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Evaluation of Salinity Tolerance in Sorghum Genotypes (*Sorghum bicolor* L. Moench) Using PCA Biplot

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ABSTRACT: Sorghum, an important cereal crop that is similar to millets to some extent, is severely challenged by salinity stress, which negatively impacts its growth and yield. To examine the impacts of salinity, we treated: a control, hydropriming, and four concentrations of sodium chloride (50 mM, 100 mM, 150 mM, and 200 mM). Important parameters were taken to determine the influence of salinity on seed performance. These parameters were germination percentage, root length, shoot length, seedling total length, vigor index, seedling fresh weight, and dry weight of the seedlings. PCA, together with biplot, was employed to sort sorghum genotypes according to their tolerance to salinity. The high degree of variance explained by the first two principal components in all treatments varied more than 76%. This approach allowed us to effectively visualize and interpret the data, determining the most important characteristics that are related to salinity tolerance. Our study results indicated that some genotypes, namely IS 6710 (G_{34}), IS 6312(G_1), IS 6313(G_2), IS 6315(G_{26}) and IS 6316(G_{50}) performed well at all the tested salinity levels. The discovery of these salt-tolerant sorghum lines provides new opportunities for future study, especially with their physiological and biochemical mechanisms in response to salinity stress.

Keywords: Principal component analysis, biplots, salinity tolerance, vigour index.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is becoming ever more important as a key grain crop with huge potential to enhance the sustainability of agriculture and improve global food security. The fifth most significant cereal globally, it is native to Africa and is a member of the Poaceae family that includes several important crops. Sorghum has a genome size of around 730 megabases (Rajasekharan and Kumar 2023) and its natural resistance to stressful conditions like drought and moderate salinity makes it an essential crop for semi-arid regions where traditional crops are likely to face hardships because of limited water supply (Hussain *et al*., 2019). Worldwide, sorghum production has registered good growth, going up to 58.28 million metric tons, an improvement from 57.53 million metric tons in the previous year. Sorghum production is dominated by the United States with a production of approximately 8.07 million metric tons, followed by Nigeria at 6.4 million metric tons, with significant contributions from India, Mexico, and Brazil (FAO, 2023; USDA, 2023).

In India, where the output of sorghum stood at 4.74 million metric tons, Tamil Nadu played an important role, especially during the kharif season. A good quality of one of sorghum's characteristic features is its tolerance to moderate salinity, and this is a valuable chance to overcome the problem of salinity in agriculture (Yamazaki *et al*., 2020; Afzal *et al*., 2023). In addition, there has been increased interest in the genetic diversity found in sorghum germplasm as a potential route to improving salt-tolerant varieties.

Salinity is recognized as a significant abiotic stress factor that hinders plant growth and productivity. Various types of salts are found in both soil and water, and their salinity can either promote or hinder crop development. The salts exist in different forms, such as NaCl, KCl, $MgCl_2$, and $CaCl_2$. Salinity negatively impacts crop growth and development through a range of complex mechanisms, including osmotic stress, ion toxicity, mineral deficiencies, and various physiological and biochemical issues (Jilukara Soujanya *et al*., 2022).

Through the characterization and analysis of various genotypes, scientists are able to identify the genetic processes responsible for salt tolerance, eventually leading to the breeding of better cultivars that can thrive under saline conditions (Afzal *et al*., 2023). Besides, sorghum plants have demonstrated the capacity to accumulate compatible solutes like proline, carbohydrates, and amino acids to sustain osmotic regulation and water retention against stress, thus increasing their potential to withstand salinity-associated problems (Garcia-Caparros *et al*., 2021) Salinity is a challenging and complex problem to agricultural production, distinguished by the surplus deposition of soluble salts in soil and irrigation water.

