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Morpho-Physiology and Anatomical Responses of Sorghum Seedlings as Affected by Salinity in Hydroponic Culture

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Abstract

An experiment was conducted at the growth chamber of Department of Crop Botany in the Bangladesh Agricultural University, Mymensingh, Bangladesh during March 2018 to observe morpho-physiology and anatomical response of sorghum. Six genotypes were grown in hydroponics with a full nutrient solution (NH_4NO_3-500 µM; $Ca(NO_3)_2$-500 µM; $MgSO_4$-200 µM; KH_2P0_4 -100 µM; $FeC1_3$-2µM; H_2B0_3-11 µM; $MnC1_2$-2µM; $ZnC1_2$-0.35µM; $CuCl_2$-0.2µM; $(NH_4)_6Mo_70_4$-0.1 µM) and 100 mM salinity was imposed on 14 days seedlings. Data on morpho-physiological and anatomical parameters from seedlings were collected after 21 days and stress tolerant indexes of shoot and root were analyzed. Anatomical parameters like metaxylem and protoxylem thickness were also investigated. The results indicated that all the parameters *viz.* root length, shoot length, fresh and dry weight of shoot and root, stress tolerance index of root, and shoot, dry weight of shoot and root, relative chlorophyll content, photochemical efficiency (Fv/Fm), proline concentration, total root area, vascular cylinder area and root diameter were decreased with increasing salinity levels except leaf proline content. Genotypes BD 750 and BD 686 showed better performance considering tolerant indicators while the poor performance was exhibited by BD 747 and BD 753. Thus, based on overall observation BD 750 and BD 686 might be salt tolerant.

Keywords: Sorghum seedling, salinity, hydroponic culture, morpho-physiology and anatomy.

1. Introduction

Sorghum (*Sorghum bicolor* L.) known as great millet belongs to the family Poaceae is originated in Africa, and is now cultivated widely in tropical and subtropical regions (FAO 2015).

Globally, a total land area of 831 million hectares is salt affected. 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. Soil salinity is an increasing problem in the world and main obstacle to agricultural productivity especially in areas where irrigation is necessary. Salinity is a common problem of Bangladesh. It is one of the major environmental stresses affecting plant growth and development results in severe agricultural losses (Tareq, 2009). These adverse physiological effects may be attributed to non-availability to water, reduction in photosynthesis through loss of turgidity impeded nutrient uptake causing deficiency and ion toxicity to plants. Salt stress may also impair synthesis of biochemical substances such as enzymes, sugars and protein (Singh *et al.,* 2001). Crop plants growing under salt stress show reduction in dry matter accumulation and grain yield, which is invariably accompanied with pronounced changes in their ionic composition. 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. Salt tolerance in various crops has been associated with their ability to exclude sodium or chloride from shoot (Hossain et al., 2008). Selective uptake of non-toxic ions into the vascular saps and compartmentalization of toxic ions into the older plant parts and in to the vacuoles are considered to be important basis of tolerance of salinity (Flowers, 2004).

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, it 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} (Sultana, 2008). The study was designed to investigate the morpho-physiology and anatomical responses of sorghum seedlings to salinity in hydroponic culture.

2. Materials and Methods

2.1 Plant growth, environment, and treatments

The experiment was conducted at the growth chamber of Department of Crop Botany in Bangladesh Agricultural University, Mymensingh, Bangladesh during March 2018. The experiment was conducted using six sorghum genotypes namely BD 686, BD 687, BD 693, BD 753, BD 750 and BD 747 to test their morpho-physiology and anatomical attributes against salinity (0 and 100 mM) at osmotic and ionic phases. The experiment was designed by using Completely Randomized Design (CRD) having three replications. Seeds were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur.

The composition of the full strength nutrient solution was prepared according to Pitann *et al*. (2009). Seeds were surface sterilized with 5% sodium hypochlorite for 30 min and washed three to four times with distilled water. Then the seeds were soaked overnight with water for imbibition. For salinization, NaCl was dissolved with nutrient solution to reach 100mM salinity. The salinity level was measured through EC using Sens ION EC-5 meter (Hach, Loveland, Colorado, US). At the age of 14 days of sorghum seedlings, saline treatments were imposed for next 8 days.

2.2 Chlorophyll determination

An index of the relative leaf chlorophyll content was measured by a chlorophyll meter (SPAD-502, Konica Minolta, Japan). Readings were taken along the middle of the four leaves of one plant and the mean value was used for analysis. The measurements were made on ten plants from each treatment.

Chlorophyll fluorescence was measured with Pocket-PEA (Hansatech, Norfolk, UK). The maximal photo-chemical efficiency of PSII, $F_v/F_m = (F_m - F_o)/F_m$ (Genty *et al.*, 1989) was measured in dark-adapted (30 min) leaves at 8th day. Well-developed 4th leaves were selected for measurement.

