

Effect of individual and interactive alkalinity and salinity on physiological, biochemical and nutritional traits of Marvel grass

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Soil salinization and alkalization frequently co-occur in nature. However, only few studies focus on the interactive effects of mixed salt and alkali stresses on plants. To find supplementary feed source under arid and semiarid conditions, *Dichanthium* (Forsk.) Stapf. seeds and root cuttings were collected from extreme saline sodic Kachchh plains, Bhuj (Gujrat), and established at ICAR-Central Soil Salinity Research Institute, Karnal. The experiment was designed in RBD having nine different treatments i.e. control (pH₂: 7.1; ECe: 0.43), alkaline (pH₂: 9.5 and 10.0), saline (ECe: 15, 25, 35 dS m⁻¹) and saline-alkaline (pH₂ 9.0 with ECe: 10, 15, 20 dS m⁻¹). Under alkaline conditions, *Dichanthium* maintained their plant height but reduction was observed in chlorophyll concentration at both the stresses. Highest photosynthetic rate (Pn) was recorded in control treatment i.e. 36.05 μmol CO₂ m⁻² s⁻¹ which was decreased with the intensified stress. Reductions were also noticed in the rates of stomatal conductance (gS) and transpiration rate (E) under different stress levels. *Dichanthium* restrict Na⁺ accumulation (mean Na⁺ 0.27%) in root zone whereas in shoots, mean Na⁺ was 4.58%. *Dichanthium* maintained or increased shoot K⁺ concentration under saline and mixed stress condition to mitigate the injurious effect of high Na⁺ concentration. Among fodder quality parameters, *Dichanthium* contained 5.15% mean crude protein (CP). Stress treatment caused 10-25% reduction in the CP content over all the stress treatments. Alkalinity, salinity and mixed saline sodic stress caused reduction in neutral detergent fiber (NDF) content but maximum reduction was observed under salinity stress condition. ADF (Acid detergent fiber) content was higher in control (47.44%) and decreased with increasing salt stress. ADL followed the same the trend as shown by ADF.

Keywords: Abiotic stress, Alkalinity, *Dichanthium annulatum*, Fodder quality, Gas exchange attributes, Ionic relations, Kachchh plains, Salinity

Salinity is one of the important environmental stresses affecting agricultural productivity, by its effect on plant growth and metabolism. This environmental problem is becoming more prevalent throughout the world due to intensification of agriculture and global climate change. Soil salinization and alkalization frequently co-occur in nature and the conditions in natural salt-alkali soils are very complex. Some salt-alkali soils have high salinity but low pH, while some have low salinity but high pH¹⁻³. Neutral salts (NaCl and Na₂SO₄) and alkaline salts (NaHCO₃ and Na₂CO₃) in soils are two distinct stresses for plants, and are termed as salt and alkali stress, respectively²⁻⁴. When saline soil contains CO₃²⁻ and/or HCO₃⁻, it causes injury to plants not only through salt stress but also through alkali stress⁵. Globally, more than 900 million hectares of land, approx. 20% of the total

agricultural land⁶ and 6% of the world's total land area are affected by salt. In India, SAS occupy an area of about 6.73 million ha, of which saline and sodic soils constitute roughly 40 and 60%, respectively⁷. Salt-affected soils (SAS) are widespread in irrigated arid and semi-arid regions of the world where irrigation is essential to increase agricultural production to satisfy food requirements. Soil salinity is an increasing problem for agriculture, affecting the most productive crop areas of the world, those cultivated under irrigation in arid and semiarid regions which represent less than 15% of global arable land, but produce more than 40% of world food⁸. The shortage of water and high salinity is major factors hindering plant growth in these areas. Owing to the extreme salinity characteristics associated with these soils, salt tolerant halophytes forms predominant vegetation in the region. This ecosystem support many flowering plants, shrubs, climbers, herbs, trees and grasses as reported by Ishnava *et al.*⁹ and supply

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fuel, fodder and timber for local people and livestock. Saline lands are not suitable for growth of traditional crops because of extreme salinity and other adverse factors. If plant salt tolerance cannot be improved, then vast areas of saline soils may be left uncultivated. It severely threatens the national food security and biomass energy production. To maximize crop productivity, these areas should be brought under utilization where there are options for removing salinity or using the salt tolerant crops. Use of salt tolerant crops does not remove the salt whereas halophytes that have capacity to accumulate and exclude the salt in an effective way¹⁰.