Poor quality irrigation water, particularly its salinity and alkalinity, is harmful to soil and crop health. The introduction of irrigation is regarded as the primary factor contributing to increased salinity in the western regions of India (Lakshmi *et al.,* 2022). This effect can negatively impact plant health, causing lower production and degraded quality. Crops under saline conditions experience osmotic stress that disrupts their water uptake and causes ion toxicity, mainly from sodium (Na^+) and chloride (Cl^-), interfering with essential physiological processes. The effects of salinity stress are complex, bringing about nutrient imbalances capable of seriously hindering plant growth and development (Fadl *et al*., 2023). Excess production of reactive oxygen species (ROS) under conditions of salinity stress can lead to oxidative damage that undermines cellular integrity and functionality. Such negative impacts are most significant in arid and semi-arid environments, coastal regions prone to saltwater intrusion, where rampant evaporation enhances the process of salinization of croplands (Mukhopadhyay *et al*., 2021). Principal Component Analysis (PCA) is a multivariate technique employed for analyzing a data table in which observations are described by multiple quantitative interrelated dependent variables.

Its main purpose is to draw meaningful information out of the statistical data and represent it in the form of a set of orthogonal new variables, referred to as principal components. The method helps in the visualization of patterns of similarity between the observations and variables using graphical plots. Mathematically, PCA is based on the eigen-decomposition of positive semi-definite matrices and singular value decomposition (SVD) of a rectangular matrix, with the process characterized by eigenvectors and eigenvalues related to square matrices (Mishra *et al*., 2017). The aim of this study is to delineate sorghum genotypes that possess improved salinity tolerance and assess the adaptive features that allow for their survival in saline environments.

Different biometrical tools can be used to analyze the genotypes for salinity tolerance. These will give the accurate and reliable data. Therefore, the current study employs 2D biplot and principal component analyses to avoid the least significant variable and provide exact results. The current study will help to explain how these traits can be manipulated for improving agriculture in salinity-affected environments.

MATERIALS AND METHODS

A. Experimental site

The laboratory experiment was performed in the 2025 Department of Genetics and Plant Breeding of SRM College of Agricultural Sciences, SRM Institute of Science and Technology, Baburayenpettai, Chengalpattu district, Tamil Nadu, India. The aim of this experiment was to assess the germination and seedling characteristics of 100 sorghum genotypes. Out of these, 87 genotypes were collected by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad whereas the remaining 13 were procured from diverse locations in and around Tamil Nadu.

B. Treatment details

The research work covered various treatments that were meant to study the impacts of salinity on physiological seed quality traits. Treatments comprised a control set (T0) using distilled water, Hydropriming (T1), and sodium chloride (NaCl) at different concentrations: T2 (50 mM), T3 (100 mM), T4 (150 mM), and T5 (200 mM). NaCl was dissolved in distilled water to obtain treatment solution. Each of the treatments was imposed in trays containing sterilized sand with a length of 18 cm, arranged based on a Factorial Complete Randomized Design (FCRD) with four replications.

The seeds were sterilized at the surface and soaked under initial stress conditions at various concentrations for 8 hours, using 25 seeds in each treatment. The seeds were then sown in trays for salinity screening. The sterilized sand was then subjected to different salinity levels corresponding to the concentration of each treatment. Germination numbers were methodically counted throughout the experiment until all the seeds had either germinated or died, as well as assessments of physiological seed quality factors to obtain information about how seeds react under varied salinity stresses.

The following observations were accurately recorded according to International Seed Testing Association (ISTA) guidelines made in 2012 (ISTA, 2012). The Germination Percentage (G%) represents the percentage rate at which seeds germinate successfully, reflecting seed viability. Total Seedling Length (TSL) has a unit of centimeters (cm) and encompasses the total seedling height. Root Length (RL) and Shoot Length (SL), in units of centimeters, give an indication of the development of the root system of the seedling and above ground plant, respectively. Fresh Weight (FW) and Dry Weight (DW) are measured in milligrams (mg) to determine the seedlings' mass at various states of moisture. The Vigour Index (VI), determined according to the method described (Abdulbaki and Anderson 1973) is a useful measure of seedling viability and potential for growth, considering criteria such as germination and seedling growth.

