Pak. J. Bot., 54(5): 1697-1706, 2022. DOI: http://dx.doi.org/10.30848/PJB2022-5(29)

PHYSIO-BIOCHEMICAL ANALYSES OF SELECTED HALOPHYTES FROM THE SALINE REGIONS OF PAKISTAN AND THEIR POTENTIAL FOR BIOSALINE AGRICULTURE IN ARID ENVIRONMENTS

MUHAMMAD ADEEL GHAFAR[1], NUDRAT AISHA AKRAM[1*], BILQUEES GUL[2] AND HADI PIRASTEH-ANOSHEH[3]

[1]*Department of Botany, Government College University, Faisalabad, 38000, Pakistan*
[2]*Institute of Sustainable Halophyte Utilization, University of Karachi, University Road, Karachi 75270, Pakistan*
[3]*National Salinity Research Center, Agricultural Research, Education and Extension Organization, Yazd 8917357676, Iran*
[*]*Corresponding author's email: nudrataauaf@yahoo.com*

Abstract

Biosaline agriculture is used for growing of salt tolerant crops by using high saline water and soils, especially in the arid lands. Halophytes, due to their high salinity tolerance can be used directly as forage after multilateral evaluations. In Pakistan, many areas are highly salt affected including Salt Range (SR) and some areas of Faisalabad region. In the current study, different plant species such as *Aeluropous lagopoides* (L.), *Cenchrus ciliaris* (L.), *Heliotropium crispum*, *Heliotropium curassavicum*, *Cyperus arenarius* (Retz.), *Heliotropium europaeum* (L.) and *Dichanthium annulatum* were collected from the Salt Range as well as Faisalabad region. The results showed that *C. ciliaris*, *C. arenarius* and *H. crispum* exhibited higher biomass than the other plant species. Spatial variation between species of both regions was significant in terms of biomass production, chlorophyll contents, osmolytes production (glycinebetaine, proline) and activities of enzymatic antioxidants (catalase, peroxidase and superoxide dismutase). Specimens of Faisalabad region (*Cenchrus ciliaris*, *Cyperus arenarius* and *D. annulatum*) showed high fresh and dry biomass production than that of Salt Range area. On the other side, specimens of Salt Range showed elevated level of oxidative defense mechanism (SOD, POD, and CAT) due to high salt level in the soil. *H. europaeum*, *Cyperus arenarius* and *Cenchrus ciliaris* were highly responsive to saline conditions of Salt Range area. Above all, *H. europaeum, Cyperus arenarius* and *Cenchrus ciliaris* were highly adaptive to saline conditions in terms of plant biomass and oxidative stress tolerance.

Key words: Antioxidative defense system, Drought, Halophytes, Reactive oxygen species, Salinity.

Introduction

Arid and semi-arid areas are exposed to different environmental constraints. Of them salinity and drought stresses are the major constraints (Alfarrah & Walraevens, 2018). Soil salinization is increasing day by day due to unsuitable irrigation system, poor water quality and cultivation of same crops continuously (Brewster, 2018; Xuan, 2018). More than 831 million hectares of land in the world is salt affected and losing its crop production capability (Rahman *et al*., 2017). Accumulation of high salts in rhizosphere causes alteration in the growth pattern of plant and may leads to changes at physiological, molecular, cellular and anatomical levels (Safdar *et al*., 2019). Plants exposed to abiotic stresses have adopted specialized mechanisms to cope with drought and salinity stresses. These changes may cause cell and/or even plant death (Ghori *et al*., 2019). Plants respond to these changes by modifying metabolic processes and morphological changes. In these arid lands, various forage producing plants i.e. shrubs, herbs and grasses could normally grow. These forage plants act as buffer against annual and seasonal forage production of rangeland areas. These wild herbs and shrubs are used as biofuel and fodder for grazing livestock (Vlaeminck *et al*., 2010). Over long periods, plants of these stressed areas had developed specialized mechanisms to perceive and respond to salinity and drought stresses (Hasanuzzaman *et al*., 2013).

Salinity affects seed germination, plant growth, and also causes water stress by changing osmotic potential of soil and plants in the rhizosphere (Shrivastava & Kumar, 2015; Blum, 2018). Salinity level rises in soil due to natural conditions and coastal areas (LeMonte *et al*., 2017; Ackerman, 2017). High saline areas are usually considered as wasteland for cultivation of conventional crops but wild plant of these areas are genetically salt tolerant species having fiber and medicinal properties (Qadir *et al*., 2014). These salt tolerant plants have potential to thrive under high saline soils and complete their life cycle. Sustainable development of non-conventional crops and species for forage potential is now adopting all over the world due to increasing salinity and water scarce conditions (Levidow *et al*., 2014; Avellan *et al*., 2018). Osmotic adjustment is a specialized mechanism for salinity tolerance in some halophytes. These plants accumulate sugars, organic acid, inorganic ions and amino acids (Slama *et al*., 2015). Plants tolerate salinity by changing their lipid membrane composition those were irrigated by saline water (Hanin *et al*., 2016). Level of unsaturated fatty acids increases in membranes of halophytes to cope with drastic effects of salinity stress. Unsaturated fatty acids are beneficial to keep fluidity of membrane and also stabilize the complexes of proteins of thylakoid membranes in photosystem II (He *et al*., 2018). These wild plants are source of unsaturated fatty acids i.e. α-linolenic acid found in their leaves are useful for human health. Halophytes (*Salicornia* and *Sarcocornia*) contain high potential of ions and osmotic homeostasis (Barreira *et al*., 2017).

Halophytes are those plants which can survive, grow and complete their life cycle under high salt concentration (Cairncross & Feachem, 2018; Maleki *et al*., 2019). Halophytes also play vital role in maintaining the

functioning of ecosystem (Flowers & Colmer, 2015; Parihar *et al*., 2015). These halophytes are major source of forage for livestock in the Salt Range areas, where other forage crops cannot grow well due to high salts in the rooting zone (Glenn *et al*., 2013).

The Salt Range, Punjab, Pakistan (32.5980° N and 72.3701° E) contains world's second largest salt reserves and soil of this area is highly affected by salts. The objective of the present research was the comparative investigation of adaptive mechanisms in some selected plant species from Salt Range as well as of Faisalabad region.

Material and Methods

In the present study, total 7 halophyte species were collected at their vegetative stage along with their rhizospheric soil (0-15 cm depth) and water from two different locations: Salt Range area and Faisalabad region (31.6391° N, 73.2345° E), Punjab, Pakistan (Table 1) with minimum human interference during April and May 2018. Leaves and roots were stored at -70°C for further analyses. All the analyses were carried-out in the Stress Biology Lab, Department of Botany, Government College University, Faisalabad, Pakistan. The examined species were *Aeluropous lagopoides* (L.), *Cenchrus ciliaris* (L.), *Heliotropium crispum*, *Heliotropium curassavicum*, *Cyperus arenarius* (Retz.), *Heliotropium europaeum* (L.) and *Dichanthium annulatum* (Forssk.).

