

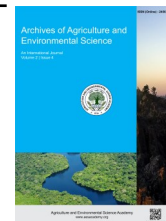


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ORIGINAL RESEARCH ARTICLE



## Screening and evaluation of sorghum germplasm for salinity tolerance using physiological indices in hydroponic culture

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### ABSTRACT

Salinity is a major abiotic stress limiting crop productivity globally, particularly in coastal and deltaic regions prone to saltwater intrusion. This study aimed to screen and evaluate eighteen *Sorghum bicolor* L. germplasm for salinity tolerance using physiological and biochemical indices under controlled hydroponic conditions. The experiment was conducted in a completely randomized design with two salinity treatments (0 and 12 dS m<sup>-1</sup>), replicated thrice, in the physiology laboratory of Bangladesh Agricultural University. Morphological (shoot/root length, leaf number), physiological (SPAD value, Fv/Fm, proline content), and biochemical traits (Na<sup>+</sup>, K<sup>+</sup>, and K<sup>+</sup>/Na<sup>+</sup> ratio) were assessed to quantify salinity responses. Significant genotypic differences ( $p \leq 0.001$ ) were found in key indices: shoot length stress tolerance index ranged from 74.16% (BD-738) to 92.10% (BD-713), while root-shoot ratio reached a maximum of 135.34 in Safal 999. The highest K<sup>+</sup>/Na<sup>+</sup> ratio was observed in sorghum BD-731 (10.44), whereas sorghum BD-701 showed the lowest (2.16), indicating high susceptibility. Proline accumulation increased under stress, notably in sorghum BD-713 and Hybrid Sorgho, suggesting osmotic adjustment mechanisms. Based on visual SES scoring, five genotypes (Sorghum BD-703, Sorghum BD-706, sorghum BD-713, sorghum BD-730, sorghum BD-737) were classified as tolerant, and seven as moderately tolerant. Hydroponic screening coupled with physiological indices provided a robust and reproducible method to identify salinity-tolerant sorghum lines. These findings highlight sorghum BD-703, Sorghum BD-713, Sorghum BD-706, and Hybrid Sorgho as promising candidates for further field evaluation in salt-affected environments.

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### INTRODUCTION

Soil salinity is an increasingly critical abiotic stress affecting global agricultural sustainability. It is estimated that more than 20% of irrigated land worldwide is adversely impacted by salinity, with projections indicating a further rise due to climate change, sea-level rise, and unsustainable land use practices

(Shrivastava & Kumar, 2015; FAO, 2021). Salinity imposes a dual stress mechanism on plants-osmotic stress, which limits water uptake, and ion toxicity, primarily from sodium (Na<sup>+</sup>) and chloride (Cl<sup>-</sup>) ions, which disrupt cellular homeostasis and enzymatic activity (Munns & Tester, 2008). In contrast to drought, which predominantly limits water availability, or heat stress, which damages proteins and membranes (Wahid et al., 2007),

salinity uniquely induces both water deficit and ion imbalance, culminating in inhibited growth, reduced photosynthesis, and yield losses (Negrão et al., 2017). This challenge is especially severe in deltaic and coastal regions, including Bangladesh, where approximately 1.05 million hectares of arable land are affected by varying degrees of salinity (Mahmuduzzaman et al., 2014; Shawkhatuzamman et al., 2023). The resulting decline in agricultural productivity exacerbates food insecurity and economic vulnerability, particularly for resource-poor farming communities.

In response to this pressing issue, the development and deployment of salt-tolerant crop species is a critical adaptive strategy. Sorghum (*Sorghum bicolor* L. Moench) has emerged as a promising candidate due to its physiological plasticity, resilience to multiple abiotic stresses, and suitability for cultivation in marginal environments. As a C<sub>4</sub> cereal crop with high photosynthetic and water-use efficiency, sorghum ranks as the fifth most important cereal globally and is widely cultivated for food, forage, and biofuel purposes (Reddy et al., 2009; Dahlberg et al., 2011; Kumar & Chopra, 2013). Importantly, sorghum has shown substantial potential to withstand salinity levels ranging from 12 to 18 dS m<sup>-2</sup>, although notable genotypic variation exists in stress tolerance (Liang et al., 2026). Identifying and selecting elite sorghum genotypes with enhanced salt tolerance are, therefore, essential to expand its cultivation in saline-prone ecosystems. Previous studies have underscored the importance of screening cereal crops for salinity tolerance using morphological and physiological criteria (Khan et al., 2024; Banerjee et al., 2025). Traits such as relative water content, chlorophyll concentration, proline accumulation, membrane stability index (MSI), and Na<sup>+</sup>/K<sup>+</sup> ratio have been effectively utilized as physiological indicators to distinguish tolerant genotypes under salt stress (Parida & Das, 2005; Negrão et al., 2017). While numerous soil-based studies have attempted to evaluate sorghum germplasm under saline conditions, such approaches often suffer from inconsistencies due to soil heterogeneity, microbial variability, and uneven salt distribution (Rajabi et al., 2024). In contrast, hydroponic systems offer a precise, controlled, and reproducible environment for early-stage screening. These systems ensure uniform salinity application, facilitate accurate measurement of stress responses, and allow direct root observation and timely nutrient adjustments (Li et al., 2026; Negrão et al., 2017).

