

## ROLE OF PHYSIOLOGICAL PLASTICITY IN ADAPTABILITY OF SOME NATIVE GRASSES TO HYPER-SALINE ENVIRONMENTS

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### Abstract

Six grasses *Ochthochloa compressa* (Oc), *Lasiurus scindicus* (Ls), *Panicum antidotale* (Pa), *Cymbopogon jwarancusa* (Cj), *Leptochloa fusca* (Lf), *Aeluropus lagopoides* (Al) collected from the study range of Kallar Kahar (North Punjab) to Cholistan (South Punjab) were tested for role of plasticity in physiological attributes in salt-adaptability. Samples of each species collected from three different sites varying in soil salinity along with their rhizospheric soil. Soil samples collected from depths of 0-5cm, 15-20cm and 25-30cm and packed in polythene zipper bags with proper labeling. Higher Na and K concentrations noted in soil samples in most of the sites (HsP, KKr, KnP, PkA and 87A) building higher soil ECe. Results showed general decreasing trend of ECe and Na with increasing depths of soil in all sites. However, variable results for soil moisture contents (MC), pH, organic matter, K, Ca, Mg, P, NO<sub>3</sub><sup>-</sup>, NH<sub>3</sub> and Cl<sup>-</sup> recorded. Among bio-chemical parameters of shoot, Na, Na/K ratio, Ca and P increased with higher levels of salinity while Mg and Cl behaved variably. Shoot Na, Na/K ratio, Ca, Mg and P contents positively correlated with ECe and Na concentration in soil. In comparison, shoot FW, DW, K and N showed a negative correlation with soil ECe and Na. In conclusion, hyper accumulation of Na, K, organic osmotica, anti-oxidative enzymes (SOD, POD, and CAT) identified as possible adaptability mechanisms for survival of the tolerant grasses under saline environments.

**Key words:** Physiological plasticity, Hyper-saline environments, Native grasses, Varying soil depth, Rhizospheric soil.

**Abbreviations for sites:** 1 = Noorpur Thal (NpT); 2 = Khanpur (KnP); 3 = Kallar Kahar (KKr); 4 = Khushab (Ksb); 5 = 87A-Feroza (87A); 6 = Hasilpur (HsP); 7 = Perrowal (Pwl); 8 = Khanewall (Kwl); 9 = Paka Anna (PkA); 10 = Noorpur Thal (NpT); 11 = Yazman (Yaz); 12 = Khanpur (KnP); 13 = Chak#70-Feroza (H70); 14 = Sahianwala (Shw); 15 = 87A-Feroza (87A); 16 = Noorpur Thal (NpT); 17 = Khushab (Ksb); 18 = Kallar Kahar (KKr)

**Abbreviations for parameters:** S-FW = Shoot fresh weight; S-DW = Dry weight, S-Na = Sodium, S-K = Potassium, S-Na-K = Sodium-potassium ratio, S-Ca = Calcium, S-Mg = magnesium, S-P = Phosphorus, S-N = Nitrogen, S-Prol = Shoot Proline; S-Prot = Protein; S-Sug = Total soluble sugars; S-SOD = Superoxide dismutase; S-POD = Peroxide dismutase; S-APX = Ascorbate peroxidase; S-AA = Total amino acids, S-CAT = Catalase

### Introduction

All abiotic components (temperature, water, radiations, nutrients, soil, wind etc.) prevail within optimal range for the growth of the natural plant populations. However, plants often grow in environments exhibiting sub- or supra-optimal intensities of the essential environmental components. Deviation of these abiotic components from their normal regimes is stress. The most common stresses endeavored by plants are heat and cold (temperature), drought and water-logging, salinity, heavy metals and many other pollutants (Nouri, *et al.*, 2017). Plants growing in environmentally stressed regions show a number of tolerance mechanisms. These mechanisms regulate growth by activating genes involved in signaling of ion homeostasis, free radicals disposing and scavenging of toxic ions (Ahanger & Agarwal, 2017). These plants develop certain modifications in growth, flowering and fruiting patterns by regulating certain physiological, anatomical and morphological mechanisms to cope with such environmental adversities (Mustafa *et al.*, 2019).

Grasses are the largest group of the flowering plants with about 10,000 species in 610 genera (Farooq *et al.*, 2009). Grasses are widely distributed throughout the world as compared to other flowering plants. Different grasses form a large proportion of vegetation in the semi-

arid regions. Some species of grasses provide food as cereals for human and fodder for animals (Farooq *et al.*, 2009). The grasses selected in study included *Leptochloa fusca*, *Panicum antidotale*, *Cymbopogon jwarancusa*, *Lasiurus scindicus*, *Aeluropus lagopoides* and *Ochthochloa compressa*. These grasses are widely found in the saline and arid region of Pakistan and form dense populations. A wide variety of fauna located in the desertified regions relies on these grasses for food, forage and shelter. These grasses had co-evolved with the local climate and soil type. Such native grasses provide optimum habitat conditions to native fauna and have high value as fodder (Chaudhry *et al.*, 2006).

Physiological adjustments to survive involve plasticity in certain morphological and physiochemical attributes such as accumulation of osmoprotectants like proline in plants growing in saline environments. This shown to correlates positively with the degree of salt adaptability by various researchers and is a function of the genetic makeup of different species (Hameed *et al.*, 2008; Bibi *et al.*, 2021). Many antioxidants such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) been reported to increase in grasses during salt stress (Ma *et al.*, 2011). The salt tolerance ability of these grasses had a clear linked to the higher activities of the anti-oxidative defense system (Esfandiari *et al.*, 2007).

Adaptability mechanisms in plants are complex involving various morphological, anatomical, molecular and genetic aspects. Efforts are under way to explore these adaptability mechanisms for salt tolerance in natural vegetation using conventional and advanced methods. In this study, plasticity in conventional morphological characters along with some biochemical and physiological aspects were used as marker to explore the adaptability mechanisms in selected grasses collected from hyper-saline environments.

## Materials and Method

Different salt affected sites selected from three distinct eco-regions of Punjab including Cholistan, Salt range and Thal desert. Though all six grasses selected in this study are perennial, they become dormant during cold and dry weather of winter, therefore, the collection of plant samples done at vegetative stage during active growing season from April to October of 2017 and 2018. Rhizospheric soil of each plant species was also sampled at three depths, *i.e.*, at 0-5, 10-15 and 25-30 cm depths. Soil physicochemical characteristics were measured for samples collected from various habitats. Plants uprooted with the help of soil auger for the study of soil and plant shoot biochemical (Table 1).

Among soil physicochemical parameters, ionic content ( $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ), saturation percentage, extractable N, P, K, moisture content, organic matter, soil texture, moisture contents, ECe and pH measured from saturation paste extract using the standard protocols. The concentrations of Na, K and Ca in soil were analysed by Flame Photometer (Jenway PFP-7, England). The Mg content of the soil extract was determined by atomic absorption spectrophotometer (AAAnalyst 300, Perkin Elmer, Germany). Phosphate contents in samples determined by using the method proposed by Yoshida (1976). The N estimated by converting it into ammonium sulphate by titration method after Allen (1953). Soluble chloride contents were measured by silver nitrate titration method following Richards (1954). Organic contents of the soil measured with the method of Allison & Black (1965).

Dried plant material (shoot) was grinded in a dry-mill. One gram of this dried material digested with sulfuric acid and hydrogen peroxide after the Wolf method (1982) and concentrations of Na, K, and Ca in shoot analyzed by flame photometer (Jenway PFP-7, Japan). Activity of SOD was calculated by measuring its ability to stop the photo reduction of nitro-blue-tetrazolium (NBT) following the method of Giannopolitis & Ries (1977). While activities of CAT and POD determined by using the method of Chance & Machly (1955). Protein content of the extract measured following Bradford (1976). Total free amino acids were calculated according to Hamilton & van-Slyke (1973). Free proline was spectrophotometrically determined using the protocol of Bates *et al.*, (1973). Soluble sugar contents were determined by following the Yemm & Willis (1954) method.

Table 1. Habitat details of the six grasses collected from various eozones of Punjab Province.

