

# Effect of salinity stress on chlorophyll, carotenoid content, and proline in *Salicornia prostrata* Pall. and *Suaeda prostrata* Pall. subsp. *prostrata* (Amaranthaceae)

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**Abstract** *Salicornia prostrata* Pall. and *Suaeda prostrata* Pall. subsp. *prostrata* occur together on saline soils in Kızılırmak Delta in Samsun, Turkey. The effects of salinity stress on photosynthetic pigments and proline were investigated in these two species in natural habitats. With the increasing soil salinity, a decrease in chlorophyll (Chl) and carotenoid contents was observed in both taxa. However, in *Salicornia prostrata*, photosynthetic pigments levels were lower than those in *Suaeda prostrata*. Proline contents significantly increase with increase in salinity level in both species, whereas *Salicornia prostrata* produced less proline than *Suaeda prostrata*. Proline and Chl *a* contents were significantly different between examined species according to soil salinity. Based on obtained data, we can conclude that at in situ conditions, *Salicornia prostrata* is more salt tolerant compared with *Suaeda prostrata*.

**Keywords** Photosynthetic pigments · Salt stress · Turkey

## Introduction

Among environmental factors limiting plant productivity and growth, soil salinity is a major adverse environmental factor (Li et al. 2004; Munns 2005). An estimated 80,000,000 ha of cultivated land is adversely affected by high salinity (Munns 2002; Askaril et al. 2006). Under salt

stress conditions, plants are exposed to osmotic stress by limiting absorption of water from soil (Youssef 2009). High salt stress can affect numerous physiological and biochemical processes at both the cellular and the whole-plant levels (Wenxue et al. 2003; Aghaleh et al. 2009).

Most halophytes have the ability to adapt to salinity by adjusting the osmotic potential of their internal tissues, which mechanism is well known (Aghaleh et al. 2009; Flowers and Colmer 2008). Adaptation of halophytes to salinity is related to the accumulation of osmoregulators solutions that include sugars and free proline (Bohnert et al. 1995). Accumulation of sugars and free proline prevents the loss of water and ion toxicity (Ashraf and Foolad 2007). Within organisms, osmoregulation permits to take up additional water from the environment by lowering water potentials (Kumar et al. 2003). It has been reported that one of the adaptations of plants to salinity and water deficit is high proline levels (Kumar et al. 2000; Ramanjulu and Sudhakar 2001). Matysi et al. (2002) also reported that proline has protected plants against free radicals.

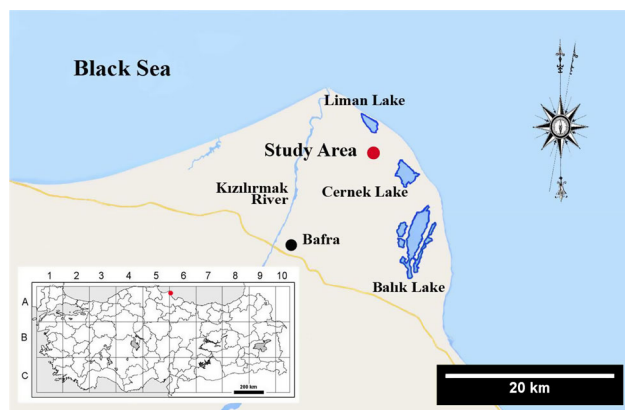
Reduction in photosynthesis activity under salt stress is due to reduction of chlorophyll (Chl) and also reduction of CO<sub>2</sub> absorption (Francisco et al. 2002). Kato and Shimizu (1985) reported that the loss of Chls in salinity is related to photoinhibition or reactive oxygen species formation. Plants grown under high salinity have a lower stomatal conductance in order to conserve water (Mafakheri et al. 2010). Consequently, CO<sub>2</sub> fixation is reduced and photosynthetic rate decreases. In *Salicornia persica* Akhiani and *Salicornia europaea* L., a decrease in photosynthetic pigments induced by salt has been reported (Aghaleh et al. 2009).

