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Assessment of Salinity Tolerance in Barnyard millet (*Echinochloa frumentacea* Roxb.) Genotypes using Dendrogram and PCA Biplot

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ABSTRACT: Barnyard millet (*Echinochloa frumentacea*) is a minor millet known for its nutritional value and adaptability to adverse environments. However, soil salinity significantly hinders its early growth, germination, and productivity. The present study evaluated 100 genotypes of Barnyard millet under six salinity treatments (0, hydro-primed, 50, 100, 150, and 200 mM NaCl) using a factorial completely randomized design. Key early growth parameters including percentage of germination, root and shoot length, fresh weight, dry weight and vigour index were assessed. Dendrogram clustering and principal component analysis revealed genotypic variability in response to salinity stress. The genotypes G_{34} (IEc 688), G_{25} (IEc 675), G_{18} (IEc 154), G_{44} (IEc 360) and G_{43} (IEc 356) performed well consistently upon exposure to salt levels. Certain genotypes maintained higher germination and vigour under stress, indicating physiological adaptations such as osmotic regulation and reactive oxygen species (ROS) detoxification. PCA identified vigour index and root length as discriminative traits for salinity tolerance. The study highlights the importance of genotype screening and phenotypic clustering in identifying salt-tolerant lines, aiding breeding programs aimed at enhancing millet productivity in salt-prone regions.

Keywords: Salinity stress, genotype screening, vigour index, dendrogram clustering, Principal Component Analysis.

INTRODUCTION

Millets, often referred to as “miracle crops,” are gaining importance due to their resilience to various abiotic and biotic stresses, coupled with their nutritional benefits and adaptability to marginal environments (Adhikari *et al.*, 2021). Among them, *Echinochloa frumentacea* (Barnyard millet) excels in its nutritional profile, including high dietary fibre, iron content, low glycaemic index, and gluten-free properties. Notably, it is one of the oldest domesticated millets, predominantly grown in arid and semi-arid regions of Asia and Africa, where environmental stresses such as salinity are prevalent (Jha *et al*., 2022).

Soil salinity has emerged as a major constraint to agricultural productivity in these regions, adversely affecting seed germination, early seedling establishment, and ultimately crop yield. In Barnyard millet, salinity stress has been reported to reduce seedling length, disrupt water uptake, and impair physiological processes critical to early plant development (Nedunchezhiyan *et al.*, 2020). High salinity levels lead to osmotic stress, ionic imbalance and the excessive production of reactive oxygen species (ROS), that collapse cellular structure and cell metabolisms (Upadhyay *et al.*, 2022). Furthermore, elevated salt concentrations can suppress the synthesis of essential metabolites like flavonoids, amino acids, and proteins, contributing to compromised growth and grain quality (Negrão *et al.*, 2017).

Plants respond to salt stress through including osmotic adjustment through accumulation of compatible solutes (such as proline and sugars), hormonal regulation (notably abscisic acid), and enhanced activity of antioxidant defense systems that scavenge ROS (Saha *et al.*, 2022). Genetic variability among different genotypes plays a major role in determining the degree of tolerance or sensitivity to salinity, especially during early developmental stages (Mukhthambica *et al.*, 2023). Thus, identifying and characterizing salt-tolerant genotypes is essential for improving millet productivity under stress-prone conditions (Vijayalakshmi *et al.*, 2014).

The present study investigates the differential response of selected Barnyard millet genotypes to incremental levels of salt stress. Salinity was imposed by pre-soaking seeds in sodium chloride (NaCl) solutions of 50 mM, 100 mM, 150 mM and 200 mM concentrations, while untreated seeds served as the control. Key early growth parameters such as root and shoot length, germination percentage, fresh and dry biomass, and vigour index were evaluated to assess the physiological and biochemical impacts of salinity. This work aims to identify genotypic variation in salt tolerance, which may inform breeding strategies for stress-resilient millet cultivars. The salt-tolerance in genotypes is analysed by principal component analysis and dendrogram clustering.

