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Original Article

Assessment and assortment of tomato genotypes against salinity at vegetative stage

Abul Fazal Mohammed Shamim Ahsan[1*], Al Harun Md. Motiur Rahman Talukder[1], Shamsun Nahar Mahfuza[1], Faruque Ahmed[1], Mohammad Amdadul Haque[2], Mohammad Abdul Goffar[2], Mohammad Masuduzzaman Masud[3], Ahmed Khairul Hassan[4]

[1]Plant Physiology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[2]Horticulture Research Centre, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[3]Soil Science Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[4]Department of Agronomy, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

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**Corresponding author email:*
shamim.agro@gmail.com

Abstract

Salinity affects growth of salt-sensitive vegetable crops at an early stage. So, selection of vegetable crops at an early vegetative stage is a significant step in improving salt tolerance. In this study, twenty-four tomato genotypes were subjected to two different salinity stress viz., control, and 15 dS m^{-1} at 35 days after emergence under hydroponic culture. Visual scoring of salt injury and morpho-physiological traits (length, fresh and dry weights of root and shoot, leaf area, membrane stability, and content of Na^+, K^+, Ca^{2+}, K^+: Na^+ and Ca^{2+}: Na^+) were investigated. Analysis of variance revealed that specific and interaction effects of both salinity and genotype for all measured traits were significant ($P<0.05$), suggesting a wide range of diversity in these genotypes. On the basis of visual scoring, genotypes G4, G7, G14, and G16 were found in lower injury scale classes 1 and 2. They also had the least reduction of root length, leaf area, and total biomass under salinity. At 15 dS m^{-1} salinity level, the genotypes G1, G4, G7, G14, and G16 showed the least cell membrane stability index CMSI compared to the control genotype. Moreover, these genotypes also uptake less Na^+ with higher % of K^+ and Ca^{2+}, which resulted in higher K^+: Na^+ and Ca^{2+}: Na^+ ratios than others, that expressed their tolerance to salinity. Tomato genotypes were classified into four clusters, where, G1, G4, G5, G7, G14, and G16 genotypes were found in cluster 3 and cluster 1, with the maximum mean values and top-ranking scores in their measured morphological traits. On the other hand, G13, G20, G22, G23, and G24 were in cluster 4, with the lowest mean values and bottom-ranking scores. The results of the study consistently confirmed that G4, G7, G14, and G16 genotypes are salt-tolerant at the vegetative stage.

Keywords: Tomato, Genotype, Salinity, Vegetative, Hydroponic

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Introduction

Gratitude for its nutritional value; its numerous uses; and alluring properties related to touch taste, and aroma, tomato (*Solanum lycopersicum* L.) is the most important and popular vegetable in Bangladesh (Schreinemachers et al., 2016). It also acts as a recuperation agent of various types of human diseases (Ballon-Landa and Parsons, 2017). The global tomato production is now about 180 million tons (FAOSTAT, 2019) and it subsidizes 60% of the world's total fresh vegetable production (Mitra and Sharmin, 2019). In Bangladesh, it ranks second after brinjal in terms of both production area and yield, and the national average yield (14.57 tons/ha) is very low (BBS, 2021). As a tropical plant, tomato is suitable for almost all climate zones around the world; but, abiotic stresses are the most significant constraints to its yield potential (Loudari et al., 2020). Among the abiotic stresses, soil salinity is an important suffering factor that constrains vegetable productivity mainly in semi-arid or coastal areas (Bünemann et al., 2018).

The salinized areas are increasing at an annual rate of 10% for various reasons (Gorji et al., 2020) and more than 50% of the arable land would be salinized by the year 2050 (Shrivastava and Kumar, 2015). The area under saline land in the coastal belt of Bangladesh is also increasing day by day and is being affected with varying levels of salinity ranging from 3.63-27.67 dS m^{-1}. Shrivastava and Kumar (2015) also reported that the productivity of most crops is significantly reduced by soil salinity when the value of electric conductivity approaches 4.0 dS m^{-1}. About 58.5% of the cultivated land of the coastal and offshore regions of Bangladesh is affected above this threshold level of salinity 4.01->16 dS m^{-1}. In the short term, salinity stress causes osmotic stress due to a decrease in water availability, and in the long term, ion toxicity due to an imbalance of cytosol nutrients (Sheteiwy et al., 2019). A high concentration of exogenous salt causes an ionic imbalance in the cells which leads to ion toxicity and osmotic stress (Chakraborty et al., 2018), nutrient imbalances, membrane damage, and reduced photosynthetic activities (Chourasia et al., 2021), and alteration of NO_3^- uptake by plants, which affect plant growth and yield (Yasuor et al., 2017).

