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Research Paper

Physiological responses of Bajra-Napier hybrids and a tri-specific hybrid to salinity stress

Respuestas fisiológicas de los híbridos Bajra-Napier y de un híbrido tri-específico al estrés por salinidad

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Abstract

Physiological responses of 3 Bajra-Napier (*Cenchrus* spp., syn. *Pennisetum* spp.) hybrid varieties, viz. BNH-3, BNH-6, BNH-10, and 1 tri-specific hybrid (TSH) were tested under different gradients of soil salinity, i.e. Control, 4, 6 and 8 dS/m electric conductivity (ECe), in a pot trial. The experiment was laid out in a factorial completely randomized design with 3 replications. Shoot dry weight, root dry weight, root:shoot ratio and chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentrations were reduced with increasing salinity level as compared with Control. However, the concentration of Na^+ in leaves increased and K^+ concentration decreased with increasing salinity level. Physiological parameters, i.e. relative water content (RWC), membrane stability index (MSI), chlorophyll stability index, carotenoid stability index and K^+: Na^+ ratio, in leaves tended to be higher in the BNH-3 variety than in other varieties. Shoot dry weight showed highly positive significant correlation with RWC, MSI, K^+ concentration and K^+:Na^+ ratio, while it was negatively correlated with Na^+ concentration ($P<0.01$). All BN hybrid varieties and the tri-specific hybrid studied were susceptible to salinity stress, showing marked reductions in growth as the level of salinity increased above 4 dS/m. However, even at salinity levels producing EC of 8 dS/m these varieties still produced 25–44% DM yields. There are prospects for improving forage yields from saline soils by planting these hybrids but further breeding studies are warranted to identify germplasm with greater tolerance of saline conditions if these soils are to be utilized effectively to contribute more to supplying forage to support the world's ruminant population.

Keywords: *Cenchrus americanus*, *Cenchrus purpureus*, *Cenchrus squamulatus*, dry matter yields, *Pennisetum* hybrids, salt-tolerance, tropical grasses.

Resumen

Se examinaron las respuestas fisiológicas de 3 variedades híbridas de Bajra-Napier (*Cenchrus* spp., syn. *Pennisetum* spp.), a saber, BNH-3, BNH-6, BNH-10, y 1 híbrido ttri-específico (TSH) bajo diferentes gradientes de salinidad del suelo: Control, 4, 6 y 8 dS/m de conductividad eléctrica (EC), en un ensayo en macetas. El experimento se realizó en un diseño factorial completamente al azar con 3 repeticiones. El peso seco del brote, el peso seco de la raíz, la relación raíz:brote y las concentraciones de clorofila a, clorofila b, clorofila total y carotenoides se redujeron con el aumento del nivel de salinidad en comparación con el Control. Sin embargo, la concentración de Na^+ en las hojas aumentó y la de K^+ disminuyó con el aumento del nivel de salinidad. Los parámetros fisiológicos: contenido relativo de agua (RWC), índice de estabilidad de la membrana (MSI), índice de estabilidad de la clorofila, índice de estabilidad de los carotenoides y la

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relación K^+: Na^+, en las hojas tendieron a ser más altos en la variedad BNH-3 que en otras variedades. El peso seco de los brotes mostró una correlación significativa altamente positiva con el RWC, el MSI, la concentración de K^+ y la relación K^+:Na^+, mientras que se correlacionó negativamente con la concentración de Na^+ (P<0.01) Todas las variedades híbridas BN y el híbrido tri-específico estudiado fueron susceptibles al estrés por salinidad, mostrando marcadas reducciones en el crecimiento a medida que el nivel de salinidad aumentaba por encima de 4 dS/m. Sin embargo, incluso a niveles de salinidad que producían una EC de 8 dS/m, estas variedades seguían produciendo un rendimiento de 25–44% de materia seca. Hay perspectivas de mejorar los rendimientos de forraje de los suelos salinos mediante la siembra de estos híbridos, pero se justifica la realización de más estudios de mejoramiento para identificar el germoplasma con mayor tolerancia a las condiciones de salinidad si se quiere utilizar estos suelos de manera eficaz para contribuir más al suministro de forraje para mantener a la población mundial de rumiantes.

Palabras clave: *Cenchrus americanus*, *Cenchrus purpureus*, *Cenchrus squamulatus*, gramíneas tropicales, híbridos de *Pennisetum*, rendimiento de materia seca, tolerancia a la sal.

Introduction

Salinity is one of the major abiotic stresses of arid and semi-arid regions that affect crop growth, development and productivity (Pons et al. 2011). About 20% of the world's cultivated area and about half of the world's irrigated lands are affected by salinity stress (Sairam and Tyagi 2004). More than 800 million hectares of land throughout the world are adversely affected by high salinity (Munns and Tester 2008). In India, salt-affected soils occupy an area of about 6.73 Mha of which saline and sodic soils constitute about 40 and 60%, respectively (Singh et al. 2010).

