

Genetic evaluation and characterization using cluster heat map to assess NaCl tolerance in tomato germplasm at the seedling stage

Fazal Rehman^{1*}, Asif Saeed¹, Muhammad Yaseen², Amir Shakeel¹, Khurram Ziaf¹, Hassan Munir¹, Sultan Ali Tariq³, Muhammad Ahsan Raza¹, and Awais Riaz¹

¹University of Agriculture, Faculty of Agriculture, Faisalabad 38040, Pakistan. *Corresponding author (fazlpbg@gmail.com). ²University of Agriculture, Faculty of Sciences, Faisalabad 38040, Pakistan.

³Pakistan Agricultural Research Council (PARC), National Agricultural Research Center (NARC), Park Road, Islamabad 44000, Pakistan.

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ABSTRACT

Salinity is a serious problem that limits crop growth and yield. The present study used plotting to evaluate 25 tomato (*Solanum lycopersicum* L.) accessions for various morpho-physiological parameters at the seedling stage and identify significantly salt-tolerant tomato lines at three different salinity levels. The pot experiment had a completely randomized design with three replicates in a factorial arrangement under greenhouse conditions. Tomato plants were exposed to 0 (control), 8, and 12 dS m⁻¹ as NaCl stress at the seedling stage. The morpho-physiological traits, such as root and shoot length, root/shoot ratio, number of leaves, fresh and dry shoot weight, fresh and dry root weight, leaf area, Na⁺ and K⁺ concentrations, K⁺/Na⁺ ratio, and tolerance index, were recorded to examine salt tolerance. According to principal component analysis (PCA), there were six principal components (PCs) with Eigen values > 1 and 77.2% of total cumulative variability. The PC1 (24.3%) revealed the highest variability followed by PC2 (16.2%). Meanwhile, the PCA biplot and cluster heat map analyses indicated that Subarctic, Raad-Red, Naqeeb, Pakit, Tommy-Toe, and BL-1076 were salt-tolerant, whereas PBLA-1401, PB-017902, CLN-2413, BL-1078, BL-1174, and BL-1079 were the most susceptible accessions based on their performance under stress.

Key words: Cluster heat map, pot experiment, principal component analysis, salinity, *Solanum lycopersicum*, tomato seedling.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important world commercial crop consumed as fresh salad and in processed forms, such as pulp, paste, ketchup, and juice, while tomato soup is a good remedy for patients suffering from constipation, and it is a good appetizer (Alda et al., 2009). Tomato is a healthy source of phytochemicals, such as lycopene, beta carotene, flavonoids, vitamin C, and essential nutrients, which significantly contribute to prevent malnutrition in humans (Alda et al., 2009; Martí et al., 2018). Salinity is considered to be the most serious environmental challenge that causes large-scale crop loss. Most factors such as salt water irrigation, low rainfall, larger surface evaporation, improper agricultural practices, and dirty water for irrigation lead to salinity (Foolad, 2004).

Seed germination can be affected by either salinity stress or osmotic stress; in this way, seed water uptake is restricted or toxic effects of sodium and chloride ions on the germinating seed also occur (Khajeh-Hosseini et al., 2003). Nutrient imbalance and deficiencies can be due to mineral nutrient and salt interactions. At the cellular level, most stresses are accumulated as ionic, osmotic, and oxidative stress due to saline toxicity (AbdElgawad et al., 2016; Akram et al., 2017).

All the main processes such as photosynthesis, protein synthesis, and energy and lipid metabolism are affected by the development and beginning of plant salinity stress. As a result, leaf surface expansion is reduced and then terminated as stress increases.