Tomato is moderately sensitive to salinity (Zushi and Matsuzoe, 2017), and cannot endure or tolerate with very low yields. Salinity level above 3-5.5 dS m^{-1} markedly reduces leaf area index, total chlorophyll and also reduces tomato yield by 12-32% (Zhai et al., 2015). Salt stress influences a series of major physiological processes such as photosynthesis, ion partitioning as well as Na^+: K^+ ratio, Reactive Oxygen Species (ROS), and hydraulic conductivity which affects the bioenergetic processes of the electron transport chain (Almeida et al., 2017). Furthermore, salt stress seems to affect root anatomy and morphology parameters (Robin et al., 2016). Earlier researchers investigated the response of salinity on different vegetables (Taïbi et al., 2016; Kumar et al., 2017; Raza et al., 2017), where they observed stressed plants with significantly reduced the biomass, leaf area, and growth. Root and shoot weight, taproot length, chlorophyll content, and transpiration rate are some of the morph-physiological traits that can be employed to develop salt-tolerant cultivars (Taïbi et al., 2016). Among the physiological markers, selective ion uptake is the important indicator for salinity tolerance, with tolerant cultivars having enhanced K^+:Na^+ ratio and maintained low Na^+ (Liu et al., 2017; Ahsan et al., 2020). However, scientists around the world have screened up to 20 dsm^{-1} at vegetative stage and developed salt-tolerant tomato varieties (Dasgan et al., 2002; Kumar et al., 2017; Raza et al., 2017). Similarly, scientists in Bangladesh also conducted research, but their activities were confined to released varieties only (Moniruzzaman et al., 2013; Shimul et al., 2014; Rashed et al., 2016). The results of the studies showed that these varieties were not able to give such promising yields in coastal areas. Therefore, it has become imperative to develop salt-tolerant tomato varieties for use in uncultivated areas due to soil salinity including meeting the food demands of growing population. In this connection, plant breeders have developed some new advance lines and hybrids, which are expected to be suitable for cultivation in coastal areas. Moreover, recalling the adverse impacts of climate change on the farm sector and according to "The 2030 Agenda for Sustainable Development" adopted by the UN's general assembly in 2015, more emphasis should be given on the development of resilience and high-yielding genotypes. So, the present study was initiated in hydroponic systems subjecting to salt stress of some newly developed tomato parents and their crosses at the early vegetative stage for detecting salt tolerance, which could be useful for potential breeding programs to develop salt-tolerant tomato variety. This research will also help to recognize the appropriate genotypes for salt stress-prone areas in Bangladesh.

Material and Methods

Plant material and growth condition

The study was conducted in the hydroponic (ambient environment) of plant physiology division, Bangladesh Agricultural Research Institute (BARI), Gazipur during the winter season (November) of 2019. A total of 24 hydroponically grown tomato genotypes (6 parents, 15 F_1 hybrids, and 3 commercial varieties) were screened-out (Table 1) against different salinity levels viz. control (2.0-2.5 dS m^{-1}), and 15 dS m^{-1}. Seeds were collected from the Olericulture Division of Bangladesh Agricultural Research Institute (BARI). Healthy and equal-sized seeds of each genotype were chosen, then surface-sterilized for 3 minutes with a 70% ethanol solution, then washed several times with sterile distilled water. The seeds were planted in cell trays (35 cm × 35 cm × 5.5 cm; 36 cells/tray with drainage holes) with the potting mixture (2/3rd parts of cocopeat, 1/6th part of unfilled rice seed, and 1/6th part of vermicompost) and kept in a shade with a moist cover. Subsequently, two-week-old seedlings (second-true leaf) were transplanted into cork sheet holes floating on ½ strength nutrient solution culture (Dasgan et al., 2002) in a 160 L plastic container. A sponge plug was placed around the base of the stem to hold the plants in the holes and roots were submerged in the nutrient solution. The nutrient solution was protected from sunlight by a cork sheet with white polythene. The pH value was maintained at 6±0.5. Continuous aeration in the container was ensured through an aquarium bubble stone by a diaphragm pump (RESUN LP60 50W) with a flow rate of 140 L/min. After one-week, homogenous seedlings were transplanted in three other hydroponic containers, containing ½ strength nutrient solution and seventy-four seedlings were grown in each container. Salinity treatment was imposed on 35 days-old seedlings and the desired level of salinity were achieved in each container within the next nine days, and the seedlings were grown for the next 10 days at respective salinity stress. Throughout the study period, a group of plants was grown in a similar type of container without saline solution for comparisons. The salinity levels were monitored with the help of an EC meter (soil probe; HI 993310, Hanna, Romania).

Measurement of morphological and physiological traits

The salt tolerance of the seedlings was evaluated visually after 10 days of salt stress following method described by Dasgan et al. (2002). The intensity of salt susceptibility was measured on a scale of 1 to 5 for each plant (Table 2).

Table-1: Genotypes name and working code of genetic materials

Working code	Genotypes name
G1	SL0303
G2	SL0304
G3	SL0307
G4	SL0308
G5	SL0313
G6	SL 0423
G7	SL0303 × SL0304
G8	SL0303 × SL0307
G9	SL0303 × SL0308
G10	SL0303 × SL0313
G11	SL0303 × SL 0423
G12	SL0304 × SL0307
G13	SL0304 × SL0308
G14	SL0304 × SL0313
G15	SL0304 × SL 0423
G16	SL0307 × SL0308
G17	SL0307 × SL0313
G18	SL0307 × SL 0423
G19	SL0308 × SL0308
G20	SL0308 × SL0313
G21	SL0313 × SL 0423
G22	BARI Hybrid Tomato-4
G23	BARI Hybrid Tomato-8
G24	BARI Hybrid Tomato-10

Table-2: Score sheet of phenotypic evaluation for salt stress.

