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ORIGINAL RESEARCH ARTICLE

Salinity tolerance in tomato genotypes at an early plant growth stage: Morphological and physiological responses

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ABSTRACT

Salinity is a significant factor restricting plant development at various stages, resulting in lower yield and productivity. The current study was carried out to investigate and assess the tolerance of several tomato genotypes to salty conditions. Thirty (30) tomato genotypes were cultivated in pots and tested for salinity at three levels: 5 ds/m NaCl, 10 ds/m NaCl, and 15 ds/m NaCl, in comparison to the control (0 mM NaCl). Two weeks after treatment, several morphological and physiological parameters were measured. The effects of salt stress on tomato genotypes included a considerable reduction in leaf area, chlorophyll content, shoot and root length, shoot and root biomass, and relative water content. Different tomato genotypes responded differently to salinity severity score (SSS). Reduction of shoot dry weight (0.27 to 0.44) and leaf area (0.33 to 0.45) were positively correlated with SSS at moderate (10 ds/m) to higher (15 ds/m) salinity levels, respectively. Based on the experiment results, the genotypes BARI Tomato 4, BARI Tomato 14, BARI Tomato 15, SAU Tomato 2, AV_0T_0 1228, and NS 501 were found to be more salinity tolerant than other genotypes. The results showed that measuring shoot length, leaf area, and shoot fresh and dry weight was better for evaluating salinity stress and screening salt-tolerant tomato genotypes.

Keywords: tomato; salinity, tolerance; relative water content; leaf area

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1. Introduction

Salinity is consequently one of the most serious environmental factors impacting agricultural productivity globally[1]. Salinity stress, depending on the severity and duration of the stress, produces alterations in many physiological and metabolic systems, ultimately reducing crop production[2]. Plant growth is suppressed by soil salinity due to osmotic stress, which is followed by ion toxicity[3]. During the early phases of salinity stress, the osmotic stress of excessive salt accumulation in soil and plants lowers root system water absorption capacity and accelerates water loss from leaves. Thus, salinity stress is also known as hyperosmotic stress[4].

Tomato (*Solanum lycopersicum*) is one of the most significant vegetable plants in the world. Tomatoes can be consumed raw, boiled, or processed into sauces such as juice, pulp, or paste[5]. Salinity has an impact on a number of physiological processes in plants, including accelerated respiration, altered plant development, and mineral distribution. Salinity inhibits shoot growth by reducing cell division and cell expansion in growing points. According to reports, salt tolerance is a developmental phenomenon in many crop species that is governed by stage[5]. This indicates that tolerance at a particular stage of plant development is not always indicative of tolerance at a later stage. The early plant stages are often the focus of physiological studies and salinity screening techniques in many plant species, including tomato. Numerous investigations have demonstrated that modifications in the kind of plant or the saline concentration have a negative or positive impact on the fresh and dry weights of the shoot system[7]. Studies indicating that many plants undergo osmotic regulation when subjected to salt stress by raising the negative of the osmotic potential of the leaf sap demonstrate changes in the water relations of plants affected by salinity[8]. The results of the research that examined how salt stress affected growth show a connection between a reduction in plant length and an increase in sodium chloride concentration[9]. Previous studies indicated that varying NaCl concentrations had a negative effect on leaf area[10].

One of the most effective strategies to prevent salinity is to introduce salt-tolerant crops or to breed salt-tolerant varieties/hybrids. It has been noted that there are variances in salt tolerance not just across species but even within species[11]. When it comes to salt tolerance, tomato genotypes exhibit a great deal of genetic heterogeneity. The intricacy of the trait, a lack of efficient selection areas, and a lack of genetic and physiological knowledge of tolerance-related traits have all impeded salt tolerance breeding initiatives. Selecting and breeding for salt tolerance can be a smart strategy to reduce salinity impacts and improve production efficiency.

Environmental factors like temperature and humidity can easily alter the complex effects of salinity on plants[12]. Under field conditions, determining the critical parameters would be difficult because any environmental change could drastically alter the plant's response to salinity. This study aimed to evaluate salt tolerance among genotypes in shed house environments. The determined salt tolerance criteria and evaluation method can subsequently be used to field breeding practices. The primary goal of this study was to establish predictive specifications by assessing the correlation between genotypes' outward appearances that might be used in tomato plants' early developmental stages. The study's secondary goal was to ascertain how 30 tomato genotypes from Bangladesh responded differently to salinity stress.