Biotic and abiotic stresses act as production limiting factors because tomato imports have increased during the last decade. To deal with these stresses, there is a demand to develop the most suitable varieties and a more nutritive tomato with good tonnage. Tomato is quite sensitive to salinity (Lycoskoufis et al., 2005), which affects tomato growing areas of both arid and semi-arid climates (Qadir et al., 2006; Azevedo-Neto et al., 2006). Munns and Tester (2008) reported that salinity stress has affected more than 45 million ha of land and approximately 1.5 million ha will become wasteland each year worldwide because of high salinity. A survey conducted by the Water and Power Development Authority (WAPDA; Lahore, Pakistan) showed that Pakistan has 16.795 million ha of land for crop cultivation, which consists of 73% non-saline soil, 10% slightly saline, 4% moderately saline, 7% highly saline, and 6% miscellaneous land types (Khan et al., 2012). Plants also survive in a harsh environment by activating their physiological and metabolic defense systems through structural modifications (Gong et al., 2009). Under stress, plants respond in an adaptive way to the phenotypic variation of a particular species trait; plants also change their behavior according to the innate mechanism due to different environmental stresses (Raza et al., 2016). Therefore, this study was conducted to assess the effects of salinity on tomato seedling growth, measure physiological and morphological parameters, and genetically identify tomato lines and cultivars that significantly contribute to salinity tolerance.

MATERIALS AND METHODS

Twenty-five tomato accessions, namely, Tiny Tim, Way Ahead, Naqeeb, LA-0164, LA-1789, Pakit, Subarctic, Raad Red, Earliana, Oregon Spring, Tommy Toe, CLN-1621L, BL-1076, 017859, PBLA-1401, PBLA-1932, BL-1078, BL-1079, CLN-2413, CLN-2418A, CLN-2366A, CL-519-93D4, BL-1174, BL-1175, and PB-017902 were collected from the Vegetable Research Institute, Faisalabad (30°-31.5° N, 73°-74° E; 188 m a.s.l.) and the University of Agriculture, Faisalabad, Pakistan.

Experiment layout

The experiment was conducted in the greenhouse of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Using a germplasm collection, a tomato nursery bed was sown in the winter season (October). Before sowing the nursery bed, the seed surface was sterilized with 2% calcium hypochlorite for 5 min and then washed with distilled water before nursery planting (Habib, 2009). The 30-d-old seedlings were exposed to three levels of NaCl stress. Three levels of electrical conductivity (EC) were prepared in soil media to properly check each accession behavior: 0 (control), 8.0, and 12.0 dS m⁻¹. The exact amount of NaCl required for both salinity levels, other than the control, was calculated according to the formula provided by the US Salinity Laboratory Staff (1954):

(g) NaCl salt per kg of soil =
$$\frac{\text{TSS} \times \text{eq. wt. of NaCl salt} \times \text{SP}}{1000 \times 100}$$

where TSS is total soluble salts and SP is the saturation percentage of soil. After 30 d of germination in the tomato nursery, small pots (1 kg soil medium, 12.75 and 15.24 cm width and height, respectively) were filled with a homogenous 1:1 sand:silt mixture along with the calculated NaCl concentration processed by a mechanical mixer to reach the exact EC level in the medium. All pots were transferred to the greenhouse where transplanting was done under controlled conditions, and six plants per pot were maintained. Healthy 30-dold tomato seedlings from the October sowing were selected for transplanting. The experiment had three replicates in a completely randomized block design with factorial arrangements and a total of 75 (25 accessions \times 3 salinity levels) treatments were prepared for each replicate. The experiment was subjected to controlled greenhouse conditions (60%-70% RH, 23 \pm 3 °C, and a photoperiod of 12 \pm 1 h).

Morphological trait determination

The data of the following traits were recorded 60 d after seedlings were transplanted. The morphological parameters, such as root length, shoot length, root/shoot ratio, fresh root weight, dry root weight, fresh shoot weight, dry shoot weight, and number of leaves per plant, were estimated by the following standard procedures.

