J. Farm Sci., 38(4): (448-451) 2025

RESEARCH PAPER

Mechanisms of salinity tolerance in Pearl Millet Napier (PMN) hybrids [*Pennisetum glaucum* (L.) R. Br.] x [*Pennisetum purpureum* (K.) Schum]

M. AMULYA[1*], M. B. DODDAMANI[1], EDNA ANTONY[2] AND V. B. KULIGOD[3]

[1]Department of Crop physiology, [3]Department of Soil science and Agricultural Chemistry, College of Agriculture, Dharwad University of Agricultural Sciences, Dharwad - 580 005, India

[2]Department of Plant Physiology, ICAR-IGFRI-SRRS, Dharwad

*E-mail: amulyamallur0202@gmail.com

(Received:January, 2023 ; Accepted:November, 2025)

DOI: 10.61475/JFS.2025.v38i4.25

Abstract: Soil salinity affects various physiological and biochemical processes, reducing plant biomass production. Salinity stress affects the physiology of whole plants at cellular levels through osmotic and ionic stress. The study was undertaken at Main Agricultural Research Station (MARS), Dharwad, to evaluate the four Pearl millet Napier (PMN) hybrids *viz*., DHN6, BNH14, COBN5 and Phule Yashwanth under saline conditions and also to understand the physiological and biochemical mechanisms in [*Pennisetum glaucum* (L.) R. Br.] × [*Pennisetum purpureum* (K.) Schum]. The experiment was laid out in a factorial, completely randomised design with 5 replications in pot trials. The treatments are 0ECe (control) and 12ECe (salinity). A combination of salts of NaCl, Na_2SO_4, $MgCl_2$ and $CaSO_4$ in the ratio 13:7:1:2, respectively, was used to prepare saline soils. Changes in membrane stability and total biomass were recorded at each harvest. At 12ECe, the highest and lowest biomass was recorded in Phule Yashwant and DHN6, respectively. The fresh stem weight of all genotypes, except DHN 6, increased under salinity compared to the control but the dry weight in all genotypes decreased under salinity in all the genotypes. The membrane stability of all the genotypes increased as the plant matured at 12ECe in both harvests. COBN5 and Phule Yashwant have high tissue tolerance, and the genotype DHN6 recorded the lowest tissue tolerance. The sensitive genotype DHN 6 had the highest leaf succulence under salinity. PMN hybrids increase the stem biomass under salinity conditions to store salts in the stem, a non-photosynthetic tissue to protect the leaves, which contain photosynthetic machinery.

Key words: Membrane stability, Salinity, Tissue tolerance

Introduction

Salinity is one of the significant constraints challenging crop production worldwide. Around 6.7mha in India is salt affected. Salinity harms plant growth by decreasing leaf water potential and inducing morphological and physiological changes (Khan *et al.,* 2014). Plant growth is affected by reduced water uptake, lack of nutrients, and accumulation of toxic sodium and chloride ions (Gehan, 2015).Restoration of saline lands is moving forward very slowly. Growing grasses are one of the reclamation methods to reduce salinity in soils. Among the grasses, Pearl Millet Napier is an artificial hybrid developed by crossing common Napier grass [*Pennisetum purpureum* (K.) Schum] with Bajra (pearl millet) [*Pennisetum glaucum* (L.) R. Br.]. It is an interspecific triploid (AA'B genomes) hybrid with 2n=3x=21 chromosomes (7 from Pearl millet and 14 from Napier Grass).It is a perennial grass with a height of 3-4m, a heavy tiller, pearl millet-like leaves, and exceptionally stiff and hairy leaves close to the stem.It is a multi-cut forage grass with high biomass, nutritional quality and palatability. (Kaur *et al.,* 2019).

When cropping is impossible due to the increase in soil salinity, pasture production or fodder cultivation in saline soils can turn around the land use pattern and improve animal husbandry as an additional income (Munns and Gilliham, 2015). The livestock sector is one of the most promising sectors in agriculture.About 20.5 million people depend upon livestock for their livelihood. Livestock contributed 16% to the income of small farm households and provided livelihood to two-thirds of the rural community. It also employs about 8.8% of the population in India. The livestock sector contributes 4.11% to the GDP and 25.6% to the total Agriculture GDP.Forage crops undoubtedly play a significant part in maintaining a large livestock population and meeting the rising demand for milk and meat.