C. Statistical Analysis

Sorghum genotypes and different salinity levels were treated as two independent factors with Principal Component Analysis (PCA). The statistical analysis was done on SPSS version 16.0 (SPSS Inc., Chicago, USA)., which seeks to attain the dimensionality reduction of a dataset with as much variance retained as possible. This is done by converting the original variables into a new set of uncorrelated variables referred to as principal components, where each component records the direction of maximum variance. The largest variance is captured by the first principal component, the second largest by the second principal component (orthogonal to the first), and so on. In this study, biplots based on the described methodology (Gabriel, 1971) were produced separately for every level of salinity. PCA transformed the raw data into unitless variables that allocated variability among various factors or principal components and picked out only those factors with an eigenvalue of more than one to be studied further. The biplot, a scatter diagram in two dimensions, showed the patterns of distribution of the traits and genotypes and highlighted the major factors that were responsible for the large majority of the variability.

RESULTS AND DISCUSSION

Principal Component Analysis (PCA) was a useful multivariate approach to assess trait variation and genotype performance in controlled, hydroprimed, and rising salinity environments. The large variance being explained by the first two principal components in all treatments more than 75% at 50 mM salinity demonstrates PCA's ability to spot significant sources of variation in the dataset (Goswami and Patel 2024).

The PCA biplot of the control demonstrates sample distribution and influence of variables by the first principal component PC1, which accounts for 65.8% variance and PC2 accounts for 11.1% collectively represent 76.9% of the variability. The majority of the genotypes are concentrated close to the origin signifying comparable growth responses, while genotypes G_{34}, G_1, G_{26}, G_{50}, and G_2 are clearly differentiated along Dim1, which represents greater values in aspects such as Dry Weight, Fresh Weight, and Vigor Index. This distinction is indicative of a possible genetic component of vigor that can be utilized in breeding schemes (Foolad, 2004). Red arrows demonstrate high positive correlations between these variables. In the Hydropriming treatment, 70.2% of the variance is explained by PC1 and 11.7% by PC2, summing up to 81.9% of the variability of the data.

Red arrows symbolize extremely influential characteristics like DW, FW, Germination Percentage, Root Length, Vigor Index, and Shoot Length. The genotypes G_{34}, G_1, G_{26}, G_{50}, and G_2 were found to be the standout performers, consistent with previous studies that priming treatments can enhance seedling establishment and resistance to stress (Ashraf and Foolad 2005). Traits such as vigour index, germination percentage, and seedling length, which had high loadings on PC1, played a central role in separating these genotypes, establishing their importance in early growth vigor (Farooq and Azam 2006). The biplot of the 50 mM treatment identifies the genotype variation and the effect of major traits under salt stress with the first principal component (PC1) responsible for 75.3% of the variance and the second component (PC2) for 9.6%, both explaining 84.9% of the variation.

The red arrows for characteristics such as Dry Weight, Germination Percentage, Root Length, Vigor Index, Total Shoot Length, and Shoot Length demonstrate good positive correlation with PC1. Concurrently, Fresh Weight exhibits a negative contribution. The genotypes G_{34}, G_1, G_{26}, G_{50}, and G_2 lie away from the general cluster along PC1, implying that they are possibly more tolerant or superior-performing genotypes under salt stress, while the majority of other genotypes group close to the origin, indicating typical trait values. (Munns and Tester 2008). This biplot highlights differentiation in traits among genotypes and the important roles of dry weight and vigour index in differentiating performance under 50 mM salinity. the 100 mM salinity treatment, the PCA biplot is used in this case to represent the distribution of the genotypes under stress, with PC1 accounting for 71.2% of the variance and PC2 contributing 9.5%, making the total 80.7%. The genotypes G_{34}, G_1, G_2, G_{26}, and G_{50} are outliers along PC1, which presents higher traits and possibly greater performance at high salinity, while the core cluster is still concentrated at the origin representing average traits.

Interestingly, vigour index and dry weight became the major traits under salinity in accordance with results (Munns and Tester 2008; Shekhawat *et al*., 2024) which emphasize the significance of biomass and vigor traits in salinity tolerance mechanisms. The analysis clearly points out a few genotypes that pass 100 mM stress, proving the significance of vigor and biomass traits in obtaining variation and enhancing future breeding for salt stress tolerance. The PCA biplot under 150 mM salinity stress indicates the discrimination between genotypes, with PC1 accounting for 71% and PC2 accounting for 10.7% of the total variance, together representing 81.7% of the data variance. The morphological characteristics that are seen contribute significantly along PC1, especially fresh weight having a distinct vector direction that describes its singular contribution to the overall variance.

