

RESEARCH ARTICLE

GERMINATION-BASED SCREENING OF SORGHUM GERMPLASM UNDER SALINITY STRESS

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ARTICLE DETAILS

ABSTRACT

Article History:

Received 17 April 2025

Revised 21 May 2025

Accepted 23 May 2025

Available online 16 June 2025

Sorghum (*Sorghum bicolor* L. Moench), known for its resilience to abiotic stresses, has the potential to be reintroduced in Bangladesh for food and fodder, particularly in saline-prone coastal areas where major crops often fail. Salinity, a major abiotic stress, is expanding globally, including in the arid and coastal regions of Bangladesh, posing a severe threat to crop productivity. With a view to assess the impact of salinity on the germination of 35 sorghum germplasm, a study was carried out at the Plant Physiology Laboratory, Department of Crop Botany, Bangladesh Agricultural University (BAU), Mymensingh, from 12 to 19 November 2015. The experiment followed a Completely Randomized Design (CRD) with four treatments, each replicated three times. Four treatments were 0, 6, 12, and 18 dS m⁻¹. Salinity treatments were prepared using fresh seawater adjusted to target EC values using an EC meter. Germination parameters such as Germination Stress Tolerance Index (GSI), Root Length Stress Index, and Shoot Length Stress Index were evaluated. The results of the present study revealed that GSI values ranged from 21.81-98.84, 7.51-65.78, and 0-30.66 at 6, 12, and 18 dS m⁻¹, respectively. Based on GSI under moderate salinity (6 and 12 dS m⁻¹), 18 germplasm were shortlisted, including Sorghum BD-737, Sorghum BD-726, Sorghum BD-713, and Safal 999. In a subsequent hydroponics trial, Sorghum BD-737 and Hybrid Sorgo recorded the highest Sorghum BD-726, Sorghum BD-713 grain yields. These findings suggest that selected germplasm, especially Sorghum BD-737 and Hybrid Sorgo, may be promising candidates for further field trials and breeding programs aimed at developing Sorghum BD-737 salinity-tolerant sorghum varieties suited to coastal ecosystems.

KEYWORDS

Sorghum germplasm, screening, salinity level, germination

1. INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is the 5th most important cereal after wheat, maize, rice and barley in worldwide, primarily grown in arid and semi-arid regions due to its Adaptability to Drought, heat, and salinity (Foolad, 2004; Bhadha et al., 2019). Its multi-purpose usage as food, fodder, bioethanol, and industrial raw material further enhances its value (Sairam and Tyagi, 2004). It is a self-pollinated diploid C₄ grass with a high photosynthetic efficiency. However, its cultivation remains limited in Bangladesh, with only 187 hectares under production and an average yield of 1.36 t/ha (FAOSTAT, 2014). It is a source of energy, protein, vitamins and minerals. Besides, it is a promising crop in Bangladesh due to grow it any kind of climate (Bhadha et al., 2019). That is why it is suitable for Bangladeshi weather. It also able to grow with moderate salinity. Now a days, salinity is increasing in soil day by day in Bangladesh. So, sorghum is promising crop to combat salinity in our country. As sorghum can tolerate salinity, making it suitable for cultivation in degraded lands where most cereals fail (Mansour et al., 2000; FAOSTAT, 2014). Since, salinity is a major abiotic stress that adversely affects soil health, crop productivity, and food security worldwide. Soil is typically considered saline when its electrical conductivity (EC) exceeds 4 dSm⁻¹, often accompanied by high exchangeable sodium percentages (Flowers and Yeo, 1995). Approximately 20% of irrigated land and 6% of the total global land area are salt affected (Nayyar and Walia, 2003). In coastal zones, rising sea levels and saltwater intrusion exacerbate the problem, rendering vast areas unfit for conventional agriculture (Kausar et al., 2012; Malibari et al.,