MATERIALS AND METHODS

A. Experimental details

The experiment was conducted at the Genetics and Plant Breeding Laboratory, in affiliation with Seed Science and Technology Laboratory at SRM College of Agricultural Sciences, Baburayanpettai, Tamil Nadu, India. One hundred genotypes of barnyard millet were obtained from the International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Hyderabad. The salinity tolerance in each genotype were assessed by applying varied concentrations of NaCl across six treatments: T_0 (control, 0 mM NaCl), T_1 (hydro priming) and T_2 to T_5 (50 mM NaCl, 100 mM NaCl, 150 mM NaCl and 200 mM NaCl, respectively). The analysis was done using Factorial Completely Randomized Design (FCRD) with four replicates. The sterilized sand is used to grow the seedlings and it was placed in plastic trays. The initial stress was introduced to the genotypes by soaking seeds in distilled water and NaCl solutions for three hours. Hundred seeds per genotype are soaked for each treatment. The NaCl solutions were applied to the sanitized sand trays before sowing of the seed. The seeds are allowed to grow in the sand tray and observations are taken.

B. Growth parameters recorded

The experiment was carried out with in accordance with International Seed Testing Association (ISTA) guidelines (ISTA, 2012). The germination data was recorded every day and on the 14th day of emergence, the last counts were made and the percentage of germination was determined. Ten healthy seedlings were selected from each replication for observing the root length, on the 14th day of emergence and it was measured in 'cm'. The root length was measured starting from the surface to tip of the main root. Ten randomly selected healthy seedlings were selected from each replication, on the 14th day of emergence and it is denoted in 'cm'. The shoot length was measured from the leaf tip to the end of the shoot of seedling. Ten healthy seedlings were selected from each replication, on the 14th day of emergence and weighed immediately after taking it from the sand tray and the fresh weight of roots and shoots can be calculated. Seedlings are shade dried and kept in a hot air oven at 70ºC for 20 hours and it was weighed. Fresh and dry weights are expressed in 'mg 10 seedlings^{-1}'. The values of the vigor index were calculated by multiplying germination percentage with total seedling length and the average values were shown as whole numbers (Abdul Baki and Anderson 1973).

C. Statistical Analysis

The data from each observation were analyzed using the standard deviation from the replicates was used. The Factorial Completely Random Design (FCRD) was used for experimental set up. The principal component analysis (PCA) and Dendrogram Clustering of the growth parameters were done using SPSS version 16.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

A. Dendrogram

The clustering of genotypes was done using dendrogram to screen the genotypes for salinity tolerance. The genotypes within similar cluster represented the same extent of tolerance to the salinity stress. The clustering was done for the key traits that are used to assess the salinity tolerance in the millet seedlings and the traits include germination percentage and vigour index of the genotypes (Tanwar *et al.*, 2023).

The cluster I (red color) included genotypes G_{34}, G_{18}, G_{25}, G_{28}, G_{31}, G_{33}, G_{30}, G_{40}, G_{43} and G_{44} (Fig. 1). These clusters represent highest germination percentage among other genotypes and they exhibited similar response to the exposure of different treatments of salinity. The halophytic mechanism in certain genotypes was exhibited by maintenance of cell turgor and the enzymatic production (Afzal *et al.*, 2023). These genotypes responded significantly well, due to the regulated uptake of water, which potentially directs to increased germination percentage.

The cluster II (green) and cluster III (blue) encompasses moderately salt tolerant genotypes (Fig. 1). The cluster II included 28 genotypes and showed moderate variability in salt stress adaptation. The cluster III formed a smaller group of genotypes with comparatively lower level of tolerance to salinity exposure. The cluster IV (purple) represented the highly divergent genotypes with sensitivity to salinity (Fig. 1). It included 44 genotypes whose germination percentage is highly reduced due to the salt stress. This revealed that the barnyard millet exhibit sensitivity to various salinity levels (Mukhopadhyay *et al.*, 2021). The reduction in germination percentage was observed due to the oxidative stress and ion toxicity. The reactive oxygen species (ROS) production within the seeds will be influenced by the ionic toxicity (Nedunchezhiyan *et al.*, 2020). It disrupts the cellular components and compartmentalization.