The physiological responses of a plant to salinity are often complex and multi-faceted, which makes experiments difficult to design and interpret (Negrão et al. 2017). Salinity poses two major threats to plant growth, i.e. osmotic stress and ionic stress (Flowers and Colmer 2008). The responses to these changes are often accompanied by a variety of symptoms, such as a decrease in leaf area, an increase in leaf thickness and succulence, abscission of leaves, necrosis of roots and shoots and a decrease in internode lengths (Parida and Das 2005). Roots, being a primary organ, are directly exposed to saline environments, but their growth is less vulnerable to salinity than that of shoots (Picchioni et al. 1990). The accumulation of Na^+ in roots is an adaptive response used by various woody species to avoid its toxicity in shoots (Picchioni et al. 1990; Gucci and Tattini 1997).

Livestock production is the backbone of Indian agriculture and it has been projected that the livestock population will increase to around 286.5 million adult cattle units by 2050 (IGFRI Vision 2050). The major concern is to ensure sufficient green fodder is available throughout the year, as there is a deficiency of green fodder and concentrate feed (Semple et al. 2003). Cultivation of cereals and cash crops has resulted in the reduction in the area of land for fodder production for livestock, which is the major constraint in green fodder production. There is a need to use degraded lands, particularly saline soils, by identifying salt-tolerant crops and grasses, which could be used as fodder for grazing livestock (Kumar and Sharma 2020).

Bajra-Napier (BN) hybrid is an interspecific hybrid between bajra [*Cenchrus americanus* (L.) Morrone, the name currently accepted by the GRIN taxonomy (npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch) for *Pennisetum glaucum*) and Napier grass (*Cenchrus purpureu*s (Schumach.) Morrone, syn. *Pennisetum purpureum*). Bajra-Napier hybrid and tri-specific hybrid (*Cenchrus americanus* × C. *purpureus* × C. *squamulatu*s; syn. *Pennisetum glaucum* × *P. purpureum* × *P. squamulatum*) are perennial, multi-cut forage grasses with high biomass and high nutritional quality coupled with high palatability (Singh et al. 2018). BN hybrids can withstand drought for a short spell and currently about one hundred thousand hectares are grown in India. Considering the adverse effects of salt stress on crop growth and productivity, the development of salt-tolerant genotypes and more particularly salt-tolerant BN hybrids and tri-specific hybrids could play a major role in sustaining livestock production in the salt-affected lands and would also be helpful in future breeding programs. We hypothesize that these hybrids are salt-tolerant and should produce well in saline soils. Keeping in view the above facts, the present experiment was conducted to evaluate the physiological responses in 3 BN hybrids and 1 tri-specific hybrid (TSH) grown under saline conditions in a glasshouse.

Materials and Methods

Experimental design

This pot study was conducted at Crop Improvement Division of ICAR - Indian Grassland and Fodder Research

Institute, Jhansi (25°45' N, 78°58' E; 243 masl), during Rabi (winter season, October–March) 2018 in a complete randomized block design. Root slips of 4 varieties, viz. BNH-3, BNH-6, BNH-10 and TSH were collected from ICAR-IGFRI Technology Demonstration Block and planted in pots containing 6 kg of soil at 4 different (Control, 4, 6 and 8 dS/m) levels of salinity and 3 replications. The initial properties of the collected soil were: slightly alkaline with pH 7.62; electrical conductivity (ECe) 1.12 dS/m; and low in organic carbon (0.49%). The total nitrogen, available phosphorus and potassium concentrations in the soil were 213, 13.8 and 191 kg/ha, respectively. Saline conditions were created by adding a mixture of NaCl, Na_2SO_4, $MgCl_2$ and $CaSO_4$ (in ratio 13:7:1:4) to pots to provide electrical conductivity of treated soils of 4, 6 and 8 dS/m at 30 days after transplanting with a Control (1.12 dS/m) for comparison. Plants were harvested at 30 and 55 days after stress was imposed.

Shoot dry weight and root dry weight

At each harvest, i.e. at 60 and 85 days of age, above-ground material was removed, placed in paper bags and oven-dried at 45 °C until a constant weight was reached after about 72 hours to determine shoot dry weight (SDW). At the 85-day harvest, roots were also collected and dried (RDW). Root:shoot ratio (RSR) was determined based on the shoot and root values measured.

Physiological parameters

The acetone method was applied to green leaf samples (200 mg fresh weight) from the 3rd leaf from top portion to extract chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Total Chl) and carotenoid (Car) and 100 mg leaf (green leaf) samples were used to determine membrane stability index (MSI) (3rd leaf from top portion) according to the method of Premachandra et al. (1990). The relative water content (RWC) of 100 mg leaf samples (3rd leaf from top portion) was analyzed by the method of Weatherley (1950). Sodium (Na^+) and potassium (K^+) concentrations in 1 g dry leaf (sampled from young shoot leaves) samples were determined by the flame photometer method of Jackson (1973). Chlorophyll stability index (CHSI) was calculated by following the method described by Sairam et al. (1997) using the formula: (Total chlorophyll in salt-stressed plants/Total chlorophyll in Control plants) × 100; a similar formula was used to determine carotenoid stability index (CARSI).