2008). Bangladesh, with a 710 km long coastline and more than 35 million inhabitants in coastal zones, is highly vulnerable. Currently, about 1.05 million hectares of land in the coastal region are affected by various degrees of salinity (Ghoulam and Fares, 2001). The increasing salinization of coastal lands has led to a decline in agricultural productivity, threatening livelihoods and contributing to food insecurity (Munns and Tester, 2008). Studies report a 50-60% reduction in the diversity of summer and winter crop varieties in southwestern coastal districts of Bangladesh due to salinity from 1975 to 2006 (Munns et al., 2006). In this context, identifying alternative crops capable of withstanding salinity is vital for sustainable agriculture in affected areas. Sorghum is one of them. Salinity affects germination and seedling growth through osmotic stress and ion toxicity, leading to reduced water uptake, membrane instability, nutrient imbalance, and oxidative stress (Munns & Tester, 2008; Ashraf et al., 2003; Ali et al., 2012). Sodium (Na⁺) competes with potassium (K⁺), disrupting cellular homeostasis and impairing growth and productivity (Ali et al., 2012; Chaves et al., 2009). Early growth stages, particularly germination, are critical indicators of salinity tolerance. Hence, screening for germplasm based on seedling traits under saline conditions is vital. Some researcher identified physiological indices through their experiments such as the Germination Stress Tolerance Index (GSI), Shoot Length Stress Index (SLSI), and Root Length Stress Index (RLSI) as effective screening tools for salinity tolerance (Kausar et al., 2012). Traits like Na⁺ exclusion, higher K⁺/Na⁺ ratios, and vacuolar compartmentalization are associated with salt tolerance (Flowers & Yeo, 1995; Parida and Das, 2005). Developing or identifying salinity-tolerant

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DOI:
10.26480/trab.02.2025.77.81

sorghum varieties through large-scale screening would provide a resilient crop alternative for coastal agriculture in Bangladesh. Keeping the above facts in mind, the present study was conducted to evaluate the germination performance of 35 sorghum germplasm under different salinity levels, and to identify salinity-tolerant sorghum germplasm at the germination stage for future breeding and field trials.

2. MATERIALS AND METHODS

2.1 Experimental Site, Period and Design

With a view to evaluate and screening sorghum germplasm for salinity tolerance based on germination traits, the experiment was conducted at the Plant Physiology Laboratory, Department of Crop Botany, Bangladesh Agricultural University (BAU), Mymensingh, during December-January 2015. A Completely Randomized Design (CRD) was followed with two factors and three replications. Factor A consisted of four salinity levels such as 0 (control), 6, 12, and 18 dS m⁻¹ and factor B consisted of 35 sorghum germplasm, including 30 genotypes collected from the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh & 5 high-yielding exotic varieties sourced from Japan and Sudan. In total, 420 petriplates (35 genotypes × 4 salinity levels × 3 replications) were used.

2.2 Preparation of Salinity Solution

Salinity levels were prepared by diluting seawater collected from the Bay of Bengal (initial EC: 36.9 dS m⁻¹) using distilled water and confirmed using an electrical conductivity (EC) meter (model: HI98331, Hanna Instruments). The control treatment was maintained using distilled water only.

2.3 Procedure of Germination Test and Collection of Related Data

Germination tests were carried out in sterilized petriplates lined with Whatman No. 1 filter paper. Prior to sowing, seeds were surface sterilized using 5% HgCl₂ solution for two minutes to prevent fungal contamination and then rinsed thoroughly with distilled water. The sterilized seeds were stored at 4°C for 24 hours to break dormancy and synchronize germination. Ten seeds of each germplasm were sown in petriplates premoistened with the respective salinity solutions. The petriplates were kept in the dark at room temperature (approximately 25±2°C) for six days to facilitate germination. Germination was recorded daily, and seeds were considered germinated when radicle emergence reached or exceeded 2 mm. The Final Germination Percentage (FGP) was calculated on the 5th day using the following formula (Ashraf et al., 2003).

The germination percentage is an estimate of the viability of seeds. Germinated seeds were counted every day for five days. The seeds were considered to have germinated upon the emergence of radicles (≥ 2mm). Final Germination Percentage (FGP) was calculated with the following

formula:

$$\text{FGP (\%)} = \frac{\text{Number of seeds germinated at 5th day}}{\text{Number of total seeds}} \times 100$$

The root (radicle) and shoot (plumule) length (mm) were measured on day three, four and five of germination experiment. The root and shoot length at 5th day five was considered as final length of root and shoot.

