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Effect of salinity on seed germination and seedlings growth of sorghum (*Sorghum bicolor* l.)

Manobendra Nath Sarkar[1], AKM Zakir Hossain[1], Shahanara Begum[1], Syed Nazrul Islam[2], Sanjoy Kumar Biswas[3] and Md. Zablul Tareq[3]

[1]Dept. of Crop Botany, Bangladesh Agricultural University, Mymensingh, Bangladesh
[2]Jute Research Sub Station, Bangladesh Jute Research Institute, Monirumpur, Jessore, Bangladesh
[3]Jute Agriculture Experimental Station, Bangladesh Jute Research Institute, Jagir, Manikganj, Bangladesh

✉ For any information: ask.author@journalbinet.com
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ABSTRACT

A laboratory experiment was conducted to investigate salt tolerant sorghum genotypes based on morphological characters of nine sorghum genotypes during germination and seedling growth. The treatments were consisted of 0mM and 100mM NaCl and nine genotypes were BD 686, BD 687, BD 693, BD 699, BD 700, BD 747, BD 748, BD 750, BD 753. The experiment was laid out in a CRD with 3 replications. The plants were grown hydroponically in the plant physiology laboratory, Department of Crop Botany, BAU, Mymensingh. Under salinity stressed (0 and 100 mMNaCl) condition, tolerant genotypes BD 750 and BD 686 showed higher germination percentage, different stress tolerance index (GSTI, SLSI, RLSI,FSTI and DSTI), shoot and root length, fresh weight , dry weight, leaf area (LA), vigor index than those in sensitive genotype BD 747 and BD 753. The underlying mechanism to these changes was described in details.

Key Words: *Salinity, Seed germination, Seedling growth and Sorghum*

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I. Introduction

Sorghum (*Sorghum bicolor* L.) is the world's fifth most important cereal crop after *Oryza* spp. (rice), *Triticum* spp. (wheat), *Zea mays* L. (maize), and *Hordeum vulgare* L. (barley) (FAO, 2015). Sorghum grain is used mainly for human consumption in Asia and Africa while it is used as animal feed in the America, China and Australia. 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), thus it is an important crop in semi-arid and arid regions of the world. In India, the rainy season sorghum grain is used mostly for animal/poultry feed while the post rainy season sorghum grain is used primarily for human consumption. In Bangladesh, 254 tons of sorghum grains are produced annually from about 187 ha of land average yield is 1.36 tons per hectare (FAOSTAT, 2013). In Bangladesh about 1.5 million hectares out of 2.85 million hectares of

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coastal and offshore area is affected by different degrees of salinity which is equivalent to that constitute about 53% of the cultivable area in 13 districts of the South-east part.

Sorghum is a crop of world-wide importance and is unique in its ability to produce under a wide array of harsh environmental condition (House, 1995). Among agricultural crops, sorghum is naturally drought and salt-tolerant crop that can produce high biomass yields with low input. Also, it can thrive in places that do not support corn, sugarcane and other food crops. In terms of salinity tolerance degree it also be known as a moderately tolerant Crop with the almost salinity tolerance of 6.8 dSm^{-1} (Greenway and Munns, 1980). This research work was the attempt to evaluate salinity tolerance of different sorghum varieties in Bangladesh. Thus, to provide more information about variation in salinity tolerance and to improve crop productivity on salt affected lands of the country, the relative salt tolerance of nine sorghum genotypes was determined at germination and seedling stage based on morphological parameters in hydroponic culture. From the above facts the experiment was designed to investigate the effect of salinity on germination of seed and morphological characteristics of sorghum seedlings.

II. Materials and Methods

Plant materials and growth condition: Nine sorghum genotypes with diversified genetic background were used in the experiment. These Germplasm were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur. The following genotypes were used in the experiment (Table 01).