2.3 Proline determination

The proline content of the supernatant was determined according to the method described by Zhang and Huang (2013). In brief, for the determination of proline content 0.50 g fresh leaves were collected from the 120-day old sorghum plants. The leaves were homogenized on a mortal-pastel with 4.0 mL of 3% sulfosalicylic acid and centrifuged at 11,500 × g for 15 min. The supernatant was used for the proline estimation.

2.4 Anatomical data collection

The stressed plant roots were collected 14 days after salinization. For root anatomical studies, 1 cm root segments were collected after discarding 2 cm from root tip. Both well-developed 4th leaf and root segments were kept in ethanol till transverse sections were made to observe the differences in anatomical structure.

Root and leaf anatomy of stressed and controlled plants were observed by light microscope (Axioscop; Carl Zeiss, Oberkochen, Germany). Here 10x and 40x of images were observed and captured. Images were processed for taking various anatomical parameters using the ZEISS ZEN digital imaging software for light microscopy. For root, metaxylem thickness and protoxylem thickness were measured from the same xylem strand. Vascular cylinder area and total root diameter were also measured. In each cell three positions were selected to take thickness values and then the average was calculated.

2.5 Statistical analysis

The collected data were subjected to statistical analysis following ANOVA technique. Differences among treatment means were adjusted by Duncan's Multiple Range Test with the help of a computer based statistical package program MSTAT-C (Gomez and Gomez, 1984).

3. Results and Discussion

3.1 Effects of salinity on morphological characters

3.1.1 Shoot length and its stress tolerance index (SLSTI)

The variation in shoot length among the studied genotypes was statistically significant at $P \leq 0.01$ (Table 1). The highest shoot length was recorded in genotype BD 686 (41.98 cm) and BD 747 had the shortest shoot length (15.11 cm).

For SLSI, the highest tolerance index was found in BD 686 (97.16%) followed by BD 750 (96.28%) and the lowest one was in BD 747 (87.10%).

The effect of salinity on shoot length was significant (Table 2). The longest shoot length was recorded in control (29.96 cm) and the shortest was recorded in plants under 100 mM (26.08 cm) salinity.

Table 1. Effects of genotypes on morphological characteristics at vegetative stage

Genotypes	Shoot length (cm)	Shoot length stress tolerance index (%)	Root length (cm)	Root length stress tolerance index (%)	Shoot fresh weight (g/hill)	Shoot fresh weight stress tolerance index (%)
BD 686	41.98a	97.16a	21.90c	92.41c	4.27b	93.77c
BD 687	24.02d	91.28c	19.65d	91.41c	3.48c	90.39d
BD 693	31.21c	93.70b	29.55a	96.90a	4.42b	94.48b
BD 747	15.11f	87.10d	14.40e	87.28d	3.58c	90.64d
BD 750	38.81b	96.28a	27.45b	95.31b	5.13a	97.20a
BD 753	16.99e	88.29d	12.73f	86.63d	2.86d	87.81e
LSD	0.793	1.69	0.733	1.38	0.169	0.736

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

Table 2. Effects of salinity level on morphological characteristics at vegetative stage

Salinity level	Shoot length (cm)	Shoot length stress tolerance index (%)	Root length (cm)	Root length stress tolerance index (%)	Shoot fresh weight (g/hill)	Shoot fresh weight stress tolerance index(%)
0 mM	29.96a	100.00 a	22.62a	100.00 a	4.26a	100.00a
100 mM	26.08b	84.60 b	19.28b	83.31 b	3.65b	84.76b
LSD	0.458	0.975	0.423	0.796	0.097	0.425

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

Table 3. Combined effects of genotypes and salinity level on morphological characteristics at vegetative stage

Interaction of genotype to salinity	Shoot length (cm)	Shoot length stress tolerance index (%)	Root length (cm)	Root length stress tolerance index (%)	Shoot fresh weight (g/hill)	Shoot fresh weight stress tolerance index (%)
T_1V_1	43.23a	100.0a	23.70d	100.0a	4.55c	100.00a
T_1V_2	26.31f	100.0a	21.50e	100.0a	3.85e	100.00a
T_1V_3	33.31d	100.0a	30.50a	100.0a	4.68c	100.00a
T_1V_4	17.35i	100.0a	16.50h	100.0a	3.95de	100.00a
T_1V_5	40.31b	100.0a	28.80b	100.0a	5.28a	100.00a
T_1V_6	19.25h	100.0a	14.70i	100.0a	3.25f	100.00a
T_2V_1	40.72b	94.33b	20.10f	84.82d	3.98de	87.53d
T_2V_2	21.72g	82.55d	17.80g	82.81e	3.11f	80.79e
T_2V_3	29.11e	87.40c	28.60b	93.81b	4.16d	88.96c
T_2V_4	12.87k	74.20e	12.30j	74.56f	3.21f	81.28e
T_2V_5	37.31c	92.56b	26.10c	90.63c	4.98b	94.40b
T_2V_6	14.74j	76.57e	10.77k	73.26f	2.46g	75.62f
LSD	1.12	2.39	1.04	1.95	0.238	1.042

In a column, figures having similar letter(s) do not differ significantly 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 747; V_5= BD 750; V_6= BD 753; T_1= 0 mM (control); T_2= 100 mM NaCl concentration.

