Physiological and Biochemical Analysis of the Selected Halophytes of District Mardan, Pakistan

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***Abstract*—During the present study four halophytes: *Suaeda fruticosa* Forsk, *Atriplex leucoclada* Boiss, *Haloxylon salicornicum* (Moq.), *Salicornica verginica.* L along with their rhizospheric soil were collected from different locations of District Mardan. Electrical conductivity of rhizospheric soil was 4.2dS/m and pH: 8.3-9.3. *Haloxylon salicornicum* had highest concentration of K^+, Ca^{2+} and Mg^{2+} ions in its leaves as compared to other 3 selected halophytes, whereas Fe^{+2}, Cu^{+3}, Zn^{+2} and heavy metals like Co^{+3} and Ni^{+3} were higher in *Atriplex leucoclada*. Significantly lower production of protein, chlorophyll and carotenoid contents were observed in plants collected from saline areas of District Mardan. *Atriplex leucoclada* possessed higher chlorophyll a/b ratio and protein content. The *Salicornica verginica* and *Haloxylon salicornicum* appear to use sugar as osmolyte while proline was found significantly higher in *Suaeda fruticosa* and *Atriplex leucoclada*. The production of abscisic acid (ABA) was significantly higher as compared to indole acetic acid (IAA) in all the selected plants. *Atriplex leucoclada Suaeda fruticosa* and *Salicornica verginica* possessed higher concentration of ABA. It is inferred that halophytes differ in their adaptability and use different compound as osmoprotectant. Na^+/K^+ ratio considered an index of salt tolerance and correlation present between Na^+/K^+, ABA/IAA ratio and proline content. Among the selected halophytes *Atriplex leucoclada* have maximum ABA/IAA ratio as well as higher Na^+/K^+ ratio and higher proline and protein contents.**

***Index Terms*—Abscisic acid, Proline, Salinity, Halophytes.**

I. Introduction

Soil salinity is becoming a major problem due to various natural and man caused factors in almost all the regions of the world and especially in arid areas. Soil salinity is characterized by a high concentration of soluble salts in soil. Soils are considered saline when it's EC = 4dS/m or more [1]. The annual loss in the economy of Pakistan due to soil salinity has been estimated 300 US million dollars per year [2]. According to [3] adaptation of plants to soil salinity are of three various types (1) Osmotic stress tolerance, (2) Na^+ or Cl^- exclusion, (3) high accumulation of Na^+ or Cl^-.

The decrease in chlorophyll a, chlorophyll b, and carotenoid content in leaves under salinity has been reported by Gebauer and Ebert 2003. 5-aminolaevulinic acid (ALA) is precursor of all tetrapyrroles converted to protochlorophyllides which in turn converted into chlorophyll when exposed to light. ALA is made from glutamate that was reported to decrease in salt stressed leaves [4].

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It is reported that proteins level decreased under salinity is due to low uptake of nitrate ions [5]. High proline content can be considered beneficial to stressed plants. Significant correlation between enhanced tolerance and proline accumulation in plants under saline condition has been reported [6]. Accumulation of selective ions ensures osmotic adjustments in plants, which occur through mass action, and it enables the plants to increase water retention and sodium exclusion [7]. ABA referred as a "stress hormones" because its level increases in response to various environmental stresses, mostly in drought condition [8],[9] reported that reduction in leaf growth under drought and salinity is due to a considerable accumulation of ABA.

The present study was aimed to determine the protein, sugar, chlorophyll, carotenoid, proline, ABA and IAA contents of the selected halophytes *Suaeda fruticosa* Forsk, *Atriplex leucoclada* Boiss, *Haloxylon salicornicum* (Moq.) and *Salicornica verginica* L. concomitant with the determination of soil characteristics collected from district Mardan. Tannins, Cardicglycoside, Phlobetannins, Flavonoids, Terpenoids, Alkaloids, Anthraquinone, Cumarins, Steriods and Saponins were also included in the present study.