Table 01. List of genotypes used for study

Sl	Genotypes	Symbol	Origin	Sl	Genotypes	Symbol	Origin
1.	BD 686	V_1	BARI	6.	BD 747	V_6	BARI
2.	BD 687	V_2	BARI	7.	BD 748	V_7	BARI
3.	BD 693	V_3	BARI	8.	BD 750	V_8	BARI
4.	BD 699	V_4	BARI	9.	BD 753	V_9	BARI
5.	BD 700	V_5	BARI				

Sorghum seeds were sterilized with 5% sodium hypochlorite for 30 min. and washed thoroughly with distilled water. These seeds were then the soaked in water and were imbibed for 24 hours and then seeds were placed in Petri dishes containing filter paper to allow them to germinate. Each Petri dish contained 10 sorghum seeds. In case of control (T_1) 4 ml of distilled water was added to the Petri dish. Filter papers were moistened with 4ml of respective salt solutions to develop the respective level of salinity stress (T_2: 100 mM) treatments. Each treatment was performed three times. The sorghum seeds were allowed to germinate at around 25°C.

Treatments: The treatments were consisted of 0 and 100mM NaCl and nine sorghum genotypes (Table 01). The experimental petridishes were laid out in Completely Randomized Design (CRD) with two factors using three replications, giving a total of 54 petridishes.

Germination percentage: The number of sprouted seeds was counted daily commencing from 1st day to 7th day. After 7th day, final count was done and Germination Percentage (GP) of final day was calculated by the following formula:

Percent germination (PG) = (Total no. of seeds germinated)/ (Total no. of seeds taken for germination) ×100

Shoot and root length: Shoot and root length of all sprouting from each replication were measured 7th day. Shoot length was measured from shoot base to the tip of the longest leaf and root length was measured from root base to the root tip. Different physiological indices like Germination Stress Tolerance index (GSTI), Root Length Stress Tolerance Index (RLSI), Plant Height Stress Tolerance Index (PHSI), Shoot Fresh Weight Stress Tolerance Index (SFSI), Root Fresh Weight Stress Tolerance Index (RFSI), Shoot Dry Weight Stress Tolerance Index (SDSI) and Root Dry Weight Stress Tolerance Index (RDSI) were estimated according to Ashraf *et al.* (2008).

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Statistical analysis: The collected data on various parameters under study were statistically analyzed. Analysis of variance was calculated using the computer software program MSTATC (Gomez and Gomez, 1984). The mean differences were evaluated by Duncan's Multiple Range Test.

III. Results

Germination percentage

Germination percentage showed significant differences among the genotypes due to salinity (Table 02). The highest percentage of germination was recorded in BD 750 (93%). In contrast, BD 700 had the lowest germination percentage (65.5%). Results revealed that germination percent was decreased with increasing salinity levels. The highest germination was recorded in control (82.22%) and the lowest was recorded at 100 mM (74.4%). Result revealed that the decrement of germination due to combined effects of salinity and genotypes was less in BD 750 (Table 03). The highest germination percentage observed in BD 750 (95%) at control and in treatment (91%). The lowest value showed by BD 700 (60%) at 100 mM salinity.

Germination stress tolerance index (GSTI)

Like germination percentage, GSTI was also significantly varied among sorghum genotypes P ≤ 0.01 (Table 02). BD 750 exhibited maximum GSTI values (97%) and BD 700 showed minimum GSTI values (80%) followed by BD 699 (80.50%). GSTI showed significant variation due to salinity (Table 02). The highest GSTI value was obtained at control (100.0%) and lowest at 100 mM (75.33%). Germination stress tolerance index (GSTI) was significantly reduced by the combined effects of genotypes and salinity. In each case, GSTI was 100 in control condition. But the maximum GSTI value was recorded in BD 750 at 100 mM (94.0%) and minimum GSTI was recorded in BD 700 (60%) at 100 mM (Table 03).

Table 02. Effects of genotypes and salinity levels on germination and salt stress indices