Site No.	Species	Abb.	Site name	Abb.	Habitat type	Date of sample collection	Soil texture	Latitude (°N)	Longitude (°E)	Altitude (m)	Annual rainfall (mm)	Max. Temp. (°C)
01.			Noorpur Thal	NpT	Desert	Sep., 2017	Sandy loam	31.87807	71.903099	185.325	274	42
02.	<i>Ochthochloa compressa</i>	Oc	Khanpur	KnP	Saline Arid	Oct., 2017	Sandy loam	28.633183	70.657369	87.354	112	42.9
03.			Kallar Kahar	KKr	Saline Semi-Arid	Sep., 2017	Clay loam	32.769991	72.706833	644.469	485	39
04.			Khushab	Ksb	Semi Semi-Arid	Oct., 2017	Clay loam	32.294956	72.320033	189.373	400	42
05.	<i>Lasiurus scindicus</i>	Ls	87A-Feroza	87A	Desert	Oct., 2017	Loamy sand	28.790937	70.930894	91.813	112	42.9
06.			Hasilpur	HsP	Desert	Oct., 2017	Loamy sand	29.703066	72.584096	137.974	166	43
07.			Perrowal	Pwl	Arid	Oct., 2017	Loamy sand	30.331765	72.035366	140.000	166	42.3
08.	<i>Panicum antidotale</i>	Pa	Khanewall	Kwl	Arid	Oct., 2017	Loamy sand	30.292049	71.944729	135.103	166	42.3
09.			Paka Anna	PkA	Saline Semi-arid	Oct., 2017	Clay loam	31.244301	72.793989	175.755	328	41.4
10.			Noorpur Thal	NpT	Desert	Sep., 2017	Loamy sand	31.880548	71.911339	185.633	274	42
11.	<i>Cymbopogon jwarancusa</i>	Cj	Yazman	Yaz	Desert	Sep., 2017	Loamy sand	29.006427	71.744056	114.581	137	42.4
12.			Khanpur	KnP	Desert	Sep., 2017	Loamy sand	28.638908	70.662519	88.950	112	42.9
13.			Chak#70-Feroza	H70	Semi-arid	Mar., 2018	Clay loam	28.756381	70.806421	93.843	101	43.4
14.	<i>Leptochloa fusca</i>	Lf	Sahianwala	Shw	Saline Semi-arid	Mar., 2018	Clay loam	31.639083	73.234515	192.875	335	40.8
15.			87A-Feroza	87A	Desert	Mar., 2018	Sandy loam	28.791087	70.93716	93.302	112	42.9
16.			Noorpur Thal	NpT	Desert	Apr., 2018	Loamy sand	31.993848	71.982407	187.441	274	42
17.	<i>Aeluropus lagopoides</i>	Al	Khushab	Ksb	Saline Semi-arid	Apr., 2018	Loamy sand	32.292199	72.321406	186.901	400	42
18.			Kallar Kahar	KKr	Saline Semi-Arid	Apr., 2018	Clay loam	32.777808	72.715343	647.995	485	39

**Statistical analysis:** The data were analyzed statistically using analysis of variance (ANOVA). The LSD values so calculated used to determine the significance of soil physico-chemical and biochemical attributes of different grass species at varying soil depths or habitat types (whichever applicable). A Redundancy Analysis (RDA) performed using CONACO for Windows (v 4.5). RDA biplots (species with environmental variables) and Response Curves (RC) were prepared in CanoDraw (v 4.0) package supplied with CANOCO. The Pearson correlation coefficient ( $r$ ) of various morpho-anatomical attributes of individual grasses with soil physico-chemical attributes was also determined. For presentation of data in graphical form, all sites for given species were arranged in order of increasing salinity gradients and ECe values rounded to nearest zero. Capital letters (A, B, C) represented significance of species means and reflected by different colors of alphabets. The small letters (a, b, c) represented significance of habitat means for a given species.

## Results

Shoot fresh weight of six grasses collected from various saline habitats showed a general decrease with increasing salinity. *Cj* exhibited the maximum shoot fresh weight that differed non-significantly from *Pa*, *Lf* and *Al*. *Oc* showed the lowest but highly significantly different shoot fresh weight. Shoot dry weight showed significant differences among various habitats as well as within grass species parallel to the shoot fresh weight. The maximum shoot dry weight exhibited by *Cj* differed non-significantly from *Pa* but significantly from all other species (*Ls*, *Lf*, *Oc* and *Al*). Among three sites of *Cj*, the ecotypes collected from the least saline habitat NpT (31 dS m<sup>-1</sup>) showed the maximum shoot dry weight that decreased significantly in ecotypes from habitats with higher soil salinities i.e. Yaz (38 dS m<sup>-1</sup>) and KnP (42 dS m<sup>-1</sup>). A similar gradual decrease in shoot dry weight was observed in all three ecotypes of *Oc* (NpT, KnP, KKr), *Ls* (Ksb, 87A, HsP), *Pa* (Pwl, Knw, PkA), *Lf* (H70, Shw, 87A) and *Al* (NpT, Ksb, KKr) that corresponded to the soil salinities of their respective habitats (Fig. 1).

Shoot Na concentration of all six species of saline habitats differed significantly from each other. Among all species, the maximum Na concentration as noted in *Al* differed significantly from other grasses. The second highest Na concentration noted in *Lf* differed significantly with *Pa* and *Ls*, though both these species exhibited statistically non-significant differences for shoot Na concentration. The least statistically non-significant shoot Na concentration found in two grasses *Cj* and *Oc*. The maximum K concentration among shoots of six grasses species noted in H70 habitat of *Lf* differed significantly from all other grass species. Grasses like *Ls*, *Pa*, *Cj* and *Al* generally showed non-significant differences, while *Oc* showed the lowest but significantly different shoot K concentration. The shoot Na/K ratio calculated for all six species of grasses showed the maximum Na/K ratio exhibited by *Al* which differed significantly from all other grasses i.e. *Lf*, *Pa*, *Ls*, and *Oc*. However, all these grasses differed non-significantly for Na/K ratio among each other. The least significantly lower Na/K ratio among all six grasses observed for *Cj* (Fig. 2).

Generally, the ecotypes collected from hyper-saline habitats exhibited higher Na/K ratio that was significantly lower in less saline habitats. The maximum Na/K ratio noted in KKr ecotype of *Al* growing at 48 dS m<sup>-1</sup> salinity level and minimum in Yaz ecotype of *Cj* with soil salinity level of 38 dS m<sup>-1</sup>. The comparison of different *Oc* ecotypes showed that the maximum Na/K ratio as noted in KKr ecotype of highly saline habitat (55 dS m<sup>-1</sup>) differed significantly from ecotypes of NpT and KnP habitats with relatively lower salinity levels. In *Ls*, the maximum Na/K ratio observed in HsP (45 dS m<sup>-1</sup>). The Na/K ratio of PkA ecotype of *Pa* differed significantly from ecotypes of other two sites. The 87A ecotype of *Ls* and Pwl, and Knw ecotypes of *Pa* differed significantly from the rest of ecotypes. All three sites of *Cj* (NpT, Yaz, and KnP) showed non-significant differences regardless of soil salinity levels. A greater Na/K value as noted in Yaz and KnP habitats of *Lf* with higher salinity levels differed non-significantly from each other but significantly from least saline H70 habitat (33 dS m<sup>-1</sup>). The maximum Na/K ratio as noted in KKr habitats of *Al* ecotype differed significantly from other two lesser saline habitats i.e. NpT and Ksb (Fig. 2).

Of all six grasses, the maximum Ca content in shoot as noted in *Al* ecotype of highly saline KKr habitat differed non-significantly from *Ls*. However, it was significantly different from *Oc*, *Pa*, *Cj* and *Lf*. All three ecotypes of *Oc* showed non-significant differences in shoot Ca concentration regardless of the soil salinity levels of native habitats. In comparison, the soil salinity of the parent habitat did not showed much significant influence on Mg concentration of the ecotypes under study. The shoot Mg content in all six species differed non-significantly among species (Fig. 3). Shoot P concentration significantly influenced by soil salinity of the parent habitat. Overall, shoot P content increased with increase in soil salinity of different habitats except *Lf* showing significant decrease in shoot P content with increase in soil salinity levels. The maximum P content as noted in *Lf* differed non-significantly to *Pa* while it differed significantly from *Oc*, *Ls* and *Cj* ecotypes. The least P contents recorded in *Al* specie (Fig. 4). Shoot N concentration in populations of six species collected from various saline sites of Punjab province varied significantly among various habitats as well as with soil depth. Results showed that the maximum N<sub>2</sub> accumulation as noted in *Ls* and *Pa* differed non-significantly to each other but significantly from all other grass species including *Oc*, *Cj* *Lf* and *Al*. The *Oc* plants collected from NpT, KnP and KKr sites showed non-significant differences in shoot nitrogen concentration regardless of soil salinity levels of their habitats. While all *Ls* (Ksb, 87A, and HsP habitats), and, *Pa* ecotypes (Pwl, Knw, and PkA habitats) showed a significant decrease in shoot N<sub>2</sub> concentration with increasing soil salinity levels. The *Cj* ecotype from moderately saline Yaz (38 dS m<sup>-1</sup>) habitat differed significantly in N<sub>2</sub> concentration from the least saline NpT (31dS m<sup>-1</sup>) and hyper-saline KnP (42dS m<sup>-1</sup>) ecotypes, both of which differed non-significantly to each other. Similarly, *Lf* ecotypes from the least saline H70 (33 dS m<sup>-1</sup>) differed significantly from Shw (40dS m<sup>-1</sup>) and 87A (43 dS m<sup>-1</sup>) ecotypes. However, *Lf* from Shw and 87A sites differed non-significantly to each other. The shoot N concentration in all three ecotypes of *Al* decreased significantly with gradual increase in soil salinity levels from 31 dS m<sup>-1</sup> (NpT) to 41 dS m<sup>-1</sup> (Ksb) and further to 48 dS m<sup>-1</sup> (KKr) of habitats (Fig. 4).