II. Material and Methods

A. Collection of Plant Samples

In the present study four plant species: *Suaeda fruticosa* Forsk, *Atriplex leucoclada* Boiss, *Haloxylon salicornicum* (Moq.), *Salicornica verginica* L. collected in triplicate from different locations of Mardan district. Mardan is a district in the North-West Frontier Province of Pakistan (altitiude of 400 – 1,700 m a.s.l;). The district lies from 34° 05' to 34° 32' north latitudes and 71° 48' to 72° 25' east longitudes. The physicochemical nature of rhizospheric soil collected at a depth of 6 inches was studied; leaves of selected halophytes collected at vegetative stage were used to study the biochemical and physiological characters.

B. Soil pH and Soil Electrical Conductivity (EC)

The pH of rhizospheric soil was measured by preparing 1:1 (soil: water) suspension [10],[11]. Electrical conductivity meter recorded the EC of extract. Readings were measured in microsiemens per centimeter (µS/cm).

C. Nutrients Analysis of Rhizospheric Soil and Leaves of Selected Plants

The rhizospheric soil was analyzed for macro, micro and heavy metals following the Ammonium Bicarbonate-DTPA method developed by [12].

The availability of different elements in the collected plants was determined by Perchloric-acid digestion method [13].

D. Physiological Characterization

Protein content of leaves was determined following the method [14].

Sugar estimation of fresh leaves was done following method of [15].

Chlorophyll and carotenoid content of leaves was determined by the method of [16].

The proline contents of leaves were measured by the method of [17].

The extraction and purification of ABA and IAA was made following the method of [18].

E. Biochemical Analysis

Tannins was determined in the plant by [19].

Saponins test was performed by the method of [20].

Determination of flavonoids by [20] and [19].

Test for terpenoids (Salkowski test) according to [20].

Test for alkaloids according to [20].

Identification of phlobatannins according to [21]..

Cardiac glycosides determination [21].

Coumarins identification [21].

Anthraquinone detection according to [21].

F. Statistical Analysis

The data were analyzed statistically by Analysis of Variance technique (Steel and Torrie, 1980) and comparison among treatment means was made by Duncan's Multiple Range Test (DMRT) (Duncan's, 1955).

III. Results

Macronutrients content of rhizospheric soil and leaves of selected halophytes (µg/g)

Table I indicated that there was hyper accumulation of Na^{+}, Ca^{++}, Mg^{++}, and K^{+} ions in leaves of halophytes collected from saline areas of District Mardan. The plant genera ranked for Na^{+} content as *Haloxylon salicornicum* > *Atriplex leucoclada* > *Salicornica verginica* > *Suaeda fruticosa*. P and No_3^{-} were found in the range of 0.4-0.8 µg/g in the soil.

The concentration of Ca^{+2} ions was higher in the leaves of both *Haloxylon salicornicum* and *Salicornica verginica* as compared to *Atriplex leucoclada*. *Suaeda fruticosa* leaves have the minimum concentration of Ca^{+2} ions. Ranking of plant genera for K^{+} ion concentration were, *Haloxylon salicornicum* > *Salicornica verginica* > *Suaeda fruticosa* > *Atriplex leucoclada*. Higher concentration of Mg^{+2} ions was found in *Atriplex leucoclada* and *Haloxylon salicornicum* while least was found in *Salicornica verginica.* The Fe^{+2}, Cu^{+3} and Zn^{+2} ion concentration were higher in *Atriplex leucoclada,* followed by *Suaeda fruticosa* while *Salicornica verginica* showed minimum concentration of these three elements.