Phenotypes of the seedlings	Score
Plants with or without subtle inward curly leaves that are normally green in color	1
Plants green and complete innermost twisted leaves	2
All leaves are curly, dry leaves from reasonable to severe damages	3
About 50-80% leaves are drying with damages	4
All leaves are damages	5

Every genotype's tolerance scale was the sum of three replications. Subsequently, plants were sampled, separated into leaves, shoots, and roots, and leaf area was measured using an automated leaf area meter (LI-3100 C; LI-Cor, USA). Promptly plant fresh weight, as well as their root and shoot lengths were measured. Plants were dried in an oven for 72 hours at 70°C before being weighed on an analytical scale to determine the total dry matter. Atomic spectrometry was used to measure Na^+, K^+, and Ca^{2+} concentration in plant, a well-mixed plant sample (0.5 g) was weighed and burned in a muffle furnace at 550°C for 7-8 hours followed by dry-ashing method (Islam et al., 2021), The CMSI was determined by estimating ion leaching from leaf tissue into distilled water based on the procedure of Ahsan et al. (2020). Briefly, 1 g leaf was placed into 10 ml deionized water in sets of closed vials. The first set was incubated at 25 ℃ for 12 hours, and electrical conductivity (E_1) of the bathing solution was determined by EC meter (HI 993310, Hanna, Romania). The second set was put in a boiling water bath for 10 minutes and its electrical conductivity (E_2) was also measured. The cell membrane stability index (CMSI) was calculated using the following formula-

$$\%\ \text{CMSI} = 1 - \left(\frac{E_1}{E_2}\right) \times 100$$

Ranking and grouping of morphological traits

The relative values of the examined morphological traits viz., root length (RL), shoot length (SL), total fresh weight (TFW), total dry weight (TDW) and leaf area (LA) for each genotype were used to calculate ranked scores and grouping. Top ranking genotypes had the maximum mean values, while bottom ranking genotypes had the minimum means. The rank means were also estimated from the rank score of all studied traits. Relative values, rank-sum (RS), and standard deviation of ranks (SDR) for genotypes were calculated by using the following formula:

$$\text{Relative value} = \frac{\text{Value in salinity stress}}{\text{Value in control}} \times 100$$

Rank sum (RS) = Rank mean (RM) + Standard deviation of rank (SDR) and SDR= $(S_i^2)^{0.5}$

Statistical analysis

The collected data were analyzed by the analysis of variance technique to determine significant varietal differences among the 24 genotypes using Statistix 10. The mean values were separated by the least significant difference (LSD) at 5% level of probability. Correlation coefficients were estimated among morphological traits and multivariate cluster analysis of the genotypes was performed by STAR 2.0.1 (STAR, 2014). Treatments in the experiment were arranged in a completely randomized design (CRD), with three replications in a single container and single plant was considered as one replication.

Results

Visual assessment

All the parameters showed substantial differences under salt stress which indicated that seedling growth of the tomato is hindered by increased salinity stress, and thereafter, the salt injury was observed as a particular indication in tomato leaves. Visual appearance also showed that tomato genotypes reacted differently to salinity stress. However, the majority of genotypes were moderate to highly sensitive, with a rating of 4 to 5 (Table 3). Among the screened genotypes, G4 and G14 in scale class 1 (rating <1.5) were less affected at 15 dS m^{-1} salinity stress. Two genotypes (G7 and G16) in scale class 2 (ranking 1.5 to 2.0) were mildly affected. Plants of this scale were almost average in size or slightly smaller, and the edge of the lamina was slightly wilted. Six genotypes (G1, G5, G10, G11, G17 and G21) in class 3 were moderately (rating 2.1 to 3.0) affected with leaf wilting symptom. The older leaves of these plants wilted to various degrees, whereas the younger leaves wilted partially. Fourteen genotypes [(G3, G6, G8, G12, G15 and G18 in rating 3.1 to 4) and (G2, G9, G13, G19, G20, G22, G23 and G24 in rating scale 4.1 to 5)] in scale classes 4 and 5 were severely affected by salt stress and showed partial or full wilting symptom with mostly or fully dry leaves.

Effect of salinity on growth of tomato genotypes

Analysis of variance was calculated for different morpho-physiological traits of tomato genotypes. Regarding mean square of genotype, salinity, and their interaction significantly varied for all studied morpho-physiological attributes. Analysis of variance indicated that genotypes tended to possess genetic variability for several traits; hence these genotypes may be selected for further breeding programs. However, the interaction effect of genotype and salinity on the morphological (RL, DSL, TFW, TDW, and LA) and physiological (Na^+, K^+, Ca^{2+}, K^+: Na^+, and Ca^{2+}: Na^+) parameters are described below.

Leaf area

The leaf area of tomato seedlings was significantly affected by salinity (Figure 1). The highest relative leaf area (RLA) was discovered in G14 (25%), followed by G4 (21%) genotypes, and the lowest was measured in G24 (7%). On the other, the minimum leaf area reduction percentage from the control (LARC) was observed in G14 (74%), which was also significantly different from others. However, the maximum value of LARC was recorded in G24 (92%) genotypes.

Table-3: Standard evaluation method for noticeable salt damage in tomato genotypes

Genotypes	Scale (1-5)[A]	Score
G1	2.2 b-d	3
G2	5 j	5
G3	3.2 fg	4
G4	1.4 ab	1
G5	2.2 b-d	3
G6	3.4 f-h	4
G7	1.8 a-c	2
G8	3.2 fg	4
G9	4.6 ij	5
G10	2.4 c-e	3
G11	2.8 d-f	3
G12	4 hi	4
G13	4.4 ij	5
G14	1.3 a	1
G15	3.4 f-h	4
G16	1.6 ab	2
G17	2.2 b-d	3
G18	3.6 gh	4
G19	4.4 ij	5
G20	4.6 ij	5
G21	3 e-g	3
G22	4.8 j	5
G23	5 j	5
G24	4.8 j	5
LSD (0.05)	0.606	-
CV (%)	1.99	-

[A] Increasing scale class from 1-5 indicates increases in salt damages. Means in the same column followed by a different letter(s) differ significantly at $p<0.05$

Root and shoot length

Root and shoot growth were drastically decreased in all tomato genotypes under salinity stress compared to control (Figure 2 and Figure 3). Moreover, reduction percent was more prominent in the shoot length than the root length of all the tested genotypes. Reduction of root length from control (RLRC) in all tomato genotypes ranged from 21.82 to 50.89%, whereas it ranged 57.61- 78.27% in shoot length. A similar trend was also observed in both relative values of root and shoot length.

