cell Environ **29:** 1220–1234

Khan MIR, Asgher M, Iqbal N, Khan NA (2013) Potentiality of sulphur-containing compounds in salt stress tolerance. *In* Ecophysiology and Responses of Plants under Salt Stress. Springer, New York. pp 443–472

Khan MS, Haas FH, Samami AA, Gholami AM, Bauer A, Fellenberg K, Reichelt M, Hänsch R, Mendel RR, Meyer AJ, Wirtz M, Hell R (2010) Sulfite reductase defines a newly discovered bottleneck for assimilatory sulfate reduction and is essential for growth and development in *Arabidopsis thaliana*. Plant Cell **22:** 1216–1231

Király L, Künstler A, Höller K, Fattinger M, Juhász C, Müller M, Gullner G, Zechmann B (2012) Sulfate supply influences compartment specific glutathione metabolism and confers enhanced resistance to Tobacco mosaic virus during a hypersensitive response. Plant Physiol Biochem **59:** 44–54

Kopriva S (2006) Regulation of sulfate assimilation in Arabidopsis and beyond. Ann Bot (Lond) **97:** 479–495

Koprivova A, Kopriva S (2008b) Lessons from investigation of regulation of APS reductase by salt stress. Plant Signal Behav **3:** 567–569

Koprivova A, North KA, Kopriva S (2008a) Complex signaling network in regulation of adenosine 5′-phosphosulfate reductase by salt stress in Arabidopsis roots. Plant Physiol **146:** 1408–1420

Laby RJ, Kincaid MS, Kim D, Gibson SI (2000) The Arabidopsis sugar-insensitive mutants sis4 and sis5 are defective in abscisic acid synthesis and response. Plant J **23:** 587–596

Lang C, Popko J, Wirtz M, Hell R, Herschbach C, Kreuzwieser J, Rennenberg H, Mendel RR, Hänsch R (2007) Sulphite oxidase as key enzyme for protecting plants against sulphur dioxide. Plant Cell Environ **30:** 447–455

Lappartient AG, Touraine B (1996) Demand-driven control of root ATP sulfurylase activity and SO_{42}- uptake in intact canola (the role of phloem-translocated glutathione). Plant Physiol **111:** 147–157

Lappartient AG, Vidmar JJ, Leustek T, Glass ADM, Touraine B (1999) Inter-organ signaling in plants: regulation of ATP sulfurylase and sulfate transporter genes expression in roots mediated by phloem-translocated compound. Plant J **18:** 89–95

Lee BR, Huseby S, Koprivova A, Chételat A, Wirtz M, Mugford ST, Navid E, Brearley C, Saha S, Mithen R, Hell R, Farmer EE, et al (2012) Effects of fou8/fry1 mutation on sulfur metabolism: is decreased internal sulfate the trigger of sulfate starvation response? PLoS One **7:** e39425 10.1371/journal.pone.0039425

Leustek T (2002) Sulfate metabolism. Arabidopsis Book **1:** e0017

Leustek T, Martin MN, Bick JA, Davies JP (2000) Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies. Annu Rev Plant Physiol Plant Mol Biol **51:** 141–165

Lewandowska M, Sirko A (2008) Recent advances in understanding plant response to sulfur-deficiency stress. Acta Biochim Pol **55:** 457–471

Llanes A, Bertazza G, Palacio G, Luna V (2013) Different sodium salts cause different solute accumulation in the halophyte *Prosopis strombulifera*. Plant Biol (Stuttg) **15(Suppl 1):** 118–125

Llanes A, Masciarelli O, Ordoñez R, Isla M, Luna V (2014) Differential growth responses to sodium salts involve different abscisic acid metabolism and transport in *Prosopis strombulifera*. Biol Plant **58:** 80–88

López-Berenguer C, Carvajal M, Garcéa-Viguera C, Alcaraz CF (2007) Nitrogen, phosphorus, and sulfur nutrition in broccoli plants grown under salinity. J Plant Nutr **30:** 1855–1870

Manchenko GP (2002) Handbook of Detection of Enzymes on Electrophoretic Gels. CRC Press, Boca Raton, FL

Martin NM, Maricle BR (2015) Species-specific enzymatic tolerance of sulfide toxicity in plant roots. Plant Physiol Biochem **88:** 36–41

Martin MN, Tarczynski MC, Shen B, Leustek T (2005) The role of 5′-adenylylsulfate reductase in controlling sulfate reduction in plants. Photosynth Res **86:** 309–323

Maruyama-Nakashita A, Inoue E, Watanabe-Takahashi A, Yamaya T, Takahashi H (2003) Transcriptome profiling of sulfur-responsive genes in Arabidopsis reveals global effects of sulfur nutrition on multiple metabolic pathways. Plant Physiol **132:** 597–605

Mulholland M, Otte M (2000) Effects of varying sulphate and nitrogen supply on DMSP and glycine betaine levels in *Spartina anglica*. J Sea Res **43:** 199–207