Plant growth attributes: By using quadrat method, the samples of above-mentioned species were collected. For growth attributes, length of roots and shoots were recorded manually by using the scale. After recording fresh and dry biomass of root and shoot of plant samples, they were kept in an oven at 70ºC for 72h and data for dry weights were measured by using the electrical balance.

Chlorophyll and carotenoid contents: Chlorophyll and carotenoid contents were determined in plant samples using method proposed by Arnon (1949). Fresh leaf (0.5 g) of each replicate was extracted using 80% (v/v) acetone. The samples were placed overnight at 4ºC. All extracts were centrifuged at 5000 *rpm* for 5 min and then absorbance was taken at 480, 645 and 663 nm.

Glycinebetaine (GB) contents: Leaf GB contents were quantified following Grieve & Grattan (1983). Took 0.5 g fresh leaf and simultaneously grinded in 10 ml solution of 0.5% toluene and filtered. After it, 1 ml filtrate was taken in the test tube and mixed with 1 ml of 2*N* H_2SO_4. Then, 0.5 ml of supernatant was added to 0.2 ml of potassium tri-iodide, shaken the mixture and cooled it on ice bath for 90 min. The ice cooled distilled water (2.8 ml) along with 6 ml of 1,2 dichloroethane (cooled at -10°C) poured into the sample containing tubes. Two layers were formed in the test tubes by passing the stream of air for 2 min, during this process the tubes were kept remained on ice bath. The reaction was completed and separated the organic aqueous layer and measured its optical density at 365 nm using spectrophotometer.

Proline contents: Fresh leaf (0.5 g) was extracted in 10 ml of 3% (w/v) sulfosalicylic acid. Following the procedure proposed by Bates *et al*., (1973), the absorbance of sample was recorded at 520 nm and free proline contents were quantified.

Total phenolics: A method proposed by Julkenen-Titto (1985) was used to determine the total phenolic contents in the leaf extract. Fresh leaf (0.1 g) was homogenized in 5 ml acetone (80%). Centrifuged the samples at 10,000 *rpm* for 10 min. Then, 100 µl of supernatant, 2 ml of dH_2O, 1 ml of Folin-Phenol Ciocalteu's reagent, and 5 ml of 20% sodium carbonate were mixed together. The final volume made up to 10 ml with distilled water and total phenolics in leaf tissues were estimated at 750 nm.

Ascorbic acid: Fresh leaf (0.25 g) was homogenized with trichloroacetic acid (10 ml; 6%). Each 4 ml of the extract was mixed with 2 ml of dinitrophenyl hydrazine. One drop of 10% thiourea was added and boiled the mixture at room temperature. Following it, 5 ml of 80% (v/v) H_2SO_4 were added to the mixture. The absorbance of the sample was recorded at 530 nm (Mukherjee & Choudhuri, 1983).

Hydrogen peroxide (H_2O_2): Hydrogen peroxide contents were determined following the method of Velikova *et al*., (2000). Fresh leaf (0.5 g) were extracted in an ice bath with 5 ml (0.1%; w/v) trichloroacetic acid. The extract was centrifuged at 12000 × *g* for 15 min and 0.5 ml of the extract was added to 0.5 ml of 10 mM potassium phosphate buffer (pH, 7.0) and 1 ml of 1 M potassium iodide. The absorbance of the supernatant was read at 390 nm.

Malondialdehyde (MDA): Cakmak & Horst (1991) proposed a method for the estimation of malondialdehyde contents in the leaf samples. Fresh leaf (0.5 g) homogenized in 5 ml of TCA (5% w/v) and centrifuged at 12,000 *rpm* for 15 min. Equal volume of supernatant and TBA (0.5% in 20% w/v TCA) were mixed. Boiled the mixture at 100ºC for 25 min. Then, recorded OD at 532 nm and 600 nm and calculated the MDA contents.

MDA (nmol ml^{-1}) = Absorbance (sample) – Absorbance (blank)/1.56 x 1000000

Total free amino acids: A method proposed by Sanada *et al*., (1995) was adopted for the estimation of free amino acids. Free amino acids in the supernatant were determined by using amino acid analyzer.

Table 1. Physico-chemical properties of experimental soil taken from two different sites.

Site	pH	EC dS/m	O.M g/kg	Saturation %	Sand %	Silt %	Clay %	P ppm	K ppm
Salt range area	7.9	6.2	0.91	38	65.5	12.5	22	2.9	180
Faisalabad region	7.8	3.4	3.2	40	57	18.6	24.4	7.3	290

Total soluble proteins: Bradford (1976) method was used to determine the total soluble proteins contents in the leaf extract. Fresh leaf (0.5 g) was extracted in 5 ml of 50 mM potassium phosphate buffer (pH 7.8) prepared in an ice bath. The extract was centrifuged at 10, 000 × g for 15 min at 4ºC and recorded the readings.

Total soluble sugars: A method proposed by Yemm and Willis (1954) was used to determine the total soluble sugars. A leaf (0.1 g) was extracted in 10 ml of 80% ethanol. The supernatant (0.1 ml) was mixed with 3 ml of anthrone reagent in the test tubes. The reaction mixture was boiled for 10 min and then cooled at room temperature. The total soluble sugars were determined at 630 nm against glucose curve.

Activities of enzymatic antioxidants: Following the method of Giannopolitis and Ries (1977), the activity of superoxide dismutase (SOD) enzymes was determined. The reaction mixture contained 20 mM phosphate buffer (pH, 5.0), 13 mM methionine, 75 µM triton-X, 50 µM nitroblue tetrazolium, 0.1 ml enzyme extract and 1.3 µM riboflavin. The absorbance was measured at 560 nm. For the determination of activity of catalase (CAT) enzyme, the method devised by Maehly & Chance (1955) was adopted. The enzyme extract (0.1 ml) was mixed with 1.9 ml potassium phosphate buffer and 1 ml of H_2O_2 (5.9 mM). Then the absorbance of the reaction mixture was measured at 240 nm. Similarly, following Maehly & Chance (1955) method, the activity of peroxidase (POD) enzyme was carried-out. The enzyme extract (50 µl) added to 100 µl of H_2O_2 (40 mM) in a cuvette. After it, 100 µl of guaicol (20 mM) and 750 µl of potassium phosphate buffer were mixed and the absorbance noted at 470 nm.

Statistical analyses

The obtained data for each parameter were subjected to two-way analysis of variance (ANOVA) and correlation among different attributes by using the statistical software CoStat (version 6.2).

Results

Plant growth: Shoot fresh and dry weights of halophytic species collected from the Salt Range area were lower than the Faisalabad region. This difference might be due to high salinity conditions in the Salt Range area, Punjab Pakistan (Fig. 1A-B). Maximum fresh and dry weights were observed in *C. arenarius* and *C. ciliaris* L. collected from both regions. Specimens from the Salt Range area were more influenced in this attribute than the species of Faisalabad region. *C. arenarius*, *H. curassavicum* and *D. annulatum* showed high root fresh and dry biomass from both collection sites (Fig. 1C-D). Shoot and root lengths of halophytes from highly saline region (Salt Range) was lower than the species of less saline area (Faisalabad region). The maximum shoot length was recorded in *C. arenarius* from Faisalabad region. Root length was high of *H. curassavicum* of Salt Range area. Root and shoot lengths of *C. ciliaris* also showed significantly better response to both experimental sites (Fig. 1E-F).