The interaction effect of salinity levels to genotypes at vegetative stage had a significant effect on shoot length and SLSI (Table 3). Results revealed that shoot length was less affected in T_1V_1 (43.23cm). It indicates that the genotype had salinity tolerance in growth and development than the others. In contrast, shoot length was affected severely in all the genotypes at 100mM and shortest shoot length was recorded in T_2V_4 (12.87 cm). Similar results were reported by Szalai and Janda (2009); Farooq *et al.*, (2015); Hamid *et al.* (2008).

Again SLSI was significantly reduced due to salinity stress. However, it varied in different sorghum cultivars under different levels of salinity. In each case, SLSI was 100 in control . But the maximum SLSI value was recorded in T_2V_1 (94.33%) and lowest SLSI (74.20%) was found in T_2V_4 followed by T_2V_6 (76.57%) (Table

3). Similar results were reported by Szalai and Janda (2009); Farooq *et al*. (2015); Hamid *et al.*, (2008).

3.1.2 Root length and its stress tolerance index (RLSI)

The variation of root length among the genotypes was significant at $P \leq 0.01$ (Table 1). The highest shoot length was 29.55 cm in BD 693 () and the lowest one was in BD 747 (12.73 cm). For RLSI, the highest tolerance index was in BD 693 (96.90%) and the lowest one was in BD 753 (86.63%)

The effect of salinity on root length was found significant (Table 2). The longest root length was recorded in control (22.62 cm) and the shortest one was recorded in 100 mM (19.28 cm). For SLSI no reduction occurred in control condition while 100 mM exhibited the highest reduction (83.31%).

The interaction between salinity levels and genotypes at vegetative stage had a significant effect on root length and RLSI (Table 3). Results revealed that root length was less affected in T_1V_3 (30.5cm). In contrast, root length was affected severely in all the genotypes at 100 mM and shortest root length was recorded in T_2V_6 (10.77 cm). Again RLSI was significantly reduced due to the effect of salinity. The maximum RLSI value was recorded in T_2V_3 (93.81%) and T_2V_6 had the lowest RLSI (73.26%) followed by T_2V_4 (74.56%). Similar results were reported by Szalai and Janda (2009); Farooq *et al*.(2015); Hamid *et al*.(2008).

3.1.3 Shoot fresh weight and its stress tolerance index (SFSTI)

The shoot fresh weight varied significantly among the genotypes (Table 1). The highest shoot fresh weight was recorded in BD 750 (5.13 g/hill) followed by BD 686 (4.27 g/hill) and BD 693 (4.42 g/hill). The lowest shoot fresh weight was in BD 753 (2.86 g/hill). For SFSTI, the highest tolerance index was obtained in BD 750 (97.2%) and the lowest one was in BD 747 (87.81%).

The effect of salinity on shoot fresh weight was found statistically significant (Table 2). The highest shoot fresh weight was recorded in control (4.26 g/hill) and the lowest was recorded in 100 mM (3.65 g/hill). In case of SFSTI, no reduction occurred in control condition while 100 mM exhibited the highest reduction (84.76%).

The interaction of salinity levels to genotypes at vegetative stage had a significant effect on shoot fresh weight and SFSTI (Table 3). The shoot fresh weight was less affected in T_1V_5 (5.28 g/hill). In contrast, shoot fresh weight was severely affected in all the genotypes at 100 mM and shortest shoot fresh weight was recorded in T_2V_6 (2.46 g/hill).

Similarly salinity affected SFSTI significantly which varied in sorghum cultivars. Considering SFSTI 100 in control the SFSTI value was in T_2V_5 (94.40%)and in T_2V_6 (75.62%) (Table 3). Similar results were reported by Szalai and Janda, 2009; Farooq *et al*., 2015; Hamid *et al*., 2008.

3.1.4 Root fresh weight and its stress tolerance index (RFSTI)

The variation in root fresh weight among the six genotypes was significant at $P \leq 0.01$ (Table 4). The highest root fresh weight was in BD 750 (1.26 g/hill) and the lowest one was in BD 747 (0.51 g/hill). For RFSTI, the highest tolerance index was in BD 686 (97.97%) and the lowest one was in BD 747 (84.14%).