Reduction in performance relative to Controls (%ROC) was calculated as follows:

$$\%ROC = \frac{\text{Value for Control-Value for stressed plants}}{\text{Value for Control}} \times 100$$

Statistical analysis

The study was conducted as a factorial experiment based on a completely random design with 3 replications. The data were analyzed by Microsoft Excel and SAS 9.3 statistical analytical tool and the significance of differences between treatment means was checked with Duncan's multiple range test at P<0.05.

Results

Significant to highly significant interactions were found between variety and level of salinity for SDW and RWC at the first harvest and for MSI and carotenoids at the second harvest, whereas highly significant interactions were found for K^+ and Na^+ concentrations and K^+:Na^+ ratio at the first harvest and for SDW, RDW, RWC, Chl a, Chl b, Total Chl, K^+ and Na^+ concentrations at the second harvest (Table 1).

Effects of salt stress on shoot dry weight, root dry weight and root:shoot ratio

Shoot dry weight (SDW), root dry weight (RDW) and root:shoot ratio (RSR) declined for all varieties as level of salinity increased (Table 2). While an ECe level of 4 dS/m had no significant effect on growth at the first harvest, at the highest salinity level reduction in SDW over Controls ranged from 56% for BNH-3 to 75% for BNH-6, and at the second harvest from 61% for BNH-3 to 72% for BNH-10. Reductions in RDW over the Controls at the second harvest were more pronounced than for SDW with reductions of 19–33% at 4 dS/m and 71–78% at 8 dS/m. As a result, RSR declined from 0.42–0.54:1 for Controls to 0.33–0.39:1 at the highest salinity level (Table 2). At the first harvest, SDW showed positive significant correlations (P<0.01) with RWC, MSI, K^+ and K^+:Na^+ ratio and negative correlations with Chl a, Chl b, Total Chl, carotenoid and Na^+ concentrations. At the second harvest, SDW indicated positive significant correlations with RDW, RSR, RWC, MSI and Chl b, Total Chl, carotenoid and K^+ concentrations, while Na^+ concentrations showed negative significant correlations (P<0.01) with RSR, MSI and K^+:Na^+ ratio (Table 6).

Table 1. ANOVA results of the effects of salt stress on SDW, RDW, RWC, MSI, Chl a, Chl b, Total Chl, Car, K^+, Na^+ and K^+:Na^+ ratio of *Cenchrus* hybrid varieties.

First Harvest Mean square												
Variable	df	SDW	RWC	MSI	Chl a	Chl b	Total Chl	Carotenoids	Chl a+b	K^+	Na^+	K^+:Na^+ ratio
V	3	4.43*	90*	301**	0.18**	0.10**	0.55**	0.008**	0.004NS	0.069**	0.268**	0.081**
ECe	3	164.45**	569**	363**	0.02*	0.02*	0.09*	0.002NS	0.001NS	1.859**	0.085**	0.765**
V×ECe	9	1.59*	7*	2NS	0.004NS	0.003NS	0.004NS	0.001NS	0.08NS	0.027**	0.075**	0.010**
Error	30	0.726	74.564	46.657	0.008	0.005	0.021	0.001	0.045	0.004	0.004	0.002

Second Harvest Mean square													
		SDW	RDW	RWC	MSI	Chl a	Chl b	Total Chl	Carotenoids	Chl a+b	K^+	Na^+	K^+/Na^+ ratio
V	3	4.14*	2.99**	85**	110*	0.15**	0.07**	0.4**	0.02**	0.03NS	0.183**	0.035**	4.123 NS
ECe	3	146.75**	41.60**	4669**	159*	0.40**	0.21**	1**	0.01**	0.03NS	17.843**	0.575**	1237.96 NS
V×ECe	9	1.50**	0.14**	48**	14*	0.01**	0.01**	0.04**	0.0005*	0.01NS	0.170**	0.014**	0.860 NS
Error	30	0.155	0.005	0.826	36.355	0.002	0.0012	0.006	0.0001	0.018	0.004	0.003	1.779

SDW - shoot dry weight
RDW - root dry weight
RWC - relative water content
MSI - membrane stability index
Chl a - chlorophyll a
Chl b - chlorophyll b
Total Chl -total chlorophyll
Car - carotenoid
K^+ - potassium
Na^+ - sodium
K^+:Na^+ ratio - potassium to sodium ratio
V - variety
ECe - electrical conductivity of the extract of a saturated soil-paste.

Table 2. Effects of salt stress on shoot dry weight (g/pot), root dry weight (g/pot) and root:shoot ratio of *Cenchrus* hybrid varieties at 60 days (1st harvest) and 85 days (2nd harvest) of age.

