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OAS-TLs degrade L-Cys in *Salicornia* & *Sarcocornia*

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Title:
Higher degradation of L-Cys by O-acetylserine-thiolyases in Sarcocornia than Salicornia

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Summary:
The low level of organic S in Sarcocornia as compared to Salicornia is the result of a higher L-cysteine degradation rate by O-acetylserine-(thiol) lyases, especially when supplemented with sulfate.

Footnotes:

List of author contributions:
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 immunoprecipitation assay; A.S. participated in qRT-PCR; A.A. performed preliminary experiment with Salicornia and Sarcocornia grown on perlite; Y.V. compared plants grown on 100 mM sodium sulfate with those grown on 100 or 200 mM sodium chloride; M.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.

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ABSTRACT

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, while 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 Sarcocornia: the sulfate reductive pathway that generates cysteine and L-cysteine desulphydrase that degrades cysteine to \( \text{H}_2\text{S}, \text{NH}_3 \) and pyruvate. The major function of \( O\)-acetylserine-(thiol) lyase (OAS-TL; EC 2.5.1.47) is the formation of L-cysteine, but our study shows that the OAS-TL A and B of both halophytes are enzymes that also degrade L-cysteine to \( \text{H}_2\text{S} \). 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-cysteine 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-cysteine degradation rate, resulting in higher net cysteine biosynthesis. These results present an initial road map for halophyte growers to attain better growth rates and nutritional value of Salicornia and Sarcocornia.
INTRODUCTION

Soil salinity is one of the oldest and most important abiotic stresses affecting agricultural productivity globally. According to the Food and Agricultural Organization, roughly 800 million hectares of land worldwide, constituting 6% of the world’s total land area, are affected by salt. It has further been predicted that about 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. In order 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 non-saline 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 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 [5-30mM in interstitial water (Howarth and Giblin, 1983; Davy et al., 2001; Davy et al., 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; Davy et al., 2006) and several Salicornia species are already used as 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 since 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 (CoA) and S-adenosyl- methionine, peptides such as glutathione and phytochelatins, secondary metabolites such as allyl cysteine sulfoxides and glucosinolates as well as the sulfur-containing amino acids cysteine (Cys) and methionine (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, 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, EC 1.8.3.1.) or reduced to sulfide by the chloroplastic sulfite reductase (SiR, EC 1.8.7.1). Sulfide, together with O-acetylserine (OAS) whose biosynthesis is catalyzed by serine acetyltransferase (SAT, EC 2.3.1.30), is then incorporated into Cysteine in a reaction catalyzed by the O-acetylserine-(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). Cysteine homeostasis is controlled by the cytosolic L-cysteine desulphydrase 1 (DES1, EC 4.4.1.1) which catalyzes the breakdown of cysteine 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 threefold. Interestingly, an increase in APR activity was correlated with a higher rate of Cys biosynthesis to regulate the increased demand for glutathione in response to the salinity stress as a defense response to reactive oxygen species [ROS (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 Brassica napus (Ruiz and Blumwald, 2002) and barley (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 glutathione (GSH) and dimethylsulfiniopropionate (DMSP) (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 0-1.6 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, Brassica and tobacco 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 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; Reginato et al., 2014; Llanes et al., 2013; Llanes et al., 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 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-around cultivation was demonstrated, as well as the importance of molybdenum for improving total biomass.
accumulation in Salicornia grown in seawater (Ventura et al., 2010; Ventura et al., 2011b; Ventura and Sagi, 2013; Ventura et al., 2015).

Employing RNA and protein sequences of *Salicornia* (being highly similar to *Sarcocornia*) allow 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 *Salicornia europaea* (ecotype RN) in response to sulfate supplementation, while *Sarcocornia fruticosa* (ecotype VM) accumulated higher sulfate, but showed no increase in biomass. The sulfate reductive pathway and the L-cysteine desulphhydrase (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-cysteine, but we found that OAS-TL also functions as a desulphhydrase, degrading L-cysteine to H₂S. We attribute the higher organic-S and greater biomass accumulation in *Salicornia* to the significantly lower L-cysteine DES activity of OAS-TL A and 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-cysteine. 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 to 30 mM (Howard & Giblin 1983). Interestingly, irrigation with a solution containing 50% seawater improved biomass accumulation in both genera as compared with the absence of seawater (Ventura 2011a). These results led us to examine the effect of supplementation of high sulfate levels such as 10 mM, since optimization of sulfur nutrition may not only affect biomass but also the organic sulfur accumulation in plants. Since 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 Na₂SO₄ supplementation to the ½ MS (containing 0.87 mM sulfate) growth medium was carried out in the presence of either 30 or 180 mM NaCl, so that both levels include 50 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 and 200 mM NaCl solution supplemented with ½ Hoagland nutrient solution, containing 0 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 ½ MS localized in a growth-room or grown in perlite supplied with ½ Hoagland nutrient solution and localized in a controlled greenhouse (see the Methods section), did not affect the biomass accumulation response to high sulfate in *Salicornia* and *Sarcocornia*. 
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, http://www.jmp.com/). Different uppercase 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 fifteen different experiments that yielded similar results.

