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 (NH₄NO₃-500 µM; Ca(NO₃)₂-500 µM; MgSO₄-200 µM; KH₂PO₄-100 µM; FeCl₃-2µM; H₂B₃O₃-11 µM; MnCl₂-2µM; ZnCl₂-0.35µM; CuCl₂-0.2µM; (NH₄)₆Mo₇O₄₀-0.1 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, $Fv/Fm= (Fm− Fo)/Fm$ (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 mortar-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 ≤ 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 (%) |
|-------------------------------------|-------------------|-----------------------------------------|------------------|----------------------------------------|-----------------------------|-----------------------------------------------|
| T1V1                                | 43.23a            | 100.0a                                  | 23.70d           | 100.0a                                 | 4.55c                       | 100.00a                                       |
| T1V2                                | 26.31f            | 100.0a                                  | 21.50e           | 100.0a                                 | 3.85e                       | 100.00a                                       |
| T1V3                                | 33.31d            | 100.0a                                  | 30.50a           | 100.0a                                 | 4.68c                       | 100.00a                                       |
| T1V4                                | 17.35i            | 100.0a                                  | 16.50h           | 100.0a                                 | 3.95de                      | 100.00a                                       |
| T1V5                                | 40.31b            | 100.0a                                  | 28.80b           | 100.0a                                 | 5.28a                       | 100.00a                                       |
| T1V6                                | 19.25h            | 100.0a                                  | 14.70i           | 100.0a                                 | 3.25f                       | 100.00a                                       |
| T2V1                                | 40.72b            | 94.33b                                  | 20.10f           | 84.82d                                 | 3.98de                      | 87.53d                                        |
| T2V2                                | 21.72g            | 82.55d                                  | 17.80g           | 82.81e                                 | 3.11f                       | 80.79e                                        |
| T2V3                                | 29.11e            | 87.40c                                  | 28.60b           | 93.81b                                 | 4.16d                       | 88.96c                                        |
| T2V4                                | 12.87k            | 74.20e                                  | 12.30j           | 74.56f                                 | 3.21f                       | 81.28e                                        |
| T2V5                                | 37.31c            | 92.56b                                  | 26.10c           | 90.63c                                 | 4.98b                       | 94.40b                                        |
| T2V6                                | 14.74j            | 76.57e                                  | 10.77k           | 73.26f                                 | 2.46f                       | 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 \( T_1V_1 \) (43.23 cm). 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 100 mM 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 55 Physiology & anatomy of sorghum at salinity).
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 ≤ 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 T1V3 (30.5cm). In contrast, root length was affected severely in all the genotypes at 100 mM and shortest root length was recorded in T2V6 (10.77 cm). Again RLSI was significantly reduced due to the effect of salinity. The maximum RLSI value was recorded in T2V3 (93.81%) and T2V6 had the lowest RLSI (73.26%) followed by T2V4 (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 T1V4 (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 T2V6 (2.46 g/hill).

Similarly salinity affected SFSTI significantly which varied in sorghum cultivars. Considering SFSTI 100 in control the SFSTI value was in T2V3 (94.40%) and in T2V6 (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 ≤ 0.01 (Table 4). The highest root fresh weight was in BD 700 (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                                       |

In a column figures having similar letter(s) do not differ significantly at 5% level of probability. V1 = BD 686; V2 = BD 687; V3 = BD 693; V4 = BD 747; V5 = BD 750; V6 = BD 753. T1 = 0 mM (control); T2 = 100 mM NaCl.