The interest in search for alternative/additional feed ingredients is of paramount importance in developing countries, mainly because of the acute shortage of traditional feed materials. Introduction of saline agriculture production systems in salt affected regions effectively saves fresh water for human and animal consumption while the saline water could be used for animal feed production^{11,12}. The major concern in developing a sustainable dairy sector is to ensure availability of green fodder throughout the year to feed the animals¹³. However, the scarcity of green fodder is severe and at present, India alone faces a net deficit of net deficit of 35.6% of green fodder, 26% of dry crop residues and 41% of concentrate feed ingredients in India¹⁴. The ever increasing cultivation of cereals and cash crops resulted in shrinking the land available for fodder cultivation. A promising but not yet deeply investigated field is represented by the use of halophytic plants in association with crops cultivated in conditions of salt stress: the salt uptake and accumulation performed by the halophytes can reduce the severity of the stress at rhizospheric level, providing better conditions for the growth of the agricultural species and thereby, better yields. Many halophyte grasses and non-grasses having potential to be used as fodder, grows in the region that exhibit inherent potential to survive salt concentration even greater than the seawater¹⁵. They survive saline environments by developing mechanisms to overcome water deficit in the root zone arising from low water potential, ion toxicity and nutrient imbalances. These plants have special physiological adaptations that enable them to grow in salt affected soils under seawater irrigation and can produce relatively high consumable biomass in saline areas where non-halophytic species did not grow or have low dry matter yields¹⁶.

Therefore, halophytes may be considered as a supplementary feed source under arid and semiarid conditions but only few studies focus on the interactive effects of various salt and alkali stresses on plants. In this study, we have made an attempt to evaluate the physiological responses of *Dichanthium* grass on salt affected soils (alkaline/saline).

Materials and Methods

Experimental details (Plant material and growth conditions)

Seeds as well as root slips of *Dichanthium annuatum* (Forsk.) Stapf. (Grass halophyte; Poaceae) were collected from extreme saline sodic Kachchh plains, Bhuj, Gujarat and established in pots under controlled conditions. After establishment, these grasses transferred to micro plots (2.5×1.5×0.5 m) facilities of Crop Improvement Division, Central Soil Salinity Research Institute (CSSRI), Karnal (29°43'N, 76°58'E, and 245 m above the mean sea level), Haryana, India. Different treatments of alkalinity/salinity were imposed in these microplots separately (pH₂: 9.5 and 10.0 and ECe: 15, 25, 35 dS m⁻¹) and in combination (pH₂: 9.0 with ECe: 10, 15, 20 dS m⁻¹) with 3 replications. The net house was covered with a high quality polythene sheet to avoid the entry of rain water and the desired salt stress was maintained in the microplots as per treatments.

Physiological parameters

The following physiological parameters were studied thrice (one month interval) 30 days after the imposition of stress treatments. Photosynthetic rate (Pn), transpiration (E), and stomatal conductance (gs) were measured with an infrared open gas exchange system (LI-6400, LICOR Inc., Lincoln, NE, USA). The photochemical efficiency of plants was obtained from the fluorescent analysis of the chlorophyll. The measurements were made on the same leaves that were evaluated for gas exchange. The maximum photochemical efficiency (*Fv/Fm*), quantum photochemical yield [Y(II)] of photosystem II were determined using a portable pulse modulated fluorescence measurer (Junior PAM chlorophyll fluorometer, Germany) after adapting the leaves to the dark for 5 min via special leaf clips. The readings were made after saturating 1 s light pulses to promote the closing of the photosystem II reaction center. The chlorophyll content was determined using DMSO (Dimethyl sulphoxide) as described by Hiscox and Israelstam¹⁷. Freshly harvested plants were weighed

and analyzed for proline¹⁸. For Na⁺ and K⁺ content, 100 mg of oven dried and well ground plant material was digested with 10 mL of HNO₃ : HClO₄ (3:1) di-acid mixture and readings were taken with flame photometer (PFP7, Jenway, Bibby Scientific, UK) using standard NaCl and KCl.

Fodder quality

Protein content was determined as N content multiplied by 6.25. Different proximate components of *Dichanthium* i.e. cell wall constituents (NDF, ADF and ADL) were estimated as per procedure described by Goering and Van Soest¹⁹.

Statistical analysis

All the data were subjected to variance analysis using the SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA). Duncan's multiplication range test was applied at 5% probability level to compare the mean differences. Correlation analysis was performed to determine the relationship between the traits using the Pearson coefficient procedure.

