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Osmotic Adjustment of Natural Vegetation in Saline Soils

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Leaf samples of natural vegetation collected in selected areas of variable salinity (ECe - 4, 12, 22 and 36 dS m^{-1}) were assayed for different parameters *viz*. photosynthetic pigments, relative water content (RWC), nitrate reductase activity (NRA), proline, membrane injury index (MII) and ionic ratios to understand the osmoregulation mechanisms in plants. The results showed that the biochemical parameters like photosynthetic pigments, nitrate reductase activity and RWC followed a declining trend while, proline and MII increased with increase in salinity from 4 to 36 dS m^{-1}. The concentration of proline was maximum in *P. oleracea* at 36 dS m^{-1} of conductivity while, it was least in *C. sparsiflorus* at 4 dS m^{-1}. The content of magnesium in leaves decreased whereas, potassium and calcium followed an increasing trend resulting in fall of Na^+/K^+ ratio and rise in Ca^{2+}/Mg^{2+} ratio with increase in salinity. The correlations revealed that the electrical conductivity was positively and significantly correlated with proline, MII and Ca/Mg ratio, whereas it was negatively correlated with photosynthetic pigments, RWC, NR activity and Na/K ratio at 1% level of significance.

*(**Key words:** Chlorophyll, Nitrate reductase, Osmotic adjustment, Proline, Saline soils)*

Salinity stress is a major environmental constraint to crop productivity in the arid and semiarid regions of the world. In India, out of 6.72 million ha of salt affected soils, 2.95 million ha (44%) are saline in nature (including coastal sands). About 1.75 M ha is under inland salinity and 1.2 M ha is distributed in the east and west coasts of India. In undivided Andhra Pradesh out of 2,74,207 ha of salt affected soils, 77,598 ha are saline (Mandal *et al.,* 2010). The most serious problem in saline areas is the scarcity of water. The type of irrigation systems and quality of irrigation water contribute to a large extent in development of substantially or partially unproductive saline soils (Ashraf and McNeilly, 2004). High concentrations of salts cause ion imbalance and osmotic stress in plants.

High salt stress disrupts homeostasis in water potential and ion distribution. This disruption of homeostasis occurs at both the cellular and the whole plant levels. Drastic changes in ion and water homeostasis lead to molecular damage, growth arrest and even death (Zhu, 2001). Saline environments affect the plant growth in different ways such as a decrease in water uptake, an accumulation of ions to toxic levels, and a reduction of nutrient availability.

However, a small group of higher plants can grow under saline conditions. The plant species that live under conditions of high salinity exhibit succulence, which might resort to other physiological adaptations to overcome the adverse saline environment in the soil. Their extreme tolerance to salinity is related to their ability to maintain a high salt concentration within their cells. Plant species growing in saline area may provide useful information regarding the degree of salinization and consequent soil deterioration. Such information may be helpful in more effective planning for practical uses of wastelands. Strogonov (1974) assumed that the survival of plants in saline environments depends upon altered biochemical reactions and on a quantitative ratio between the toxic and protective (*i.e.*, proline) compounds. Keeping this in view, a study was conducted to identify the natural vegetation at different salinity levels and characterize them for mechanisms of osmoregulation.

MATERIALS AND METHODS

Leaf samples of natural vegetation existing in fields having ECe values of 4.14, 11.84, 21.86 and 36.14 dS m^{-1} were collected and categorized into three groups based on their survival at different ECe levels. The ECe values were rounded off to 4, 12, 22 and 36 dS m^{-1} for convenience. The ECe was determined in saturation extract (ECe) by using Wheatstone Conductivity Bridge and expressed as dS m^{-1} (Jackson, 1973). The fresh leaf

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samples were subjected to biochemical assay. The photosynthetic pigments in leaves were estimated colorimetrically by dimethyl sulphoxide (DMSO) method as described by Hiscox and Stam (1979). The relative water content was determined by the method described by Slatyer and Mcllroy (1961) while, nitrate reductase activity in the leaf was estimated as per Hageman and Flesher (1960). The nitrite formed is estimated by the method given by Nicholas *et al.* (1976). The proline content was estimated by the method of Bates *et al.* (1973) and membrane injury index was determined according to the method given by Premachandra *et al.* (1990). The plant samples were shade dried initially and then oven dried at 60°C and powdered. The samples were digested using diacid mixture. The potassium and sodium in the extract were estimated using flame photometer while, calcium and magnesium by EDTA method as described by Tandon (1999).