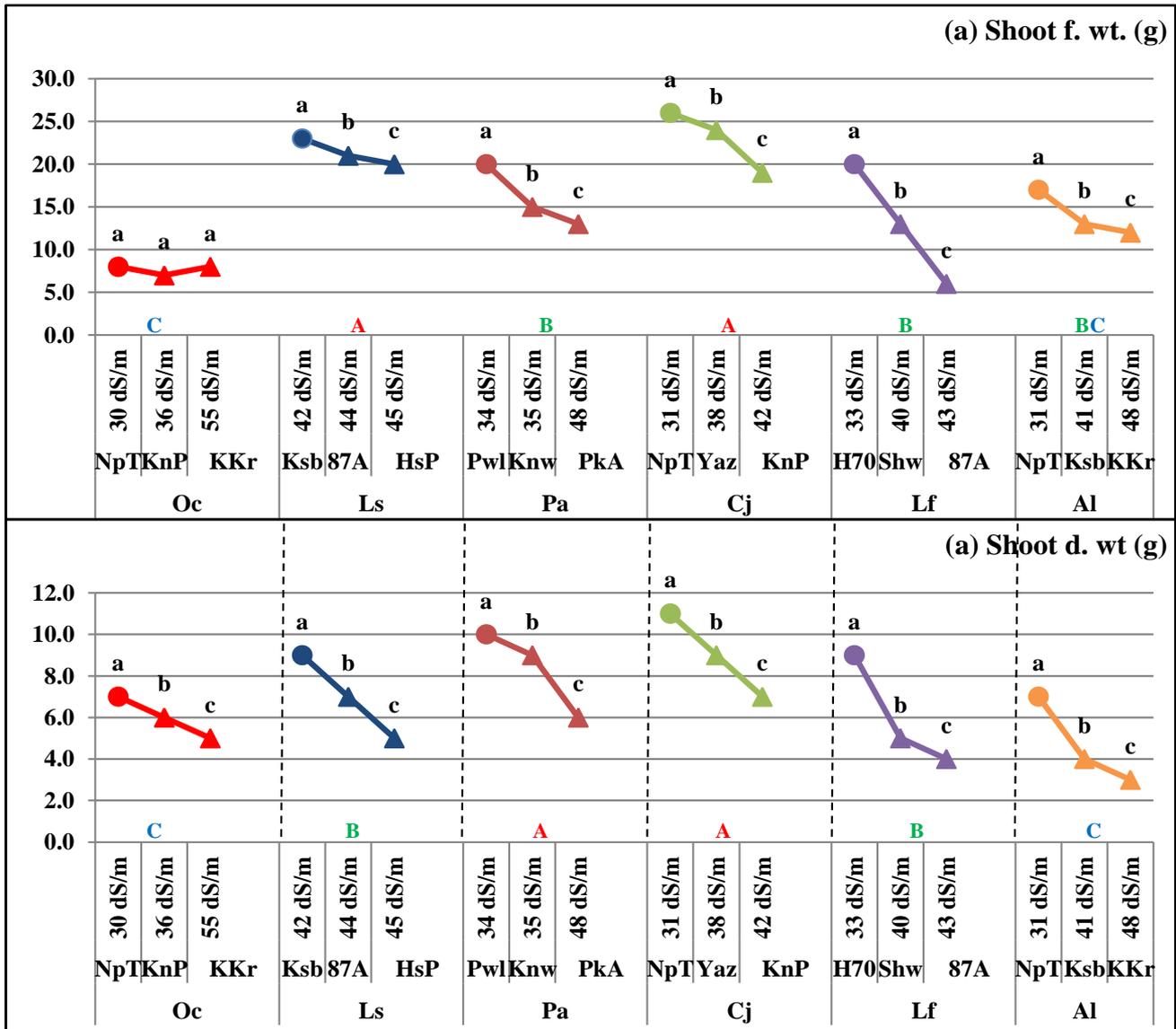


Fig. 1. Mean values for shoot fresh and dry weight of the plant samples collected from various saline habitats of the Punjab province.

Among various grasses, the maximum shoot proline as noted in *Cj* differed non-significantly from *Oc* and *Lf*, while it differed significantly from other three grasses i.e. *Ls*, *Pa* and *Al*. The ecotypes of *Oc*, *Ls* and *Pa* from all three habitats exhibited a general increasing trend of proline with increasing salinity while it was not true for *Cj*, *Lf* and *Al* showing a decreasing trend with increasing salinity of parent habitat. Among *Oc* ecotypes, the maximum proline content as noted in the plants collected from highly saline *KKr* site differed significantly from ecotypes collected from lesser saline habitat *KnP* and *NpT*. Similarly, the maximum shoot proline concentration noted in *Ls* ecotypes from *HsP* and *Pa* ecotypes of *PkA*, both of which collected from the habitat of highest salinity levels. These ecotypes differed significantly in *Ls* ecotype from relatively lower soil salinities (*87A* and *Ksb*), and *Pa* of (*Knw* and *Pwl*), respectively (Fig. 5).

The total protein contents showed a little difference among various grasses collected from different habitats. The *Oc*, *Pa* and *Lf* exhibited non-significant differences while the *Ls*, *Cj* and *Al* exhibited significantly differences in shoot protein contents. Total amino acid

of six species collected from different habitats of Punjab exhibited statistically non-significant differences for inter-specific and inter-habitat terms. Overall, the maximum total amino acid contents as noted in *Pa* and *Ls* decreased significantly from all other species. The total amino acids of *Oc*, *Ls*, *Cj* and *Lf* had non-significant differences among each other (Fig. 6). The *Oc* ecotypes collected from all three sites (*NpT*, *KnP*, and *KKr*) and *Cj* ecotypes (*NpT*, *Yaz*, and *KnP*) differed non-significantly to each other regardless of the salinity levels of their respective habitats. The *Ls* from ecotype from *Ksb* (42 dS m<sup>-1</sup>), *Pa* ecotype from *Pwl* (34 dS m<sup>-1</sup>) and *Lf* ecotype from *H70* (33dS m<sup>-1</sup>), all of which were collected from least saline habitats, showed the maximum accumulation of total amino acids that decreased significantly in ecotypes from more saline habitats i.e. *Ls* (*87A*, *HsP*), *Pa* (*Knw*, *PkA*) and *Lf* (*Shw*, *87A*). Among *Al* ecotypes collected from moderately saline *Ksb* habitat (41 dS m<sup>-1</sup>) showed the maximum total amino acids contents that decreased significantly from less saline *NpT* (31 dS m<sup>-1</sup>) and hyper-saline *KKr* (48 dS m<sup>-1</sup>) ecotypes (Fig. 6).

