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EVALUATION OF TOMATO GERMPLASM FOR SALINITY TOLERANCE AT THE SEEDLING STAGE

N. FATIMA[1*], A. SAEED[1*], A. SHAKEEL[1], and F.S. AWAN[2]

[1]Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan
[2]Centre of Agriculture Biochemistry and Biotechnology, University of Agriculture Faisalabad, Pakistan
*Corresponding authors' email: norfatima44@yahoo.com, drasifpbg@gmail.com
Email addresses of co-authors: dramirpbg@gmail.com, awanfaisal@yahoo.com

SUMMARY

Salinity consists of critical abiotic stress adversely affecting tomato growth and development. Given the increase in saline areas, breeders endeavor to develop crops that can tolerate salinity. It indicates the importance of genotypes that can grow in salt-affected soil to cope with the problem. This study focused on identifying salt-tolerant and salt-sensitive genotypes using Principal Component Analysis (PCA). This study used a two-factor factorial under a complete randomized design, with three replications and three levels (T_0 = control, T_1 = 6 dS/m, T_2 = 12 dS/m) of salt (NaCl) treatment. Data collection ensued at the seedling stage. Data for various morphological and biochemical attributes were recorded and subjected to analysis of variance and PCA to check the variation in germplasm and identification of suitable genotypes. Analysis of variance showed significant results for all attributes indicating the presence of variability in germplasm. Using PCA identified tolerant and non-tolerant tomato genotypes. Based on the results obtained from PCA analysis, genotypes AUT-318, CLN-2498-A, 17884, Picendanto, 17260, 17256, 17263, and 17266 showed as salt tolerant, whereas the 19903, 19908, Target-66, H-24, 17255, Nadir, and Peelo displayed as salt-sensitive genotypes. Selected genotypes suit further use for the development of breeding material.

Keywords: Tomato germplasm, salinity, principal component analysis, osmolytes, screening, selection

Key findings: Based on results obtained from the analysis, the genotypes AUT-318, CLN-2498-A, 17884, Picendanto, 17260, 17256, 17263, and 17266 proved salt-tolerant. These selected genotypes suit future use for salt-tolerant varieties and hybrid development.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the second most important vegetable after potato (Quinet *et al.*, 2019). It belongs to the family Solanaceae which includes 3,000 species (Iqbal *et al.*, 2019). It is a diploid plant with 12 pairs of chromosomes (Salava *et al.*, 2021). Its genome size measures approximately 900 Mb. It is a self-pollinating crop. It is known as a "protective food" because of its enriched vitamins, minerals, and various antioxidant contents

In tomatoes at the pre-flowering stage,

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the salinity of 6 dS/m and 12 dS/m reduce the plant height, flower cluster, primary branches, fruit cluster, number of fruits, and fruit yield plant^{-1}, and in leaves, it reduces the content of the amino acid and increase the total and reducing sugars (Moniruzzaman *et al.*, 2013; Rai *et al*., 2016). At salinity stress, the tomato plant reduces the dry biomass of roots, leaves, stems, and fruits (Ladewig *et al.*, 2021). Salinity causes a considerable reduction in the yield of tomato crops. Salt stress causes biochemical and physiological changes in tomato plant growth. Osmotic and ionic stresses caused by the salinity stress affect the tomato plant at the cellular level. The tomato plant is sensitive to salt stress, with yield reduction observed above EC of 2.5 dS m^{-1} (Siddiky *et al.*, 2012; Ladewig *et al.*, 2021). At the global level, salinity affects irrigated and dryland agriculture, estimating 19.5% and 2.1%, respectively. About 326.6 million ha of land in Asia is salt-affected, with 4.2 million ha of agricultural land in Pakistan affected by salinity. More than 60% of its soil area is sodic soil, which causes more salinity in Pakistan (Rehman *et al.,* 2021). The reasons for lower production consist of the lack of good quality seeds, high temperature at the time of harvesting, salinity, and frost during the early-growth stages of the tomato plant. In tomatoes, above 5 dS/m caused a 7.2% yield reduction observed per unit increase in salinity (Anjum *et al.*, 2019). Salt-affected soils are the saline or sodic soils that include about 6% of total land. About 45 m/ha of irrigated land and 32 m/ha of dry land are salt-affected (Tahir *et al.*, 2018). According to one estimation, about 316.5 million ha of land is salt-affected in Asia, while only 4.2 million ha of agricultural area in Pakistan harbored salinity. Moreover, Pakistan`s total soil area consists of sodic soil (>60%), which causes more salinity stress (Plaut, 1995; Raza *et al.*, 2017). In the world, 50% of soil is sodic and saline-sodic (Rehman *et al.*, 2021).

Genetic characterization of germplasm is the first step in breeding for the development of hybrids and cultivars (Tahir *et al.*, 2018; Al-Khayri *et al*., 2022). Germplasm evaluation can use multivariate analysis. PCA (principal component analysis) is an analytical breeding technique for evaluating and identifying genotypes that have variability for a large number of observations, which is a tedious task if done through manual selection (Mukul *et al.*, 2022). The seedling and germination stage of tomato is selected as the most sensitive stage for salinity (Rehman *et al.,* 2021). The genotypes, selected through the PCA based on various morphological (plant length, shoot length, root length, root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight) and biochemical attributes (Na^+ content, K^+ content, and Na^+/K^+ ratio), can help further in the hybridization program for the development of improved hybrids and genotypes, including their characteristics (Sinha *et al.*, 2021). It is easy to select genotypes through PCA analysis on morphological and physiological attributes because these characters show the level of tolerance under salinity stress. The latest research focuses on tomato germplasm screening against salt stress and selected genotypes utilization for hybrid development. Therefore, this study aimed to a) evaluate the tomato germplasm for various morphological and biochemical attributes against salinity and b) select different genotypes more tolerant to salt stress.

Salinity affects the tomato yield and quality, assessed by various morphological, physiological, and biochemical parameters. Using different biometrical techniques (PCA) can provide access to the variability in germplasm based on traits that are affected by salinity.

MATERIAL AND METHODS

Collection of germplasm

Germplasm of *Solanum lycopersicum* L. consists of 101 genotypes collected from the Plant Genetic Resources Institute, NARC Islamabad, Nuclear Institute for Agriculture and Biology, and Ayub Agricultural Research Institute, Faisalabad, Pakistan.

Experimental conditions

A research experiment on tomato germplasm screening against salt stress proceeded in the greenhouse of the Faculty of Agriculture, University of Agriculture Faisalabad, Pakistan.

Germplasm screening against salt stress

The germplasm screening experiment proceeded in a two-factor factorial under a completely randomized design with three repetitions. Sowing in the nursery took place in Oct 2019 in the nursery growing area of the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan, and transplanted into polythene bags at the three-leaf stage. The bags, filled with sand,

received a Hoagland Solution of 200 ml (Hoagland and Arnon, 1950) application per bag on alternative days. Application of stress proceeded after one week of nursery transplantation, with three levels of salt treatment, T_0 = control, T_1 = 6 dS/m, and T_2 = 12 dS/m (above threshold). The latter two levels are above the threshold level. Recording of the date of five plants per genotype per treatment per replication occurred after 40 days of stress for the following attributes.