Previous study on *Salicornia* and *Suaeda* species involved some physiological responses to salinity (Aghaleh et al. 2009, 2011; Youssef 2009; Zhang et al. 2010). But up

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**Fig. 1** Map indicating Kızılırmak Delta at Samsun, Turkey

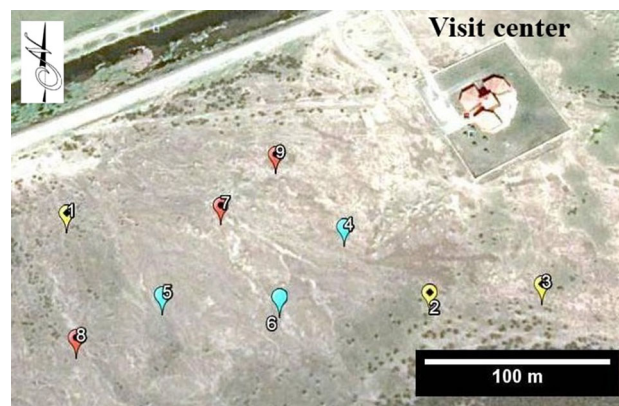
to now, no investigation has considered the comparative physiological responses of *Salicornia prostrata* and *Suaeda prostrata* subsp. *prostrata* to salt stress in their natural environment.

In this study, we first investigated the Chl, carotenoid, and proline contents of *Salicornia prostrata* and *Suaeda prostrata* subsp. *prostrata* as a response to soil salt gradient. We then examined the response to soil salt stress between these species.

## Materials and methods

The coastline of the Central Black Sea Region located in the North of Turkey represents the study area (Figs. 1, 2). This area belongs floristically to the Euxine Province, which is a province of the Euro-Siberian phytogeographical region. The study area consists of alluvial sediment soils carried by the Kızılırmak River. Soil is typically dark grayish brown (vertisol) and soil depth is mainly 1 m (Yakupoglu et al. 2010). On average, soil texture is 48 % clay, 33 % silt, and 19 % sand (Yalcin et al. 2014). The mean annual temperature and annual rainfall are 13.9 °C and 722.5 mm, respectively. The current climate in the region is semihumid (Demir et al. 2009). In the Kızılırmak Delta, numerous lagoons and wetlands exist formed by successional dunes and forests. Nowadays, groundwater drainage and soil salinity problems in Kızılırmak Delta are present, raised by excessive irrigation water use, seepage from canals, inefficient irrigation methods, and inadequate or malfunctioning drainage systems (Arslan 2012).

*Salicornia prostrata* and *Suaeda prostrata* subsp. *prostrata* (Amaranthaceae) were selected as plant materials in this study. *Salicornia prostrata* and *Suaeda prostrata* subsp. *prostrata* have been distributed together on saline soils in Kızılırmak Delta in Samsun, Turkey. Diagnosis of plant species was given according to the Flora of Turkey (Ball 1966; Aellen 1966). Twenty-eight locations were



**Fig. 2** Map of detailed localities in Kızılırmak Delta

determined according to different soil salinities in August 2009 in the study area. In these localities, we pay attention to the coexistence of both species. These locations have the same soil and microclimatic properties. Soil samples were collected as a profile from the root zone of each locality, and transported to the laboratory in polyethylene bags. They were air dried and sieved to pass through a 2 mm mesh prior to analysis. Electrical conductivity ( $\text{dS m}^{-1}$ ) was determined in soil–water extracts at 1:1 (w:v) using a Hanna 215 electrical conductivity meter. Nine localities that had different soil salinities were selected from 28 locations according to the results of soil salinity analysis, which were 2.0, 3.9, 4.2, 9.3, 10.1, 10.7, 18.4, 23.5, and 26.2  $\text{dS/m}$ , respectively. Species were sampled in the same day from different localities from August of 2009, 2010, and 2011. Thirty individuals of both species were collected from nine localities, transported to the laboratory, and stored at 4 °C.