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), while 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*.

**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, http://www.jmp.com/). Different uppercase 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.
The generation of reactive oxygen species (ROS), such as superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$), 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 pre-mature senescence (Yarmolinsky et al., 2014; Brychkova et al., 2015), 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$_2$O$_2$ 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 Fig. 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 200ppm commercial N–P–K fertilizer [20–20–20 + microelements, Haifa Chemicals Ltd., Israel 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 glutathione-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 (STs) 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).

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 (O₂) content in Salicornia and Sarcocornia. The values are means ± SE (n = 4). B. Hydrogen peroxide (H₂O₂) 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, http://www.jmp.com/). Different uppercase 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.
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), while in *Sarcocornia*, sulfate supplementation had no significant effect (Fig. 5B).

Sulfide, the substrate for cysteine biosynthesis, is the product of sulfite reduction by sulfite reductase, and/or a product of sulfur containing metabolite degradation (Yarmolinsky et al., 2014;...
Brychkova et al., 2015). Sulfide levels were unaffected by salinity increase in both plants (Fig. 5C). A significant reduction in H₂S content was seen in *Salicornia*, but not in *Sarcocornia* plants supplemented with 10 mM sulfate at the low salinity (Fig. 5C).

**The effect on cysteine and glutathione**

Cysteine is the final product of the S assimilation pathway, and is the rate-limiting factor for glutathione and methionine biosynthesis. Glutathione 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 cysteine in *Salicornia* was unaffected by salinity and sulfate treatments, whereas in *Sarcocornia*, cysteine level decreased with salinity, being significantly lower than in *Salicornia*. Sulfate supplementation resulted in cysteine enhancement at the lowest salinity level tested (Fig. 6A). Sulfate supplementation increased total glutathione 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 inserts 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 significant 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 cysteine degradation. These possibilities were further examined to uncover the factor/s responsible for these differences.
Figure 6. The effect of sodium (50, 200 mM) and sulfate (0, 10 mM) concentrations on cysteine and glutathione content in the shoots of Salicornia and Sarcocornia. Sulfate is applied as sodium sulfate and the remainder sodium as sodium chloride. A. Cysteine levels in Salicornia and Sarcocornia. Error bars indicate SE (n = 4). The data are from 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 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, http://www.jmp.com/). Different uppercase 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.

Adenosine 5'-phosphosulfate reductase expression
Chloroplast-localized adenosine 5'-phosphosulfate (APS) reductase (APR) is known to be a key regulatory point in 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, 4).
A reduction in APR transcripts was evident in response to 10 mM sulfate treatment in both *Salicornia* and *Sarcocornia*, while APR transcript abundance was significantly higher in *Salicornia* grown in 200 mM NaCl without sulfate supplementation than in either halophyte exposed to other treatments (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.

**Sulfite reductase expression**

Chloroplast-localized sulfite reductase (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 (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.