However, despite growing interest in sorghum improvement under salinity stress, several research gaps remain. First, few studies have systematically integrated hydroponic culture with multi-trait physiological assessments for early-stage sorghum screening. Second, existing research has largely focused on soil-based systems, which are less reliable for dissecting salinity tolerance mechanisms at the seedling stage. Third, little attention has been directed toward evaluating sorghum genotypes specifically for their potential adaptability in coastal and saline agroecosystems like those in South Asia. These gaps limit the precision of phenotyping and delay the identification of truly resilient germplasm for saline-prone regions. The primary challenge in salinity screening lies in maintaining uniform stress

exposure across treatments and accurately quantifying physiological traits under dynamic field conditions. Hydroponics overcomes these barriers by allowing tight regulation of salinity levels, minimizing external variability, and enabling high-throughput screening of diverse germplasm. Additionally, physiological indices such as shoot-to-root ratio, chlorophyll retention, Na<sup>+</sup>/K<sup>+</sup> selectivity, and osmolyte accumulation (e.g., proline) provide robust metrics for identifying adaptive traits linked to stress tolerance (Munns & Gilliam, 2015). The present study is significant in that it combines hydroponic culture with physiological trait analysis to screen and evaluate sorghum germplasm for salinity tolerance. The identification of tolerant genotypes can inform breeding programs aimed at improving sorghum productivity in saline-affected environments, particularly in low-lying coastal zones. Furthermore, the use of hydroponics in conjunction with physiological indices offers a scalable, cost-effective, and scientifically rigorous method for salinity stress evaluation. The novelty of this research lies in its integrated approach leveraging hydroponic screening with multi-dimensional physiological assessments to identify salt-tolerant sorghum genotypes suitable for salinity-prone agroecosystems. To the best of our knowledge, this is one of the first studies to apply such a methodology to sorghum in the context of Bangladesh's coastal agricultural systems.

## MATERIALS AND METHODS

### Experimental site and period

The two factors (Factor A and Factor B) hydroponic experiment was conducted following Complete Randomized Design (CRD) with eighteen sorghum germplasms to observe their responses to salinity treatments (0 dSm<sup>-1</sup> & 12 dSm<sup>-1</sup>) having three replications in plant physiology laboratory of the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh-2202, Dhaka, Bangladesh during the period of 1 to 23 June of 2016. Factor A consist of two salinity level i.e., control (Tap water and 12dSm<sup>-1</sup>) and Factor B consist of 18 sorghum varieties i.e., Hybrid Sorgo, Hybrid Sudan (Debgiri Co.), Sorghum BD-703, Sorghum BD-701, Sorghum BD-706, Sorghum BD-707, Sorghum BD-713, Sorghum BD-720, Sorghum BD-725, Sorghum BD-726, Sorghum BD-727, Sorghum BD-728, Sorghum BD-730, Sorghum BD-731, Sorghum BD-733, Sorghum BD-737 and Sorghum BD-738. In case of salinity treatments, the different salinity levels (Control-1.42 dSm<sup>-1</sup> and 12 dSm<sup>-1</sup>) were obtained by dissolving crude salt collected until the treatment level was reached by checking the EC meter. The control treatment was maintained using tap water only. Salinity was imposed at 6 days after sowing and continued up to 21 days after sowing.

### Experimental set up, details of treatments and data collection

The experiment was conducted using plastic trays with one salinity treatment and a control. Eighteen sorghum germplasms were randomly assigned to two trays, each containing nine germplasms. A total of 12 plots (trays) were required for the study. Seeds of all germplasms were sown on 1st June 2024

after surface sterilization with 5% sodium hypochlorite solution for 30 minutes, followed by three to four rinses with distilled water. Salinity levels were prepared by dissolving crude salt, collected from the seashore, into water until the desired electrical conductivity (EC) was attained, as measured by an EC meter. The control treatment (EC 0) was maintained using distilled water only. Salinity was imposed at 6 days after sowing (DAS) and continued up to 21 DAS. A full-strength nutrient solution was applied according to the formulation of Pitann *et al.* (2009). Three seedlings per germplasm per treatment were uprooted for evaluation. In the case of salt-sensitive germplasms, seedlings were uprooted once they had completely perished. Data were collected on seedling height (cm), total root length (cm), number of green leaves per plant, green leaf length per plant (cm), shoot length (cm), shoot fresh weight (g), root fresh weight (g), root dry weight (mg), shoot dry weight (mg), and total dry matter (mg). Total dry matter per seedling was computed as the sum of root and shoot dry weights for each treatment. After harvest, sorghum plants were separated into roots and shoots, which were oven-dried at 70°C until constant weight was achieved. The dried samples were ground using a Wiley Hammer Mill, passed through a 40-mesh screen, thoroughly mixed, and stored in plastic vials for analysis. The Na<sup>+</sup> and K<sup>+</sup> contents of the plant samples were determined from acid-digested material prepared using the micro-Kjeldahl digestion method as described by Thomas *et al.* (1967). Elemental analysis was performed using a flame photometer (Model PERKIN-ELMER, 2380), and the K<sup>+</sup>/Na<sup>+</sup> ratio was calculated from the respective concentrations in the plant tissues. Seedling height was measured from the root collar to the tip of the longest leaf at the time of uprooting. Root length was measured using a ruler for each seedling and averaged over nine seedlings. The number of green leaves was counted and averaged in a similar manner. Roots were carefully separated and washed with tap water followed by distilled water, then oven-dried at 70°C to constant weight. Root dry weight per seedling (mg) was then recorded. Shoots were similarly separated, washed, oven-dried, and their dry weights were measured. Total seedling dry matter (mg) was calculated by summing the root and shoot dry weights for each treatment. Morphological indices for each parameter relative to the control were calculated for each genotype following the formula described by Ashraf *et al.* (2003). Seedling height, root

and shoot length (cm), number of green leaves per plant, and root and shoot fresh and dry weights (g) were measured using a measuring tape and a digital weighing balance, respectively. In case of physiological traits, relative chlorophyll content was measured by a chlorophyll meter (SPAD-502, Konica Minolta, Japan) and maximum photochemical efficiency of PSII ( $F_v/F_m$ ) was also measured from the 3<sup>rd</sup> leaf of 21 days old seedlings with the help of a Pocket-PEA (Hansatech, Norfolk, UK) according to the formula of photochemical efficiency of PSII,  $F_v/F_m = (F_m - F_o)/F_m$  (Genty *et al.*, 1989). On the contrary, chemical, and bio-chemical constituent analysis was done in this study.