Leaf area. Five plants were randomly selected per treatment for each replicate. Leaf area was determined according to Easlon and Bloom (2014). One leaf from each plant and a red colored, square-shaped calibration scale with a 4 cm² area was scanned with a flatbed HP Deskjet Scanner (Model 1510, Hewlett-Packard, Palo Alto, California, USA) and images were produced at 200px resolution. Scanned images were then processed with a Windows application (Easy Leaf Area software, Version 2.0; Lobet, 2017) to determine leaf area. Mean values for each replicate were calculated.

Na⁺ and K⁺ concentration. The Na and K concentration was determined from cell sap. Cell sap was extracted from leaves according to Gorham et al. (1997). Five plants from each treatment in each replicate were randomly selected. The collected sap was diluted 40 times and the Na concentration was determined with a Sherwood Flame Photometer (Model 410, Sherwood Scientific Ltd., Cambridge, UK) and the mean concentration was calculated for each replicate. The K⁺/Na⁺ ratio was also calculated by dividing their values.

Statistical analysis

The recorded data of the abovementioned traits were subjected to ANOVA following the method described by Steel et al. (1997) to determine significant genotypic differences. Data were further analyzed by the multivariate approach, principal component analysis (PCA) (Ogunbayo et al., 2005), and cluster analysis (Everitt et al., 2001). All statistical analyses were performed with the R software version 3.2.0. (Gentleman, 2006; Kabacoff, 2012) (http://www.r-project.org).

RESULTS

The ANOVA was significant among all seedling traits and accessions were found to be genetically diverse. Simple correlation coefficient values demonstrated significant positive correlations for the number of leaves, root and shoot length, fresh and dry shoot weight, fresh and dry root weight; this reflects that an increase in any of these traits increased salinity tolerance in tomato (Table 1). Therefore, it is proposed that these morphological traits can be used to select salt-tolerant accessions.

The PCA was performed to obtain more reliable information on how to identify groups of accessions that have desirable yield traits for plant breeding. The PCA technique was applied to observe the interrelationship among variables (Rencher, 2002); according to PCA results, of 16 principal components (PCs), only six PCs had Eigen values > 1 and contributed to 77.2% of total cumulative variability among the different accessions. The contribution of PC1 for variability was higher (24.3%) than other PCs (Table 2). The PC1 showed positive factor loadings for all traits, except root and shoot dry weight,

Attributes	NL	RL	SL	R/S ratio	FSW	DSW	FRW	DRW	LA	Na+	K+	K+/Na+ ratio	RDW TI	SDW TI	Ca++/Na+ TI
RL	0.350**														
SL	0.566**	0.166													
R/S ratio	-0.203	0.451**	-0.384**												
FSW	0.382**	0.244*	0.253*	0.000											
DSW	0.295*	0.263*	0.225*	-0.038	0.779**										
FRW	0.232*	0.315**	0.180	0.079	0.612**	0.500**									
DRW	0.117	0.217	0.120	0.019	0.486**	0.495**	0.865**								
LA	-0.048	0.076	-0.011	-0.080	0.354**	0.203	0.188	0.178							
Na ⁺	-0.050	0.177	-0.032	0.116	-0.124	-0.089	0.188	0.096	-0.136						
K ⁺	0.110	-0.006	0.142	0.039	0.141	0.093	-0.007	0.048	0.133	-0.318**					
K+/Na+ ratio	0.068	-0.122	0.037	-0.029	0.207	0.118	-0.083*	0.019	0.203	-0.856**	0.677**				
RDW TI	0.036	-0.136	-0.028	-0.169	0.317**	-0.260*	-0.414**	-0.307**	-0.093	-0.198	0.112	0.177			
SDW TI	-0.127	-0.136	-0.170	-0.115	-0.379**	-0.213	-0.374**	-0.292*	0.089	-0.034	0.137	0.046	0.697**		
Ca++/Na+ TI	0.001	-0.050	0.166	-0.191	0.019	0.049	-0.010	-0.058	-0.206	0.036	0.060	-0.090	-0.296*	0.239*	
K+/Na+ TI	-0.057	-0.217	0.038	-0.230*	-0.219*	-0.049	-0.343**	-0.335**	-0.127	-0.089	-0.019	-0.072	0.102	0.154	0.525**

Table 1. Correlation	analysis	among	morphological	and	physiological	parameters	of	tomato	in	response	to	three
salinity levels.												