**Sulfite oxidase 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 co-factor-containing peroxisomal sulfite oxidase (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 *Sarcocornia* (Fig. S4C). Importantly, salinity level had little effect on SO activity, whereas sulfate addition decreased SO activity in either plant (Fig. 8C). 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 catalyzes the biosynthesis of cysteine using O-acetylserine and sulfide generated by the sulfate reduction pathway and/or as a result of the degradation of thiol-containing metabolites (Álvarez et al., 2010; Álvarez et al., 2012).
The genes encoding OAS-TL A and 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 contrast, *Sarcocornia* showed a significant increase in the abundance of both *OAS-TL A* and *B* transcripts in response to sulfate supplementation. We observed an increase in both *OAS-TL A* and *B* transcript abundance in *Sarcocornia* at the higher salinity level, while in *Salicornia*, the increase was observed mainly in the abundance of *OAS-TL B* transcripts (Fig. S4D 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 cysteine 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; Carfagna et al., 2016), as the organic-S and biomass accumulation in *Salicornia* were indeed significantly increased at the highest level of supplemental sulfate (Figs.7B and Fig. 2). The results indicate also a higher capacity of cysteine 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-cysteine desulphhydrase (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-cysteine degradation activity was monitored by sulfide production using both in gel and kinetic assays (Fig. 9B and C respectively). Significantly, *Sarcocornia* exhibited a higher L-cysteine-degrading activity level (L-cysteine desulphhydrase) than did *Salicornia* and exhibited a significant increase when supplemented with high sulfate, whereas in *Salicornia* L-cysteine desulphhydrase activity was decreased with the low salinity and did not change much with high salinity when supplemented with enhanced sulfate (Fig. 9B and 9C). The results of both in-gel and kinetic assays that detected sulfide production, show that the L-cysteine-degrading activity is higher in *Sarcocornia* than in *Salicornia* and that it shows a more marked increase in response to sulfate supplementation in *Sarcocornia*.
Identification of the cysteine desulfhydrase activity source by trypsinization of the activity bands in *Salicornia* and *Sarcocornia*

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

Identification of the cysteine desulphhydrase 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, B and C, 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 B proteins can be explained by their 81 and 78% sequence identity, respectively (Fig. S6A and B).

Immunoprecipitation analysis of Salicornia and Sarcocornia protein extract was performed employing OAS-TL A and B antibodies (see method section). The L-cysteine 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-cysteine DES activity bands whereas the proteins that were incubated at the absence of the antibody in the pull-down assay exhibited desulphhydrase activity bands (Fig. 10D). Considering the relatively close identity of Salicornia OAS-TL A and B to the Arabidopsis proteins [Fig. S6A and B (GenBank/EMBL data libraries, accession numbers P47998 and P47999, respectively)], as well as the absence of L-cysteine...
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 cysteine desulphhydase activity as shown in Fig. 8B. The activity bands were then sliced and were fractionated by 12.5% SDS polyacrylamide gel electrophoresis (PAGE) together with Arabidopsis Wild-type (Col) crude extract and then 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 desulphhydase activity employing antibodies raised against Arabidopsis OAS-TL A and OAS-TL B, respectively. Plus sign indicates with, and minus sign is without antibody, red pointer indicates activity band.
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 source 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 ½ MS growth medium resulted in a significant increase in biomass accumulation in Salicornia, while in Sarcocornia 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 et al., 1983). While Salicornia europaea is found in areas exposed to frequent seawater tidal flooding, Sarcocornia 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 10-20% 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 the current study, both Salicornia and Sarcocornia exhibited similar sulfate accumulation ratios of ca. 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 Fig. 5A and Fig. 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, while 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 H2O2 in both Salicornia and Sarcocornia treated with the highest salinity (Fig. 3 and 4B). Interestingly, the enhanced sulfate
supplementation resulted in an increase in anthocyanins and H$_2$O$_2$ in *Sarcocornia* (Fig. 3 and 4B). The H$_2$O$_2$ enhancement is most likely the cause for anthocyanin production, serving as a ROS scavengers 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, while 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 *Prosopis 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 (Fig. S7). Since 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 B exhibit significant L-cysteine desulphhydrase activity in *Sarcocornia* especially in the presence of high sulfate

The synthesis of cysteine and its degradation should be well coordinated. L-cysteine desulphhydrase activity results in the release of sulfide, while OAS-TL consumes sulfide for cysteine biosynthesis. It has been claimed that the kinetic properties of the OAS-TLs A, B and C in *Arabidopsis* most likely do not allow a significant reverse reaction (Wirtz et al., 2004). Yet, free H$_2$S was shown to be partially released by OAS-TLs (Papenbrock et al., 2007), acting not only in L-cysteine 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 on chilling stress (Fu et al., 2013). These indicate the feasibility of certain conditions where L-Cysteine desulphhydrase activities of OAS-TL can be affected at least to a certain level.

Sulfide for cysteine biosynthesis can be derived from cysteine 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; Birke et al., 2015b). Yet, this
appears to be in doubt since 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-cysteine desulphhydrase 1 (DES1) that degrades cysteine 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 desulphhydrase 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-cysteine degradation by the Sarcocornia OAS-TL (Fig. 9A and B). Several lines of evidence support this notion. Firstly, 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\(_2\)S (Fig. 9B and C). Finally, the DES activity was shown to be attributable directly to OAS-TL A and B by peptide identification in the L-cysteine 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 B antibodies (Fig. 10). The complete pull down of the L-cysteine 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 co-migrated with the OAS-TLs still the vast majority of DES activity is of OAS-TL A and 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 adenosine 5-phosphosulfate (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* (Fig. 5A and Fig. 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 cysteine 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 cysteine and methionine) 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-cysteine.