The salinity affected root fresh weight significantly (Table 5). The root fresh weight was 0.91 g/hill in control (Table 5) which reduced to 0.76 g/hill in 100mM. (Table 5). Similarly RFSTI reduced to 80.80%at 100 mM salinity.

Table 4. Effects of genotypes on morphological characteristics at vegetative stage

Genotypes	Root fresh weight (g/hill)	Root fresh weight stress tolerance index (%)	Shoot dry weight (g/hill)	Shoot dry weight stress tolerance index (%)	Root dry weight (g/hill)	Root dry weight stress tolerance index (%)
BD 686	1.09b	97.97a	1.51ab	93.03b	0.39ab	93.94b
BD 687	0.77c	88.95d	1.21ab	88.11d	0.31b	89.70e
BD 693	0.82c	89.56c	1.27ab	89.23c	0.33b	90.28d
BD 747	0.51e	84.14f	0.92b	84.20f	0.23c	85.61f
BD 750	1.26a	96.19b	1.74a	97.39a	0.45a	97.89a
BD 753	0.57d	85.59e	0.97b	84.90e	0.25c	86.13e
LSD	0.053	0.689	0.100	6.105	0.038	1.857

Table 5. Effects of salinity on morphological characteristics at vegetative stage

Salinity level	Root fresh weight (g/hill)	Root fresh weight stress tolerance index (%)	Shoot dry weight (g/hill)	Shoot dry weight stress tolerance index (%)	Root dry weight (g/hill)	Root dry weight stress tolerance index(%)
0 mM	0.91a	100.00a	1.40a	100.00a	0.35a	100.00a
100 mM	0.76b	80.80b	1.13b	78.95b	0.29b	81.18b
LSD	0.031	0.398	0.058	3.525	0.022	1.072

Table 6. Combined effects of genotypes to salinity level on morphological characteristics at vegetative stage

Interaction of genotype to salinity	Root fresh weight (g/hill)	Root fresh weight stress tolerance index (%)	Shoot dry weight (g/hill)	Shoot dry weight stress tolerance index (%)	Root dry weight (g/hill)	Root dry weight stress tolerance index (%)
T_1V_1	1.11c	100.00a	1.62c	100.00a	0.41	100.00a
T_1V_2	0.86d	100.00a	1.37d	100.00a	0.34	100.00a
T_1V_3	0.91d	100.00a	1.42d	100.00a	0.36	100.00a
T_1V_4	0.60f	100.00a	1.09ef	100.00a	0.27	100.00a
T_1V_5	1.31a	100.00a	1.79a	100.00a	0.46	100.00a
T_1V_6	0.66ef	100.00a	1.14e	100.00a	0.29	100.00a
T_2V_1	1.07c	95.94a	1.39d	86.06c	0.36	87.88c
T_2V_2	0.67ef	77.90b	1.04f	76.22d	0.27	79.40d
T_2V_3	0.72e	79.12b	1.11e	78.47d	0.29	80.56d
T_2V_4	0.41g	68.28c	0.74g	68.40e	0.19	71.22e
T_2V_5	1.21b	92.38a	1.69b	94.78b	0.44	95.78b
T_2V_6	0.47g	71.18bc	0.79g	69.79e	0.21	72.26e
LSD	0.075	0.974	0.141	8.634	0.053	2.626

In a column figures having similar letter(s) do not differ significantly at 5% level of probability. V_1= BD 686; V_2= BD 687; V_3= BD 693; V_4= BD 747; V_5= BD 750; V_6= BD 753. T_1= 0 mM (control); T_2= 100 mM NaCl.

The interaction between salinity levels and genotypes at vegetative stage had significant effects on root fresh weight and RFSTI (Table 6). Results revealed that root fresh weight was less affected in T_1V_5 (1.31 g/hill). In contrast, root fresh weight was severely affected in all the genotypes at 100mM and shortest root fresh weight was recorded in T_2V_4 (0.41 g/hill) followed by T_2V_6 (0.47 g/hill). In each case, RFSTI was 100 in control condition. But the maximum RFSTI value was recorded in T_2V_1 (95.94%) followed by T_2V_5 (92.38%) and T_2V_4 had lowest RFSTI value (75.62%). These results are agreement with Dafalla *et al.*, (2014); Kafi *et al.*, (2013); Farooq *et al.*, (2015); Queiroz *et al.*, (2012).

3.1.5 Shoot dry weight and its stress tolerance index (SDSTI)

The variation in shoot dry weight among the studied genotypes was statistically significant at $P \leq 0.01$ (Table 4). The highest shoot dry weight was recorded in BD 750 (1.74 g/hill). BD 747 had the lowest shoot dry weight (0.92 g/hill) followed by BD 753 (0.97 g/hill). Again in case of SDSTI, the highest tolerance index was shown by BD 750 (97.39%) and the lowest tolerance was shown by BD 747 (84.20%). The effect of salinity on shoot dry weight was found statistically significant (Table 5). The highest shoot dry weight was recorded in control (1.4 g/hill) and the lowest was recorded in 100mM (1.13 g/hill). In case of SFSTI, no reduction occurred in control while 100 mM exhibited the highest reduction (78.95%).