Variety/	Shoot dry weight (1st harvest)				Shoot dry weight (2nd harvest)				Root dry weight (2nd harvest)				Root:shoot ratio			
Treatment	TSH	BNH-6	BNH-3	BNH-10	TSH	BNH-6	BNH-3	BNH-10	TSH	BNH-6	BNH-3	BNH-10	TSH	BNH-6	BNH-3	BNH-10
Control	11.3±0.6	12.0±0.9	11.2±0.5	12.4±0.6	10.8±0.1	10.0±0.5	10.1±0.2	12.4±0.2	5.5±0.1	4.8±0.0	6.0±0.1	5.2±0.0	0.51	0.48	0.54	0.42
ECe4	11.0±0.5	11.5±0.2	10.9±0.3	12.0±0.3	9.6±0.2	9.5±0.1	8.7±0.1	11.2±0.4	4.5±0.0	3.2±0.0	4.7±0.0	4.1±0.0	0.47	0.34	0.43	0.36
ROC%	2	4	2	3	11	5	14	10	19	33	22	21				
ECe6	6.2±0.1	5.7±0.0	7.8±0.1	6.8±0.2	5.1±0.1	4.2±0.1	5.8±0.0	5.6±0.1	1.9±0.0	1.4±0.0	2.9±0.0	1.9±0.0	0.37	0.33	0.37	0.34
ROC%	45	53	30	45	52	58	42	55	65	71	52	63				
ECe8	4.5±0.1	3.0±0.1	4.9±0.0	4.0±0.1	3.7±0.1	3.3±0.0	3.9±0.0	3.4±0.0	1.4±0.0	1.1±0.0	1.7±0.0	1.1±0.0	0.39	0.33	0.35	0.33
ROC%	60	75	56	68	66	68	61	72	74	78	71	79				

Mean (n = 3)
ROC% - per cent reduction over Control for ECe of 4, 6 and 8 dS/m.

Effects of salt stress on Relative water content and Membrane stability index

Relative water content (RWC; %) and Membrane stability index (MSI; %) were considered reliable parameters to assess the salt stress and tolerance of crop species. RWC of leaf declined in all varieties with increasing salinity, with percentage reduction relative to Controls at the highest salinity level ranging from 48 to 63% for the different varieties at the first harvest and from 50 to 69% at the second harvest (Table 3). Membrane stability index (MSI) for all varieties also declined with increasing salinity at first ($P<0.01$) and second ($P<0.05$) harvests. RWC showed highly significant positive correlations with SDW, MSI, K^+ and K^+:Na^+ ratio at the first harvest, and highly significant positive correlations with SDW, RDW, MSI and K^+ and moderately significant correlation with K^+:Na^+ ratio at the second harvest (Table 6). MSI showed highly significant positive correlations with SDW, RWC, K^+ and K^+:Na^+ ratio at the first harvest, and with SDW, RDW, RSR, RWC, K^+ and K^+:Na^+ ratio at the second harvest.

Effects of salt stress on photosynthetic pigments and Chlorophyll stability index and Carotenoid stability index

Data in Table 4 show that chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentrations decreased as salinity increased at both harvests with the main part of the decline occurring between 6 and 8 dS/m. Chlorophyll stability index (CHSI) and carotenoid stability index (CARSI) also declined as salinity level increased, with the major reduction occurring between 6 and 8 dS/m (Table 5). Photosynthetic pigments (Chl a, Chl b and Total Chl) showed significant positive correlations with each other and carotenoid concentrations at both first and second harvests (Table 6).

Effects of salt stress on K^+ and Na^+ concentrations and K^+:Na^+ ratio in leaves

Potassium concentrations in leaves at the first and second harvests declined as salinity levels increased (Table 7) but differences failed to reach significance ($P>0.05$) despite reductions in concentrations at 8 dS/m ECe being about 52 and 70%, respectively. In contrast, sodium concentrations showed little consistent response at the first harvest ($P>0.05$) but increased markedly for TSH, BNH-3 and BNH-6 and decreased for BNH-10 at the second harvest with again no significant responses ($P>0.05$). In general K^+:Na^+ ratio declined as level of salinity increased at both harvests with the effect being much more pronounced at the second harvest (except for BNH-10) but again differences were not significant ($P>0.05$).

In addition to correlations mentioned earlier, K^+ concentrations showed significant positive correlations with K^+:Na^+ ratio at the first and second harvests, while Na^+ concentration showed significant negative correlations with K^+:Na^+ ratio in first and second harvests (Table 6).

Table 3. Effects of salt stress on Relative water content and Membrane stability index in *Cenchrus* hybrid varieties.

