

## GROWTH PARAMETERS AND ANTIOXIDANT ENZYMES ACTIVITIES IN SELECTED HALOPHYTIC GRASS SPECIES FROM CHOLISTAN RANGELAND, PAKISTAN UNDER SALINITY STRESS

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### Abstract

The study was conducted to evaluate the antioxidant enzymes activities, chlorophyll contents, growth parameters in four halophytic grasses Khawi (*Cymbopogon jwarancusa*), Kalarghaa (*Aeluropus lagopoides*), Morat (*Panicum antidotale*) and Dhaman (*Cenchrus setigerus*) from Cholistan Rangeland, Pakistan. The experiment was conducted hydroponically in the research area of department of Forestry, Range and Wildlife Management, The Islamia University of Bahawalpur, Pakistan. The results revealed that biomass production in the selected halophytes increased with increasing salinity while under highly saline conditions. *Aeluropus lagopoides* produced highest number of leaves (19.6 A) at 140 mM NaCl while at 70 mM NaCl *Cymbopogon jwarancusa* had the maximum number of leaves (24 A). Highest chlorophyll contents were recorded in *Aeluropus lagopoides* (1.14 A) at 140 mM NaCl while at 70 mM NaCl *Cenchrus setigerus* produced more chlorophyll contents (0.93 A). Shoot length was recorded (80.23 A) in *A. lagopoides* under 140 mM NaCl and it reduced its length (27.63 C) at 210 mM NaCl while at 70 mM NaCl *C. jwarancusa* had the maximum shoot length (72.8 A). Under 70 mM NaCl root length (48.93 A) was measured in *P. antidotale* and has reduced to (29.06 C) at 210 mM NaCl. At 140 mM NaCl highest root length (43.05 A) was observed in *A. lagopoides*. Root length in *C. jwarancusa* was (16.9 D) at 210 mM NaCl while it had root length (47.41 A) at 70 mM NaCl salt level. Antioxidant enzymes activities of SOD enzymes activity was found maximum in *A. lagopoides* (40.67 A) with the minimum value was in *C. setigerus* (10.67 D). While the CAT enzyme activity was at highest value at 0 mM NaCl (35.67 A) in *A. lagopoides* and its value was decreased with the increasing salinity in all four species and it was (8.33 D).

**Key words:** Rangeland, Halophytes, Chlorophyll, Antioxidants, Catalase.

### Introduction

Abiotic stress is described as factors enforced by the environment on the optimum functions of an organism. Stress factors including scarcity of water, salinity, life-threatening (extremely high/low) temperature, chemical toxicity and oxidation stresses are severe intimidations of vegetation and cause degradation of land on earth (Jamil *et al.*, 2011). Salinity has severe effects on plants production and this is due to the different stresses induced by salinity (Modarresi *et al.*, 2013). Reactive oxygen species (ROS) are the chemically toxicity produced during different biotic and abiotic stresses. Salinity stress also leads to the construction of these ROS such as Singlet oxygen (<sup>1</sup>O<sub>2</sub>), super oxide anions (O<sub>2</sub><sup>-</sup>), radicals of hydroxyl (OH<sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Plants have also evolved self-protective mechanism against ROS. Superoxide dismutase (SOD), Catalase, Peroxidase (POD) and Glutathione Reductase (GR) are the key antioxidant enzymes that tragedy the major role against the Reactive Oxygen Species (ROS). Superoxide Dismutase (SOD) is an effective and first defensive enzymes against the ROS produced during stresses. Salinity affects the activity rate of SOD in plants. In this experiment activity of Superoxide Dismutase (SOD) changed with the changing concentrations of salts in arid region with high/low temperature and severe drought conditions (Jithesh *et al.*, 2006).