### Scoring of saline injured seedlings based on visual symptoms

Eighteen sorghum (*Sorghum bicolor*) germplasm lines were evaluated under both control and saline stress conditions to assess their tolerance to salinity at the seedling stage. The assessment was performed using a modified version of the Standard Evaluation System (SES) for rice developed by the International Rice Research Institute (IRRI), which was adapted for sorghum based on visual symptom expression under saline stress (Table 1). According to this system, plants were classified into five tolerance categories based on observable growth characteristics and leaf injury symptoms induced by salinity. A score of 1 denoted highly tolerant genotypes exhibiting normal growth without visible damage; a score of 3 indicated tolerant lines showing near-normal growth with slight leaf tip whitening or rolling; a score of 5 represented moderately tolerant plants with markedly stunted growth and rolling of most leaves; a score of 7 described susceptible lines characterized by growth arrest, leaf drying, and partial mortality; while a score of 9 reflected highly susceptible genotypes where the majority of seedlings either died or were on the verge of death (IRRI, 1996; Gregorio *et al.*, 1997).

### Data analysis

The collected data were statistically analyzed using the Statistix-10 software based on a Completely Randomized Design (CRD). Analysis of variance (ANOVA) was employed to assess the significance of treatment effects, and mean comparisons were performed using Tukey's Honest Significant Difference (HSD) test. All graphs were done with the help of Microsoft Excel worksheet 2010.

**Table 1.** Modified IRRI standard evaluation scoring (SES) system based on the visual symptoms of saline injury at seedling stage of sorghum (*S. bicolor*).

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves turn whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; few elongated	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants died	Susceptible
9	Almost all plants died or dying	Highly susceptible

## RESULTS AND DISCUSSION

### Shoot length and shoot length stress tolerance index (SLSI)

Significant ( $p \leq 0.001$ ) variation in final shoot length was present among the germplasm (Table 2). Shoot length at control condition was always higher than the salinity stress condition. Shoot growth of all the germplasm was reduced by the stress conditions. Maximum shoot length index was observed in Sorghum BD-713 (92.10) followed by Sorghum BD-706 (92.03), Hybrid Sorghum Sudan (90.17) and Sorghum BD-720 (89.07); while the minimum was recorded from Sorghum BD-738 (74.16) followed by Sorghum BD-728 (74.63). Sensitive genotypes exhibited a relatively higher leaf elongation rate index (Table 3), which sug-

gests that stress-sensitive germplasm exhibit faster initial growth that may lead to later-stage stress symptoms due to ion accumulation (Wolf et al., 1990; Cherry, 2011). Root length and root length stress tolerance index (RLSI) Statistically significant variation ( $p \leq 0.001$ ) was present in the root length of studied germplasm (Table 2). Root length decreased with salinity stress. The highest root length index was recorded in Sorghum BD-703 (107.0) followed by Hybrid Sorghum Sudan (106.13) and Sorghum BD-713 (106.2). Sorghum BD-730 had the lowest root length index (82.0), indicating sensitivity. Sensitive lines showed initial higher root elongation but developed leaf drying symptoms and died later, likely due to excessive  $\text{Na}^+$  accumulation (Netondo et al., 2004a; Lacerda et al., 2001).

**Table 2.** Analysis of Variance (ANOVA) for various traits of eighteen Sorghum germplasm in hydroponics under different salt concentrations.

Parameters	Sources of variation			Per cent Relative value (Stress/Control*100)		
	Germplasm (G)	Salt stress (S)	G x S	RV (G)	LSD	CV
Shoot length (cm)	0***	0***	0.73 <sup>NS</sup>	0.30 <sup>NS</sup>	14.54	10.35
Root length (cm)	0.0007***	0.0021***	0.83 <sup>NS</sup>	0.61 <sup>NS</sup>	25.82	16.73
Root Shoot ratio	0***	0.0013***	0.68 <sup>NS</sup>	0.60 <sup>NS</sup>	34.88	18.95
Number of leaves	0.0124*	0***	0.07 <sup>NS</sup>	0.12 <sup>NS</sup>	19.60	13.89
SPAD value	0.19 <sup>NS</sup>	0.10 <sup>NS</sup>	0.59 <sup>NS</sup>	0.27 <sup>NS</sup>	20.20	11.75
Fv/Fm	0.0047**	0.0001***	0***	0***	4.36	2.59
PI	0.0118*	0***	0.05*	0.07 <sup>NS</sup>	44.74	21.41
Leaf elongation rate (cm/day)	0.39 <sup>NS</sup>	0.0001***	0.48 <sup>NS</sup>	0.17 <sup>NS</sup>	104.67	73.05
Shoot Fresh weigh (g)	0***	0***	0.008**	0.011*	17.36	14.91
Root fresh weight (g)	0.0033**	0.33 <sup>NS</sup>	0.32 <sup>NS</sup>	0.10 <sup>NS</sup>	66.78	36.44
Shoot dry weight (g)	0***	0***	0.25 <sup>NS</sup>	0.55 <sup>NS</sup>	38.91	29.55
Root dry weight (g)	0.41 <sup>NS</sup>	0.32 <sup>NS</sup>	0.51 <sup>NS</sup>	0.21 <sup>NS</sup>	43.35	26.01
Total dry weight (g)	0***	0.52	0.07	0.07	44.74	21.41
Root K <sup>+</sup> content (g)	0.23 <sup>NS</sup>	0***	0.21 <sup>NS</sup>	0***	22.20	26.72
Root Na <sup>+</sup> content (g)	0***	0***	0***	0***	350.71	24.54
Shoot K <sup>+</sup> content (g)	0***	0***	0.015*	0.68 <sup>NS</sup>	19.10	29.38
Shoot Na <sup>+</sup> content (g)	0.035*	0***	0.18 <sup>NS</sup>	0.17 <sup>NS</sup>	585.70	34.12
Total content K <sup>+</sup> (g)	0.18 <sup>NS</sup>	0***	0.20 <sup>NS</sup>	0***	18.88	24.69
Total content Na <sup>+</sup> (g)	0***	0***	0***	0***	298.50	21.35
Potassium/Sodium ratio (K <sup>+</sup> -Na <sup>+</sup> )	0***	0***	0***	0***	0.81	7.74
Proline mg/100g	0***	0***	0***	0***	3.06	0.5

RV= Relative Value, \* = Significant at 5% level, \*\* = Significant at 1% level, \*\*\* = Significant at 0.1% level.