Variety/ Treatment	Relative Water Content (%)							
	1st Harvest				2nd Harvest			
	TSH	BNH-6	BNH-3	BNH-10	TSH	BNH-6	BNH-3	BNH-10
Control	82.7±1.21a	78.4±2.40a	74.6±1.70a	78.7±2.04a	77.1±2.23a	70.0±2.31bc	68.0±1.73cd	72.0±1.79b
4 dS/m	78.7±1.91ab (5)	70.0±2.31b (11)	73.0±1.15a (2)	74.0±1.73a (6)	67.0±1.73d (13)	61.0±2.31e (13)	60.0±1.15e (12)	66.0±0.58d (8)
6 dS/m	52.0±2.19c (37)	49.0±2.48c (37)	60.0±2.19a (20)	56.0±1.50c (29)	44.0±1.15g (43)	38.0±1.73h (46)	48.0±1.04f (29)	48.0±1.44f (33)
8 dS/m	38.0±1.44d (54)	29.0±2.31de (63)	39.0±0.92d (48)	36.0±1.73d (54)	24.0±1.33k (69)	23.0±2.31k (67)	34.0±1.73i (50)	30.0±2.19j (58)
Variety/ Treatment	**Membrane Stability Index (%)**							
	1st Harvest				2nd Harvest			
	TSH	BNH-6	BNH-3	BNH-10	TSH	BNH-6	BNH-3	BNH-10
Control	65.0±1.44a	54.2±1.54ab	64.7±1.20a	58.3±0.96a	53.0±3.18a	48.0±2.6a	50.0±2.89a	49.0±3.76a
4 dS/m	57.7±1.50a (11)	46.2±2.17abc (15)	61.5±1.17a (5)	53.3±1.80ab (9)	33.09±1.8b (26)	32.0±2.5b (33)	37.0±1.73b (26)	35.0±1.2b (28)
6 dS/m	42.73±1.92abc (34)	31.72±2.26c (41)	42.11±0.87abc (35)	38.16±1.25bc (35)	34±1.62c (37)	30±2.48bc (38)	35±1.45bc (30)	32±2.37bc (35)
8 dS/m	24.21±1.45c (63)	19.31±2.13c (64)	28.32±1.97c (56)	24.71±1.84c (58)	26±1.82d (51)	14±2.23d (70)	26±1.51cd (48)	25±1.62d (49)

Means within column(s) followed by the same letter(s) are not significantly different ($P>0.05$). N=3. Values in parenthesis depict per cent reduction over control (ROC%).

Table 4. Effects of salt stress on chlorophyll and carotenoid concentrations (mg/g fresh weight) in *Cenchrus* hybrid varieties

Variety/Treatment		First harvest				Second harvest			
		Chl a	Chl b	Total Chl	Car	Chl a	Chl b	Total Chl	Car
TSH	Control	0.60+0.0 4abc	0.47+0.048 abc	1.06+0.004 cbd	0.22+0.03 a	0.56+0.02 bcd	0.43+0.017 edf	0.99+0.038 ed	0.16+0.009 bcd
BNH-6		0.81+0.05 ab	0.65+0.028 ab	1.46+0.083 ab	0.19+0.02 ab	0.75+0.06 a	0.59+0.021 ab	1.34+0.076 ab	0.11+0.028 fhig
BNH-3		0.88+0.05 a	0.69+0.038 a	1.57+0.008 a	0.25+0.02 a	0.78+0.06 a	0.61+0.035 a	1.39+0.052 a	0.22+0.030 a
BNH-10		0.62+0.03 abc	0.49+0.042 abc	1.11+0.076 abcd	0.19+0.02 ab	0.57+0.04 bcd	0.45+0.015 cde	1.03+0.023 cde	0.15+0.007 cdef
TSH	4 dS/m	0.56+0.04 bc (7)	0.42+0.0 4bc (9)	0.99+0.00 4cbd (7)	0.20+0.018 ab (7)	0.51+0.02 cde (10)	0.37+0.015 efg (14)	0.88+0.03 efg (11)	0.15+0.008 cde (8)
BNH-6		0.73+0.05 ab (9)	0.57+0.02 ab (12)	1.33+0.078 ab (9)	0.17+0.016 ab (9)	0.66+0.05 abc (13)	0.49+0.017 cd (22)	1.14+0.07 bcd (15)	0.10+0.006 hifg (15)
BNH-3		0.84+0.04 a (5)	0.65+0.03 a (6)	1.50+0.007 a (5)	0.23+0.023 a (6)	0.71+0.03 ab (8)	0.54+0.018 abc (12)	1.25+0.05 ab (10)	0.20+0.016 ab (9)
BNH-10		0.58+0.03 abc (6)	0.46+0.03 abc (7)	1.04+0.072 bcd (6)	0.18+0.016 ab (7)	0.51+0.03 cde (11)	0.40+0.013 defg (14)	0.91+0.02 edf (12)	0.12+0.006 b (18)
TSH	6 dS/m	0.46+0.05 c (23)	0.39+0.016 bc (17)	0.85+0.031 cd (17)	0.18+0.01 ab (16)	0.35+0.03 fg (38)	0.30+0.023 g (29)	0.65+0.052 g (34)	0.13+0.012 cdefg (20)
BNH-6		0.61+0.04 abc (25)	0.49+0.038 abc (25)	1.10+0.006 abcd (25)	0.15+0.01 b (20)	0.47+0.01 def (38)	0.41+0.023 defg (30)	0.88+0.037 efg (34)	0.08+0.005 fhig (28)
BNH-3		0.72+0.05 a (18)	0.58+0.029 ba (17)	1.30+0.076 ab (17)	0.23+0.02 a (8)	0.65+0.03 abc (16)	0.49+0.040 bcd (19)	1.14+0.068 bcd (18)	0.17+0.004 bc (22)
BNH-10		0.49+0.03 bc (21)	0.41+0.017 bc (16)	0.90+0.018 cd (16)	0.18+0.01 ab (8)	0.40+0.02 ef (30)	0.34+0.012 fg (24)	0.74+0.029 fg (28)	0.13+0.012 defg (10)
TSH	8 dS/m	0.30+0.03 c (50)	0.26+0.016 c (44)	0.56+0.019 d (44)	0.14+0.01 ab (36)	0.21+0.01 gh (62)	0.20+0.007 h (53)	0.41+0.020 h (59)	0.08+0.003 hij (51)
BNH-6		0.32+0.03 c (60)	0.28+0.029 c (57)	0.60+0.054 d (57)	0.1+0.01 b (37)	0.12+0.02 h (84)	0.12+0.030 h (80)	0.24+0.029 h (82)	0.04+0.020 j (64)
BNH-3		0.54+0.02 bc (39)	0.43+0.019 bc (38)	0.97+0.005 bcd (38)	0.2+0.02 b (35)	0.46+0.00 def (41)	0.35+0.023 fg (43)	0.81+0.027 efg (41)	0.12+0.014 defg (46)
BNH-10		0.31+0.03 c (50)	0.25+0.019 c (49)	0.56+0.010 d (49)	0.1+0.02 b (37)	0.21+0.02 gh (63)	0.19+0.004 h (58)	0.43+0.026 h (58)	0.09+0.004 hig (39)