2. Materials and methods

2.1. Experimental site

The experiment was carried out in a shed house at Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh with thirty tomato genotypes subjected to natural lighting conditions and varying day/night temperatures. According to the National Mapping Organization of Bangladesh, Dhaka is located at at 23°42′37″ N (Latitude), 90°24′26″ E (Longitude) and it has an average elevation of 4 m (13.12 ft.). During the trial period of November 2020 to January 2021, the average minimum and maximum temperatures were 17.2 ℃ and 28.2 ℃, respectively, with an average relative humidity of 60%.

2.2. Seed collection, seed sowing and transplanting

Thirty (30) tomato seeds were obtained from the Bangladesh Agricultural Research Institute (BARI) in Gazipur and Siddique Bazar in Dhaka, Bangladesh (**Table 1**). The seeds were planted in PVC tanks ((1.2 × 0.6 × 0.6 m)) with a soil mixture containing mixed fertilizers (Thrive; Yates) at a rate of 2 g per tank. The experiment used a completely randomized design with three replications. Seedlings were transplanted to the maintained pots (5 seedlings/pot) at 25 days after sowing (DAS), filled with soil, and given the recommended fertilizer doses[13].

Table 1. Name, source, and type of 30 tomato genotypes used in the present study.

Sl. No.	Tomato genotypes	Source	Germplasm type
1	BARI Tomato 2	PGRC, BARI	Variety
2	BARI Tomato 4	PGRC, BARI	Variety
3	BARI Tomato 8	PGRC, BARI	Variety
4	BARI Tomato 10	PGRC, BARI	Variety
5	BARI Tomato11	PGRC, BARI	Variety
6	BARI Tomato14	PGRC, BARI	Variety
7	BARI Tomato15	PGRC, BARI	Variety
8	BARI Tomato16	PGRC, BARI	Variety
9	BARI Tomato17	PGRC, BARI	Variety
10	BARI Tomato18	PGRC, BARI	Variety
11	BARI Tomato19	PGRC, BARI	Variety
12	BARI Tomato20	PGRC, BARI	Variety
13	BARI Tomato21	PGRC, BARI	Variety
14	BARI F_1 Tomato 4	PGRC, BARI	Hybrid
15	BARI F_1 Tomato 5	PGRC, BARI	Hybrid
16	BARI F_1 Tomato 7	PGRC, BARI	Hybrid
17	BARI F_1 Tomato 8	PGRC, BARI	Hybrid
18	AvTo 1228	PGRC, BARI	Line
19	AvTo 1217	PGRC, BARI	Line
20	AvTo 1229	PGRC, BARI	Line
21	AvTo 1318	PGRC, BARI	Line
22	SAU Tomato 1	PGRC, SAU	Variety
23	SAU Tomato 2	PGRC, SAU	Variety
24	SAU Tomato 3	PGRC, SAU	Variety
25	Cherry Tomato	Australia	Variety
26	Golden Purna	PGRC, SAU	Variety
27	Pathorkuchi	Local market	Hybrid
28	NS501	India	Variety
29	Heroplus	Russia	Hybrid
30	Red Star	Thailand	Variety

PGRC = Plant Genetic Resource Centre, BARI = Bangladesh Agricultural Research Institute, SAU = Sher-e-Bangla Agricultural University.

2.3. Treatments and sample collection

Four salinity treatments were used in the pot experiment: control (no salinity added), 5, 10, and 15 ds m^{-1} were given to wash through the pots repeatedly until the solution had a constant salt concentration. To achieve the required salinity level, NaCl solutions were applied to 35-day-old seedlings (vegetative stage) for seven days. For each treatment, three replications were used. Two weeks after salinity treatments, all morphological and physiological parameters were measured.

2.4. Scores for salinity stress tolerance

From each of the thirty genotypes, five seedlings (20 days old) were transplanted into each pot with three replications and grown under the identical shed house conditions as mentioned before. Four salinity treatments

(0, 5, 10, and 15 ds/m) were applied for two weeks before recording and scoring the degree of leaf injury and the number of surviving plants. A scale from 0 to 4 was used to grade the severity of the leaf damage (score 0, whole plant without symptoms; score 1, about 20% leaf has discoloration/wilting; score 2, >20%–40% leaf shows yellowing/wilting; score 3, >40–60% leaf shows yellowing/wilting; score 4; >60% leaf shows yellowing/wilting (**Figure 1**).

