Salt tolerance mechanisms in perennial fodder grasses

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Keywords: Biomass; double bond index; salt excretion; succulence; Tissue tolerance; total antioxidants;

Abstract

Salinity stress is one of the most damaging stresses in crop plants. It reduces the productivity of the soil and makes it unsuitable for crop cultivation. Fodder crops are considered the best alternative in such uncultivable land. Using salinity-affected land for pasture development is the best alternative to utilize such lands. However, the extent of salinity tolerance varies among different grass species. In this study, Pearl millet Napier hybrids (PMN hybrid) and guinea grass varieties were studied for salinity tolerance in artificially created saline soils in the ratio of 13:7:1:2 (NaCl: Na_2SO_4: MgCl: $CaSO_4$, respectively) to understand the salinity tolerance mechanisms existing in perennial fodder grasses.

Morphologically, the plant height increased in saline-tolerant PMN hybrid varieties, creating more space in nonphotosynthetic tissues to store accumulated salts away from photosynthetic tissues. Whereas in guinea grass tolerant varieties, tiller number increased under salinity. The fresh weight was highest under salinity in the PMN hybrids. In contrast, dry weight was high in control (no salt) plants, implying more water accumulation in PMN hybrids under salinity to dilute the concentration of salts absorbed by the plant. In Guinea grass, varieties like DGG1 had lower leaf succulence than control and high salt excretion through leaf hairs. Tissue tolerance in PMN hybrids was less compared to guinea grass. Membrane stability was maintained in saline-tolerant varieties. The double bond index increased in tolerant PMN hybrid varieties under salinity compared to control, implying fatty acid remodelling for maintaining the stability of membranes under salinity. Fodder grasses adopt various saline tolerance mechanisms based on their growth habit and morphology.

Introduction

Saline-affected soils are increasing due to errors of humanity and climate change. Salinity is devastating stress to plants as it affects their productivity. As more saline-affected lands become barren and uncultivable, pasture development is the best viable option for utilizing such lands. Enhancing the availability of green fodder to livestock reduces the cost of rearing. Cultivating saline-tolerant fodder crops helps in animal husbandry and milk production, as fresh grass minimizes the cost of the concentrates. However, crop species differ significantly in salinity tolerance due to variations in tolerance mechanisms. Hence, this study was carried out to understand the salinity tolerance mechanisms in guinea grass and Pearl millet Napier (PMN) hybrid grass. The popular varieties of these grasses differ significantly in their growth habits and biomass production. A suitable variety combined with high yield and salinity tolerance is a solution for recuperating salinity-affected barren lands**.**

Methods

The experiments to study the salinity tolerance in guinea grass and PMN hybrids (Pearl millet Napier hybrids) were initiated in 2015 and continued to date. The salinity was artificially created using a combination of salts NaCl, Na_2SO_4, $MgCl_2$, and $CaSO_4$ in the ratio of 13:7:1:2. The experiments were conducted in grow bags containing 60 kg of soil. The salts required to create 12 ECe was divided and applied in three doses to one-month-old plants to emulate the slow accumulation of salts in the soil. The samples were harvested every 45 days, and fresh and dry biomass were recorded. Membrane stability (Sairam *et al.* 2002), *Ex-situ* tissue tolerance by the leaf clip method (Chakraborty *et al*. 2020), and the total antioxidants (Benzie and Strain 1999) were estimated. Leaf succulence was recorded as the ratio of fresh weight to dry weight of leaf discs taken on the 4th leaf in PMN hybrids and the 3rd leaf in guinea grass. The double bond index (DBI) was calculated using the formula (Larkindale and Huang 2004) after obtaining the fractionation of Fatty acids through GC-MS.

Results and Discussion

In PMN hybrids, an increase in plant height was observed at 12 ECe (Table 1). In guinea grass, tolerant varieties like DGG1, BG2, and BG1 had a more leaf-to-stem ratio at 12 ECe than Co1, a susceptible variety (Fig 1). Compared to Guinea grass, the leaf-to-stem ratio of PMN hybrids was low. In two tolerant PMN hybrids, Phule Yashwant and BNH-14, the total fresh biomass was higher at 12 ECe compared to the control. In these genotypes, the fresh biomass increased due to more fresh stem weight than dry weight. However, fresh and dry biomass in guinea grass was consistently higher in control plants (Table 2). Salinity-induced total antioxidant activity to high levels in PMN hybrids compared to guinea grass. COBN 5 had higher tissue tolerance compared to the tolerant varieties among PMN hybrids. Whereas in guinea grass, the increase in tissue tolerance was much higher in 12 ECe plants compared to the control. DGG1, a saline-tolerant variety, recorded the highest tissue tolerance amongst all the guinea grass genotypes, followed by Co1. The Na ion content in stems and leaves supports this observation. The membrane stability improved after several days of salinity application in PMN hybrids, with a concomitant increase in DBI in tolerant varieties.