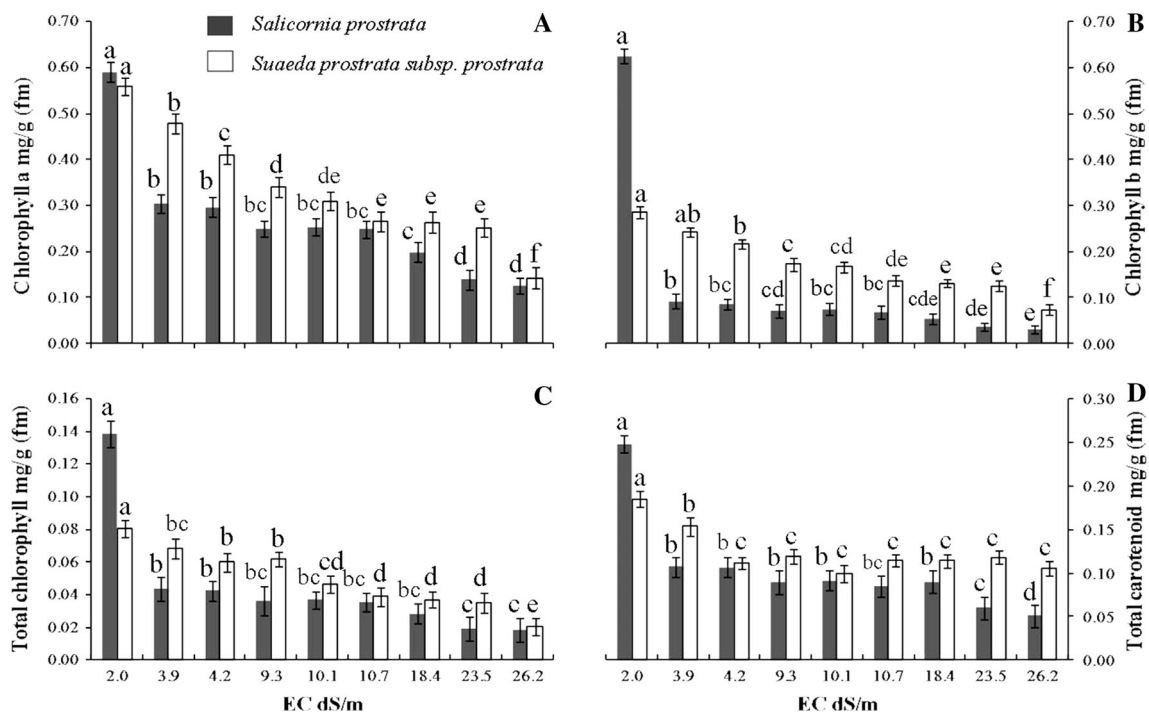
Then, chlorophylls (Chl *a* and *b*) and carotenoids ( $\text{mg g}^{-1}$ ) were extracted by 100 % acetone from fresh plant samples and quantified spectrophotometrically. Supernatants were used for the analysis of pigments. Absorbances were determined at 645, 652, 662, and 470 nm, respectively, and the following equations were used for calculations (Lichtenthaler and Wellburn 1983):

$$\text{Total Chl: } A_{652} \times 27.8 \times 20/\text{mg leaf weight},$$

$$\text{Chl } a : (11.75 \times A_{662} - 2.35 \times A_{645}) \times 20/\text{mg leaf weight},$$

$$\text{Chl } b : (18.61 \times A_{645} - 3.96 \times A_{662}) \times 20/\text{mg leaf weight},$$

$$\text{Car: } [(1000 \times A_{470} - 2.27 \times \text{Chl } a - 81.4 \times \text{Chl } b)/227] \times 20/\text{mg leaf weight}.$$



**Fig. 3** Chlorophyll *a* (a), chlorophyll *b* (b), total chlorophyll (c), and total carotenoid (d) contents of *Salicornia prostrata* and *Suaeda prostrata* under NaCl stress. Mean  $\pm$  SE of three replicates. Different letters indicate significant differences ( $P < 0.01$ )

Free proline content ( $\text{mmol g}^{-1}$ ) was determined according to the method from Bates et al. (1973). L-Proline was used as a standard. Determination of absorbents was made using a UV-visible spectrometer (Termo He $\lambda$ ios  $\gamma$ ), liquid phase was read at a wave length of 520 nm a standard calibration curve and calculate on a fresh mass.

All analyses were conducted as three replicates ( $n = 3$ ). Data were expressed as mean  $\pm$  standard deviation and submitted to analysis of variance using SPSS (version 10). The significance of differences was determined according to Tukey's test. Comparisons with  $P$  values  $< 0.01$  and  $0.05$  were considered significantly different.