Heavy metal analysis of leaves of selected halophytes and rhizospheric soil (µg/g)

Table II indicated that there was comparatively greater accumulation of Ni^{+3}, Li^{+1}, Pb^{+4} and Cd^{+2} ions in the leaves of selected halophytes as compared to that of soil. The concentration of Ni^{+3} ranged from 23-30µg/g in all plants. Similar accumulation of Li^{+1}, Pb^{+4}, Cd^{+2} was found in all the four selected halophytes. *Atriplex leucoclada* had accumulated higher concentration of Fe^{+2}, Zn^{+2} and Cu^{+3} Co^{+3} and Ni^{+3} ions, while *Suaeda fruticosa* and *Salicornica verginica* had lower Co^{+3} accumulations. *Suaeda fruticosa* is the maximum accumulator of Pb^{+4} and Mn^{+2}. The results indicated that halophytes species differ in the accumulation of micronutrients. It is also inferred that halophytes can be used as a phytoremediator of heavy metals from polluted soils.

Chlorophyll and carotenoid contents (mg/g)

Fig. 1 indicated that plants growing in saline soils contained significantly lower chlorophyll a and chlorophyll b contents as compared to other glycophytes; the chlorophyll a being significantly higher then chlorophyll b. Among the selected halophytes chlorophyll a content was found maximum in *Salicornica verginica* while it was minimum in *Atriplex leucoclada*. Whereas chlorophyll b was found maximum in *Haloxylon salicornicum*. The magnitude of chlorophyll a/b ratio was found higher in Atriplex *leucoclada* while total chlorophyll was found maximum in both *Haloxylon salicornicum* and *Salicornica verginica*. While *Atriplex leucoclada, Haloxylon salicornicum* and *Salicornica verginica* have higher carotenoid content. Minimum amount of carotenoid was found in *Suaeda fruticosa*. (Fig. 2)

Sugar contents (mg/g)

Higher sugar content was found both in *Haloxylon salicornicum* and *Salicornica verginica*, while *Suaeda fruticosa* and *Atriplex leucoclada* have minimum sugar content (Fig. 3).

Protein and Proline Contents (mg/g) Fig. 4 revealed that protein contents were higher in *Atriplex leucoclada*, while *Haloxylon salicornicum* and *Salicornica verginica* have lower but statistically similar protein content; minimum protein content was found in *Suaeda fruticosa*. The higher proline content was found in *Suaeda fruticosa* and *Atriplex leucoclada*, while *Haloxylon salicornicum* and *Salicornica verginica* have lower proline content.

Abscisic acid (ABA) and Indole acetic acid (IAA) contents (µg/g)

Fig. 5 showed that both ABA and IAA content was found higher in *Haloxylon salicornicum, while* both *Salicornica verginica* and *Suaeda fruticosa* showed lower ABA and IAA content. *Atriplex leucoclada* has ABA content lower than *Haloxylon salicornicum* but higher than all other 3 halophytic species.

Qualitative analysis of metabolites in arial parts and root extract of selected halophytes

Results in Table III,IV showed qualitative analysis of secondary metabolites in arial parts and root extract of selected halophytes. Result revealed that alkaloids and flavonoids are present in all most all of the selected plant extracts. Only 20% of the plant extract have no tannins, phlobetannins and cardic glycoside, whereas terpenoids and cumarins are absent in 30% of plant extracts among the selected halophytes while, anthraquinone and steriods are absent in 40% of the plant extracts selected for the study.

TABLE I: MACRO AND MICRONUTRIENT CONTENT (μG/G) OF RHIZOSPHERIC SOIL AND LEAVES OF SELECTED HALOPHYTES COLLECTED AT VEGETATIVE STAGES OF DISTRICT MARDAN

Metals	Na^{+}	Ca^{+2}	K^{+}	Mg^{+2}	K^{+}/Na^{+}	Fe^{+2}	Zn^{+2}	Cu^{+3}
Soil	14.8 +/-0.4	15 +/-0.1	0.08 +/-0.002	3.6 +/-0.07	185 +/-2.3	0.4 +/-0.01	0.07 +/-0.03	1.5+/- 0.05
S. fruticosa	2774 +/-14	15356 +/-24	9.1 +/-0.17	1708 +/-38	304 +/-2.6	635 +/-2.8	21 +/-0.04	215 +/-2
A.leucoclada	3565 +/-33	19582 +/-30	5.4 +/-0.20	4604 +/-49	659 +/-3.1	885 +/-3	26 +/-0.5	249 +/-1.2
H.salicornicum	3854 +/-32	24613 +/-28	19.4 +/-0.5	6125 +/-49	199 +/-2.6	278 +/-4.0	24 +/-1	167 +/-1.1
S. verginica	3293 +/-6	26144 +/-40	12.4 +/-0.5	1239 +/-18	265 +/-1.7	375 +/-3	19 +/-0.5	207 +/-3.4