RESULTS AND DISCUSSION

The details of natural vegetation observed and used for bio-chemical estimations at various salinity levels are presented in Table1. The plants were divided into three groups based on their existence at different salinity levels. Group one comprised of *Cynodon dactylon, Croton sparsiflorus, Parthenium hysterophorus* and *Portulaca oleracea* which were found at all salinity levels (4, 12, 22 and 36 dS m^{-1}) studied. *Tribulus terrestris* was observed only at lower conductivity levels of 4 and 12 dS m^{-1} (Group II) while, *Cressa critica* and *Abutilon indicum* were observed at only higher EC levels of 22 and 36 dS m^{-1}(Group III).

Photosynthetic pigments

The data related to leaf photosynthetic pigments content (chlorophyll a, chlorophyll b, carotenoids and total chlorophyll) are presented in table 2. The chlorophyll a content among various plant species in Group I varied from 0.455 in *C. dactylon* to 0.675 mg g^{-1} fresh weight in *P. hysterophorus* at the lowest salinity level of 4 dS m^{-1}. At the highest EC studied (36 dS m^{-1}) the value ranged from 0.302 in *P. hysterophorus* to 0.409 mg g^{-1} fresh weight in *C. sparsiflorus*. The per cent decline in chlorophyll a varied from 27.03 to 55.25 with the highest and lowest values of fall recorded by *C. dactylon and P. hysterophorus,* respectively. Chl b and carotenoids also recorded similar fall in their contents. The maximum per cent decline of chl b was observed in *P. hysterophorus* (62.95%) and minimum was observed in *P. oleracea* (29.87%) while the maximum (35%) and minimum (19.16%) carotenoids decline was observed in *P. oleracea* and *C. dactylon,* respectively. The data pertaining to total chlorophyll in group I plants indicated that *C. dactylon* recorded the lowest reduction (15.26%), while the highest (23.26%) was observed in *C. sparsiflorus.*

T. terrestris placed in group II (growing at 4 and 12 dS m^{-1}), recorded drop in chl a, chl b, carotenoids and total chl to a tune of 25.20, 50.56, 30.85 and 26.09 per cent, respectively with increase in salinity.

Among Group III plants surviving at high salinity levels (22 and 36 dS m^{-1}) maximum decline in chl a (33.37%) and chl b (20.07%) was observed in *A.indicum*, while, per cent reduction in carotenoids and total chl was maximum (17.37 and 29.75) in *C. critica*.

Azooz *et al.* (2004) reported that chlorophyll pigment drastically decreased with increase of salinity in maize. Soil salinization reduced total chlorophyll content particularly more at later stages and the percent reduction was 2.9 to 44.4 in different varieties of barley (Singh *et al.,* 2007). As the chloroplast is membrane bound, its stability is dependent on the membrane stability which under high salinity condition seldom remains intact due to which reduction in chlorophyll may have occurred (Ali *et al*., 2004).

Table 1*. Natural vegetation at different ECe levels*

Category	Weed name	ECe dS m^{-1}			
		4.0	12.0	22.0	36.0
Group I	*Cynodon dactylon*	✓	✓	✓	✓
	Croton sparsiflorus	✓	✓	✓	✓
	Parthinium hysterophorus	✓	✓	✓	✓
	Portulaca oleracea	✓	✓	✓	✓
Group II	*Tribulus terrestris*	✓	✓	X	X
Group III	*Cressa critica*	X	X	✓	✓
	Abutilon indicum	X	X	✓	✓