Naturally occurring salt-tolerant plants, halophytes, which survive at high salt concentrations, provide a unique source of traits for tolerance.To grow in saline conditions, plants need physiologically adapted mechanisms. Plants tolerate or resist salinity through morphological, physiological, anatomical and molecular mechanisms. The parental materials of both pearl millet and napier have now been improved worldwide, making it possible to develop better hybrids in terms of quality and yield. During 1989 and 2000, various forage breeding institutions developed vast Pearl millet x Napier accessions.Scientists selected and bred high-yielding pearl millet napier hybrid varieties over the years of forage development. None of the varieties was evaluated for their ability to produce green fodder under saline conditions.Hence, a study is conducted to better understand the adaptability of pearl millet napier hybrid varieties to saline conditions and also the physiological mechanisms involved in pearl millet napier hybrids to confer salinity.

Material and methods

Study site and experimental design

The experiment was conducted at Main Agricultural Research Station (MARS), Dharwad, from September 2021 to

August 2022 to evaluate the salinity tolerance in Pearl millet napier hybrid genotypes in a pot culture experiment. Artificial saline soils were prepared based on the USDA Agriculture handbook no 60. Containers which can hold 23 kg of soil were used for each treatment. The containers were without drainage holes to ensure the retention of salts in the soils and not be washed away due to irrigation.The desired treatments are 0 and 12 ECe. Salts of NaCl, Na_2SO_4, $MgCl_2$ and $CaSO_4$ in the salt ratio 13:7:1:2, respectively, were used to prepare saline soils. The soil used for the experimental purpose was dried properly without any moisture content, and its initial ECe was checked. The dried soil was weighed and filled in the container.The soil salinity was created based on a percentage of salt ratio and uniformly distributed in the soil in the container by dissolving the salts in water. The salts added were dissolved and dispersed in the soil due to repeated irrigation and drying.After the dispersal of salt, single-rooted slips of DHN6, BNH14, COBN5 and Phule Yashwant were planted in the soil bags. The experiment was designed for factorial RBD with four genotypes, two salt levels and five replications.

Total biomass

Plants were harvested at 15cm above the stem every 45 days.The tillers from the bags were all harvested individually. After separating the leaf and stem portions, the fresh weight was calculated.Total fresh biomass was used to represent the accumulated weights. After oven drying, the materials were weighed more and expressed as dry biomass.All the tillers present in a bag were counted manually.

Physiological parameters

Membrane stability Index

The membrane stability index (MSI) was calculated by taking the electrical conductivity (EC) of leaf samples in double distilled water at 40°C and 100°C by following the method of Sairam (1994).

A mature leaf was cut into small pieces, and 0.5g of sample was taken in a test tube with 10 ml of double distilled water and kept at 40°C in a water bath for 60 mins, and electrical conductivity(C_1) was measured. After taking EC, water was changed by adding 10 ml of double distilled water and kept at 100° C in the water bath for 10 min, and the electrical conductivity(C_2)was measured by a conductivity meter. Then the membrane stability index was calculated by using the formula,

$$\text{Membrane stability index (MSI)} = 1 - \frac{C_1}{C_2} \times 100$$

Where C_1 is the conductivity at 40° C and C_2 is the conductivity at 100° C

Tissue tolerance

Tissue tolerance was estimated by *Exsitu* leaf clip assay to measure the pigment retention capacity of plants under similar sodium load in mesophyll tissues. Leaves (2 grams of leaves) were collected and cut into pieces of about 5cm and placed in Petri dishes containing saline water of 500 mM concentration for 14days. The chlorophyll and tissue ion content (Na+) were estimated daily. The chlorophyll content was estimated by the DMSO method, and the ion content was estimated using a flame photometer. (Chakraborty *et al.,* 2020). From the collected data, daily chlorophyll degradation rate and tissue tolerance (the amount of sodium concentration where half of the chlorophyll pigments were destroyed) were calculated for each genotype.

Statistical analysis

The data collected from the experiment were subjected to statistical analysis in GRAPES (General R-shiny based Analysis Platform Empowered by Statistics), a web application based on R software developed by the Department of Agricultural Statistics, College of Agriculture, Vellayani.

Results and discussion

Effect of salinity on total biomass and related attributes

In perennial grasses, the economic part of the crop is fresh leaves and stems. In general, yield is the net result of the interaction of various metabolic activities occurring at various growth stages, primarily influenced by environmental factors.The highest total fresh biomass was recorded in Phule Yashwant (1131.88g plant^{-1}), and the lowest was in the case of DHN 6 (147.00g plant^{-1}) under salinity (Table 1). Salinity stimulated the growth of Secale cereal. (Morant-Manceau *et al.*, 2004). The highest total fresh biomass of Phule Yashwant significantly differed with DHN 6, COBN 5 and BNH 14 under salinity and control conditions. Tolerant genotypes may use an increase in biomass to tolerate and respond to mild stress conditions. Salt tolerance could be affected by several factors, such as the type of salts in the soil solutions, growth conditions, age and plant genotype (Moisender *et al.*, 2002, Sheekh-El *et al.*, 2002).