Results and Discussion

In this study, we evaluated the physiological responses and nutritional quality aspects of the halophyte grass, *Dichanthium annulatum* under salt affected environments. Per cent germination in *Dichanthium* was very poor i.e. 15-20%. So, root cutting were used for growth and maintenance. The

results of DMRT test showed significant effect of salinity and sodicity alone or mixed saline sodic stresses. Significant variability ($P < 0.01$) were observed in all studied parameters among different treatments, as indicated by mean sum of squares.

Growth and physiological responses

Plant height is a reliable growth indicator to reflect plant stress resistance. Under sodic/alkaline conditions, *D. annulatum* maintained their plant height or showed marginal decrease i.e. decrease in plant height with 1.8 % at pH₂ 9.5 and 3.78 % at pH₂ 10.0 was observed compared to the control (Table 1). In case of saline stress alone and mixed saline sodic stress, plant height showed decreased pattern (Table 1). While salinity alone or in combination with sodicity caused reduction in plant height i.e. 63.1% at ECe 35 dS m⁻¹ and 67.73% at pH₂ 9.0 + ECe 20 dS m⁻¹ compared to the control. Regardless of the salt concentration used, salt stress has different degrees of inhibition on the growth of plants^{20,21}. Kattach and Ouda²² also reported that increasing salt concentration also caused a proportional decline in plant height but there were genotypic differences with different salinity levels. There are few studies on the physiology of salt-alkali tolerance of halophytes. Reduction in plant growth might be due to an osmotic effect of salt stress, resulting from low water potential²³ or it could be partially attributed to the reduction of carbon assimilation under stress²⁴. Ion

Table 1 — Effect of salt stress (alkalinity/salinity) on physiological and nutritional (fodder quality) properties of *Dichanthium annulatum*

	DF	Plant height (cm)	BM	PC	Na ⁺ (R)	Na ⁺ (S)	K ⁺ (R)	K ⁺ (S)	CC	CP	NDF	ADF	ADL
Replication	2	110.0139	14.2222	0.009	0.0001	0.006	0.0007	0.0017	0.133	0.0174	142.77	0.204	0.0057
Treatment	8	3056.61**	1128.27**	10.15**	0.0136**	23.34**	0.0245**	0.538**	64.24**	0.5761**	179.48	14.238**	0.334**
Error	16	11.1201	0.5660	0.0021	0.0000	0.0758	0.0004	0.0008	0.1224	0.0079	147.99	0.063	0.044
Response of <i>Dichanthium annulatum</i> under salt affected environments													
	Treatments	PH	BM	CC	PC	Na ⁺ (R)	Na ⁺ (S)	K ⁺ (R)	K ⁺ (S)	CP	NDF	ADF	ADL
Control	pH ₂ : 7.1 and ECe: 0.43	136.1 ^A	106.75 ^A	44.85 ^A	0.86 ^H	0.178 ^I	1.10 ^F	0.635 ^A	2.15 ^A	5.64 ^A	76.42	47.44 ^A	6.24 ^A
Sodic Stress	pH ₂ : 9.5	133.65 ^A	94.5 ^B	41.25 ^B	2.84 ^F	0.252 ^F	2.23 ^E	0.575 ^C	1.95 ^B	5.50 ^{AB}	73.07	43.51 ^B	5.73 ^B
	pH ₂ : 10.0	130.95 ^A	86.0 ^C	32.85 ^F	3.66 ^D	0.317 ^C	1.34 ^F	0.450 ^E	1.87 ^C	4.95 ^D	70.30	45.26 ^C	5.57 ^{BC}
Saline Stress	ECe: 15 dSm ⁻¹	69.5 ^B	50.83 ^{EF}	40.75 ^B	1.62 ^G	0.214 ^G	5.72 ^C	0.520 ^D	0.97 ^H	5.45 ^B	72.14	46.46 ^B	5.47 ^{BC}
	ECe: 25 dSm ⁻¹	60.73 ^{BC}	53.17 ^E	38.55 ^D	3.40 ^E	0.282 ^E	6.98 ^B	0.462 ^E	0.99 ^H	5.25 ^C	69.31	45.31 ^C	5.48 ^{BC}
	ECe: 35 dSm ⁻¹	48.63 ^{CD}	45.83 ^F	32.45 ^F	4.88 ^B	0.333 ^B	3.30 ^D	0.352 ^F	1.66 ^E	4.83 ^D	49.81	44.48 ^D	5.28 ^{CD}
Saline-Sodic Stress	pH ₂ : 9.0 + ECe: 10 dSm ⁻¹	71.95 ^B	61.17 ^D	39.92 ^C	3.88 ^C	0.196 ^H	5.70 ^C	0.611 ^{AB}	1.23 ^G	5.53 ^{AB}	73.15	43.52 ^E	5.51 ^{BC}
	pH ₂ : 9.0 + ECe: 15 dSm ⁻¹	52.7 ^{CD}	49.17 ^F	35.52 ^E	4.90 ^B	0.294 ^D	5.47 ^C	0.585 ^{BC}	1.37 ^F	4.85 ^D	72.38	42.12 ^F	5.23 ^{CD}
	pH ₂ : 9.0 + ECe: 20 dSm ⁻¹	42.53 ^D	43.33 ^G	31.61 ^G	7.01 ^A	0.381 ^A	9.42 ^A	0.527 ^D	1.71 ^D	4.32 ^E	70.62	40.79 ^G	5.09 ^D
	Mean	82.97	65.56	37.53	3.67	0.272	4.58	0.524	1.54	5.15	69.69	44.65	5.51
	CV (%)	4.02	1.15	0.932	1.23	2.52	6.00	3.66	1.86	1.72	17.46	0.563	3.82