**High sulfate accumulation and sulfite oxidase 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 DeKok, 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 cysteine and methionine, whereas a significant enhancement of sulfate was evident in the presence of active SO in wild-type plants (Brychkova et al., 2013; Brychkova et al., 2015). This notion is supported by the high SO activity rate in *Sarcocornia*, since 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 non-toxic 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; Brychkova et al., 2012).

**CONCLUSIONS**
By exploring the sulfate reductive pathway that generates L-cysteine and the L-cysteine desulphhydrase (DES) activities to identify factors affecting organic-S and sulfate levels, we identified a major role for OAS-TLs, enzymes known to catalyze L-cysteine as the final step of the sulfate reductive pathway. We showed that OAS-TL A and B exhibit significant L-cysteine 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 cysteine degrading activity of OAS-TL A and B, likely followed by SO oxidation of sulfite originated from protein bound sulfur amino acid degradation. The low cysteine 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-cysteine DES activities in *Salicornia* is suggestive for its higher net cysteine 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 which 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 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 Sde Boqer Campus, Ben-Gurion University of the Negev, under the following conditions: 14-h light/10-h dark, 25°C, 75-85% relative humidity and under light intensity of 150 µmol m⁻² sec⁻¹.

Seeds were germinated and grown in standard 90 mm Petri dishes on ½ MS medium supplemented 1% plant agar and 1% sucrose for 10 days. The seedlings were transferred to large Petri dishes of 155x30 mm diameter and height respectively, supplied with ½ MS medium ([Murashige and Skoog, 1962] containing 0.87 mM sulfate level) supplemented with either 50 or 200 mM NaCl without the addition of Na₂SO₄. The NaCl concentration was 30 or 180 mM NaCl when 10 mM Na₂SO₄ was supplemented. All treatments were performed in three replicates.
The weight of shoot biomass accumulation was determined 14 days after the treatment onset for *Salicornia europaea* (RN) and 21 days for *Sarcocornia fruticosa* (VM). Results were expressed as average plant growth rate in mg day$^{-1}$ plant$^{-1}$. Samples were immediately frozen in liquid nitrogen and stored at -80ºC for further use.

To estimate biomass accumulation in the preliminary experiments, *Salicornia* and *Sarcocornia* seeds were germinated in pots (0.4 liter) filled with the highly air permeable and high water capacity perlite [up to 2 mm particles size (Agrekal Habonim Industries Ltd, Moshav Habonim, Israel)]. After germination, seedlings were thinned out to similar numbers of 40 seedlings per pot in 4 replicas and subjected to 100 and 200 mM NaCl in half strength Hoagland’s nutrient solution (Hoagland and Arnon, 1950) supplemented with 0 or 10 mM sulfate. Plants were grown in controlled greenhouses in Sede Boqer campus, Ben Gurion University of the Negev, Israel, under ca 13.5-h sunlight (May, 2012), day temperature 25 to 31°C, night temperature ca 20°C, relative humidity was ca 60% and light intensity ranged between 400 to 600 µmol m$^{-2}$sec$^{-1}$. The biomass accumulation data from this experiment is presented in supplemental Fig. S3.

For RNA sequencing, *Salicornia europaea* (RN) seeds were germinated in plastic trays on Metromix-360 garden soil in a greenhouse at KAUST (King Abdullah University of Science and Technology, Biological and Environmental Science and Engineering Division (BESE), Thuwal, 23955-6900, Saudi Arabia) and the seedlings were maintained under natural day light conditions until sampled.

**RNA sequencing and transcriptome de novo assembly**

Four - month old *Salicornia 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 *Salicornia europaea* RNA using Illumina TruSeq RNA Library Prep Kit v2 to prepare RNAsq libraries using manufacturers low sample protocol and libraries were sequenced on Illumina HiSeq2000 platform (Illumina, San Diego, CA, USA). A total of ~550 million paired-end reads of insert size 100 bp were produced. Low quality reads and adapter sequences were filtered using Trimmomatic (Bolger et al., 2014), leaving ~500 million high quality reads for downstream analyses. Transcriptome de novo assembly was performed using Trinity (Grabherr et al., 2011) and annotated following the Trinotate pipeline (Haas et al., 2013). A total of 56107 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.
Total RNA extraction, complementary DNA synthesis and the quantitative analysis of transcripts of Salicornia and Sarcocornia shoots grown in ½ MS medium was performed as previously described (Brychkova et al., 2007) employing the primers shown in 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, while 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 Fig. S8). ACT (88% identity to Beta vulgaris subsp. vulgaris actin-related protein 7-like, Accession XR_789363.1| PREDICTED) and TFIID (85% identity to Beta 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$_2$SO$_4$ 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, OAS-TL in Salicornia and Sarcocornia shoots grown in ½ 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 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 Desulfhydrase activity