The fresh stem weight of all genotypes, except DHN 6, increased under salinity compared to the control but the dry weight in all genotypes decreased under salinity in the genotypes (Table 2). Dry weight increased in a few genotypes of maise at lower salinity levels, while dry biomass decreased at higher salinity levels (Ashraf *et al.,* 1999). The decrease in dry weight designated the role of water or moisture in the stem,which helped to dilute the salts, thereby reducing the ion concentrations that would have negatively affected the plants under salinity.

Membrane stability index (%)

The membrane stability index is a crucial physiological parameter that identifies damage to cell membranes and

Table 1. Effect of soil salinity on total fresh biomass in PMN hybrids

Genotype	Salinity levels		
	0ECe	12ECe	Mean
DHN6	460.00	147.00	303.50^{b}
BNH14	705.60	898.83	802.22^{ab}
COBN5	697.40	704.50	700.95^{b}
Phule Yashwant	838.40	1131.88	985.14^{a}
Mean	675.35	720.55	
	S. Em±	CD at 5%	
Genotype	137.1	397.2	
Salinity	96.96	NS	
Genotype x Salinity levels	193.9	NS	

Table 2. Effect of soil salinity on Stem fresh weight and Stem dry weight (g plant^{-1}) of PMN hybrids

Genotype	Salinity levels					
	Stem fresh weight			Stem dry weight		
	Control	Salinity	Mean	Control	Salinity	Mean
DHN6	226.20	107.00	166.60^{c}	46.80	22.00	34.40^{c}
BNH14	395.20	471.00	433.10^{ab}	109.40	92.00	100.70^{ab}
COBN5	326.20	395.50	360.85^{b}	83.40	62.50	72.95^{bc}
Phule Yashwant	415.60	711.25	563.43^{a}	144.40	139.00	141.70^{a}
Mean	340.80	421.19		96.00	78.88	
	S.Em±	C.D. at 5%		S.Em±	C.D. at 5%	
Genotype	61.35	177.71		14.44	41.82	
Salinity	43.38	NS		10.21	NS	
Genotype x Salinity levels	86.757	NS		20.41	NS	

Table 3. Effect of salinity on membrane stability index (%) in PMN hybrids

Genotypes	Salinity levels											
	First harvest						Second harvest					
	30DAYS			45DAYS			30DAYS			45DAYS		
	Control	Salinity	Mean	Control	Salinity	Mean	Control	Salinity	Mean	Control	Salinity	Mean
DHN6	68.95	62.01	65.48	71.48	60.60	66.00^{b}	76.23	61.29	68.76^{b}	78.61	66.77	72.69^{c}
BNH14	75.95	64.97	70.46	83.02	71.21	77.11^{a}	86.67	68.89	77.78^{a}	85.92	76.54	81.23^{b}
COBN5	74.50	64.97	69.73	79.12	66.64	72.88^{ab}	83.61	72.85	78.23^{a}	84.81	75.10	79.95^{b}
Phule Yashwant	78.77	69.43	74.10	81.51	75.45	78.48^{a}	85.33	79.57	82.45^{a}	89.14	84.97	87.04^{a}
Mean	74.5^{a}		65.3^{b}	78.7^{a}		68.4^{b}	82.9^{a}		70.6^{b}	84.6^{a}		75.8^{b}
	S.Em ±		CD at 5%	S.Em±		CD at 5%	S.Em±		CD at 5%	S.Em±		CD at 5%
Genotypes	2.13		NS	2.5		7.24	1.79		5.19	1.02		2.96
Salinity levels	1.50		4.36	1.77		5.12	1.29		3.67	0.72		2.09
Genotype x	3.01		NS	3.54		NS	2.53		NS	1.45		NS

