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Effect of salt stress on growth of sorghum germplasms at vegetative stage

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Abstract

Soil salinity is an increasing problem in the world and main obstacle to agricultural productivity especially in areas where irrigation is necessary. It adversely affects plant growth and development. Adoption of salt tolerant variety is more important here and so screening of salt tolerant germplasms is essential. For this reason, a germination test under salinity and a hydroponic experiment were conducted at Plant Physiology Laboratory, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh to investigate the effect of NaCl on germination, morphological characters and growth of sorghum seedlings. The experiment comprised of two levels of NaCl concentration *viz.* Control (without NaCl and EC 1.36 dSm^{-1} in hydroponic culture) and 80 mM NaCl and five sorghum germplasms *viz.* BD 700, BD 703, BD 707, BD 710 and BD 726. There were 10 (5×2) treatments in a completely randomized design (CRD) with three replications. Results showed that germination percentage, hypocotyls and epicotyls length, root and shoot length, leaf length, leaf sheath length, fresh and dry mass production were influenced by NaCl stress. However, among tested germplasms, BD 707 showed the best performance considering the seedlings growth, germination percentage and other parameters. BD 710 and BD 703 showed the highest sensitivity to NaCl stress based on the above parameters studied. These results suggested that sorghum is able to grow with moderate salinity.

Introduction

Sorghum (*Sorghum bicolor* L.) is the fourth most important cereal crop grown in the world. Sorghum is grown on approximately 44 million hectares in 99 countries. In Bangladesh, 254 tons of sorghum grains are produced annually from about 187 ha of land with an average of 1.36 tons per hectare (FAOSTAT, 2013). Sorghum has potential uses such as: food (grain), feed (grain and biomass), fuel (ethanol production), fiber (paper), fermentation (methane production) and fertilizer (utilization of organic byproducts). In India, during rainy season sorghum grain is used mostly for animal/poultry feed while in post rainy season sorghum grain is used primarily for human consumption. Moreover, sorghum has the biological nitrification inhibition capacity (Hossain *et al.,* 2008) which can reduce the use of nitrogen fertilizer. Sorghum is a principal source of energy, protein, vitamins and minerals for millions of the poorest people in the semi arid regions (Khaton *et al*., 2016). The protein content of sorghum (11.3%) is nearly equal and is comparable to that of wheat and maize. Average starch content of the seeds range from 56 to 73% and is relatively rich in iron, phosphorous and vitamin B-complex (ICRISAT, 2013).

Salinity is one of the major abiotic stresses in agriculture worldwide, limiting crop productivity (Munns and Tester, 2008). Globally, a total land area of 831 million hectares is affected by salinity (Turkan and Demiral, 2009; Munns, 2005). Salt accumulation is mainly related to a dry climate, salt rich parent materials of soil formation, insufficient drainage and saline groundwater or irrigation water (Almodares *et al.,* 2008). Salts in soils are chlorides and sulfates of sodium, calcium, magnesium, and potassium. Among them sodium chloride has the highest negative effect on the plant growth and development that results in severe agricultural losses (Ashraf and Wahed, 1993). The adverse physiological effects may be attributed to unavailability to water, reduction in photosynthesis through loss of turgidity, impeded nutrient uptake causing deficiency and ion toxicity to plants (Niu *et al.,* 2012; Munns and Tester, 2008; Netondo *et al.,* 2004a, 2004b). Salt stress may also impair synthesis of biochemical substances such as enzymes, sugars and protein (Singh *et al.,* 2001). During salinity stress, decrease in K^+ and Ca^{2+} and accumulation of Na^+ and Mg^{2+} ions in both roots and shoots occurs in plant body (Farooq *et al*., 2015; Yasmeen *et al.,* 2013) causing reduction in dry matter accumulation and grain yield (Flowers, 2004).

Salinity is a common problem of Bangladesh. In Bangladesh about 1.5 million hectares out of 2.85 million hectares of coastal and offshore area is affected by different degrees of salinity which is equivalent to 53% of the cultivable land in 13 districts of the south-east part. Agricultural land uses in these areas are very poor and cropping intensity is very low (62% only) where the country average cropping intensity is 190%

(MoA, 2007). The impact of such salinity severely affects the economy of Bangladesh. So, finding out possible solutions is essential in order to alleviate the adverse effects of salinity for crop production.