Morphological attributes

The uprooted seedlings underwent washing and cleaning (with tissue paper) to remove the sand from the roots. The measurement of roots and shoot length used a measuring scale. The fresh weight of shoots and roots of uprooted seedlings received measuring with an electronic balance. For dry weight, roots and shoots were dried in the oven at 65°C for three to four days and ground to measure dry weight.

Biochemical attributes

The Na^+ and K^+ concentrations in shoot and root samples gained measurement using the wet digestion method. The placing of 0.5 g of the dry weight of the sample (root and shoot separately) proceeded into a 50ml flask. Adding 5ml of HNO_3 and $HClO_4$ (3:1) took place, then covering the flask with aluminum foil and left overnight. The next day the sample underwent heating on a hotplate until the formation of fumes began, and it turned colorless. Adding distilled water increased the extract volume to 50ml, then filtered with Whatman filter paper. The concentration used a flame photometer for measurement (Jenway Model UK, 1998).

The following data to calculate indices used the succeeding formula (Wu *et al.,* 2019):

Salt-tolerance index = Value for the NaCl-treated plant/Value for the control

Biometrical analysis

Analyzing recorded data used the analysis of variance (Steel *et al.*, 1997). Salinity-tolerant and salinity-susceptible genotypes identification used principal component analysis (PCA) given by Jolliffe (2002).

RESULTS AND DISCUSSION

Analysis of variance

All genotypes showed highly significant results for all morphological and biochemical attributes. The notable results showed the genetic variation among genotypes for salt stress (Table 1). Genotype × treatment mean square showed significant results (α = 0.01) for all the attributes. Relevant results for morphological traits (root length, shoot length, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, and dry mass ratio) and biochemical attributes (K^+/Na^+ ratios and chlorophylls [a,b]) were also reported by Alam *et al.* (2021) against salinity. Devi and Arumugam (2019) calculated the data at the germination and seedling stage and showed significant results for the germination %, shoot length, seedling length, and root length.

Table 2 presents the descriptive statistics of characters under salinity. Standard deviation measures the amount of variability within a sample. Reduction in shoot length and shoot fresh weight resulted in plants under salinity. Mean values for shoot length showed 41.51cm under control treatment, but under stress conditions, T_1 and T_2, values showed 39.55 and 39.71 cm, respectively. The standard deviation was 7.6, 8.2, and 7.2 for T_0, T_1, and T_2, respectively. The mean values for shoot fresh weight under normal conditions achieved 1.3 g, but under stress conditions T_1 and T_2, mean values revealed 5.33 g and 4.93 g, respectively. The standard deviation for shoot fresh weight indicated 1.3, 2.2, and 1.8 for T_0, T_1, and T_2, respectively. The shoot dry weight was not so much affected by salinity. The experiments conducted by Habibi *et al.* (2021) and Alam *et al.* (2021) also showed a reduction in tomato shoot length under high salt stress. Kayess *et al.* (2020) also observed a decrease in shoot fresh weight with a rise in salinity level. Kadoglidou *et al.* (2021) observed a significant reduction in shoot fresh weight and shoot dry matter content under salt stress. Roots play an essential role in plant growth and development. Genotypes with longer roots can withstand salt stress. The root and shoot lengths declined during the salt stress, yet the roots showed more damage. Root length under normal conditions displayed a mean value of 26.67 cm, but under stress conditions, T_1 and T_2, at 22.6 cm and 28.08 cm, respectively. The mean value for root fresh

Table 1. Analysis of variance for different morphological and biochemical traits under salt (NaCl) stress conditions.

SOV	d.f.	SL	SFW	SDW	RL	RFW	RDW	K shoot	K root	Na Root	Na Shoot	PFW	PDW	PL	Na/K root	Na/K shoot
Genotypes	100	294.42**	18.34**	0.24**	252.41**	5.61**	0.09**	5.94×10^{8}**	4.7×10^{8} **	5.58×10^{9}**	2.3×10^{9}**	39.17**	0.58**	720.31**	3587.91**	959.84**
Treatments	2	357.28**	46.77**	0.48**	3177.76**	8.48**	0.26**	3.03×10^{9}**	1.2×10^{8} **	3.81×10^{10} **	2.7×10^{10} **	27.85**	0.05**	4089.94**	1396.68**	709.43**
Gen × Treat	200	119.62**	6.40**	0.09**	128**	1.95**	0.05**	5.43×10^{8}**	2.8×10^{8} **	5.36×10^{9} **	2.1×10^{9} **	11.22**	0.22**	286.44**	3562.18**	989.34**
Error	606	28.22	0.50	0.0036	41.63	0.20	0.0034	4366007	1.7×10^{7}	1.62×10^{9}	4.6×10^{8}	0.73	0.0068	83.20	285.75	42.30

**, * = Significant at 1% and 5% probability levels, respectively, SOV = Source of variation, DF = Degree of freedom, SL = Shoot length, SFW = Shoot fresh weight, SDW= Shoot dry weight, RL = Root length, RFW = Root fresh weight, RDW= Root dry weight, K shoot = K^+ concentration in shoot, K root = K^+ concentration in root, Na root = Na^+ concentration in shoot, Na shoot = Na^+ concentration in shoot, PFW = Plant fresh weight, PDW= Plant dry weight, PL = Plant length, Na/K root = Na^+/K^+ ratio in the root, Na/K shoot = Na^+/K^+ ratio in the shoot

Table 2. Descriptive statistics for different morphological and biochemical traits under salinity.