Significance level: **highly significant (P < 0.01), *significant (P > 0.01 or P < 0.05).

NL: Number of leaves; RL: root length; SL: shoot length; RS ratio: root/shoot ratio; FSW: fresh shoot weight; DSW: dry shoot weight; FRW: fresh root weight; DSW: dry root weight; LA: leaf area; Na⁺: Na⁺ concentration: K⁺: K⁺ concentration: K⁺/Na⁺ ratio; RDW TI: root dry weight tolerance index; Ca⁺⁺/Na⁺ TI: Ca⁺⁺/Na⁺ ratio tolerance index; K⁺/Na⁺ TI: K⁺/Na⁺ ratio tolerance index.

Parameters	PC1	PC2	PC3	PC4	PC5	PC6
Eigen value	1.973	1.609	1.416	1.251	1.139	1.007
Proportion of variance	0.243	0.162	0.125	0.098	0.081	0.063
Cumulative proportion	0.243	0.405	0.530	0.628	0.709	0.772
NL	0.216	-0.145	-0.249	-0.462	-0.261	0.116
RL	0.234	0.112	0.139	-0.254	-0.511	-0.323
SL	0.177	-0.142	-0.397	-0.358	-0.101	0.210
RS ratio	0.047	0.174	0.419	0.179	-0.520	-0.209
FSW	0.421	-0.151	-0.024	0.028	0.089	-0.148
DSW	0.371	-0.121	-0.075	-0.005	0.104	-0.328
FRW	0.433	0.093	0.069	-0.010	0.165	0.011
DRW	0.387	0.035	0.110	0.022	0.246	0.039
LA	0.140	-0.182	0.202	0.030	0.381	-0.349
Na ⁺	0.031	0.503	-0.020	-0.228	0.075	-0.148
K^+	0.041	-0.422	0.051	0.108	-0.242	-0.092
K ⁺ /Na ⁺ ratio	0.026	-0.561	0.120	0.227	-0.140	0.130
RDW TI	-0.274	-0.240	0.107	-0.434	0.057	-0.121
SDW TI	-0.281	-0.163	0.159	-0.354	0.182	-0.420
Ca++/Na+ TI	0.011	0.062	-0.517	0.327	-0.139	-0.248
K+/Na+ TI	-0.188	-0.035	-0.447	0.156	-0.005	-0.492

 Table 2. Principal component (PC) analysis of morpho-physiological traits among 25 tomato genotypes.

NL: Number of leaves; RL: root length; SL: shoot length; RS: root/shoot ratio; FSW: fresh shoot weight; DSW: dry shoot weight; FRW: fresh root weight; LA: leaf area; Na⁺: Na⁺ concentration; K⁺: K⁺ concentration; K⁺/Na⁺ ratio: RDW TI: root dry weight tolerance index: SDW TI: shoot dry weight tolerance index; Ca⁺⁺/Na⁺ TI: Ca⁺⁺/Na⁺ ratio tolerance index; K⁺/Na⁺ TI: K⁺/Na⁺ ratio tolerance index.

tolerance index (TI), and K⁺/Na⁺ TI, while PC2 indicated positive factor loading for root length, root/shoot ratio, fresh and dry root weight, Na⁺ concentration, and Ca⁺⁺/Na⁺ TI. Thus, PC1 can be referred to as having effective salt-tolerant factors based on the variation (24.3%). The PC2 explained a high value for Na⁺ (0.50). The PC3 explained the highest root/shoot ratio (0.42) value. Therefore, the stress-tolerant accessions could be distinguished from the non-stress conditions in PC1.