Protein extraction of Salicornia and Sarcocornia shoots grown in ½ MS medium and desalting for DES was performed as described before for STs (Brychkova et al., 2013). DES activity was detected based on direct detection of H$_2$S formation in the presence of L-cysteine as described previously (Riemenschneider et al., 2005). The modified assay solution contained 0.1 M Tris-HCl, pH 9.0, 2.5 mM dithiothreitol, 0.8 mM L-cysteine and 10 μg of desalted protein in a total volume of 0.2 mL. After incubation at 37 °C for 30 min, H$_2$S was detected according to Bloem et al. (2004) with 30 mM
FeCl₃ 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 (http://www.biotek.com/spectrophotometer.htm). Na₂S*9H₂O was used as a standard.

Measurement of sulfur-containing metabolites.

To determine total sulfur content, ∼10 mg dried and powdered Salicornia and Sarcocornia shoots grown in ½ MS medium were placed in tin containers in an elemental analyzer (EA1108 CHNS/O; Fisons Instruments, Milan, Italy) and the amounts of total S in each sample were quantified according to a standard calibration curve prepared with reduced glutathione.

Sulfate and sulfite from Salicornia and Sarcocornia shoots grown in ½ MS medium were determined as previously described (Brychkova et al., 2012). Briefly, for sulfate determination, the leaf samples were extracted in 24 mM formaldehyde in 2 mM Na₂CO₃/0.75 mM NaHCO₃ solution (1:100, w/v) and determined employing Ion-Chromatograph DIONEX LC20 with ED 50 Electrochemical Detector using IonPak® AS9-SC Analytical (4 mm × 250 mm). Sulfite was determined by employing chicken SO using NADH-peroxidase dependent determination of H₂O₂ generated as a by product 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 Na₂HPO₄, and 1 mM salicylic acid, pH 5.0 in 1:40 (w:v) ratio and detected by employing the microsensor ISO-H2S-100 for H₂S according to Yarmolinsky et al. (2014). Cysteine and total glutathione 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 ½ MS medium were ground using a pestle and mortar in extraction buffer (ratio 1:4) containing 0.25 M sucrose, 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 were 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 H₂S (Manchenko, 2002). Lead acetate was employed to detect the generated H₂S, producing dark brown lead sulfide bands. The reaction solution contained 0.15 M Tris–HCl, pH 8.5, 1 mM Dithiothreitol (DTT), 50 mM
β-mercaptoethanol, 20 mM L-cysteine, 0.1 mM pyridoxal 5-phosphate, 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-cysteine desulphydrase 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) 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 2 peptides. High confidence peptides passed the 1% FDR threshold. (*FDR = false discovery rate, is the estimated fraction of false positives in a list of peptides). Semi quantitation 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 Proteome Discoverer™ Software ver. 1.4.1.14 (Thermo Fisher Scientific Inc. https://www.thermofisher.com/order/catalog/product/IQLAAEGABSFAKJMAUH) 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-cysteine desulphydrase.**

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 polyacrylamide gel electrophoresis (PAGE) as previously described (Brychkova et al., 2013) and blotted onto polyvinylidene difluoride membranes (PVDF, Immobilon 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). Phosphate-buffered saline diluted (1:5000) secondary antibodies (anti-rabbit IgG; Sigma; http://www.sigmaaldrich.com) was followed. Protein bands were visualized in ChemiDoc™ Touch Imaging system (Bio-Rad, www.bio-rad.com/) after staining with an ECL detection system, using the SuperSignal West Pico Chemiluminescent Substrate (Pierce, www.piercenet.com/) according to the manufacturer’s instructions. Band intensities were quantified by using ImageJ software (http://rsbweb.nih.gov/ij/).

For the immunoprecipitation assay, fifty µg of protein from Salicornia and Sarcocornia were incubated with 30 µl of the OAS-TL A or OAS-TL B antibodiesin Tris-buffer saline (TBS) 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 2h with continuous shaking and then centrifuged at 10,000g 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 ½ MS medium was crushed in 600 µL methanol acidified with 1% HCl. The extract was centrifuged for 10 sec at 4000g. 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 sec followed by centrifugation at 4000 g for 2 min. The total anthocyanin in the aqueous phase was determined by detecting the optical density (OD) at A530 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 ½ 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, Norwood, MA).