Genotypes (G)	Germination percentage	GSTI	Root length (cm)	RLSI	Shoot length (cm)	SLSI
BD 686	87.50b	93.50b	6.30c	88.76b	8.95b	87.76b
BD 687	79.00d	89.00d	5.15d	83.08d	7.20d	83.73d
BD 693	85.50c	92.50c	7.95a	87.38c	8.60c	86.88c
BD 699	67.50h	80.50g	3.05h	74.33f	3.15g	73.19g
BD 700	65.50i	80.00g	2.35i	73.33g	2.95g	71.87h
BD 747	74.00g	84.00f	3.50g	77.76e	4.05f	76.39f
BD 748	75.50f	84.50f	3.98f	78.59e	5.65e	79.58e
BD 750	93.00a	97.00a	6.75b	92.50a	11.15a	91.41a
BD 753	77.50e	88.00e	4.45e	82.42d	5.85e	80.14e
LSD	1.171	0.828	0.192	0.996	0.228	0.681
Level of sig.	**	**	**	**	**	**
Salinity level						
0 mM	82.22a	100.00a	5.77a	100.00ab	7.67a	100.00a
100 mM	74.44b	75.33b	3.89b	64.04b	5.12b	62.43b
LSD	0.552	0.390	0.091	0.469	0.18	0.393
Level of sig.	**	**	**	**	**	**

In a column, within either genotype or salinity, figures having similar letter(s) do not differ significantly at 5% level of probability. ** = Significant at 1% level of probability, * = Significant at 5% level of probability

Root length and root length stress tolerance index (RLSI)

The variation in root length and root length stress tolerance index among the studied genotypes was statistically significant at P ≤ 0.01 (Table 02). The highest root length was recorded in BD 750 (7.95 cm). In contrast, BD 700 had the shortest root length (2.35 cm). Again in case of RLSI, the highest tolerance index was shown by BD 750 (92.5%) and the lowest tolerance was shown by BD 700 (73.33%).

The effect of salinity on root length and RLSI was found statistically significant (Table 02). Result revealed that root length and RLSI was decreased with increasing salinity levels. The longest root

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length was recorded in control (5.77 cm) and the lowest was recorded in 100 Mm (3.89 cm). In case of RLSI, no reduction occurred in control condition and highest reduction observed at 100 mM (64.04%).

Table 03. Combined Effects of genotypes and salinity level on morphological characteristics based on germination

T × G	Germination percentage	GSTI	Root length (cm)	RLSI	Shoot length (cm)	SLSI
T_1V_1	90.00b	100.00a	7.10b	100.00a	10.20b	100.00a
T_1V_2	81.00fg	100.00a	6.20d	100.00a	8.60c	100.00a
T_1V_3	88.00c	100.00a	9.10a	100.00a	9.90b	100.00a
T_1V_4	73.00j	100.00a	4.10h	100.00a	4.30h	100.00a
T_1V_5	71.00k	100.00a	3.20j	100.00a	4.10h	100.00a
T_1V_6	80.00g	100.00a	4.50g	100.00a	5.30g	100.00a
T_1V_7	82.00ef	100.00a	5.07f	100.00a	7.10e	100.00a
T_1V_8	95.00a	100.00a	7.30b	100.00a	12.20a	100.00a
T_1V_9	80.00g	100.00a	5.40e	100.00a	7.30e	100.00a
T_2V_1	85.00d	87.00c	5.50e	77.52c	7.70d	75.52c
T_2V_2	77.00h	78.00e	4.10h	66.16e	5.80f	67.46e
T_2V_3	83.00e	85.00d	6.80c	74.76d	7.30e	73.76d
T_2V_4	62.00m	61.00h	2.00m	48.67h	2.00j	46.39h
T_2V_5	60.00n	60.00h	1.50n	46.66i	1.80j	43.74i
T_2V_6	68.00l	68.00g	2.50l	55.52g	2.80i	52.79g
T_2V_7	69.00l	69.00g	2.90k	57.19f	4.20h	59.15f
T_2V_8	91.00b	94.00b	6.20d	85.01b	10.10b	82.81b
T_2V_9	75.00i	76.00f	3.50i	64.84e	4.40h	60.27f
LSD	1.656	1.171	0.272	1.408	0.323s	1.179
Level of sig.	**	**	**	**	**	**
CV %	*1.28*	*0.81*	*3.41*	*1.04*	*3.05*	*0.88*

In a column, within either genotype or salinity, figures having similar letter(s) do not differ significantly at 5% level of probability. ** = Significant at 1% level of probability, * = Significant at 5% level of probability
Here, "V" represents the Variety or Genotypes, V_1= BD 686; V_2= BD 687; V_3= BD 693; V_4= BD 699; V_5= BD700; V_6= BD 747; V_7= BD 748; V_8= BD 750; V_9= BD 753. T_1= 0 mM (control); T_2= 100 mMNaCl concentration.