Halophytes are the major plants species that can produce well and has produced strong defense against ROS. Native halophytic grasses; *Panicum antidotale*, *Aeluropus lagopoides*, *Cenchrus setigerus* and *Cymbopogon jwarancusa* were checked for antioxidant enzymes against ROS (Wariss *et al.*, 2013). These are considered as highly endurable saline species. Enzymes reduce salt stress and minimize it by converting toxic Singlet oxygen (<sup>1</sup>O<sub>2</sub>), super oxide anions (O<sub>2</sub><sup>-</sup>), radicals of hydroxyl (OH<sup>-</sup>) into low toxic ROS hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in different plants species. Cholistan desert is situated in sub-tropical region and its semi-arid climate, extreme temperatures, that influenced periodically by long droughts. The humidity of these areas is very low with high evaporation rate (Ashraf *et al.*, 2007). The average rainfall per year varies from 100 mm to 250 mm. The average temperature in summer is 34°C - 38°C and the average temperature in winter is 15°C - 20°C. The highest temperature in this area may reach over 51.6°C (Arshad *et al.*, 2006). Very little work has been conducted on halophyte plant species of Cholistan rangeland. Due to little or no work on the species and there is a need for prevention of production losses by plants and to make good use of saline soils by understanding that how plants respond in this situation and get adoption to this stress (Yıldıztuğay *et al.*, 2011), were selected for antioxidant enzymes activities.

## Material and Methods

A hydroponic experiment was conducted at experimental area of Forestry, Range & Wildlife Management, University College of Agriculture & Environmental Sciences, The Islamia university of Bahawalpur during the month of September to October, 2018. The grass stumps of the *Cymbopogon jwarancusa*, *Panicum antidotale*, *Cenchrus setigerus* and *Aeluropus lagopoides* were collected from the Cholistan rangeland. The plants were trimmed and placed in the Hoglands solution (1979) for different treatments. The plants were kept under green shed of the research area at 35°C and pH was maintained at range of 5.5 to 7.0. The hydroponic medium was changed once a week to maintain the nutrients in proper proportion and pH of Hoglands solution was maintained at range of 5.5 to 7.0. Pots were arranged in a Completely Randomized Design (CRD) with four blocks. Each species has the 4 treatment with 3 replications (Kong, 2014). The solutions were aerated during the whole experiment by using air pumps. A total of 72 plants stumps were tested in the experiment. 4 stubbles were grown in each pot which represented one replicate. Different doses of NaCl 0, 70, 140, 210 mM NaCl were given to the treatments.

**Number of leaves:** Three plants were selected from each replication for every species to count the number of leaves.

**Shoot/Root length:** Root and Shoot were separated from whole plant. Root/Shoots were washed with running water followed by the distill water and lengths were measured by using measuring tape.

**Determination of Chlorophyll contents:** Chlorophyll contents were measured by using chlorophyll measuring meter (SPAD-502).

**Antioxidant enzymes activities:** For sampling 0.5g plant extract from each treatment for every species was taken after washing the plant with distilled water. The extracted material was cooled on ice. 2-3ml buffer solution of phosphate was added with pH of 7.8  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  (16.385g) +  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (0.663g) and volume of 1000ml by using distill water. Samples were homogenized on ice and 5ml buffer solution was added. At 4°C samples were centrifuged at 8000-13000 rpm for 20 minutes. Samples were preserved at 4°C (Jones *et al.*, 1991).

**Enzyme assay for superoxide dismutase (SOD):** We took 2.725mL of RS + 0.25ml  $\text{H}_2\text{O}_2$  + 0.025 ml Enzyme in 25 ml glass beaker. Controlled the experiment in 100% light-containing 2.75 ml of RS + 0.25ml  $\text{H}_2\text{O}$  (CK reading). For zero reading, control the samples in 100% Dark, containing 2.75 ml of RS + 0.25ml  $\text{H}_2\text{O}$ .

Place the control light and all other samples beakers under light conditions at 4000 lux for 20 minutes while control dark sample in 100% dark condition.

Read the samples by using Photo-spectrometer at 560nm.

SOD activity (U/g FW):  $\{(AcK-Ae) \times V\} \div \{0.5 \times AcK \times W \times Vt\}$

**Enzyme assay for catalase (CAT):** We took 2.8ml BPS, 0.1 ml of enzyme and 0.1 ml of  $\text{H}_2\text{O}_2$ . Gently shaken and took reading at 240 nm through photo-spectrometer. For Zero reading 2.8ml of BPS, 0.1ml of  $\text{H}_2\text{O}$  and 0.1 ml of  $\text{H}_2\text{O}_2$  was taken. Measure the rate by using the formula:

CAT (mM/g FW) activity =  $(\text{activity} \times A \times V/a) / (E \times \text{FW})$