S.fruticosa:Suaeda fruticosa, A.leucoclada:Atriplex leucoclada, H.salicornica: Haloxylon salicornicum, S. verginica: Salicornica verginica. Leaves of selected halophytes along with rhizospheric soil (EC: 4.2dS/m; pH: 8.3-9.3) at a depth of 6 inches were collected with three replicates.

TABLE II: HEAVY METAL ANALYSIS (μG/G) OF RHIZOSPHERIC SOIL AND LEAVES OF SELECTED HALOPHYTES COLLECTED AT VEGETATIVE STAGES OF DISTRICT MARDAN

Metals	Ni^{+3}	Li^{+1}	Pb^{+4}	Cd^{+2}	Cr^{+3}	Co^{+3}	Mn^{+2}
Soil	0.05+/-0.02	0.05+/-0.03	1.3+/-0.03	0.1+/-0.002	0.05+/-0.09	0.1+/-0.02	0.5+/-0.01
S. fruticosa	28+/-1.7	9.2+/-0.34	370+/-5.2	26+/-1.6	31+/-0.8	10+/-0.3	184+/-2.9
A.leucoclada	30.5+/-1.5	9.2+/-0.1	318+/-4.6	25+/-0.5	26+/-1.4	14+/-0.5	152+/-2.0
H.salicornicm	25.4+/-0.6	9.3+/-0.12	350+/-2.02	26+/-1.1	34+/-1.7	13+/-0.4	117+/-1.7
S. verginica	23.4+/-1.2	9.4+/-0.08	346+/-4.9	27+/-1.4	49+/-2.3	11+/-1.0	143+/-2.8

S.fruticosa:Suaeda fruticosa, A.leucoclada:Atriplex leucoclada, H.salicornica: Haloxylon salicornicum, S. verginica: Salicornica verginica. Leaves of selected halophytes along with rhizospheric soil (EC: 4.2dS/m; pH: 8.3-9.3) at a depth of 6 inches were collected with three replicates.

TABLE III: UALITATIVE ANALYSIS OF METABOLITES IN ARIAL PARTS AND ROOT EXTRACT OF SELECTED HALOPHYTES

Plants	Tannins	C. glycoside	P. batannins	Flavonoids	Terpenoids
Salicornica. A	+	+	±	+	+
Salicornica. R	+	+	+	+	+
Atriplex.A	+	+	+	+	+
Atriplex.R	-	+	+	+	+
Haloxylon.A	+	+	+	+	+
Haloxylon.R	+	-	+	+	-
Lespedeza.A	+	+	-	+	+
Lespedeza.R	-	+	+	+	±
Suaeda.A	+	+	+	+	-
Suaeda.R	+	-	-	+	-

Salicornica. A= Salicornica Arial, Salicornica.R= Salicornica root, Atriplex.A= Atriplex arial, Atriplex.R= Atriplex root, Haloxylon.A= Haloxylon arial, Haloxylon.R= Haloxylon root, Lespedeza.A= Lespedeza arial, Lespedeza.R= Lespedeza root, Suaeda.A= Suaeda arial, Suaeda.R= Suaeda root, (+) for present, (-) for absent, (±) for weakly present