Findings indicate that the majority of the traits remain positively correlated even under extreme salinity stress. Of particular interest, genotypes G_{34}, G_1, G_2, G_{26}, and G_{50} always exhibit better performance or salt tolerance, clustering on the extreme right along PC1, signifying their capability for high adaptability towards rising salt stresses (Mishra *et al*., 2024). The study at 200 mM salinity stress, the PCA biplot still shows distinct discrimination among genotypes, with PC1 accounting for 68.6% and PC2 11.6% of the total variance, combined to account for 80.2%. Even with the higher salt concentration, the grouping of trait vectors like fresh weight, dry weight, germination percentage, root length, shoot length, total seedling length and vigour index close together reflects a positive relationship between these traits.

Fresh weight remains a prevailing trait, still functioning as a sensitive marker of salt stress impact. The same genotypes G_{34}, G_1, G_2, G_{26}, and G_{50} once again differentiate themselves along PC1, displaying their strength and superior performance under high salinity levels. The majority of the other genotypes congregate close to the origin, identifying potential genetic improvement areas and stress adaptation mechanisms. The increasing significance of PC2, especially through factors like fresh weight and dry weight, predicts a shift in stress response dynamics, with biomass partitioning becoming more important under extreme stress (Flowers *et al.*, 2010). Such shifts can be interpreted in terms of physiological strategies like osmotic adjustment and biomass partitioning that are helpful in maintaining growth under high salinity. Overall, this PCA analysis highlights tolerance in some genotypes to severe salinity and demonstrates the significance of biomass and vigor-related traits as key discriminators, setting the stage for further research and development of salt-tolerant genotypes. The eigenvalues and percentage of variance explained by the first seven principal components (PCs) in the treatments, which include control, hydropriming, and salinity stress treatments (50 mM, 100 mM, 150 mM, and 200 mM). PC1 is the major contributor to variance, accounting for important variation in salinity-responsive characteristics: 65.79% control, 70.18% hydropriming, and reaching a maximum of 75.32% with 50 mM salinity.

Its contribution does weaken with greater salinity stress, however, 71.20% at 100 mM, 70.95% at 150 mM, and 68.55% at 200 mM, showing a more spread-out distribution of variability among other components. The first five PCs account for more than 99% of the cumulative variance, with the importance of PC2 and PC3 increasing at increased salinity, pointing to the intricacy of response traits. The PCA loadings were used to reinforce that vigour index and total seedling length significantly loaded in PC1 for all treatments consistently, emphasizing their significance in initial vigor and as selection indices of stress tolerance (Table 1). At the same time, the increasing contribution of FW and DW to PC2 at high salinities reflects the capacity of genotypes to preserve biomass as an essential characteristic for sustained performance under stress (Bandehagh and Taylor 2020). The Principal Component Loading values of treatments Control, Hydropriming, and salinity levels of 50 mM, 100 mM, 150 mM, and 200 mM reveal that characters such as germination percentage, root length, shoot length, total shoot length, and vigor index load highly positive on PC1, indicating that they are critical for seedling vigor. VI and TSL reveal the highest loadings on PC1 in all cases, signifying their potential to differentiate genotypic responses.

Conversely, fresh weight and dry weight both have variable contributions with more loading on PC2 at higher salinity (150 mM and 200 mM), indicating a change in the influence of traits (Shashibhushan *et al.*, 2022). Dry weight influences PC1 moderately but has higher loadings on PC2, which implies that it represents biomass variation under stress. Their frequent differentiation along PC1 and better performance on characteristics such as VI and DW suggest their suitability for breeding programs aiming to increase salt stress tolerance (Vetriventhan and Upadhyaya 2019). The stability of these characteristics also justifies their selection as criteria in upcoming genetic improvement programs (Table 2).