## Results and Discussions

**Number of leaves:** Halophytic plants can tolerate and minimize the effect produced by salinity (Glenn *et al.*, 1999). These plants have ability to produce a series of adaptations like anatomical, physiological and morphological adaptations for example an extensive root system and specialized glands for salt secretion on the leaf surface (Hameed *et al.*, 2008). Mean comparison of number of leaves produced in four halophytic grasses under different treatments are shown in table 1. More number of leaves were produced under treatment T2 that were (24.0 A) followed by T3 that were (16.6 B) and the treatments T4 and T1 that are (12.6 C) and (11.3 C) respectively. Similarly the mean number of leaves produced during the experiment in *C. setigerus* at T2 was maximum (13.3 A) but its numbers was decreased with elevated salinity under T1, T3 and T4 salinity treatments that were (8.3 BC) and (7.3 C) respectively while at T1 number of leaves has (10.16 AB). *A. lagopoides* results were highly significant and produced more number of leaves under T3 that are (19. A). Number of leaves produced against T2, T4 and T1 were (15.3 AB), (11.0 BC) and (9.3 C) while *P. antidotale* produced highest numbers of leaves at T2 (21.3 A). Under T3 the numbers of leaves produced were (15.3 B) followed by the treatments T1 and T4 that were (10.6 C) and (10.0 C) respectively. Moreover when we compare the four species, *A. lagopoides* produced well and produced more number of leaves (19.6 A) against high salinity treatment T3. At treatment T2 *C. jwarancusa* produced (24.0 A) number of leaves. Least number of leaves were produced by the *C. setigerus* and produced (13.3 A) and (7.3 C) against T2 and T4 respectively. Fig. 1 showed number of leaves of halophytic grasses i.e *C.j*, *A.l*, *C.s* and *P.a* under different NaCl treatments.