Traits	T_0 = Control				T_1 = 6 dS/m				T_2 = 12 dS/m			
	Minimum	Maximum	Mean	Std. deviation	Minimum	Maximum	Mean	Std. deviation	Minimum	Maximum	Mean	Std. deviation
SL (cm)	24.00	63.67	41.51	7.60	13.00	56.00	39.55	8.24	22.67	52.67	39.71	7.22
SFW (g)	1.62	7.37	4.54	1.36	0.58	11.07	5.33	2.26	1.47	14.50	4.93	1.86
SDW(g)	0.11	0.91	0.43	0.17	0.05	1.21	0.49	0.25	0.10	1.41	0.51	0.22
RL (cm)	13.33	46.00	26.67	6.45	5.00	44.33	22.43	8.37	5.33	48.00	28.80	7.60
RFW(g)	0.34	4.11	1.76	0.84	0.09	5.43	1.53	1.07	0.17	7.19	1.86	1.13
RDW(g)	0.02	0.73	0.25	0.16	0.005	0.73	0.20	0.15	0.02	0.70	0.20	0.12
K^+ Shoot (mg/L)	215.05	56129.03	30273.86	11334.59	2365.59	81058.72	36112.62	14246.63	160.50	84086.02	35300.74	15113.70
K^+ Root (mg L^{-1})	215.05	61505.37	11791.93	9711.12	215.05	64467.00	10978.73	11340.34	215.05	48362.94	12221.53	10990.92
Na^+ Root (mg L^{-1})	5279.83	110490.87	32576.89	16569.37	3011.45	622058.00	51210.17	63727.23	633.57	260618.32	52697.38	33159.96
Na^+ Shoot (mg L^{-1})	633.57	96051.09	51568.38	16850.30	8935.11	149324.69	65435.17	19901.71	19265.85	405917.38	69734.65	38797.83
Na^+/K^+ Root	0.51	226.02	13.64	35.48	1.16	192.27	17.19	35.09	0.01	190.24	17.50	32.86
Na^+/K^+ Shoot	0.86	250.60	4.37	24.80	0.97	12.32	2.08	1.25	0.62	148.31	4.98	19.04
PFW (g)	2.72	11.48	6.30	1.91	0.73	15.31	6.86	3.10	1.93	19.74	6.79	2.68
PDW (g)	0.14	1.64	0.69	0.31	0.11	1.64	0.69	0.37	0.17	1.84	0.71	0.31
PL (cm)	41.00	96.33	68.18	10.73	18.00	89.67	61.99	14.16	36.17	93.17	68.51	10.72

weight under normal conditions scored at 1.7 g, but under stress conditions, T_1 and T_2, values bared 1.5 g and 1.8 g, respectively. As for the root dry weight under normal conditions, the mean value achieved 0.25g, but with T_1 and T_2 stress conditions gave scores at 0.205g and 0.201g, respectively. The standard deviation values for root length under T_0, T_1, and T_2 consisted of 6.45, 8.37, and 7.60, respectively. The standard deviation for root fresh weight under T_0, T_1, and T_2 included 0.84, 1.07, and 1.13, respectively, whereas for root dry weight under T_0, T_1, and T_2, 0.16, 0.15, and 0.12, respectively. Devi and Arumugam (2019) reported a reduction in root length. An increase in the root thickness and number of lateral roots resulted more comparatively to shoots when applied with salt stress (Colla *et al.,* 2010). Salt stress caused the reduction in root fresh weight and dry weight reported by Kayess *et al.* (2020). Kadoglidou *et al.* (2021) further reported that the decline in tomato root fresh weight and root dry matter occurred from the salinity effect. Plants under saline conditions did not grow properly. Salinity caused the restriction in plant growth, especially in roots. The mean value of 30273.86mg/L showed for K^+ content in shoot under normal conditions but scored 36112.624mg/L and 35300.74mg/L under stress conditions, T_1 and T_2, respectively. The K^+ content in root under normal conditions, mean value is 11791.93mg/L, but for T_1 and T_2 stress conditions, at 10978.73mg/L and 12221.53mg/L, respectively. The Na^+ content in root under normal conditions gave a mean value of 32576.89mg/L, but, under T_1 and T_2 stress conditions, values disclosed 51210.177mg/L and 52697.38mg/L, respectively. For the shoot, Na^+ content under normal conditions showed a value of 51568.38mg/L, and under stress conditions, T_1 and T_2, scored 65435.17mg/L and 69734.65mg/L, respectively. The standard deviation for K^+ contents in shoot under T_0, T_1, and T_2 are 11334.59, 14246.63, and 15113.7. For root, values under T_0, T_1, and T_2 showed 9711.12, 11340.34, and 10990.92, respectively. The standard deviation for Na^+ contents in root under T_0, T_1, and T_2 are 16569.37, 63727.23, and 52697.38, while in the shoot, these are 16850.30, 19901.71 and 38797.83, respectively. Rahman *et al.* (2021) also observed an increase in Na^+ contents in roots and shoots in tomato plants. As the salinity level intensified, Na^+ increased in the roots and shoot, which reduced the K^+ contents in the roots and shoot. Reduction in K^+ content in the shoot happened in genotypes exposed to salt stress (Raza *et al.,* 2017; Rahman *et al.,* 2021). Tandra *et al.* (2022) further reported as K^+ content decreased in roots, tomato plants showed a high amount of Na^+ content in the root and shoot, where they observed restricted growth. Higher concentrations of Na^+ in the shoot or root increase as the salinity escalate, which affects the osmotic potential and decreases the water uptake, but little changes occur in K^+ concentration as compared with Na^+ under a saline environment (Singh *et al.,* 2012). The Na^+/K^+ ratio in root under normal conditions gave a mean value of 13.64, but under stress conditions, T_1 and T_2, the values were 17.19 and 17.50, respectively. The Na^+/K^+ ratio in shoot under normal conditions showed a mean value of 4.37, and under stress conditions, T_1 and T_2 scored mean values of 2.08 and 4.98, respectively. The standard deviation of Na^+/K^+ root ratio of tomato plants under T_0, T_1, and T_2 showed 35.48, 35.09, and 32.86, respectively, while under T_0, T_1, and T_2, the deviation showed 24.80, 1.25, and 19.04, respectively. Studies reported observations of the increase in Na^+/K^+ root and Na^+/K^+ shoot ratio under the saline condition in tomato plants (Singh *et al.,* 2012: Raza *et al.,* 2017: Rahman *et al.,* 2021: Tandra *et al.,* 2022). Plant fresh weight under normal conditions showed a mean value of 6.30 g, but under stress conditions, T_1 and T_2 provided mean values of 6.86 g and 6.79g, respectively. Plant dry weight under normal conditions revealed the mean value of 0.69 g, but under stress conditions, T_1 and T_2 scored values of 0.69 g and 0.71 g, respectively. Plant length under normal conditions had a mean value of 68.18 cm, but T_1 and T_2 stress conditions showed mean values of 61.99 cm and 68.51 cm, respectively. Kadoglidou *et al.* (2021) reported a significant reduction in shoot length, leaf number, and shoot thickness at exposure to salt stress.

Analysis of variance showed the presence of variation for all the characters. Salinity caused the reduction in shoot, root, and plant length. Salinity-sensitive genotypes showed more reduction in root and shoot (fresh and dry) weight as compared with salinity-tolerant genotypes. The salinity-sensitive genotypes displayed a higher Na^+ content and low K^+ content in the roots and shoots. Inversely, the salinity-tolerant genotypes presented a higher K^+ content and low Na^+ content in the roots and shoots.