electrolyte leaks from cells. The MSI of all the genotypes decreased under salinity compared to the control (Table 3). Under stress, ROS and O^{-} increase electrolyte leakage and affect membrane stability (Hu *et al*., 2016). The membrane stability of the plant was recorded at 30 and 45days of the first and second harvests. In the first harvest under control condition (0ECe), the highest membrane stability was recorded by Phule Yashwant (78.77%) and BNH 14 (83.02%) at 30days and 45days, respectively, whereas in salinity, the highest membrane stability was recorded at Phule Yashwant (74.54%) and the least was observed in DHN 6 (60.60%) at 45 days after salinity (Table 3). The same trend was observed in all the genotypes in the second harvest at 30 and 45 days.The genotype Phule Yashwant had the highest membrane stability under salinity, and as the plant matured, the membrane stability increased under salinity.DHN 6 had lower membrane stability than other genotypes (Table 3). The effect of salinity on the plasma membrane is primarily due to the action of salt ions (Mansour, 1998).This membrane stability finding suggested that the regenerated tillers remembered the salinity stress and prepared for the following generations with higher membrane stability, suggesting a transcriptional memory impacting the salinity response in plants.

Tissue tolerance- way to understand salinity tolerance of PMN hybrid genotypes

The ability of tissues to tolerate high salt concentrations found in leaves but compartmentalised at the cellular and intercellular levels (particularly in the vacuole) is achieved through the combination of ion transporters, proton pumps, and the production of appropriate solutes.The genotype DHN 6 required far less tissue sodium to destroy half of the initial chlorophyll content, and its 50 per cent chlorophyll degradation was seen on the sixth day of the stress period (Fig 1). Phule Yashwant recorded the most negligible chlorophyll degradation and had a high tissue tolerance ability, as its 50 per cent chlorophyll degradation was observed on the ninth day of the stress period. Though the genotype COBN 5 had a higher sodium content (2.85 mg g^{-1} DW) than Phule Yashwant, its 50

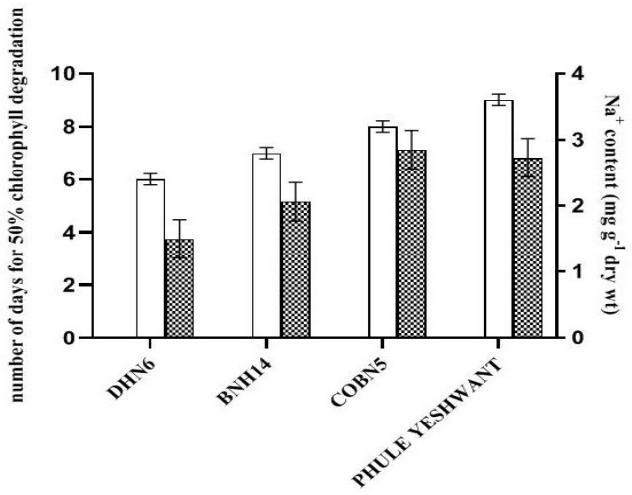

Fig 1. *Ex situ* leaf tissue tolerance in PMN hybrid genotypes under 500m M salt stress

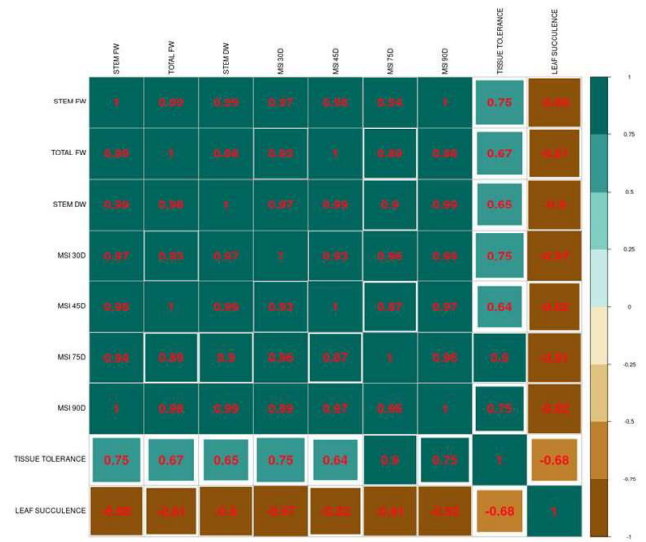

Fig 2. Heat map of Pearson's correlation coefficients of morphological and physiological parameters calculated for four PMN hybrid gernotypes under salinity. n=4; at 5% P=0.95. FW=fresh weight, DW= dry weight, MSI = Membrane stability index

percent chlorophyll degradation was seen on the eighth day of the stress period. This study confirms that the salts taken up from the soil by plants were stored in stem, non-photosynthetic tissue and also, the genotype COBN 5 recorded more leaf succulence than Phule Yashwant under salinity (Table 4), which confirms the storage or compartmentalisation of salts into vacuoles. The genotype Phule Yashwant had the lowest leaf succulence under salinity, and its tolerance to salinity stress is attributed to its growth habit, the formation of side tillers, which mainly arise from the top nodes of the main tiller. This formation