TABLE IV: QUALITATIVE ANALYSIS OF METABOLITES IN ARIAL PARTS AND ROOT EXTRACT OF SELECTED HALOPHYTES

Plants	Alkaloids	Ant.Quinone	Cumarins	Steriods	Saponins
Salicornica. A	+	-	+	-	-
Salicornica. R	+	+	+	+	+
Atriplex.A	+	+	+	-	+
Atriplex.R	+	-	±	+	+
Haloxylon.A	+	+	+	+	+
Haloxylon.R	+	±	-	-	+
Lespedeza.A	+	+	±	+	+
Lespedeza.R	+	±	-	+	+
Suaeda.A	+	-	+	-	+
Suaeda.R	+	-	-	+	+

Salicornica. A= Salicornica Arial, Salicornica.R= Salicornica root, Atriplex.A= Atriplex arial, Atriplex.R= Atriplex root, Haloxylon.A= Haloxylon arial, Haloxylon.R= Haloxylon root, Lespedeza.A= Lespedeza arial, Lespedeza.R= Lespedeza root, Suaeda.A= Suaeda arial, Suaeda.R= Suaeda root, (+) for presence, (-) for absence, (±) for weakly present

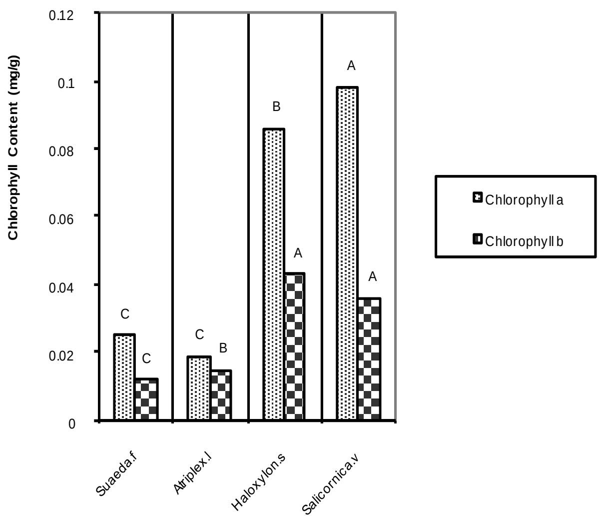

Fig. 1. Showed comparison of the chlorophyll a and chlorophyll b content (mg/g) of selected halophytes of District Mardan all bars which share same letters are non-significantly different at 5% level of significance

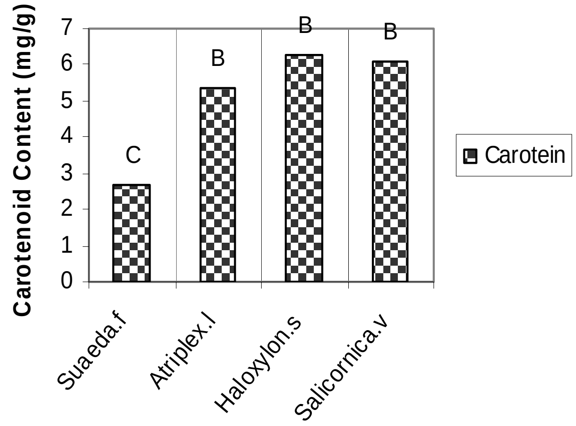

Fig. 2. Showed comparison of carotenoids content (mg/g) of selected halophytes of District Mardan All bars which share same letters are non-significantly different at 5% level of significance

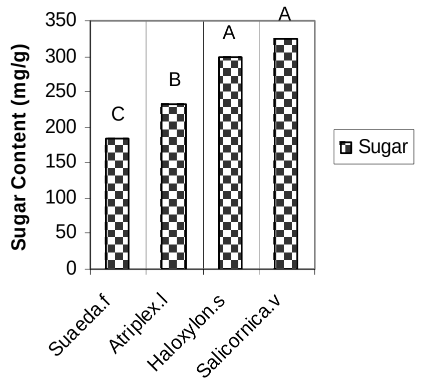

Fig. 3. Showed comparison of sugar content (mg/g), of selected halophytes of District Mardan All bars which share same letters are non-significantly different at 5% level of significance