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	PC7
Eigenvalue	**Control**	4.60	0.77	0.66	0.54	0.40	0.00	0.00
	Hydropriming	4.91	0.81	0.52	0.43	0.31	0.00	0.00
	50mM	5.27	0.67	0.45	0.35	0.23	0.00	0.00
	100mM	4.98	0.66	0.59	0.42	0.32	0.00	0.00
	150mM	4.96	0.74	0.64	0.34	0.28	0.00	0.00
	200mM	4.79	0.81	0.69	0.40	0.28	0.00	0.00
Variability (%)	Control	65.79	11.09	9.47	7.83	5.79	0.00	0.00
	Hydropriming	70.18	11.67	7.43	6.16	4.50	0.03	0.00
	50mM	75.32	9.64	6.46	5.13	3.37	0.05	0.00
	100mM	71.20	9.53	8.45	6.13	4.62	0.05	0.00
	150mM	70.95	10.69	9.23	4.96	4.10	0.04	0.00
	200mM	68.55	11.57	9.99	5.78	4.07	0.00	0.00
Cumulative variability (%)	Control	65.79	76.88	86.36	94.19	99.99	99.99	99.99
	Hydropriming	70.18	81.85	89.29	95.45	99.96	100.00	100.00
	50mM	75.32	84.97	91.43	96.57	99.94	100.00	100.00
	100mM	71.20	80.73	89.19	95.32	99.94	100.00	100.00
	150mM	70.95	81.64	90.85	95.85	99.95	100.00	100.00
	200mM	68.55	80.13	90.13	95.91	99.99	99.99	99.99