Means in column (s) followed by the same letter (s) are not significantly different (P>0.05). N=3. Values in parenthesis depict per cent reduction over control (ROC%).

Table 5. Effects of salt stress on chlorophyll stability index and carotenoid stability index in *Cenchrus* hybrid varieties.

Variety/ Treatment	Chlorophyll stability index (%)					
	1st harvest			2nd harvest		
	EC4	EC6	EC8	EC4	EC6	EC8
TSH	93	80	53	88	66	41
BNH-6	91	75	41	85	66	18
BNH-3	95	82	61	91	83	87
BNH-10	94	81	50	94	79	21
Variety/ Treatment	**Carotenoid stability index (%)**					
	1st harvest			2nd harvest		
	EC4	EC6	EC8	EC4	EC6	EC8
TSH	93	84	64	92	80	49
BNH-6	91	80	63	85	72	36
BNH-3	94	92	65	91	78	54
BNH-10	93	92	63	82	90	61

Table 6. Correlations among different parameters in *Cenchrus* hybrid varieties subjected to salinity stress.

PM	1st Harvest										
	SDW	RWC	MSI	Chl a	Chl b	Total_Chl	Car	Chl a:b	% K^+	% Na^+	K^+:Na^+ ratio
SDW	—										
RWC	0.977***	—									
MSI	0.930***	0.964***	—								
Chl a	-0.139	-0.041	-0.059	—							
Chl b	-0.130	-0.033	-0.042	0.996***	—						
Total Chl	-0.130	-0.036	-0.054	0.999***	0.998***	—					
Car	-0.173	-0.034	-0.027	0.801***	0.792***	0.791***	—				
Chl a:b	-0.261	-0.179	-0.243	0.682**	0.622*	0.660**	0.633**	—			
% K^+	0.796***	0.817***	0.841***	-0.312	-0.310	-0.314	-0.004	-0.251	—		
%Na^+	-0.178	-0.223	-0.300	0.337	0.331	0.341	0.056	0.269	-0.368	—	
K^+:Na^+ ratio	0.720**	0.751***	0.799***	-0.351	-0.349	-0.355	0.007	-0.258	0.976***	-0.548*	—

PM	2nd Harvest												
	SDW	RDW	RSR	RWC	MSI	Chl a	Chl b	Total Chl	Car	Chl a:b	% K^+	% Na^+	K^+:Na^+ ratio
SDW	—												
RDW	0.927***	—											
RSR	0.651**	0.858***	—										
RWC	0.963***	0.936***	0.710**	—									
MSI	0.808***	0.880***	0.802***	0.874***	—								
Chl a	-0.258	-0.194	0.016	-0.123	-0.139	—							
Chl b	-0.264	-0.184	0.032	-0.133	-0.150	0.993***	—						
Total Chl	-0.274	-0.201	0.018	-0.140	-0.152	0.998***	0.997***	—					
Car	-0.424	-0.287	0.040	-0.264	0.020	0.734**	0.717**	0.732**	—				
Chl a:b	-0.260	-0.194	0.039	-0.120	-0.074	0.839***	0.782***	0.815***	0.695**	—			
% K^+	0.779***	0.838***	0.764***	0.786***	0.816***	-0.199	-0.189	-0.203	-0.151	-0.158	—		
% Na^+	-0.596*	-0.679**	-0.598*	-0.714**	-0.614*	0.132	0.161	0.148	0.243	0.001	-0.379	—	
K^+:Na+ ratio	0.572*	0.732**	0.857***	0.663**	0.778***	-0.041	-0.076	-0.060	0.115	0.200	0.654**	-0.711**	—

PM - parameters; SDW - shoot dry weight; RDW - root dry weight; RSR - root:shoot ratio; RWC - relative water content; MSI - membrane stability index; Chl a - chlorophyll a; Chl b - chlorophyll b; Total Chl - total chlorophyll; Chl a:b - Chl a:Chl b ratio; Car - carotenoid; K^+ - potassium; Na^+ - sodium; K^+:Na^+ ratio - potassium: sodium ratio.