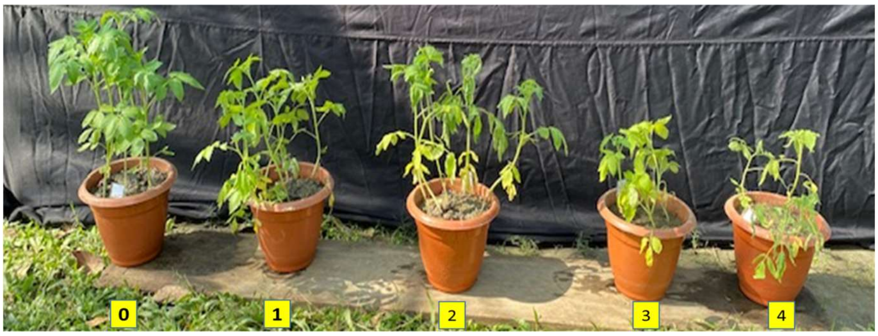

Figure 1. Salinity severity score in tomato seedlings. Score 0, whole plant without symptoms; score 1, about 20% leaf has discoloration/wilting; score 2, >20%–40% leaf shows yellowing/wilting; score 3, >40–60%, leaf shows yellowing/wilting; score 4, >60%, leaf shows yellowing/wilting.

2.5. Determination of shoot and root growth

Three plants from each treatment and replication were chosen at random to measure shoot and root length two weeks after salinity stress. To separate the substrates, the roots were carefully washed with tap water. The longest root length (cm plant^{-1}) was calculated by measuring the distance from the soil surface to the end of the longest root (cm plant^{-1})[14].

2.6. Biomass production

Three plants (above and below ground) were randomly selected from each treatment and replicated two weeks following salt exposure. Plant samples were placed in a 65 °C oven for 72 h before the matter weight was collected[15].

2.7. Measurement of SPAD value

The SPAD-502 chlorophyll meter (Minolta, Tokyo, Japan) was used to measure the chlorophyll content of the first fully expanded leaves. Each salinity-treated and control plant had measurements taken from the middle of the leaf lamina[16].

2.8. Relative water content measurement

The relative water content (RWC) was determined following the procedure given by Smart and Bingham[17]. Three leaves were pooled for each replicate, and their fresh weights (FW) were calculated. The leaves were then immersed in water at room temperature for twelve hours to regain turgidity; the turgid tissue was quickly blotted to remove excess water, and their turgid weights (TW) were determined. The samples were then dried for 24 h in an oven at 65 °C to determine the dry weights (DW). The RWC was calculated using the formula below:

$$\text{RWC}\ \% = \frac{\text{FW-DW}}{\text{TW-DW}} \times 100$$

2.9. Measurement of leaf area

Every leaf sample was measured using a ruler for its maximum width (W) and length (L). The breadth of a tomato plant was measured on the widest leaflet, while the length was measured along the rachis from where the initial leaflet inserted to the distal end[18].

2.10. Data analysis

SPSS 20.0 was used to analyze the data. Means among treatments were considered statistically significant when $P < 0.05$. To show the results, the mean SE from the replicates was used. The significance of correlations between various parameters was determined using bivariate correlations based on Pearson's correlation (2-tailed). The graphs were made using Microsoft Excel.

3. Results and discussion

3.1. Shoot and root length

Shoot and root length are important traits to be considered under any abiotic stress condition. As influenced by NaCl, the shoot length of tomato genotypes delineated significant differences in all the treatments. The reduction of shoot length ranged from 3 to 30% (5 ds/m of NaCl), 10 to 42% (10 ds/m of NaCl), and 14 to 57% (15 ds/m of NaCl). Among the genotypes, the lowest reduction of shoot length was found in AV_0T_0 1228 (14%), followed by BARI Tomato 4 (16%) and SAU Tomato 2 (21%). In contrast, the highest reduction of shoot length was recorded in BARI Tomato 18 (57 %) at the highest stress level (15 ds/m of NaCl) (**Figure 2**).