Principal component analysis

The principal component analysis is a non-parametric method. Its objective seeks to obtain a small number of factors that account for the maximum variation out of the total variation. Biplots of T_0, T_1, and T_2, i.e., PCA1 for T_0 and PCA2 for T_1 and PCA3 for T_3, are presented in Figures 1, 2, and 3, respectively. The scientists also used the principal component analysis to analyze the data. Based on PCA of 15 traits, tomato germplasm under normal conditions showed 100% diversity (Kadoglidou *et al.,* 2021: Habibi *et al.,* 2021: Tandra *et al.,* 2022). However, five PCs had >1 Eigenvalue, which signifies the maximum variability among variables with the 76.23% diversity percentage (Table 4), thus, further explaining these five PCs. PC1 showed a 31.54% variation, while PC2, 3, 4, and 5 showed 16.7%, 11.21%, 8.81%, and 7.95% of total variability, respectively.

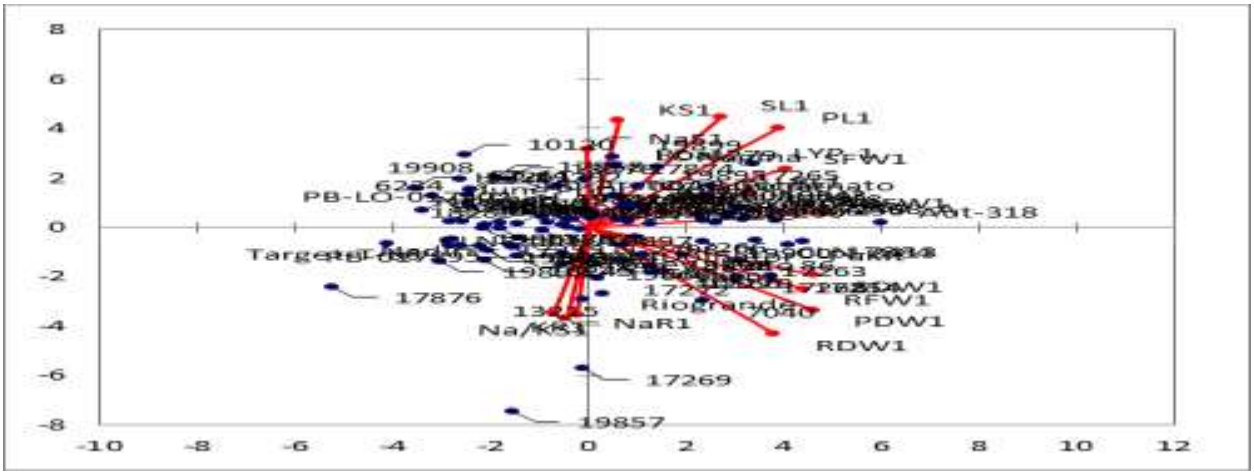

Figure 1. PCA1 of germplasm under normal conditions.

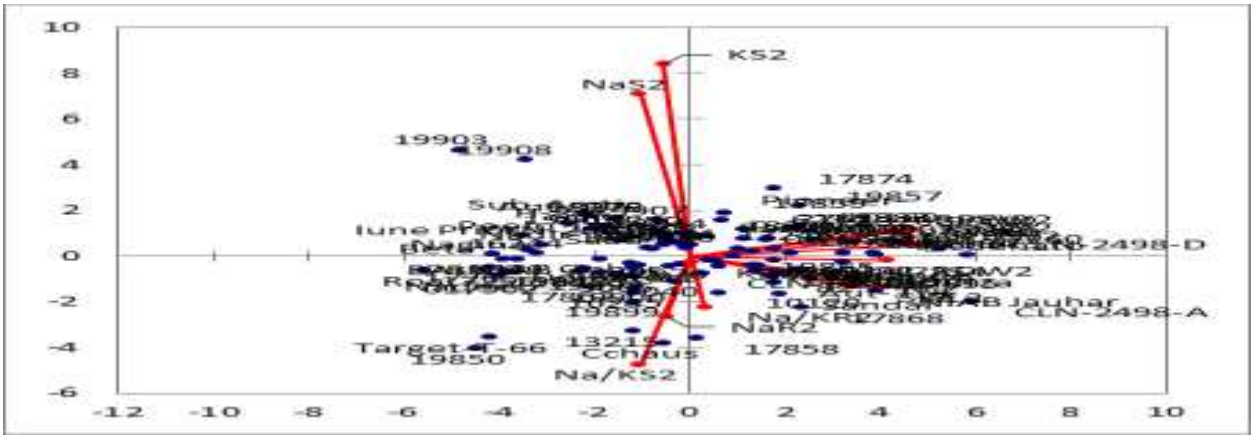

Figure 2. PCA2 of germplasm under 6dS/m.

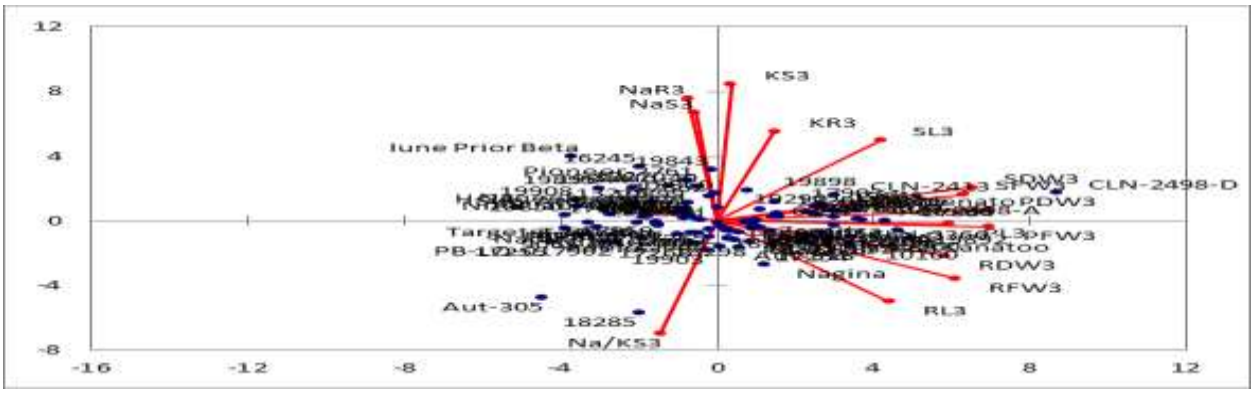

Figure 3. PCA3 of germplasm under 12dS/m.

Brejda *et al.* (2000) reported that Eigenvalue >1 showed at least a 10% variation. So calculating for higher Eigenvalues served as a suitable representative of system character in PC. Table 3 presents the eigenvalue of the principal component of germplasm under normal condition. Bases on PC with 15 attributes of 100% variation, it formed the 13-principal component. Put of 13 principal component, five PCs showed the eigenvalue more than one, which signifies the maximum variation among the variables. These five PCs represented the 76.23% variation (Table 3). In PCA1, quadrant I contained the characters K^+ shoot, shoot length, plant length, shoot fresh weight, root length, and plant fresh weight. Quadrant II included Na^+/K^+ root and Na^+ shoot. Quadrant III has Na^+ root, K^+ root, and Na^+/K^+ shoot, but in Quadrant IV, shoot dry weight, root fresh weight, plant dry weight, and root dry weight were present. In PCA1, genotypes Aut-318, CLN-2498-A, Picendanto, 17256, 19843, Lyp-1, 17260, and 17868 comprised quadrant I, and performance proved better for most of the morphological character under normal conditions. But the genotypes Target-T-66, PB-017895, Nadir, 17876, and 19892 present in quadrant III showed poor performance for most of the morphological attributes and are found as salinity-susceptible lines.