The interaction between salinity levels and genotypes at vegetative stage had a significant effect on shoot dry weight and SDSTI (Table 6). Results revealed that shoot dry weight was less affected in T_1V_5 (1.79 g/hill). It indicates that this genotype had salinity tolerance in growth and development than the others. In contrast, shoot dry weight was severely affected in all the genotypes at 100 mM and smallest shoot dry weight was recorded in T_2V_4 (0.74 g/hill) followed by T_2V_6 (0.79 g/hill). Again SDSTI was significantly reduced by the application of salt. In each case, SDSTI was 100 in control condition. But the maximum SDSTI value was recorded in T_2V_5 (94.78%) and T_2V_4 had lowest SDSTI (68.4%) followed by T_2V_6 (69.79%).

3.1.6 Root dry weight and its stress tolerance index (RDSTI)

The variation in root dry weight among the six genotypes was statistically significant at $P \leq 0.01$ (Table 4). The highest root dry weight was recorded in BD 750 (0.45 g/hill). BD 747 had the lowest root dry weight (0.23 g/hill) followed by BD 753 (0.25 g/hill). Again in case of RDSTI, the highest tolerance index was shown by BD 750 (97.89%) and the lowest tolerance was shown by BD 747 (85.61%).

The effect of salinity on root dry weight was found statistically significant (Table 5). The highest root dry weight was recorded in control (0.35 g/hill) and the lowest was recorded in 100mM (0.29 g/hill). In case of RDSTI, no reduction occurred in control condition while 100 mM exhibited the highest reduction (81.18%).

There was no significant interaction between salinity levels and genotypes in terms of root dry weight. But RDSTI was significantly reduced by the application of salinity (Table 6). In each case, RFSTI was 100 in control condition. But the maximum RDSTI value was recorded in T_2V_5 (95.78%) and T_2V_4 had lowest RFSTI (71.22%) followed by T_2V_6 (72.26%). These results are agreement with Dafalla *et al.,* (2014); Kafi *et al.,* (2013); Farooq *et al.,* (2015); Queiroz *et al.,* (2012).

3.2 Effects of salinity on physiological characters

3.2.1 Relative chlorophyll content (SPAD index)

The relative chlorophyll content significantly differed among six genotypes at $P \leq 0.01$. BD 750 (35.92) has the highest relative chlorophyll content than the others followed by BD 686 (34.82) while the lowest content was observed in

BD 687 (27.14; Table 7). Relative chlorophyll content was significantly affected due to salinity at $P \leq 0.01$. The highest content was in control condition (33.82) and the lowest content was observed at 100 mM salinity level (28.94; Table 8).

The interaction between salinity levels and genotypes had a significant effect on relative chlorophyll content (SPAD) (Table 9). Results showed that SPAD index value was high in T_1V_5 (36.57) followed by T_1V_1 (36.07) in controlled condition. In contrast, SPAD index was severely affected in all the genotypes at 100 mM and smallest SPAD index was recorded in T_2V_2 (23.21). Reduced relative chlorophyll content at salt stress may be due to the immediate effect of salinity on plant leaf while at the later stage plant might be regaining its tolerance. A similar result was found by Niu *et al.*, (2012).

3.2.2 Maximum photochemical efficiency of PSII (Fv/Fm)

The variation in Fv/Fm value among the six genotypes was statistically significant at $P \leq 0.01$ (Table 7). The highest Fv/Fm value was recorded in BD 693 (0.775). BD 747 had the lowest Fv/Fm value (0.720). FV/Fm value was significantly affected due to salinity at $P \leq 0.01$ (Table 8). The maximum value was recorded in control condition (0.769). In contrast minimum was in 100 mM (0.730).Maximum photochemical efficiency of sorghum decreased with increasing salinity level but there is no interaction effect of genotypes and salinity on Fv/Fm value. These results are in agreement with the results of the previous researches that salinity may affect maximum photochemical efficiency of PS II (Fm/Fv) (Niu *et al.*, 2012; Netondo *et al.*, 2004b; Akram *et al.*,2011).