NaCl concentration in the medium significantly affected the root length of tomato. The reduction in root length value was the highest at approximately 38% in BARI Tomato 18, followed by BARI Tomato 16 (32%), and the lowest root length reduction was found at approximately 1% in BARI Tomato 2, followed by BARI Tomato 5 (about 3%) with low salinity level (5 ds/m of NaCl). However, at the highest salinity level (15 ds/m of NaCl), the lowest reduction of root length was found in BARI Tomato 4 (17%), followed by BARI Tomato 15 (19%), and the highest reduction was observed at approximately 66% in BARI Tomato 18 (**Figure 3**).

Increased salt may limit plant elongation by delaying water intake, which could be another reason for this decline[19]. Cell elongation is primarily determined by cell turgidity, which is lowered by salt stress, resulting in a reduction in tomato shoot length[20]. Salinity can rapidly inhibit root growth, water uptake capacity, and essential mineral nutrition from soil[21]. According to Cuartero and Fernández-Muñoz[5], roots are typically exposed first when plants are exposed to salt stress. Salt stress causes changes in the growth, morphology, and physiology of the roots, affecting water and ion intake as well as the synthesis of signals (hormones) that can send information to the shoot. When the roots grow in a saline medium, the entire plant suffers.

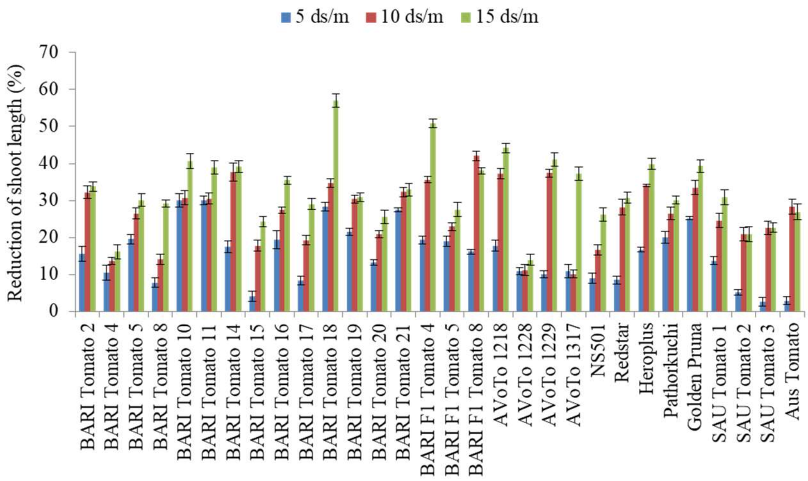

Figure 2. Reduction of shoot length in different salinity-treated plants of tomato genotypes at the seedling stage (expressed as a percentage of the control).

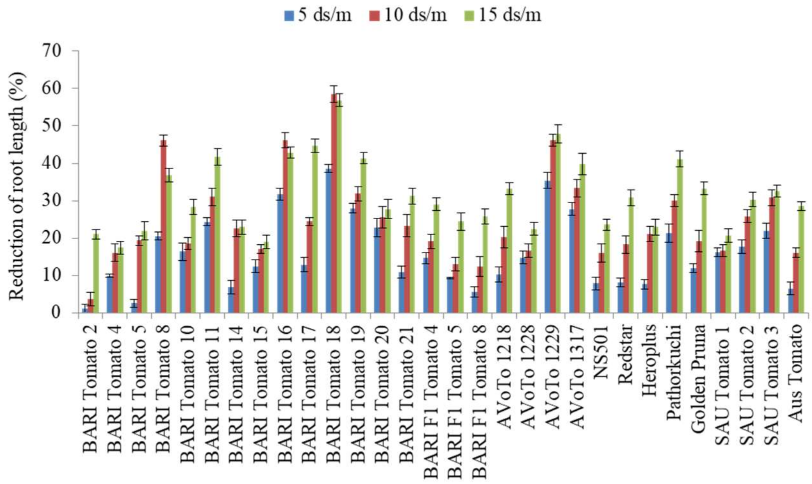

Figure 3. Reduction of root length in different salinity-treated plants of tomato genotypes at the seedling stage (expressed as a percentage of the control).