Photosynthetic pigments: Analysis of variance showed a significant reduction ($p \leq 0.01$) in chlorophyll *a* and *b* contents under high saline conditions (Fig. 1G-H). Specimens of Faisalabad region had high chlorophyll contents than that of Salt Range area. Chlorophyll *a* contents were higher in *C. arenarius* and *H. curassavicum* of both research areas. Total chlorophyll contents of halophyte species were also significantly affected by high saline conditions in the Salt Range area than the plants of Faisalabad region (Fig. 2A). *C. arenarius* and *H. curassavicum* exhibited maximum chlorophyll contents as shown in Fig. 2A at both study sites. Carotenoid contents also varied significantly in both the samples and maximum concentration was observed in the plants collected from the Salt Range (Fig. 2B).

Accumulation of osmoprotectants: Glycinebetaine (GB) contents of halophyte species from both regions significantly responded (Fig. 2C). The species of the Salt Range area contained higher GB contents than the Faisalabad region. From Faisalabad region *C. arenarius* and *C. ciliaris* accumulated more GB contents than the other five species. From Salt Range area, *C. arenarius* and *C. ciliaris* also showed more GB contents than the other five species. It was observed by analysis of variance that halophyte species respond significantly ($p \leq 0.001$) to proline contents (Fig. 2D). Plants of Salt Range area had more proline contents than that of Faisalabad region. *C. ciliaris* and *C. arenarius* had more proline contents than the other five species from the Salt Range area. While in case of Faisalabad region, *A. lagopoides*, *D. annulatum* and *C. arenarius* indicated elevated levels of proline.

Non-enzymatic antioxidants (total phenolics and AsA): Total phenolic contents were higher in *Cyperus* species from both study sites. *Cyperus* species of Salt Range area exhibited more phenolic contents compared to Faisalabad region (Fig. 2E). It was also observed that ascorbic acid contents significantly improved in halophyte species. Species of Salt Range area contained more ascorbic acid contents than the plants of Faisalabad region. Of all plant species *C. ciliaris*, *H. europaeum* and *C. arenarius* contained more ascorbic acid contents than the other species (Fig. 2F).

Oxidative system: Data presented in Fig. 2G indicated that the species of the Salt Range area accumulated high level of hydrogen peroxide contents due to high salinity level in the soil. *C. ciliaris*, *D. annulatum*, and *A. lagopoides* contained maximum level of hydrogen peroxide contents than the other species. Species of Salt Range area showed higher malondialdehyde (MDA) contents than the species of Faisalabad region. *C. ciliaris* and *C. arenarius* accumulated higher quantity of MDA than the other five species of halophytes from the Salt Range area in this study. While from Faisalabad region *A. lagopoides* and *D. annulatum* contained higher level of MDA than the other five species of halophytes (Fig. 2H).

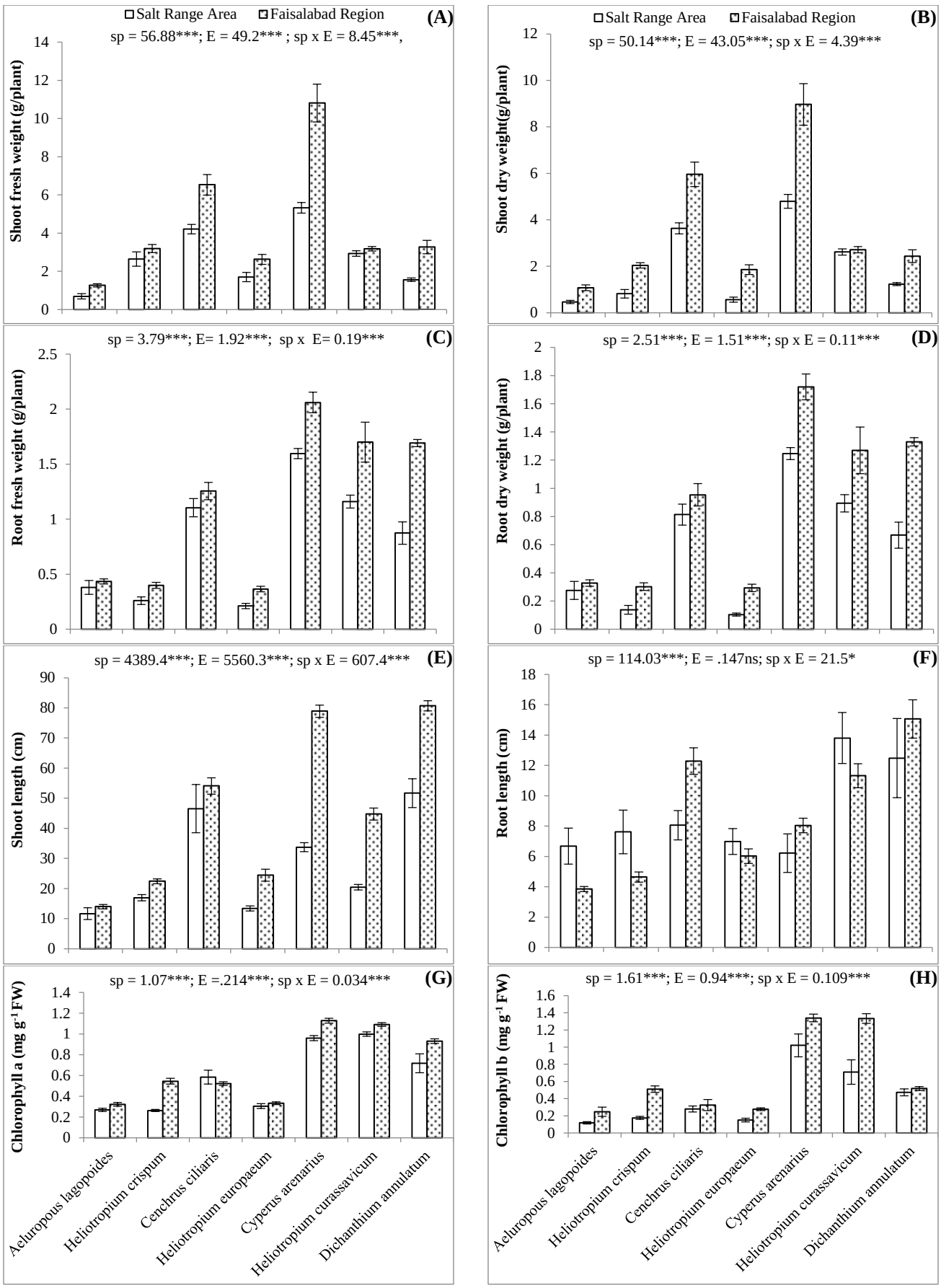

Fig. 1. (A-H): Shoot and root fresh weights, dry weights, lengths, chlorophyll contents of halophytic species collected from Salt Range area and Faisalabad region Punjab, Pakistan (Mean ± S.E.). sp = Species, E= Ecotypes; ns, non-significant, *,** and ***, significant at 0.05, 0.01 and 0.001 levels, respectively.