So morphologically speaking, salinity stress up to 12 ECe enhances plant height and the number of leaves based on the grass type. PMN hybrids are grasses with central stems. Hence increasing fresh biomass and plant height is a safe way to induce salinity tolerance. The tolerance mechanism is achieved by storing excess salt in nonphotosynthetic tissues and protecting the photosynthetic surface. An increase in stem fresh weight at 12 ECe compared to control implies the accumulation of water in stems to dilute the salts. The tissue tolerance of PMN hybrids was less compared to guinea grass. PMN hybrids having more stem areas prefer to store salts in stems than in leaves. In guinea grass, tolerant varieties had higher tissue tolerance than low-tolerant varieties. Guinea grass had less stem area than PMN hybrids. The salts need to be stored in leaves after being taken up. Hence having high tissue tolerance coupled with leaf succulence (Table 2) helps to tolerate salt accumulation in leaves. However, in guinea grass, leaf succulence decreased under 12 ECe in genotypes DGG1 and BG4.

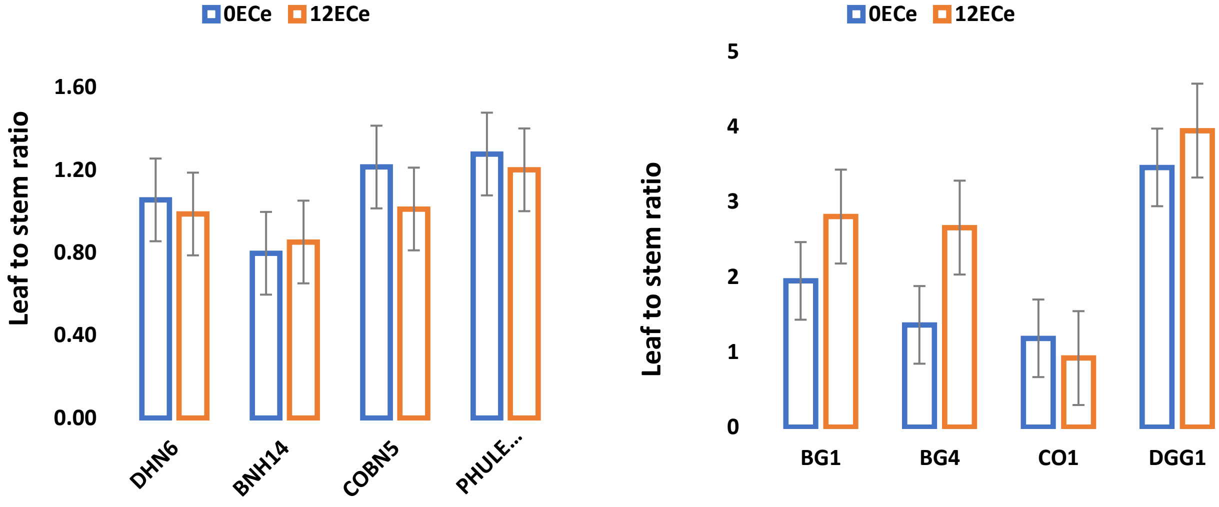
In an earlier study, the genotypes BG4 and DGG1 that recorded low leaf succulence also recorded more leaf salt excretion through micro leaf hairs (Antony *et al*., 2021). In the tolerant genotypes of PMN hybrids, the Double Bond Index (DBI) was much higher at 12 ECe (Fig3). The increase in unsaturated fatty acids is reflected in the double bond index. The increase was very evident when compared with 0 ECe. The remodelled unsaturated fatty acids help maintain membrane stability after several days of salinity application. The genotype BNH10, a saline-tolerant PMN hybrid, recorded the same amount of unsaturated fatty acids under control as in 12 ECe.

Conclusion

Different grasses exhibit different types of tolerance mechanisms to salinity. When used for growing fodder in salinity-affected soils, a combination of grass genotypes with varying tolerance mechanisms would be viable as they adjust to salinity tolerance with different tolerance mechanisms to yield better performance under such abiotic stress.