Table 4 presents the eigenvalue of the principal component of germplasm under saline conditions (T_1 = 6dS/m). Based on PC with 15 attributes of 100% variation, it formed 13 principal components. Out of 13 principal components, four PCs showed the eigenvalue >1, which signifies the maximum variability among the variables. These four PCs showed 74.85% diversity (Table 4). In PCA2, quadrant I consisted of root fresh weight, plant fresh weight, shoot fresh weight, shoot dry weight, and plant dry weight. Meanwhile, quadrant II held Na^+ shoot and K^+ shoot. The Na^+ root and Na^+/K^+ shoot were in quadrant III, but root dry weight, root length plant length, shoot length, K^+ shoot, and Na^+/K^+ root were in quadrant IV. In PCA2, the genotypes CLN-2498-D, 17260, Picendanto, Legend, 17884, 17263, 17266, 17260, and Peto 86 in quadrant I achieved selection as salinity tolerant lines, but genotypes Target-T-66, 19850, 17255, 17265, Roma, 19842 and Cchaus from quadrant III showed as salt susceptible genotypes (Figure 2).

The Eigenvalue of the principal component of germplasm under saline conditions (T_2 = 12 dS/m) displays in Table 5. Based on PC with 15 attributes of 100% variation, it formed 14 principal components. Out of 14 principal components, five PCs showed the Eigenvalue >1, which signified the maximum variability among the variables. These five PCs showed a 75.72% diversity (Table 5). PCA3, quadrant I included plant dry weight, shoot fresh weight, shoot dry weight, shoot length, K^+ root, and K^+ shoot. Quadrant II of PCA3 contained Na^+ root, Na^+ shoot, and Na^+/K^+ root. Quadrant III has only one trait– Na^+/K^+ shoot. Quadrant IV consisted of the plant length, plant fresh weight, root dry weight, root fresh weight, and root length. In PCA3, the genotypes CLN-2498-D, 19907, CLN-2498-A, 17261, 18278, 17876, Picendanto, CLN02413, 17884, 17266 and 19868 from quadrant I underwent selection as salt tolerant genotypes, but in quadrant III, Target-T-66, 17255, PB-LO-017902, Nadir, Riogrande, 17265, 19888, 17268, 18298 and 16244 revealed as salinity-sensitive genotypes (Figure 3).

Table 3. Eigenvalue and contribution of the principal component axes toward variation in germplasm under controlled conditions (T_0 = control).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13
Eigenvalue	4.7312	2.5051	1.6823	1.3227	1.1940	0.9935	0.8701	0.8144	0.3233	0.2601	0.1712	0.1319	0.0002
Variability (%)	31.5415	16.7009	11.2153	8.8178	7.9598	6.6233	5.8007	5.4294	2.1556	1.7342	1.1412	0.8794	0.0010
Cumulative %	31.5415	48.2423	59.4577	68.2755	76.2353	82.8586	88.6593	94.0886	96.2442	97.9784	99.1196	99.9990	100.0000

Table 4. Eigenvalue and contribution of the principal component axes toward variation in germplasm under saline conditions (T_1 = 6dS/m).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13
Eigenvalue	6.1718	1.9934	1.6125	1.4513	0.9534	0.8841	0.6908	0.4716	0.3339	0.2245	0.1238	0.0884	0.0006
Variability (%)	41.1453	13.2896	10.7497	9.6752	6.3557	5.8941	4.6054	3.1438	2.2261	1.4967	0.8251	0.5894	0.0038
Cumulative %	41.1453	54.4349	65.1846	74.8598	81.2155	87.1096	91.7150	94.8588	97.0849	98.5817	99.4068	99.9962	100.000

Table 5. Eigenvalue and contribution of the principal component axes toward variation in tomato germplasm under saline conditions (T_2 = 12dS/m).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14
Eigenvalue	5.702	1.89	1.6012	1.1448	1.0137	0.9655	0.7624	0.6367	0.4506	0.3457	0.3058	0.1750	0.0001	0.0000
Variability (%)	38.013	12.645	10.6748	7.6318	6.7578	6.4368	5.0825	4.2447	3.0040	2.3044	2.0386	1.1665	0.0004	0.0000
Cumulative %	38.013	50.658	61.332	68.964	75.722	82.159	87.241	91.486	94.490	96.794	98.833	99.999	100.000	100.000

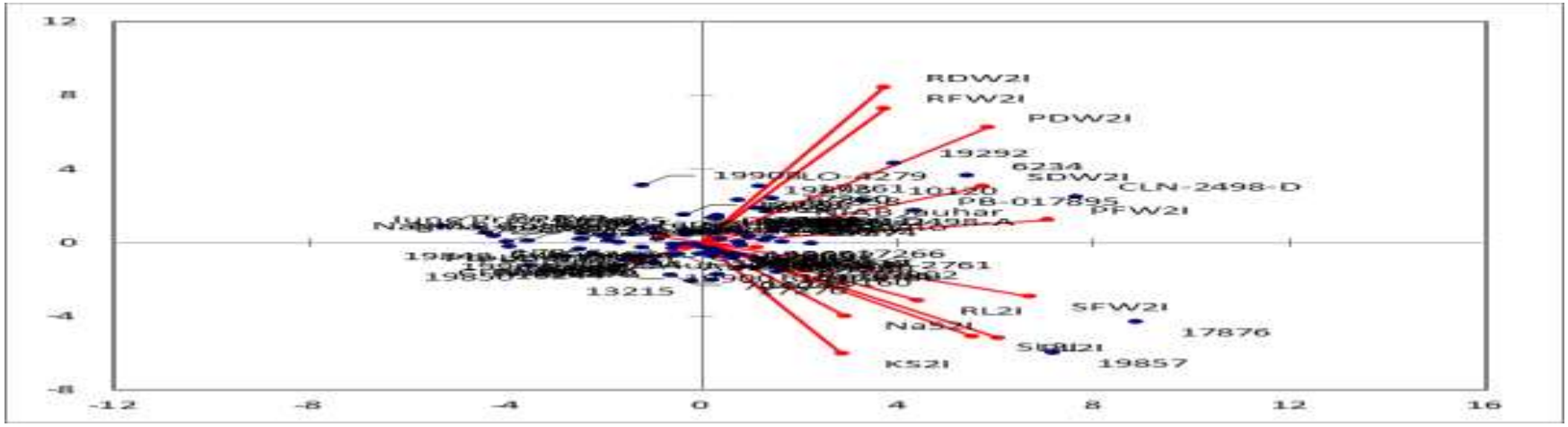

Figure 4. PCA4 of tomato germplasm for stress indices of T2 (6dS/m).