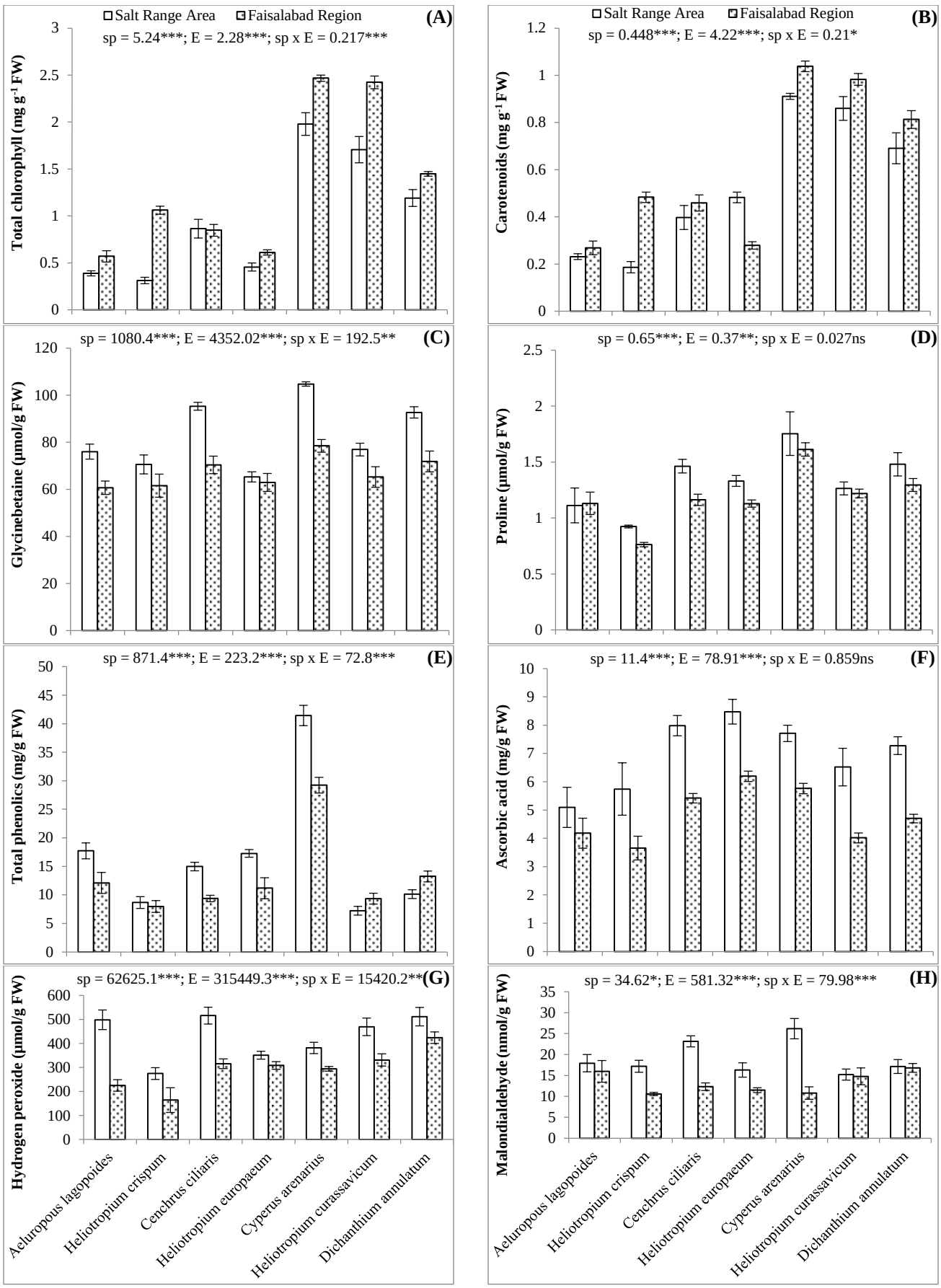

Fig. 2. (A-H): Total chlorophyll, carotenoid, glycinebetaine, proline, total phenolics, ascorbic acid, hydrogen peroxide and malondialdehyde contents of halophytic species collected from Salt Range area and Faisalabad region Punjab, Pakistan (Mean ± S.E.). sp= Species, E= Ecotypes; ns, non-significant, *,** and ***, significant at 0.05, 0.01 and 0.001 levels, respectively.