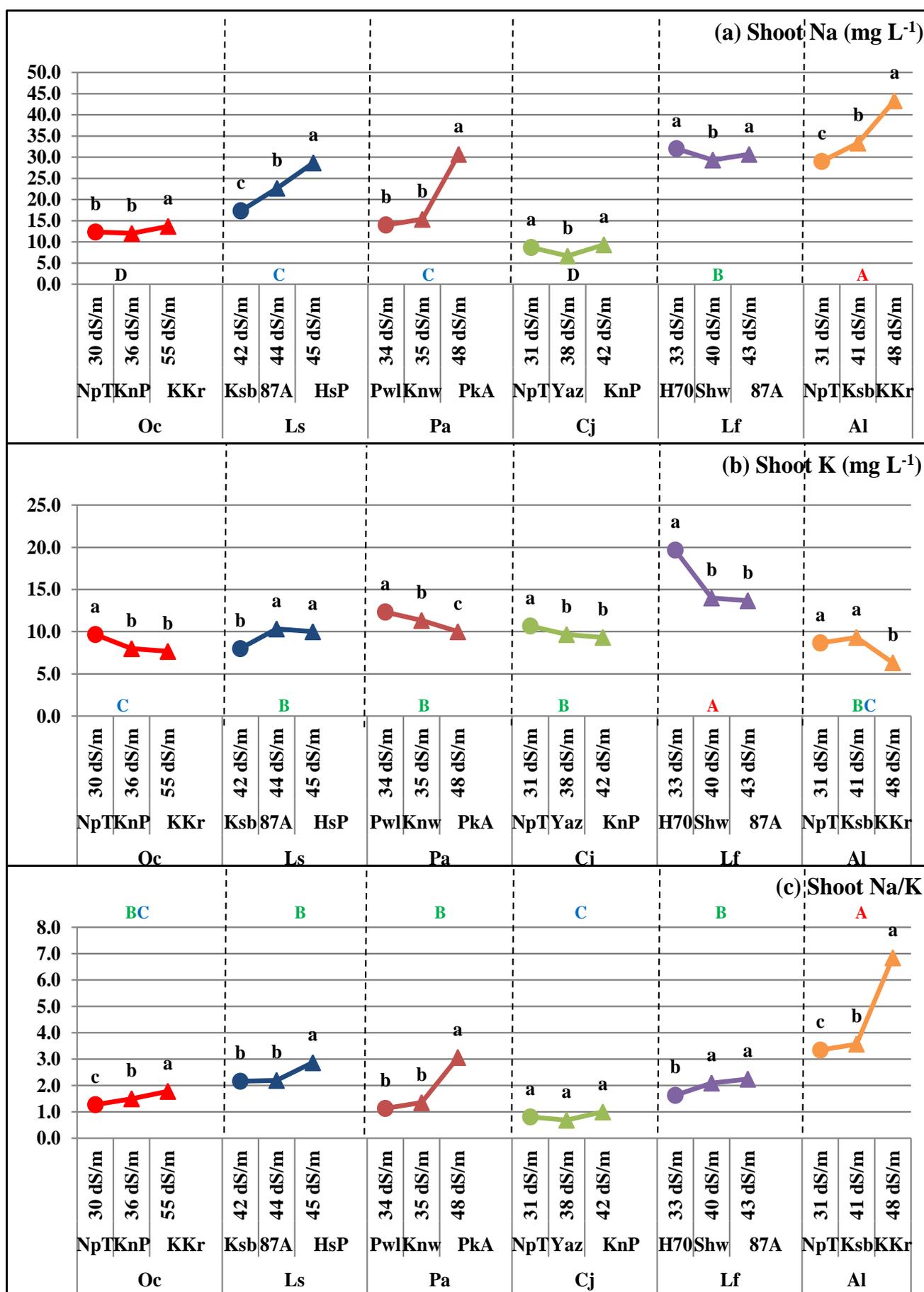


Fig. 2. Mean values for shoot Na, K and Na/K ratio of the plant samples collected from various saline habitats of the Punjab province.

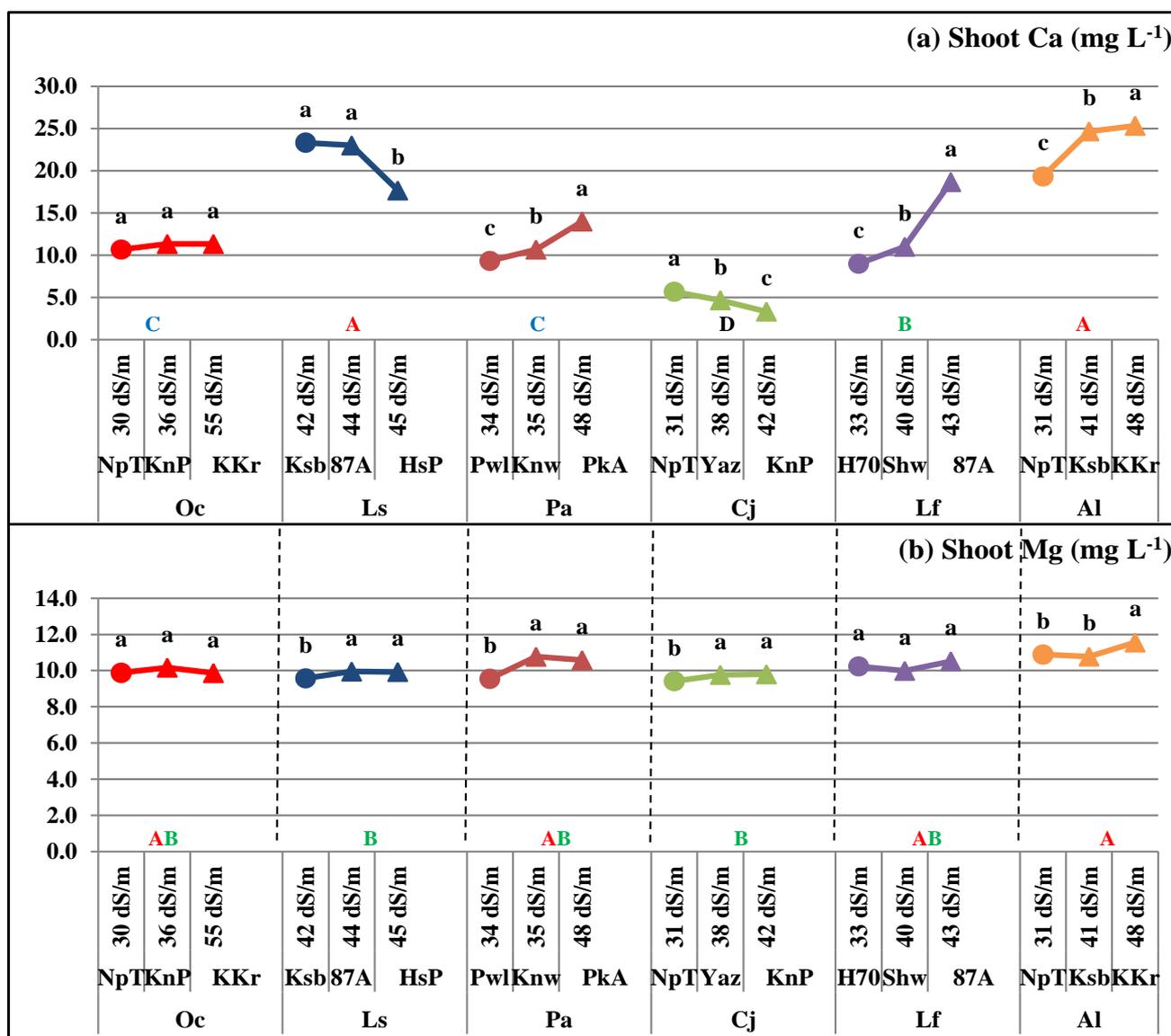


Fig. 3. Mean values for shoot Ca and Mg contents of the plant samples collected from various saline habitats of the Punjab province.

The APX activity increased gradually with increasing soil salinity of the parent habitats. Among all six species of various grasses collected from different ecozones of Punjab, the maximum APX activity as noted in *Pa* differed significantly from all other species. The *Oc*, *Ls*, *Cj* and *Al* species differed non-significantly from each other. Out of all six grasses, the *Lf* species exhibited significantly lower APX activity. Among three sites of *Oc*, the ecotype from highly saline habitat *KKr* showed the maximum APX activity that was significantly lower in ecotypes collected from less saline habitat *KnP* that further decreased to the least saline habitat *NpT*. Similarly *Ls* ecotype of *HsP*, *Lf* ecotype of *87A* and *Al* ecotype of *KKr*, all of which belonged to hyper saline habitats, exhibited the maximum APX activity that decreased significantly in ecotypes from moderately to least saline habitats of *Ls* (*87A*, *Ksb*), *Lf* (*Shw*, *H70*) and *Al* (*Ksb*, *NpT*), respectively. The APX activity in *Pa* ecotypes of the least saline habitats *Pwl* and *Knw* exhibiting almost parallel regimes of soil salinity differed non-significantly to each other while it increased significantly in *PkA*

ecotype collected from the hyper-saline habitat with 48 dS m<sup>-1</sup> soil salinity. Similarly, *Cj* ecotype of moderately saline habitats i.e. *NpT* and *Yaz* differed non-significantly to each other but was significantly higher in the highly saline *KnP* ecotype (Fig. 7).

Among all six grasses, the maximum CAT activity as noted in *Pa* differed significantly from all other species (*Oc*, *Ls*, *Cj*, *Lf* and *Al*) though CAT activity among *Oc*, *Ls*, *Cj* and *Lf* grasses differed non-significant to each other. *Al* showed the minimum CAT activity with respect to all other species. The SOD enzyme activity differed significantly in all ecotypes of the six grass species collected from various ecozones of Punjab varying in salinity levels. The maximum SOD activity noted in *Pa* differed non-significantly from *Cj* and *Oc*, while it differed significantly from SOD activity observed for *Ls*, *Lf* and *Al*. However, SOD activity differed non-significantly among *Ls*, *Lf* and *Al*. Some grasses like *Oc*, *Cj* and *Lf* showed a general decreasing trend with increasing salinity. However, *Ls*, *Pa* and *Al* exhibited increasing trends with increasing salinity (Fig. 7).