One of the strategies to face the challenges is to introduce new but tolerant crops. One such crop could be Sorghum. Because in terms of salinity it is a moderately salt tolerant crop with the tolerance of 6-8 dSm^{-1} (Greenway & Munns, 1980) that can also produce high biomass yields with salinity than other crops. Among cereals, rice is the staple food of the people of Bangladesh. Rice is moderately salt sensitive crop species (Mass and Hoffman, 1977). It has been reported that rice at initial level of salinity (4 dSm^{-1}) might give normal straw yield (Venkateswarlu *et al.,* 1970). Rice or other crops cannot be grown in many salt affected areas where sorghum cultivation may become a solution. Hence to reduce the risk of loss from salinity superior genetic resources with improved tolerance to salinity are essential and it requires the identification of easily measureable traits related to salinity tolerance.

The study for identifying traits of saline tolerance is very scanty and the physiological mechanisms that mediate tolerance are poorly understood. So the present study is aimed at evaluating the effect of NaCl salinity on sorghum germplasms based on their germination and seedling growth.

Materials and Methods

A germination test using petri dish and a hydroponic experiment were conducted at Plant Physiology Laboratory, Department of Crop Botany, BAU, Mymensingh. Five germplasms of sorghum viz. BD 700, BD 703, BD 707, BD 710 and BD 726 collected from Bangladesh Agricultural Research Institute (BARI) were used as experimental materials. In this research, petri dishes were used for germination experiment and plastic pots (8L) were used for hydroponics experiment. Cleaned and dried petri dishes were taken with filter papers. The black colored pots were taken with Hogland's full nutrient solutions and an aeration pump was attached for supplying oxygen to the nutrient medium.

The two factorial experiments were laid out in Completely Randomized Design (CRD) with three replications. Treatment combinations were five varieties and two levels of salt concentration. The germplasms of sorghum were imposed into two levels of salt stress *viz.* control (without NaCl) and salt stress (80mM NaCl). Each treatment was received equal amount of macro and micro nutrients. Thus, in this study 30 (5×2×3) pots were used. The individual pot size was 8L. All the experiments were conducted in a growth room at 25°C under a 12h light and 12h dark regime, 70% relative humidity and pH 6.5.

The seeds were washed and soaked with distilled water and kept in refrigerator for 24 hrs. Fifteen imbibed seeds were placed for germination on filter papers in each petri dish and germinated seeds were placed in net with water for hydroponic experimental set up. One-week-old seedlings were transferred to continuously aerated nutrient solution (Hogland's solution) in 8L plastic pot on Styrofoam blocks with 4 holes and three plants per hole, supported with sponge. In petri dish 2 ml NaCl solution of 80mMconcentrations was given to the seeds and in hydroponics experiments salt concentration was incorporated directly. The composition of the nutrient solution was 500 µM NH_4NO_3, 500µM $Ca(NO_3)_2$, 200µM $MgSO_4$, 100µM KH_2P0_4, 2µM $FeC1_3$, 11 µM H_2B0_3, 2µM $MnC1_2$, 0.35µM $ZnC1_2$, 0.2µM $CuCl_2$, 0.1 µM $(NH_4)_6 Mo_70_4$. After imposition of nutrient solution EC of the culture solution became 1.36 dSm^{-1}.

Seed germination and growth were frequently observed and pH of the culture solutions was closely monitored at 6.5 and adjusted at two days interval. The nutrient solution was renewed with fresh nutrient solutions at 5 days interval. Data of hypocotyls length, epicotyls length and germination percentage were collected at 4, 6, and 8 DAS in germination experiment and root length, shoot length, leaf length, leaf sheath length, fresh weight and dry weight were collected at 10, 15 and 20 DAS in hydroponics experiment.

Data were statistically analyzed for analyses of variance (ANOVA) using the M−STAT C software in accordance with the principles of Completely Randomized Design. Duncan's Multiple Range Test (DMRT) was used to compare variations among the treatments.

Results

Performance of sorghum genotypes under artificial salt stress at germination

Germination percentage

The interaction effect of variety and NaCl level on germination percentage at 2, 6 and 8 DAS was found significant (Fig. 1). The highest germination percentage was recorded in BD 707 at control at all growth stages (88.53%) and the lowest germination percentage was observed in BD 710 (55.35%) and BD 703 (62.23%) under salt stress at all growth stages.