3.2. Shoot and root dry weight

All genotypes under different treatments showed significant differences in the dry weight of the shoot (**Figure 4**). The reduction of shoot dry weight ranged from 3.13% to 42.90% in 5 ds/m of NaCl; 16% to 58% in 10 ds/m of NaCl, and 27% to 70% in 15 ds/m of NaCl (**Figure 4**). The highest reduction of dry seedling weight was detected in the genotype Heroplus (86%), followed by BARI F_1 Tomato 8 (68%), and the lowest reduction of shoot dry weight was found in SAU tomato 2 (27%), followed by BARI Tomato 15 (32%) at the increased salinity level in 15 ds/m of NaCl.

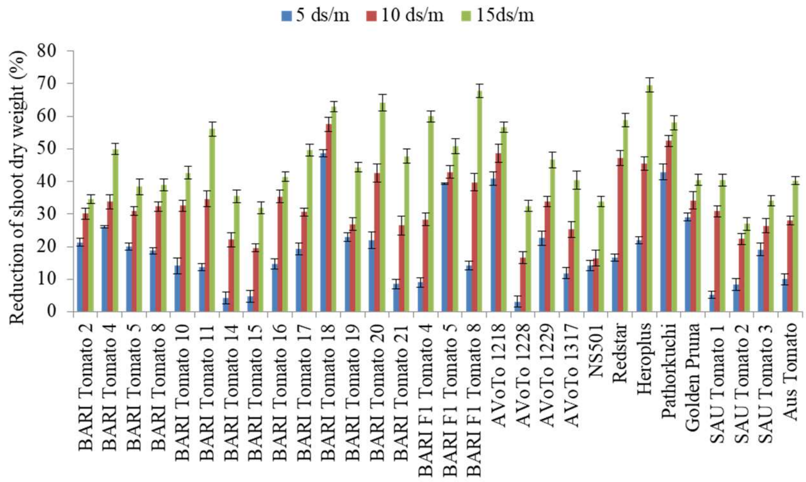

Figure 4. Reduction of shoot dry weight in different salinity-treated plants of tomato genotypes at the seedling stage (expressed as a percentage of the control).

The reduction of root dry weight varied from 2.77% to 39.73% in 5 ds/m of NaCl and varied from 7 to 73% in 10 ds/m of NaCl, and it varied from 24% to 81% in 15 ds/m of NaCl (**Figure 5**). The maximum reduction of root dry weight (80.84%) was recorded in the genotype AV_0T_0 1218, followed by BARI F_1 Tomato 4 (78%) and AV_0T_0 1229 (80%) genotype and the minimum reduction of root dry weight (24.44%) were recorded in BARI Tomato 15 followed by NS 501 (30%) in the highest salinity stress (15 ds/m of NaCl). Salt stress significantly reduced the dry weights of shoots and roots[22]. Osmotic stress in the salinity treatment caused a decrease in cellular water content and shoots lengthening[23]. Salinity lowered the growth parameters of tomato cultivars, such as shoot and root dry weights and pre-harvesting growth stages[24]. NaCl stress's effect on tomato plants' growth was reflected in lower dry weights of shoot and root[25]. The reduction in dry weights caused by increased salinity could be due to a combination of osmotic and specific ion effects of Cl^- and Na^+[26].

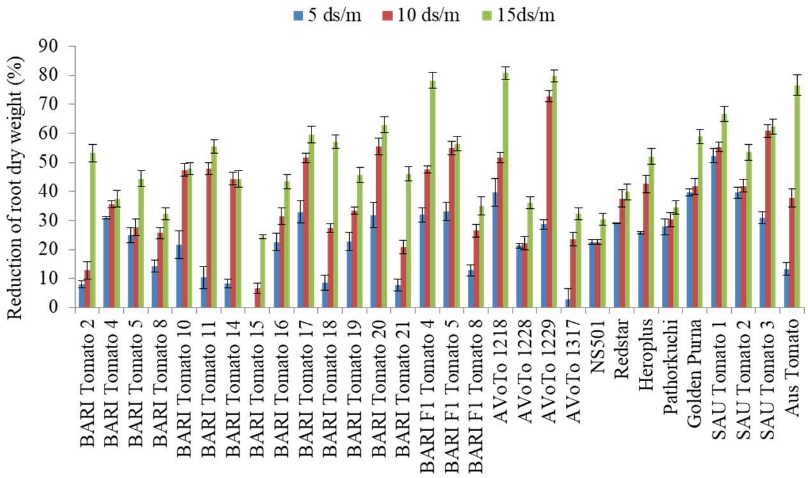

Figure 5. Reduction of root dry weight in different salinity-treated plants of tomato genotypes at the seedling stage (expressed as a percentage of the control).

