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Seasonal physiological response of *Salicornia europaea* L.

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Research Article

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ABSTRACT

Terminal shoots of a succulent true halophyte *Salicornia europaea* was collected from two sites along the Damietta- Port Said coastal road, Egypt during winter and summer, 2019 to determine osmotic potential (op), water content, ash content, Na, K, Ca, Mg, Cl, proline and soluble sugars. Also, associated soil was taken from rhizosphere area to investigate soil physical and chemical characters. Water content, op, Ca, Mg and soluble sugars were reduced in summer, while Na, K, Cl and proline were increased in summer season. All determined parameters were higher in *S. europaea* growing at site 2 than those growing at site 1 except in water content did not change significantly. A strong negative correlation was detected between EC of soil and both water content and Mg content in shoot of *S. europaea*. In contrast, strong positive correlations were detected between Na and EC, soil moisture, Cl and ash content. Moreover, strong positive correlations were detected among Ca & op, Mg & water content, proline & K, soluble sugars with soil moisture and Ca. Due to high accumulation of ash content, *S. europaea* can be invested in saline soil reclamation.

INTRODUCTION

Salicornia is an important genus including in Amaranthaceae family. All plant species in this genus are succulent annuals joins from 25 to 30 species present in Eurasia, North America and Africa [1]. *Salicornia* and *Sarcocornia* species are excellent investment for saline agriculture due to their high tolerance to salinity [2]. The Salicornioideae are suitable for cultivation as vegetables in highly saline habitats (Ventura et al. 2011) and as a rich source of some secondary and antioxidant compounds [3]. S. *europaea* is the only species in this genus present in Egypt which distributes in salt marshes, coastal mud flats and estuaries where the meeting of fresh water and salty sea creates unique and dynamic habitats [4]. Therefore, it is commonly distributing at Elmanzala lake.

Salicornia europaea appeared non-significant change in shoot fresh and dry weights and root dry weight up to 400 mM NaCl [5]. *Salicornia europaea* have the ability to keep on a higher turgor and relative water content at low leaf water potentials that accompanied with a greater capacity for osmotic adjustment [6]. Drought and salinity are among the most common abiotic stresses experienced by plants [7]. Mostly, osmotic potentials decrease in plant tissues affected by drought or salinity, largely as a result of compatible solutes accumulation in the cytoplasm or inorganic ions accumulation in vacuoles [8,9]. The relative contribution of organic and inorganic solutes to osmotic adjustment varies from plant species to other or even within different tissues of the same plant [10]. Regarding halophytes, they based mainly on inorganic ions sequestration within the vacuoles to avoid their toxicity in the cytosol and to assert their osmolarity with low energy cost [11]. In addition to these organic solutes are concentrated by halophytes in the cytoplasm to alleviate adverse effects on metabolism [12]. Also, leaf Ca^{++}, Mg^{++}, and K^{+} contents decreased as salinity increased in *Suaeda fruticosa*, and *Atriplex prostrata* [13,14]. On the other hand, the succulence, Na/K ratio, and the content of proline, sodium, pigment, carbohydrates, and protein in *S. europaea* increased as salinity increased [15]. Proline is correlated with osmotic adjustment to alleviate salinity stress. Moreover, high concentrations of proline have been observed in halophytic plants growing at higher salinity [16,9].

In the current paper, physiological behavior of S. *europaea* was investigated by determining water content, op, ash content, Na^{+}, K^{+}, Ca^{++}, Mg^{++}, Cl, proline and total soluble sugars parameters of plants shoot's growing naturally at Damietta- Port Said coastal road during winter and summer seasons.

MATERIALS AND METHODS

Fresh aerial parts of *Salicornia europaea* from those growing naturally at Damietta- Port Said coastal road and their accompanied soil were collected from two sites in late February (winter season) and late June (summer season), 2019. The GPS reading for site 1 is N: 31° 12.222, E: 32° 17.117 and the GPS reading for site 2 is N: 31 17.618, E: 32 09.680. Meteorological data for the studied area were collected from the nearest station (from January to June 2019) and represented in Table 1 [17]. The soil samples were collected at 0-20 cm depth, dried and crushed gently with wooden wallet and passed through 2 mm sieve. Some of the fresh plant samples were used in osmotic potential and proline estimation and the rest were dried at 60 ºC till constant weight and ground to fine powdered to estimate total ash, Na^+, K^+, Ca^{++}, Mg^{++}, Cl^- and soluble sugars.