The vigour index of the genotypes was computed and subjected to dendrogram analysis (Fig. 2). The cluster IV (purple) consisted of genotypes that show higher vigour index. The genotypes G_{30}, G_{43}, G_{34}, G_{44}, G_{18} and G_{25} showed highest degree of variability. The consistency in seedling establishment can be positively related with the growth parameters like germination percentage (Powell, 2022). The cluster III (blue) was the smallest cluster with fewer genotypes, showing moderate variability. The cluster II (green) also showed moderate variability & vigour index, but this cluster includes majority of the genotypes. The cluster I (red) expressed lesser variability among all the clusters. This result proves that the vigor index of genotypes varied across treatments and showed moderate divergence. The moderate divergence showed distinct clusters, which indicates medium level of dissimilarity (Saddiq *et al.*, 2021).

According to results of both the dendrogram, vigour index was considered as the discriminative trait among other growth parameters. The vigour of the seedlings is not only important for filed emergence, but also correlates with stress mitigation mechanisms (Williams *et al.*, 2019). The results of clustering pattern will help the breeders to identify elite genotypes, resilient to salinity stress.

B. Principal Component Analysis (PCA)

The Principal Component Analysis was used to evaluate the variation in the barnyard millet genotypes, which were subjected to different salinity levels. This PCA was used to identify the principal components and distinguishing traits, that contribute to the salt tolerance mechanisms in the barnyard millet genotypes (Prabu *et al*., 2020). Germination percentage, root length, shoot length, total seedling length, fresh weight, dry weight and vigour index are the traits subjected to the principal component analysis.

The principal components PC_1 and PC_2 of the control (T_0) contributed to 70.87% and 10.75% accounted to 81.62% of cumulative variance (Table 1). The genotype differentiation was observed in shoot length, root length, total seedling length and vigour index in PC_1. The PC_2 was influenced by fresh weight (Fig. 3). It was observed that the biomass accumulation across genotypes was more in this treatment. It is because of the non-stress growth conditions, that the metabolism of seed germination and establishment is well maintained (Mishra *et al.*, 2024). The hydro primed seeds (T_1) showed 68.15% variance in PC_1 with root length, shoot length, total seedling length and vigour index as distinguishing traits (Fig. 3). These traits had higher loading values. In PC_2, the fresh weight was clearly dominant. Hydropriming possibly enhanced water uptake and vigour of the seedlings, due to improved rate of metabolism (Nedunchezhiyan *et al.*, 2020).

Under 50 mM NaCl concentrations, the fresh weight dominated PC_2 and germination percentage contributed moderately to PC_2. The PC_1 & PC_2 contributes to 75.47% and 8.15% respectively to the cumulative variance (Table 1). The mild salt stress interrupts the osmotic balance, reduces the water uptake and influences other growth parameters. The genotypes showing positive associations with growth parameters showed considerable tolerance. The principal component PC_1 and PC_2 accounted for 72.45% and 9.93% of total variation in 100 mM NaCl treatment. The major contributes to PC_1 were vigour index, root length and shoot length. The dry weight showed very lower loading value in PC_2. Fresh weight continued to influence PC_2 in this treatment also (Fig. 3). The total seedling length was increased but the biomass was reduced due to the increased salt concentration (Narasimhulu *et al*., 2022).