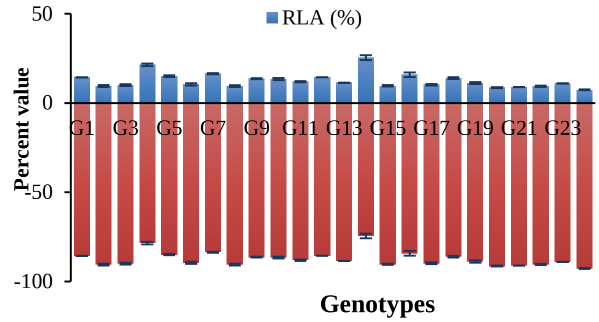

Figure-1: Relative leaf area (RLA) and leaf area reduction from control (LARC) of the tomato genotypes at 15 ds m^{-1} salinity stress

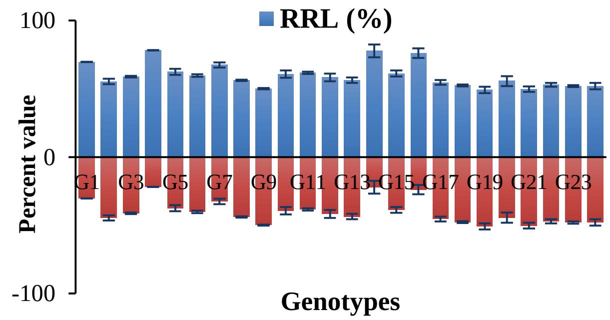

Figure-2: Relative root length (RRL) and root length reduction from control (RLRC) of the tomato genotypes at 15 ds m^{-1} salinity stress

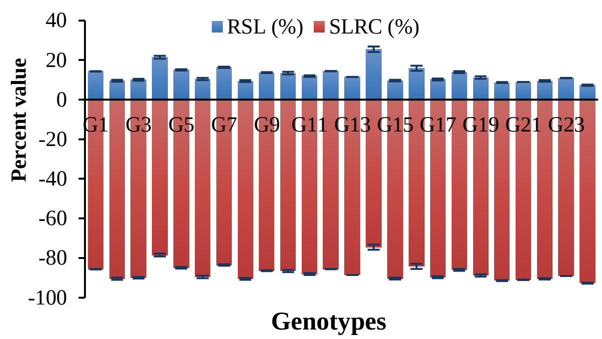

Figure-3: Relative shoot length (RSL) and shoot length reduction from control (SLRC) of the tomato genotypes at 15 ds m^{-1} salinity stress

Total biomass production

Plant biomass (fresh and dry) in the tomato genotypes was significantly reduced ($p<0.05$) by salinity stress (Figure 4 and Figure 5). However, the reduction percentage was higher in total fresh weight than total dry weight compared to control. Results showed that total fresh weight reduction compared to control (TFWRC) ranged from 73.52 to 93.96% in all studied genotypes, and total dry weight reduction ranged from 66.57 to 89.79%. Among the genotypes, plant growth was much more noticeable in the G14 genotype as it produced maximum relative values of total fresh (26.47%) and dry biomass (33.42%) per plant.

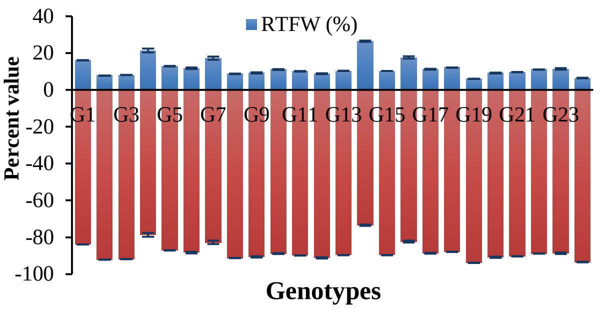

Figure-4: Relative total fresh weight (RTFW) and total fresh weight reduction from control (TFWRC) of the tomato genotypes at 15 ds m^{-1} salinity stress

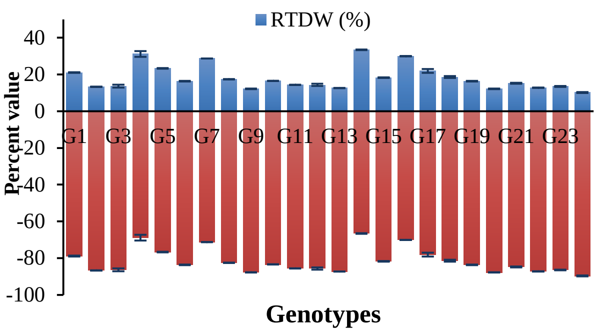

Figure-5: Relative total dry weight (RTDW) and total dry weight reduction from control (TDWRC) of the tomato genotypes at 15 ds m^{-1} salinity stress