***Table 2**. Photosynthetic pigments in natural vegetation at different salinity levels (mg g^{-1} fresh weight)*

Category	Weed name	ECe dS m^{-1}	Chl a	Chl b	Carotenoids	Total Chl
Group I	*C. dactylon*	4.0	0.455	0.650	0.167	1.47
		12.0	0.409	0.480	0.164	1.27
		22.0	0.406	0.466	0.152	1.26
		36.0	0.332	0.371	0.135	1.24
		CV	1.79	0.21	0.31	0.15
	C. sparsiflorus	4.0	0.612	0.791	0.184	1.87
		12.0	0.597	0.747	0.178	1.75
		22.0	0.446	0.503	0.163	1.46
		36.0	0.409	0.379	0.143	1.43
		CV	2.36	0.26	0.08	0.10
	P. hysterophorus	4.0	0.675	0.772	0.173	1.71
		12.0	0.656	0.711	0.161	1.55
		22.0	0.417	0.329	0.120	1.44
		36.0	0.302	0.286	0.114	1.36
		CV	1.83	0.25	0.17	0.19
	P. oleracea	4.0	0.600	0.646	0.180	1.69
		12.0	0.558	0.474	0.140	1.58
		22.0	0.437	0.467	0.120	1.47
		36.0	0.405	0.453	0.117	1.32
		CV	1.83	0.21	0.17	0.21
Group II	*T. terrestris*	4.0	0.373	0.439	0.175	1.73
		12.0	0.279	0.217	0.121	1.28
		CV	0.13	0.07	0.05	0.03
Group III	*C. critica*	22.0	0.670	0.738	0.213	1.28
		36.0	0.564	0.715	0.176	1.81
		CV	0.03	0.04	0.03	0.11
	A. indicum	22.0	0.728	0.513	0.207	1.83
		36.0	0.485	0.410	0.176	1.39
		CV	0.07	0.36	0.04	0.05

Relative water content (RWC) (%)

The data related to relative water content in natural vegetation are presented in table 3. Among different plants in Group I the RWC varied from 78.26 to 85.18 per cent at low salinity level of 4 dS m^{-1} while, it ranged from 59.09 to 66.66 per cent at maximum salinity studied (36 dS m^{-1}). The lowest RWC at all salinity levels was recorded by *C. sparsiflorus*. The per cent decline in relative water content with increase in salinity from 4 to 36 dS m^{-1} was highest (29.45) in *P. hysterophorus* and lowest (18.51) in *C. dactylon*.

T. terrestris (Group II) recorded the RWC of 76.0 and 68.18 per cent, respectively at 4 and 12 dS m^{-1}. The values indicated that the RWC dropped by 10.28 per cent.

The values of RWC registered by *C. critica* and *A. indicum* (Group III) at salinity levels of 22 and 36 dS m^{-1} were 78.57, 74.19 and 70.83, 65.21, respectively with a per cent decline of 5.57 and 7.93. The data indicated that *C. critica* with high RWC at highest salinity level has its higher tolerance to salinity.

The decrease in RWC indicated a loss of turgor that resulted in limited water availability for cell expansion process (Katerji *et al.,* 1997). Bastias *et al*. (2004) have reported that high concentrations of salts, disrupt homeostasis in water relations and change the ion distribution at both cellular and whole plant levels. The existing osmotic stress might have affected the water status of the plants. Salinity induced significant decrease in RWC, but the pattern of decrease varied at different salinity levels. Maximum reduction was observed at high salinity levels as compared to the control. The results are in tune with Fahmy and Esmat (2014).

Nitrate reductase activity (μM $g^{-1}h^{-1}$)

The data related to nitrate reductase activity are presented in table 3. The effect of salinity on NR activity in Group I was paramount (2.09 μM $g^{-1}h^{-1}$) in

Table 3. *RWC, NR activity and proline content in natural vegetation at different salinity levels*

Category	Weed name	ECe dS m^{-1}	RWC (%)	NRA (μM g^{-1} h^{-1} Fw)	Proline (μg g^{-1} Fw)
Group I	*C. dactylon*	4.0	81.81	1.60	177
		12.0	73.68	1.4	191
		22.0	69.23	1.19	252
		36.0	66.66	1.05	336
		CV	0.35	0.28	0.07
	C. sparsiflorus	4.0	78.26	2.09	185
		12.0	71.42	2.01	207
		22.0	62.5	1.26	265
		36.0	59.09	0.98	351
		CV	0.46	0.18	0.15
	P. hysterophorus	4.0	85.18	1.76	171
		12.0	82.14	1.49	193
		22.0	66.66	1.37	282
		36.0	60.09	1.27	315
		CV	0.45	0.26	0.16
	P. oleracea	4.0	83.87	1.61	174
		12.0	71.42	0.95	197
		22.0	75.0	0.86	330
		36.0	66.66	0.60	356
		CV	0.40	0.39	0.17
Group II	*T. terrestris*	4.0	76	1.03	162
		12.0	68.18	0.99	196
		CV	0.08	0.15	0.04
Group III	*C. critica*	22.0	78.57	1.19	218
		36.0	74.19	0.65	305
		CV	0.12	0.08	0.08
	A. indicum	22.0	70.83	1.5	213
		36.0	65.21	0.55	322
		CV	0.11	0.12	0.08