**Table 3.** Relatives value (%) of shoot & root length and root-shoot ratio of eighteen Sorghum germplasm grown under stress and control condition in hydroponics.

Germplasm	Shoot Length Index	Root Length Index	Root Shoot Ratio	Leaf elongation
Hybrid Sorgho	84.69 <sup>a-d</sup>	99.07 <sup>a</sup>	119.0 <sup>abc</sup>	53.26b
Hybrid Sorghum Sudan	90.17 <sup>ab</sup>	106.13 <sup>a</sup>	121.06 <sup>abc</sup>	113.82b
Safal 999	75.64 <sup>bcd</sup>	102.22 <sup>a</sup>	135.34 <sup>a</sup>	246.12a
Sorghum BD-701	86.0 <sup>a-d</sup>	95.44 <sup>a</sup>	109.91 <sup>abc</sup>	86.51b
Sorghum BD-703	84.77 <sup>a-d</sup>	107.0 <sup>a</sup>	126.38 <sup>ab</sup>	102.77b
Sorghum BD-706	92.03 <sup>a</sup>	84.13 <sup>a</sup>	90.98 <sup>c</sup>	78.42b
Sorghum BD-707	82.08 <sup>a-d</sup>	94.78 <sup>a</sup>	116.02 <sup>abc</sup>	74.57b
Sorghum BD-713	92.10 <sup>a</sup>	106.20 <sup>a</sup>	115.35 <sup>abc</sup>	94.45b
Sorghum BD-720	89.08 <sup>abc</sup>	89.31 <sup>a</sup>	100.90 <sup>abc</sup>	71.34b
Sorghum BD-725	81.14 <sup>a-d</sup>	86.10 <sup>a</sup>	107.0 <sup>abc</sup>	63.26b
Sorghum BD-726	83.88 <sup>a-d</sup>	85.94 <sup>a</sup>	103.58 <sup>abc</sup>	69.22b
Sorghum BD-727	88.41 <sup>a-d</sup>	96.44 <sup>a</sup>	111.28 <sup>abc</sup>	80.58b
Sorghum BD-728	74.63 <sup>cd</sup>	88.08 <sup>a</sup>	117.95 <sup>abc</sup>	84.70b
Sorghum BD-730	81.80 <sup>a-d</sup>	82.0 <sup>a</sup>	100.17 <sup>bc</sup>	87.26b
Sorghum BD-731	88.93 <sup>abc</sup>	88.99 <sup>a</sup>	100.57 <sup>abc</sup>	77.39b
Sorghum BD-733	84.74 <sup>a-d</sup>	93.33 <sup>a</sup>	109.90 <sup>abc</sup>	47.60b
Sorghum BD-737	88.55 <sup>a-d</sup>	82.05 <sup>a</sup>	94.21 <sup>bc</sup>	40.97b
Sorghum BD-738	74.16 <sup>d</sup>	87.34 <sup>a</sup>	117.13 <sup>abc</sup>	82.07b
LSD	14.54	25.81	34.88	104.67
CV (%)	10.35	16.73	18.95	73.05

Means bearing the dissimilar letter within the column differ significantly.

The effect of salinity on root shoot ratio was statistically significant ( $p \leq 0.001$ ). Maximum root shoot ratio was recorded from Safal 999 (135.34) indicating a potential escape mechanism in short-term salt stress (Table 3). This aligns with the observation that sorghum restricts  $\text{Na}^+$  and  $\text{Cl}^-$  transport to shoots under stress (Lacerda et al., 2001). The index of leaf elongation rate did not differ significantly except for Safal 999 (246.12) (Table 3). Sensitive genotypes demonstrated numerically greater leaf elongation, possibly linked to ion toxicity and imbalance in expanding tissues (Bernstein et al., 1993). Number of leaves per plant Significant differences in the number of leaves were observed under control conditions (9 to 11.89), but no statistically significant difference was noted under stress (8.33 to 10.44) (Table 4). Hybrid Sorgho produced more leaves under stress, showing a stress-adaptive response, consistent with Li et al. (2026) regarding developmental-stage dependent tolerance. No significant variation in chlorophyll content was observed among germplasm (Table 4).

SPAD values under stress were highest in Safal-999 (31.78) and lowest in Sorghum BD-738 (25.03). Salinity affects chlorophyll synthesis and stomatal function, impacting photosynthesis (Netondo et al., 2004b). Maximum photochemical efficiency of PSII ( $F_v/F_m$ )  $F_v/F_m$  values ranged between 0.69–0.77 (control) and 0.76–0.79 (stress) (Table 4), consistent with Hammami et al. (2024). The slight increase under stress suggests photo protection mechanisms in tolerant lines. Performance Index (PI) PI varied significantly in control but not under stress (Table 4). PI increased under stress, suggesting higher stress tolerance as per Taiz et al. (2015). High PI in tolerant genotypes indicates efficient energy dissipation and reduced ROS formation. Symptomatic evaluation SES score-based grouping classified four tolerant, nine moderately tolerant, and five sensitive germplasm (Table 5). This is consistent with Gregorio et al. (1997) and Brady & Weil (2002), affirming visual symptom-based selection under salinity.

**Table 4.** Number of leaves per plant, SPAD, photochemical efficiency and performance index of 18 Sorghum germplasm under control and stress condition grown in hydroponics.

