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Improving salt tolerance and weight percent reduction in tomato by exploiting physio-agronomic seedling traits

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Salinity being a serious limitation to crop production is an established fact since ages. It has adversely affected the adaptive behavior of our field crops particularly at seed germination and seedling stages. Identification of particular plant traits conferring salinity tolerance is important for inducing genetic variation among the target traits and adjusting the selection pressure for them in field. This experimental study was conducted to explore percent (%) weight loss of roots and shoots at increasing salt stresses (control, 10 dS m^{-1} and 15 dS m^{-1}) along with certain dry weight and cationic ratio tolerance indices. The experiment was conducted in the glasshouse to screen seedlings of 25 tomato genotypes. Principal component analysis and correlation analysis were used to screen the genotypes for variability and salt tolerance. Based on associative interactions for salt tolerance traits and highly negative response towards weight percent (%) reductions, three genotypes were identified as salt tolerant; BEAVER LODGE SLICER, ZARNITZA, and FORME DE COEUR. Two genotypes GLACIER and Rio-GRANDE were highly positive for K^+/Na^+ and Ca^{2+}/Na^+ ratios tolerance indices. Based on these findings, the genotypes BEAVER LODGE SLICER, ZARNITZA and FORME DE COEUR are suggested to be planted in salt affected area. The six genotypes (ANAHU, LO-2707, 17860, UOVO ROSEO, NAGINA and LA-2821) showed significant negative behavior towards weight % reduction, and a little positive towards salt tolerance indices were considered as moderately salt tolerant.

Key words: Weight percent reduction, NaCl, tomato seedling, physio-agronomic, salt tolerance.

INTRODUCTION

Over 800 million hectares of land (>6%), including one third portion of the cultivated land, throughout the world are salt affected (FAO, 2008; Naz et al., 2010; Kosová et al., 2013). Worldwide, out of 230 million ha of total irrigated land, 45 million ha (about 20%) are salt affected (FAO, 2008). Although there is merely 15% land that is irrigated out of total cultivated, but it is producing world's one third food and has productivity twice in contrast to rainfed (FAO, 2008; Kosová et al., 2013). Approximately 2% (32 million ha) of 1500 million ha dryland used for

agricultural purposes is affected with varying degrees of secondary salinity (Munns and Tester, 2008).

Field vegetables like tomato (*Solanum lycopersicum* L.) are prominently found in arid and semiarid climates where salinity is a major problem (Qadir et al., 2006; Azevedo-Neto et al., 2006). In semiarid and arid regions, salts move from basal rocks and accumulate over the upper layer of soil because of prevalent water evaporation (Kosová et al., 2013). Saline areas continue to expand in semiarid and arid regions due to improper cultural practices, use of saline water, insufficient irrigation and excessive fertilization; which consequently promises a decline in crop production over the period (Shahid et al., 2012).

Globally, irrigated and cultivated land areas of arid and semi-arid regions have limited agricultural productivity mainly because of saline conditions (Azevedo-Neto et al., 2006; Nawaz et al., 2010; Shahid et al., 2012; Kosová et al., 2013; Maurya and Gothandam, 2014)) along with other stresses (Abbas et al., 2010; Bhantana and Lazarovitch, 2010; Siringam et al., 2012). Fifty percent of the total irrigated arable land is undergoing the salinization, and this area contributes to one third of the total global food productivity (Munns, 2002; Munns and Tester, 2008). Under saline environment, plant growth reduction has been reported in tomato in a number of studies (Romero-Aranda et al., 2001; Fujita et al., 2006).

Saline soils composition varies due to different types and concentrations of salts where plants show specific responses against particular salts at their different developmental stages (Cuartero et al., 2006). Tomato (*S. lycopersicum* L.) also unevenly tolerates salt stresses at altered growth stages though it has natural sensitivity at its seedling stage (Al-Taisan 2010). Principally, if the seedling stage has been adversely affected with saline conditions, this could limit plant growth and will translate into poor economic yield (Maas, 1986). Although plants differ in their ability to cope with adverse saline conditions (Kosová et al., 2013), there are certain attributes to assess salinity tolerance that is, reduction in rate of plant relative growth (biomass reduction) or as survival of plant (index of salt tolerance), at defined concentrations of salts (Munns, 2002).