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 (%) |
|------------------------------------|-----------------------------|--------------------------------------------|--------------------------|---------------------------------------------|------------------------|---------------------------------------------|
| T1 V1                             | 1.11c                       | 100.00a                                    | 1.62c                    | 100.00a                                      | 0.41                   | 100.00a                                      |
| T1 V2                             | 0.86d                       | 100.00a                                    | 1.37d                    | 100.00a                                      | 0.34                   | 100.00a                                      |
| T1 V3                             | 0.91d                       | 100.00a                                    | 1.42d                    | 100.00a                                      | 0.36                   | 100.00a                                      |
| T1 V4                             | 0.60f                       | 100.00a                                    | 1.09ef                   | 100.00a                                      | 0.27                   | 100.00a                                      |
| T1 V5                             | 1.31a                       | 100.00a                                    | 1.79a                    | 100.00a                                      | 0.46                   | 100.00a                                      |
| T1 V6                             | 0.66ef                      | 100.00a                                    | 1.14e                    | 100.00a                                      | 0.29                   | 100.00a                                      |
| T2 V1                             | 1.07c                       | 95.94a                                     | 1.39d                    | 86.06c                                      | 0.36                   | 87.88c                                      |
| T2 V2                             | 0.67ef                      | 77.90b                                     | 1.04f                    | 76.22d                                      | 0.27                   | 79.40d                                      |
| T2 V3                             | 0.72e                       | 79.12b                                     | 1.11e                    | 78.47d                                      | 0.29                   | 80.56d                                      |
| T2 V4                             | 0.41g                       | 68.28c                                     | 0.74g                    | 68.40e                                      | 0.19                   | 71.22e                                      |
| T2 V5                             | 1.21b                       | 92.38a                                     | 1.69b                    | 94.78b                                      | 0.44                   | 95.78b                                      |
| T2 V6                             | 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. V1 = BD 686; V2 = BD 687; V3 = BD 693; V4 = BD 747; V5 = BD 750; V6 = BD 753. T1 = 0 mM (control); T2 = 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 T1V5 (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 T2V4 (0.41 g/hill) followed by T2V6 (0.47 g/hill). In each case, RFSTI was 100 in control condition. But the maximum RFSTI value was recorded in T2V5 (95.94%) followed by T2V4 (92.38%) and T2V3 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 ≤ 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 (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%).

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 T1V5 (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 T1V5 (0.74 g/hill) followed by T1V6 (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 T1V5 (94.78%) and T1V4 had lowest SDSTI (68.4%) followed by T1V6 (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 ≤ 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%).

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 T1V5 (95.78%) and T1V4 had lowest RFSTI (71.22%) followed by T1V6 (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 ≤ 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 ≤ 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 T1V5 (36.57) followed by T1V1 (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 T2V2 (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 ≤ 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 ≤ 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 (1.28 mg/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) |
|------------------------------------|----------|-------|-----------------------|
| T1V1                               | 36.07a   | 0.780 | 1.05g                 |
| T1V2                               | 31.07d   | 0.760 | 1.47efg               |
| T1V3                               | 34.07c   | 0.807 | 0.80g                 |
| T1V4                               | 31.57d   | 0.737 | 1.50efg               |
| T1V5                               | 36.57a   | 0.780 | 1.23fg                |
| T1V6                               | 33.57c   | 0.750 | 1.60d-g               |
| T2V1                               | 33.57c   | 0.747 | 3.63b                 |
| T2V2                               | 23.21h   | 0.727 | 2.50cd                |
| T2V3                               | 29.11e   | 0.743 | 2.80bc                |
| T2V4                               | 24.37g   | 0.703 | 2.10c-f               |
| T2V5                               | 35.27b   | 0.740 | 5.70a                 |
| T2V6                               | 28.12f   | 0.720 | 2.77cde               |
| LSD                                | 0.880    | 0.031 | 0.075                 |

In a column figures having similar letter(s) do not differ significantly at 5% level of probability. V1 = BD 686; V2 = BD 687; V3 = BD 693; V4 = BD 747; V5 = BD 750; V6 = BD 753. T1 = 0 mM (control); T2 = 100 mM NaCl.

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

| Genotypes | Total root area (µm²) | Vascular cylinder area (µm²) | 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 (µm²) | Vascular cylinder area (µm²) | 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
The interaction between salinity levels and genotypes had a significant effect on leaf proline (Table 9). Results revealed that proline content was higher in T1V5 (5.70 mg/100g FW). In contrast, leaf proline was the lowest in T1V1 (0.80 mg/100g FW); T1V4 (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 µm²). In contrast, BD 747 had the lowest total root area (43451.75 µm²).

#### 3.3.2 Vascular cylinder area (VCA)

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 µm²) and the lowest was recorded in 100 mM salinity level (56300.90 µm²).

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 T1V5 (87549.30 µm²) while the lowest total root area was observed in T2V4 (35451.70 µm²) (Table 12).
had the lowest vascular cylinder area (11698.80 µm²) (Table 10). The vascular cylinder area was decreased with increasing salinity levels. The highest vascular cylinder area was recorded in control (17573.42 µm²) and the lowest was recorded in 100 mM salinity (14623.53 µm²) (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 µm²) while the lowest vascular cylinder area was observed in \( T_2V_4 \) (9688.90 µm²) (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 \( T_2V_4 \) (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 (Karaharai 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 (Karaharai 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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