Promptness Index (PI) was calculated using the following formula described by Ashraf et al., (2008)

$$\text{PI} = \text{nd1 (1.0)} + \text{nd2 (0.75)} + \text{nd3 (0.5)} + \text{nd4 (0.25)}$$

Where, PI = Promptness Index; nd1, nd2, nd3 and nd4 are the number of seeds germinated on day one, two, three and four, respectively.

Then Germination Stress Tolerance Index (GSTI) as percentage was calculated using the following formula described by Kausar et al., 2012)

$$\text{GSTI (\%)} = \frac{\text{PI of stressed seeds}}{\text{PI of control Seeds}} \times 100$$

The Root Length Stress Index (RLSI) and Shoot Length Stress Index (SLSI) were calculated using the following formula described by Kausar et al., 2012)

$$\text{RLSI (\%)} = \frac{\text{Root length of stressed seeds at day five}}{\text{Root length of control seeds at day five}} \times 100$$

$$\text{SLSI (\%)} = \frac{\text{Shoot length of stressed seeds at day five}}{\text{Shoot length of control seeds at day five}} \times 100$$

2.4 Data Analysis

Microsoft Excel 2010 was used to record, tabulate, process, and compile all experimental data for statistical analysis. All data were analyzed statistically using Statistix 10 software (Analytical Software, 2013). Analysis of variance (ANOVA) was performed, and treatment means were separated using Tukey's Honest Significant Difference (HSD) test at a 5% significance level (Gomez and Gomez, 1984). All graphs of this study were done using the software of Microsoft Excel 2010.

3. RESULTS

The analysis of variance (Table 1) revealed that significant differences (P<0.001) among sorghum germplasms, salinity treatments, and their interactions for all measured parameters (Final Germination Percentage, Root Length, Shoot Length, Germination Stress Tolerance Index (GSI), Root Length Stress Index-RLSI, and Shoot Length Stress Index-SLSI). The significant Genotype (G) × Salinity (S) interaction for most traits indicates differential salinity response across genotypes, suggesting the potential for selecting salt-tolerant germplasm at the germination stage.

Table 1: Analysis of Variance (ANOVA) for various traits of 35 Sorghum germplasm

Parameters	Sources of variation					
	Germplasm (G)		Salt Stress (S)		G × S	
	Sig.	LSD	Sig.	LSD	Sig.	LSD
Final Germination Per cent (%)	***	25.16	***	5.72	*	58.27
Root Length (mm)	***	0.061	***	0.014	***	0.143
Shoot Length (mm)	***	0.0115	***	0.0038	***	0.023
GSI (%)	***	13.82	***	4.04	*	23.95
RLSI (%)	***	3.64	***	1.06	**	6.31
SLSI (%)	***	0.2409	***	0.0705	***	0.417

* = Significant at 5% level, ** = Significant at 1% level *** = Significant at 0.1% level

3.1 Germination Stress Tolerance Index (GSI)

The results of Figure 1 revealed that salinity had a strong inhibitory effect on the GSI across all germplasms. As salinity levels increased from 6 to 18 dSm⁻¹, GSI declined markedly. In case of 6 dSm⁻¹, Sorghum BD-713 (98.94) and BD-737 (96.54) produced the highest GSI values. Besides, under 12 dSm⁻¹, Sorghum BD-737, Safal 999 (65.78), BD-726 (65.61), and Hybrid Sorgho (63.08) performed well. Interestingly, Sorghum BD-726 the varieties of Hybrid Sorgho (30.66) and Safal 999 (29.60) retained the highest GSI, while Sorghum BD-700, Sorghum BD-704, Sorghum BD-705, and Hybrid Sudan Grass showed complete failure Sorghum BD-700, Sorghum BD-704, Sorghum BD-705 (GSI = 0) under the extreme condition of salinity (18 dSm⁻¹). These results indicate that germination tolerance varies widely and can be a reliable screening trait under saline conditions.