Mullineaux PM, Rausch T (2005) Glutathione, photosynthesis and the redox regulation of stress-responsive gene expression. Photosynth Res **86:** 459–474

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant **15:** 473–497

Nakamura T, Yamaguchi Y, Sano H (2000) Plant mercaptopyruvate sulfurtransferases: molecular cloning, subcellular localization and enzymatic activities. Eur J Biochem **267:** 5621–5630

Nazar R, Iqbal N, Masood A, Syeed S, Khan NA (2011b) Understanding the significance of sulfur in improving salinity tolerance in plants. Environ Exp Bot **70:** 80–87

Nazar R, Iqbal N, Syeed S, Khan NA (2011a) Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. J Plant Physiol **168:** 807–815

Nguyen X-V, Klein M, Riemenschneider A, Papenbrock J (2014) Distinctive features and role of sulfur-containing compounds in marine plants, seaweeds, seagrasses and halophytes, from an evolutionary point of view. *In* Sabkha Ecosystems. **IV:** Cash Crop Halophyte and Biodiversity Conservation. Springer, New York, pp 299–312

Nikiforova V, Freitag J, Kempa S, Adamik M, Hesse H, Hoefgen R (2003) Transcriptome analysis of sulfur depletion in *Arabidopsis thaliana*: interlacing of biosynthetic pathways provides response specificity. Plant J **33:** 633–650

Nocito FF, Lancilli C, Dendena B, Lucchini G, Sacchi GA (2011) Cadmium retention in rice roots is influenced by cadmium availability, chelation and translocation. Plant Cell Environ **34:** 994–1008

Ohkama-Ohtsu N, Zhao P, Xiang C, Oliver DJ (2007) Glutathione conjugates in the vacuole are degraded by γ-glutamyl transpeptidase GGT3 in Arabidopsis. Plant J **49:** 878–888

Papenbrock J, Riemenschneider A, Kamp A, Schulz-Vogt HN, Schmidt A (2007) Characterization of cysteine-degrading and H2S-releasing enzymes of higher plants—from the field to the test tube and back. Plant Biol (Stuttg) **9:** 582–588

Papenbrock J, Schmidt A (2000) Characterization of a sulfurtransferase from *Arabidopsis thaliana*. Eur J Biochem **267:** 145–154

Parida AK, Jha B (2010) Antioxidative defense potential to salinity in the euhalophyte *Salicornia brachiata*. J Plant Growth Regul **29:** 137–148

Rao AC, Reddy AR (2008) Glutathione reductase: a putative redox regulatory system in plant cells. *In* Sulfur Assimilation and Abiotic Stress in Plants. Springer, New York. pp 111–147

Ravina CG, Barroso C, Vega JM, Gotor C (1999) Cysteine biosynthesis in *Chlamydomonas reinhardtii*. Molecular cloning and regulation of O-acetylserine(thiol)lyase. Eur J Biochem **264:** 848–853

Reginato M, Sgroy V, Llanes A, Cassán F, Luna V (2012) The American halophyte *Prosopis strombulifera*, a new potential source to confer salt tolerance to crops. *In* Crop Production for Agricultural Improvement. Springer, New York, pp 115–143

Reginato M, Sosa L, Llanes A, Hampp E, Vettorazzi N, Reinoso H, Luna V (2014) Growth responses and ion accumulation in the halophytic legume *Prosopis strombulifera* are determined by Na_2SO_4 and NaCl. Plant Biol (Stuttg) **16:** 97–106

Riemenschneider A, Riedel K, Hoefgen R, Papenbrock J, Hesse H (2005) Impact of reduced O-acetylserine(thiol)lyase isoform contents on potato plant metabolism. Plant Physiol **137:** 892–900

Rogel JA, Ariza FA, Silla RO (2000) Soil salinity and moisture gradients and plant zonation in Mediterranean salt marshes of southeast Spain. Wetlands **20:** 357–372

Romero LC, Domínguez-Solís JR, Gutiérrez-Alcalá G, Gotor C (2001) Salt regulation of O-acetylserine(thiol)lyase in *Arabidopsis thaliana* and increased tolerance in yeast. Plant Physiol Biochem **39:** 643–647

Rouached H, Secco D, Arpat B, Poirier Y (2011) The transcription factor PHR1 plays a key role in the regulation of sulfate shoot-to-root flux upon phosphate starvation in Arabidopsis. BMC Plant Biol **11:** 19

Ruiz JM, Blumwald E (2002) Salinity-induced glutathione synthesis in *Brassica napus*. Planta **214:** 965–969

Schüssler MD, Alexandersson E, Bienert GP, Kichey T, Laursen KH, Johanson U, Kjellbom P, Schjoerring JK, Jahn TP (2008) The effects of the loss of TIP1;1 and TIP1;2 aquaporins in *Arabidopsis thaliana*. Plant J **56:** 756–767

Steffen S, Ball P, Mucina L, Kadereit G (2015) Phylogeny, biogeography and ecological diversification of *Sarcocornia* (*Salicornioideae*, *Amaranthaceae*). Ann Bot (Lond) **115:** 353–368

Stribling JM (1997) The relative importance of sulfate availability in the growth of *Spartina alterniflora* and *Spartina cynosuroides*. Aquat Bot **56:** 131–143

Stulen I, De Kok LJ (1993) Whole-plant regulation of sulfur metabolism, a theoretical approach and comparison with current ideas on regulation of nitrogen-metabolism. LJ De Kok, I Stulen, H Rennenberg, C Brunold, WE Rauser, eds. *In* 2nd Workshop on Sulfur Metabolism in Higher Plants. SPB Academic Publishing, The Hague, The Netherlands.