Table 1. Effect of salinity on plant height in PMN Hybrid genotypes

Genotypes	***Salinity levels***	
	0ECe	12ECe
DHN 6	92.83[b]	87.83[c]
DHN 15	111.11[a]	121.31[ab]
COBN 5	116.10[a]	121.74[a]
CO 6	110.32[a]	112.12[b]
BNH 10	119.37[a]	126.86[a]
IGFRI 7	116.75[a]	122.37[a]
PHULE YASHWANT	112.37[a]	123.42[a]
MEAN	111.26	116.58
	S.Em ±	CD at 5%
Genotypes	2.11	8.7
Salinity levels	1.59	4.65
Genotype x Salinity levels	4.23	12.30

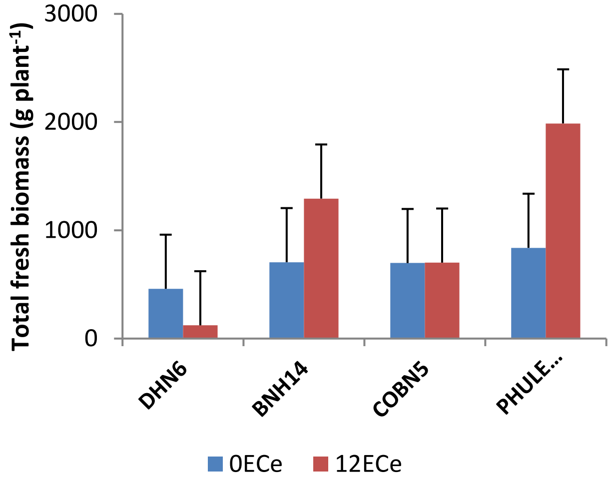


A B

Fig1. Leaf-to-stem ratio in (A) PMN hybrid and (B) Guinea grass genotypes

Table 2. Total antioxidants, leaf succulence, and Tissue tolerance in PMN hybrids and guinea grass varieties under salinity

	TOTAL ANTIOXIDANTS (U/mg)		**LEAF SUCCULENCE (gH_2O/g Fr. Wt)**		**TISSUE TOLERANCE**
PMN HYBRID var	**0ECe**	**12ECe**	**0ECe**	**12ECe**	**12ECe**
DHN6	3.00	14.25	1.85^{c}	2.43^{a}	6.2
BNH14	1.00	32.18	1.78^{c}	2.43^{a}	7.2
COBN5	3.00	22.96	2.16^{b}	2.40^{a}	8.11
PHULE YASHWANTH	3.00	32.95	2.52^{a}	2.33^{ab}	6.22
Mean	**16.98^{b}**	**25.58^{a}**	**2.08^{b}**	**2.39^{a}**	
Genotype	**SEM±**	**CD AT 5%**	**SEM±**	**CD AT 5%**	
	1.78	**5.16**	**0.03**	**0.11**	
GUINEA GRASS var	**0ECe**	**12ECe**	**0ECe**	**12ECe**	**12ECe**
BG1	12.38	21.33	1.51^{bc}	1.96^{ab}	4.7
BG4	17.16	27.39	2.3^{a}	1.56^{bc}	3.6
CO1	12.61	20.77	1.39^{c}	2.12^{a}	6.8
DGG1	17.12	18.43	1.56^{bc}	1.33^{c}	7.7
Genotype	**SEM±**	**CD AT 5%**	**SEM±**	**CD AT 5%**	
	2.08	**6.02**	**0.112**	**0.325**	



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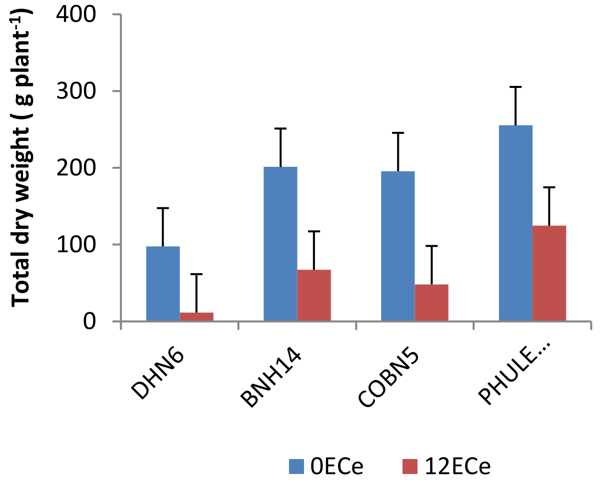