3.3. Leaf area

At higher concentrations, salinity stress significantly reduces the leaf area of tomato plants because it affects nitrogen levels, slows photosynthesis, and reduces leaf area[27]. Leaf area was significantly ($p < 0.01$) reduced in all tomato genotypes when treated with lower salinity level (5ds/m of NaCl) (**Figure 6**). BARI Tomato 17 showed the most significant reduction (39%). In comparison, BARI Tomato 15 showed the lowest reduction (3%), followed by SAU Tomato 1 (4%) (**Figure 6**). The higher salinity level (15ds/m of NaCl) caused more reduction in leaf area, being 51%–86% for tomato genotypes. In this salinity stress, the lowest reduction of leaf area was found in SAU Tomato 2 (17%), followed by BARI Tomato 14 and BARI Tomato 2 in approximately 27%, and the highest reduction was observed in Golden Purna (approximately 54%). the growth rate of tomato plants decreased as salt concentration increased[28]. The results indicated that plant growth and development are slowed by salt stress, resulting in a slower rate of leaf area expansion development. The decreased leaf area caused by salinity stress is due to toxic ion accumulation and decreased water availability[29]. As the salt concentration increases, the growth rate slows.

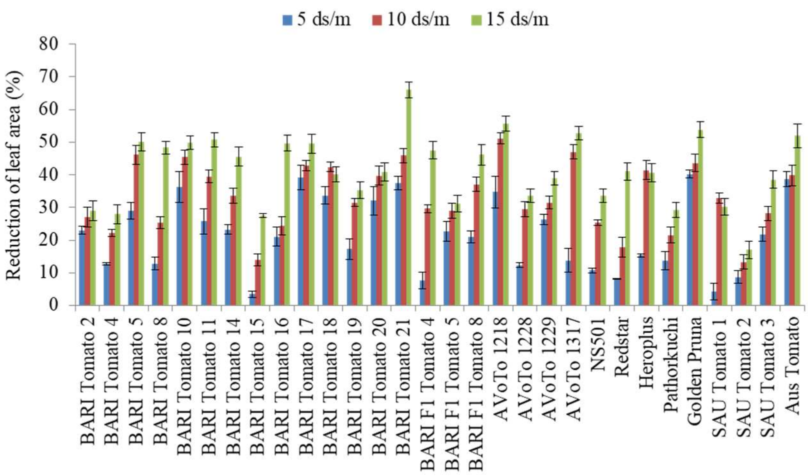

Figure 6. Reduction of shoot leaf area in different salinity-treated plants of tomato genotypes at the seedling stage (expressed as a percentage of the control).

3.4. Relative water content (RWC)

Low salinity level (5 ds/m of NaCl) caused less reduction in RWC, being 2%–28% for tomato genotypes. The higher salinity level (15 ds/m of NaCl) caused more reduction in RWC, being 10%–34% for tomato genotypes. In contrast, the lowest reduction of leaf area was found in BARI Tomato 4 (10%), followed by BARI Tomato 11 and NS501, both in approximately 12%. The highest reduction was observed at about 34% in SAU Tomato 1, followed by AV_0T_0 1228 (about 32%) (**Figure 7**). Under salt stress, Sairam et al.[30] found that the salt-sensitive plant had a larger decline in RWC than the tolerant plant. According to Neocleous and Vasilakakis[31], RWC decreases with increasing salt concentration. The primary sign of water stress is this decline in the RWC of the cells, which restricts water flow to new cell elongation sites in tomato[32] and peach (*Prunus persica* L.)[33].