Higher salinity causes serious, and in many cases, irreversible damage to the plants. It includes stomatal closure and reduction in leaf expansion due to deficient osmotic conditions, overall drop in photosynthesis and biomass production (Rahnama et al., 2010; James et al., 2011). Both Cl^- and Na^+ ions in excessive forms are a unique cause of leaf scorching and firing that leads to stunted growth of plants (Shannon et al., 2000). Elevated levels of Na^+ may be responsible for shortage of other essential elements and osmolytes such as Ca^+ and K^+, and could disturb K^+-dependent processes which eventually lead to conformational changes in proteins (Mahajan and Tuteja, 2005).

To identify salt tolerant genotypes a selection criterion should be devised that best explains the behavioral retort of genotypes over multiple saline conditions. Formerly, physio-agronomic plant traits; (K^+/Na^+ and Ca^{2+}/Na^+ ratios) (Dasgan et al., 2002; Juan et al., 2005; Ahmadi et al., 2009; Turhan and Seniz, 2012), root fresh and dry weights, shoot fresh and dry weights at early plant stages were preferred as screening criterion for salt tolerance (Ibrahim, 2003). Shoot biomass production under salinity (Kumar et al., 2012; Bolarin et al., 1991; Foolad, 1996) and selectivity of K^+ over Na^+ are some of the best salt tolerance indicators to study cultivated and even wild species of tomato (Cuartero et al., 1992).

This study aims to phenotype the seedlings based on numerically descriptive parameters such as weight % reductions of roots and shoots along with tolerance indices that will be derived from dry weights and inorganic osmolytes (K^+, Na^+ and Ca^{2+}), collectively to find salt tolerant tomato genotypes.

MATERIALS AND METHODS

Plant materials

The germplasm consisting of 25 tomato genotypes was collected from Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad (UAF) and Vegetable Research Institute (VRI), Faisalabad, Punjab, Pakistan.

Saline soil preparation, layout and growth conditions

The experiment was conducted in a glasshouse at the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad (31°26'00.6" N 73°04'19.6" E). Seeds of each genotype were surface sterilized with 2% bleach by dipping for 5 min, and then washing with distilled water. The seedlings were evaluated to record their response against salinity by artificially producing three levels of salinity (S_0 = control, S_{10} = 10 dS m^-1 and S_{15} = 15 dS m^{-1}) in soil media. A homogenous mixture of sand to silt (50:50) ratio was used as control having 1.7 dS m^{-1} salinity, but to prepare other two salinity levels, exact amount of NaCl salt was determined using the formula of U.S. Soil Salinity Lab (1954):

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

(*TSS* is total soluble salts and *SP* is saturation percentage of soil)

The calculated amounts of salt (one for each S_{10} and S_{15}) were

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mixed separately with the soil media by mechanical ways. Salinity of each level was confirmed after power-driven mixing of soil and salt. Small pots (with capacity of 1 kg soil media, height of 6 inches and diameter of 4 inches) were filled with prepared saline soil. Thirty days old healthy tomato seedlings from October sown nursery were selected for transplantation. Transplanting was done under controlled conditions and 10 plants per pot were maintained. The experiment had three replications in a randomized complete block design with factorial arrangements. A total of 75 (25 genotypes × 3 salinity levels) treatments were prepared for each replication. The experiment was subjected to controlled conditions (humidity 60-70%, temperature 23±3 °C and photoperiod of 12±1 hours) within glasshouse. Irrigations were applied at 60% field capacity of the soil mixture on weekly basis before permanent wilting point (8.2% moisture) arrived. There was no application of fertilizers at all. Data of the following traits was recorded after 60 days of seedling transplantation.

Scoring the seedling tolerance

Fresh and dry weight percent (%) reductions (FWPR and DWPR)

Ten plants were chosen randomly from each treatment within each replication and uprooted carefully after heavy irrigation to minimize any root loss. Plants were washed with tap water, dried with paper towel and cut into shoots and roots with particularity at crown (the root-shoot junction). Fresh roots weight (FRW) and fresh shoots weight (FSW) were measured with a digital balance. Average values of FRW and FSW were calculated for each treatment. Roots and shoots of each plant were first sundried in paper bags for 3 days then placed in an oven (70°C) for 72 h for complete drying. Dry roots weight (DRW) and dry shoots weight (DSW) were recorded. Average values of DRW and DSW were calculated for each treatment. FWPR and DWPR were determined using the following formula as given by El-Goumi et al. (2014):

$$FWPR\ \% = 100 \times [1 - (FW_{salt\ stress}/FW_{control})]$$

$$DWPR\ \% = 100 \times [1 - (DW_{salt\ stress}/DW_{control})]$$

Where:

FW = Fresh weight of roots or shoots, and *DW* = Dry weight of root or shoot

Taking in consideration the aforementioned formula, four types of FWPRs were calculated including two fresh shoot weight % reductions ($FSWPR_{10}$ using observations of S_{10} and S_0, and $FSWPR_{15}$ using observations of S_{15} and S_0) and two fresh root weight % reductions ($FRWPR_{10}$ using observations of S_{10} and S_0, and $FRWPR_{15}$ using observations of S_{15} and S_0). Four types of DWPRs were also calculated including two dry shoot weight % reductions ($DSWPR_{10}$ using observations of S_{10} and S_0, and $DSWPR_{15}$ using observations of S_{15} and S_0) and two dry root weight % reductions ($DRWPR_{10}$ using observations of S_{10} and S_0, and $DRWPR_{15}$ using observations of S_{15} and S_0).

Ratios of K^+/Na^+ and Ca^{2+}/Na^+

Na, K and Ca concentrations were determined from cell sap. Cell sap was extracted from leaves according to Ghanem et al. (2010). Five plants from each treatment within each replication were randomly selected, their leaves were harvested chopped into small pieces separately, and placed in perforated falcon tubes for flash freezing in liquid N. The samples were then thawed at room temperature to rupture cell membranes. The freezing-thawing cycle was repeated three times, and tubes were encased in an intact second falcon tube which was centrifuged at 9000 rpm at 4°C for 10 min. The collected sap was diluted 40 times and concentration of Na^+, K^+ and Ca^{2+} was determined by Sherwood Flame Photometer (Model 410, Sherwood Scientific Ltd., Cambridge, UK) and average concentration was calculated for each replication. Ratios of K^+/Na^+ and Ca^{2+}/Na^+ were calculated after concentrations of all three elements were determined from cell sap of same plant. Average values of both of ratios (K^+/Na^+ and Ca^{2+}/Na^+) for each replication were calculated from all five plants. These inorganic osmolytic ratios of tomato seedlings were further used to determine tolerance indices.

Tolerance indices

Tolerance indices being individual genotypic response towards salt treatments were calculated using the formula of LaRosa et al. (1989):

$$Tolerance\ index\ (TI) = 100 + \Sigma^n\ [X\ (T_x/T_0)100]$$

Where:

n = number of salinity levels;
X = NaCl concentration (g L^{-1}) in soil;
T_x= value of seedling trait on stressed plants;
T_0= value of seedling trait on control plants.

Four types of tolerance indices were determined for every genotype including two dry weight tolerance indices (RDWTI for roots and SDWTI for shoots) and two ratio based K^+/Na^+ and Ca^{2+}/Na^+ tolerance indices (K.Na.TI and Ca.Na.TI) following the study of Turhan and Seniz (2012).

Statistical analysis

After taking data for all the seedling traits, it was subjected to analysis of variance (ANOVA) following the study of Steel et al. (1997) to sort out significant differences among genotypes using their interactions with salinity levels subsequent of complete randomized design. Using RStudio software Version 0.98.1102, RStudio, Inc. (R Core Team), principle component analysis (PCA) was performed to obtain more reliable information on how to identify groups of genotypes that have desirable salt tolerance traits for breeding. The graphical data representation of salinity and plant interactions whenever provided by PC-biplot is so informative that it requires only a look to understand the potential salt tolerance of genotypes (Raza et al., 2016). PCA was obtained following the method as given by Husson et al. (2011).

RESULTS AND DISCUSSION

Variability in germplasm and associations among seedling tolerance traits

Hitherto, different crop species were observed under saline conditions for fresh and dry weights of roots and shoots (Liem et al., 1985; Azhar and McNeilly, 1989; Noori and McNeilly, 2000; Akinci et al., 2004) along with salt affected cationic ratios (K^+/Na^+ and Ca^{2+}/Na^+) (Dasgan et al., 2002; Juan et al., 2005; Ahmadi et al.,

Table 1. Mean square table for seedling tolerance traits.

Source of variation	Genotype	Replication	Error
Degree of freedom	24	2	150
Fresh shoot weight % reduction at S_{10} ($FSWPR_{10}$)	3732.80**	3.200	0.600
Fresh shoot weight % reduction at S_{15} ($FSWPR_{15}$)	8430.90**	0.500	1.800
Dry shoot weight % reduction at S_{10} ($DSWPR_{10}$)	21135.30**	27.800	2.900
Dry shoot weight % reduction at S_{15} ($DSWPR_{15}$)	23524.90**	23.000	7.000
Fresh root weight % reduction at S_{10} ($FRWPR_{10}$)	14335.40**	0.600	1.800
Fresh root weight % reduction at S_{15} ($FRWPR_{15}$)	12952.20**	1.300	2.700
Dry root weight % reduction at S_{10} ($DRWPR_{10}$)	189644.00**	206.000	23.000
Dry root weight % reduction at S_{15} ($DRWPR_{15}$)	46445.00**	82.200	9.300
K^+/Na^+ ratio tolerance index (K.Na.TI)	17.96**	111.620	1.080
Ca^{2+}/Na^+ ratio tolerance index (Ca.Na.TI)	131.18**	120.420	1.150
Shoot dry weight tolerance index (SDWTI)	8.84**	108.190	1.050
Root dry weight tolerance index (RDWTI)	4.90**	107.220	1.027