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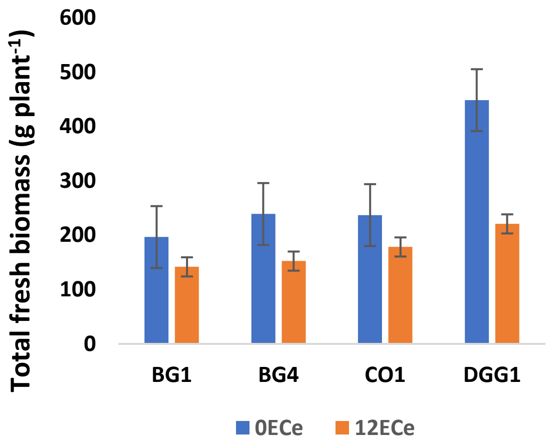

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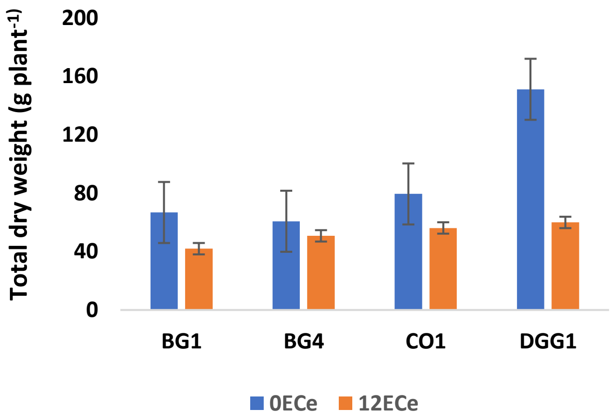

D

Fig 2. Fresh and Dry biomass of PMN hybrids (A&B, respectively) and Guinea grass genotypes (C&D, respectively)

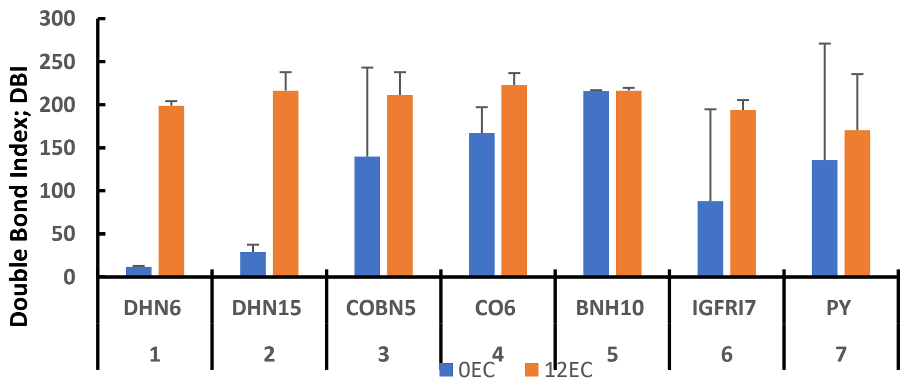

Fig 3. Double bond index in PMN hybrids under salinity (12 ECe)

Acknowledgements

The authors thank the Director of the Indian Grassland and Fodder Research Institute, Jhansi, for providing research facilities. The authors acknowledge the help of SAIF IIHR, Bengaluru, for Fatty acid estimation.

References

Antony E, Apoorva B Kawadikai, Savitha Hullur, K. Sridhar ,Seva Nayak and V.K. Yadav.2021. Biomass repartitioning, tiller regeneration, and salt secretion through micro leaf hairs for salinity tolerance in guinea grass (*Megathyrsus maximus* Jacq.) Range Management and Agroforestry 42 (2): 246-254

Benzie, F.F and Strain, J.J. 1999 Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in enzymology. 299:15-23

Chakraborty, K., Mondal, S., Ray, S., Samal, P., Pradhan, B., Chattopadhyay, K., Kar, M.K., Swain, P. and Sarkar, R.K., 2020. Tissue tolerance coupled with ionic discrimination can potentially minimize the energy cost of salinity tolerance in rice. Frontiers in plant science. *11*:p.265. https://doi.org/10.3389/fpls.2020.00265

Larkindale J and Huang B. 2004. Changes in lipid composition and saturation level in leaves and roots for heat-stressed and heat-acclimated creeping bentgrass (*Agrostis stolonifera*). Environmental and Experimental Botany. 51: 57–67

Sairam, R.K., Rao, K.V. and Srivastava, G.C., 2002. Differential response of wheat genotypes to long-term salinity stress with oxidative stress, antioxidant activity and osmolyte concentration. Plant Science: *163*(5): 1037-1046