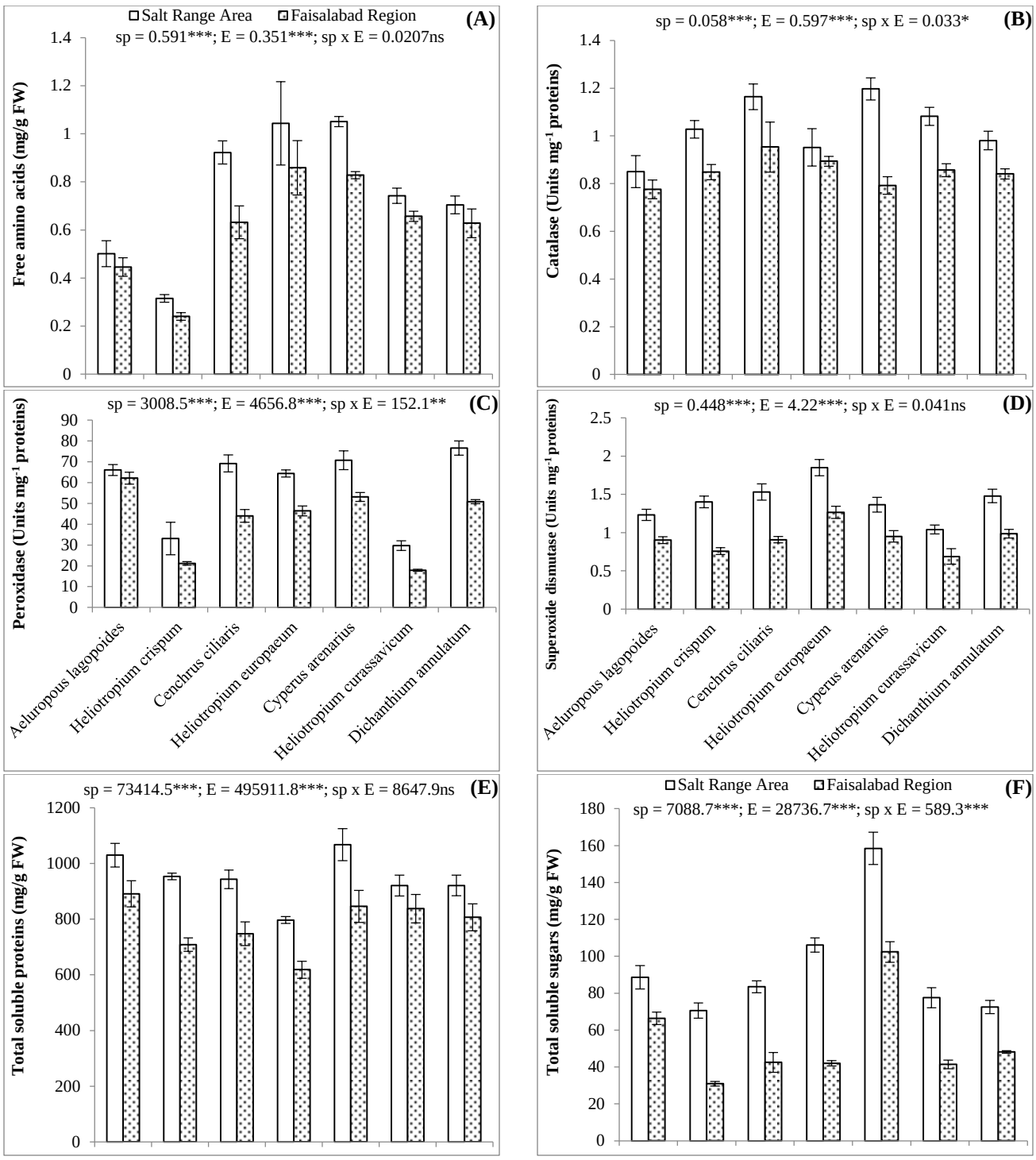

Fig. 3. (A-F): Free amino acids, total soluble proteins, total soluble sugars, activities of catalase, peroxidase and superoxide dismutase enzymes of halophytic species collected from Salt Range area and Faisalabad region Punjab, Pakistan (Mean ± S.E.). sp = Species, E= Ecotypes; ns, non-significant, *,** and ***, significant at 0.05, 0.01 and 0.001 levels, respectively.

Total free amino acids, soluble proteins and soluble sugars: Total free amino acid contents were significantly ($p \leq 0.001$) higher in the species collected from the Salt Range area. *H. europaeum*, *C. arenarius* and *C. ciliaris* showed maximum free amino acids than the other five species from the Salt Range area (Fig. 3A). Total soluble protein contents differed significantly at both sites and plants of Faisalabad region accumulated more total soluble protein contents than the plants of Salt Range (Fig. 3B). Plant species respond significantly ($p \leq 0.001$) in terms of total soluble sugar contents. *C. arenarius* and *D. annulatum* accumulated higher quantity of soluble sugars in the Salt Range area (Fig. 3C).

Activities of enzymatic antioxidants: Activities of enzymatic antioxidants (catalase, peroxidase and superoxide dismutase) differed ($p \leq 0.001$) significantly in the plant species collected from two different sites (Fig. 3D-F). Halophyte species of Salt Range area indicated higher activities of catalase, peroxidase and superoxide dismutase

enzymes. *C. arenarius* and *C. ciliaris* showed maximum activity of catalase enzyme collected from the Salt Range area. In case of Faisalabad region, *H. euroapeum* and *C. ciliaris* showed highest activity of catalase enzyme. From the Salt Range area, *D. annulatum* was highest in the activity of peroxidase enzyme. *H. europaeum* showed maximum activity of superoxide dismutase enzyme than the species collected from the Salt Range.

Correlation

Correlation test was carried-out to analyze the relationship between various physio-biochemical parameters to characterize the salinity tolerance in selected halophyte species. Shoot fresh weight was significantly correlated with shoot dry weight, root dry weight and shoot length (r = 0.6*-0.9***). Ascorbic acid contents considerably correlated with the activity of superoxide dismutase enzyme (r = 0.65*). Glycinebetaine contents was highly correlated with total soluble sugars (r = 0.603*) and proline contents significantly correlated with free amino acids (r = 0.61*).

Discussion

Global population is increasing and expected to be double in 2050 (Myers *et al*., 2015). Food requirement will also be increased in a similar fashion. Production of crops has to be increased to meet this high demand in future (Soest, 2018) and alarming due to land degradation caused by salinity, drought and urbanization (DeLong *et al*., 2015). Soil salinity is common in arid and semi-arid lands. Salinity in soils is increased due to poor quality of water, improper irrigation and use of synthetic fertilizers. Seashores and rock salts also cause the salinity in soil of their surrounding area (Goudie, 2018). Halophyte species are source of salt tolerant genetic makeup that could be used for salinity tolerance markers (Fita *et al*., 2015). Now a days, halophytes are being used as forage stuff in many salty areas. Livestock use halophyte species as feed and grazing purpose (Alam & Sharma, 2017). Salt tolerance mechanism involves compartmentalization of ions, accumulation of osmolytes, osmotic adjustment, selective uptake and transport of ions, regulation of redox and water status (Polle & Chen, 2015).

In our results, shoot fresh and dry weights of plants species was lower of Salt Range area due to high salinity level. Maximum fresh and dry weights were observed in *C. arenarius* and *C. ciliaris* L. Both of these species from the Faisalabad region have high biomass production than the Salt Range area. Root biomass was higher in *C. arenarius*, *H. curassavicum* and *D. annulatum* collected from the both research sites. Gul *et al*., (2014) demonstrated that fresh plant weight of *Alhagi pseudoahagi* was reduced under increasing salinity level. Root and shoot fresh weight of *H. mucronatum* (found in coastal areas of Karachi) decreased when salinity level was increased. Osmotic potential, water potential and stomatal conductance became negative on increasing salinity applied to *Phragmites australis* plants (Gorai *et al*., 2010).

The response of *Panicum turgidum* has been observed in terms of photosynthetic attributes and plant growth and suggested that it can be cultivated as a fodder crop on saline soil irrigated by brackish water (Hussain., 2015). Different halophyte species are being used as fodder crops in different countries i.e. *Atriplex* in Australia and Argentina (Salem *et al*., 2010), *Spartina alterniflora* and *S. bigelovii* in the United States (Proffitt *et al*., 2005) and *Haloxylon* and *Kochia* in Iran (Moghaddam & Koocheki, 2003). It has been studied that high salinity level (250 mM NaCl) decreased the photosynthetic pigments in *Plantago* spp. (Darbandi *et al*., 2018). In quinoa (*Chenopodium quinoa* Willd.), salt stress (200 mM) negatively affected the photosynthetic rate (Eisa *et al*., 2012). In this study, chlorophyll *a* and *b* contents were significantly affected by high salt level at both studying sites. Moreover, chlorophyll *a* contents were higher in *C. arenarius* and *H. curassavicum*.