Length of hypocotyls

Sorghum genotypes showed significant variation on hypocotyls length against application of NaCl (Table 1). The longest hypocotyls length was found in BD 707 at control (4.80, 5.70 and 6.60 cm for 4, 6 and 8 DAS, respectively) followed by BD 700 at control (4.70, 5.40 and 6.20 cm for 4, 6 and 8 DAS, respectively). On the other hand, the shortest hypocotyls were observed in BD 710 under salt stress (1.80, 2.20 and 2.50 cm for 4, 6 and 8 DAS, respectively).

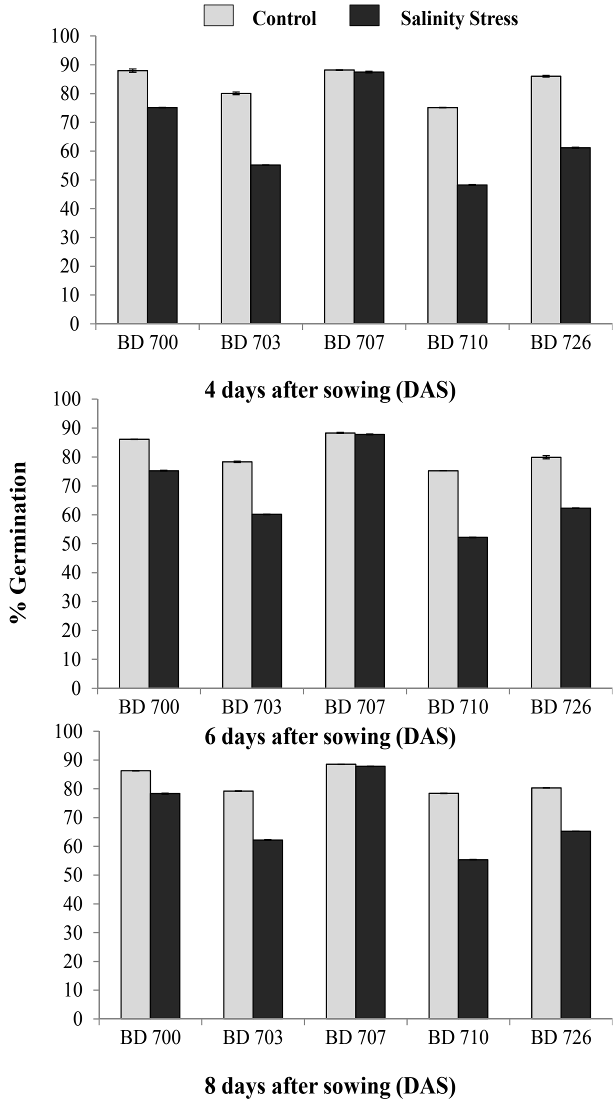

Fig. 1. Effect of salinity on germination percentage of 5 sorghum germplasms grown on petri dishes. Bar represents standard error of mean (n=3).

Length of epicotyls

The epicotyls lengths of sorghum genotypes varied significantly due the imposition of salt (NaCl) (Table 1). At 8 DAS, the longest epicotyls were recorded in BD 707 at control (4.20 cm) and the shortest was recorded in BD 710 under salt stress (2.20 cm).

Effect of salt stress on sorghum genotypes grown in hydroponic

Root length

Significant variation was found in root lengths when NaCl artificially imposed in hydroponic culture, growing media of 5 sorghum genotypes (Table 2). After 20 days of culture, the highest root length was observed in BD 707 at control (14.70 cm) and the lowest was recorded in BD 710 under salt stress (8.60 cm).

Shoot length

The shoot length at 10, 15 and 20 DAS was significantly affected by application of artificial salinity imposition (NaCl) on different genotypes of sorghum in hydroponic system (Table 2). At 20 DAS, the highest shoot length was recorded in BD 707 at control (7.20 cm). The lowest shoot length was recorded in BD 710 under salt stress (4.70 cm).

Leaf blade length

Leaf blade length of different sorghum genotypes varied significantly due to artificial salinity stress condition in hydroponic culture (Table 3). The longest leaf blade was recorded in BD 707 at control at 20 DAS (12.20 cm). On the other hand, the shortest leaf blade was observed in BD 710 under salt stress (9.20 cm at 20 DAS).