## Results and discussion

A decrease in photosynthetic pigment content of *Salicornia prostrata* and *Suaeda prostrata* subsp. *prostrata* was observed along soil salinity gradient in this study (Fig. 3a–d). In the case of Chl *a*, there was decrease in response to higher salinity. The highest rate of Chl *a* was observed at locality where salinity level was determined as EC 2.0 dS  $\text{m}^{-1}$ . The lowest rate of this value was determined in the most higher salinity level (EC 26.2 dS  $\text{m}^{-1}$ ). *Suaeda prostrata* subsp. *prostrata* produced more Chl except for locality with EC 2.0 dS  $\text{m}^{-1}$  salinity level when compared to *Salicornia prostrata* (Fig. 3a). Chl *b* and total Chl were level declined by high salinity (Fig. 3b, c). The results

obtained in this study are in agreement with those studies of Aghaleh et al. (2009) for *S. persica* and *S. europaea* L. in which salt treatment decreased photosynthetic pigments. Reduction in Chl content under salt stress could be attributed to the destruction of Chl pigments (Levitt 1980). Beinsan et al. (2009) reported that the decrease in Chl content was due to the increase in the activity of Chls enzyme and instability of protein complexity of pigments.

Based on soil salinity stress, it was observed statistically that *Suaeda prostrata* subsp. *prostrata* was divided into more groups according to the contents of Chls *a*, *b*, and total Chl (Fig. 3a–c) as a comparison to *Salicornia prostrata*. Among other pigments, increased soil salinity-based stress-related Chl *b* decrease in *Salicornia prostrata* is found to be the highest. Chl *b* is the most decreased pigment in *Salicornia prostrata* related to increasing soil salinity stress. The contents of chlorophylls (Chls *a*, *b*) and carotenoids decreased more proportionally in *Salicornia prostrata* than *Suaeda prostrata* subsp. *prostrata*, while no significant difference in proline ratio was observed in both species. The Chl content of the examined species was significantly different (Table 1). These results indicated that *Suaeda prostrata* subsp. *prostrata* was more sensitive to soil salinity than *Salicornia prostrata* in terms of Chl content. Salinity reduces net photosynthetic rate (Burman et al. 2003). Nunes et al. (2008) reported that in saline habits, soil salinity and arid climate greatly affected the synthesis of pigment components of plants. Morsy et al.

**Table 1** The percentage change of traits examined in plant samples by soil salinity

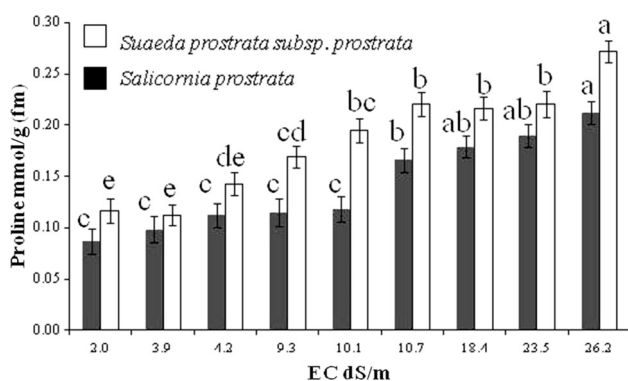
	<i>Salicornia prostrata</i>	<i>Suaeda prostrata</i> subsp. <i>prostrata</i>
Chl <i>a</i> (%)	-21.18	-25.40
Chl <i>b</i> (%)	-4.96	-25.17
Tot-Chl. (%)	-12.94	-25.00
Tot-Car. (%)	-20.56	-53.51
Proline (%)	+41.03	+41.17

(2008) concluded that Chls *a*, *b*, and carotenoids remarkably decreased in summer on desert plants. We noted that *Suaeda prostrata* subsp. *prostrata* appears as small groups in the study region, while *Salicornia prostrata* covers the whole area.

Free proline content of both investigated species is represented in Fig. 4. The free proline content significantly increased along soil salinity gradient. Free proline content was significantly higher in *Suaeda prostrata* subsp. *prostrata* than in *Salicornia prostrata* under salinity. Moghaieb (2004) demonstrated that under salt stress, higher proline accumulation occurred in *Suaeda maritima* subsp. *richii* (Fern.) Bassett & C.W. Crompton than of *S. europaea*. Slama et al. (2007) reported that *S. europaea* is characterized by apparently higher ratio of succulence; this is considered as a mechanism through which certain halophytes are adapted to high salinity. Youssef (2009) observed that all halophytes collected during summer from high salinity site tended to retain higher soluble protein, sugar, and proline as well as higher levels of total organic osmolytes, compared to those parameters of the other groups of halophytes in low salinity site. However, he stated that high succulence in salinity can be considered as an adaptive response in halophytic plant species. Similarly, in this study, *Salicornia prostrata* showed lower accumulation of proline than the other investigated halophytic species. Therefore, proline content of *Suaeda prostrata*

subsp. *prostrata* was also higher in all localities between investigated species. These results suggest that *Salicornia prostrata* is potentially more tolerant to salt damage.