3.2 Root Length Stress Tolerance Index (RSTI)

All tested salinity level had significantly affected on root length stress tolerance index (RSTI) and the results are presented in Figure 2. From the results of present study revealed that, root length declined progressively with increasing salt levels. At 6 dSm⁻¹, root length ranged from 1.80-14.0 mm; at 12 dSm⁻¹, from 0.83-7.90 mm; and at 18 dSm⁻¹, from 0.06-3.0 mm. From the results of present study, it was concluded that Sorghum BD-733, Sorghum BD-736, and Sorghum BD-727 recorded the highest RSTI at 6, 12, and 18 dSm⁻¹ Sorghum BD-733, Sorghum BD-736 and Sorghum BD-727, respectively, suggesting these genotypes generated better root development under the mentioned salinity condition. Conversely, genotypes like Sorghum BD-707 and Sorghum BD-709 exhibited the lowest RSTI at higher salt levels, confirming their sensitivity. The variation in RSTI suggests root elongation is more affected at higher stress and may serve as an early marker for salinity tolerance.

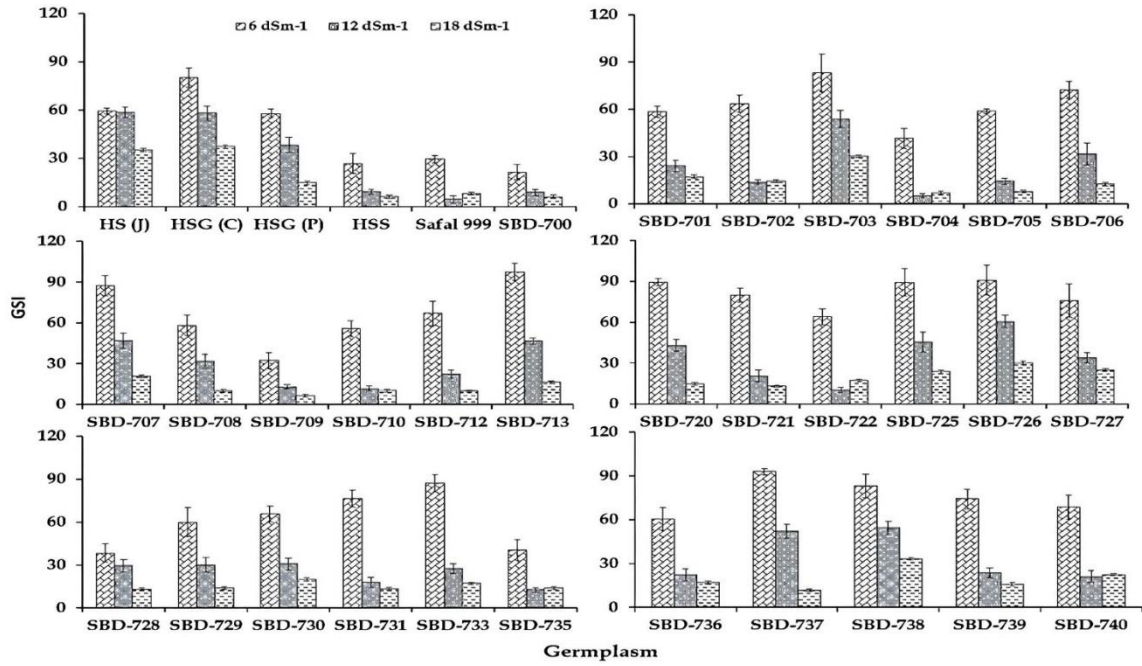


Figure 1: Germination stress tolerance index (GSI) of 35 Sorghum germplasm at four levels (control, 6, 12 and 16 dSm⁻¹) of salt stress