Germplasm	Number of leaves		SPAD value		PSII ( $F_v/F_m$ )		PI (performance index)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Hybrid Sorgho	9.0 <sup>f</sup>	9.33 <sup>abc</sup>	27.81 <sup>a</sup>	27.06 <sup>bcd</sup>	0.77 <sup>a</sup>	0.77 <sup>abc</sup>	2.12 <sup>ab</sup>	2.85 <sup>a</sup>
Hybrid Sorghum Sudan	10.0 <sup>cf</sup>	8.56 <sup>c</sup>	28.08 <sup>a</sup>	30.64 <sup>abc</sup>	0.76 <sup>ab</sup>	0.77 <sup>abc</sup>	1.83 <sup>bcd</sup>	2.30 <sup>b-f</sup>
Safal 999	9.44 <sup>ef</sup>	8.78 <sup>bc</sup>	26.51 <sup>a</sup>	31.78 <sup>a</sup>	0.76 <sup>ab</sup>	0.77 <sup>abc</sup>	1.77 <sup>bcd</sup>	2.64 <sup>a-d</sup>
Sorghum BD-701	11.0 <sup>a-d</sup>	10.44 <sup>a</sup>	26.31 <sup>a</sup>	29.88 <sup>abc</sup>	0.77 <sup>ab</sup>	0.76 <sup>bc</sup>	2.07 <sup>abc</sup>	2.25 <sup>c-f</sup>
Sorghum BD-703	11.44 <sup>abc</sup>	10.0 <sup>ab</sup>	28.79 <sup>a</sup>	31.48 <sup>ab</sup>	0.75 <sup>ab</sup>	0.76 <sup>c</sup>	1.85 <sup>bcd</sup>	2.51 <sup>a-f</sup>
Sorghum BD-706	11.33 <sup>a-d</sup>	8.78 <sup>bc</sup>	28.67 <sup>a</sup>	31.46 <sup>ab</sup>	0.77 <sup>ab</sup>	0.77 <sup>abc</sup>	2.39 <sup>a</sup>	2.52 <sup>a-f</sup>
Sorghum BD-707	10.22 <sup>b-f</sup>	9.0 <sup>bc</sup>	26.43 <sup>a</sup>	26.41 <sup>cd</sup>	0.75 <sup>b</sup>	0.77 <sup>abc</sup>	1.53 <sup>d</sup>	2.24 <sup>c-f</sup>
Sorghum BD-713	11.11 <sup>a-d</sup>	8.89 <sup>bc</sup>	25.72 <sup>a</sup>	29.52 <sup>abc</sup>	0.77 <sup>ab</sup>	0.78 <sup>abc</sup>	2.10 <sup>ab</sup>	2.82 <sup>ab</sup>
Sorghum BD-720	9.89 <sup>def</sup>	9.22 <sup>abc</sup>	28.14 <sup>a</sup>	28.40 <sup>a-d</sup>	0.76 <sup>ab</sup>	0.77 <sup>abc</sup>	1.73 <sup>bcd</sup>	2.56 <sup>a-e</sup>
Sorghum BD-725	11.56 <sup>ab</sup>	9.22 <sup>abc</sup>	27.16 <sup>a</sup>	26.57 <sup>cd</sup>	0.76 <sup>ab</sup>	0.78 <sup>ab</sup>	1.93 <sup>bcd</sup>	2.28 <sup>b-f</sup>
Sorghum BD-726	11.44 <sup>abc</sup>	9.11 <sup>bc</sup>	26.50 <sup>a</sup>	26.31 <sup>cd</sup>	0.77 <sup>ab</sup>	0.77 <sup>abc</sup>	2.13 <sup>ab</sup>	2.18 <sup>c-f</sup>
Sorghum BD-727	10.67 <sup>a-e</sup>	8.56 <sup>c</sup>	28.84 <sup>a</sup>	28.13 <sup>a-d</sup>	0.77 <sup>ab</sup>	0.76 <sup>bc</sup>	2.08 <sup>abc</sup>	2.41 <sup>a-f</sup>
Sorghum BD-728	10.0 <sup>cf</sup>	8.89 <sup>bc</sup>	28.70 <sup>a</sup>	27.27 <sup>bcd</sup>	0.69 <sup>c</sup>	0.79 <sup>a</sup>	1.64 <sup>cd</sup>	2.08 <sup>ef</sup>
Sorghum BD-730	11.44 <sup>abc</sup>	9.11 <sup>bc</sup>	28.83 <sup>a</sup>	28.23 <sup>a-d</sup>	0.76 <sup>ab</sup>	0.77 <sup>abc</sup>	2.04 <sup>abc</sup>	1.99 <sup>f</sup>
Sorghum BD-731	11.44 <sup>abc</sup>	8.78 <sup>bc</sup>	26.44 <sup>a</sup>	26.70 <sup>cd</sup>	0.77 <sup>a</sup>	0.78 <sup>abc</sup>	1.89 <sup>bcd</sup>	2.16 <sup>def</sup>
Sorghum BD-733	11.22 <sup>a-d</sup>	8.78 <sup>bc</sup>	29.58 <sup>a</sup>	29.30 <sup>a-d</sup>	0.77 <sup>ab</sup>	0.76 <sup>abc</sup>	1.64 <sup>cd</sup>	2.16 <sup>def</sup>
Sorghum BD-737	11.89 <sup>a</sup>	9.33 <sup>abc</sup>	28.12 <sup>a</sup>	28.70 <sup>a-d</sup>	0.76 <sup>ab</sup>	0.77 <sup>abc</sup>	2.07 <sup>abc</sup>	2.71 <sup>abc</sup>
Sorghum BD-738	11.11 <sup>a-d</sup>	8.33 <sup>c</sup>	27.0 <sup>a</sup>	25.03 <sup>d</sup>	0.77 <sup>a</sup>	0.77 <sup>abc</sup>	2.41 <sup>a</sup>	2.29 <sup>b-f</sup>
LSD	1.54	1.24	4.04	4.48	0.02	0.02	0.45	0.54
CV (%)	8.63	8.25	8.8	9.48	1.78	1.73	13.8	13.67

**Table 5.** Visual categorization of eighteen hydroponically grown sorghum germplasm based on modified IRR standard evaluation system Gregorio et al. (1997) SES score.