It was concluded that the synthesis of proline copes with increasing salinity stress in halophytic plants (Wang et al. 2007; Pagter et al. 2009; Siddiqui et al. 2009). *Salicornia prostrata* had lower proline content than *Suaeda prostrata* subsp. *prostrata* in our study. Unlike *Salicornia prostrata* which spreads to quite large area even at increased soil salinity rates, at same conditions, *Suaeda prostrata* subsp. *prostrata* formed small clusters (Figs. 5, 6). These results suggested that *Suaeda prostrata* subsp. *prostrata* has proline synthesis mechanism in order to deal with salinity stress. On the other hand, *Salicornia prostrata* may have different mechanisms along with proline synthesis. For instance, it may have different anatomical structures and strategies. Ashraf (2004) also said that proline accumulation not only occurred by soil salinity but also arose due to drought in plant tissues.



**Fig. 4** Effects of different NaCl concentrations on proline content in *Salicornia prostrata* and *Suaeda prostrata* subsp. *prostrata*. Mean  $\pm$  SE of three replicates. Different letters indicate significant differences ( $P < 0.01$ )



**Fig. 5** General appearance of *Salicornia prostrata*





**Fig. 6** *Suaeda prostrata* subsp. *prostrata*

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## References

- Aellen P (1966) *Suaeda* L. In: Davis PH (ed) Flora of Turkey and the east Aegean Island, vol 2. University Press, Edinburgh, p. 321–324
- Aghaleh M, Niknam V, Ebrahimzadeh H, Razavi K (2009) Salt stress effects on growth, pigments, proteins and lipid peroxidation in *Salicornia persica* and *S. europaea*. Biol Plant 53:243–248
- Aghaleh M, Niknam V, Ebrahimzadeh H, Razavi K (2011) Effect of salt stress on physiological and antioxidative responses in two species of *Salicornia* (*S. persica* and *S. europaea*). Acta Physiol Plant 33:1261–1270
- Arslan H (2012) Spatial and temporal mapping of groundwater salinity using ordinary kriging and indicator kriging: the case of Bafra Plain, Turkey. Agric Water Manag 113:57–63
- Ashraf M (2004) Some important physiological selection criteria for salt tolerance in plants. Flora Morphol Distrib Funct Ecol Plants 199:361–376
- Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59:206–216
- Askaril H, Edqvist J, Hajheidaril M, Kafi M, Salekdeh GH (2006) Effects of salinity levels on proteome of *Suaeda aegyptiaca* leaves. Proteomics 6:2542–2554
- Ball PW (1966) *Salicornia* L. In: Davis PH (ed) Flora of Turkey and the east Aegean Island, vol 2. University Press, Edinburgh, p. 321–324
- Bates LS, Wadern RP, Teare ID (1973) Rapid estimation of free proline for water stress determination. Plant Soil 39:205–207
- Beinsan C, Camen D, Sumalan R, Babau M (2009) Study concerning salt stress effect on leaf area dynamics and chlorophyll content in four bean local landraces from Banat area. In: Florijančić T, Luzaić R (eds) 44th Croatian and 4th international symposium on agriculture, 16–20 February, pp 416–419
- Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptations to environmental stresses. Plant Cell 7:1099–1111
- Burman U, Garg BK, Kathju S (2003) Water relations, photosynthesis and nitrogen metabolism of Indian mustard (*Brassica juncea* Czern. & Coss.) grown under salt and water stress. J Plant Biol 30:55–60
- Demir Y, Erşahin S, Güler M, Cemek B, Günel H, Arslan H (2009) Spatial variability of depth and salinity of groundwater under irrigated ustifluents in the Middle Black Sea Region of Turkey. Environ Monit Assess 158:279–294
- Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. N Phytol 179:945–963
- Francisco G, Jhon L, Jifon S, Micaela C, James PS (2002) Gas exchange, chlorophyll and nutrient contents in relation to Na<sup>+</sup> and Cl<sup>-</sup> accumulation in sunburst mandarin grafted on different root stocks. Plant Sci 35:314–320
- Kato M, Shimizu S (1985) Chlorophyll metabolism in higher plants. VI. Involvement of peroxidase in chlorophyll degeneration. Plant Cell Physiol 26:1291–1301
- Kumar RG, Shanb K, Dubey RS (2000) Salinity induced behavioral changes in malate dehydrogenase and glutamate dehydrogenase activities in rice seedlings of differing salt tolerance. Plant Sci 156:23–24
- Kumar SG, Reddy AM, Sudhakar C (2003) NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. Plant Sci 165:1245–1251
- Levitt J (1980) Responses of plant to environmental stress chilling, freezing, and high temperature stresses, 2nd edn. Academic, New York
- Li PH, Wang ZL, Zhang H, Wang BS (2004) Cloning and expression analysis of the B subunit of V-H<sup>+</sup>-ATPase in the leaves of *Suaeda salsa* under NaCl stress. Acta Bot Sin 46:93–99
- Lichtenthaler H, Wellburn AR (1983) Determination of total carotenoids and chlorophyll *a* and *b* of leaf extracts in different solvents. Biochem Soc Trans 603:591–593
- Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi Y (2010) Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aust J Crop Sci 4:580–585
- Matysi J, Bhalu BA, Mohanty P (2002) Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Curr Sci 82:525–532
- Moghaieb R (2004) Effect of salinity on osmotic adjustment, glycine betaine accumulation and the betaine aldehyde dehydrogenase gene expression in two halophytic plants, *Salicornia europaea* and *Suaeda maritima*. Plant Sci 166:1345–1349
- Morsy AA, Youssef AM, Mosallam HA, Hashem AM (2008) Assessment of selected species along Alamein-Wadi El-Natrun Desert Road. Egypt J Appl Sci Res 4:1276–1284
- Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239–250
- Munns R (2005) Genes and salt tolerance: bringing them together. N Phytol 167:645–663
- Nunes C, de Sousa S, da Silva JM, Feveireiro MP, da Silva AB (2008) Physiological responses of the legume model *Medicago truncatula* cv. Jemalong to water deficit. Environ Exp Bot 63:289–296
- Pagter M, Bragato C, Malagoli M, Brix H (2009) Osmotic and ionic effects of NaCl and Na<sub>2</sub>SO<sub>4</sub> salinity on *Phragmites australis*. Aquat Bot 90:43–51
- Ramanjulu S, Sudhakar C (2001) Alleviation of NaCl salinity stress by calcium is partly related to the increased proline accumulation in mulberry (*Morus alba* L.) callus. J Plant Biol 28:203–206