[BM, Biomass (g/plant); CC, Chlorophyll conc. (µg/mL); PC, Proline content (mg/g FW); Na⁺ (R), Root Na⁺ conc. (%); Na⁺ (S), Shoot Na⁺ conc. (%); K⁺ (R), Root K⁺ conc. (%); K⁺ (S), Shoot K⁺ conc. (%); CP, Crude protein (%); NDF, Neutral detergent fibre (%); ADF, Acid detergent fibre (%); and ADL, Acid detergent lignin (%)]

toxicity and ionic imbalance caused by salinity also disrupted several cellular functions and physiological processes²⁵, resulting a retarded growth; however, halophytes either have stimulated growth at lower salinities or relatively less affected under salt stress. In terms of biomass accumulation, *D. annulatum* showed 11.48% decrease at pH₂ 9.5 and 19.44% increase at pH₂ 10.0 over their respective control whereas saline stress as well as mixed stress of sodicity and salinity caused higher reduction in biomass (Table 1). Maximum reduction in biomass was observed at ECe 35 dS m⁻¹ i.e. 32.55 % reduction and at pH₂ 9.0 + ECe 20 dS m⁻¹ (36.23% reduction) in comparison to control treatment (61.17 g/plant).

Total chlorophyll concentration has been known as an index for evaluation of source, therefore decrease in concentration can be considered as a stomata non-limiting factor under stress conditions. Under control conditions, chlorophyll concentration was 44.85 µg/mL in *D. annulatum* (Table 1). Reduction in chlorophyll concentration were observed on all the three types of stresses i.e. 27.04% reduction at pH₂ 9.5 (sodic stress), 28.1% reduction at ECe 35 dS m⁻¹ (saline stress) and 29.27% reduction at pH₂ 9.0 + ECe 20 dS m⁻¹ (mixed saline sodic stress). Chlorophyll is a membrane bound pigment and many factors account for its loss under stress conditions. The decrease of chlorophyll is mainly attributed to the destruction of chlorophyll 'a' which is considered to be more sensitive to salinity than chlorophyll 'b'²⁶. This corresponding decrease in chlorophyll content with increasing stress conditions implied a lower capacity of leaf tissues for light harvesting and production of reactive oxygen species which is mainly driven by excess energy absorption in the photosynthetic apparatus; this might be avoided by degrading the absorbing pigments²⁷. Proline accumulation is an important physiological index for plant response under abiotic stresses. Data presented in Table 1 shows increased accumulation of proline content which might counteract the adverse effects of toxic salt ions in cell vacuoles. Under control conditions, *Dichanthium* showed 0.86 mg/g F.W. proline content accumulation. However, 4.23 folds increased accumulation was observed at pH₂ 10.0, 5.62 folds at ECe 35 dS m⁻¹ and 8.07 folds at pH₂ 9.0 + ECe 20 dS m⁻¹ (Table 1) over the respective control treatments. Proline is a potent osmoregulator molecule and counteracts the adverse effects of toxic salt ions in cell vacuoles, contributes to membrane stability and mitigates the effect of NaCl on cell disruption²⁸.