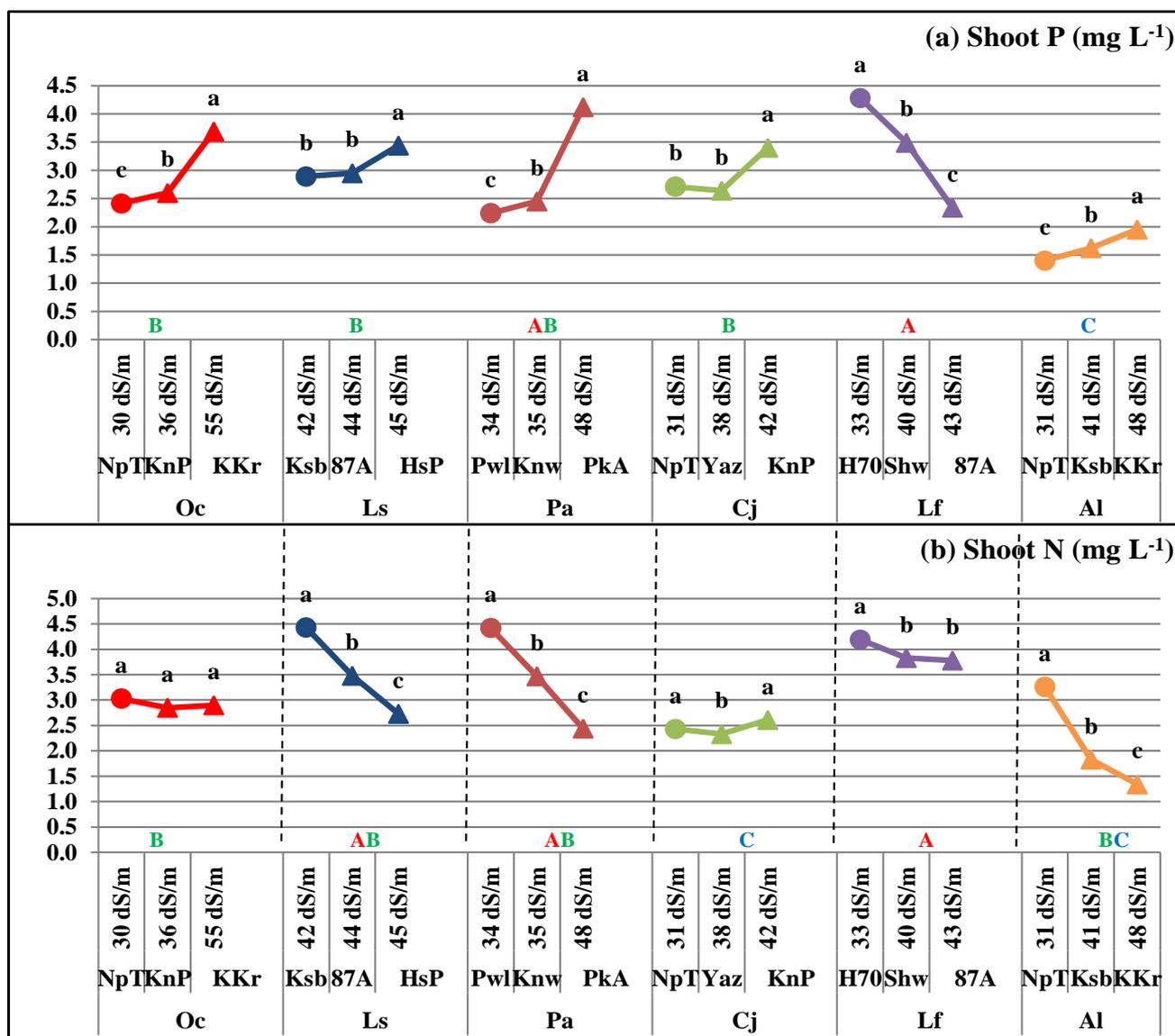


Fig. 4. Mean values for shoot P and N of the plant samples collected from various saline habitats of the Punjab province.

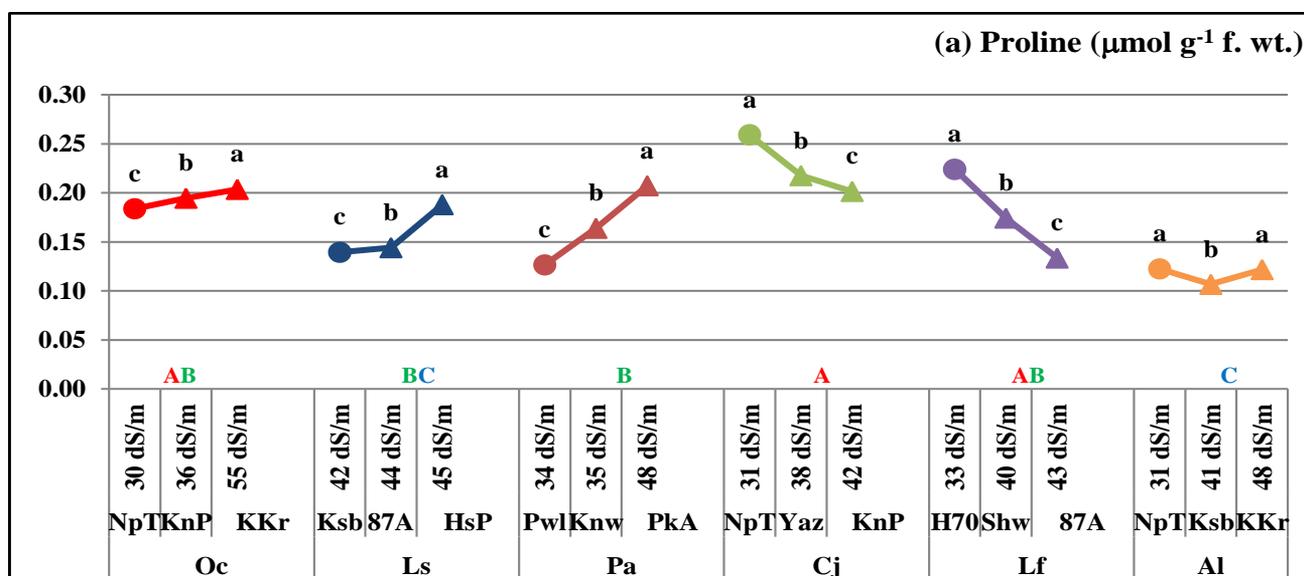


Fig. 5. Mean values for shoot proline of the plant samples collected from various saline habitats of the Punjab province.

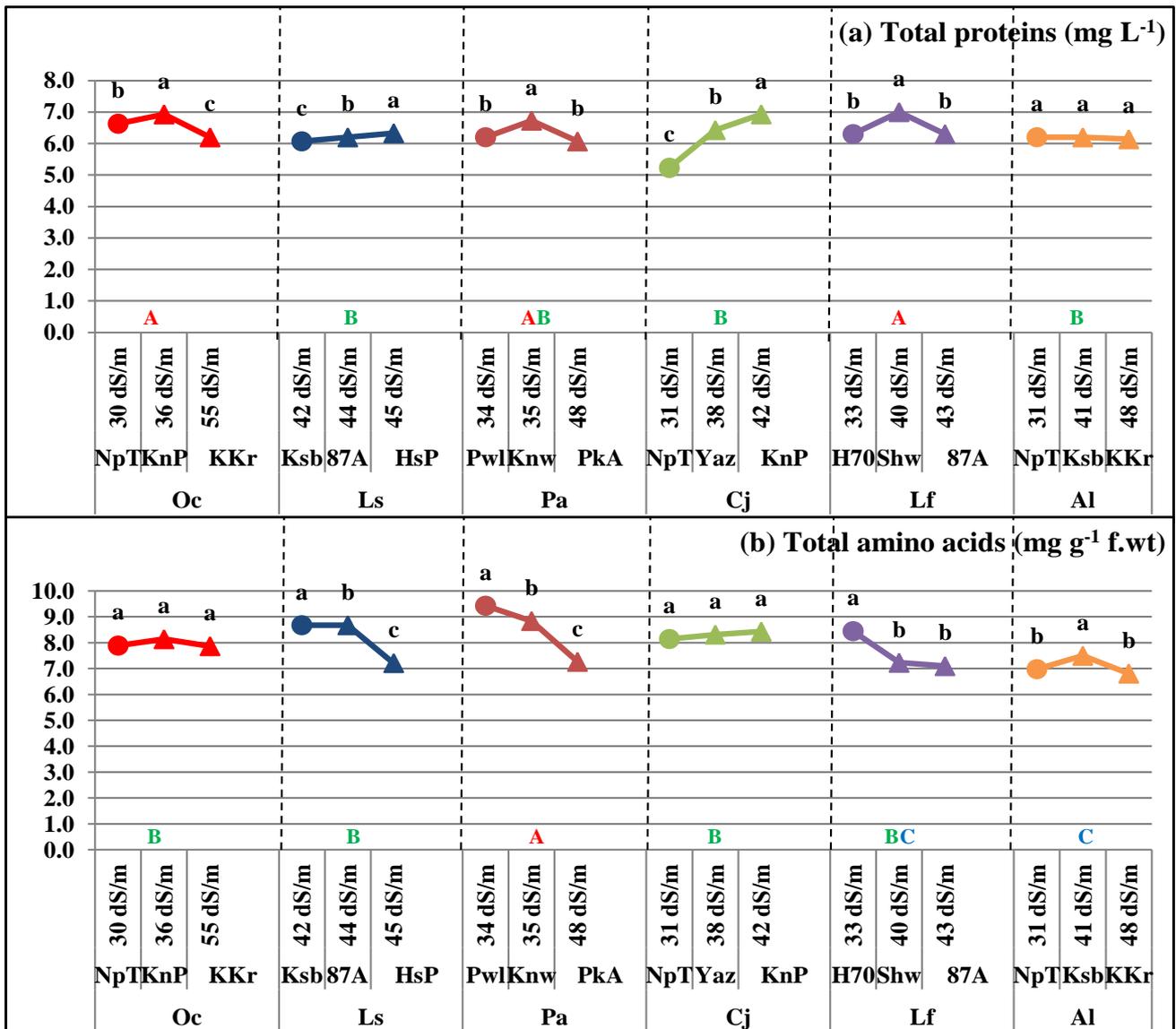


Fig. 6. Mean values for shoot total proteins and total amino acids of the plant samples collected from various saline habitats of the Punjab province.