Germplasm	Score of Control	Score of Stress	Rank	Category
Hybrid Sorgho	1	3.67	8	Moderately tolerant
Hybrid S. Sudan	1	7	17	Susceptible
Safal 999	1	5	12	Susceptible
Sorghum BD-701	1	3.67	9	Moderately tolerant
Sorghum BD-703	1	1.67	1	Tolerant
Sorghum BD-706	1	1.67	2	Tolerant
Sorghum BD-707	1	5.67	14	Susceptible
Sorghum BD-713	1	1.67	3	Tolerant
Sorghum BD-720	1	3.67	10	Moderately tolerant
Sorghum BD-725	1	5.67	15	Susceptible
Sorghum BD-726	1	5	13	Moderately tolerant
Sorghum BD-727	1	5.67	16	Susceptible
Sorghum BD-728	1	7	18	Susceptible
Sorghum BD-730	1	2.33	4	Tolerant
Sorghum BD-731	1	3	6	Moderately tolerant
Sorghum BD-733	1	3	7	Moderately tolerant
Sorghum BD-737	1	2.33	5	Tolerant
Sorghum BD-738	1	4.33	11	Moderately tolerant

Tolerant (1.1-3), Moderately tolerant (3.1-5), Susceptible (5.1-7), Highly susceptible (7.1-9).

**Shoot fresh weight and shoot fresh weight index (SFWI)**

Highly significant ( $p \leq 0.001$ ) variations were observed in shoot fresh weight (Table 6). Higher SFWI in Hybrid Sorgho and Hybrid Sorghum Sudan indicates better tolerance, reflecting superior dry matter retention under salt stress. Shoot dry weight and shoot dry weight index (SDWI) SDWI varied significantly, with Sorghum BD-713 showing the highest index (108.26) (Table 6). High SDWI reflects better adaptation through reduced  $\text{Na}^+$  uptake or improved ionic regulation (Munns & Tester, 2008). Root fresh weight and root fresh weight index (RFWI) RFWI differed significantly; Hybrid Sorgho showed the highest index (189.23) (Table 6), suggesting enhanced osmotic adjustment or root tissue tolerance (Tester & Davenport, 2003). Root dry weight and root dry weight index (RDWI) RDWI was highest in Sorghum BD-701 (129.41), indicating root biomass retention under stress, similar to Wambua et al. (2010). Total dry matter content and total dry matter index (TDMI) TDMI was highest in Sorghum BD-701 (124.91) (Table 6). High TDMI suggests better tolerance mechanisms, such as osmolyte accumulation and reduced  $\text{Na}^+$  uptake (Munns, 2002).  $\text{Na}^+$  content index in root and shoot  $\text{Na}^+$  content varied significantly

(Table 7). Higher  $\text{Na}^+$  in roots indicates restricted translocation, a tolerance mechanism (Cherry, 2011). Sorghum BD-701 and Sorghum BD-713 showed contrasting patterns reflecting divergent salt tolerance strategies.  $\text{K}^+$  content index in root and shoot  $\text{K}^+$  content differed among germplasm (Table 7). High root  $\text{K}^+$  content in Sorghum BD-701 suggests superior ion homeostasis, vital for metabolic function (Greenway & Munns, 1980).  $\text{K}^+ : \text{Na}^+$  ratio index  $\text{K}^+ : \text{Na}^+$  ratio varied, with Sorghum BD-731 showing the highest (10.44) (Table 7), confirming its high salinity tolerance potential (Krishnamurthy et al., 2007). Proline content Proline content increased under stress, highest in Sorghum BD-713 and Sorghum BD-701 (Figure 1), indicating osmoprotection (Netondo et al., 2004b). This is a key biochemical marker for salt tolerance. Overall, ten genotypes Hybrid Sorgho, Sorghum BD-701, Sorghum BD-703, Sorghum BD-706, Sorghum BD-713, Sorghum BD-720, Sorghum BD-730, Sorghum BD-731, Sorghum BD-733, Sorghum BD-737 demonstrated superior performance across morpho-physiological and biochemical traits, recommending them for further evaluation under field conditions to develop salinity-tolerant Sorghum cultivars.

**Table 6.** Index of Shoot and root fresh and dry weight of Sorghum germplasm grown under control and stress condition in hydroponics.