A PCA biplot showed that variables are superimposed on the plot as vectors; relative vector length represents the relative variability proportion in each illustrated variable and provides the best method for visualizing the interaction pattern among treatments and lines. The biplot graph indicated that the accessions BL-1076, Subarctic, Raad-Red, Naqeeb, Pakit, and Tommy Toe were tolerant because of the high concentrations of K and most of the studied traits, while low Na occurred at the highest salinity stress level (Figure 1).

Cluster analysis is used to define and represent pairwise structural dissimilarities between objects. The accessions or variables, such as environments and traits, are grouped by clustering methods, which are somewhat similar to each other. Two-way clustering partitioned the data in two directions (accessions and environments/traits). The merit of two-way clustering is that certain objects are clustered together and their variables are also clustered at the same time (Hageman et al., 2012). The PCA results were further verified by cluster analysis. In the present study, 25 accessions with three salinity levels were distributed into nine clusters (Figure 2). Cluster 1 consisted of 14 accessions, followed by 2, 18, 3, 8, 5, 8, 7, and 10 in clusters 2, 3, 4, 5, 6, 7, 8, and 9, respectively. The heat map depicts two-way trait and accession classification; seedling traits under salinity stress were divided into five groups. The cluster heat map shows that the accessions Tommy Toe and PBLA-1932 belonged to clusters 1, 3, and 7. Meanwhile, Pakit, Raad Red, Subarctic, BL-1076, and Naqeeb belonged to clusters 6 to 9, which were considered as salt-tolerant lines due to their better performance under 0 (control), 8, and 12 dS m⁻¹ salinity levels for all the studied traits. Moreover, these accessions can be considered for use in future breeding programs. The PBLA-1401, PB-017902, CLN-2413, BL-1078, BL-1174, and BL-1079 accessions belong to cluster 5, which is considered as the most susceptible under the highest salinity stress conditions.



Figure 1. Biplot of morpho-physiological traits of 25 tomato accessions at three salinity levels for principal components PC1 and PC2.

NL: Number of leaves; RL: root length; SL: shoot length; RS: root/shoot ratio; FSW: fresh shoot weight; DSW: dry shoot weight; FRW: fresh root weight; LA: leaf area; Na*: Na* concentration, K*: K* concentration; K*/Na* ratio; RDW TI: root dry weight tolerance index; SDW TI: shoot dry weight tolerance; index; Ca**/Na* TI: Ca**/Na* ratio tolerance index; K*/Na* TI: K*/Na* ratio tolerance index.

DISCUSSION

The correlation analysis of morpho-physiological traits under various salinity levels revealed strong significant positive correlations for number of leaves, root and shoot length, fresh and dry shoot weight, and fresh and dry root weight; this reflects that an increase in any of these traits will increase salinity tolerance in tomato. Therefore, it is proposed that the studied traits can be used to select salt-tolerant accessions. Similar results have also been reported by Liu et al. (2014) and Raza et al. (2018). The PCA indicated six PCs had Eigen values > 1 and contributed 77.2% of total cumulative variability among the different accessions. The contribution of PC1 toward variability was the highest (24.3%), followed by PC2, PC3, PC4, PC5, and PC6, which contributed 16.2%, 12.5%, 9.8%, 8.1%, and 6.3% variability, respectively.

Figure 2. Cluster heat map of 25 accession performance at all three salinity levels. Hierarchical clustering analysis expresses the visual representation of accessions indifferent groups based on similarity as well as the correlation with different traits. A heat map showing color pattern.



NL: Number of leaves; RL: root length; SL: shoot length; RS: root/shoot ratio; FSW: fresh shoot weight; DSW: dry shoot weight; FRW: fresh root weight; LA: leaf area; Na⁺: Na⁺ concentration; K⁺: K⁺ concentration; K⁺/Na⁺ ratio; RDW TI: root dry weight tolerance index; SDW TI: shoot dry weight tolerance index; Ca⁺⁺/Na⁺ TI: Ca⁺⁺/Na⁺ ratio tolerance index; K⁺/Na⁺ TI: K⁺/Na⁺ ratio tolerance index.