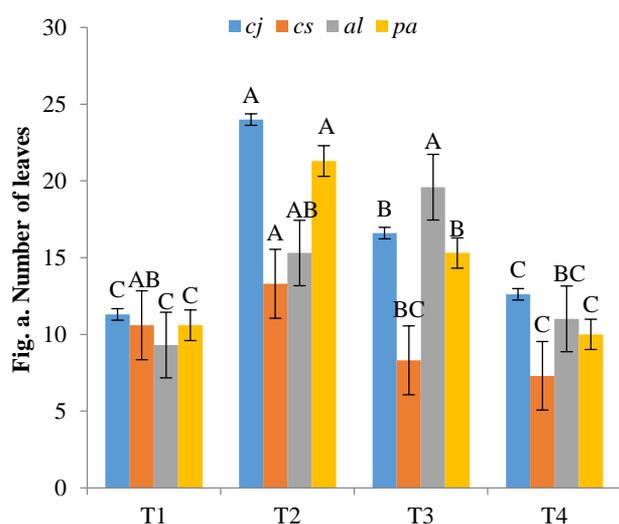
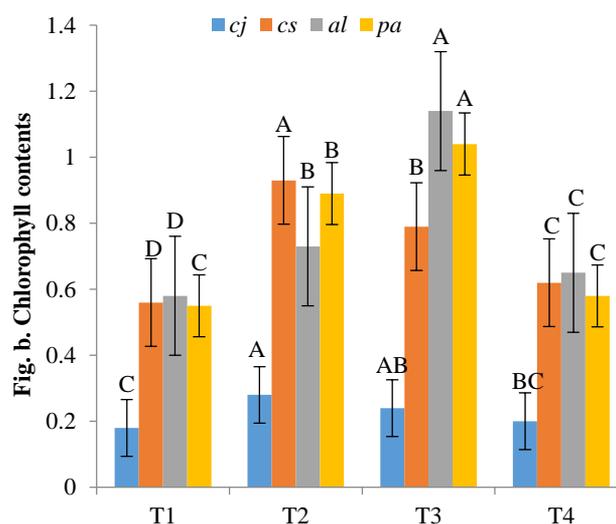
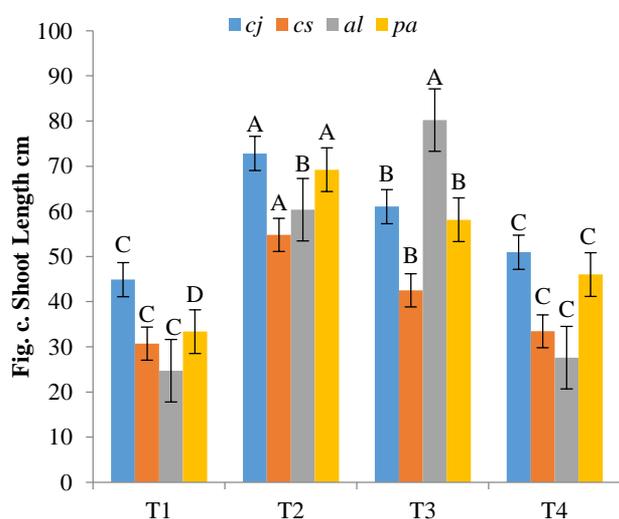
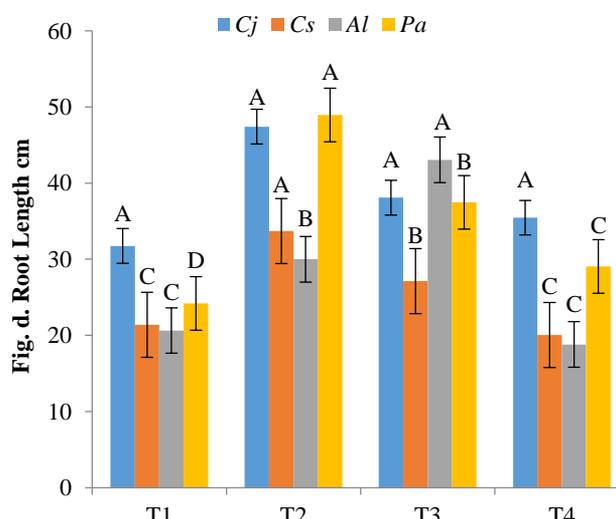
Salinity has great impact on plants production that not only reduces the total productions but may leads to severe damage and even death of plants occurs (Glenn *et al.*, 1999). Salt levels have significant consequences on leaves of halophytes. These results correlate the study conducted by (Alamgir & Ali, 2006), number of leaves decreased with the increasing salinity in *Oryza sativa*. Similar results were also found in wheat crop by (Hu & Schmidhalter, 2007). Germination of *Cleome viscosa* seeds also showed some resistance against salinity stress investigated by the Javaid *et al.*, (2020).

**Table 1.** Number of leaves in *C. jwarancusa*, *A. lagopoides*, *C. setigerus* and *P. antidotale* against salt stress.

Treatments	mM NaCl	<i>Cymbopogon jwarancusa</i>	<i>Aeluropus lagopoides</i>	<i>Cenchrus setigerus</i>	<i>Panicum antidotale</i>
T1	0	11.3 ± 0.68	9.3 ± 0.93	10.6 ± 0.93	10.6 ± 0.93
T2	70	24.0 ± 1.34	15.3 ± 0.93	13.3 ± 0.68	21.3 ± 1.125
T3	140	16.6 ± 0.68	19.6 ± 1.36	8.3 ± 0.68	15.3 ± 0.68
T4	210	12.6 ± 0.93	11.0 ± 0.89	7.3 ± 0.25	10 ± 0.44

**Table 2.** Chlorophyll contents in *C. jwarancusa*, *A. lagopoides*, *C. setigerus* and *P. antidotale* against salt stress.

Treatments	mM NaCl	<i>Cymbopogon jwarancusa</i>	<i>Aeluropus lagopoides</i>	<i>Cenchrus setigerus</i>	<i>Panicum antidotale</i>
T1	0	0.18 ± 0.0089	0.58 ± 0.0089	0.56 ± 0.013	0.55 ± 0.089
T2	70	0.28 ± 0.0089	0.73 ± 0.011	0.93 ± 0.0989	0.89 ± 0.089
T3	140	0.24 ± 0.016	1.14 ± 0.089	0.79 ± 0.089	1.04 ± 0.024
T4	210	0.20 ± 0.0044	0.65 ± 0.089	0.62 ± 0.089	0.58 ± 0.011

Fig. 1. Number of leaves of halophytic grasses i.e., *C.j*, *A.l*, *C.s* and *P.a* under different NaCl treatments.Fig. 2. Chlorophyll contents of halophytic grasses i.e., *C.j*, *A.l*, *C.s* and *P.a* under different NaCl treatments.Fig. 3. Shoot lengths of halophytic grasses i.e., *C.j*, *A.l*, *C.s* and *P.a* under different NaCl treatments.Fig. 4. Root lengths of halophytic grasses i.e., *C.j*, *A.l*, *C.s* and *P.a* under different NaCl treatments.