Ion concentration

The scenario of Na^+, K^+, Ca^{2+}, K^+: Na^+ and Ca^{2+}: Na^+ ratio of tomato leaf exposing to salt stress was significant (Figure 6-10). Generally, salt concentration within the growth medium increased, the concentration of toxic ion (Na^+) also increased whereas the concentration of essential ions (K^+, Ca^{2+}) and their ratios with Na^+ (K^+: Na^+ and Ca^{2+}: Na^+) deceased. In comparison to respective control plants, the least growing value of Na^+ was observed in G4, G5, G7, G14, and G16 genotypes by 624, 296, 401, 326, and 323%, respectively (Figure 6). However, the concentration of potassium and calcium ions of all tomato genotypes decreased under 15 dS m^{-1} solution. Among the genotypes, the lowest K^+ reduction rate was recorded by 59%, 49%, 61%, and 55% in G1, G4, G5, and G14, respectively (Figure 7). Results also showed that G2, G17, G20, and G24 genotypes reduced calcium ions by more than 60%, while G1, G5, G7, G14, and G16 genotypes reduced by below 30% of Ca^{2+} as compared with control plants (Figure 8). K^+: Na^+ and Ca^{2+}: Na^+ in leaf tissue were drastically reduced due to salinity stress. Among the genotypes, the highest values of K^+: Na^+ (0.2) and Ca^{2+}: Na^+ (0.45) ratios were observed in the G14 genotype and the lowest in the genotype G17 (0.02 and 0.07, respectively) under 15 dS m^{-1} salt. Moreover, genotypes that accumulate maximum K^+: Na^+ and Ca^{2+}: Na^+ ratios showed smaller scale classes and minimum injury at salinity stress.

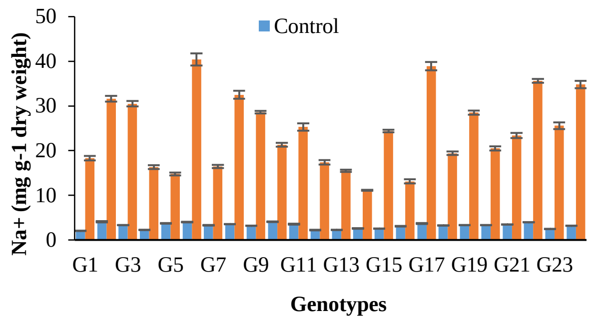

Figure-6: Concentration of sodium (Na^+) ions of different tomato genotypes grown in control and salinity stress

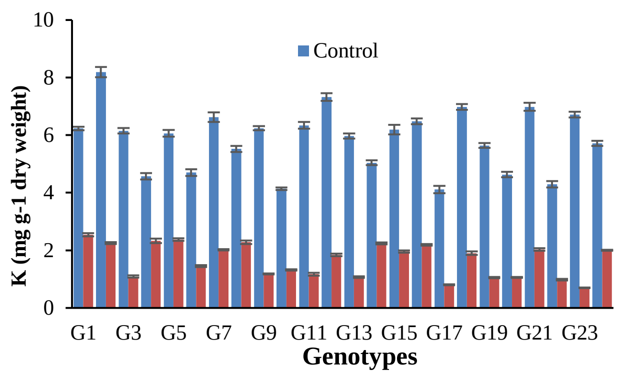

Figure-7: Concentration of potassium (K^+) ions of different tomato genotypes grown in control and salinity stress

Cell membrane stability index

Cell membrane stability index (CMSI) varied with the genotypes and salinity levels (Figure 11). Under salinity, all genotypes showed a significant reduction in CMSI values compared to controls. More than 60% reduction in CMSI was observed in the G3, G20, and G24 (salt-sensitive) genotypes compared to controls. On the other hand, below 25% reduction in CMSI was observed in G4, G5, G14, and G16 (salt-tolerant) genotypes which were 18, 22, 14, and 15%, respectively.

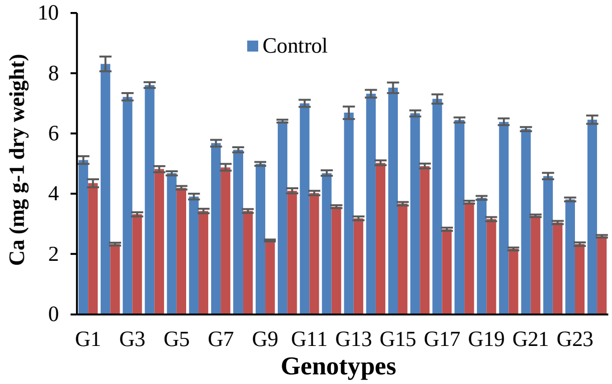

Figure-8: Concentration of calcium (Ca^{2+}) ions of different tomato genotypes grown in control and salinity stress

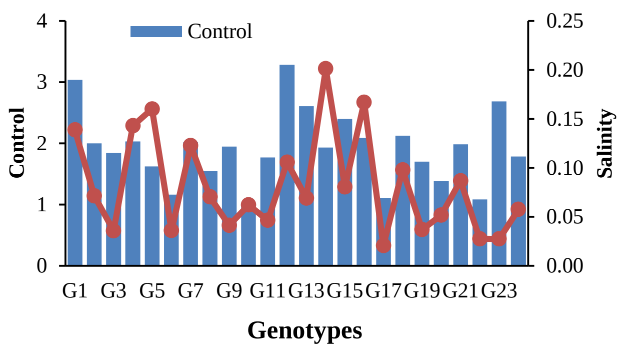

Figure-9: The ratio of potassium: sodium (K^{+}: Na^{+}) ions of different tomato genotypes grown in control and salinity stress