Kaya et al. (2002) revealed that accessions with higher PC1 and lower PC2 values had high yields, while accessions with lower PC1 and higher PC2 values had low yields; this is consistent with our findings. The PC1 showed positive factor loadings for all traits, except root and shoot dry weight tolerance index (TI) and K⁺/Na⁺ TI, while PC2 indicated positive factor loadings for root length, root/shoot ratio, fresh and dry root weight, Na⁺ concentration, and Ca⁺⁺/Na⁺ TI. It was reported that PC1 explained 66% of the variation and was associated with yield and stress tolerance (Farshadfar and Sutka, 2002; Raza et al., 2016). The PC1 explained 52.97% of the variation in shoot length and PC2 explained 59.72% of the variation in dry root weight. However, PC1 can be distinguished on the basis of stress-tolerant accessions from non-stress conditions. These results are similar to the present findings (Khajeh-Hosseini et al., 2013). Higher plant exposure to salinity leads to higher accumulation of Na⁺ concentration and restricts the flow of the K⁺ ion, which is very important for the cellular, protein, and photosynthetic activity in plants. Our results are similar to those found by Siddiqui et al. (2017).

The different trait combinations using a multivariate approach can help subsequent research to assimilate full genome expression patterns under salinity stress (Pandit et al., 2010; Cotsaftis et al., 2011; Baisakh et al., 2012; Cuin et al., 2012). A PCA biplot showed that the first two PCs contributed 40.5% toward total variance and were plotted as PC1 on the x-axis and PC2 on the y-axis to detect the association between different clusters. Accessions which lay close to a variable

vector had high mean values for that trait and showed fewer variations. It was concluded from the biplot that accessions 17859, LA-0164, PBLA-1401, PB-017902, BL-1079, BL-1174, CLN-2413, and BL-1078 performed worse than the control at the highest salinity level of 12 dS m⁻¹. There was a significant relationship at 8 dS m⁻¹ for Na, root/shoot ratio, and Ca⁺⁺/Na⁺ TI, which indicated that these accessions were susceptible due to high Na levels and low K concentrations. Meanwhile, accessions BL-1076, Subarctic, Raad red, Naqeeb, Pakit, and Tommy Toe were considered as tolerant due to high concentrations of K and most of the studied traits and low Na at the highest salinity stress level. The results are in accordance with a previous study conducted by Raza et al. (2016). Related results were also discussed by Kahriman et al. (2016) in which a maize (*Zea mays* L.) line × tester breeding design was evaluated for yield and oil and protein contents by the PCA biplot graphical approach.

Similar studies about salinity-linked traits have been discussed by Khajeh-Hosseini et al. (2003) and Golparvar (2011). A study investigated vectors for the number of fruits per plant, fruit clusters per plant, and fresh fruit yield per plant; they were close to each other and therefore strongly associated (Agong et al., 2004). A related study by Saleem et al. (2011) discussed screening for drought- tolerant and drought-sensitive accessions using biplot analysis.

The PCA results further verified by two-way cluster analysis simultaneously clustered into accessions and traits. Accessions with similar behavior in relation to traits could be easily detected on the heat map. The accessions belonging to cluster 1 and 8 had high values for seed and oil yield and short vegetative growth, but cluster 9 had a moderate to low value for vegetative duration and moderate to high values for seed and oil yield; based on the obtained results, accessions should be further exposed to a breeding program (Jankulovska et al., 2014). Genetic divergence is assessed by multivariate analysis in tomato to select superior accessions in a breeding program (Iqbal et al., 2014).