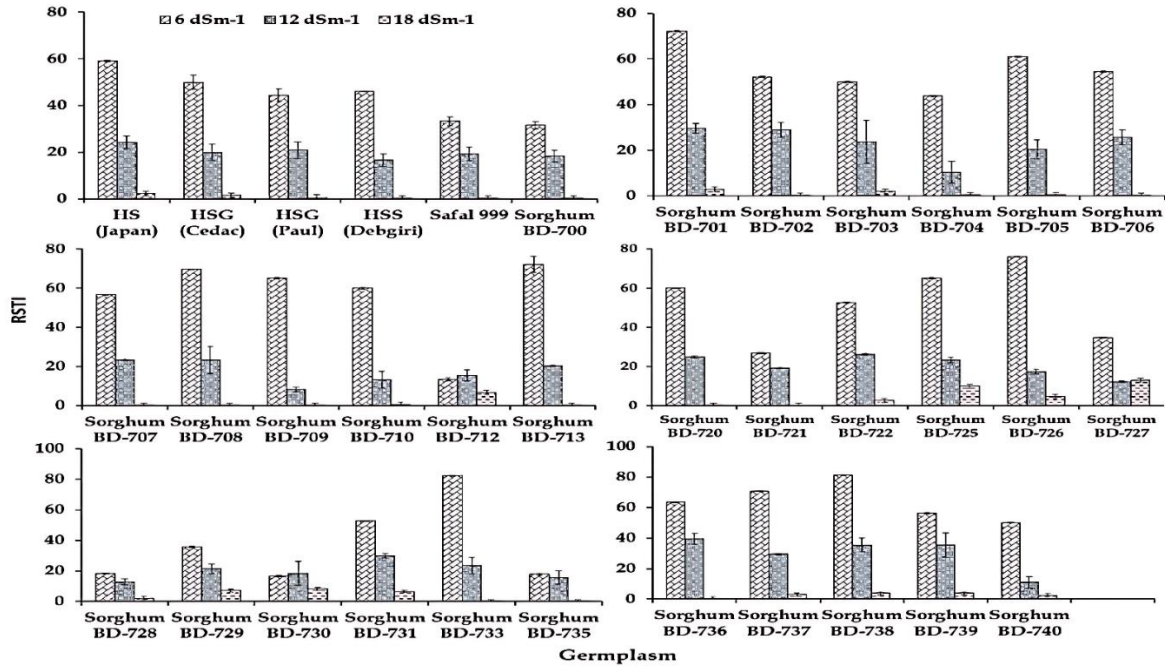


Figure 2: Root-length Stress Tolerance Index (RSTI) of 35 Sorghum germplasm at four levels (control, 6, 12 and 16 dSm⁻¹) of salt stress.

3.3 Shoot Length Stress Tolerance Index (SLTI)

Results from the Table 2 revealed that shoot length was even more sensitive to salinity than root length. SLTI values sharply decreased across all salinity levels. Among all tested germplasms, Sorghum BD-702 and Sorghum BD-710 showed 100% SLTI under 6 dSm⁻¹, while Sorghum BD-702 and Sorghum BD-710 (75%) and BD-701 (66.67%) also performed well. On the contrary, Sorghum BD-709 and Sorghum BD-701 at 12 dSm⁻¹,

HSS-Debgiri (39.99%) and BD-739 (39.87%) showed the best performed whereas, most of genotypes fell Sorghum BD-739 below 1% SLTI, indicating severe shoot growth inhibition. Notably, BD-727 (12.50%) and BD-730 (18.67%) recorded the highest SLTI values at 18 dSm⁻¹, demonstrating relative tolerance. These findings confirm that shoot growth is a critical parameter affected by salinity and can discriminate between tolerant and sensitive genotypes.