Takahama U (1992) Hydrogen peroxide scavenging systems in vacuoles of mesophyll cells of *Vicia faba*. Phytochemistry **31:** 1127–1133

Thalmann MR, Pazmino D, Seung D, Horrer D, Nigro A, Meier T, Kölling K, Pfeifhofer HW, Zeeman SC, Santelia D (2016) Regulation of leaf starch degradation by abscisic acid is important for osmotic stress tolerance in plants. Plant Cell tpc.00143.2016

Tsakraklides G, Martin M, Chalam R, Tarczynski MC, Schmidt A, Leustek T (2002) Sulfate reduction is increased in transgenic *Arabidopsis thaliana* expressing 5′-adenylylsulfate reductase from *Pseudomonas aeruginosa*. Plant J **32:** 879–889

Vauclare P, Kopriva S, Fell D, Suter M, Sticher L, von Ballmoos P, Krähenbühl U, den Camp RO, Brunold C (2002) Flux control of sulphate assimilation in *Arabidopsis thaliana*: adenosine 5′-phosphosulphate reductase is more susceptible than ATP sulphurylase to negative control by thiols. Plant J **31:** 729–740

Ventura Y, Eshel A, Pasternak D, Sagi M (2015) The development of halophyte-based agriculture: past and present. Ann Bot (Lond) **115:** 529–540

Ventura Y, Sagi M (2013) Halophyte crop cultivation: the case for *Salicornia* and *Sarcocornia*. Environ Exp Bot **92:** 144–153

Ventura Y, Wuddineh WA, Ephrath Y, Shpigel M, Sagi M (2010) Molybdenum as an essential element for improving total yield in seawater-grown *Salicornia europaea* L. Sci Hortic (Amsterdam) **126:** 395–401

Ventura Y, Wuddineh WA, Myrzabayeva M, Alikulov Z, Khozin-Goldberg I, Shpigel M, Samocha TM, Sagi M (2011a) Effect of seawater concentration on the productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia* halophytes as leafy vegetable crops. Sci Hortic (Amsterdam) **128:** 189–196

Ventura Y, Wuddineh WA, Shpigel M, Samocha TM, Klim BC, Cohen S, Shemer Z, Santos R, Sagi M (2011b) Effects of day length on flowering and yield production of *Salicornia* and *Sarcocornia* species. Sci Hortic (Amsterdam) **130:** 510–516

Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta **218:** 1–14

Wipf D, Mongelard G, van Tuinen D, Gutierrez L, Casieri L (2014) Transcriptional responses of *Medicago truncatula* upon sulfur deficiency stress and arbuscular mycorrhizal symbiosis. Front Plant Sci **5:** 680

Wirtz M, Droux M, Hell R (2004) O-acetylserine (thiol) lyase: an enigmatic enzyme of plant cysteine biosynthesis revisited in *Arabidopsis thaliana*. J Exp Bot **55:** 1785–1798

Yamasaki H, Sakihama Y, Ikehara N (1997) Flavonoid-peroxidase reaction as a detoxification mechanism of plant cells against H_2O_2. Plant Physiol **115:** 1405–1412

Yarmolinsky D, Brychkova G, Fluhr R, Sagi M (2013) Sulfite reductase protects plants against sulfite toxicity. Plant Physiol **161:** 725–743

Yarmolinsky D, Brychkova G, Kurmanbayeva A, Bekturova A, Ventura Y, Khozin-Goldberg I, Eppel A, Fluhr R, Sagi M (2014) Impairment in sulfite reductase leads to early leaf senescence in tomato plants. Plant Physiol **165:** 1505–1520

Yesbergenova Z, Yang G, Oron E, Soffer D, Fluhr R, Sagi M (2005) The plant Mo-hydroxylases aldehyde oxidase and xanthine dehydrogenase have distinct reactive oxygen species signatures and are induced by drought and abscisic acid. Plant J **42:** 862–876

You J, Chan Z (2015) ROS regulation during abiotic stress responses in crop plants. Front Plant Sci **6:** 1092

Zechmann B, Müller M, Zellnig G (2008) Modified levels of cysteine affect glutathione metabolism in plant cells. *In* Sulfur Assimilation and Abiotic Stress in Plants. Springer, New York, pp 193–206