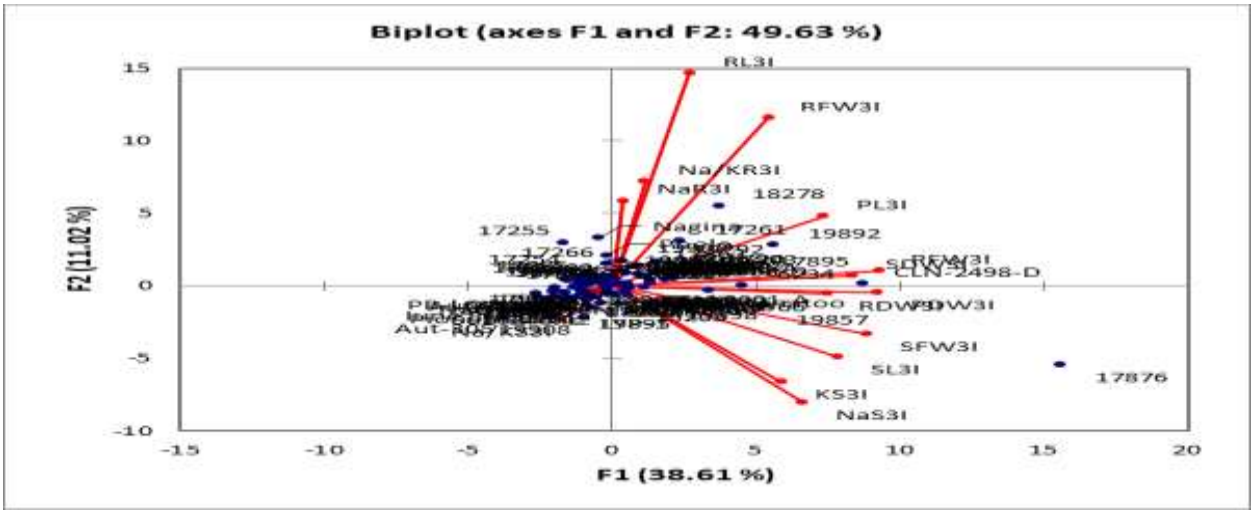

Figure 5. PCA5 of tomato germplasm for stress indices of T3 (12dS/m).

PCA of salt-tolerance index

Biplots for T2 and T3 indices, i.e., PCA4 for T2 and PCA5 for T3 indices, are presented in Figures 4 and 5, respectively. In PCA4, the genotypes–19292, CLN-2493-D, PB-017895, 10120, LO-4279, 19895, 17261, 17260, 17858, and 6234 present in Quadrant I–all the indices had positive responses toward salinity tolerance for the root dry and fresh weight, plant dry and fresh weight, and shoot dry weight. In Quadrant II inclusive of 19908, Roa, NIAB Gohar, Nagina, 17255, Lune Prior Beta, and 17265 genotypes, the indices of Na^+ content in root showed the negative response toward salinity tolerance. In Quadrant III, containing genotypes 19843, 19850, Peelo, 19903, 19900, Aut-305, and Aut-318, the Na^+/K^+ root ratio and Na^+/K^+ shoot ratio showed a negative response toward salinity tolerance. In quadrant IV, present with 17876, 19857, 17266, 17902, 17270, 16245, 10160, Peto-86, and Riogrande genotypes, the K^+ content in root and shoot, shoot fresh weight, root length, shoot length, and plant length showed the positive response toward salinity tolerance. So, the genotypes in quadrants I and IV exhibited salinity tolerance, but quadrants II and III revealed salinity-susceptible genotypes. In PCA5, the genotypes 18278, 19892, CLN-2498-D, 17261, PB-017895, 17903, and 17838 present in Quadrant I obtained all the indices with a positive response toward salinity tolerance for the root length, root fresh weight, plant fresh weight, shoot dry weight, plant length, plant fresh weight, and negative for the Na^+ content root and Na^+/K^+ root. In quadrant II, 17255, 17266, 17254, 18285, and 17859 existed. In Quadrant III containing Zarnita, LYP-1, Aut-305, 19908, 19895 Pioneer-2761, the K^+ content in the root had a positive response, but the Na^+/K^+ shoot had a negative reaction toward salinity. The genotypes Kanatoo, 19857, 13205, and 19898 found in Quadrant IV showed indices of plant dry weight, root dry weight, shoot fresh weight, shoot length, and K^+ content in the shoot with positive responses, but Na^+ content shoot had negative response toward salinity.

Correlation analysis

The use of correlation analysis checked the strength of the association between variables. It also helps determine the actual attributes with an indirect selection of suitable genotypes. Calculating correlation among 15 attributes under stress and non-stress conditions took place. The blue color in the correlation tables showed a strong correlation value of 1, while the yellow color assigned for 0 correlation and red for negative correlation -1. Under non-stress conditions, shoot length showed a strong positive association with plant length (0.81) but a weak negative association with Na^+ root (-0.23); strong positive association of shoot fresh weight with plant fresh weight (0.92) but a weak negative association with Na^+ root (-0.24) and Na^+/K^+ shoot (-0.23). Plant dry weight showed a strong positive relationship with shoot dry weight (0.92) and root dry weight (0.91). Na^+

shoot showed a positive relationship with K^+ shoot (0.74) (Table 6).

Under stress conditions (T_1 = 6 dS/m) strong positive association of shoot length showed with plant length (0.85). Plant fresh weight showed a strong positive association with shoot fresh weight (0.97), root fresh weight (0.85), and shoot dry weight (0.80). Plant dry weight showed a positive association with plant fresh weight (0.79), shoot fresh weight (0.72), shoot dry weight (0.96), root fresh weight (0.78), and root dry weight (0.89). Na^+ root showed a positive association with Na^+/K^+ root (0.50). A strong positive association also existed between K^+ shoot and Na^+ shoot (0.76) (Table 7).

Under high-stress conditions (T_2 = 12 dS/m), plant length showed a strong positive association with shoot length (0.71), shoot fresh weight (0.57), shoot dry weight (0.54), root length (0.74), and plant fresh weight (0.62). Plant dry weight showed a strong positive relation with shoot fresh weight (0.69), shoot dry weight (0.95), root fresh weight (0.67), root dry weight (0.83), and plant fresh weight (0.76). The Na^+/K^+ shoot showed a negative relation with shoot length (-0.23) and K^+ shoot (-0.37). Likewise, the Na^+/K^+ root showed a negative association with shoot dry weight (-0.21), root length (-0.20), and K^+ root (-0.45) (Table 8).