The interaction between salinity levels and genotypes at germination stage had a significant effect on root length and RLSI (Table 03). Results revealed that root length was less affected in BD 693 (9.1 cm) at control and lowest root length found in BD 700 (1.5 cm) at 100 mM. In each case RLSI was 100 in control condition. But the maximum RLSI value was recorded in BD 750 (85.01%) at 100 mM and like root length minimum RLSI was recorded in BD 700 (46.66%) at 100 mM salinity.

Shoot length and shoot length stress tolerance index (SLSI)

The variation in root length and root length stress tolerance index among the studied genotypes was statistically significant at P ≤ 0.01 (Table 02). The highest shoot length was recorded in BD 750 (11.15 cm) and BD 700 had the shortest shoot length (2.95 cm). Again in case of SLSI, the highest tolerance index was shown by BD 750 (91.41%) and the lowest tolerance was shown by BD 700 (71.87%). The effects of salinity on shoot length and SLSI was found statistically significant (Table 02). Result revealed that shoot length was decreased with addition of salinity. The longest shoot length was recorded in control (7.67 cm) and the lowest was recorded in 100 mM (5.12 cm). In case of SLSI, no reduction occurred in control condition while 100 mM exhibited the highest reduction (62.43 cm).

The interaction between salinity levels and genotypes at germination stage had a significant effect on shoot length and SLSI (Table 03). Results revealed that maximum shoot length recorded in BD 750 (12.20 cm) in the controlled condition and minimum value was recorded in BD 700 (1.80 cm) followed by BD 699 (2 cm) at 100mM. Again SLSI was significantly reduced by the application of salinity. In each case, SLSI 100 was in control condition. But the maximum SLSI value was recorded in BD 750 (82.81%) at 100 Mm and minimum SLSI was recorded in BD 700 (43.74%) (Table 03).

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Fresh weight and fresh weight stress tolerance index (FSTI)

Fresh weight and fresh weight stress tolerance index significantly varied among sorghum genotypes (Table 04) at P ≤ 0.01. BD 686 exhibited maximum fresh weight and FSTI values (1.97 g/10 seedling and 92.06%) and BD 700 showed minimum value (1.13 g/10 seedling & 77.93%). Fresh weight and FSTI showed significant variation due to salinity (Table 04). Results revealed that fresh weight & FSTI was decreased by salt addition. The highest fresh weight &FSTI value was obtained at control (1.69 g/10 seedling & 100%) and lowest (1.17 g/10 seedling & 68.19 %) at 100 mM salinity.

Table 04. Effects of genotypes and salinity levels on morphological characteristics based on germination

Genotypes	Fresh weight (g/10 seedling)	FSTI	Dry weight (g/10 seedling)	DSTI	Leaf area (cm^2)	Vigor index (VI)
BD 686	1.97a	92.06a	0.55b	87.33c	4.01b	2158.33ab
BD 687	1.46c	85.09d	0.57b	88.32b	4.68a	1730.82bcd
BD 693	1.59b	87.85c	0.45c	83.99d	3.41c	1994.17abc
BD 699	1.15f	78.23g	0.25e	76.51g	2.09e	1042.67e
BD 700	1.13f	77.93h	0.22e	75.78g	1.98e	937.67e
BD 747	1.21e	80.87f	0.33d	79.26f	2.69d	1388.17de
BD 748	1.29d	81.65e	0.34d	79.76f	2.75d	1634.67cd
BD 750	1.62b	88.26b	0.70a	92.70a	4.03b	2338.67a
BD 753	1.44c	84.91d	0.43c	82.71e	3.30C	1731.00bcd
LSD	0.052	0.218	0.052	0.721	0.128	367.386
Level of sig.	**	**	**	**	**	**
Salinity level						
0 mM	1.69a	100.00a	0.50a	100.00a	3.56a	2001.29a
100 mM	1.17b	68.19b	0.35b	65.86b	2.88b	1322.30b
LSD	0.025	0.126	0.025	0.416	0.060	212.111
Level of sig.	**	**	**	**	**	**

In a column, within either genotype or salinity, figures having similar letter(s) do not differ significantly at 5% level of probability. ** = indicates significant at 1% level of significance.