Table 2: Shoot-length stress tolerance index (STI) of 35 Sorghum germplasm of *Sorghum bicolor* (L.) at 4 levels (control, 6, 12 and 18 dSm⁻¹) of salt stress

Germplasms	Shoot Length Stress Tolerance Index-SLTI (%)		
	6 dSm ⁻¹	12 dSm ⁻¹	18 dSm ⁻¹
Hybrid Sorgo	50.0 ^e	16.66 ^f	0.28 ^d
Safal 999	60.0 ^d	0.33 ^k	0.33 ^d
HSS (Debgiri)	40.0 ^f	39.99 ^a	0.33 ^d
HSG (Cedac)	0.41 ⁿ	0.41 ^k	0.41 ^d
HSG (Paul)	16.66 ^l	16.66 ^f	0.28 ^d

Table 2 (cont): Shoot-length stress tolerance index (STI) of 35 Sorghum germplasm of *Sorghum bicolor* (L.) at 4 levels (control, 6, 12 and 18 dSm⁻¹) of salt stress

Sorghum BD-700	0.41 ⁿ	0.41 ^k	0.41 ^d
Sorghum BD-701	66.67 ^c	0.28 ^k	0.28 ^d
Sorghum BD-702	100.0 ^a	0.33 ^k	0.33 ^d
Sorghum BD-703	28.57 ^h	28.57 ^c	0.24 ^d
Sorghum BD-704	24.99 ⁱ	0.41 ^k	0.41 ^d
Sorghum BD-705	20.83 ^j	0.34 ^k	0.34 ^d
Sorghum BD-706	33.33 ^e	16.66 ^f	0.28 ^d
Sorghum BD-707	50.0 ^e	33.33 ^b	0.28 ^d
Sorghum BD-708	28.57 ^h	28.57 ^c	0.24 ^d
Sorghum BD-709	75.0 ^b	24.99 ^d	0.41 ^d
Sorghum BD-710	100.0 ^a	0.55 ^k	0.55 ^d
Sorghum BD-712	19.99 ^k	20.0 ^e	9.99 ^c
Sorghum BD-713	40.0 ^f	20.0 ^e	0.33 ^d
Sorghum BD-720	40.0 ^f	20.0 ^e	0.33 ^d
Sorghum BD-721	33.33 ^e	33.33 ^b	0.28 ^d
Sorghum BD-722	33.33 ^e	0.28 ^k	0.28 ^d
Sorghum BD-725	14.28 ^m	14.28 ^g	0.24 ^d
Sorghum BD-726	60.0 ^d	20.0 ^e	0.33 ^d
Sorghum BD-727	25.0 ⁱ	12.50 ^h	12.50 ^b
Sorghum BD-728	0.28 ⁿ	0.28 ^k	0.28 ^d
Sorghum BD-729	20.0 ^k	9.99 ⁱ	0.33 ^d
Sorghum BD-730	0.41 ⁿ	12.44 ^h	18.67 ^a
Sorghum BD-731	59.80 ^d	19.93 ^e	9.96 ^c
Sorghum BD-733	0.33 ⁿ	9.96 ⁱ	0.33 ^d
Sorghum BD-735	0.33 ⁿ	0.33 ^k	0.33 ^d
Sorghum BD-736	19.93 ^k	9.96 ⁱ	0.33 ^d
Sorghum BD-737	0.28 ⁿ	8.31 ^j	0.28 ^d
Sorghum BD-738	19.93 ^k	9.96 ⁱ	0.33 ^d
Sorghum BD-739	0.33 ⁿ	39.87 ^a	0.33 ^d
Sorghum BD-740	16.62 ^l	0.28 ^k	0.28 ^d
LSD (5%)	0.418	0.409	0.337
CV (%)	0.82	1.87	11.85

Means bearing the dissimilar letter within the column differ significantly.

However, the finding of the present study suggests that the germination for all germplasms affected by applied different type of salinity levels. Genotypes such as Hybrid Sorgo, Sorghum BD-727, Sorghum BD-701, Sorghum BD-739, Sorghum BD-731, and Sorghum BD-730 were consistently tolerant, showing higher values across multiple traits.