- Siddiqui MH, Mohammad F, Khan MN (2009) Morphological and physio-biochemical characterization of *Brassica juncea* L. Czern. & Coss. genotypes under salt stress. *J Plant Interact* 4:67–80
- Slama I, Ghnaya T, Hessini K, Messedi D, Savoure A, Abdelly C (2007) Comparative study of the effects of mannitol and PEG osmotic stress on growth and solute accumulation in *Sesuvium portulacastrum*. *Environ Exp Bot* 61:10–17
- SPSS (1999) Statistical Package for the Social Sciences, SPSS version 10.0. Chicago
- Wang CQ, Chen M, Wang BS (2007) Betacyanin accumulation in the leaves of C3 halophyte *Suaeda salsa* L. is induced by watering roots with H<sub>2</sub>O<sub>2</sub>. *Plant Sci* 172:1–7
- Wenxue W, Bilsborrow PE, Hooley P, Fincham DA, Lombi E, Forster BP (2003) Salinity induced differences in growth, ion distribution and partitioning in barley between Marythorpee and its derived mutant golden promise. *Plant Soil* 250:183–191
- Yakupoğlu T, Sarioğlu F, Dengiz O (2010) Morphology, physico-chemical characteristics and classification of two vertisols in Bafra and Çarşamba Delta Plains. *Ondokuz Mayıs Univ Anadolu J Agric Sci* 25:67–73
- Yalcin E, Kilinc M, Kutbay HG, Bilgin A, Korkmaz H (2014) The lowland meadow vegetation of the central Black Sea Region of Turkey. *Ekoloji* 23:36–51
- Youssef AM (2009) Salt tolerance mechanisms in some halophytes from Saudi Arabia and Egypt. *Res J Agric Biol Sci* 5:191–206
- Zhang H, Irving LJ, McGill C, Matthew C, Zhou D, Kemp P (2010) The effects of salinity and osmotic stress on barley germination rate: sodium as an osmotic regulator. *Ann Bot* 106:1027–1035