Table 6. Correlation analysis among morphological and biochemical attributes under non-stress conditions.

Traits	SL1	SFW1	SDW1	RL1	RFW1	RDW1	KS1	KR1	NaR1	NaS1	Na/KR1	Na/KS1	PFW1	PDW1	PL1
SL1	1.00														
SFW1	0.51	1.00													
SDW1	0.30	0.39	1.00												
RL1	0.16	0.25	0.34	1.00											
RFW1	0.00	0.49	0.59	0.44	1.00										
RDW1	0.01	0.18	0.69	0.26	0.64	1.00									
KS1	0.24	0.07	0.01	0.12	-0.03	-0.03	1.00								
KR1	-0.11	-0.09	0.04	-0.13	0.03	0.15	-0.12	1.00							
NaR1	-0.23	-0.24	0.02	-0.05	0.03	0.10	-0.01	0.47	1.00						
NaS1	0.06	-0.07	-0.04	0.11	-0.07	-0.05	0.74	-0.05	-0.01	1.00					
Na/KR1	-0.10	0.03	-0.09	-0.05	-0.04	-0.10	0.04	-0.33	0.21	0.04	1.00				
Na/KS1	-0.19	-0.23	-0.06	-0.07	0.07	0.27	-0.29	0.04	0.08	0.00	-0.03	1.00			
PFW1	0.36	0.92	0.53	0.37	0.79	0.41	0.03	-0.05	-0.16	-0.08	0.00	-0.13	1.00		
PDW1	0.17	0.31	0.92	0.33	0.67	0.91	-0.01	0.10	0.06	-0.04	-0.10	0.11	0.51	1.00	
PL1	0.81	0.51	0.42	0.72	0.26	0.16	0.24	-0.16	-0.19	0.11	-0.10	-0.18	0.48	0.32	1.00

Table 7. Correlation analysis among morphological and biochemical attributes under stress conditions (T_1 = 6 dS/m).

Traits	SL2	SFW2	SDW2	RL2	RFW2	RDW2	KS2	KR2	NaR2	NaS2	Na/KR2	Na/KS2	PFW2	PDW2	PL2
SL2	1.00														
SFW2	0.57	1.00													
SDW2	0.54	0.76	1.00												
RL2	0.45	0.43	0.45	1.00											
RFW2	0.27	0.70	0.71	0.49	1.00										
RDW2	0.35	0.51	0.72	0.50	0.75	1.00									
KS2	-0.09	0.00	-0.03	-0.10	-0.05	-0.14	1.00								
KR2	0.17	-0.10	0.00	0.09	-0.12	0.07	0.00	1.00							
NaR2	0.03	-0.07	-0.09	-0.02	-0.14	-0.13	-0.05	0.10	1.00						
NaS2	-0.13	-0.09	-0.11	-0.15	-0.06	-0.22	0.76	0.03	-0.09	1.00					
Na/KR2	0.05	0.13	0.04	0.01	0.05	-0.05	-0.07	-0.34	0.50	-0.11	1.00				
Na/KS2	-0.02	-0.22	-0.18	-0.15	-0.14	-0.13	-0.50	0.02	-0.01	-0.01	-0.04	1.00			
PFW2	0.51	0.97	0.80	0.48	0.85	0.63	-0.02	-0.11	-0.10	-0.09	0.11	-0.21	1.00		
PDW2	0.50	0.72	0.96	0.51	0.78	0.89	-0.08	0.03	-0.12	-0.16	0.01	-0.17	0.79	1.00	
PL2	0.85	0.59	0.58	0.86	0.45	0.50	-0.11	0.16	0.01	-0.17	0.04	-0.10	0.58	0.59	1.00

Table 8. Correlation analysis among morphological and biochemical attributes under stress conditions (T_2 = 12 dS/m).

Traits	SL3	SFW3	SDW3	RL3	RFW3	RDW3	KS3	KR3	NaR3	NaS3	Na/KR3	Na/KS3	PFW3	PDW3	PL3
SL3	1.00														
SFW3	0.55	1.00													
SDW3	0.46	0.68	1.00												
RL3	0.05	0.28	0.33	1.00											
RFW3	0.13	0.58	0.58	0.61	1.00										
RDW3	0.25	0.52	0.62	0.39	0.67	1.00									
KS3	0.15	0.18	0.05	-0.14	-0.04	-0.09	1.00								
KR3	0.17	0.04	0.29	0.08	0.03	0.04	0.02	1.00							
NaR3	0.09	-0.08	-0.01	-0.13	-0.16	-0.12	0.13	0.32	1.00						
NaS3	-0.09	-0.05	0.02	-0.06	-0.11	-0.08	0.26	0.24	0.19	1.00					
Na/KR3	-0.07	-0.02	-0.21	-0.20	-0.17	-0.03	0.06	-0.45	0.17	-0.03	1.00				
Na/KS3	-0.23	-0.16	-0.18	0.08	-0.10	-0.12	-0.37	0.00	-0.10	-0.10	-0.08	1.00			
PFW3	0.43	0.94	0.72	0.46	0.83	0.64	0.11	0.04	-0.12	-0.08	-0.09	-0.16	1.00		
PDW3	0.42	0.69	0.95	0.38	0.67	0.83	0.00	0.23	-0.06	-0.02	-0.16	-0.18	0.76	1.00	
PL3	0.71	0.57	0.54	0.74	0.52	0.44	0.00	0.17	-0.03	-0.10	-0.19	-0.10	0.62	0.55	1.00

Correlation analysis is used for the indirect selection of desirable traits, where the selection is done for one trait, and the other traits positively correlated will automatically be selected and enhanced. Selection done for shoot length will automatically increase the plant length, negatively correlated with Na^+ contents and positively correlated with K^+ contents. In other words, high plant and shoot lengths show an indirect indication of salinity tolerance. Results showed that shoot length has a significant positive association with plant length. Root length showed a negative association with Na^+ contents, but a positive association with K^+ contents, where selection for higher root length will lead toward salinity tolerance. However, negative associations between characters cause a reduction in one trait when other traits get selected.

CONCLUSIONS

The identification and development of salt-tolerant tomato genotypes long required implementation to overcome the challenges of tomato production in saline areas of Pakistan. In this study, tomato germplasm proceeded to evaluation against salinity. Based on the principal component analysis results, the genotypes AUT-318, CLN-2498-A, 17884, Picendanto, 17260, 17256, 17263, and 17266 gained selection as salinity-tolerant genotypes while the 19903, 19908, Target-66, H-24, 17255, Nadir, and Peelo as salinity-sensitive genotypes. In these genotypes, salinity showed less effect. These salinity-tolerant genotypes can highly benefit tomato hybrid breeding programs or the development of salt-tolerant varieties.

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REFERENCES

Alam MS, Tester M, Fiene G, Mousa MAA (2021). Early growth stage characterization and the biochemical responses for salinity stress in tomato. *Plants* 10: 712.