Table 7. Effects of salt stress on Na^+ and K^+ concentrations in leaves of *Cenchrus* hybrid varieties over 2 harvests.

Variety	Treatment	1st Harvest			2nd Harvest		
		% K^+	%Na^+	K^+:Na^+ ratio	% K^+	% Na^+	K^+:Na^+ ratio
TSH	Control	1.81±0.06bd	1.67±0.03ca	1.09±0.02bd	1.93±0.03cd	0.08+0.00a	24.08+0.40bd
	ECe4	1.12±0.02bc	1.90±0.05c	0.59±0.01bc	1.53±0.02c	0.23±0.01ab	6.71±0.19bc
	ECe6	0.95±0.03b	1.80±0.06cb	0.53±0.03b	0.51±0.02bc	0.56±0.01ac	0.92±0.06b
	ECe8	0.88±0.03ab	1.83±0.03cb	0.48±0.01ab	0.26±0.01ac	0.67±0.01ad	0.39±0.01ab
BNH-6	Control	1.67±0.05ad	1.65±0.03ad	1.01±0.05ad	1.22±0.01ad	0.05±0.005ab	24.48±2.18ad
	ECe4	1.00±0.07ac	1.98±0.01cd	0.50±0.03ac	0.65±0.01ac	0.23+0.01b	2.78+0.10ac
	ECe6	0.82±0.01ab	2.00±0.06bd	0.41±0.02ab	0.59±0.01ab	0.59±0.02bc	1.01±0.02ab
	ECe8	0.76±0.02a	2.27±0.04bd	0.34±0.00a	0.49±0.02a	0.70±0.01bd	0.70±0.04a
BNH-3	Control	1.80±0.04ad	1.64±0.03a	1.10±0.04d	1.82±0.01bd	0.09±0.00a	20.90±0.78abd
	ECe4	1.13±0.01ac	1.64±0.04ac	0.69±0.01cd	0.76±0.02bc	0.20±0.01ab	3.70±0.36abc
	ECe6	1.12±0.01ab	1.70±0.02ab	0.66±0.00bd	0.67±0.01b	0.30+0.01ac	2.21+0.17ab
	ECe8	0.93±0.08a	1.50±0.03ab	0.62±0.04ad	0.58±0.01ab	0.46±0.01ad	1.27±0.03aab
BNH-10	Control	1.62±0.03bd	1.70±0.02ab	0.95±0.03bd	2.31±0.01ac	0.59±0.01ab	3.92±0.05abd
	ECe4	1.33±0.03bc	1.95±0.03bc	0.68±0.02bc	0.68±0.02c	0.23±0.01b	3.00±0.05abc
	ECe6	0.99±0.01b	1.67±0.04b	0.59±0.01b	0.61±0.01bc	0.29±0.02bc	2.13±0.18ab
	ECe8	0.75±0.02ab	1.60±0.04b	0.47±0.004ab	0.48±0.02ac	0.30±0.02bd	1.60±0.04ab

Means in columns followed by the same letter (s) are not significantly different (P>0.05), where letter "a" represents the least value. N = 3.

Discussion

Salinity stress affects growth and productivity in plants by altering physiological mechanisms like water relations, metabolism, ion accumulation, nutrient imbalance and Reactive Oxygen Species (ROS) generation. While salinity tolerance in annual forages and plants is well defined (Roy and Chakraborty 2014; Munns et al. 2020a, 2020b; Rahimi et al. 2021), this is not the case for perennial grasses and plants. Salts are common and necessary components of soil and many salts (e.g. sodium nitrate, potassium carbonate, bicarbonate and potassium chloride) are essential plant nutrients at low concentrations.

Grasses are quite variable in their tolerance of salinity in terms of growth (Khan et al. 1999; Hester et al. 2001; Muscolo et al. 2003; Joshi et al. 2004). Muscolo et al. (2003) reported that the biomass of kikuyu grass (*Cenchrus clandestinus* formerly *Pennisetum clandestinum*) leaves and roots was affected by 150 mM NaCl and extensively reduced at high concentration of NaCl (200 mM) compared with Control, while growth was little affected at lower concentrations of NaCl (50 mM).

Our results showed that shoot dry weight, root dry weight and root:shoot ratio declined for all varieties as the level of salinity increased, while the low level of 4 dS/m had very little or no effect on growth and dry matter yield. These results agreed with Al-Ghumaiz et al. (2017), who reported that dry fodder yield declined at high levels of salinity (8,000 ppm NaCl) with very little or no effect on growth and dry fodder yield at the low level of salinity (4,000 ppm NaCl) in perennial ryegrass, tall fescue and orchard grass.