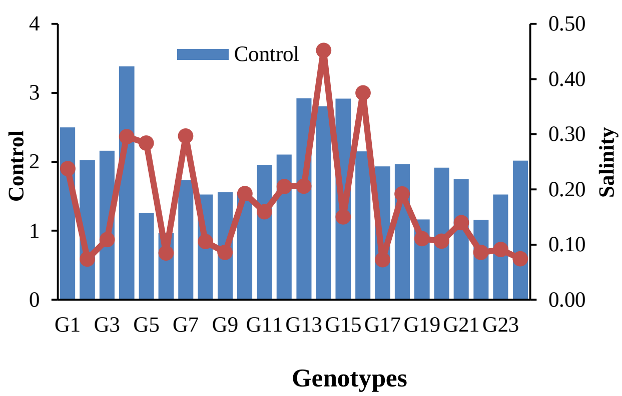

Figure-10: The ratio of calcium: sodium (Ca^{2+}: Na^{+}) ions of different tomato genotypes grown in control and salinity stress

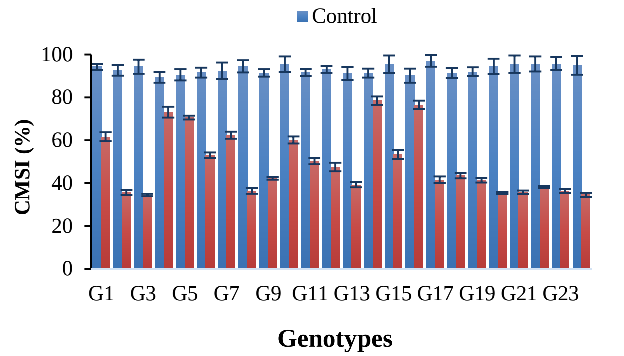

Figure-11: Cell membrane stability index of different tomato genotypes grown in control and salinity stress

Ranking and grouping of the genotypes

Salinity-tolerant genotypes were identified by ranking and multivariate cluster analysis based on the morphological parameters (Table 4 and Figure 12). Each of the 24 tomato genotypes was scored based on a combination of the relative values of all morphological traits. The best five rankings were observed in G1, G4, G7, G14, and G16 genotypes with their rank mean 6.0, 1.8, 4.0, 1.2, and 3.2, respectively. On the other hand, the worst five rankings 20.4, 20.2, 18.2, 17.6, and 17.4 were recorded in G24, G20, G2, G9, and G21 genotypes, respectively. Results also revealed that the higher five topmost standard deviations of ranks were found in G19 (6.61), G24 (5.94), G12 (5.85), G23 (5.74), and G18 (5.50) while, the lower five values of 0.45, 0.46, 0.45, 0.55, and 0.71 were found in G14, G4, G16, G5, and G7 genotypes, respectively. However, best ranking of the rank sum (RS) was recorded in G14 (1.65), G4 (2.25), G16 (3.65), G7 (4.71), and G5 (5.95), while worst five RS in G24 (26.34), G20 (23.76), G19 (23.41), G9 (22.92), and G23 (22.7).

Furthermore, tomato genotypes were classified into four clusters based on the 0.877 phenotypic correlation coefficient using Ward's procedure and interval squared Euclidean distance subsequent discriminate analysis (Figure 12).

Table-4: Rank (R), rank mean (RM), standard deviation of ranks (SDR), and Rank sum (RS) of morphological traits of the tomato genotypes

Genotypes	RVRL	R	RVSL	R	RVTFW	R	RVTDW	R	RVLA	R	RM	RS	SDR
G1	69.68	4	31.34	7	16.12	5	21.07	7	14.35	7	6	7.41	1.41
G2	55.29	16	30.41	15	7.81	22	13.26	19	9.50	19	18.2	20.97	2.77
G3	58.90	11	27.55	20	8.07	21	13.57	17	10.01	17	17.2	21.10	3.90
G4	78.18	1	42.16	2	21.30	2	31.07	2	21.43	2	1.8	2.25	0.46
G5	62.41	6	33.25	5	12.90	6	23.34	5	15.08	5	5.4	5.95	0.55
G6	59.87	10	27.34	21	11.68	8	16.31	13	10.38	15	13.4	18.43	5.03
G7	67.41	5	33.53	4	17.19	4	28.70	4	16.37	3	4	4.71	0.71
G8	56.14	14	28.38	19	8.68	20	17.41	10	9.44	21	16.8	21.46	4.66
G9	50.12	22	29.85	18	9.22	17	12.21	22	13.63	9	17.6	22.92	5.32
G10	60.61	9	31.09	8	11.10	12	16.53	11	13.40	10	10	11.58	1.58
G11	61.59	7	30.26	17	10.12	15	14.36	15	11.89	11	13	17.00	4.00
G12	58.24	12	33.03	6	8.80	19	14.32	16	14.40	6	11.8	17.65	5.85
G13	56.26	13	24.64	23	10.33	13	12.61	21	11.45	12	16.4	21.58	5.18
G14	77.66	2	42.39	1	26.48	1	33.43	1	25.39	1	1.2	1.65	0.45
G15	61.15	8	30.59	14	10.30	14	18.29	9	9.62	18	12.6	16.70	4.10
G16	75.96	3	34.56	3	17.63	3	29.93	3	15.73	4	3.2	3.65	0.45
G17	54.60	17	30.70	12	11.27	10	21.80	6	10.20	16	12.2	16.69	4.49
G18	52.38	19	30.33	16	12.11	7	18.55	8	13.90	8	11.6	17.10	5.50
G19	49.11	24	30.92	11	6.04	24	16.36	12	11.18	13	16.8	23.41	6.61
G20	55.40	15	24.68	22	9.14	18	12.16	23	8.62	23	20.2	23.76	3.56
G21	49.65	23	30.70	12	9.66	16	15.21	14	8.95	22	17.4	22.28	4.88
G22	52.94	18	31.05	9	11.13	11	12.78	20	9.46	20	15.6	20.82	5.22
G23	52.03	20	21.73	24	11.31	9	13.50	18	10.91	14	17	22.74	5.74
G24	51.91	21	31.04	10	6.51	23	10.21	24	7.28	24	20.4	26.34	5.94

RVRL: Relative value of root length, RVSL: Relative value of shoot length, RVTFW: Relative value of the total fresh weight, RVTDW: Relative value of the total dry weight, RVLA: Relative value of leaf area.