4. DISCUSSION

Sorghum (*Sorghum bicolor* L. Moench), ranked as the 5th most important cereal globally after wheat, rice, maize, and barley, is well-recognized for its ability to thrive under drought-prone and marginal conditions, especially in semi-arid and arid environments (ICRISAT, 2019). However, its cultivation in Bangladesh has declined due to the dominance of other high-yielding cereals, cash crops and salinity. Given its tolerance to abiotic stresses, especially salinity and drought, Sorghum presents a promising option for diversification in the saline-prone southern and coastal belts of Bangladesh. In the present study, 35 Sorghum germplasms were evaluated under varying salinity conditions (6, 12, and 18 dS m⁻¹) to assess their germination potential and early seedling vigor. The analysis of variance (Table 1) revealed significant differences ($P < 0.001$) among germplasm, salinity treatments, and their interactions for all studied traits (final germination percentage, root length, shoot length, GSI, RLSI, and SLSI). This confirms both genotypic variability and differential response to salinity stress across genotypes.

4.1 Germination Stress Tolerance Index (GSI)

The GSI significantly declined with increasing salinity levels, indicating the strong inhibitory effect of salt stress on seed germination. This is in line with earlier findings by researcher who reported a consistent reduction in

GSI with increased NaCl concentrations in *Sorghum bicolor* (Kausar, 2012). At 6 dS m⁻¹, germplasm such as Sorghum BD-713 (98.94) and Sorghum BD-737 (96.54) produced the highest GSI, suggesting their relative tolerance during germination. At more severe levels (12 and 18 dS m⁻¹), Hybrid Sorgo and Safal 999 outperformed others, maintaining germination under high salinity (Figure 1). These results suggest these lines have better osmotic adjustment or ionic tolerance during seed imbibition and early radicle emergence stages (date is mental). In contrast, genotypes such as Sorghum BD-704, Sorghum BD-705, Sorghum BD-700, and Hybrid Sudan Grass showed zero GSI at 18 dS m⁻¹, indicating complete failure to germinate under extreme salt conditions. The Sorghum BD-705, Sorghum BD-700 range of GSI values across genotypes underlines the necessity of screening genetic materials before introducing them into stress-prone environments (Ashraf, 2003). Similar type of result was found by (Ghoulam and Fares, 2001).

4.2 Root and Shoot Length Stress Tolerance Indices (RLSI and SLSI)

Root and shoot lengths were significantly reduced with salinity stress. Root length is often more tolerant than shoot under salt stress due to its protective osmotic and anatomical adaptations (Munns and Tester, 2008). In this study, root length at control was the highest in Sorghum BD-707 (29.9 mm) and the lowest in Sorghum BD-710 (9.83 mm). At 6 dS m⁻¹, Sorghum BD-733 showed the highest Sorghum BD-710 (82.35), while Sorghum BD-712 was the least (13.32). At higher salinity (18 dS m⁻¹), only a few germplasms, like Sorghum BD-727 (13.04), maintained some root elongation, indicating the critical nature of salt tolerance for root development (Figure 2). The results are in line with the findings of Munns et al., 2006. On the other hand, shoot length stress index (SLTI) values were more drastically affected than roots, with many genotypes showing SLTI values below 1.0 under severe salt conditions (Table 2). Only

Sorghum BD-727 (12.50), Sorghum BD-730 (18.67), and Sorghum BD-731 (9.96) showed moderate shoot elongation at 18 dS m⁻¹, indicating that shoot tissues are more sensitive Sorghum BD-727, Sorghum BD-730 and Sorghum BD-731 to salt-induced osmotic and ion toxicity than roots. These findings align with earlier work by who reported a greater reduction in shoot biomass than root under saline stress in Sorghum due to ionic imbalances and membrane instability. The results of present study are agreed with (Ali et al., 2012; Ghoulam and Fares, 2001).

5. CONCLUSION

The present study suggests that the germination for all germplasm affected by applied different type of salinity levels. It may be concluded from the finding of the study that the stress indices (GSI, RSTI, and SLTI) consistently declined with increasing salinity. Significant differences among genotypes suggest genetic variation in salt stress responses, especially in root and shoot development. Genotypes such as Hybrid Sorgho, Sorghum BD-727, Sorghum BD-701, Sorghum BD-739, Sorghum BD-731, and Sorghum BD-730 were consistently tolerant, showing Sorghum BD-727, Sorghum BD-701, Sorghum BD-739, Sorghum BD-731 and Sorghum BD-730 higher values across multiple traits.

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