Soil mechanical analysis was carried out by hydrometer method while soil chemical analysis was estimated in soil water extract (1:5) where pH, soil moisture content, EC, Na^+, K^+, Ca^{++} and Mg^{++} were determined according to [18,19]. Chlorides were assayed by titration with 0.01N $AgNO_3$ in presence of potassium chromate 5% an indicator as described by [20]. Sulphates were assayed by the turbidity method [21]. Bicarbonate was estimated by titration with sulphuric acid 0.01N [22].

PLANT ANALYSES

Water content percentage of plant leaves was determined as the difference between Fw and Dw divided on Fw and the result multiplied by 100. Osmotic potential was measured in press sap of shoot system using a cryo osmometer (Osmomat 030, Genotec GMBH, Berlin). A standard solution of 300 mosmol NaCl used for the calibration of the device. Readings obtained were then converted into MPa unit [23]. For total ash content, plant dry material was ignited at 500 ˚C in a muffle furnace for 6 hours, and calculated as in A.O.A.C [24]. To determine chloride contents, plant ashes was dissolved in nitric acid and titrated with $AgNO_3$ according to [25] . Sodium and potassium were estimated in acid digested samples using flame photometer as described by [26] Calcium and magnesium were measured in acid digested samples by inductively coupled plasma optical emission mass spectrometer (ICP) (POEMSIII, thermo Jarrell elemental company USA), using 1000 mg/l (Merck) as stock solution for standard preparation. Free proline was estimated as described [27]. A known weight of fresh shoots was grinded in 3% sulfosalicylic acid, then centrifuged at 18.000g for 5 min. Equivalent volumes from the extract, acid- ninhydrin reagent and glacial acetic acid (1:1:1) were added in a test tube, then incubated in water bath at 96 ˚C for 1 h. After cooling in ice bath 4 ml of toluene were added and vortexed. The upper phase was measured at 520 nm on spectrophotometer. Soluble sugars were estimated in boiled 80% ethanolic extract for 10 min at 90 ˚C according to Land häusser et al. and quantified with acid phenol-sulfuric acid method as described [28,29].

STATISTICAL ANALYSIS

According to Casella, the given data for each season were subjected to analysis of variance (ANOVA) using SAS, (2003) [30]. The Duncan's multiple range at $P \leq 0.05$ was used for separating the treatment means. Additionally, all possible coefficients of simple correlation (r) were calculated according to Snedecor & Cochran between the tested traits [31].

RESULTS

Firstly, soil chemical analyses were changed significantly by seasonal changes while physical properties did not change (Table 2). Soil supporting *Salicornia* was sandy at site 1 and sandy loam at site 2. pH value ranged from slightly acidic in summer season to slightly alkaline in winter season. Electrical conductivity was greatly increased in summer season recording the highest value (23.5 ds m^{-1}) at site 1. Like EC, soluble cations and anions were increased in summer.

Osmotic potential was decreased negatively in summer season recording the lowest value in those growing at site 1 as shown in Table 3. Generally, the water content in *S. europaea* was higher in winter than in summer. Regarding ash content, plants at site 2 was higher than those at site 1 recording the highest value in plants growing at site 2 in summer (38.80 %). Seasonal effect had no significant change in terms of ash content. Both Na and K were affected significantly by seasons, sites and their interaction recording the highest value in those growing at site 2 during summer season. On the other hand, Ca content was higher in winter than in summer and higher in site 2 than in site 1. Like Ca, Mg content tended to increase in winter recorded the highest content in *Salicornia* growing at site 1 during winter. The maximum Cl content was observed in those growing at site 2 during summer (18.71% dry wt.). For proline content, *Salicornia* was more than two folds in summer as compared to winter. Nevertheless, proline does not seem to be the main compatible solute in this study. The highest proline content was detected in *Salicornia* at site 2 in summer (11.36 µmole proline/g f. wt.), followed by those at site 1 in winter (4.39 µmole proline/g f. wt.) and the lowest value (2.89 µmole proline/g f. wt.) in those at site 2 in winter. A different pattern was observed in soluble sugars between two studied sites during winter and summer in *S. europeae*. The maximum value of total soluble sugars was detected in *Salicornia* growing at site 2 during winter (2.51% dry wt.).