Leaf sheath length

The interaction effect of variety and NaCl level on leaf sheath length at 10, 15 and 20 DAS was significant (Table 3). The longest leaf length was recorded in BD 707 with control at 20 DAS (4.80 cm). On the other hand, the shortest leaf length was observed in BD 710 under salt stress (2.40 cm at 20 DAS).

Table 1. Effect of salinity on hypocotyls and epicotyls length of sorghum germplasms at germination test

Genotypes	Salinity Treatment	Hypocotyls length (cm)			Epicotyls length (cm)		
		4 DAS	6 DAS	8 DAS	4 DAS	6 DAS	8 DAS
BD 700	Control	4.70a	5.40b	6.20b	2.60b	2.90b	3.80b
	Stress	3.00f	4.00e	4.60d	2.10d	2.30d	3.20e
BD 703	Control	3.80c	4.40d	4.90d	1.90e	2.60c	3.10f
	Stress	2.50h	3.10g	3.60e	1.20g	1.90f	2.30h
BD 707	Control	4.80a	5.70a	6.60a	2.80a	3.20a	4.20a
	Stress	3.40d	4.10e	4.90d	2.30c	2.79b	3.50d
BD 710	Control	3.20e	3.30f	3.80e	1.80ef	2.30d	2.90g
	Stress	1.80i	2.20h	2.50f	1.10g	1.70g	2.20i
BD 726	Control	4.30b	5.10c	5.80c	2.30c	2.80b	3.60c
	Stress	2.80g	3.40f	3.90e	1.70f	2.10e	2.90g
Significance		*	**	*	*	*	*

** = Significant at 1% level of probability; * = Significant at 5% level of probability

Table 2. Variation of root length and shoot length of sorghum genotypes grown with artificial salt stress condition in hydroponic culture

Genotypes	Salinity Treatment	Root length (cm)			Shoot length (cm)		
		10 DAS	15 DAS	20 DAS	10 DAS	15 DAS	20 DAS
BD 700	Control	7.13b	9.30b	12.30b	4.30c	5.20c	6.10c
	Stress	6.90bcd	8.90bc	10.30c	3.90e	4.60e	5.50d
BD 703	Control	6.80bcde	8.80bcd	11.60b	3.90e	4.50e	5.50d
	Stress	6.50def	8.40cd	9.10de	3.50h	4.10g	5.30d
BD 707	Control	8.20a	11.20a	14.70a	4.90a	5.80a	7.20a
	Stress	7.00bc	10.90a	14.10a	4.60b	5.60b	6.70b
BD 710	Control	6.40ef	8.70bcd	10.10c	3.60g	4.30f	5.40d
	Stress	6.20f	8.20d	8.60e	3.10i	3.90h	4.70e
BD 726	Control	6.90bcd	8.90bc	11.80b	4.20d	4.90d	6.00c
	Stress	6.60cdef	8.50cd	9.70cd	3.70f	4.50e	5.30d
Significance		**	*	*	*	*	*

** = Significant at 1% level of probability; * = Significant at 5% level of probability

Table 3. Effect of salinity on leaf length and leaf sheath length of different sorghum genotypes in hydroponic culture

Genotypes	Salinity Treatment	Leaf blade length (cm)			Leaf sheath length (cm)		
		10 DAS	15 DAS	20 DAS	10 DAS	15 DAS	20 DAS
BD 700	Control	8.20bc	10.30ab	11.90ab	2.30ab	3.50ab	4.40b
	Stress	7.70e	9.50c	11.20c	1.80d	2.50f	3.40e
BD 703	Control	7.90d	9.20cd	10.70d	1.90d	2.90de	3.70d
	Stress	7.50f	8.80d	9.600e	1.50e	2.10g	2.90g
BD 707	Control	8.50a	10.50a	12.20a	2.40a	3.70a	4.80a
	Stress	8.30b	10.20ab	11.50bc	2.20bc	3.30bc	4.20c
BD 710	Control	6.90g	8.20e	9.60e	1.80d	2.70ef	3.10f
	Stress	6.40h	7.30f	9.20e	1.20f	1.70h	2.40h
BD 726	Control	8.10c	10.00b	11.80ab	2.10c	3.10cd	3.70d
	Stress	7.60ef	9.00d	10.40d	1.60e	2.20g	2.90g
Significance		*	*	*	**	**	*

** = Significant at 1% level of probability; * = Significant at 5% level of probability

Fresh weight

The sorghum variety showed significant difference in case of fresh weight plant^{-1} under 80mM NaCl level at 10, 15 and 20 DAS (Fig. 2). The highest fresh weight plant^{-1}was recorded in treatment combination of BD 707 with control at 10, 15 and 20 DAS (130.3, 390.6, and 497.7 mg plant^{-1}). On the other hand, the lowest fresh weight was observed in BD 710 under salt stress (50, 90.67 and 141.5 mg plant^{-1} for 10, 15 and 20 DAS, respectively).