C. sparsiflorus and smallest (1.60 μM $g^{-1}h^{-1}$) in *C. dactylon* at minimal salinity level of 4 dS m^{-1}. At maximum salinity level (36 dS m^{-1}) the lowest NR activity (0.6 μM $g^{-1}h^{-1}$) was recorded by *P. oleracea* while, the highest activity was observed in *P. hysterophorus* (1.27 μM $g^{-1}h^{-1}$). The per cent decline was extreme (62.73%) in *P. oleracea.*

The NR activity in *T. terrestris* of Group II was 1.03 and 0.99 μM $g^{-1}h^{-1}$at 4 and 12 dS m^{-1}, respectively. The percent decline was only 3.88 with increase in EC from 4 to 12 dS m^{-1}.

Among Group III plants, *C. critica* recorded maximum NR activity at both salinity levels (22 and 36 dS m^{-1}). However the per cent decline (63.33%) was maximum in *A. indicum.* and NR activity in *C. critica* dropped by 45.37 percent with increase in salinity from 22 to 36 dS m^{-1}.

Presence of salts in the rooting medium inhibits nitrate uptake and consequently at lower nitrate concentration in the leaves, the NRA decreases (Heidari *et al.,* 2011). The decrease of NRA could be due to the low content of nitrate reductase protein and/or a limiting nitrate transport to shoots. Thus, the effect of salt stress on NRA inhibition could be explained by the limited nitrate availability and/or the toxicity exerted by Na^+ and Cl^-. Reduced nitrate uptake might be responsible for low NR activity as the enzyme is substrate inducible.

Proline content (μg g^{-1} fresh weight)

The data on leaf proline content in different natural vegetation are presented in 3. In Group I, *C. sparsiflorus* recorded the maximum proline content of 185 and 207 μg g^{-1} fresh weight at lower salinity levels i.e. 4 and 12 dS m^{-1}, respectively. Whereas at higher salinity levels (22 and 36 dS m^{-1}), maximum proline content (330 and

356 μg g^{-1} fresh weight, respectively) was observed in *P. oleracea*, which recorded highest per cent rise in proline (51.12%). The proline in *T. terrestris* shoot up to 196 from 162 as the salinity increased up to 12 dS m^{-1} with a per cent increase of 20.87.

Among Group III, *C. critica* recorded the maximum proline content (218 to 305 μg g^{-1} fresh weight) followed by *A. indicum* (213 to 322 μg g^{-1} fresh weight) at higher salinity levels (22 and 36 dS m^{-1}). The per cent increase was highest (51.17%) in *A. indicum*.

Proline, an important osmoprotectant, contributes to osmotic adjustment, protecting enzymes from oxidative damage under saline condition (Gupta and Huang, 2014). Proline accumulated in response to high concentration of inorganic ions and leaf proline is increased under salinity conditions (Ashraf *et al.*, 1998). Proline accumulation presents advantage as compared to other compatible solutes because it has less metabolic reactions besides being a short-chain amino acid (Burg and Ferraris, 2008).

Membrane injury index (MII) (%)

The data pertaining tomembrane injury index (%) are presented in table 4. The MII within Group I plants at minimum salinity level (4 dS m^{-1}) was found to be lowest (20.66%) in *P. hysterophorus* while, highest (26.35%) was observed in *C. dactylon.* Similar trend was followed at maximum salinity level of 36 dS m^{-1} where, *P. oleracea* recorded the highest MII (40.26%) and *C. sparsiflorus* the least (32.07%).

The MII values recorded by *T. terrestris* belonging to Group II were 21.98 and 23.09 per cent at 4 and 12 dS m^{-1}, respectively. Among Group III, *A. indicum* recorded the maximum MII values of 33.58 and 36.89 per cent at salinity levels of 22 and 36 dS m^{-1}, respectively while, the values were 32.38 and 35.90 per cent in *C. critica.* The per cent increase was higher (10.87 %) in *C. critica.* Farooq and Azam (2006) reporteda rise in cell membrane injury under salt stress in different wheat varieties and Rozbeh *et al*. (2012) in *Brassica napus* L.