The POD activity showed a highly significant variation among all grasses under study collected from different habitats. Generally, POD activity decreased in most of the species under study with increasing salinity level of their native habitats. However, it was not true for *Ls* and *Pa* where it significantly increased in *Ls* but remained unaffected in *Pa* at different salinity level (Fig. 8).

Total soluble sugars showed statistically significant variation among six species of the Punjab. Generally, total soluble sugars increased in all grasses with increase in soil salinity of different habitats. The maximum sugar contents noted in *Pa* differed significantly from all other species. *Ls*, *Oc*, *Cj*, and *Lf* differed non-significantly to each other. The minimum concentration of total soluble sugars observed in *Al* (Figs. 8 and 9).

**Discussion**

Some plants show adverse effects on growth and physiology in the presence of relatively low levels of salts (salt sensitive plants) in soils while others can survive at high soil salinity (salt-tolerant plants). Some plants called

halophytes flourish under hyper-saline conditions. Adaptability mechanisms in plants are complex involving various morphological, anatomical, molecular and genetic aspects. Efforts are under way to explore these adaptability mechanisms for salt tolerance in natural vegetation using conventional and advanced methods (Hasegawa *et al.*, 2000). In current study, some physiological mechanisms of grasses explored to determine the adaptability mechanisms in selected grasses collected from hyper-saline environments.

Present study revealed a variable response of six grass species to soil salinity of their habitats. The grasses selected in this study are common and native to this region. Most of the adverse effects of salt stress as observed in this study are directly linked to the toxic effects of salt stress on crucial cellular and biochemical attributes hindering plant growth and survival in hyper-saline environments (Munns *et al.*, 2002). In the present study, different ecotypes of grasses (*Ochthochloa compressa*, *Lasiurus scindicus*, *Panicum antidotale*, *Cymbopogon jwarancusa*, *Leptochloa fusca*, and *Aeluropus lagopoides*) collected from habitats with varying soil salinity ranging from 30 dS m<sup>-1</sup> to 55 dS m<sup>-1</sup>. Results indicated that the distribution of *Pa* (Knw, PkA), *Ls* (HsP)

and *Al* (*Kkr*) ecotypes strongly influenced by soil  $E_{ce}$ , Na, P,  $NH_3$ ,  $NO_3$ , OM, pH, SP, and MC. The soil  $Cl^-$  content had a strong influence on distribution of *Cj* of *KnP* and *Oc* of *NpT*. *Al* (*NpT*) was mainly influenced by soil Ca content. Since these populations grow in these hyper-saline environments over long time-periods, they must have evolved specific adaptation at physiological and biochemical levels as explored in this study (Hameed *et al.*, 2010).

The drastic effects of soil salinity in hyper-saline soils seem to be directly linked to the alterations in nutritional imbalance especially those of  $Ca^{2+}$ ,  $K^+$ , Mg, and N thereby restricting photosynthesis and other critical metabolic pathways involved in energy assimilation (Munns *et al.*, 2002). As observed from results of the present study, shoot Na, Na/K ratio, Ca, Mg and P were positively correlated with soil  $E_{ce}$  gradients ( $r = 0.32, 0.43, 0.37, 0.18$  and  $0.36$ , respectively). While shoot K and N were negatively correlated with soil  $E_{ce}$  having  $r = -0.35$  and  $0.29$ , respectively. Other reasons include stunted leaf growth leading to reduction in leaf number,

size and area, mostly, because of reduced cellular expansion through effects on cell turgor due to lower water potentials of plants growing in the highly saline areas (Hameed *et al.*, 2009).

Elevated concentration of sodium and chloride in the rhizospheric soil has been shown to exert negative effects on plant growth and development (Zhang *et al.*, 2010). The same was observed in terms of strong negative correlation between shoot fresh and dry weights and soil  $Cl^-$  content ( $r = -0.57$  and  $-0.53$ , respectively) of present study. In past studies, a marked reduction in shoot and root lengths was more significant than shoot length (Hameed *et al.*, 2008). Under highly saline environments, reduction in shoot and root length is mainly attributed to decreased water potential in the rooting zone leading to cell turgor loss. Such reduced cell turgor directly inhibits cell division, expansion and elongation. Additionally, cell differentiation and proliferation are limited that reduces plant biomass accumulation in saline environments (Ashraf *et al.*, 2008).

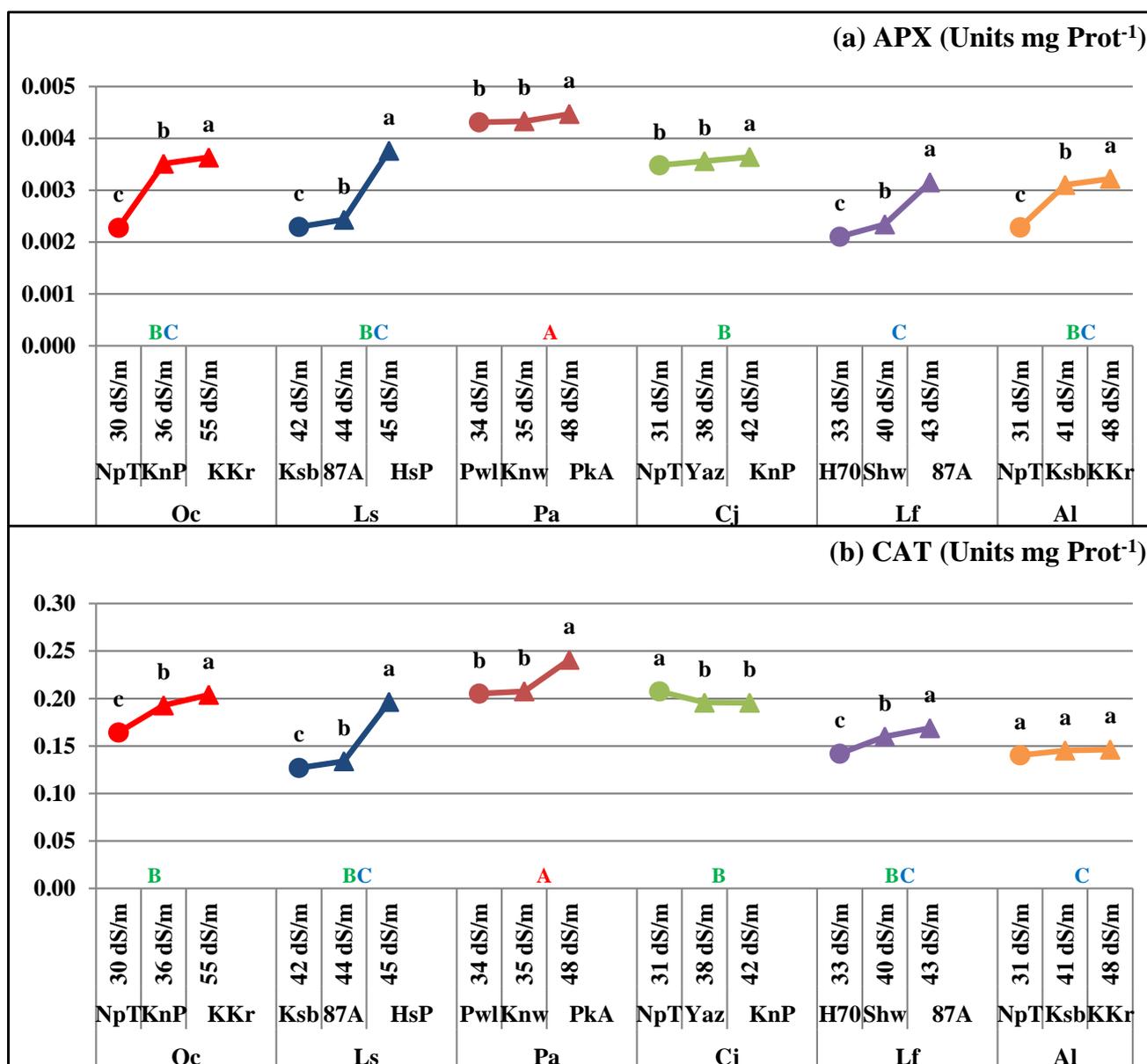


Fig. 7. Mean values for shoot APX and CAT of the plant samples collected from various saline habitats of the Punjab province.