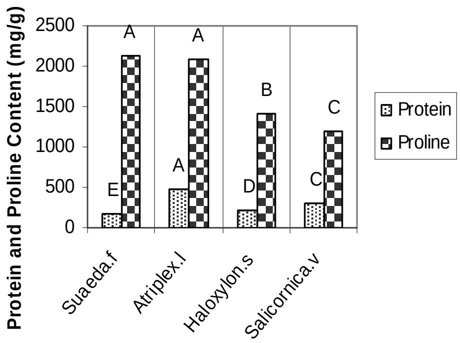

Fig. 4. Showed comparison of proteins and proline content (mg/g), of selected halophytes of District Mardan All bars which share same letters are non-significantly different at 5% level of significance

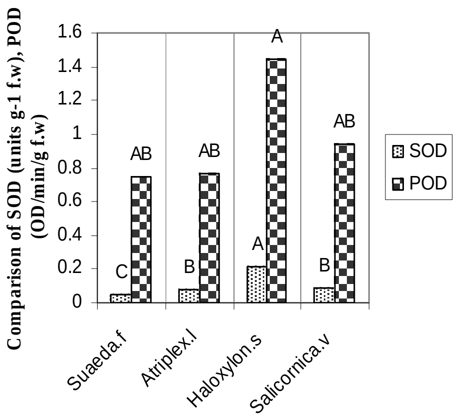

Fig. 5. Showed comparison of SOD (units g^{-1} f.w) and POD (OD/min/g f.w) of selected halophytes of District Mardan All bars which share same letters are non-significantly different at 5% level of significance

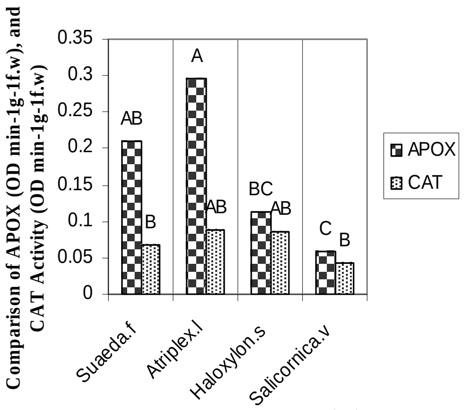

Fig. 6. Showed comparison of APOX (OD $min^{-1}g^{-1}$f.w) and CAT activity (OD $min^{-1}g^{-1}$f.w) of selected halophytes of District Mardan All bars which share same letters are non-significantly different at 5% level of significance

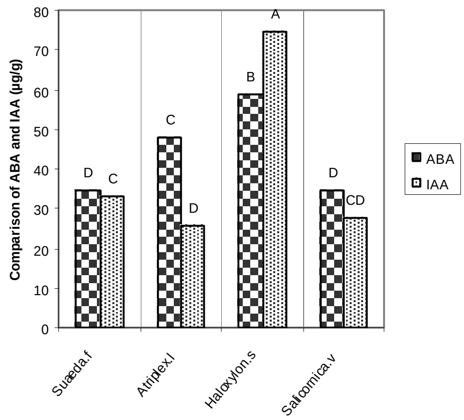

Fig. 7. Comparison of the ABA, IAA (µg/g) of Selected Halophytes of District Mardan All bars which share same letters are non-significantly different at 5% level of significance

IV. Discussion

Salinity leads to the reduction of P, No_3^- and Fe^{+2} ions in the soil due to the presence of high Na^+ and Cl^- ions concentration in the soil. Gadallah, (1999) reported that salinity increase the uptake of Na^+, Ca^{2+}, and Cl^- whereas the ratio of K^+/Na^+ decreases in saline condition. Na^+/K^+ ratio considered an index of salt tolerance (Alian *et al* 2000), was higher in *Atriplex leucoclada* having lower Ca^{2+} content but *Haloxylon salicornicum* which has least Na^+/K^+ ratio has relatively higher Ca^{2+} than *Atriplex leucoclada*. Ca^{2+} ion act as secondary messenger (Sanders *et al* 1999) and hence it appears that *Haloxylon salicornicum* has better signal transduction mechanism.