At 150 mM NaCl, PC_1 had something loading values from root length, shoot length, fresh weight, total seedling length and germination percentage. The PC_1 contributed to 73.29% of variance, whereas PC_2 contributed to 10.07% of variance (Table 1). The strong loading value in PC_2 was observed in dry weight. The above results indicate that some genotypes were able to germinate well and maintain the biomass, under salinity (Gupta and Khandelwal 2022). But few genotypes germinate well and fail to accumulate biomass. This phenomenon of reduction in accumulation of biomass is commonly observed in saline environments (Mukhthambica *et al*., 2023).

At the maximum concentration of 200 mM NaCl, the PC_1 and PC_2 explained 71.08% and 9.45% of total variance (80.52%) (Table 1). The PC_1 influenced vigour index, shoot length, root length and total seedling length. PC_2 was governed by germination percentage and dry weight (Fig. 3). The biplot revealed wider variability that is expressed due to salinity imposition (Durge *et al.*, 2022). The reduction in growth of seedlings across the salinity treatments may be due to interruptions in membrane compartmentation, osmotic imbalance and ionic toxicity (Ladumor *et al.*, 2021).

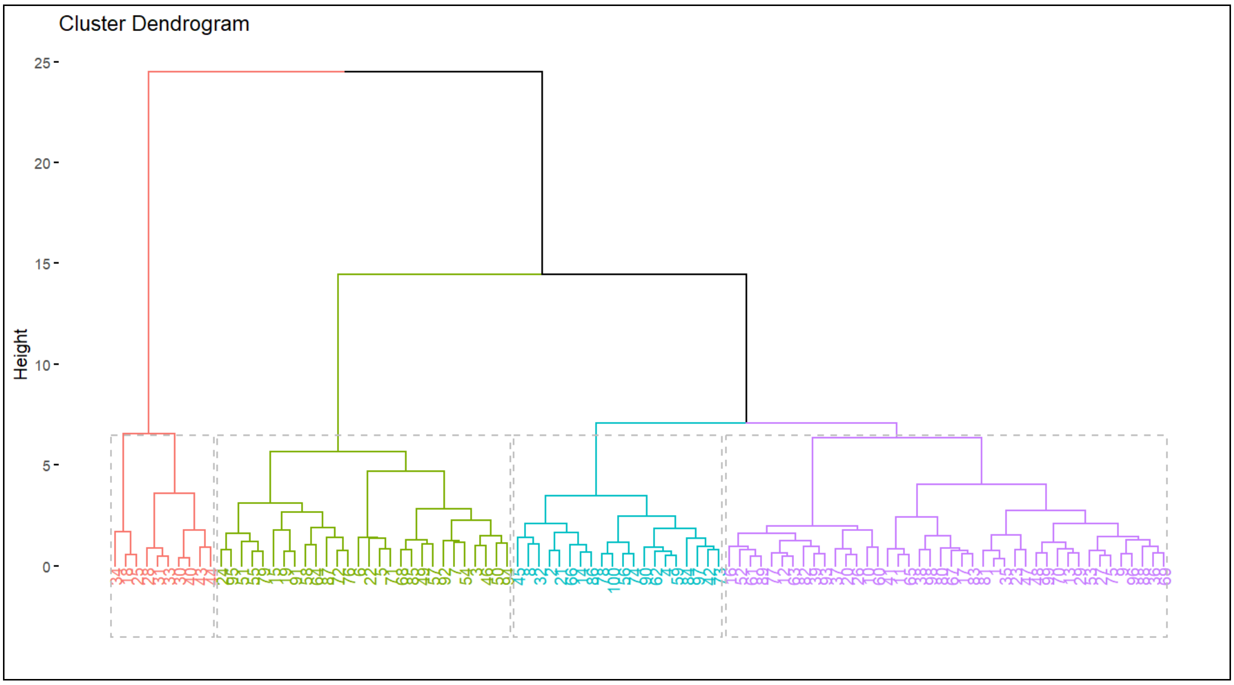

Fig. 1. Cluster dendrogram of germination percentage of Barnyard millet genotypes under various levels of salinity: Control (T_0), Hydropriming (T_1), and 50 mM NaCl (T_2), 100 mM NaCl (T_3), 150mM NaCl (T_4) and 200mM NaCl (T_5).