**ROS Determination**

For detecting O$_2^-$ and H$_2$O$_2$, frozen shoots of *Salicornia* and *Sarcocornia* were extracted in 50 mM phosphate buffer (pH 7.5) at a ratio of 1:8 (w/v) and centrifuged (Eppendorf 5417R http://www.eppendorfna.com) 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 previously described (Yesbergenova et al., 2005). Absorbance was measured at 480 nm employing an Epoch Microplate Spectrophotometer supported by Gen5 1.10 software (http://www.biotek.com/ spectrophotometer.htm).

The reaction mixture for detecting H$_2$O$_2$ consisted of 0.85 mm 4-aminooantipyrine, 3.4 mm 3,5-dichloro-2-hydroxobenzene sulfonate, 4.5 U ml$^{-1}$ HRP in 2 ml of 50 mM phosphate buffer (pH 7.5) in the presence or absence of 2 mM tungstic acid and 100 µM DPI as previously described (Yesbergenova et al., 2005). Absorbance was measured after 5 min at 515 nm as described above.

**Accession Numbers**

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

Primers were designed based on similarity analysis to Beta vulgaris XR_789363.1 (ACT), XM_010690649 (TFIID), XM_010688518.1 (APR), XP_010673651.1 (SIR), XP_010674891.1 (OAS-TL A), XM_010674145.2 (OAS-TL B) and to Populus trichocarpa XP_002300104.1 (SO).

Supplemental Figures

Supplemental Figure S1. Effect of NaCl (100 and 200 mM) and Na$_2$SO$_4$ (100mM) 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, 200mM) and sulfate (0 and 10mM) levels on biomass accumulation of Salicornia and Sarcocornia.

Supplemental Figure S4. Effect of sodium (50, 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 Spinach 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 thaliana’s OAS-TL A and OAS-TL B protein sequence.

Supplemental Figure S7. Effect of sodium (50, 200 mM) and sulfate (0, 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 Tables

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.
ACKNOWLEDGMENT:

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 B antibodies.

FIGURE LEGENDS

Figure 1. Schematic representation of the Sulfate reduction and Cys degradation pathways in Arabidopsis plants. ATP sulfurylase (ATPS) catalyzes the adenylation of sulfate to adenosine 5′-phosphosulfate (APS) using ATP as an electron donor. Then, APS is reduced by the plastidic enzyme APS reductase (APR) to sulfite in the presence of two molecules of reduced glutathione, which acts as an electron donor. The generated sulfite can be oxidized to sulfate by sulfite oxidase (SO) with the formation of $\text{H}_2\text{O}_2$ as a byproduct or further be reduced to sulfide by the Sulfite Reductase (SiR) employing 3 molecules of reduced ferredoxin. The sulfide together with O-acetyl-L-Serine are the substrates for Cys biosynthesis catalyzed by O-acetylsersine-(thiol) lyase (OAS-TL). Cysteine homeostasis is controlled by L-cysteine desulphhydrase (DES1, EC 4.4.1.1), which catalyzes the breakdown of cysteine 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.

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, http://www.jmp.com/). Different uppercase 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 fifteen different experiments that yielded similar results.

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, http://www.jmp.com/). Different uppercase 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.

**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$_2$O$_2$) 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, http://www.jmp.com/). Different uppercase 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 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, http://www.jmp.com/). Different uppercase 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 6.** The effect of sodium (50, 200 mM) and sulfate (0, 10 mM) concentrations on cysteine and glutathione content in the shoots of *Salicornia* and *Sarcocornia*. Sulfate is applied as sodium sulfate and the remainder sodium as sodium chloride. A. Cysteine 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, http://www.jmp.com/). Different uppercase 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.
letters indicate significant difference between *Salicornia* and *Sarcocornia* subjected to the same
treatment.