Salinity caused significant decrease in chlorophyll a, chlorophyll b, and carotenoid content in leaves (Parida *et al*., 2002, Gebauer and Ebert 2003 and Hernandez *et al*., 1999). The *Haloxylon salicornicum* showed maximum carotenoid, chlorophyll a and chlorophyll b concentration; the chlorophyll a/b ratio of *Atriplex leucoclada* was highest but carotenoid content was less than *Haloxylon salicornicum*. Significantly lower amount of carotenoid was found in *Suaeda fruticosa* and *Atriplex leucoclada*. In *Salicornica verginica* chlorophyll a and chlorophyll b concentration was found higher but its carotenoid content was less than *Haloxylon salicornicum*. According to Abdel-Kader (2000) the decrease in chlorophyll a and b content is due to the increase in their degradation under saline condition. The changes occur in the chlorophyll a/b ratio are used as an indicator for relative photosystem stoichiometry (Pfannscmidt *et al.*, 1999). Higher Chlorophyll a/b ratio in wheat (Moharekar *et al*., 2003) suggesting that light harvesting antenna size in their photosystem was smaller in comparison with mong bean. According to Paulsen (1997) carotenoid play a structural role for chlorophyll binding proteins of antenna system and reaction center. This indicates that different species of halophytes have different mechanisms to adapt under saline condition. The role of carbohydrates under saline condition in plants has been repoted as osmoprotectants, osmotic adjustment and radical scavenging (Singh *et al*., 2000; Kerepesi and Galiba, 2000).

Present result showed that significantly higher amount of sugar was found in *Salicornica verginica* and *Haloxylon salicornicum*. *Suaeda fruticosa* showed lower sugar content.

A strong correlation reported between sugar accumulation and osmotic adjustment during abiotic stress condition (Gilmour *et al*., 2000 and Taji *et al*., 2002).

Agastian *et al*., (2000) reported that proteins level decreased under salinity is due to low uptake of nitrate ions. During the present study, higher protein content was found in *Atriplex leucoclada* as compared to other selected halophytes, while it was found least in *Suaeda fruticosa*.

High level of proline content can be considered beneficial to stressed plants (Hyun *et al*., 2003). Significant correlation between enhanced tolerance and proline accumulation in plants under saline condition has been reported (Ashraf and Foolad, 2007). Proline content was found higher in *Suaeda fruticosa* and *Atriplex leucoclada*. *Salicornica verginica* and *Haloxylon salicornicum* showed lower amount of proline contents but higher suger content. Lower proline content but higher content of sugar in *Salicornica verginica* and *Haloxylon salicornicum* suggest that sugar can be used as osmolyte in these plants. The roles of proline and betaine as osmoprotactant in plant cells under salt stress have already been reported (Sairam *et al*., 2002; Khedr *et al*., 2003; Demiral and Turkan, 2004).

Abscisic acid plays a key role in plants under salinity condition (Zhang *et al*., 2006). *Atriplex leucoclada, Suaeda fruticosa* and *Salicornica verginica* possessed higher concentration of ABA as compared to IAA.

There is a strong interaction present between Na^+/K^+ ratio, ABA/IAA ratio and proline content. Among the selected halophytes *Atriplex leucoclada* have maximum ABA/IAA ratio as well as higher Na^+/K^+ ratio and higher proline and protein contents. While *Haloxylon salicornicum* and *Salicornica verginica* have lower Na^+/K^+ ratio, lower ABA/IAA ratio as well as lower proline and protein content.

V. Conclusion

Na^+/K^+ ratio, ABA and proline production interaction can be treated as a key character for salt tolerance. The various halophytes species also have ability to decontaminate soil from heavy metals. Of all the macro and essential micronutrient elements K^+ accumulation was least whereas Ca^{+2} accumulations was higher than glycophytes.

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