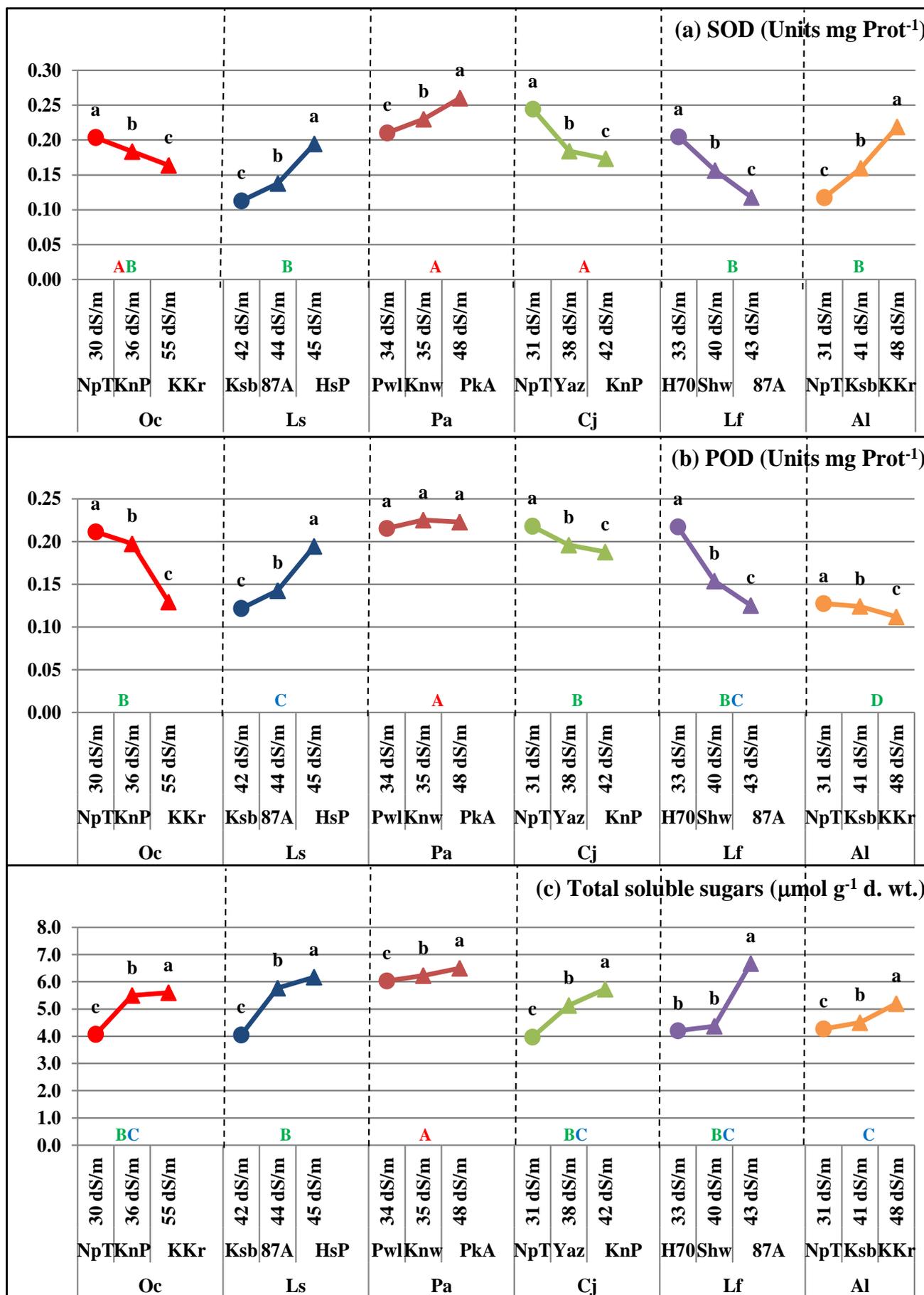


Fig. 8. Mean values for shoot SOD, POD and total soluble sugars of the plant samples collected from various saline habitats of the Punjab province.

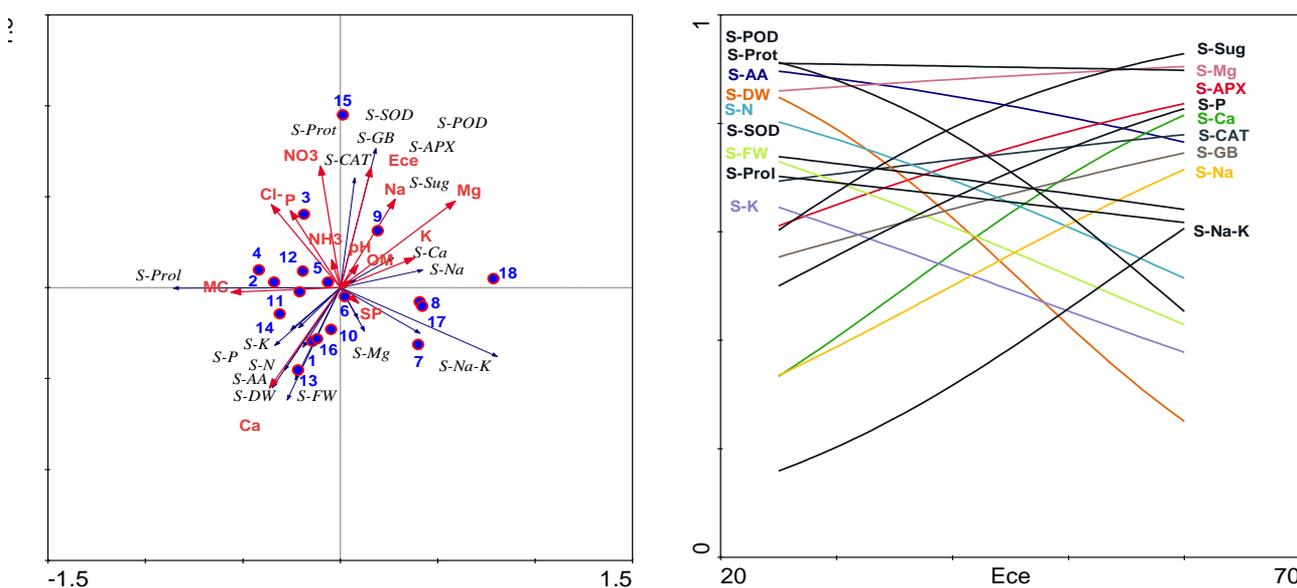


Fig. 9. Redundancy analysis (RDA) and response curves for various biochemical attributes of six grass populations collected from habitats with varying soil salinity across Punjab. The direction of arrow below Ece ordination axis shows changes in species response along increasing salinity gradients. Sites and species abbreviation are presented below Abstract.

In present study, shoot and root fresh and dry weights reduced progressively with increasing salinity gradients and was found to negatively correlated with soil moisture content, Ece, Na, K, Mg,  $\text{NO}_3$  and Cl- (Mantri *et al.*, 2012). The maximum reduction in root fresh weight for species *Cj*, *Pa*, *Lf*, *Al* and *Oc* observed at high salinity gradients of the most saline habitats (PkA, KKr, and Ksb). It might be due to accumulation of salts in the rooting zone that resulted in lowering of osmotic potential thereby possibly reducing water uptake and increased the respiration leading to reduction in plant growth (Karlberg *et al.*, 2006). Adaptability and stress tolerance are inherited characters (Niknam & McComb, 2000), and are partially due to adjustment of ions in shoot and root tissues, ensuring salts exclusion by roots or by minimizing the translocation of salts to areal parts (Flowers & Colmer, 2008, Voronkova *et al.*, 2008). The continuity of the growth even in an area having 40 and above Ece level is the indication of high degree of adaptability to salt stress as exhibited by all grasses i.e. *Oc*, *Ls*, *Pa*, *Cj*, *Lf* and *Al* in this study.

Growth and survival of the plants are two indicators of survival under highly saline environments (Niknam & McComb, 2000). Growth depends on the cell division, turgor pressure and cell enlargement along with cell wall extensibility. Inorganic ions when accumulate in exceptionally high concentrations triggers accumulation of organic solutes in salt adopted ecotypes that is seen as an important physiological adaptation against salinity stress (Wyn Jones & Gorham, 2002). Both organic and inorganic solutes are essential for osmoregulation in salt-adapted ecotypes, especially growing in highly saline habitats (Hameed *et al.*, 2008). Similar variable behavior exhibited by six species as they responded variably to habitats, species and even ecotypes of same species showed variable behavior to varying soil depths. Various compatible osmolytes accumulate in higher plants such as amino acids, soluble proteins and sugars. Arginine (Arg), leucine (leu), glycine (gly), Alanine (ala), valine (Val) and serine (Ser), along with the imino acid citrulline (cit), proline (pro) and ornithine (orn) are shown to accumulate in the plants under salt stress (Mansour, 2000) as a

principal strategy in many plant species to adopt saline environments (Martino *et al.*, 2003). A similar accumulation in proline been reported in large amounts in many grasses under study, which however was not the true in all ecotypes investigated (Abraham *et al.*, 2003).

Solute transport via xylem vessels in saline environments shows some degree of selectivity, particularly between Na and K (Ashraf *et al.*, 2008). Ion imbalance, particularly by P, Mg and K is the most important and widely occurring character affected by salt stress, which is directly influenced by the uptake of sodium and chloride ions (Munns *et al.*, 2006). Sustaining K and Ca concentrations in cells and reduction in Na uptake are vital for the salt adaption in grasses as observed in this study (Karmoker *et al.*, 2008). Effects of soil salinity in hyper-saline soils seems to be directly linked to the alterations in nutritional imbalance especially those of  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ , and N thereby restricting photosynthesis and other critical metabolic pathways involved in energy assimilation (Zhang *et al.*, 2010). To prevent toxicity of mineral ions in the cytoplasm, grasses use vacuoles as storage site and then excreted by the salt glands present on leaves (Munns, 2005; Saqib *et al.*, 2005).