*C.j*= *Cymbopogon jwarancusa*, *A.l* = *Aeluropus lagopoides*, *P.a*= *Panicum antidotale* and *C.s*= *Cenchrus setigerus*

**Chlorophyll contents:** For the growth of any species chlorophyll contents are very important as photosynthesis occurs in chlorophyll. Any biotic or abiotic stress leads to the reduction in chlorophyll contents that cause hindrance in plant growth. Salts stress also induce changes in chlorophyll contents (Baunthiyal & Sharma, 2014). Salinity had significant effects on chlorophyll contents of *C. jwarancusa* species. Table 2 showed the highest ratio of chlorophyll was measured at treatment T2 that was (0.28 A). Lowest value of chlorophyll was measured at T1 (0.18 D) while at T3 and T4 its values were (0.24 AB) and (0.20 BC) respectively. Mean values for chlorophyll contents of *C. setigerus* at T2 treatment was (0.93 A). While at treatments T1, T3 and T4 the recorded values were (0.56 D), (0.79 B) and (0.62 C) respectively. *A. lagopoides* produced highest number of chlorophyll under treatment T3 (1.14 A). Lowest number of chlorophyll was measured at T1 treatment (0.58 C) while at treatment T2 and T4 these were (0.73 B) and (0.65 C) respectively. Mean values for chlorophyll of *P. antidotale* was (1.04 A) at treatment T3. Different treatment showed the different level of chlorophyll, at T1, T2 and T4 these values were (0.55 C), (0.85 B) and (0.58 C) respectively. Fig. 2 chlorophyll contents of halophytic grasses i.e. *C.j*, *A.l*, *C.s* and *P.a* under different NaCl treatments.

Chlorophyll plays a major role in photosynthesis that leads to plants production. Salinity has severed effects on chlorophyll contents and was attributed to the inhibition of chlorophyll synthesis, together with the activation of its degradation by the enzyme chlorophyllase (Santos, 2004). Salinity stress in our study decreased the chlorophyll contents and correlated with Jaleel *et al.*, (2008) who studied the chlorophyll contents in *Catharanthus roseus* and its values decreased at higher salinity level. Similar results also found by Azooz *et al.*, (2004) and (Taibi *et al.*, 2016) that revealed the decreased in chlorophyll in *Phaseolus vulgaris*.

**Shoot/Root lengths:** Shoot length is the total length of plant from collar region to the aerial part of plant. It determines the extent of biomass production in plants. Shoot length of halophyte is greatly affected by salinity (Jaleel *et al.*, 2008). Shoot length of *C. jwarancusa* was significantly affected under different treatments as describe in table 3. Under normal saline condition T2 *C. jwarancusa* gained its shoot length (72.8 A) and the length was decreased with elevated NaCl treatments T3 and T4 (61.07 B) and (50.96 C) respectively. At controlled environment T1 the shoot length was (44.84 C). Shoot length of *A. lagopoides* increased with the increasing salinity at T1, T2 and T3 (24.71 C), (60.36 B) and (80.23 A) while at very high salinity T4 it was reduced to (27.63 C).