3.2.3 Proline content in sorghum leaf

The variation in leaf proline among the studied genotypes was statistically significant (Table 7). The highest leaf proline content was recorded in BD 750 (3.47 mg/100g FW). In contrast, BD 747 and BD 693 had the lowest leaf proline (1.80 mg/100g FW). The effect of salinity on leaf proline was found statistically significant (Table 8). Result revealed that the highest leaf proline content was recorded in saline condition at 100 mM (3.17 mg/100g FW) and the lowest was recorded in control condition (1.28mg/100g FW)

Table 7. Effects of genotypes on physiological characteristics at vegetative stage

Genotypes	SPAD	Fv/Fm	Proline (mg/100 g FW)
BD 686	34.82b	0.763ab	2.34b
BD 687	27.14f	0.743bc	1.98c
BD 693	31.59c	0.775a	1.80c
BD 747	27.97e	0.720d	1.80c
BD 750	35.92a	0.760ab	3.47a
BD 753	30.85d	0.735cd	1.93c
LSD	0.623	0.022	0.053

Table 8. Effects of salinity level on physiological characteristics at vegetative stage

Salinity level	SPAD	Fv/Fm	Proline (mg/100 g FW)
0 mM	33.82a	0.769a	1.28b
100 mM	28.94b	0.730b	3.17a
LSD	0.359	0.013	0.031

In a column figures having similar letter(s) do not differ significantly at 5% level of probability.

Table 9. Combined effects of genotypes to salinity level on physiological and biochemical characteristics at vegetative stage

Interaction of genotype to salinity	SPAD	Fv/Fm	Proline (mg/100 g FW)
T_1V_1	36.07a	0.780	1.05g
T_1V_2	31.07d	0.760	1.47efg
T_1V_3	34.07c	0.807	0.80g
T_1V_4	31.57d	0.737	1.50efg
T_1V_5	36.57a	0.780	1.23fg
T_1V_6	33.57c	0.750	1.60d-g
T_2V_1	33.57c	0.747	3.63b
T_2V_2	23.21h	0.727	2.50cd
T_2V_3	29.11e	0.743	2.80bc
T_2V_4	24.37g	0.703	2.10c-f
T_2V_5	35.27b	0.740	5.70a
T_2V_6	28.12f	0.720	2.27cde
LSD	0.880	0.031	0.075

In a column figures having similar letter(s) do not differ significantly at 5% level of probability. V_1= BD 686; V_2= BD 687; V_3= BD 693; V_4= BD 747; V_5= BD 750; V_6= BD 753. T_1= 0 mM (control); T_2= 100 mM NaCl.

Table 10. Effects of genotypes on anatomical characteristics at vegetative stage

Genotypes	Total root area (μm^2)	Vascular cylinder area (μm^2)	Root diameter (µm)	Protoxylem thickness (µm)	Metaxylem thickness (µm)
BD 686	72450.35b	21511.46a	264.00c	3.87e	2.71e
BD 687	46451.60e	12244.07d	171.70f	6.32b	4.23b
BD 693	68950.60c	17778.33b	275.90b	4.32d	3.01d
BD 747	43451.75f	11698.80d	209.70e	5.87c	3.93c
BD 750	85049.40a	18432.04b	319.88a	1.98f	1.78f
BD 753	57451.45d	14926.17c	221.55d	7.82a	5.07a
LSD	0.012	779.50	11.916	1.192	1.053

In a column figures having similar letter(s) do not differ significantly at 5% level of probability.

Table 11. Effects of salinity level on anatomical characteristics at vegetative stage

Salinity level	Total root area (μm^2)	Vascular cylinder area (μm^2)	Root diameter (µm)	Protoxylem thickness (µm)	Metaxylem thickness (µm)
0 mM	68300.82a	17573.42a	265.01a	3.62b	2.35b
100 mM	56300.90b	14623.53b	222.57b	6.44a	4.56a
LSD	0.007	450.10	6.880	0.688	0.608

In a column figures having similar letter(s) do not differ significantly at 5% level of probability.