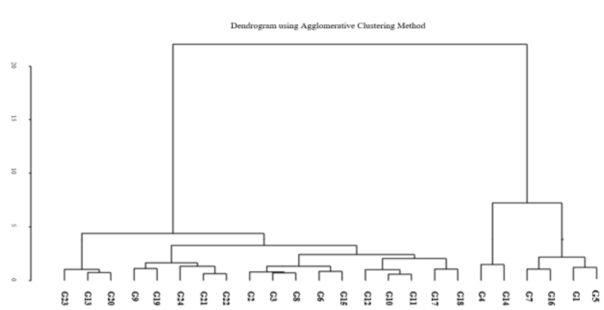

Figure-12: Dendrogram using agglomerative clustering method, summarizing data on variation among 24 tomato genotypes according to the performance of morphological traits under salinity stress at vegetative stage.

There were four genotypes (G1, G5, G7, and G16) in the first cluster; fifteen genotypes (G2, G3, G6, G8, G9, G10, G11, G12, G15, G17, G18, G19, G21, G22, and G24) in the second; two genotypes (G4 and G14) in the third; and three genotypes (G13, G20, and G23) in the fourth cluster. Mean values and standard deviation for relative values of morphological traits in four cluster groups are presented in Table 5. Cluster III recorded the highest values of the investigated metrics viz. root length (77.92), shoot length (42.28), total fresh weight (23.89), total dry weight (32.25), and leaf area plant^{-1} (23.41). However, cluster IV had the lowest values of root length (54.56), shoot length

(23.68), total fresh weight (10.26), total dry weight (12.76), and leaf area plant^{-1} (10.32). The values of all contributing morphological traits in clusters I and II were modest.

Table-5: Mean values and standard deviation for four clusters based on morphological traits of 24 tomato genotypes

Morphological traits	Cluster I	Cluster II	Cluster III	Cluster IV
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
RL	68.86±5.62	55.50±4.37	77.92±0.36	54.56±2.24
SL	33.17±1.34	30.22±1.46	42.28±0.16	23.68±1.69
TFW	15.96±2.14	9.50±1.85	23.89±3.66	10.26±1.08
TDW	25.76±4.24	15.41±2.93	32.25±1.67	12.76±0.68
LA	15.38±0.87	10.88±2.11	23.41±2.80	10.32±1.50

RL: Root length, SL: Shoot length, TFW: Total fresh weight, TDW: Total dry weight, LA: Leaf area.

Correlation among the morphological traits

There was a positive and strong correlation between studied morphological traits of tomato genotypes under different levels of salinity stress (Table 6). RL indicated positively and substantially correlated with SL (r=0.710), TFW (r=0.870), TDW (r=0.850), and LA (r=0.792). SL showed an almost similar correlation to RL. Total biomass production traits like TDW and TFW had also a strong and positive correlation with all the morphological traits, and the correlation of TFW than TDW was relatively strong among the other traits [RL (r=0.870), SL (r=0.725), TDW (0.895), and LA (r=0.894)]. Moreover, LA also showed positively and significantly correlated with RL (r=0.792), SL (0.797), TFW (r=0.894), and TDW (r=0.830).

Table-6: Correlation coefficients between five morphological traits of tomato genotypes in salinity stress

	RL	SL	TFW	TDW	LA
RL	1				
SL	0.710**	1			
TFW	0.870**	0.725**	1		
TDW	0.850**	0.779**	0.895**	1	
LA	0.792**	0.797**	0.894**	0.830**	1

RL: Root length, SL: Shoot length, TFW: Total fresh weight, TDW: Total dry weight, LA: Leaf area.