Gas exchange attributes and Chlorophyll fluorescence

Photosynthesis is one of the main physiological processes affected by salt stress, and the emission of chlorophyll fluorescence provides an indicator of the primary photochemistry of photosynthesis. Our results revealed that gas exchange parameters declined as stress conditions prevailed i.e. reduced photosynthesis, minimum transpiration, high stomatal resistance and minimum internal CO₂ concentration. Fig. 1 (A, D & E) shows the changes in gas exchange parameters [photosynthesis rate (A) Pn, transpiration rate (E), stomatal conductance gs (D)], and Fig. 1 B and C show the salt induced changes in chlorophyll fluorescence characteristics [Fv/Fm (B), Y [II] (C)]. These parameters were observed for three months at one month interval. Among gas exchange characteristics, photosynthetic rate (Pn), stomatal conductance (gS) and transpiration rate (E) consistently decreased with increased stress treatment (Fig. 1 A, D and E). The highest photosynthetic rate was recorded in control treatment i.e. 36.05 µmol CO₂ m⁻² s⁻¹ which was decreased with the intensified stress (Fig. 1A). Sodic stress alone reduced the photosynthetic rate by 22.87% at pH₂ 9.5 and 39.87% at pH₂ 10.0 whereas saline stress reduced the photosynthetic rate by 26.48% at ECe 15 dS m⁻¹, 36.42% at ECe 25 dS m⁻¹ and 44.96 % at ECe 35 dS m⁻¹ (Fig. 1A). Mixed saline sodic stress caused maximum reduction, and the minimum photosynthetic rate (17.16 µmol CO₂ m⁻² s⁻¹) was found under stress condition of pH₂ 9.0 + ECe 20 dS m⁻¹. Reductions were also noticed in the rates of stomatal conductance (gS) and transpiration rate (E) under different saline/sodic levels. Stomatal conductance was 0.649 mmol H₂O/m²/s in control and decreased to 0.409 mmol H₂O/m²/s under sodic stress of pH₂ 10.0, 0.367 mmol H₂O/m²/s under saline stress of ECe 35 dS m⁻¹ and 0.107 mmol H₂O/m²/s under combined saline sodic stress treatment (Fig. 1D). In control conditions, recorded transpiration rate was 15.89 µmol H₂O/m²/s, which was decreased to 3.24 µmol H₂O/m²/s in combined stress treatment (pH₂ 9.0 + ECe 20 dS m⁻¹). Sodicity alone caused 38.28% reduction at pH₂ 9.5 and 61.46 % at pH₂ 10.0 in transpiration rate while salinity alone lead to 53-69.3% reduction in transpiration rate in *D. annulatum* (Fig. 1E). The reduction in transpiration rate consequent to stress tends to reduce the salt load into the leaves and helps to increase the longevity by maintaining salts at subtoxic levels longer than it would occur if transpiration rates were not diminished²⁹. There is a