It was studied that glycinebetaine contents were increased under salinity stress (Parida *et al*., 2002). GB prevents the high salt induced dissociation of extrinsic regulatory proteins in oxygen evolving complex. High GB contents also plays role in protection of photosystem and regulation of membrane structure (Fariduddin *et al*., 2013). Application of GB was applied to the roots of *Arabidopsis* and found that it alleviated the OH^- induced K^+ leakage. Many halophyte species accumulated glycinebetaine contents (> 90 µmol dry weight). Glycinebetaine contents were also increased in *Haloxylon recurvum* under salinity stress (Chen & Murata, 2011). In the current experiment, GB contents in all the species were higher under saline conditions. From Faisalabad region, *C. arenarius* and *C. ciliaris* accumulated more GB contents resulting in improved stress tolerance. From Salt Range area, *C. arenarius* and *C. ciliaris* accumulated more GB contents than the other species.

Results of the current experiment showed that halophyte species of Salt Range area accumulated more proline contents. Overall, *C. ciliaris* and *C. arenarius* were better in proline contents in the Salt Range area. While from Faisalabad region, *A. lagopoides*, *D. annulatum* and *C. arenarius* accumulated elevated levels of proline. Previously it was observed that *Arabidopsis thaliana* and *Thellungiella halophile* maintained Na^+ and K^+ concentrations along with proline (Shabala & Cuin, 2008). Apart from osmolyte function of proline, it also plays its role in quenching of ROS, maintains stability of ROS scavenging enzyme and ratio of NADPH to $NADP^+$ (Hayat *et al*., 2012). It was also studied in Arabidopsis plants that exogenous applications of proline improved the plant defensive system against superoxide radicles (Miller *et al*., 2010). Higher accumulation of proline in *Thellungiella salsuginea* and *Lipidium crassifolium* was noted under salt stress conditions (Gong *et al*., 2005). Proline contents increased due to salinity in mangrove *B. parviflora* and *Pringlea antiscorbutica* (Parida *et al*., 2002).

Phenolic compounds are water soluble antioxidants those plays vital role in scavenging of reactive oxygen species (Shuyskaya *et al*., 2015). They studied that halophyte species contained high phenolic compound accumulation and their antioxidant ability. Halophytes such

as *Tamarix gallica*, *Suaeda fruticosa*, *Limoniastrum monopetalum*, *Limoniastrum guyonianum* and *Mesembryanthemum edule* exhibited double amount of phenolic compound than glycophyte species. Highest phenolic activity was observed in salt sensitive wheat and barley cultivars, while least was observed in *Desmostchya bipinnata*, *Sporobolus marginatus*, *Panicum antidotale* and *Diplachna fusca* (Bose *et al*., 2014). In this research, phenolic contents were higher in *Cyperus* species. *Cyperus* species of Salt Range area contained more phenolic contents compared to Faisalabad region. Ascorbate and glutathione are non-enzymatic antioxidants those impart stress tolerance in plants (Abogadallah, 2010). Geissler *et al*., (2010) observed that *Lycopersicon pennellii* accumulated higher ascorbate contents than the glycophytes. In this study, ascorbic acid level increased and was higher in *C. ciliaris*, *H. europaeum* and *C. arenarius*.

High level of H_2O_2 produced under salinity stress in *Arabidopsis thaliana* and *Cineraria maritima* (Ghafar *et al*., 2019). Production of H_2O_2 under stress condition initiates signaling cascade and leads to activation of antioxidant defensive mechanism (Kreslavski *et al*., 2012). Results of our study showed that the variation existed in the samples of Faisalabad and Salt range in hydrogen peroxide concentration under salt stress. *C. ciliaris*, *D. annulatum*, and *A. lagopoides* accumulated higher contents of hydrogen peroxide in response to salinity.

In leaves of tomato, amount of soluble sugars and total saccharides increased significantly under salinity stress. Sugar contents in rice plants also increased under saline stress (Radi *et al*., 2013). Halophyte species respond significantly to total soluble sugar contents. Due to high salt level in soil and water, total soluble sugar contents improved in halophyte species. High contents of soluble sugars was observed in *C. arenarius* and *D. annulatum*. In the current experiment, total free amino acid contents were improved significantly due to highly sodium affected soil. Species of Salt Range area showed higher level of free amino acid. *H. europaeum*, *C. arenarius* and *C. ciliaris* resulted maximum free amino acids than the other species from Salt Range area. Parida *et al*., (2002) observed that soluble protein concentration increased under salinity stress. Total soluble proteins decreased in *Arachis hypogea* on increase in salinity level (Chakraborty *et al*., 2016).

Superoxide dismutase converted peroxides into hydrogen peroxide and water. It act as first line of defense under stress conditions. Number of reports showed correlation between better SOD activity and salinity stress tolerance (Miller *et al*., 2010). It depicted that halophytes have remarkable ability to enhance activity of SOD enzyme in response to stress e.g., *Avicennia marina* (Himabindu *et al*., 2016). Catalase enzyme involved in catalyzing hydrogen peroxide into oxygen and water. Turnover rate of catalase is very high and one unit of catalase can catalyze millions of H_2O_2 molecules (Sisein, 2014). Peroxidase enzyme is responsible for the protection from hydrogen peroxide advarsaries (Foyer & Noctor, 2013). Results of our work showed that enzymatic antioxidants (catalase, peroxidase and superoxide dismutase) increased significantly in halophyte species. Halophyte species of Salt Range area were higher in the activities of catalase, peroxidase and superoxide dismutase enzymes. *C. arenarius* and *C. ciliaris* showed maximum activity of catalase enzyme of Salt Range area. From Faisalabad region, *H. euroapeum* and *C. ciliaris* showed highest activity of catalase enzyme. High activity of peroxidase was observed in *D. annulatum* of Salt Range area.

Conclusion

The plants of Faisalabad region exhibited higher biomass production due to less saline soil compared to Salt Range area. The plants of Salt Range area showed elevated level of defensive mechanism (enzymatic and non-enzymatic antioxidants) to cope with high salt level. Overall, *C. ciliaris, H. europaeum* and *C. arenarius* showed higher biomass production and also other defensive mechanism (osmolyte accumulation).

Acknowledgements

Authors are thankful to Pakistan Science Foundation, Islamabad, Pakistan (PSF) for financial support through the project No. PSF-MSRT/Bio/P-GCUF (04) in collaboration with National Salinity Research Center, Yazd, Iran for this study.

References:

Abogadallah, G.M. 2010. Insights into the significance of antioxidative defense under salt stress. *Plant Signal. Behav*., 5: 369-374.

Ackerman, R.A. 2017. The nest environment and the embryonic development of sea turtles. In: *The Biol. Sea Turtles*, 1: 83-106, CRC Press, UK.

Alam, A. and V. Sharma. 2017. Potential of glycophytes and halophytes in phytoremediation of salt and metal contaminated soils: A review. *Curr. Environ. Engineer*., 4: 53-65.

Alfarrah, N. and K. Walraevens. 2018. Groundwater overexploitation and seawater intrusion in coastal areas of arid and semi-arid regions. *Water*, 10: 143.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplast polyphenol oxidase in *Beta vulgaris*. *Plant Physiol*., 24: 1-15.