As a macronutrient, potassium (K^+) mostly contributes to a plant's survival when exposed to various environmental stresses such as drought, salinity and cold (Wang and Wu 2013). The positive role of K^+ in the response to salinity is due to: (1) its competitiveness with sodium (Na^+) for binding sites and maintaining relative water content (RWC) in plants (Capula-Rodríguez et al. 2016); and (2) its ability to regulate the balance between ROS and antioxidants to adjust protein synthesis and stomatal function, thereby improving a plant's photosynthetic status (Wang et al. 2013). Moreover, foliar spraying of perennial ryegrass with KNO_3 (10 mM) enhanced growth, chlorophyll concentration and K:Na ratio when grown under saline conditions. The decrease in RWC under saline conditions can be attributed to a reduction of soil water potential in the root zone (Munns et al. 2006). Sairam and Tyagi (2004) and Singh et al. (2020) suggested that reduced shoot height, leaf area and number of leaves in sensitive genotypes under saline conditions may be due to their leaves having lower relative water content and membrane stability index. In addition, the accumulation of Na^+ and Cl^- ions can lead to the production of ROS which, in turn, increases the permeability of the cell membrane and decreases MSI (Nazar et al. 2011). RWC and MSI are good indicators of leaf water status and stability of membranes and are successfully used to determine stress resistance or tolerance in many crop plants (Bangar et al. 2019;

Rahimi et al. 2021). Many reports reveal that RWC and MSI are reduced under drought and salinity (Bangar et al. 2019; Rahimi et al. 2021) and those plants that maintain high RWC and MSI under extreme stress are regarded as being more stress-tolerant (Bangar et al. 2019; Rahimi et al. 2021). In our study, the reductions in RWC at the first harvest at the highest salinity level ranged from 48 to 63% and at the second harvest from 50 to 69%, while reductions in MSI ranged from 56 to 64% at the first harvest and from 48 to 71% at the second harvest. This indicates that, while these varieties can tolerate low salinity levels, impacts on these parameters at higher levels of salinity are quite significant. In our study, shoot dry weight (SDW) was positively correlated with RWC and MSI at both harvests. The highest reductions in SDW relative to Controls at both first and second harvests occurred at the highest salinity level and ranged from 56 to 75% at the first harvest and from 61 to 72% at the second harvest, which are of comparable magnitude to the reductions in RWC and MSI. Our results are in conformity with Rahimi et al. (2021), who reported that RWC and MSI were significantly and positively correlated with K^+: Na^+ ratio and K^+ concentration in shoots and roots of rye grass under salinity stress.

Chlorophyll has been proposed as a useful biochemical indicator of salt tolerance in different plants (Akram and Ashraf 2011) as chlorophyll and carotenoids are involved in the primary step concerning energy production during photosynthesis. Since salinity affects chlorophyll and carotenoid levels, it is not surprising that the growth of plants is inhibited when grown in saline situations. Salt stress increases the activity of chlorophyllase, which promotes degradation of chlorophyll and reduces chlorophyll concentration in plants (Yang et al. 2011). Although salt stress can reduce chlorophyll concentration, the extent of the reduction depends on the salt tolerance of the particular plant species. Differences in reductions in chlorophyll concentrations between the different varieties in our study suggest that the degree of tolerance of salinity by the various varieties was relatively similar, although BNH-3 did display lower reductions relative to Control than other varieties as salinity level increased. Carotenoids play an important role as a precursor in signalling during plant development under abiotic stress as they protect the membranes from oxidative damage (Verma and Mishra 2005). While all varieties demonstrated reductions in carotenoid concentrations relative to Controls with increasing salinity, at the higher salinity levels BNH-10 showed a tendency to suffer less reduction than other varieties. These results corroborate other studies that indicate that plants subjected to increased salinity levels show decreased photosynthetic pigments (Aghaleh et al. 2009; Jampeetong and Brix 2009; Al-humaiz et al. 2017).

Numerous studies have shown that salt tolerance is ultimately manifested in plants through several physiological processes including Na^+ uptake and exclusion, in homeostasis, especially between K^+:Na^+ ratio and partitioning (Ren et al. 2005). Various studies have shown that plants increase Na^+ uptake and reduce K^+ uptake under salt stress (Horie et al. 2001; Zhu 2003). The K^+ ions are beneficial to plants and by increasing K^+ concentration, plants can reduce the absorption of Na^+ ions to a certain extent, thus improving the K^+: Na^+ ratio.

Generally, the data in Table 7 indicate that the mean percentages of Na^+ in leaves of all varieties increased with increase in salinity, while K^+ concentration declined because Na^+ effectively competes with K^+ for uptake in a common transport system, i.e. the Na^+ concentration in saline environments is usually greater than that of K^+ (Gorham et al. 1990). In other words, the decrease in K^+ resulted from the presence of excessive Na^+ in the growth medium because high external Na^+ concentrations are known to have an antagonistic effect on K^+ uptake in plants (Sarwar et al. 2003). Interestingly K^+ concentration in leaf tissue of Controls was relatively similar for both harvests, while Na^+ concentration was much lower at the second than the first harvest.

Conclusions

This study has shown that the varieties of BN hybrids and the tri-specific hybrid studied were all susceptible to salinity stress, showing marked reductions in growth as the level of salinity increased above 4 dS/m. However, dry matter yields obtained at high salinity level (ECe of 8 dS/m) were still at the range of 25–44%. There are prospects for improving forage yields from saline soils by planting these hybrids but further breeding studies are warranted to identify germplasm with greater tolerance of saline conditions if these soils are to be utilized effectively to contribute more to supplying forage to support the world's ruminant population.

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