A strong negative effect of the concentration in the shoots of *S. europaea* was detected for EC and both water content and Mg (r = -0.89 and r = -0.865), respectively. While a strong positive correlation was observed between EC and Na content as illustrated in Table 4. Additionally, positive correlation was recorded between EC and both Cl and soil moisture content. On the other hand, the Pearson coefficients confirmed that there were weak correlations between EC and op, ash, Ca and soluble sugars in the studied species. Indeed, soil moisture content correlated positively with Na (r = 0.73), Cl (r = 0.71) and soluble sugars (r = 0.78). The water content in *S. europaea* correlated significantly with op (r = 0.67), Na (r = -0.68) and soluble sugars (r = 0.94). Regarding op, strong correlations were detected for Ca (r = 0.95), Mg (r = 0.74) and soluble sugars (r = 0.78). Concerning ash, positive correlations were observed for Na (r = 0.74), Cl (r = 0.86) and proline (r = 0.62). Moreover, strong positive correlations were detected between Na & Cl (r = 0.97), K & proline (r = 0.92), Ca & sugars (r = 0.89), while mediate negative correlations were observed between Na & Mg (r = -0.64) and K & sugars (r = -0.62) and mediate positive correlation was observed between Ca & Mg (r = 0.63) as shown in Table 4.

Table 1: Meteorological data covering studied area, 2019.

Items	January	February	March	April	May	June
Precipitation (mm day^{-1})	0.38	0.78	0.72	0.15	0.00	0.00
Maximum Temperature °C	17.54	18.25	19.55	22.04	27.73	30.49
Minimum Temperature °C	10.93	11.71	13.04	15.12	19.24	23.50
Relative humidity %	61.39	66.53	68.41	64.62	59.85	63.31

Table 2. Meteorological data covering studied area, 2019.

a. Soil particle distribution

Seasons	Sites	Sand %	Silt %	Clay %	Texture
Winter	Site 1	88	4	8	Sandy
	Site 2	63	17	20	Sandy loam
Summer	Site 1	90	2	8	Sandy
	Site 2	56	28	16	Sandy loam

b. Soil chemical properties

Seasons	Sites	HCO_3-Meq/l	SO_4--Meq/l	Cl^-Meq/l	Mg^{++}Meq/l	Ca^{++}Meq/l	K^+Meq/l	Na^+Meq/l	ECds m^{-1}	pH	Moisture%
Winter	Site 1	1.0	23.98	43.5	5.3	3.0	1.54	58.7	6.96	7.01	23.1
	Site 2	1.7	56.81	105.0	15.5	9.0	4.28	134.8	16.34	7.10	62.9
Summer	Site 1	1.7	50.41	192.5	16.5	7.0	3.97	216.2	23.5	6.48	50.9
	Site 2	1.5	52.25	137.0	15.5	8.5	3.71	163.0	19.2	6.79	46.2

Table 3. Seasonal changes on water content, op, ash, Na, K, Ca, Mg, Cl, proline and soluble sugars in *S. europae.*

characters	Op Mpa	Water Content %	Ash %	Na %	K %	Ca %	Mg%	Cl%	Proline µmole proline/gf. wt.	Soluble Sugars %
Seasons effect										
Winter	-0.766a	80.45a	35.38a	3.33b	0.152b	0.881a	1.380a	15.428b	3.640b	1.981a
Summer	-0.841b	77.74b	35.99a	4.04a	0.180a	0.724b	1.076b	17.402a	7.376a	1.599b
Sites effect										
Site 1	-0.840b	79.17a	33.23b	3.30b	0.155b	0.732b	1.217	14.82b	3.891b	1.545b
Site 2	-0.769a	79.02a	38.13a	4.09a	0.177a	0.873a	1.239	18.02a	7.124a	2.035a
Seasons × Sites										
W × Site 1	-0.815b	80.97a	33.29c	2.82c	0.180b	0.769b	1.403a	13.537d	4.394b	1.452c
W × Site 2	-0.716a	79.93a	37.46b	3.84b	0.123c	0.993a	1.356b	17.320b	2.886d	2.510a
S × Site 1	-0.859b	77.36b	33.17c	3.79b	0.130c	0.695c	1.029d	16.090c	3.388c	1.639b
S × Site 2	-0.823b	78.11b	38.80a	4.31a	0.230a	0.753b	1.222c	18.710a	11.363a	1.560b

W = winter S = summer

Table 4. Correlation coefficient among studied parameters.