Discussion

Tomato genotypes responded differently to salt stress, depending on the characteristics of the genotype. Different crop varieties responded differently to salt tolerance at their different stages of growth. Salt stress significantly reduced growth-related traits root-shoot elongation, leaf area, and the total weight of biomass in all studied genotypes. Shoot and root length reduction under salt is a common feature of plants as roots experience to absorb salt in the soil firstly then supply the shoot (Abbas et al., 2018). In this study, genotypes G14 showed the least reduction of root and shoot length under salt stress over control. The leaf size is an important parameter to select salt-tolerant crops (Zhang et al., 2014). Reduced leaf area under salinity condition alters the cellular construction and net photosynthetic rate. Furthermore, biomass development under stress is a key indicator of stress tolerance (Gong et al., 2013). In this study, seedling growth was suppressed in salinized conditions and displayed various visual signs of salt injury. Genotypes showed variation in phenotypical observation ranging from scale 1 to 5. The genotype G14, G4, G16, and G7 were grouped on a scale of 1 and 2. Under salt stress, all tomato genotypes significantly reduced their fresh and dry biomass yield with the least decrease in genotypes G14 and G4, indicating salt tolerance. Salt tolerant plants express the least biomass loss and improved growth to salt stress than salt-sensitive ones (Chiconato et al., 2019). The content of Na^+ and Cl^- in tissues of plants exposed to high NaCl concentrations is one of the most injurious effects of salinity stress, In this study, a considerable amount of Na^+ and K^+ content was recorded in the genotypes G16, G14, G7, and G4. The concentration of Na^+ ion decreased against K^+ and Ca^{2+} ion in the plant tissues which revealed an inverse trend leading to improved K^+: Na^+ and Ca^{2+}: Na^+ ratios like salt tolerance ranks decreased in plants. Salinity tolerance in genotype was associated with Na^+ prohibition and improved absorption of K^+ and Ca^{2+} to sustain a helpful symmetry of K^+: Na^+ and Ca^{2+}: Na^+ ratios in the plants under salinity stress (Krishnamurthy et al., 2016). The ability of plants to reduce Na^+ uptake, and decrease K^+ efflux induced by NaCl has been accepted as the essential mechanism for salinity tolerance (Tang et al., 2019). Cell membrane stability (CMSI) index had been widely used as an indicator of salt injury and salt tolerance (Quan et al., 2021). It had been suggested that a decrease in CMSI reflects the extent of lipid peroxidation caused by reactive oxygen species (Su et al., 2019). CMSI value in this experiment differed with the salinity and genotypes which is the function of lipid peroxidation, lipoxygenase enzyme, and electrolyte leakage. Similar findings on decreased CSMI with salinity stress were reported in tomato leaves (Hoffmann et al., 2020; Tanveer et al., 2020). In this research, few parents were observed to show salt tolerant to some extent, but their hybrid did not show any tolerant to salt which might be due to lower effect of Na^+ accumulation mediated genes and general combining ability. Similar findings were reported by Munns et al. (2002); Yildirim and Bahar (2010).

The selection of salt-tolerant genotypes based on the calculated rank mean of a particular trait was inconsistent (Table 4). Therefore, the most desirable salt-tolerant tomato genotypes were selected by combining the rank mean of all morphological traits and the standard deviation of ranks of all criteria. Results revealed that the genotypes G14, G4, G16, G7, G1, and G5 showed the best of ranks mean and low standard deviation of ranks in stress condition. Hence, they were identified as the most salt-tolerant genotypes, while genotypes G24, G20, G23, and G22 as the most susceptible. Several studies also employed ranking scores to select suitable genotypes under different abiotic stresses (Farshadfar, 2012; Aslam et al., 2017). Cluster analysis indicated that seedlings within group had closer variance and genetic distance, whereas seedlings between groups had difference with greatest genetic distance (Figure 12). The measurement of the value of each cluster regarding observed traits mean deviation percent of each cluster was determined from the total mean (Table 5). In this study, the cluster 3 included G4 and G14 genotypes which had the maximum mean values in their tested traits. The cluster 1 comprised G1, G7, G5 and G16 genotypes showed moderate mean values and conveyed comparatively moderate ranking score, while the cluster 4 involved G13, G20, and G23 tomato genotypes which contained lowest mean value and bottom-ranking score regarding their tested morphological traits (Figure 12 and Table 5). As it is clearly found in Figure 12, salt tolerant (G4 and G14) and moderate tolerant (G1, G7, G5 and G16) tomato genotypes grouped in cluster 3 and cluster 1, respectively, whereas the sensitive genotypes clustered together in cluster 4. Similarly, multivariate cluster analysis has already been extensively studied as an efficient index for salt tolerance categorization in several crop species (Zafar et al., 2015; Al-Ashkar et al., 2019).

The information about the significant correlation among the attributes is important for the development of any breeding project because it contributes to select the suitable genotypes with desirable traits concurrently (Ali et al., 2009). In this study, a significant and positive correlation was observed among the morphological traits (RL, SL, TFW, TDW, and LA), which indicated that an increase in one feature might lead to an increase in other attributes. Therefore, RL, SL, TFW, TDW, and LA morphological traits can be used to screen out salt-tolerant genotypes. The observed relations were consistent with Zafar et al. (2015) who reported positive and significant correlations among different physiological traits that can be used to select the wheat genotypes for salt tolerance.

Conclusion

The results reflected that salt stress has a major impact on morpho-physiological traits of the tomato genotypes at the early vegetative stage. Most of the examined parameters showed a great variation among the studied genotypes, where shoot length reduction was more pronounced than the root in tomato seedlings. Salt injury and Na^+ uptake scores that were lower in tomato seedlings suggest that they are more tolerant to salinity stress and give a simple and effective approach to measure salt-induced damages. The morphological traits were positive and significantly related to each other and the genotypes selected from cluster analysis based on these traits were also salt tolerant. Based on the growth and morphological parameters, genotypes G4, G7, G14, G16 performed better. Among these genotypes, G14 proclaimed the best performance under normal and salinity conditions at early vegetative stage. However, physiological and biochemical responses of selected genotypes can be tested further under hydroponic culture at the reproductive stage to confirm their potentiality against salinity stress.

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Contribution of Authors

Ahsan AFMS: Conducted research work, laboratory work and manuscript write up.
Talukder AMMR: Carried out laboratory work and data collection and analysis.
Mahfuza SN: Designed research methodology, carried out laboratory work and data collection.
Ahmed F & Hassan AK: Supervised experiment and approved manuscript.
Haque MA: Statistical analysis and manuscript write up and editing.
Goffar MA: Provided experimental inputs in research and write up.
Masud MM: Data collection and laboratory analysis.