The profound role of K in plant growth and development has been known since long (Taiz & Zeiger, 2010). The most imperative roles assigned to K include in enzyme activation and cell turgidity by maintaining high hydrostatic pressure inside the vacuole (Maathuis & Amtmann, 1999). Plants supplied with low  $\text{K}^+$  show reduced chlorophyll contents leading to impaired photosynthesis activity (Zhao *et al.*, 2001). The reduction, in  $\text{K}^+$  content in plants growing in hyper saline environments as observed in this study, have been attributed to presence of excessively high Na in the rhizospheric zone that imposes negative effects on  $\text{K}^+$  uptake in plant (Sarwar & Ashraf, 2003). In this study, high salt concentration reduced the uptake of potassium in all six grasses. Generally, potassium content reduced along the increasing salinity gradient in all grasses where root and shoot accumulated the maximum potassium in the least saline Pwl habitat. Maximum potassium content noted in *Lf*

at H70 (least saline) habitat as compared to other grasses *Ls*, *Pa*, *Cj* and *Al*. *Oc* showed lowest level. Such potassium selectivity accompanied with sodium compartmentalization been related to differential salt tolerance of different species in past literature (Barrett-Lennard *et al.*, 1999).

The  $K^+/Na^+$  ratio is a good criterion of salt adaptability (Song *et al.*, 2009). Salt adaptive plants been shown to have high  $K^+/Na^+$  ratio (Shi *et al.*, 2005) reportedly due to antagonistic effect of  $K^+$  and  $Na^+$  ions uptake and transport. As salt adapted plants prefer to not to uptake  $Na^+$  (Munns *et al.*, 2006), with a concurrent enhancement in  $K^+$  and  $Ca^{2+}$  uptake and assimilation (Lutts *et al.*, 2004; Meloni *et al.*, 2008). Such restricted  $Na^+$  uptake been shown to protect the metabolic process consequently maintain high growth rate (Eker *et al.*, 2006). In comparison, salt sensitive plants show higher accumulation of  $Na^+$  inside the cells thereby inhibiting  $K^+$  uptake. Such an ionic imbalance increases the  $Na^+/K^+$  ratio. High  $Na^+$  ions in salt sensitive plants also interfere with uptake of other essential ions in plant and may decrease their concentration to critically lower quantities (Hu & Schmidhalter, 2005). In present studies, maximum  $Na$  concentration observed in highly saline KKr ecotype of *Al* (48 dS  $m^{-1}$ ), and the minimum  $Na$  concentration observed among *Al* ecotypes was noted in NpT (31 dS  $m^{-1}$ ). The least  $Na/K$  ratio among all six grasses observed for *Cj*. Generally, the ecotypes collected from higher saline habitats exhibited higher  $Na/K$  ratio that was significantly lower in less saline (Pwl) habitat. Thus, it is inferred that maintenance of lower  $Na/K$  ratio as a key strategy adopted by in salt tolerant grasses observed in this study (Eker *et al.*, 2006).

Among various enzymatic antioxidants, APX, SOD, CAT and POD are important antioxidants help in scavenging of various reactive oxygen species (Di Mascio *et al.*, 1989). It helps in alleviating the toxic effects of salt stress on plants. Results of the current study revealed that salt stress enhanced production of antioxidants activities (APX, CAT, and SOD) in almost all ecotypes as their activity highly positively

correlated with high soil ECe and  $Na$  of their respective habitats (Table 2). APX activity increased gradually along with soil salinity gradients. Among all grasses, maximum antioxidants activity observed in *Pa*, while minimum in *Lf*, *Oc*, *Ls*, *Cj* and *Al*. Maximum CAT activity recorded in *Pa* while *Al* showed minimum activity. *Oc*, *Ls*, *Lf* collected from highly saline habitat showed maximum activity. *Pa* showed the maximum SOD activity. However, some grasses like *Oc*, *Cj* and *Lf* showed a reduction at high salinity level. POD activity showed a highly significant variation among different grasses but negatively correlated to soil ECe and  $Na$ . It decreased with increasing salinity levels for all species except *Ls* and *Pa* showing an increased activity. Borghesi *et al.*, (2011) reported in some cases salinity stress led to a 2-3-fold increase in the SOD contents. Other studies also revealed that salinity enhanced SOD and POD activities (De-Pascale *et al.*, 2001). Salt tolerant species of the grasses can minimize the lethal effects of salts (ion toxicity, osmotic stress) by modifying morphological, anatomical and physiological attributes (Hameed *et al.*, 2009). Production of protective antioxidants like SOD, POD, CAT *etc.* can also be used as identifying marker in species for adaptation to saline environments (Munns & Tester 2008). Plant under salt stress shows a remarkable decrease in water potential as a primary response accompanied by a concurrent decrease in soil water and osmotic potentials. Accumulation free amino acids, proteins, sugars and other osmotically compatible solutes as observed in ecotypes collected from highly saline environments is one of the primary responses of plants exposed to low soil water potential (Al-Qurainy *et al.*, 2020; Khan *et al.*, 2020). Such an enhancement in accumulation of osmotically compatible solutes in salt-adaptive species been directly correlated with soil  $Na$  gradients and shown to reduce the toxic effect of salt stress (Lee *et al.*, 2007). Similar results were also shown in this study as well where highly tolerant ecotypes of *Lf*, *Al*, and *Oc* accumulating substantially higher amounts of organic osmotica and antioxidative enzymes (Tammam *et al.*, 2008).

**Table 2. Correlation (r) between different soil and shoot plant parameters of six grasses collected from the different sites of the Punjab.**

	SP	MC	pH	OM	Ece	Na	K	Ca	Mg	P	NO3	NH3	Cl-
S-FW	-0.37	-0.14	-0.32	0.21	-0.25	-0.45	-0.60	0.51	-0.19	0.20	-0.42	0.02	-0.57
S-DW	0.07	-0.01	-0.21	0.33	-0.61	-0.55	-0.37	0.28	-0.59	0.05	-0.11	0.14	-0.53
S-Na	0.06	-0.25	0.07	-0.21	0.32	0.31	0.17	0.07	0.48	0.00	-0.21	-0.48	0.00
S-K	0.38	0.15	-0.10	0.09	-0.35	-0.01	-0.14	0.05	-0.24	-0.03	-0.06	-0.42	0.01
S-Na-K	-0.10	-0.33	0.16	-0.22	0.43	0.25	0.23	0.03	0.61	0.03	-0.24	-0.20	-0.03
S-Ca	-0.09	-0.29	-0.21	-0.13	0.37	0.15	-0.11	0.22	0.53	-0.03	-0.05	-0.18	-0.20
S-Mg	0.14	-0.34	0.04	-0.24	0.18	0.20	0.59	-0.11	0.45	0.18	-0.10	-0.23	0.01
S-P	0.10	0.19	0.31	0.25	0.36	0.40	-0.08	-0.21	-0.41	-0.06	-0.03	-0.16	0.34
S-N	0.29	0.10	-0.19	0.24	-0.29	-0.22	-0.36	0.26	-0.43	-0.20	-0.05	-0.40	-0.08
S-Prol	0.01	0.26	0.37	0.15	-0.10	0.00	-0.01	-0.19	-0.56	0.08	0.16	0.26	0.21
S-Prot	0.00	0.17	-0.15	-0.03	-0.04	0.01	0.24	-0.06	-0.14	-0.07	-0.20	-0.15	0.45
S-AA	0.19	-0.01	-0.27	0.42	-0.32	-0.23	-0.36	0.02	-0.40	0.06	-0.13	0.12	-0.35
S-APX	-0.17	-0.54	0.37	0.36	0.26	0.36	0.35	-0.73	0.08	0.31	0.19	0.13	-0.15
S-CAT	-0.11	-0.27	0.49	0.35	0.13	0.29	0.37	-0.68	-0.25	0.18	0.29	0.16	0.03
S-SOD	0.11	-0.32	0.43	0.32	-0.11	0.13	0.31	-0.46	-0.21	0.05	-0.03	0.17	-0.27
S-POD	0.02	-0.16	0.15	0.49	-0.47	-0.22	0.06	-0.21	-0.60	0.07	-0.03	0.06	-0.26
S-Sug	-0.19	-0.53	0.20	0.53	0.49	0.46	0.32	-0.64	0.27	0.47	0.19	-0.24	0.15

 +ve correlation at  $p \leq 0.001$   
 -ve correlation at  $p \leq 0.001$   
 Non-signification correlation

 +ve correlation at  $p \leq 0.01$   
 -ve correlation at  $p \leq 0.01$

 +ve correlation at  $p \leq 0.05$   
 -ve correlation at  $p \leq 0.05$

## Conclusion

Grasses evaluated in this study showed a remarkable variation in distribution under saline areas as indicated by remarkable plasticity in physio-biochemical activities. The morphological and physio-biological attributes of *Lf* proved to be more plastic than other species, and therefore found to be widely distributed in hyper-saline area. In comparison, *Al* showed a severe reducing in all attributes observed in this study indicating lesser plasticity and hence had a restricted distribution in lesser saline areas.

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