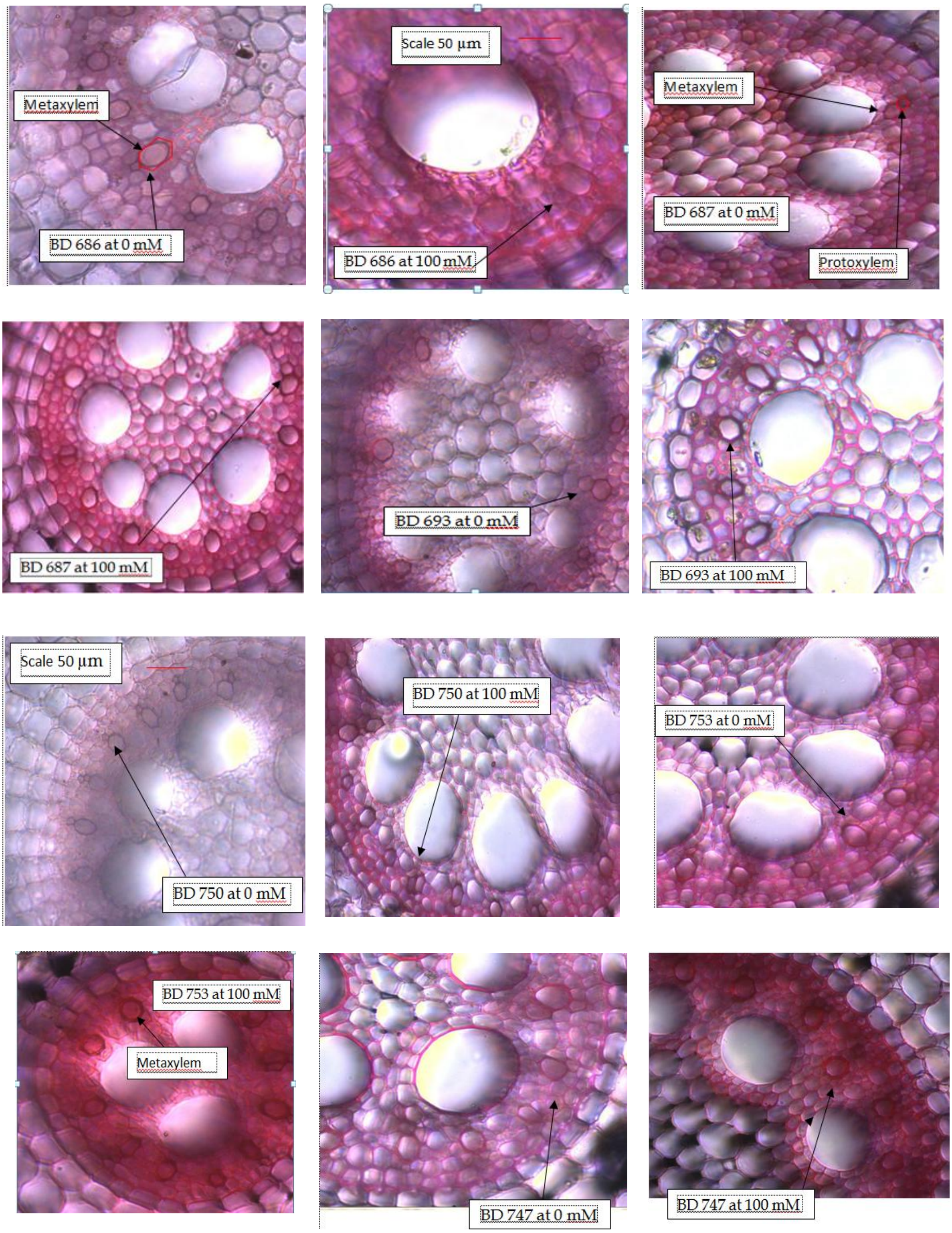

Figure 1. Anatomy of root cross section showing the protoxylem and metaxylem thickness at 0 and 100mM salinity condition of sorghum

Table 12. Combined effects of genotypes and salinity level on Anatomical characteristics at vegetative stage

Interaction of genotype to salinity	Total root area (μm^2)	Vascular cylinder area (μm^2)	Root diameter (μm)	Protoxylem thickness (μm)	Metaxylem thickness (μm)
T1V1	77450.40c	22132.29a	282.40b	2.82ef	1.85
T1V2	54451.60g	14201.80e	202.80e	4.42cde	2.85
T1V3	74450.50d	19132.70c	292.30b	3.12def	2.05
T1V4	51451.80h	13708.70e	237.80d	4.12cde	2.65
T1V5	87549.30a	19632.42c	327.25a	1.83f	1.25
T1V6	64451.30f	16632.63d	247.50cd	5.43c	3.45
T2V1	67450.30e	20890.63b	245.60cd	4.92cd	3.56
T2V2	38451.60i	10286.33f	140.60g	8.22b	5.61
T2V3	63450.70f	16423.97d	259.50c	5.52c	3.96
T2V4	35451.70j	9688.90f	181.60f	7.62b	5.21
T2V5	82549.50b	17231.67d	312.50a	2.12f	2.31
T2V6	50451.60h	13219.70e	195.60ef	10.21a	6.68
LSD	0.017	1102.00	16.852	1.685	1.489

In a column figures having similar letter(s) do not differ significantly at 5% level of probability. V_1= BD 686; V_2= BD 687; V_3= BD 693; V_4= BD 747; V_5= BD 750; V_6= BD 753. T_1= 0 mM (control); T_2= 100 mM NaCl .

The interaction between salinity levels and genotypes had a significant effect on leaf proline (Table 9). Results revealed that proline content was higher in T_2V_5 (5.70 mg/100g FW). In contrast, leaf proline was the lowest in T_1V_3 (0.80 mg/100g FW) followed by T_1V_1 (1.05 mg/100g FW); T_1V_5 (1.23 mg/100g FW). A similar trend was endorsed by Munns and Tester (2008) and Flower (2004). This increment of proline concentration was occurred by plants might be due to maintaining osmotic pressure in the cell (Munns and Tester, 2008). Thus, plants use proline as an osmolyte.