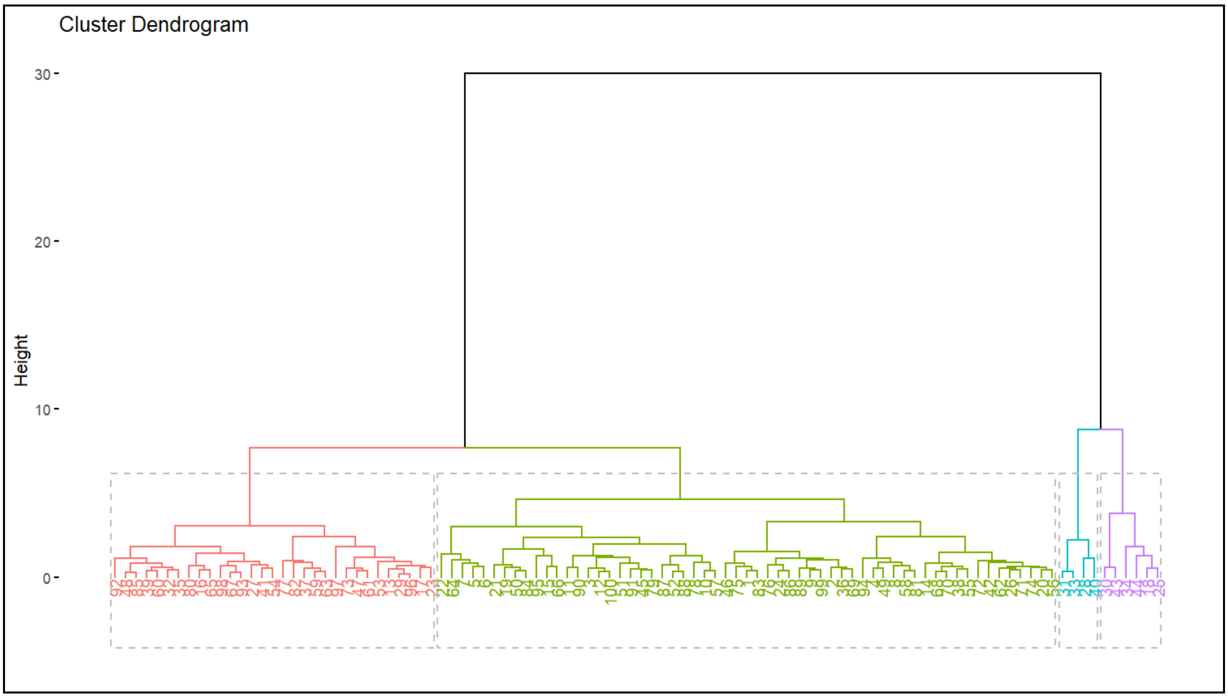

Fig. 2. Cluster dendrogram of vigour index of Barnyard millet genotypes under various levels of salinity: Control (T_0), Hydropriming (T_1), and 50 mM NaCl (T_2), 100 mM NaCl (T_3), 150mM NaCl (T_4) and 200mM NaCl (T_5).