Salinity has a pronounced effect on plasma membrane and lipid peroxidation, thereby affecting

Table 4. *MII, Na^+/K^+ and Ca^{2+}/Mg^{2+} in natural vegetation at different salinity levels*

Category	Weed name	ECe dS m^{-1}	MII (%)	Na^+/K^+	Ca^{2+}/Mg^{2+}
Group I	*C. dactylon*	4.0	26.35	1.94	1.76
		12.0	28.66	0.95	2.78
		22.0	30.12	0.92	3.15
		36.0	33.73	0.53	3.40
	C. sparsiflorus	4.0	23.85	1.50	2.58
		12.0	28.70	1.00	3.02
		22.0	31.78	0.57	3.71
		36.0	32.07	0.29	4.31
	P. hysterophorus	4.0	20.66	1.60	2.96
		12.0	23.92	0.89	3.53
		22.0	28.18	0.58	3.80
		36.0	32.50	0.49	4.71
	P. oleracea	4.0	24.35	1.04	3.22
		12.0	36.69	0.78	3.77
		22.0	39.60	0.59	4.45
		36.0	40.26	0.44	4.62
Group II	*T. terrestris*	4.0	21.98	0.63	3.04
		12.0	23.09	0.50	3.65
Group III	*C. critica*	22.0	32.38	0.94	4.64
		36.0	35.90	0.50	5.06
	A. indicum	22.0	33.58	0.61	4.11
		36.0	36.89	0.43	4.81

permeability of membranes which in turn modulates the pattern of ion leakage. It has been suggested that decrease in membrane stability reflects the extent of lipid peroxidation caused by over production of reactive oxygen species (ROS) (Sairam and Srivastava, 2002).

Ionic ratios

The data related to Na^+/K^+ and Ca^{2+}/Mg^{2+} ratio are presented in the table 4. The highest Na^+/K^+ (1.94) ratio in Group I plants at least salinity level of 4 dS m^{-1} was observed in *C. dactylon* followed by *P. hysterophorus* (1.60). The maximum percent decline (80.66%) among Group I plants was observed in *C. sparsiflorus. T. terrestris* of Group II followed similar trend with values of 0.63 and 0.50 at 4 and 12 dS m^{-1}, respectively *C. critica* in Group III scored highest Na^+/K^+ ratio of 0.94 to 0.50 at 22 and 36 dS m^{-1}, respectively. The decline in Na^+/K^+ ratio was less in *A. indicum* with a drop of 29.50 per cent whereas it was maximum (46.80) in *C. critica*. Among Group I, higher Ca^{2+}/Mg^{2+} ratios (2.96 and 4.71) at salinity levels of 4 and 36 dS m^{-1} were observed in *P. hysterophorus* while, *C. dactylon* recorded the lower values of 1.76 and 3.40, respectively. The Ca^{2+}/Mg^{2+} ratios in Group II plant *i.e. T. terrestris* were 3.04 and 3.65 at lower salinity levels (4 and 12 dS m^{-1}). Among Group III, *C. critica* recorded maximum ratios (4.64 and 5.06) while, minimum ratios (4.11 and 4.81) were seen with *A. indicum,* respectively at higher salinity levels of 22 and 36 dS m^{-1}.

The detrimental effects of salinity on plant growth may occur through an ionic imbalance, particularly of Ca^{2+} and K^+ (Kusvuran *et al.,* 2007). Some species and cultivars can maintain higher growth under saline conditions by accumulating fewer toxic ions and maintaining a high tissue Ca^{2+} and K^+ concentration (Essa, 2002). Metabolic toxicity of Na is largely due to its ability to compete with K^+ for binding site essential for cellular function (Bhandal and Malik, 1988). Potassium is major plant macro-nutrient that plays important roles related to stomatal behavior, osmoregulation, enzyme activity, cell expansion, neutralization of non-diffusible negatively charged ions, and membrane polarization. Munns and Tester (2008) reported that high Na^+ and Cl^- concentrations could disrupt electrochemical gradients across membranes, denature proteins, increase levels of reactive oxygen species (ROS), or displace cellular K^+, a vital cofactor for many enzymes.

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