Results demonstrate that salt levels have great influence on shoot length *C. setigerus*. At medium level T2 the shoot length was (54.8 A) and with the increasing salinity T3 and T4 it was reduced to (42.4 B) and (30.7 C) respectively. At non saline treatment T1 the shoot length was (30.7 C). Maximum shoot length of *P. antidotale* was maintained under treatment T2 (69.2 A) while the length was lagging behind at higher salt treatments T3 and T4 (58.0 B) and (46.0 C) accordingly. Comparison showed that at higher salt treatment *A. lagopoides* retained the highest shoot length T3 (80.23 A) while at normal saline treatment T2 *C. jwarancusa* was the best (72.80 A). Shoot length in *Cynodon dactylon* increased significantly with the increasing NaCl levels while shoot length increased at medium and started decreasing at higher levels. Root/shoot fresh and dry weights in *Cynodon dactylon* increased to some extents while at higher levels it started decreasing (Hameed & Ashraf, 2008). Root and shoot length in rice cultivars were also reduced under elevated salinity (Puvanitha & Mahendran, 2017). Fig. 3 shoot lengths of halophytic grasses i.e., *C.j*, *A.l*, *C.s* and *P.a* under different NaCl treatments.

Roots are the underground parts of plants that not only anchor the plants in soil but also absorb water and minerals for proper growth and defensive mechanism against diseases and other biotic or abiotic stress (Puvanitha & Mahendran, 2017). Root lengths in *C. jwarancusa* were highest at treatment T2 (47.41 A). Results showed that there were no significant changes occurring in root lengths under different salt treatments. At T3, T1 and T4 root lengths were measured (38.08 A), (31.75 A) and (35.47 A) correspondingly. Roots lengths in *C. setigerus* were significantly lower with increasing NaCl level. At treatment T2 it was maximum (33.70 A) and reduced at higher salts T3 and T4 (27.13 B) and (20.06 C) respectively. Due to halophytic in nature it shows low root length under control treatment T1 (21.38 C).

Because of halophytic nature *A. lagopoides* grew well and showed maximum root length at higher saline conditions T3 (43.05 A). But at very high salinity treatment T4, decrease the root length was observed (18.8 C). Under normal and controlled salinity levels T2 and T1 the species showed lower root lengths (30.0 B) and (20.63 C) correspondingly. Salinity significantly changed the root length of *P. antidotale*. Maximum root length was observed under medium salts level T2 (48.93 A) but root length was lagging behind with the elevation of NaCl level ant at T3 and T4 these were (37.46 B) and (29.06 C) respectively. Under controlled conditions it also showed retarded growth at T1 (24.2 D). Fig. 4 root lengths of halophytic grasses i.e., *C.j*, *A.l*, *C.s* and *P.a* under different NaCl treatments.

**Table 3. Shoot/root lengths in *C. jwarancusa*, *A. lagopoides*, *C. setigerus* and *P. antidotale* against salt stress.**

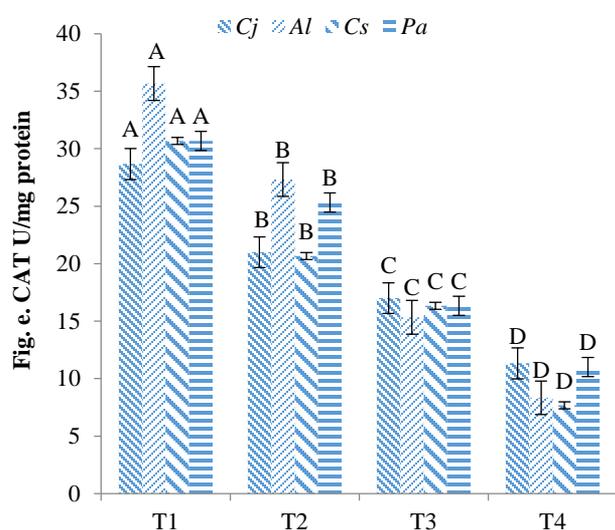
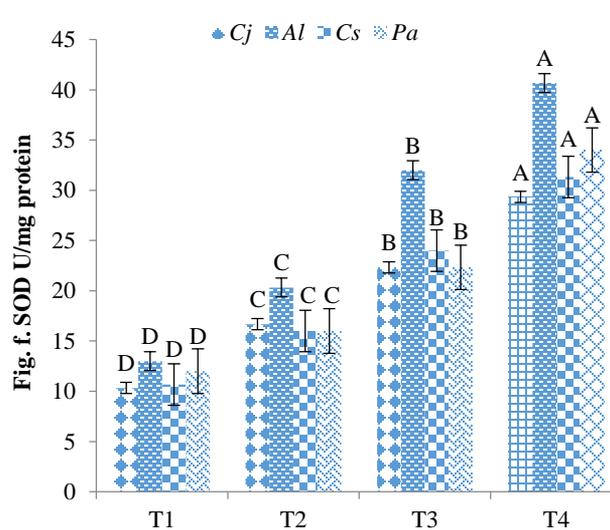
Treatments mM NaCl	<i>Cymbopogon jwarancusa</i>		<i>Aeluropus lagopoides</i>		<i>Cenchrus setigerus</i>		<i>Panicum antidotale</i>	
	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
T1 0	44.87 ± 1.93	25.82 ±	27.63 ± 0.53	20.63 ± 0.91	30.48 ± 0.47	21.38 ± 0.55	33.36 ± 0.36	24.55 ± 1.23
T2 70	72.81 ± 0.66	49.10 ±	60.36 ± 0.81	29.63 ± 1.26	55.03 ± 0.80	33.7 ± 0.47	68.58 ± 0.71	48.93 ± 1.23
T3 140	61.07 ± 2.43	42.33 ±	80.23 ± 0.43	43.05 ± 1.04	43.18 ± 0.62	27.09 ± 0.88	58.13 ± 0.69	37.46 ± 0.71
T4 210	51.0 ± 0.49	33.02 ±	24.7 ± 0.54	18.62 ± 0.63	33.02 ± 0.32	20.06 ± 0.65	46.56 ± 0.49	29.06 ± 0.79