Characters	EC	Soil moisture	W. C	op	ash	Na	K	Ca	Mg	Cl	proline	Soluble sugars
Ec	1.00											
Soil Moisture	0.69*	1.00										
W. C	-0.89**	-0.44	1.00									
Op	-0.37	0.42	0.67*	1.00								
Ash	0.22	0.49	-0.10	0.42	1.00							
Na	0.78**	0.73**	-0.68*	-0.03	0.74**	1.00						
K	-0.20	-0.48	-0.03	-0.31	0.41	0.19	1.00					
Ca	-0.22	0.52	0.48	0.95**	0.46	0.07	-0.39	1.00				
Mg	-0.86**	-0.32	0.94**	0.74**	-0.02	-0.64*	-0.10	0.63*	1.00			
Cl	0.66*	0.71**	-0.53	0.12	0.86**	0.97**	0.23	0.21	-0.47	1.00		
Proline	0.14	-0.14	-0.30	-0.29	0.62*	0.54	0.92**	-0.34	-0.34	0.56	1.00	
Soluble sugars	0.14	0.78**	0.16	0.78**	0.41	0.26	-0.62*	0.89**	0.32	0.36	-0.43	1.00

DISCUSSION

Salicornia europeae is a true halophyte lives in saline habitats those either sandy or sandy loam. Salinity degree variation relied on seasonal changes, showing an increase in EC accompanied by an increase in soluble cations and anions in summer season result in high temperature, high evaporation rate and low precipitation. The significant seasonal changes in pH and EC are in line with those obtained by Maaloul et al. who observed the soil pH decreased from 8 in wet season to 7 as the lowest pH in late August (dry season) while the reverse was obtained in EC which was four folds in dry season [32] . In general, soil supporting *S. europeae* had high moisture content indicate the plant need high water requirement as crop production. It well known that true halophytes must overcome on water stress too as soil high salinity causes physiological drought. The water content in *Salicornia* kept stability in the two studied sites ~ 79%, indicating that high salinity degrees did not severely impact *Salicornia* water status. *Salicornia europaea* is able to stay on high water content under high saline condition, proofing that the plants exhibit efficient mechanisms to absorb adequate water from the moist soil. In contrast to salinity, soil moisture stress caused significant reduction in water content accentuating that soil moisture content is more required than water quality.

Both water content and OP tended to decrease in response to summer season. Similar results were obtained by Maaloul et al. on *Limonium pruinosum* and *Limonium tunetanum* [32]. The significant reduce in op reveals that *S. europeae* adjusted its op to more negative values when soil salinity increased during the dry season. Halophytes have been mentioned to accumulate inorganic ions or osmolytes in saline conditions to decrease their cellular water potential [33]. Sodium and chloride are considered as energetically efficient osmolytes for osmotic regulation and are compartmentalized into the vacuole to alleviate cytotoxicity [34,12]. A significant increase in Na and Cl in plants at site 2 which was more saline is in agreement with those obtained by Zouhaier et al, on *Limoniastrum guyonianum* and Khan et al. on *Arthrocnemum macrostachyum* [35, 36]. This could explain the ability of *Salicornia* to accumulate higher amount of ions in their vacuoles under higher saline conditions. Ionic stress is one among major components of salinity and is achieved by excess Na^+ sequestration, especially in the shoots of plants. Since Na^+ interferes with K^+ internal balance, and especially given its entanglement in numerous metabolic processes, preserving a balanced cytosolic Na^+/K^+ ratio has become a key salinity tolerance mechanism [37].

In plant cells, K^+ is found in either the cytosol or the vacuole. K not only activates numerous enzymes but regulates the cation-anion balance as well (Marschner) [38]. Furthermore, it facilitates CO_2 diffusion from the atmosphere into chloroplasts (Jákli et al.) and co-operates with Mg in photoassimilation and photoprotection of the photosynthetic apparatus [39,40]. The high accumulation of Na^+ and K^+ as high salinity has been supported by Podar et al. in roots, stems and leaves of *Petrosimonia trianda* (family Amaranthaceae) [16] . In contrast, Yan et al. reported that plants under salt stress, suffer from potassium deficiency caused by excess salts in growth substrate [41] . The higher accumulation of Ca^{++} and Mg^{++} in plants at site 2 are in line with those recoded by Podar et al. who found Ca^{++} and Mg^{++} levels were increased by 440% and 354% in roots and 240% and 58% in stems, respectively, of *Petrosimonia trianda* grown at 4.45 dS m^{-1} as compared with those grown at 3.14 dS m^{-1} [16]. The high accumulation of Ca and Mg in shoots of *Salicornia* growing at higher salinity is to probably alleviate the damage effects of NaCl. Similar conclusion was declared by Keisham et al. Abd El-Maboud & Abdelbar [42,9]. Furthermore, endowed the importance of $Ca2^+$ to protect cell membranes from damage and its role as a regulator of plant growth and development (Hepler) its accumulation could be a part of an adaptive strategy by *S. europaea* to tolerate saline habitats [43]. The increase in Cl content in shoots of *S. europaea* coincided with an increase in EC of accompanied soil (Tables 2,3), suggesting that one of tolerance mechanism of *Salicornia* to high salinity is to accumulate chloride content in its aerial parts. In general, the high ash content shed light the capacity of *Salicornia* to accumulate salts into the cell vacuoles.