Avellan, T., R. Ardakanian, S.R. Perret, R. Ragab, W. Vlotman, H. Zainal and H.A. Gany. 2018. Considering resources beyond water: irrigation and drainage management in the context of the water–energy–food nexus. *Irrig. Drain*., 67: 12-21.

Barreira, L., E. Resek, M.J. Rodrigues, M.I. Rocha, H. Pereira, N. Bandarra, M.M.D. Silva and L. Custodio. 2017. Halophytes: Gourmet food with nutritional health benefits? *J. Food. Compos. Analy*., 59: 35-42.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, 39: 205-207.

Blum, A. 2018. *Plant Breeding for Stress Environments:* CRC press.

Bose, J., A. Rodrigo-Moreno and S. Shabala. 2014. ROS homeostasis in halophytes in the context of salinity stress tolerance. *J. Exp. Bot*., 65: 1241-1257.

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

Brewster, J.L. 2018. Physiology of Crop Growth and Bulbing. In: *Onions and Allied Crops* (pp. 53-88). CRC Press.

Cairncross, S. and R. Feachem. 2018. Environmental health engineering in the tropics. *Water Sanitation Disease,* Cont Routledge.

Cakmak, I. and W.J. Horst. 1991. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiol. Plant*., 83: 463-468.

Chakraborty, K., S.K. Bishi, N. Goswami, A.L. Singh and P.V. Zala. 2016. Differential fine-regulation of enzyme driven ROS detoxification network imparts salt tolerance in contrasting peanut genotypes. *Environ. Exp. Bot*., 128: 79-90.

Cheeseman, J. 2016. Food security in the face of salinity, drought, climate change, and population growth. In *Halophytes for Food Security in Dry Lands* (pp. 111-123). Academic Press.

Chen, T.H. and N. Murata. 2011. Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. *Plant Cell Environ*., 34: 1-20.

Darbandi, M., S. Darbandi, A. Agarwal, P. Sengupta, D. Durairajanayagam, R. Henkel and M.R. Sadeghi. 2018. Reactive oxygen species and male reproductive hormones. *Rep. Biol. Endocrinol*., 16: 87.

DeLong, C., R. Cruse and J. Wiener. 2015. The soil degradation paradox: Compromising our resources when we need them the most. *Sustainability*, 7: 866-879.

Dubo, S.M., K.A. Giles, J.K. Hamiton, P.A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substance. *Anal. Chem*., 28: 350.

Eisa, S., S. Hussin, N. Geissler and H.W. Koyro. 2012. Effect of NaCl salinity on water relations, photosynthesis and chemical composition of quinoa (*Chenopodium quinoa* 'Willd.) as a potential cash crop halophyte. *Aust. J. Crop Sci*., 6(2): 357.

Fariduddin, Q., P. Varshney, M. Yusuf, A. Ali and A. Ahmad. 2013. Dissecting the role of glycinebetaine in plants under abiotic stress. *Plant Stress*, 7(1): 8-18.

Fita, A., A. Rodríguez-Burruezo, M. Boscaiu, J. Prohens and O. Vicente. 2015. Breeding and domesticating crops adapted to drought and salinity: a new paradigm for increasing food production. *Front. Plant Sci*., 6: 978.

Flowers, T.J. and T.D. Colmer. 2015. Plant salt tolerance: adaptations in halophytes. *Ann. Bot*., 115(3): 327-331.

Foyer, C.H. and G. Noctor. 2013. Redox signaling in plants. *Antioxid. Redox Signal*., 18: 2087-2090.

Geissler, N., S. Hussin and H.W. Koyro. 2010. Elevated atmospheric CO_2 concentration enhances salinity tolerance in *Aster tripolium* L. *Planta,* 231(3): 583-594.

Ghafar, M.A., N.A. Akram, M. Ashraf, Y. Ashraf and M. Sadiq. 2019. Thiamin-induced variations in oxidative defense processes in white clover (*Trifolium repens* L.) under water deficit stress. *Turk. J. Bot*., 43(1): 58-66.

Ghori, N.H., T. Ghori, M.Q. Hayat, S.R. Imadi, A. Gul, V. Altay and M. Ozturk. 2019. Heavy metal stress and responses in plants. *Int. J. Environ. Sci. Technol*., 16(3): 1807-1828.

Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol*., 59(2): 309-314.

Glenn, E.P., T. Anday, R. Chaturvedi, R. Martinez-Garcia, S. Pearlstein, D. Soliz and R.S. Felger. 2013. Three halophytes for saline-water agriculture: An oilseed, a forage and a grain crop. *Environ. Exp. Bot*., 92: 110-121.

Gong, Q., P. Li, S. Ma, S.I. Rupassara and H.J. Bohnert. 2005. Salinity stress adaptation competence in the extremophile *Thellungiella halophila* in comparison with its relative *Arabidopsis thaliana*. *Plant J*., 44(5): 826-839.

Gorai, M., M. Ennajeh, H. Khemira and M. Neffati. 2010. Combined effect of NaCl-salinity and hypoxia on growth, photosynthesis, water relations and solute accumulation in *Phragmites australis* plants. *Flora*, 205(7): 462-470.

Goudie, A.S. 2018. *Human Impact on the Natural Environment*. John Wiley & Sons.

Grieve, C.M and S.R. Grattan. 1983. Rapid assay for determination of water-soluble quaternary ammonium compounds. *Plant Soil*, 70: 303-307.

Gul, B., R. Ansari, H. Ali, M.Y. Adnan, D.J. Weber, B.L. Nielsen and M.A. Khan. 2014. The sustainable utilization of saline resources for livestock feed production in arid and semi-arid regions: A model from Pakistan. *Emir. J. Food Agric*., 26(12): 1032-1045.

Hanin, M., C. Ebel, M. Ngom, L. Laplaze and K. Masmoudi. 2016. New insights on plant salt tolerance mechanisms and their potential use for breeding. *Front. Plant Sci*., 7: 1787.

Hasanuzzaman, M., K. Nahar, M. Alam, R. Roychowdhury and M. Fujita. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci*., 14(5): 9643-9684.

Hayat, S., Q. Hayat, M.N. Alyemeni, A.S. Wani, J. Pichtel and A. Ahmad. 2012. Role of proline under changing environments: a review. *Plant Signal. Behav*., 7(11): 1456-1466.

He, M., C.Q. He and N.Z. Ding. 2018. Abiotic stresses: general defenses of land plants and chances for engineering multi-stress tolerance. *Front. Plant Sci*., 9: 1245.

Himabindu, Y., T. Chakradhar, M.C. Reddy, A. Kanygin, K.E. Redding and T. Chandrasekhar. 2016. Salt-tolerant genes from halophytes are potential key players of salt tolerance in glycophytes. *Environ. Exp. Bot*., 24: 39-63.

Hussain, T. 2015. The effect of salinity on morphological, physiological and biochemical responses of *Panicum turgidum* Forssk. (Doctoral dissertation, University of Karachi).