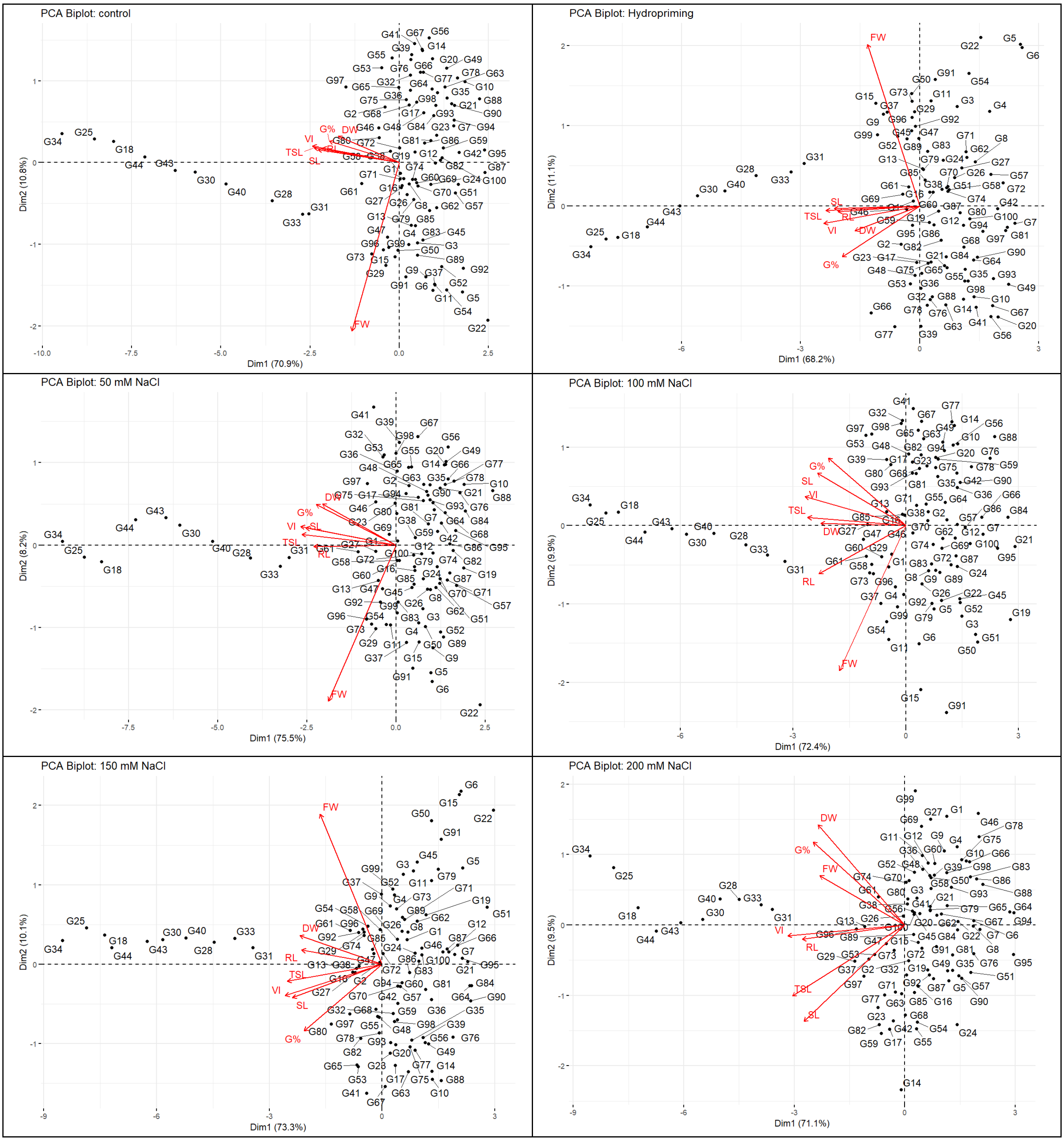

Fig. 3. Principal component biplot graphs of Barnyard millet genotypes under various levels of salinity treatments - Control (T_0), Hydropriming (water) (T_1), and 50mM (T_2), 100mM (T_3), 150mM (T_4) and 200 mM (T_5). The observed growth parameters include germination percentage (G%), shoot length (SL), root length (RL), total seedling length (TSL), fresh weight (FW), and dry weight (DW).

Table 1: Principal factors of principal component analysis and their eigenvalues, variability and cumulative variability for control, hydropriming and four different salinity treatments.