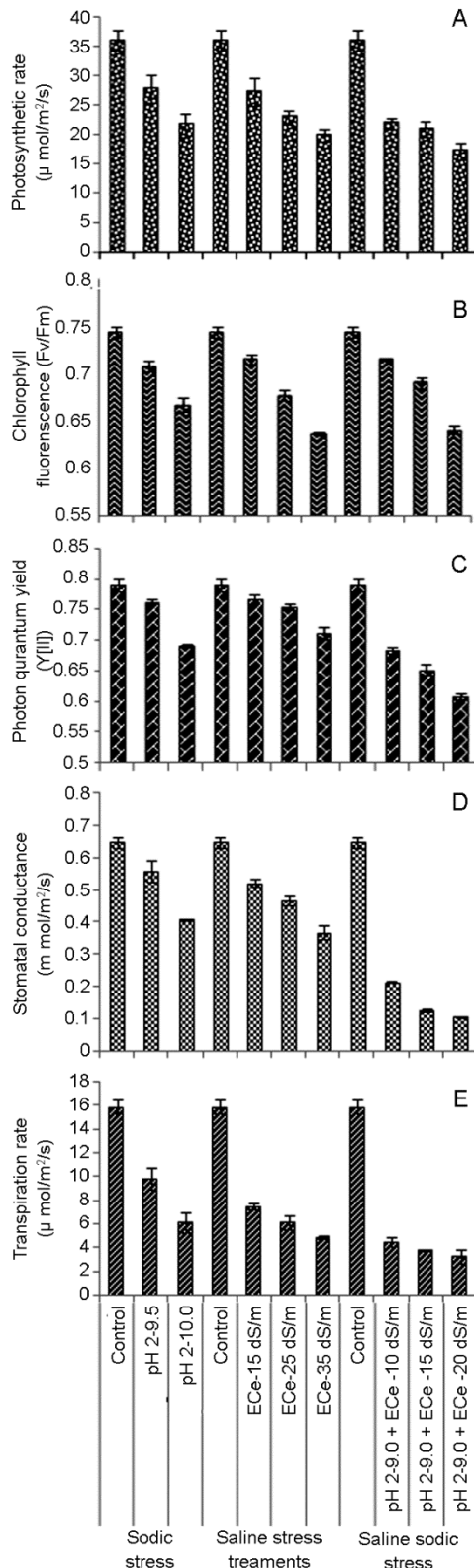


Fig. 1 — Effect of salt stress (alkalinity/salinity) on gas exchange attributes. (A) Photosynthetic rate; (D) Stomatal conductance; (E) Transpiration rate; and chlorophyll fluorescence (B) Fv/Fm; and (C) Photon quantum yield in *Dichanthium annulatum*.

strong link between photosynthesis and stomatal conductance, Stomatal closure reduced photosynthetic activity and transpiration rate could be considered as an adaptive mechanisms to cope with excessive salt, rather than merely a negative consequence of it³⁰. Perturbation in different gas exchange attributes could be associated with decreased utilization efficiency of light, photoinhibition of photosystem³¹ or might be due to increased production of reactive oxygen species (ROS), which lead to decrease in plant photosynthetic capacity. Stomatal closure and the resulting CO₂ deficit in the chloroplasts is the main cause of decreased photosynthesis under mild and moderate stresses³². Although reduced stomatal conductance imposed by high salinity restricts CO₂ diffusion, it might elevate the CO₂ partial pressure across the stomata that are utilized by leaves to maintain a consistently moderate rate of photosynthesis throughout the day, thus avoiding CO₂ starvation and photoinhibition.

Fig. 1B showed that non-stressed control plants had highest Fv/Fm ratio (0.745) while sodic stressed plants i.e. pH₂ 10.0 had the Fv/Fm ratio of 0.668. The Fv/Fm ratio in ECe 35 dS m⁻¹ salt stressed plants was 0.636 and in pH₂ 9.0 + ECe 20 dS m⁻¹ sodic saline stresses plants the ratio was 0.642. This grass halophyte showed less reduction in the Fv/Fm ratio (Fig. 1B). *Dichanthium* under stress conditions showed minimum diminution of the maximum quantum yield of Photosystem II (PSII) i.e. from 0.791 to 0.691 at pH₂ 10.0 (12.57% reduction), 0.712 at ECe 35 dS m⁻¹ (9.99% reduction), 0.609 at pH₂ 9.0 + ECe 20 dS m⁻¹ (23.03% reduction) compared to the control plant (Fig. 1C). The *in vivo* chlorophyll fluorescence technique is a powerful non-destructive and fast method to detect changes in the photosynthetic activity in leaves influenced by changes in the environment. PSII is believed to be the most stress sensitive. The ratio Fv/Fm has been shown to be reliable stress indicator and the decline in Fv/Fm ratio under severe stress reflects a reduction in the ability of PSII to reduce the primary acceptor QA³³. Decreased chlorophyll fluorescence under stress seems to indicate the occurrence of chronic photo-inhibition due to photo-inactivation of PSII centers, possibly attributable to D1 protein damage which usually limits photosynthetic activity³⁴. A decline in quantum yield of PSII observed with increasing stress conditions in our study might have resulted from the closure of stomata as induced by osmotic stress and the accumulation of salt²⁸.

Ion partitioning

Ionic toxicity is one of the components of salt stress, and one of the basic mechanisms of plant defence relies on the compartmentalisation of toxic ions in the vacuoles, which allows osmotic adjustment and, at the same time, avoids the inhibition of metabolic processes in the cytoplasm^{35,36}. Results showed that under stress condition, Na⁺ accumulation in leaves was far greater than that in roots. Concentrations of Na⁺ and K⁺ were determined in roots and shoots on dry matter basis. *Dichanthium* restrict Na⁺ accumulation in root zone and accumulated only 0.178% Na⁺ under control conditions which increased with increasing stress conditions. At pH₂ 10.0, it accumulated 0.317% Na⁺ (78.09% increase over control), 0.333% Na⁺ (87.08% increase over control) at ECe 35 dS m⁻¹ and 0.381% Na⁺ (114.05% increase over control) at pH₂ 9.0 + ECe 20 dS m⁻¹ (Table 1). Tissue-specific compartmentalisation appeared to play an important role in most of the grasses³⁷. While in shoots, non stress control plants accumulated 1.1% Na⁺ concentration which increased at pH₂ 9.5 i.e. 2.23% but it decreased at higher stress level viz. pH₂ 10.0 had 1.34 % Na⁺ concentration (Table 1). As the stress conditions prevailed i.e. saline and mixed saline sodic condition, this grass halophyte accumulated much higher amount of Na⁺ in their leaves i.e. 5.72% at ECe 25 dS m⁻¹ (5.2 folds than control), 6.98% at ECe 35 dS m⁻¹ (6.35 folds than control) and 9.42% at pH₂ 9.0 + ECe 20 dS m⁻¹ (8.56 folds than control) as compared to Na⁺ accumulation in roots (Table 1).