In the present study, it was found that the cluster heat map showed that the accessions Tommy Toe, BL-1076, Pakit, Raad-red, Subarctic, and Naqeeb belonged to clusters 1, 3, 7, 6, and 9, respectively; these were considered as salt-tolerant accessions due to their better performance under 0 (control), 8, and 12 dS m⁻¹ salinity levels among all studied traits. The accessions PBLA-1401, PB-017902, CLN-2413, BL-1078, BL-1174, and BL-1079 belonged to cluster 5, considered as the most susceptible under the highest salinity stress conditions. The accession Tommy Toe was mostly independent of salinity stress at all given salinity stress levels. The crossing of salt-tolerant accessions from clusters 6 and 9 with salt-sensitive accessions from clusters 1 and 3 would be useful for developing salt-tolerant genotypes. Our results are related to those discussed by Iqbal et al. (2014) and Ene et al. (2016).

A similar study investigated salt tolerance in *Brassica* and other related species grown in a sand medium at the seedling stage. There was a significant positive correlation for germination rate, shoot and root length, and fresh seedling weight. Results revealed that *Raphanus sativa* 'Changfeng', *Brassica juncea* 'JC', and *B. napus* 'ZS 10' had low salt tolerance, while *B. oleracea* 'JF-1' and *Sinapis alba* 'HN-2' had high salt tolerance with respect to germination percentage, root and shoot length, and fresh seedling weight. Cluster analysis based on multiple parameters at the seedling stage, such as germination rate, shoot and root length, and fresh seedling weight, could be considered a much more reliable and efficient technique to screen for salt-tolerant accessions. Cluster analysis was regarded as the most reliable way to identify salt tolerance in crop species such as rice, wheat, tomato, and sugarcane (Su et al., 2013). There are several merits for using multivariate analysis to evaluate salt tolerance. Firstly, it allows increasing accession accuracy by simultaneous multiple trait analysis. Secondly, accessions are ranked according to different salinity levels, and tolerance varies with salinity levels. Thirdly, salt-tolerant accessions are conveniently and accurately estimated on the basis of ranking among groups under different salinity stress levels (Khrais et al., 1998; Zeng et al., 2002).

Hierarchical cluster analysis categorized ten *Agropyron desertorum* accessions into three clusters. Results indicated that selecting for the highest number of traits: roots, plumule and seedling length, and seed vigor could be beneficial for seed germination ability and salinity tolerance in A. *desertorum* accessions at the seedling stage under greenhouse conditions (Golparvar, 2011). Similar results were found by Sheikh-Mohamadi et al. (2017); some of the genotypes revealed tolerance under high salinity stress, which in in accordance with the current study results of cluster analysis. The related results observed by Khajeh-Hosseini et al. (2003) also agreed with the present results. A similar study was conducted to evaluate salinity tolerance and salt-responsive genes in tomato. Tomato accessions indicated a great degree of variation in yield and fruit quality components under salinity stress. Cluster analysis grouped the accessions into three clusters on the basis of salt tolerance; in this way, three groups were categorized as tolerant, moderately tolerant, and susceptible under salinity stress (Alsadon et al., 2013).

CONCLUSIONS

The ANOVA showed significant differences for all the traits and revealed that there were variations among accessions under salinity stress. The overall performance of accessions Raad-red, Subarctic, Naqeeb, Pakit, and BL-1076 was significantly different from all the others, and they also performed comparatively better in two NaCl concentrations for most traits, such as shoot length, number of leaves, K⁺ concentration, and K⁺/Na⁺ ratio; therefore, they exhibited more salinity tolerance. It was concluded that the accessions with higher K⁺ content uptake and higher K⁺/Na⁺ ratio were salt-tolerant and less affected by increased salinity levels. The cluster heat map showed that the accessions Tommy Toe, Pakit, Raad-red, Subarctic, BL-1076, and Naqeeb belonged to clusters 1 to 3 and 6 to 9, respectively, and were considered as salt-tolerant lines due to their better performance under 0 (control), 8, and 12 dS m⁻¹ salinity levels among all studied traits. Moreover, these accessions can be considered for use in a future breeding program. The accessions PBLA-1401, PB-017902, CLN-2413, BL-1078, BL-1174, and BL-1079 belonged to cluster 5, which was considered as the most susceptible under the highest salinity stress conditions.

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