The interaction between salinity levels and genotypes had a significant effect on fresh weight & FSTI (Table 05). Highest FW was observed in BD 686 (2.14 g/10 seedlings) at control and lowest in BD 700 (1.8 g/10 seedlings) followed by BD 699 (0.83 g/10 seedlings) at 100mM salinity. In each case, FSTI 100 was in control condition. Maximum FSTI recorded in BD 686 (84.12%) & minimum value shown by BD 700 (55.86%) at 100 mM salinity level.

Dry weight and dry weight stress tolerance index (DSTI)

The variation in dry weight among the studied genotypes was statistically significant at P ≤ 0.01 (Table 04). The highest dry weight was recorded in BD 750 (0.70 g/10 seedlings). BD 700 had the lowest shoot dry weight (0.22 g/10 seedling) followed by BD 699 (0.25 g/10 seedlings). Again in case of DSTI, the highest tolerance index was shown by BD 750 (92.70%) and the lowest tolerance was shown by BD 700 (75.78%) followed by BD 699 (76.51%).

The effect of salinity on dry weight was found statistically significant (Table 04). Result revealed that dry weight was decreased with increasing salinity levels. The highest dry weight was recorded in control (0.5 g/10 seedling) and the lowest was recorded in 100 mM (0.35 g/10 seedling). In case of DSTI, no reduction occurred in control condition while 100 mM exhibited the highest reduction (65.86%). There was no significant interaction between salinity levels and genotypes in terms of dry weight (DW). Again DSTI was significantly reduced by the application of salt (Table 05). However, it varied in different sorghum cultivars. In each case, DSTI was 100 in control condition. But the maximum DSTI value was recorded in BD 750 (85.41%) and BD 700 had lowest DSTI value (51.56%) at 100 mM salt concentration.

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Table 05. Combined Effects of genotypes and salinity levels on morphological characteristics based on germination

T × G	Fresh weight (g/10 seedling)	FSTI	Dry weight (g/10 seedling)	DSTI	Leaf area (cm^2)	Vigor index (VI)
T_1V_1	2.14a	100.00a	0.63	100.00a	4.40b	2374.00
T_1V_2	1.71c	100.00a	0.64	100.00a	5.12a	2067.63
T_1V_3	1.81b	100.00a	0.53	100.00a	3.80c	2286.67
T_1V_4	1.47ef	100.00a	0.32	100.00a	2.40f	1399.33
T_1V_5	1.45ef	100.00a	0.29	100.00a	2.20g	1379.67
T_1V_6	1.49e	100.00a	0.41	100.00a	3.00e	1816.67
T_1V_7	1.58d	100.00a	0.42	100.00a	3.10e	2020.00
T_1V_8	1.83b	100.00a	0.75	100.00a	4.42b	2590.00
T_1V_9	1.69c	100.00a	0.52	100.00a	3.60d	2077.67
T_2V_1	1.80b	84.12b	0.47	74.66d	3.62cd	1942.67
T_2V_2	1.20h	70.18e	0.49	76.63c	4.24b	1394.00
T_2V_3	1.37g	75.70d	0.36	67.97e	3.02e	1701.67
T_2V_4	0.83k	56.46h	0.17	53.01h	1.79h	686.00
T_2V_5	0.81k	55.86i	0.15	51.56i	1.77h	495.67
T_2V_6	0.92j	61.75g	0.24	58.52g	2.39f	959.67
T_2V_7	1.00i	63.29f	0.25	59.52g	2.40f	1249.33
T_2V_8	1.40fg	76.51c	0.64	85.41b	3.64cd	2087.33
T_2V_9	1.18h	69.83e	0.34	65.42f	3.01e	1384.33
LSD	0.074	0.378	-	1.248	0.181	-
Level of sig.	**	**	NS	**	*	NS
CV %	*2.89*	*0.27*	*5.31*	*0.91*	*3.34*	*23.12*

In a column, within either genotype or salinity, figures having similar letter(s) do not differ significantly at 5% level of probability. ** = indicates significant at 1% level of significance. Here, "V" represents the Variety or Genotypes, V_1= BD 686; V_2= BD 687; V_3= BD 693; V_4= BD 699; V_5= BD700; V_6= BD 747; V_7= BD 748; V_8= BD 750; V_9= BD 753. T_1= 0 mM (control); T_2= 100 mMNaCl concentration.