A good supply of K⁺ to plants can minimize injurious effects of high Na⁺ under stress conditions. In the present study, the level of K⁺ gradually decreased while that of Na⁺ dramatically increased. Mean root K⁺ concentration were 0.524% in *D. annulatum*. But sodic/alkaline and saline conditions alone caused significant higher reduction than the others. Root K⁺ concentration decreased by 9.45% at pH₂ 9.5 and by 29.13% at pH₂ 10.0 under sodic conditions and 44.76 % at ECe 35 dSm⁻¹ under saline condition alone as compared to control (Table 1). Whereas *Dichanthium* maintained or increased shoot K⁺ concentration under saline and mixed saline sodic condition to mitigate the injurious effect of high Na⁺ concentration. *D. annulatum* accumulated sufficient amount of K⁺ in the shoots to protect from the injuries of salt stress. Shoot K⁺ concentration was 1.87 % at pH₂ 10.0, 1.66 % at ECe 35 dS m⁻¹ and 1.71 % at pH₂ 9.0 + ECe 20 dS m⁻¹

while in control treatment it was (2.15% K⁺ concentration). Mean shoot K⁺ concentration was 1.54% in *Dichanthium* (Table 1). The potassium plays an essential role in the osmotic and ionic regulation by opening and closing of stomata. It is also necessary for several enzymatic functions and for metabolism of protein³⁸. The diminution of K⁺ concentration in tissue may also be due to direct competition between K⁺ and Na⁺ at plasma membrane, inhibition of Na⁺ on K⁺ transport process in xylem tissues and/or Na⁺ induced K⁺ efflux from the roots. Ion accumulation in the shoot is possibly attributable to an enhanced selective ion uptake in favour of K⁺ over Na⁺ at the root level on the one hand and a high transport capacity in favour of Na⁺ vs. K⁺ from the root to the shoot on the other hand. The diminution of K⁺ concentration in tissue may also be due to direct competition between K⁺ and Na⁺ at plasma membrane, inhibition of Na⁺ on K⁺ transport process in xylem tissues and/or Na⁺ induced K⁺ efflux from the roots. Excessive accumulation of Na⁺ in the leaves has been considered highly harmful for normal metabolism of plants, and tolerant genotypes have the capacity of successful salt exclusion.

Fodder analysis (Nutritional quality)

The proximal nutritional composition of *Dichanthium* leaves were significantly ($P < 0.001$) affected by the salinity level except NDF. Generally about 6-8% CP is required for weight maintenance in various types of ruminants^{39,40}. Highest values of crude protein (5.64%) was observed under control condition (Table 1) with mean value of 5.15% CP. Stress treatment caused reduction in the CP content and the reduction was 10-25% over all the stress treatments i.e. 12.23% reduction at pH₂ 10.0, 14.36% at ECe 35 dS m⁻¹ and 23.4% at pH₂ 9.0 + ECe 20 dS m⁻¹ (Table 1). The reports by Neumann⁴¹; Nilsen and Orcutt⁴² also support our results that an increase in salinity levels in rhizosphere leads to decrease in nitrogen uptake and accumulation. In many plants, protein synthesis is affected by the exposure of the plant to sodium chloride, and in some cases, protein hydrolysis occurs with the release and accumulation of free amino acids in the tissues⁴³. The CP contents of the grass was lower than the maintenance requirements for ruminants as recommended by Norton⁴⁴ who concluded that feeds contain less than 8% CP could not provide the ammonia levels required by rumen microbes for optimum activity and suggested supplementation of such forages with

appropriate nutrients to achieve high level of animal production.