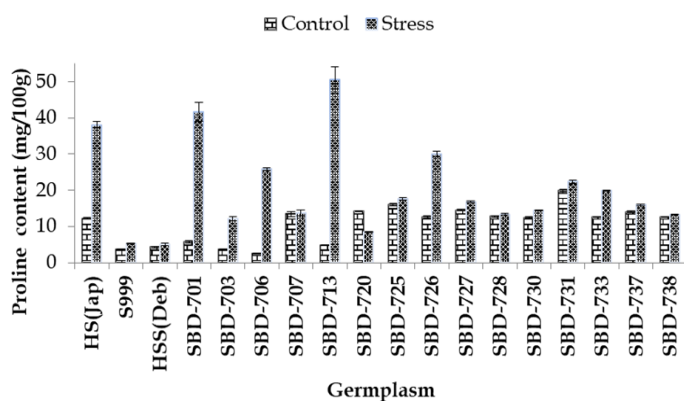
Germplasm	Shoot fresh weight index (SFWI)	Root fresh weight index (RFWI)	Shoot dry weight index (SDWI)	Root dry weight Index (RDWI)	Total dry weight index (TDWI)
Hybrid Sorgho	86.98 <sup>a</sup>	189.23 <sup>a</sup>	94.42 <sup>abc</sup>	118.79 <sup>ab</sup>	116.13 <sup>a</sup>
Hybrid S. Sudan	80.86 <sup>ab</sup>	140.71 <sup>ab</sup>	82.94 <sup>abc</sup>	74.63 <sup>c</sup>	113.47 <sup>ab</sup>
Safal 999	71.54 <sup>a-g</sup>	94.52 <sup>bc</sup>	77.10 <sup>abc</sup>	105.65 <sup>abc</sup>	103.47 <sup>abcd</sup>
Sorghum BD-701	76.22 <sup>a-f</sup>	132.56 <sup>abc</sup>	84.80 <sup>abc</sup>	129.41 <sup>a</sup>	124.91 <sup>a</sup>
Sorghum BD-703	81.27 <sup>ab</sup>	139.48 <sup>ab</sup>	93.39 <sup>abc</sup>	127.16 <sup>a</sup>	124.35 <sup>a</sup>
Sorghum BD-706	79.27 <sup>a-d</sup>	128.96 <sup>abc</sup>	75.90 <sup>abc</sup>	118.52 <sup>ab</sup>	114.86 <sup>a</sup>
Sorghum BD-707	62.81 <sup>d-g</sup>	72.11 <sup>c</sup>	74.40 <sup>abc</sup>	93.58 <sup>abc</sup>	91.71 <sup>abcd</sup>
Sorghum BD-713	77.93 <sup>a-e</sup>	137.79 <sup>abc</sup>	108.26 <sup>a</sup>	113.37 <sup>abc</sup>	112.53 <sup>ab</sup>
Sorghum BD-720	80.38 <sup>abc</sup>	100.47 <sup>bc</sup>	99.43 <sup>ab</sup>	112.39 <sup>abc</sup>	111.16 <sup>abc</sup>
Sorghum BD-725	59.26 <sup>fg</sup>	83.98 <sup>bc</sup>	68.95 <sup>bc</sup>	77.66 <sup>bc</sup>	76.84 <sup>cd</sup>
Sorghum BD-726	63.19 <sup>c-g</sup>	95.72 <sup>bc</sup>	73.54 <sup>abc</sup>	94.83 <sup>abc</sup>	93.0 <sup>abcd</sup>
Sorghum BD-727	71.43 <sup>a-g</sup>	85.54 <sup>bc</sup>	82.25 <sup>abc</sup>	98.44 <sup>abc</sup>	96.75 <sup>abcd</sup>
Sorghum BD-728	61.829 <sup>efg</sup>	82.07 <sup>bc</sup>	79.05 <sup>abc</sup>	79.84 <sup>bc</sup>	79.40 <sup>bcd</sup>
Sorghum BD-730	55.79 <sup>g</sup>	95.79 <sup>bc</sup>	57.73 <sup>c</sup>	103.02 <sup>abc</sup>	98.17 <sup>abcd</sup>
Sorghum BD-731	68.85 <sup>b-g</sup>	99.46 <sup>bc</sup>	71.31 <sup>abc</sup>	102.53 <sup>abc</sup>	99.09 <sup>abcd</sup>
Sorghum BD-733	65.87 <sup>b-g</sup>	123.09 <sup>abc</sup>	71.62 <sup>abc</sup>	102.15 <sup>abc</sup>	99.39 <sup>abcd</sup>
Sorghum BD-737	62.58 <sup>d-g</sup>	109.60 <sup>bc</sup>	60.74 <sup>bc</sup>	76.17 <sup>bc</sup>	74.77 <sup>d</sup>
Sorghum BD-738	57.11 <sup>g</sup>	76.90 <sup>bc</sup>	72.36 <sup>abc</sup>	79.55 <sup>bc</sup>	78.82 <sup>bcd</sup>
LSD	17.357	66.782	38.912	43.348	35.091
CV (%)	14.91	36.44	29.55	26.01	21.04

Means bearing the dissimilar letter within the column differ significantly.

**Table 7.** Relative per cent of  $\text{Na}^+$ ,  $\text{K}^+$  content in different plant parts, total  $\text{K}^+$  and  $\text{Na}^+$  content and  $\text{K}^+ - \text{Na}^+$  ratio of eighteen Sorghum germplasm grown in control and salt stress under hydroponic condition.