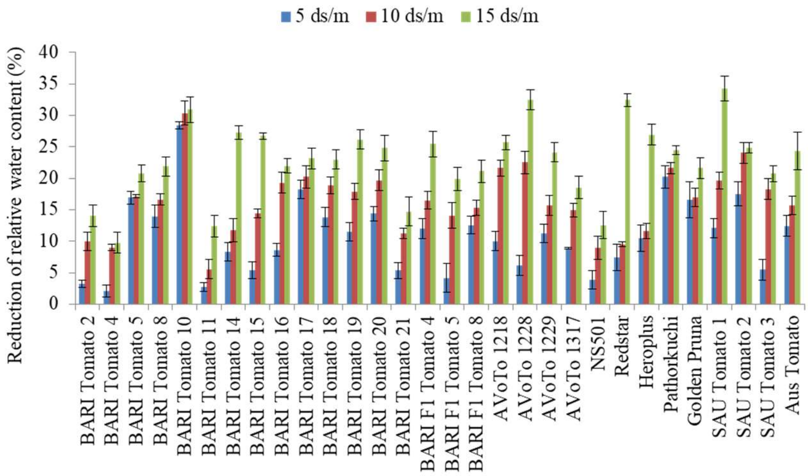

Figure 7. Reduction of relative water content in different salinity-treated plants of tomato genotypes at the seedling stage (expressed as a percentage of the control).

3.5. Chlorophyll content

Salinity stress tomato genotypes all showed a significant loss in chlorophyll content (as determined by the SPAD meter), with a greater reduction seen in plants exposed to higher salinity (15 ds/m of NaCl) than lower salinity (5 ds/m of NaCl) levels (**Figure 8**). The reduction of leaf chlorophyll was 1% to 14% for a lower level of salinity stress (5 ds/m of NaCl); 3% to 38% for a medium level of salinity stress (10 ds/m of NaCl), and 4% to 50 % for a higher level of salinity stress (15 ds/m of NaCl). In higher salinity stress (15 ds/m of NaCl), the highest reduction was recorded at approximately 50% in AV_0T_0 1229 genotype, followed by AV_0T_0 1317 (about 45%). However, a lower reduction was observed at about 4% in BARI Tomato 14 (**Figure 8**). Our results indicated significant decreases in chlorophyll content under salt stress which is in agreement with previous results of Taffouo et al.[34] on *Vigna subterranean* L. Chlorophyll content deteriorated under salt stress as a result of decreased chlorophyll biosynthesis enzymes and increased chlorophyllase activity[35]. The decrease in chlorophyll levels in salt-stressed plants has been considered a typical oxidative stress symptom[36]. The reduction of chlorophyll contents, as a result of either slow synthesis or fast breakdown, indicated that there was a photo protection mechanism through reducing light absorbance by decreasing chlorophyll contents[37].

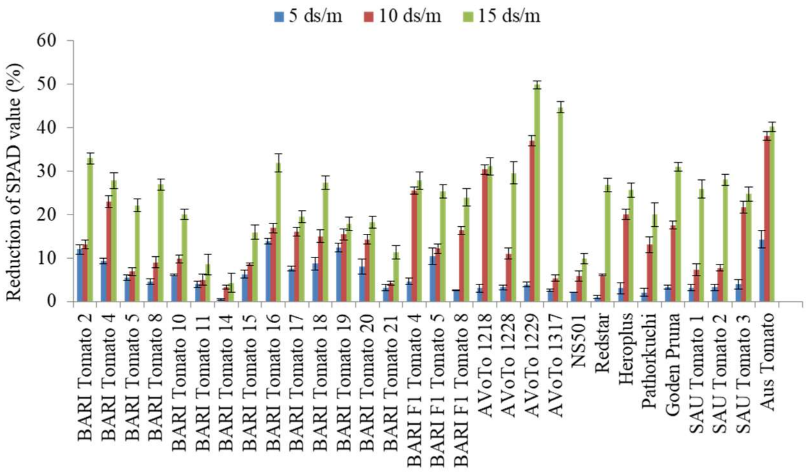

Figure 8. Reduction SPAD value in different salinity-treated plants of tomato genotypes at the seedling stage (expressed as a percentage of the control).