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](http://www.jmp.com/)). Different uppercase 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 Adenosine 5'-
phosphosulfate reductase (APR), Sulfite reductase (SiR) and Sulfite oxidase (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 *Sarcocornia*. The
values are means ± SE (n = 4). B. SiR activity was detected by cysteine appearance in *Salicornia* and
*Sarcocornia*. The values are means ± SE (n = 3). C. SO activity was detected as sulfite disappearance
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](http://www.jmp.com/)). Different uppercase 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 9. Effect of sodium (50, 200 mM) and sulfate (0, 10 mM) concentrations on O-acetylserine -
(thiol) lyase (OAS-TL) and cysteine 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 cysteine appearance in *Salicornia* and *Sarcocornia*. The values are means
± SE (n = 3). B. L-Cysteine desulhydrase (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](http://www.jmp.com/)). Different uppercase 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 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 cysteine desulphhydrase activity as shown in Fig. 9B. The activity bands were then sliced and were fractionated by 12.5% SDS polyacrylamide gel electrophoresis (PAGE) together with *Arabidopsis* Wild-type (Col) crude extract and then 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* desulphhydrase activity employing antibodies raised against Arabidopsis OAS-TL A and OAS-TL B, respectively. Plus sign indicates with, and minus sign is without antibody, red pointer indicates activity band.

**Supplemental Figures**

**Supplemental Figure S1.** Effect of NaCl (100 and 200 mM) and Na$_2$SO$_4$ (100mM) on biomass accumulation of *Salicornia* (RN) and *Sarcocornia* (VM). The experiment was carried out under controlled conditions in the greenhouse at the Ben-Gurion University in Beer Sheva. The temperature of the greenhouse was regulated by a desert cooling system which kept the maximum summer temperatures below 30°C, minimum temperatures approached ambient temperatures. A fixed light source (three 100-W incandescent bulbs in a row 1.5 m above the plants), perpendicular to the plants was used to generate artificial light to prevent flowering. Natural daylight was extended by turning on the lights earlier in the morning and by turning them off later in the evening to create a total day length of 18 h. Seeds were germinated in pots with a dimension of 6 cm x 6 cm x 8 cm filled with perlite (Agrekal Habonim Industries Ltd., Moshav Habonim, Israel; www.agrekal.co.il). Perlite was treated with 1 g of Rhizolex dissolved in 2 liters (L) of water. Rhizolex solution was applied before sowing and 3 months after seeds emergence to prevent fungi development. Pots were placed in containers (dimensions 37 cm x 16 cm x 6 cm) filled with 2 L of water to allow adequate germination. A week after the seeds emergence, seedlings were subjected to half strength Hoagland’s nutrient solution (Hoagland and Arnon, 1950). For the experiment approximately 20 seedlings per pot (0.5 Liter) filled with perlite were treated with 100 mM Na$_2$SO$_4$ as compared to 100 or 200 mM NaCl. All the saline solutions were supplied with half strength Hoagland nutrient solution in 5 replicas. Harvesting was initiated four months after sowing and subsequently carried out at least every one month. Harvesting was done four times in the NaCl salinity treatments and only three times in the Na$_2$SO$_4$ treatment within the same frame of time. The accumulated fresh shoot biomass was evaluated based on the fresh weight per unit area in kg per m$^{-2}$. Values denoted with different letters are
significantly different according to the Tukey-Kramer HSD test, \( P < 0.05 \) (JMP 8.0 software, http://www.jmp.com/). Upper case letters show significant difference between salinities within each ecotype, whiles the lower case letters show the significant difference between *Salicornia* and *Sarcocornia*. Bars represent the standard error (±SE) of the mean for five replicates (\( n = 5 \)).

**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). Sulfate is applied as sodium sulfate and the remainder sodium as sodium chloride. Photos of the treatments just before harvesting are presented in the left panels while the biomass accumulation is presented in the right panels. *Salicornia* and *Sarcocornia* plants were treated for 14 days and then were harvested and the biomass was detected. Bars represent standard error (±SE) of the mean for 30 plants (\( n = 30 \)). Biomass was calculated as plant growth per day. Values denoted with different letters are significantly different according to the Tukey-Kramer HSD test \( P < 0.05 \) (JMP 8.0 software http://www.jmp.com/). Different uppercase letters indicate significant differences between sulfate treatments in *Salicornia* or *Sarcocornia*. Different Greek letters indicate significant differences between *Salicornia* and *Sarcocornia* subjected to the same treatment.