Julkenen-Titto, R. 1985. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *J. Agric. Food Chem*., 33: 213-217.

Kreslavski, V.D., D.A. Los, S.I. Allakhverdiev and V.V. Kuznetsov. 2012. Signaling role of reactive oxygen species in plants under stress. *Russ. J. Plant Physiol*., 59(2): 141-154.

LeMonte, J.J., J.W. Stuckey, J.Z. Sanchez, R. Tappero, J. Rinklebe and D.L. Sparks. 2017. Sea level rise induced arsenic release from historically contaminated coastal soils. *Environ. Sci. Technol*., 51(11): 5913-5922.

Levidow, L., D. Zaccaria, R. Maia, E. Vivas, M. Todorovic and A. Scardigno. 2014. Improving water-efficient irrigation: Prospects and difficulties of innovative practices. *Agric. Water Manag*., 146: 84-94.

Maehly, A.C. and B. Chance. 1954. Assay of catalases and peroxidases. *Meth. Biochem. Anal*., 2: 764-817.

Maleki, P., S. Saadat, H.A. Bahrami, H. Rezaei and L. Esmaeelnejad. 2019. Accumulation of ions in shoot and seed of quinoa (*Chenopodium quinoa* Willd.) under salinity stress. *Comm. Soil Sci. Plant Anal*., 50(6): 782-793.

Miller, G., N. Suzuki, S. Ciftci-Yilmaz and R. Mittler. 2010. Reactive oxygen species homeostasis and signaling during drought and salinity stress. *Plant Cell Environ*., 33(4): 453-467.

Moghaddam, P.R. and A. Koocheki. 2003. A comprehensive survey of halophytes in Khorasan province of Iran. In *Cash Crop Halophytes*: Recent Studies (pp. 189-195). Springer, Dordrecht.

Mukherjee, S.P. and M.A. Choudhuri. 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiol. Plant*., 58(2): 166-170.

Myers, S.S., K.R. Wessells, I. Kloog, A. Zanobetti and J. Schwartz. 2015. Effect of increased concentrations of atmospheric carbon dioxide on the global threat of zinc deficiency: a modelling study. *Lancet Glob. Health,* 3(10): e639-e645.

Parida, A., A.B. Das and P. Das. 2002. NaCl stress causes changes in photosynthetic pigments, proteins, and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. *J. Plant Biol*., 45(1): 28-36.

Parihar, P., S. Singh, R. Singh, V.P. Singh and S.M. Prasad. 2015. Effect of salinity stress on plants and its tolerance strategies: a review. *Environ. Sci. Pollut. Res*., 22(6): 4056-4075.

Polle, A. and S. Chen. 2015. On the salty side of life: molecular, physiological and anatomical adaptation and acclimation of trees to extreme habitats. *Plant Cell Environ*., 38(9): 1794-1816.

Proffitt, C.E., R.L. Chiasson, A.B. Owens, K.R. Edwards and S.E. Travis. 2005. *Spartina alterniflora* genotype influences facilitation and suppression of high marsh species colonizing an early successional salt marsh. *J. Ecol*., 93(2): 404-416.

Qadir, M., E. Quillérou, V. Nangia, G. Murtaza, M. Singh, R.J. Thomas and A.D. Noble. 2014. Economics of salt-induced land degradation and restoration. *Nat. Resour. Forum*, 38(4): 282-295.

Radi, A.A., F.A. Farghaly and A.M. Hamada. 2013. Physiological and biochemical responses of salt-tolerant and salt-sensitive wheat and bean cultivars to salinity. *J. Biol. Earth Sci*., 3(1): 72-88.

Rahman, M.M., M.A. Rahman, M.G. Miah, S.R. Saha, M.A. Karim and G. Mostofa. 2017. Mechanistic insight into salt tolerance of *Acacia auriculiformis*: the importance of ion selectivity, osmoprotection, tissue tolerance, and Na^+ exclusion. *Front. Plant Sci*., 8: 155.

Reddy, M.P., M.T. Shah and J.S. Patolia. 2008. *Salvadora persica*, a potential species for industrial oil production in semi-arid saline and alkali-soils. *Ind. Crops Prod*., 28: 273-278.

Safdar, H., A. Amin, Y. Shafiq, A. Ali, R. Yasin, A. Shoukat and M.I. Sarwar. 2019. A review: Impact of salinity on plant growth. *Nature Sci*., 17(1): 34-40.

Salem, H.B., H.C. Norman, A. Nefzaoui, D.E. Mayberry, K.L. Pearce and D.K. Revell. 2010. Potential use of oldman saltbush (*Atriplex nummularia* Lindl.) in sheep and goat feeding. *Small Ruminant Res*., 91(1): 13-28.

Sanada, Y., H. Ueda, K. Kuribayashi, T. Andoh, F. Hayashi, N. Tamai and K. Wada. 1995. Novel light-dark change of proline levels in halophyte (*Mesembryanthemum crystallinum* L.) and glycophytes (*Hordeum vulgare* L. and *Triticum aestivum* L.) leaves and roots under salt stress. *Plant Cell Physiol*., 36: 965-970.

Shabala, S and T.A. Cuin. 2008. Potassium transport and plant salt tolerance. *Physiol. Plant*., 133(4): 651-669.

Shrivastava, P. and R. Kumar. 2015. Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J. Biol. Sci*., 22(2): 123-131.

Shuyskaya, E., Z. Rakhmankulova, P. Voronin, N. Kuznetsova, G. Biktimerova and I. Usmanov. 2015. Salt and osmotic stress tolerances of the C_3–C_4 xero-halophyte *Bassia sedoides* from two populations differ in productivity and genetic polymorphism. *Acta Physiol. Plant*., 37(11): 236.

Sisein, E.A. 2014. Biochemistry of free radicals and antioxidants. *Scholars Acad. J. Biosci*., 2(2): 110-118.

Slama, I., C. Abdelly, A. Bouchereau, T. Flowers and A. Savoure. 2015. Diversity, distribution and roles of osmo-protective compounds accumulated in halophytes under abiotic stress. *Ann. Bot*., 115(3): 433-447.

Soest, V.P.J. 2018. *Nutritional Ecology of the Ruminant*. Cornell University Press.

Velikova, V., I. Yordanov and A. Edreva. 2000. Oxidative stress and some antioxidant systems in acid rain treated bean plants: protective role of exogenous polyamines. *Plant Sci*., 151: 59-66.

Vlaeminck, B., P.A. Abrahamse, V. Fievez, M. Lourenco, J. Dijkstra and S. Tamminga. 2010. The effect of allocation frequency in rotational grazing systems on the fatty acid profile in milk fat of dairy cows. In *23th General Meeting of the European Grassland Federation, Kiel, Germany*, 15: 102-102.

Xuan, V.T. 2018. *Rice Production, Agricultural Research, and the Environment*. In Vietnam's Rural Transformation, pp. 185-200, Routledge.

Yemm, E.W., and A.J. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.,* 57(3): 508.

(Received for publication 10 June 2021)