Germplasm	Relative per cent of solute content (g)						Ratio of $\text{K}^+ - \text{Na}^+$
	Root $\text{K}^+$	Root $\text{Na}^+$	Shoot $\text{K}^+$	Shoot $\text{Na}^+$	Total $\text{K}^+$	Total $\text{Na}^+$	
Hybrid Sorgho	68.05 <sup>bc</sup>	852.9 <sup>bc</sup>	46.21 <sup>ab</sup>	1358.6 <sup>ab</sup>	61.52 <sup>c</sup>	870.6 <sup>bc</sup>	7.12 <sup>cd</sup>
Hybrid S. Sudan	25.62 <sup>fgh</sup>	373.9 <sup>ef</sup>	41.12 <sup>ab</sup>	1221.8 <sup>a-d</sup>	26.03 <sup>fg</sup>	389.5 <sup>fg</sup>	6.90 <sup>cde</sup>
Safal 999	50.66 <sup>cd</sup>	833.5 <sup>bc</sup>	37.74 <sup>ab</sup>	1159.4 <sup>a-d</sup>	47.63 <sup>c-e</sup>	848.8 <sup>bc</sup>	5.58 <sup>ghi</sup>
Sorghum BD-701	109.85 <sup>a</sup>	4450.4 <sup>a</sup>	41.60 <sup>ab</sup>	1126.2 <sup>a-d</sup>	92.37 <sup>a</sup>	3925.6 <sup>a</sup>	2.36 <sup>k</sup>
Sorghum BD-703	51.03 <sup>cd</sup>	685.1 <sup>b-e</sup>	46.61 <sup>ab</sup>	1328.6 <sup>abc</sup>	49.88 <sup>cd</sup>	697.5 <sup>cde</sup>	7.16 <sup>cd</sup>
Sorghum BD-706	105.55 <sup>a</sup>	1000.9 <sup>b</sup>	37.39 <sup>ab</sup>	996.5 <sup>bcd</sup>	80.49 <sup>ab</sup>	1000.8 <sup>b</sup>	8.0 <sup>b</sup>
Sorghum BD-707	44.11 <sup>d-g</sup>	714.8 <sup>b-e</sup>	35.97 <sup>ab</sup>	936.3 <sup>bcd</sup>	42.19 <sup>d-f</sup>	725.7 <sup>bcd</sup>	5.8 <sup>f-i</sup>
Sorghum BD-713	55.26 <sup>cd</sup>	637.7 <sup>c-f</sup>	51.44 <sup>a</sup>	1633.8 <sup>a</sup>	54.28 <sup>cd</sup>	665.2 <sup>c-f</sup>	8.01 <sup>b</sup>
Sorghum BD-720	57.09 <sup>bcd</sup>	857.9 <sup>bc</sup>	47.38 <sup>ab</sup>	1190.5 <sup>d</sup>	55.32 <sup>cd</sup>	868.1 <sup>bc</sup>	6.43 <sup>def</sup>
Sorghum BD-725	22.42 <sup>gh</sup>	456.9 <sup>def</sup>	32.95 <sup>ab</sup>	763.9 <sup>cd</sup>	24.05 <sup>fg</sup>	469.1 <sup>d-g</sup>	5.13 <sup>j</sup>
Sorghum BD-726	27.92 <sup>e-h</sup>	689.9 <sup>b-e</sup>	36.93 <sup>ab</sup>	824.4 <sup>bcd</sup>	29.25 <sup>e-g</sup>	695.3 <sup>c-e</sup>	4.2 <sup>j</sup>
Sorghum BD-727	49.73 <sup>cde</sup>	629.6 <sup>c-f</sup>	41.88 <sup>ab</sup>	965.5 <sup>bcd</sup>	47.53 <sup>c-e</sup>	640.8 <sup>c-f</sup>	7.43 <sup>bc</sup>
Sorghum BD-728	21.80 <sup>h</sup>	423.1 <sup>ef</sup>	40.26 <sup>ab</sup>	1014.9 <sup>b-d</sup>	24.14 <sup>fg</sup>	438.6 <sup>d-g</sup>	5.53 <sup>ghi</sup>
Sorghum BD-730	46.12 <sup>c-f</sup>	808.8 <sup>bc</sup>	29.33 <sup>b</sup>	760.8 <sup>cd</sup>	42.45 <sup>d-f</sup>	802.8 <sup>bc</sup>	5.36 <sup>hi</sup>
Sorghum BD-731	77.88 <sup>b</sup>	581.6 <sup>c-f</sup>	36.22 <sup>ab</sup>	783.9 <sup>bcd</sup>	61.7 <sup>bc</sup>	591.3 <sup>c-g</sup>	10.44 <sup>a</sup>
Sorghum BD-733	47.82 <sup>cde</sup>	792.8 <sup>bcd</sup>	34.78 <sup>ab</sup>	866.3 <sup>bcd</sup>	45.38 <sup>c-e</sup>	795.7 <sup>bc</sup>	5.72 <sup>f-i</sup>
Sorghum BD-737	24.10 <sup>fgh</sup>	395.1 <sup>ef</sup>	30.88 <sup>b</sup>	731.2 <sup>d</sup>	25.21 <sup>fg</sup>	405.0 <sup>efg</sup>	6.24 <sup>efg</sup>
Sorghum BD-738	16.17 <sup>h</sup>	320.5 <sup>f</sup>	36.54 <sup>ab</sup>	961.3 <sup>bcd</sup>	20.0 <sup>g</sup>	336.5 <sup>g</sup>	5.99 <sup>fgh</sup>
LSD	22.195	350.71	19.10	585.7	18.88	298.5	0.81
CV (%)	26.72	24.54	29.38	34.12	24.69	21.35	7.74

Means bearing the dissimilar letter within the column differ significantly.



**Figure 1.** Proline content (mg/100g) of control and stress plants in hydroponic culture.

## Conclusion

The results of the present study revealed that maximum root shoot ratio was recorded in Safal 999 (135.34) followed by Sorghum BD-703 (126.38) and Sorghum BD-728 (117.95) and the minimum from Sorghum BD-706 (90.98). Among eighteen sorghum germplasm, five tolerant (Sorghum BD-703, Sorghum BD-706, Sorghum BD-713, Sorghum BD-730 & Sorghum BD-737), seven moderately tolerant (Hybrid Sorgho, Sorghum BD-701, Sorghum BD-720, Sorghum BD-726, Sorghum BD-731, Sorghum BD-733 & Sorghum BD-738) and six became evident as salt sensitive (Hybrid S. Sudan, Safal 999, Sorghum BD-707, Sorghum BD-725, Sorghum BD-727 & Sorghum BD-728) on the basis of the evaluation score. Besides, the highest ratio of  $K^+Na^+$  index (10.44) was calculated from the germplasm Sorghum BD-731 whereas the lowest  $K^+Na^+$  ratio was measured from the germplasm Sorghum BD-701 (2.16). Higher  $K^+Na^+$  ratio index indicates more tolerant to salinity and lower  $K^+Na^+$  value indicates more susceptible to salinity. Therefore, it may be concluded that germplasm BD-701, BD-703, BD-713, and Hybrid Sorgho exhibited superior performance under salinity, making them promising candidates for future field trials in saline-prone areas.

## DECLARATIONS

**Author contribution statement:** Conceptualization: M.E.H. and A.K.M. Z.H.; Methodology: M.E.H.; Software and validation: M.S.H., M.R.A., S.C.C. and B.K.S. Formal analysis and investigation: M.E.H.; Resources: A.K.M.Z.H.; Data curation: M.S.H.; Writing—original draft preparation: M.E.H.; Writing—review and editing: A.K.M.G.S. and M.A.H.T.; Visualization: B.K.S.; Supervision: A.K.M.Z.H.; Project administration: A.K.M.G.S.; Funding acquisition: A.K.M.Z.H. All authors have read and agreed to the published version of the manuscript.

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