**Supplemental Figure S3.** Effect of sodium (100, 200 mM) and sulfate (0 and 10mM) levels on biomass accumulation of *Salicornia* and *Sarcocornia*. Sulfate is applied as sodium sulfate and the remainder sodium as sodium chloride. Photos of the treatments just before harvesting of *Salicornia* (left panels) and *Sarcocornia* (right panels) are presented in the upper panels while the biomass accumulation is presented in the lower panels. *Salicornia* and *Sarcocornia* plants were grown in perlite and supplied with Hoagland solution under greenhouse condition. Bars represent standard error (±SE) of the mean for 40 plants (\( n = 5 \)). Biomass was calculated as plant growth per plant. Values denoted with different letters are significantly different according to the Tukey-Kramer HSD test \( P < 0.05 \) (JMP 8.0 software http://www.jmp.com/). Different uppercase letters indicate significant differences between sulfate treatments in *Salicornia* or *Sarcocornia*. Different Greek letters indicate significant difference between *Salicornia* and *Sarcocornia* subjected to the same treatment.

**Supplemental Figure S4.** Effect of sodium (50, 200 mM) and sulfate (0, 10 mM) application on selected enzymes’ expression in *Salicornia* and *Sarcocornia*. Sulfate is applied as sodium sulfate and the remainder sodium as sodium chloride. Relative expression analysis of *APR* (A), *SiR* (B), *SO* (C), *OAS-TL A* (D), *OAS-TL B* (E) in *Salicornia* and *Sarcocornia*. All the relative expression levels are given after normalization to *Salicornia TFIID* [Transcription initiation factor TFIID (85% identity to the *Beta vulgaris* TFIID subunit 6 Accession XM_010690649)]. Error bars indicate se (\( n = 4 \)). Values denoted with different upper and lower case letters are significantly different according to the Tukey-Kramer HSD test \( P < 0.05 \) (JMP 8.0 software http://www.jmp.com/). Different uppercase 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*.

**Supplemental Figure S5.** CLUSTAL O (1.2.1) multiple sequence alignment of *Salicornia*’s OAS-TL A and OAS-TL B protein sequence against *Spinach oleracea*’s OAS-TL A and OAS-TL B protein sequence. A. Alignment of *Salicornia*’s OAS-TL A protein sequence against *Spinach oleracea* OAS-TL A protein sequence. B. Alignment of *Salicornia*’s OAS-TL B protein sequence *Spinach oleracea* OAS-TL B protein sequence.

**Supplemental Figure S6.** CLUSTAL O (1.2.1) multiple sequence alignment of *Salicornia*’s OAS-TL A and OAS-TL B protein sequence against *Arabidopsis thaliana*’s OAS-TL A and OAS-TL B protein sequence. A. Alignment of *Salicornia*’s OAS-TL A protein sequence against *Arabidopsis thaliana* OAS-TL A protein sequence. B. Alignment of *Salicornia*’s OAS-TL B protein sequence *Arabidopsis thaliana* OAS-TL B protein sequence.

**Supplemental Figure S7.** Effect of sodium (50, 200 mM) and sulfate (0, 10 mM) application on osmolality level in *Salicornia* and *Sarcocornia*. Sulfate is applied as sodium sulfate and the remainder sodium as sodium chloride. Error bars indicate se (n = 4). Values denoted with different upper and lower case letters are significantly different according to the Tukey-Kramer HSD test P < 0.05 (JMP 8.0 software [http://www.jmp.com/]). Different uppercase 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. mOsm – milliosmoles.

**Supplemental Figure S8.** *Salicornia* selected gene sequences and quantitative real-time PCR product verification results in multiple sequence alignment. A. *APR1*, B. *SiR*, C. *SO*, D. *OAS-TL A*, E. *OAS-TL B*, F. *Act*, G. *TFIID*. First line - *Salicornia* target gene sequence. Second line – *Salicornia* RT-PCR product sequence and identity to the target gene in %, third line – *Sarcocornia* RT-PCR product sequence and identity to the target gene in %. The identity was checked by nucleotide blast in NCBI and multiple sequence alignment by CLUSTAL O (1.2.1). Blue – forward primer, red- reverse primer.

**Supplemental Tables**

**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*. The DES activity bands of *Salicornia* and *Sarcocornia* (as shown in Fig. 9B) were sliced and fractionated in 12.5 % SDS-PAGE. Further the bands were digested by trypsin
and analyzed by LC-MS/MS as described in Materials and Methods. Yellow color – overlapped areas.

Only more than 50% overlapped unique peptides were taken

**Supplemental Table S3.** List of identified and overlapped unique peptides of OAS-TL B in *Salicornia* and *Sarcocornia*. The DES activity bands of *Salicornia* and *Sarcocornia* (as shown in Fig. 9B) were sliced and fractionated in 12.5% SDS-PAGE. Further the bands were digested by trypsin and analyzed by LC-MS/MS as described in Materials and Methods. Yellow color – overlapped areas.

Only more than 50% overlapped unique peptides were taken


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