Dramatic accumulation of proline returns to up-regulated synthesis and down-regulated degradation under a variety of stress conditions such as salt, drought and metal has been documented in many plants. Similarly, a reduction in the level of accumulated proline in the rehydrated plants is due to both decreased of proline biosynthetic pathway enzymes and motivation of proline degrading enzymes [44]. In this trend, Guo et al. found that osmotic substances including free proline were increased with increasing stress level in leaves and roots of *Lycium ruthenicum* [45]. Also, Abd El-Maboud and& Abdelbar found the highest accumulation of proline in leaves of *Limoniastrum monopetalum* growing at salt marshes [16]. Moreover, Ghanem et al. found proline content in *Salicornia europaea* was significantly increased at 200, 400 and 600 mM NaCl only, and the highest value of proline was recorded at 600 mM NaCl [5]. In this paper, proline content is independent of salinity degree. Despite large fluctuations in soil salinity and soil moisture content, no significant correlation was detected between them and proline, which is well known as not only a compatible solute contributed in osmotic adjustment but also a powerful antioxidant to counteract the damaging effects of reactive oxygen species ROS [46,9]. Proline accumulation in the presence of salinity stress is widely documented strategy of *Leptochloa fusca (*L.*)* Kunth. and *Echinochloa stagnina* (L.) P. Beauv to rectify this adverse environmental condition [47] .

The increase in total soluble sugars of *Salicornia* growing at site 2 may be result in reduced energy supply under higher saline conditions. In this respect, Ehsen et al. found that soluble sugars were increased by 30% in Haloxylon *stocksii* at 300 mm, NaCl but did not change in 100 mm compared to non-saline control [48]. The higher accumulation of soluble sugars in winter are in harmony with those obtained by Gounaris who reported that soluble sugars accumulation response to low temperature [49]. Soluble sugars (sucrose, glucose and fructose) play a pivotal role in vindicating the overall structure and growth of plants [50]. Concerning the positive correlation between soil moisture and soluble sugars accumulation, Dien et al. observed a significant reduction in leaf soluble sugar of some rice varieties affected by drought stress [51].

The positive correlation between EC and Na sequestration in the studied species suggesting that Na may contribute to its physiological salt tolerance mechanisms. In this respect, Muchate et al. found a linear raise in Na^+ accumulation as NaCl stress increased in *Spinacia oleracea* L. Also, Cai & Gao (2020) found a much greater variation (3.21-fold on average) in Na^+ content in *Chenopodium quinoa* leaves at the highest salt level relative to the control [52,53]. The positive correlation between EC and soil moisture content has been supported by Shin & Son on *Capsicum annuum* as growth proceeded to a late stage [54]. Regarding positive correlation between Na and Cl with EC indicating that both Na and Cl can contribute to salinity tolerance. In this trend, Khan et al. reported that Cl and Na contents have been increased as salinity increased in *Atriplex griffithii* plant. In the current paper, it is suggested that K has pivotal role in proline biosynthesis [55]. In this respect, Ahanger et al. concluded that potassium treated plants promoting synthesis and accumulation of free proline and free amino acids [56]. Positive correlation between proline, and K^+ concentration has been detected in *Triticum aestivum* [57]. Concerning positive correlation between Ca & Mg and Ca & soluble sugars, Hosseini et al. concluded that foliar application of Ca^{2+} in sugar beet increased the level of magnesium and silicon in the leaves, stimulated plant growth, leaf area in addition to chlorophyll level. Consequently, Ca^{2+} increased the carbohydrate levels in leaves and up-regulated the expression of *BvSUC3* and *BvTST3* genes involved in sucrose transport [58-61].

CONCLUSION

Cl and Na were not only the predominant ions in soil supporting *S. europaea* but also in the shoot of the studied species. Since, *S. europa* can be invested in reclaiming salt-affected soil in arid-zone irrigation districts. The high positive correlation between K and proline indicates a pivotal role of K in proline biosynthesis.

COMPLIANCE WITH ETHICAL STANDARDS

The author declares no conflicts of interest.

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