Vigor index (VI)
The variation in vigor index among the genotypes was statistically significant at $P \leq 0.01$ (Table 04). The highest VI value was recorded in BD 750 (2338) and BD 700 had the shortest VI value (937.67). The effect of salinity on VI was found statistically significant (Table 04). Result revealed that VI was decreased with addition of salinity stress. The highest VI was recorded in control (2001.29) and the lowest was recorded in 100 mM (1322.3). There was no significant interaction between salinity levels and genotypes in terms of vigor index (VI).

Leaf area (LA)
Leaf area significantly varied among sorghum genotypes (Table 04). BD 687 exhibited maximum values (4.68 cm^2) and BD 700 showed minimum value (1.98 cm^2) followed by BD 699 (2.09 cm^2). The effect of salinity on leaf area was found statistically significant (Table 04). Result revealed that leaf area was decreased with addition of salinity stress. The highest value was recorded in control (3.56 cm^2) and the lowest was recorded in 100 mM (2.88 cm^2). The interaction between salinity levels and genotypes at had a significant effect on leaf area (Table 05). Results revealed that highest leaf area was recorded in BD 687 (5.12 cm^2) at control and lowest leaf area was found in BD 700 (1.77 cm^2) followed by BD 699 (1.79 cm^2) at 100 mM salt concentration.

IV. Discussion
It is an established fact that tolerance at adult stage is reflected by the tolerance at seedling stage of the plant. In this experiment, germination percentage, germination stress tolerance index (GSTI) (Table 02) and vigor index of all the sorghum genotypes declined with salinity (Table 04). On the basis of germination, GSTI and vigor index BD 750 and BD 686 performed better and BD 700 showed little tolerance at 100 mM salinity levels. Generally, the success of germination was greatly hampered by salinity and so there was a significantly high reduction in final germination percentage under high NaCl concentration. These results are in agreement with the results of the previous researches that

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salinity may affect seed germination by delayed germination process or proceed at reduced rates (Dadara *et al*., 2014) and even in case of salt-tolerant plants (Khan and Ahmad, 1998).

Salt stress influences seed germination primarily by sufficiently lowering the osmotic potential of the soil solution to retard water absorption by seeds, by causing sodium and/or chloride toxicity to the embryo or by altering protein synthesis. (Farsiani and Ghobadi, 2009). Again poor seedling emergence might be caused by hypocotyls mortality associated with the salts accumulation at the soil surface (Sun *et al.,* 2014). Salinity reduces root and shoot growth and ultimately reduce the biomass of the sorghum genotypes. In this experiment, this trend was also observed. In higher salinity (100 mM), root and shoot growth was totally stunted. BD 750 and BD 686 performed better on the basis of plant height at higher salinity whereas BD 700 showed poor performance. These results are in compatible with the results of the previous researches that salinity may affect root and shoot growth (Rani *et al*., 2012). Reduction of shoot growth might be due to suppression of leaf initiation and expansion as well as internode growth and by accelerating leaf abscission (Qu *et al.,* 2012).

In our experiments, root growth was drastically affected by salinity. In all case of treatments, root length reduced in comparison to control. Because of roots, the first developing organ is sensitive to increasing levels of salinity (Kausar *et al.,* 2012). Here, very few genotypes can extend their radical at 100 mM salinity level; this might be due to lower availability of O_2 under saline conditions which deprives the plants form energy source and accumulate high level of ethylene that inhibits root growth (Akram *et al*., 2010). Again on the basis of fresh weights stress tolerance index, it was clear that salinity decreased the growth of all genotypes at 100 mM concentration. However, BD 686 produced maximum fresh biomass as compared to all others and BD 700 remained the lowest in value and showed sensitive behavior towards salinity. These findings are in agreement with those of Kausar *et al.* (2012).

V. Conclusion

Increasing salinity drastically reduces the germination percentage, shoot and root growth, seedling establishment and tolerance level of the seedlings.

VI. References

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