Al-Khayri JM, Abdel Latef AAH, Taha HSA, Eldomiaty AS, Abd-Elfattah MA, Rezk AA, Shehata WF, Almaghasla MI, Shalaby TA, Sattar MN, Awad MF, Hassanin AA (2022). In silico profiling of proline biosynthesis and degradation related genes during fruit development of tomato. *SABRAO J. Breed. Genet.* 54(3): 549-564. http://doi.org/ 10.54910/sabrao2022.54.3.8.

Anjum M, Kamran M, Saeed MQ, Sajid S, Shabbir MA, Azam M, Hanif M, Wajeeha (2019). Hydroponic assessment of tomato genotypes against salt stress. *Int. J. Biol. Res.* 2: 91-100.

Brejda JJ, Moorman TB, Karlen DL, Dao TH (2000). Identification of regional soil quality factors and indicators. I. Central and Southern High-Plains. *Soil Sci. Soc. Am. J.* 64: 2115-2124.

Colla G, Rouphael Y, Leonardi C, Bie Z (2010). Role of grafting in vegetable crops grown under saline conditions. *Sci. Hortic.* 127: 147-155.

Devi ND, Arumugam T (2019). Screening of tomato genotypes at various levels of salinity. *J Pharma. Phytochem.* 8(3): 3199-3201.

Habibi N, Sediqui N, Terada N, Sanada A, Koshio K (2021). Effects of salinity on growth,

physiological and biochemical responses of tomato. *J. Int. Soc. Southeast Asian Agric. Sci.* 27(2): 14-28.

Hoagland DR, Arnon DI (1950). The water-culture method for growing plants without soil. *Univ. California, Coll. Agric. Agric. Exp. Station* 347.

Iqbal RK, Saeed K, Khan A, Noreen I, Bashir R (2019). Tomato (*Lycopersicum esculentum*) fruit improvement through breeding. *Sch. J. Appl. Sci. Res.* 2: 21-25.

Javed A, Nawab NN, Gohar S, Akram A, Javed K, Sarwar M, Tabassum MI, Ahmad N, Mallhi AR (2022). Genetic analysis and heterotic studies in tomato (*Solanum lycopersicum* L.) hybrids for fruit yield and its related traits. *SABRAO J. Breed. Genet*. 54(3): 492-501. http://doi.org/10.54910/ sabrao2022.54.3.3.

Jolliffe IT (2002). Principal component analysis. 2nd Ed. New York. *NY Springer-Verlag*.

Kadoglidou K, Xanthopoulou A, Kalyvas A, Mellidou I (2021). Utilization of tomato landraces to improve seedling performance under salt stress. *Stresses* 1: 238-252.

Kayess MO, Rahman ML, Ahmed K, Khan MR, Hossan MS, Hossain MS, Khanam MM, Pal DC (2020). Effect of salinity stress on different root and shoot traits of selected tomato cultivars. *Asian J. Adv. Res. Rep.* 8: 1-9.

Ladewig P, Trejo-Téllez LI, Servín-Juárez R, Contreras-Oliva A, Gómez-Merino FC (2021). Growth, yield and fruit quality of Mexican tomato landraces in response to salt stress. *Not. Bot. Hortic. Agrobot. Cluj-Napoca* 49: 1-15.

Moniruzzaman M, Islam MN, Hossain MFB, Rashid MM, Ahamed KU (2013). Evaluation of tomato germplasm against salinity. *Bull. Inst. Trop. Agr., Kyushu Univ.* 36: 9-16.

Mukul S, Kumar M, Agarwal R (2022). Principal component analysis based on yield and its attributing traits in tomato (*Solanum lycopersicum* L.) genotypes. *Pharma Innov. J.* 11: 1836-1841.

Plaut Z (1995). Photosynthesis in plant/crop under water and salt stress. *Handb. Plant Crop Physiol.*

Quinet M, Angosto T, Yuste-Lisbona FJ, Blanchard-Gros R, Bigot S, Martinez JP, Lutts S (2019). Tomato fruit development and metabolism. *Front. Plant Sci.* 10: 1-23.

Rahman SU, Basit A, Ara N, Ullah I, Rehman AU (2021). Morpho-physiological responses of tomato genotypes under saline conditions. *Gesunde Pflanzen*. 73: 541-553.

Rai GK, Jamwal D, Singh S, Parveen A, Kumar RR, Singh M, Rai PK, Salgotra RK (2016). Assessment of genetic variation in tomato (*Solanum lycopersicum* L.) based on quality traits and molecular markers. *SABRAO J. Breed. Genet.* 48(1): 80-89.

Raza MA, Saeed A, Munir H, Ziaf K, Shakeel A, Saeed N, Munawar A, Rehman F (2017). Screening of tomato genotypes for salinity tolerance based on early growth attributes and leaf inorganic osmolytes. *Arch. Agron. Soil Sci.* 63: 501-512.

Rehman F, Munir H, Raza MA, Saeed A (2021). Uncover the salt tolerance potential of accessions based on photosynthetic attributes and interaction plots in tomato (*Solanum lycopersicum*). *Plant Breed.* 140: 130-141.

Salava H, Thula S, Mohan V, Kumar R, Maghuly F (2021). Application of genome editing in tomato breeding: Mechanisms, advances, and prospects. *Int. J. Mol. Sci.* 22: 1-41.

Siddiky MA, Sardar PK, Hossain MM, Khan MS, Uddin MK (2012). Screening of different tomato varieties in saline areas of Bangladesh. *Int. J. Agric. Res. Innov. Technol.* 2: 13-18.

Singh J, Sastry EVDD, Singh V (2012). Effect of salinity on tomato (*Lycopersicon esculentum* Mill.) During seed germination stage. *Physiol. Mol. Biol. Plants* 18(1): 45-50.

Sinha A, Singh P, Bhardwaj A, Verma RB (2021). Principal component analysis approach for comprehensive screening of tomato germplasm for polyhouse condition. *J. Exp. Agric. Int.* 43: 67-72.

Steel RGD, Torrie JH, Dickey DA (1997). Principles and Procedures of Statistics a Biometrical Approach. New York: McGraw Hill.

Tahir M, Zafar MM, Imran A, Hafeez MA, Rasheed MS, Bin Mustafa HS, Ullah A (2018). Response of tomato genotypes against salinity stress at germination and seedling stage. *Nat. Sci.* 16: 10-17.

Tandra SZ, Hassan L, Hannan A, Jahan J, Sagor GHM (2022). Screening and biochemical responses of tomato (*Lycopersicum esculentum* L.) genotypes for salt tolerance. A*cta Physiol. Plant.* 44: 41.

Wu H, Guo J, Wang C, Li K, Zhang X, Yang Z, Li M, Wang B (2019). An effective screening method and a reliable screening trait for salt tolerance of *Brassica napus* at the germination stage. *Front. Plant Sci*. 10:530.