3.6. Salinity severity score (SSC)

The stress tolerance index is more stable and can be considered a useful tool for screening abiotic stress-tolerant genotypes[1]. Salt severity score increased with the increased level of salt stress. Among the treatments, an increased salinity level (15 ds/m of NaCl) showed a significantly higher value of SSC, and a low salinity level (5 ds/m of NaCl) showed a significantly lower value of SSC (**Figure 9**). Under low salinity (5 ds/m of NaCl), BARI Tomato 14, BARI F_1 Tomato 5, AV_0T_0 1317, and NS 501 genotypes showed no visual symptoms. Under higher salinity level (15 ds/m of NaCl), the genotypes BARI Tomato 5, BARI Tomato 11, BARI Tomato 18, and BARI Tomato 21 genotypes showed higher salinity severity score of approximately 3.5, followed by AV_0T_0 1228, 1229, Golden Purna, SAU Tomato 1 and SAU Tomato 3. On the other hand, BARI Tomato 14, BARI Tomato 15, and NS 501 expressed a lower salinity severity score of approximately 1.0. These genotypes can be used in tomato breeding programs as donor parents and through appropriate selection to improve tomato germplasm for salt-affected areas of Bangladesh.

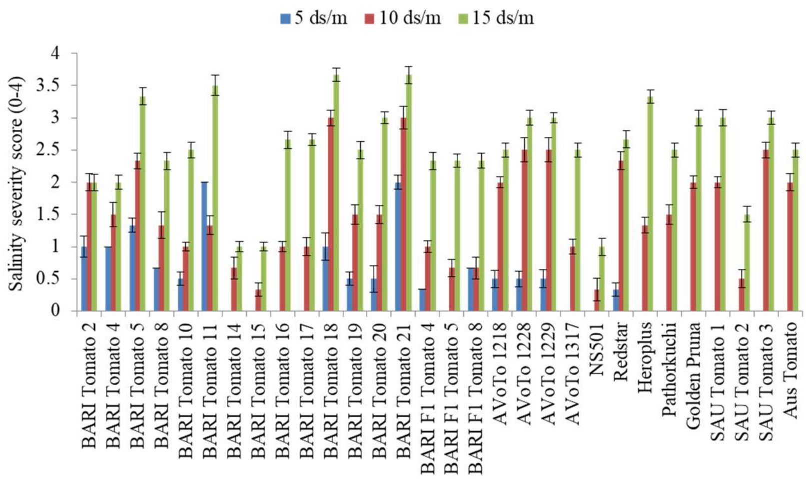

Figure 9. Salinity severity score in tomato seedlings. Score 0, whole plant without symptoms; score 1, about 20% leaf has discoloration/wilting; score 2, >20%–40% leaf shows yellowing/wilting; score 3, >40–60%, leaf shows yellowing/wilting; score 4, >60%, leaf shows yellowing/wilting.

3.7. Correlation analysis

Pearson's correlation coefficients among salinity treatments are listed in **Table 2**. All physiological and morphological parameters except root length, relative water content, and root fresh and dry weight were positively linked with salinity severity scores at three distinct salinity levels. With low salinity level (5 ds/m), shoot length, and shoot fresh weight showed the highest correlation with salinity severity score, whereas with moderate (10 ds/m) and higher (15 ds/m) salinity level, leaf area, and shoot dry weight, the highest correlation with salinity severity score.

Table 2. Correlation between salinity severity score and percentage reduction of different morphological and physiological traits of different tomato genotypes.

Traits	Salinity severity score		
	5 ds/m	**10 ds/m**	**15 ds/m**
Shoot length	0.48**	0.21**	0.29**
Root length	–0.03*	0.23**	0.37**
Shoot dry weight	0.07**	0.27**	0.44**
Root dry weight	–0.33**	0.10**	0.35**
SPAD value	0.04*	0.22**	0.20**
Leaf area	0.30**	0.33**	0.45**
Relative water content	–0.17*	-0.01*	0.05**

*$P < 0.05$; **$P < 0.01$.

4. Conclusion

In conclusion, the current study discovered that tomato genotypes varied significantly regarding morphological and physiological traits. Among the tomato genotypes studied, BARI Tomato 4, BARI Tomato 14, BARI Tomato 15, SAU Tomato 2, NS501, and AV_0T_0 1228 performed best in salinity stress and were recognized as tolerant. To produce salinity-tolerant genotypes under salinity stress, reference measures such as shoot length, shoot biomass, and leaf area could be used.

Author contributions

This work was conducted in collaboration with all authors. SC and NI conceived and designed the experiments; SC performed the experiments, analyzed the data, and contributed reagents/materials/analysis tools, prepared figures and/or tables. MRS and SA performed the experiments, reviewed drafts of the paper. SC, NI, MRS, and SA collected references, revised, and improved the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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