Dry weight

The interaction effect of variety and NaCl level on dry weight plant^{-1} at 10, 15 and 20 DAS was significant (Fig. 2). The highest dry weight plant^{-1} was recorded in BD 707 with control both at 10, 15 and 20 DAS (35.2, 95.2 and 175.9 mg plant^{-1}). On the other hand, the lowest dry weight was observed in BD 710 under salt stress (5.13, 48.3 and 80.8 mg plant^{-1} for 10, 15 and 20 DAS, respectively). But the dry weight of BD 703 was 96.2 mg plant^{-1} at 20 DAS. Again the reduction of dry mass production under NaCl was minimum in BD 707. On the other hand, the maximum reduction in dry mass production was observed in BD 710 and BD 703 indicating these two genotypes was more susceptible to NaCl toxicity than the other genotypes in sorghum.

Discussion

In this experiment, it has been found that about all the parameters were reduced due to salinity stress. Reduction in germination percentage, epicotyls and hypocotyls length in response to the stress might be due to the reduction of water taken up by the seeds or toxicity of Na and Cl ions. Generally, germination velocity is greatly retarded by salinity which causes a significantly high reduction in final germination percentage under salt stress condition even after seven days of incubation (Sagar, 2017). These results are in agreement with the results of the previous researches that salinity may affect seed germination by decreasing the ease with which water may be taken up by the seeds or toxicity of Na and Cl ions thus germination process delayed and/or proceed at reduced rates (Kazemi and Eskandari, 2011) and even in case of salt-tolerant plants (Khan and Ahmad, 1998).

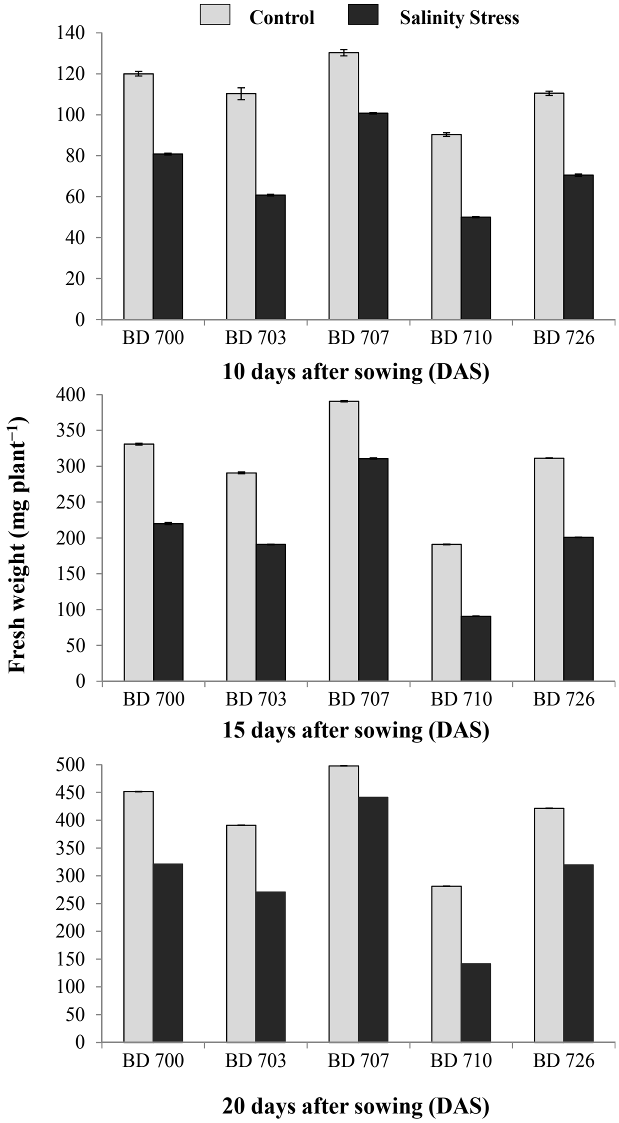

Fig. 2. Variation of fresh weight plant^{-1} of sorghum varieties at different salinity level at different DAS. Bar represents standard error of mean (n=3).