**Table 4. Superoxide Dismutase (SOD) activities in *C. jwarancusa*, *A. lagopoides*, *C. setigerus* and *P. antidotale*.**

Treatments	mM NaCl	<i>Cymbopogon jwarancusa</i>	<i>Aeluropus lagopoides</i>	<i>Cenchrus setigerus</i>	<i>Panicum antidotale</i>
T1	0	10.33 ± 0.68	13 ± 0.77	10.67 ± 0.93	12 ± 0.89
T2	70	16.67 ± 0.68	20.33 ± 0.68	16 ± 0.89	16 ± 0.89
T3	140	22.33 ± 0.68	32 ± 1.18	24 ± 0.89	22.33 ± 0.93
T4	210	29.33 ± 0.93	40.67 ± 0.68	31.33 ± 0.93	34 ± 0.89

**Table 5. Catalase (CAT) activities in *C. jwarancusa*, *A. lagopoides*, *C. setigerus* and *P. antidotale*.**

Treatments	mM NaCl	<i>Cymbopogon jwarancusa</i>	<i>Aeluropus lagopoides</i>	<i>Cenchrus setigerus</i>	<i>Panicum antidotale</i>
T1	0	28.67 ± 0.68	35.67 ± 0.51	30.67 ± 0.93	30.67 ± 0.93
T2	70	21 ± 0.77	27.33 ± 1.03	20.67 ± 0.93	25.33 ± 0.68
T3	140	17 ± 0.77	15.33 ± 0.68	16.33 ± 0.25	16.33 ± 0.93
T4	210	11.33 ± 0.68	8.33 ± 0.51	7.67 ± 0.25	11 ± 0.89

Fig. 5. Super oxide Dismutase (SOD) and Catalase (CAT) activity in halophytic grasses *C.j*, *A.l*, *C.s* and *P.a* under different NaCl treatments.Fig. 6. Super oxide Dismutase (SOD) and Catalase (CAT) activity in halophytic grasses *C.j*, *A.l*, *C.s* and *P.a* under different NaCl treatments.

**Superoxide dismutase (SOD) and Catalase (CAT) activity:** Halophytes have developed strong self-protective mechanism against abiotic stresses produced by Reactive Oxygen Species (ROS) (Sharma *et al.*, 2012). These species have diversity to bear uneven distribution of stresses. Toxicity of ROS produces during the uneven supply of ions such as superoxide radicals, H<sub>2</sub>O<sub>2</sub> and singlet oxygen. Antioxidants Superoxide dismutase (SOD) responds with superoxide radical and restrict the production of H<sub>2</sub>O<sub>2</sub> that can be removed by Catalase (CAT) and Peroxidase (POX). Superoxide dismutase (SOD) is the earliest responsive enzyme to overcome the stress and determine the number of superoxide radical and H<sub>2</sub>O<sub>2</sub>. Plants are also capable of accumulating solute against stress and compartmentalization of toxic ions (Glenn *et al.*, 1999). Homeostasis can also be maintained by these solutes that stable the protein and membrane during dehydration and (Ashraf & Foolad 2007; Ivan *et al.*, 2012). Sobhanain *et al.*, (2010) experienced the same