Variable		PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	Control	2.22	0.86	0.75	0.68	0.50	0.02
	Hydropriming	2.18	0.88	0.80	0.65	0.60	0.03
	50mM	2.29	0.75	0.68	0.66	0.48	0.03
	100mM	2.25	0.83	0.72	0.62	0.55	0.03
	150mM	2.26	0.83	0.73	0.59	0.51	0.03
	200mM	2.23	0.81	0.74	0.67	0.59	0.04
Variability (%)	Control	70.87	10.76	8.10	6.62	3.63	0.00
	Hydropriming	68.15	11.08	9.32	6.12	53.12	0.00
	50mM	75.47	81.54	6.64	6.28	3.42	0.00
	100mM	72.45	9.93	7.55	5.62	4.41	0.00
	150mM	73.29	10.07	7.72	5.08	3.81	0.00
	200mM	71.08	9.45	7.92	6.50	5.00	0.00
Cumulative variability (%)	Control	70.87	81.62	89.73	96.35	99.98	100.00
	Hydropriming	68.15	79.23	88.54	94.67	99.98	100.00
	50mM	75.47	83.62	90.26	96.55	99.98	100.00
	100mM	72.45	82.38	89.94	95.57	99.98	100.00
	150mM	73.29	83.36	91.08	96.16	99.98	100.00
	200mM	71.08	80.52	88.45	94.95	99.96	100.00

Table 2: Contribution of morphological and physiological traits in the principal factors under different salinity treatments (principal component loading values).

Character	Control		Hydropriming		50mM		100mM		150mM		200mM	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
G%	-0.35	0.12	-0.36	-0.29	-0.36	0.24	-0.33	0.38	-0.34	-0.38	-0.34	0.45
RL	-0.39	0.08	-0.38	-0.03	-0.37	-0.00	-0.37	-0.27	-0.36	0.08	-0.38	-0.07
SL	-0.41	0.07	-0.40	-0.01	-0.40	0.10	-0.38	0.29	-0.40	-0.19	-0.38	-0.52
TSL	-0.43	0.08	-0.44	-0.02	-0.42	0.06	-0.42	0.04	-0.42	-0.09	-0.42	-0.38
VI	-0.44	0.09	-0.45	-0.10	-0.42	0.11	-0.43	0.16	-0.43	-0.17	-0.44	-0.05
FW	-0.24	-0.96	-0.24	0.93	-0.30	-0.92	-0.28	-0.81	-0.27	0.86	-0.32	0.26
DW	-0.31	0.15	-0.30	-0.14	-0.33	0.24	-0.36	0.01	-0.36	0.16	-0.32	0.54

CONCLUSIONS

The present study demonstrated significant genotypic variability among Barnyard millet accessions under different salinity stress levels. The important traits such as germination percentage, root and shoot length, fresh and dry weight, and vigour index were adversely affected as salinity increased. Dendrogram analysis effectively grouped genotypes based on tolerance levels, while PCA identified vigour index as the principal component that contributes to variability. Certain genotypes consistently performed well, indicating inherent physiological and biochemical mechanisms for salt tolerance, including osmotic adjustment and ROS detoxification. The genotypes G_{34} (IEc 688), G_{25} (IEc 675), G_{18} (IEc 154), G_{44} (IEc 360) and G_{43} (IEc 356) performed well consistently upon exposure to salt levels. These findings are crucial for selecting and breeding salt-tolerant genotypes to ensure stable millet production in saline-prone regions, thereby supporting food security under environmental stress conditions.

FUTURE SCOPE

The findings from this study provide a strong foundation for future research aimed at improving salinity tolerance in Barnyard millet. The tolerant genotypes identified can be further evaluated under field conditions to validate their performance in real-world saline environments. Advanced molecular approaches such as gene expression profiling, genome-wide association studies and marker-assisted selection can be employed to identify and utilize key genes responsible for salt tolerance. By integrating the data with omics technologies will facilitate the development of elite varieties. The efforts will contribute to sustainable millet production in salt-affected regions, enhancing food security and supporting climate-resilient agriculture.

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Conflict of Interest. None.

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