Salt stress influences seed germination primarily by sufficiently lowering the osmotic potential of the soil solution to impede water absorption by seeds. It may cause sodium and/or chloride toxicity to the embryo or alter protein synthesis. Hyper-osmotic stress and toxic effects of sodium and chloride ions on germinating seeds in a saline environment may delay or inhibit germination (Farsiani and Ghobadi, 2009).

Again poor seedling emergence might be caused by hypocotyls mortality associated with the salts accumulation at the germinating surface (Sun *et al.*, 2014; Sagar, 2017). It has a great impact on root and shoot growth.

Besides this, reduction in shoot growth might be due to the suppression of leaf initiation and expansion as well as internode growth and by accelerating leaf abscission (Qu *et al.*, 2012; Sagar, 2017) resulting in reduction in leaf blade and sheath length. Root growth was also affected by salinity. It might be due to accumulation of ethylene which inhibits the root and shoot growth (Sagar, 2017). Again, due to the osmotic stress, low water availability to plant occur which retards cell division and elongation by lowering turgor pressure as well as cell growth resulting in reduction in biomass as well as reduction in dry mass (Farooq *et al.*, 2015; Sagar, 2017).

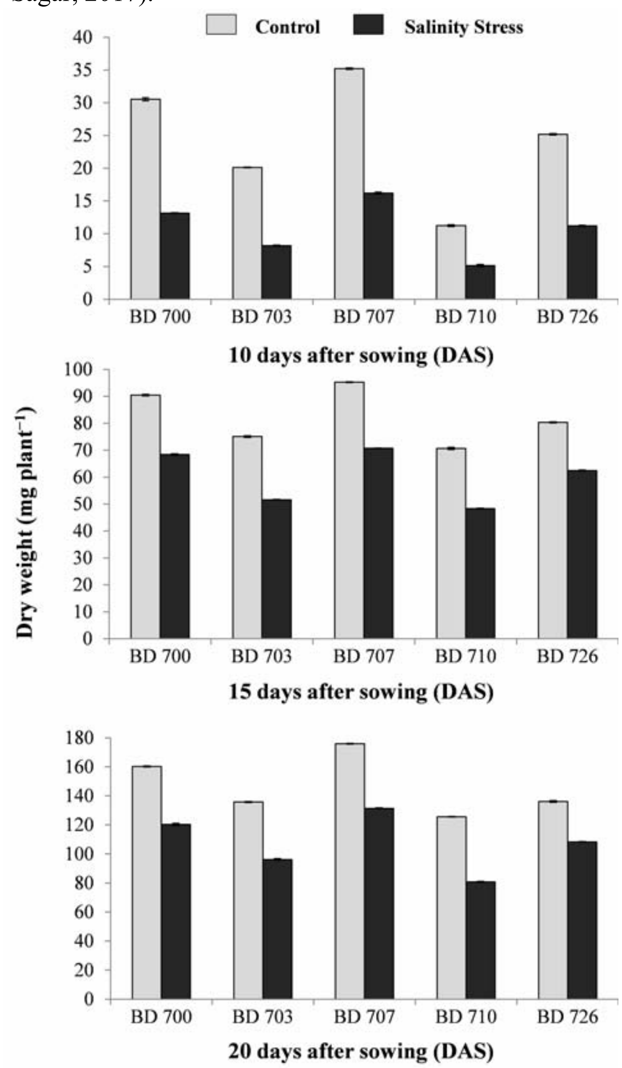

Fig. 3. Effect of salinity level on sorghum varieties considering dry weight per plant at different DAS. Bar represents standard error of mean (n=3).

Conclusion

From the present research work, it can be concluded that percent germination, hypocotyls and epicotyls length of all the varieties were reduced under increased salinity stress in germination experiment. There was also a significant effect of salinity on root and shoot length, leaf length, leaf sheath length, fresh and dry weight in hydroponics experiment. So, all the studied sorghum germplasms were variably affected in germination, growth and development of seedlings by 80 mM NaCl of salinity. In hydroponic culture, BD 707 was more tolerant at 80 mM NaCl of salinity than the other varieties in respect of growth.

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