results and reported that Antioxidant enzymes (SOD and CAT) activities were significantly higher at higher (750 mM NaCl) salt stress. CAT activity was lower at low stress while activities of SOD were increased with the increasing level of salts (Sobhanain *et al.*, 2010). Activities of antioxidants enzymes SOD and CAT were also studied by (Ivan *et al.*, 2012) in halophytes, and obtained the similar results.

SOD activity was increased with the increasing salinity in all the four species. In *C. jwarancusa* SOD value was at T4 (31.33 A) followed by the treatments T3, T2 and T1 that were (24.0 B), (16.0 C) and (10.67 D) while in *A. lagopoides* highest percentage of SOD activity at T4 that was (40.67 A). While at treatment T3 its activity was (32.0 B), monitored by the treatments T2 and T1 that were (20.33 C) and (13.0 D). SOD activity in *C. setigerus* under high salt treatment T4 was (31.33 A). Table 4 showed that SOD activity increased with increasing salts. At T3, T2 and T1

treatment conditions activity was (24.0 B), (16.0 C), and (10.67 D) respectively. SOD activity in *Panicum* was reported the highest value at T4 treatment (34.0 A) followed by the T3, T2 and T1 (22.3 B), (16.0 C) and (12.0 D). Overall comparison of four species showed that *A. lagopoides* species had the highest SOD activity (40.67 A) and was highly salt tolerant. *P. antidotale* was the second most salt tolerant and showed (34.0 A) SOD activity at T4 followed by the *C. setigerus* and *C. jwarancusa* (31.33 A) and (29.33 A) respectively. Fig. 5 Super oxide Dismutase (SOD) and Catalase (CAT) activity in halophytic grasses *C.j*, *A.l*, *C.s* and *P.a* under different NaCl treatments.

After detoxification of Singlet oxygen ( $^1O_2$ ), super oxide anions ( $O_2^-$ ) and radicals of hydroxyl ( $\cdot OH$ ) into hydrogen peroxide  $H_2O_2$  by the Superoxide Dismutase (SOD). But the excess of  $H_2O_2$  also produced toxicity. Activity of Catalase (CAT) detoxifies the  $H_2O_2$  (Bose *et al.*, 2014). Catalase (CAT) activity in *C. jwarancusa* was maximum at T1 (0 mM NaCl) (28.67 A) as describe in table 5. CAT activity in all the four species decreased with increasing salt level. Activity of catalase CAT in *C. jwarancusa* was decreased with the increasing salinity. The activity of CAT at T2, T3 and T4 reduces with the increasing salt (21.0 B), (17.0 C) and (11.3 D) respectively. Maximum CAT activity in *A. lagopoides* was measured at T1 treatment (35.67 A) and at different treatments T2, T3 and T4 activity of CAT *A. lagopoides* was (27.33 B), (16.33 C) and (8.33 D) correspondingly. CAT in *C. setigerus* was significantly reduced with salinity. At very high treatment T1 it was (30.67 A) and reduced with the increase of salt levels under T2, T3 and T4 (20.67 B), (16.33 C) and (7.67 D) accordingly. Fig. 6 Super oxide Dismutase (SOD) and Catalase (CAT) activity in halophytic grasses *C.j*, *A.l*, *C.s* and *P.a* under different NaCl treatments.

In *P. antidotale* activity of CAT at treatment T1 was (30.67 A) while its activity at T2, T3 and T4 that were (25.33 B), (16.33 C) and (11.0 D) respectively. *A. lagopoides* had the highest CAT activity (35.67 A) followed by the *P. antidotale*, *C. setigerus* and *C. jwarancusa* (30.67 A), (30.67 A) and (28.67 A) respectively.

## Conclusion

Based on results we can conclude that all species are halophytic in nature. These species do well under our normal saline environment while experienced low productivity under very high salinity. Activity of antioxidant enzyme Super oxide Dismutase (SOD) is increased with elevated salinity while activity of CAT is decreased with increasing salt levels.

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