Table 4. Effect of salinity on leaf succulence in PMN hybrids

Genotypes	Salinity levels		
	Control	Salinity	Mean
DHN-6	1.85^{c}	2.42^{a}	2.13^{bc}
BNH-14	1.78^{c}	2.41^{a}	2.09^{c}
COBN-5	2.16^{b}	2.40^{a}	2.28^{ab}
Phule Yashwant	2.52^{a}	2.33^{ab}	2.42^{a}
Mean	2.08^{b}	2.39^{a}	
	S.Em±	C.D at 5%	
Genotypes	0.05	0.16	
Salinity levels	0.04	0.11	
Genotype x salinity levels	0.08	0.22	

of side tillers contributes to the high yield under salinity in Phule Yashwant. Further, the leaf succulence had a negative correlation with leaf tissue tolerance (-0.68, fig 2). This implies that the sodium ions reach the leaves for compartmentalisation into vacuoles in meagre quantities. PMN hybrids do not carry out the high-cost energy-utilising mechanisms such as tissue tolerance.

Conclusion

The present study revealed that the genotypes Phule Yashwant and BNH 14 of PMN hybrids yield greater biomass even under saline conditions. The increased tolerance of these genotypes is attributed to the morphological parameters and physiological mechanisms of salinity tolerance like membrane stability index, leaf succulence and tissue tolerance. PMN hybrids are solid stem-containing fodder crops. This crop does not depend primarily on energy-consuming tolerance mechanisms like tissue tolerance, salt excretion and compartmentalisation into vacuoles, unlike in guinea grass. This is an added advantage for tolerant PMN hybrids to produce more biomass under salinity.

References

Ashraf M Y, Wahed R A, Bhatti A S, Sarwar G and Aslam Z, 1999, Salt tolerance potential in different Brassica species growth studies. *Halophytes Uses in Different Climates*, 11:19-125.

Chakraborty K, Mondal S, Ray S, Samal P, Pradhan B Chattopadhyay K,KumariKar M, Swain P and Sarkar R K, 2020, Tissue tolerance coupled with ionic discrimination can potentially minimise the energy cost of salinity tolerance in rice. *Frontiers in Plant Science,* https://doi.org/ 10.3389/fpls.2020.00265.

Gehan G Mostafa, 2015, Improving the growth of fennel plant grown under salinity stress using some biostimulants. *American Journal of Plant Physiology,* 10: 77-83.

Hu T, Jin Y, Li H,Amombo E, Fu J, 2016, Stress memory induced transcriptional and metabolic changes of perennial ryegrass (*Lolium perenne*) in response to salt stress. *Physiologia plantarum,* 156(1): 54-69.

Kaur A R, Kapoor Y, Vikal A K and Bhardwaj R, 2019, production of interspecific hybrids between pearl millet [*Pennisetum glaucum* (L.) R. Br.] × napier grass [*Pennisetum purpureum* (K.) Schum] and their characterisation, *International Journal of Current Microbiology and Applied Sciences*, 8(4): 1308-1313.

Khan M I R, Asgher M and Khan N A, 2014, Alleviation of salt-induced photosynthesis and growth inhibition by salicylic acid involves glycinebetaine and ethylene in mungbean (*Vigna radiata* L.), *Plant Physiology and Biochemistry,* 80: 67–74.

Mansour M M F, 1998, Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress. *Plant Physiology and Biochemistry*, 36(10): 767-772.

Morant-Manceau A, Pradier E and Tremblin G, 2004, Osmotic adjustment, gas exchanges and chlorophyll fluorescence of a hexaploid triticale and its parental species under salt stress. *Journal of plant physiology*, 161(1): 25-33.

Munns R and Gilliham M, 2015, Salinity Tolerance of Crops—What Is the Cost, *New Phytologist*, 208:668-673.https://doi.org/ 10.1111/nph.13519.

Sairam R K, 1994, effect of moisture stress on physiological activities of two contrasting wheat genotypes, *Indian Journal of Experimental Biology*, 32: 594-597.

Sheekh-El M M and Omar H H, 2002, effect of high salt stress on growth and fatty acids content of the unicellular green algae *Chlorella vulgaris*. *American Journal of Microbiology*, 55:181-191.