3.3. Effects of salinity on anatomical characteristics

3.3.1 Total root area

The variation in total root area among the studied genotypes was statistically significant (Table 10). Result revealed that the highest total root area was recorded in BD 750 (85049.40 μm^2). In contrast, BD 747 had the lowest total root area (43451.75 μm^2).

The effect of salinity on total root area was found statistically significant (Table 11). Result revealed that the total root area was decreased with increasing salinity levels. The highest total root area was recorded in control (68300.82 μm^2) and the lowest was recorded in 100 mM salinity level (56300.90 μm^2).

The interaction between salinity levels and genotypes at vegetative stage had a significant effect on total root area**.** Salinity stress significantly reduced total root area of the sorghum genotypes. Generally, in control condition, total root area was found the highest and it decreased in saline condition. Result revealed that the highest total root area was recorded in T_1V_5 (87549.30 μm^2) while the lowest total root area was observed in T_2V_4 (35451.70 μm^2) (Table 12).

3.3.2 Vascular cylinder area (VCA)

The highest vascular cylinder area was recorded in BD 686 (21511.46 μm^2). In contrast, BD 747 had the lowest vascular cylinder area (11698.80 μm^2) (Table 10). The vascular cylinder area was decreased with increasing salinity levels. The highest vascular cylinder area was recorded in control (17573.42 μm^2) and the lowest was recorded in 100 mM salinity (14623.53 μm^2) (Table 11). Salinity stress significantly reduced vascular cylinder area of the sorghum genotypes. Generally, in control condition, vascular cylinder area was found the highest and it decreased in saline condition. Result revealed that the highest vascular cylinder area was recorded in T_1V_1(22132.29 μm^2) while the lowest vascular cylinder area was observed in T_2V_4 (9688.90 μm^2) (Table 12).

3.3.3 Root diameter

The highest root diameter was recorded in BD 750 (319.88 µm). In contrast, BD 687 had the lowest root diameter (171.70 µm) (Table 10). The root diameter was decreased with addition of salt. The highest root diameter was recorded in control (265.01 µm) and the lowest was recorded in 100 mM salinity level (222.57 µm) (Table 11).Salinity stress significantly reduced root diameter of the sorghum genotypes. Generally, in control condition, root diameter was found the highest and it decreased in saline condition. The highest root diameter was recorded in T_1V_5 (327.25 µm) while the lowest vascular cylinder area was observed in T_2V_2(140.60 µm) (Table 12).

3.3.4 Protoxylem thickness (PX)

The highest protoxylem thickness was recorded in BD 753 (7.82 µm). In contrast, BD 750 had the lowest protoxylem thickness (1.98 µm) (Table 10).The protoxylem thickness was increased with addition of salt, it is due to subirization of cell wall. The highest protoxylem thickness was recorded in 100 mM salinity level (6.44 µm) and the lowest was recorded in control condition (3.62 µm) (Table 11).Salinity stress significantly increased protoxylem thickness of the sorghum genotypes. Result revealed that the highest thickness was recorded in T_2V_6(10.21 µm) while the lowest protoxylem thickness was observed in T_1V_5 (1.83µm) (Table 12). Protoxylem thickness of root cell increases with increasing of salinity level. This increase of root cell wall is due to accumulation of subirin (Figure 1). These results are agreement with Enstone *et al.*, (2002); Naseer *et al.*, (2012); Baum *et al.*, (2000) in sorghum as well as increased thickness of Casparian strip in maize roots (Karaharae *et al.*, 2004) under salt stress.

3.3.5 Metaxylem thickness (MX)

The highest metaxylem thickness was recorded in BD 753 (5.07 µm). In contrast, BD 750 had the lowest metaxylem thickness (1.78 µm) (Table 10).The metaxylem thickness was increased with addition of salt. The highest metaxylem thickness was recorded in 100 mM salinity level (4.56 µm) and the lowest was recorded in control condition (2.35 µm). Metaxylem thickness of root cell increases with increasing of salinity level. This increase of root cell wall is due to accumulation of subirin (Figure 1). These results are agreement with Enstone *et al.*, (2002); Naseer *et al.*, (2012); Baum *et al.*, (2000) in sorghum as well as increased thickness of Casparian strip in maize roots (Karaharae *et al.*, 2004) under salt stress (Table 11).

4. Conclusions

It could be concluded based on relative value at hydroponic condition, BD 759 and BD 686 can be expressed as tolerant and BD 747 and BD 753 as susceptible genotypes to salt stress. Moreover, further study is needed to evaluate the genotypes in the field condition, especially in the coastal areas in Bangladesh for their adaptability to grow.

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