Dichanthium vary greatly in their contents of fibre constituents such as NDF, ADF and ADL as summarized in Table 1. Crude fibre is the sum total of cellulose, hemicelluloses and lignin. The higher values are considered undesirable as the increased concentration of lignin in the cell wall of the plant may significantly reduce the biomass digestibility. NDF (Table 1) varied significantly ($P < 0.001$) among treatments. *Dichanthium* had the highest NDF (71.96%) under control treatment. Alkalinity, salinity and mixed saline sodic stress caused reduction in NDF content but maximum reduction was observed under salinity stress condition i.e. 34.82% reduction at $E_{c} 35 \text{ dS m}^{-1}$. Earlier reports on ryegrass⁴⁵ and bitter vetch⁴⁶ also support the results of current study and observed decrease in NDF content under salinity stress. Similarly, ADF (Table 1) content also varied significantly ($P < 0.001$) with higher values in control (47.44%) and ADF content decreased with increasing salt stress. Lignin is considered to be a major cell wall constituent that may limit nutrient availability for ruminants⁴⁷. ADL followed the same trend (Table 1) as that of ADF. Mean ADL (%) content was 5.51% with highest content i.e. 6.24% at control condition. Many halophytes contain high fibre concentration which reduces digestibility of most nutrients⁴⁸. Salt stress significantly reduced fibre content in all the three halophytes. The structural carbohydrate contents varies significantly within different species and shows a high ADL contents which reduces the use of structural carbohydrates considerably through the ruminal fermentation. The content of CF or fibre constituents in forage plays an important role in its

selection by livestock. Forages with high fibre content are usually better accepted by cattle than by sheep and goats; but this, in turn, depends on the proportions of the various components of fibre i.e. cellulose, hemicellulose, ADF, NDF, etc.

Physiological and fodder quality traits association

Association analysis (Table 2) shows significant interaction among different traits in *Dichanthium annulatum* (Grass halophyte). Highest significant and positive correlation was observed between proline content and root Na^+ concentration ($r = 0.847^{**}$) revealed that *Dichanthium* showed better osmo-protection in terms of accumulation of proline to protect the plant from salt injury. Significant and negative correlation of crude protein was observed with proline ($r = 0.879^{**}$) and root Na^+ concentration ($r = -0.921^{**}$). Significant negative correlation of biomass with shoot Na^+ concentration ($r = -0.700^{**}$) revealed that, increase in Na^+ concentration in tissues with increasing salt stress led to decrease in biomass due to decrease in photosynthates production (Table 2).

Dichanthium annulatum showed good response in terms of physiological parameters as well as nutritional quality parameters upto pH_2 : 10.0 and E_{c} : 35 dS m^{-1} stress conditions. *Dichanthium* although faced stress affects and showing more sensitivity towards saline stress than alkaline stress. Still the plant growth is maintained under stress conditions with sufficient level of crude protein and nutritional quality. Better adaptability of *Dichanthium* in comparison to crop plants under higher saline or alkaline conditions makes this grass a suitable source as potential fodder for small ruminants in salt affected regions.

Table 2—Physiological and fodder quality traits association in *Dichanthium annulatum*

Traits	Plant height	Chlorophyll conc.	Proline content	Na^+ conc. (shoot)	Na^+ conc. (root)	K^+ conc. (shoot)	K^+ conc. (root)	Biomass	Crude Protein	NDF	ADF	ADL
Plant height (cm)	1	0.377	-0.573**	-0.813**	-0.349	0.637**	0.235	0.910**	0.465*	0.184	0.647**	0.660**
Chlorophyll conc. ($\mu\text{g/mL}$)		1	-0.855**	-0.330	-0.925**	0.004	0.651**	0.077	0.907**	0.108	0.701**	0.703**
Proline content (mg/g)			1	0.600**	0.847**	-0.050	-0.323	-0.347	-0.879**	-0.078	-0.923**	-0.753**
Shoot Na^+ conc. (%)				1	0.371	-0.622**	-0.018	-0.700**	-0.483*	-0.227	-0.702**	-0.631**
Root Na^+ conc. (%)					1	0.097	-0.615**	-0.144	-0.921**	-0.158	-0.650**	-0.650**
Shoot K^+ conc. (%)						1	0.138	0.480*	-0.060	0.314	0.182	0.424*
Root K^+ conc. (%)							1	0.040	0.451*	0.267	0.057	0.388*
Biomass (mg/plant)								1	0.265	0.098	0.434*	0.433*
Crude Protein (%)									1	0.065	0.775**	0.692**
Neutral Detergent Fibre (%)										1	0.016	0.103
Acid Detergent Fibre (%)											1	0.716**
Acid Detergent Lignin (%)												1

[NDF, Neutral detergent fibre (%); ADF, Acid detergent fibre (%); and ADL, Acid detergent lignin (%). **|level of significance at 1%; and *5%]

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