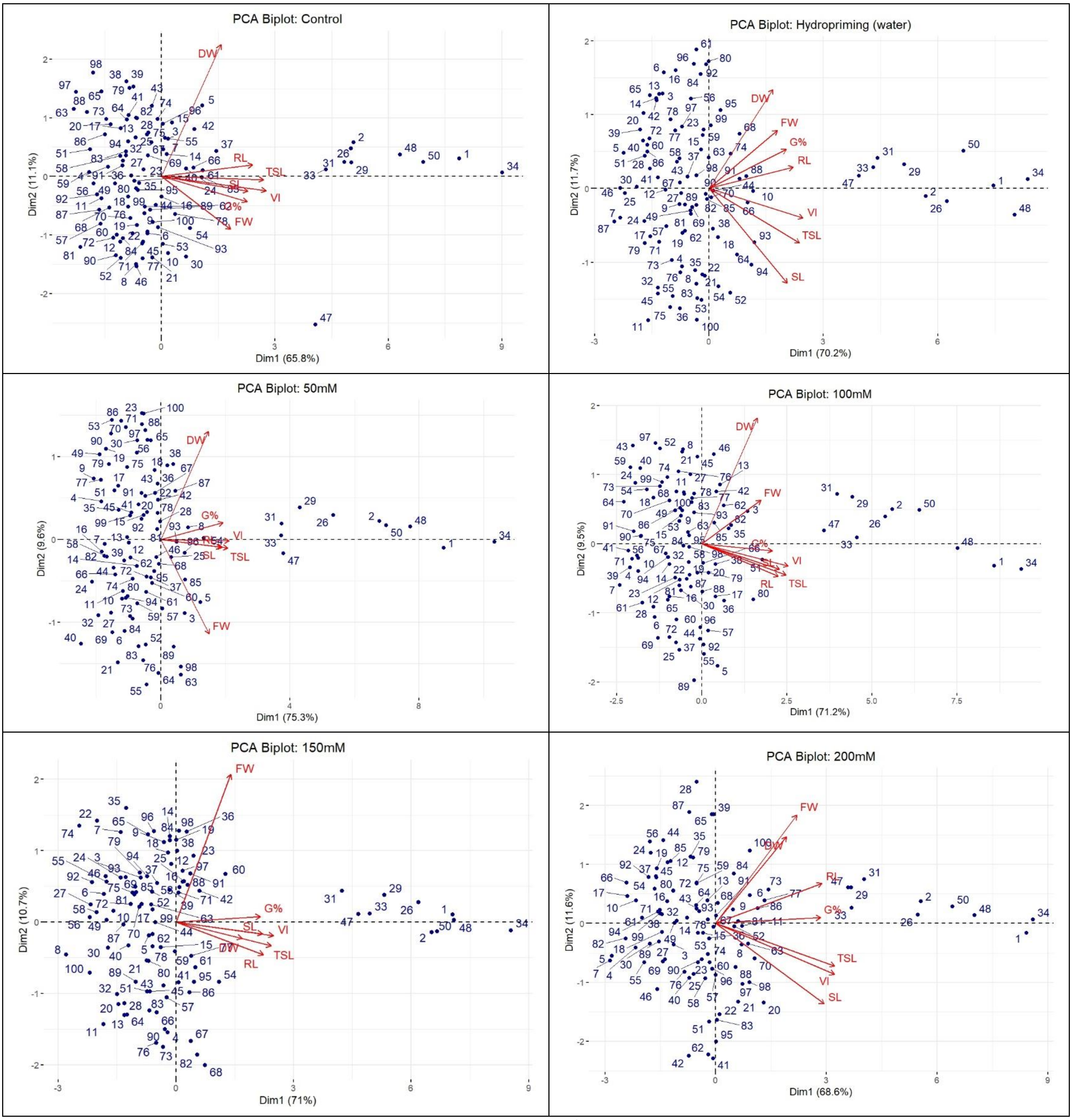

Fig. 1. 2-D principal component biplot graphs for sorghum genotypes under Control (T_0), Hydropriming (water) (T_1), and 50mM (T_2), 100mM (T_3), 150mM (T_4) and 200mM (T_5) salinity treatments. Traits include germination percentage (G%), shoot length (SL), root length (RL), total seedling length (TSL), fresh weight (FW), and dry weight (DW).

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%	0.80	-0.15	0.80	0.21	0.90	0.09	0.80	-0.04	0.85	0.03	0.84	0.02
RL	0.85	0.06	0.87	0.11	0.89	-0.04	0.87	-0.18	0.88	-0.18	0.85	0.20
SL	0.80	-0.08	0.81	-0.51	0.87	-0.04	0.89	-0.14	0.87	-0.06	0.87	-0.40
TSL	0.95	-0.01	0.94	-0.29	0.97	-0.04	0.96	-0.17	0.96	-0.13	0.95	-0.21
VI	0.97	-0.08	0.98	-0.16	0.99	-0.007	0.98	-0.12	0.98	-0.07	0.95	-0.25
FW	0.64	-0.31	0.71	0.31	0.70	-0.53	0.67	0.24	0.55	0.82	0.65	0.54
DW	0.55	0.79	0.66	0.53	0.68	0.61	0.63	0.70	0.67	-0.09	0.57	0.43

CONCLUSIONS

Based on the PCA biplots for salinity concentrations (100 mM, 150 mM, and 200 mM), some genotypes excelled and are differentiated from the main group in the positive direction of PC1, suggesting improved performance under salinity stress. In order of performance and at all levels of salinity, the top genotypes are genotypes IS 6710 (G_{34}), IS 6312(G_1), IS 6313(G_2), IS 6315(G_{26}) and IS 6316(G_{50}). They are all placed far from the origin in the same direction as desirable traits such as fresh weight, shoot length, root length, vigour index, total shoot length and germination percentage. Lastly, it can be proposed that the genotypes introduced therein be explored in future research in salinity resistance and yield.

FUTURE SCOPE

The current research identifies potential sorghum genotypes with excellent salinity tolerance, providing a platform for further improvement through breeding programs. More studies should investigate their physiological and biochemical mechanisms under field conditions. Molecular approaches like QTL mapping and transcriptome analysis can pinpoint key genes responsible for salt tolerance. Such genotypes can also be tested under other abiotic stresses for general resilience. Combining molecular information with phenotypic characteristics will accelerate the generation of better, stress-tolerant cultivars. In the long run, this work promotes the development of sorghum cultivars adaptable to salty conditions, promoting sustainable crop yields and food security.

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Conflicts of Interest. None.

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