DF indicates degrees of freedom; ** indicates significance at 1% level; S_{10} is salinity level of 10 dSm^{-1}; S_{15} is salinity level of 15 dSm^{-1}.

2009; Turhan and Seniz, 2012), that are maintained by plants. These physio-agronomic plant traits were used as worthy indicators of salt tolerance (Ibrahim, 2003) for screening tomato genotypes at seedling stages. These traits were further employed to get attributes of salt tolerance that is, weight % reductions of roots and shoots at increasing salinity levels (S_{10} and S_{15}) were recorded following El-Goumi et al. (2014) and salt tolerance indices according to Turhan and Seniz (2012).

Genotype as a source of variation was found highly significant ($p < 0.01$) for all weight % reductions (El-Goumi et al., 2014) and tolerance indices (Turhan and Seniz, 2012) (Table 1) giving an indication of a diverse genetic variability that suits in identifying tolerant and susceptible tomato genotypes. Different genotypes failed to respond in a definite and predictable response (Akinci et al., 2004) in terms of weight % reductions and tolerance indices on a given saline media. While comparing means, some genotypes had negative values of weight % reduction which means no loss of mean plant weight due to harsh saline conditions, rather an increase in overall biomass production.

One group of these genotypes including ZARNITZA, BEAVER LODGE SLICER, FORME DE COEUR, GLACIER and LO-2707, which had no weight % reduction of root and shoot at both salinity levels other than control. Second group included the genotypes (ANAHU, Rio-GRANDE, UOVO ROSEO and 17860) that showed significantly increased biomass but at highest salinity level (S_{15}). Third group consisting of NAGINA, ROMA, BL-1079, 6232, NUTYT-701 and LA-1021 genotypes produced a high fresh and dry biomass at both S_{10} and S_{15} level of salinity compared to control. All other genotypes were representative of a group with sharp decrease in plant fresh and dry biomass at saline environments other than control (Li and Stanhellini, 2001; Hajer et al., 2006; Maggio et al., 2007).

The selected trait inter-relationship positively helps in deploying the selection procedure to evaluate resilience. So, correlation analysis of seedling traits had depicted delightful results (Figure 1). It was found that root and shoot dry weight tolerance indices (RDWTI and SDWTI) have a strong negative association with all other traits particularly the weight % reductions (both fresh and dry shoots and roots weight reductions at S_{10} and S_{15} salinity levels that is, $FSWPR_{10}$, $FSWPR_{15}$, $FRWPR_{10}$, $FRWPR_{15}$, $DSWPR_{10}$, $DSWPR_{15}$, $DRWPR_{10}$ and $DRWPR_{15}$. However it does not stand true for K^+/Na^+ ratio tolerance index (K.Na.TI). At certain points, Ca^{2+}/Na^+ ratio tolerance index (Ca.Na.TI) and K^+/Na^+ ratio tolerance index (K.Na.TI) negatively correlated with other traits that is, $FSWPR_{10}$, $DSWPR_{10}$, $DSWPR_{15}$ and RDWTI (Figure 1). All weight % reductions either of roots or shoots regarding S_{10} and S_{15} levels of salinity, are strongly positive in their relationships with each other (Figure 1).

Principle component analysis (PCA) of seedling tolerance traits

The mean data were analyzed by PCA through RStudio software Version 0.98.1102, RStudio, Inc. (R Core Team). Eigen values, % variance and cumulative % variance are presented in supplemental data. Table 2 shows that the first three principal components (PCs) have Eigen values greater than 1. First two PCs contribute a cumulative variance of 69.656%, however, with first three PCs, the cumulative variance contribution was 83.731% (Table 2).

Using RStudio software two data matrices of 25 (genotypes) × 12 (PCs) and 12 (traits/variables) × 12 (PCs) were prepared for the analysis (Tables 3 and 4). Since used traits are genotypic responses with respect to

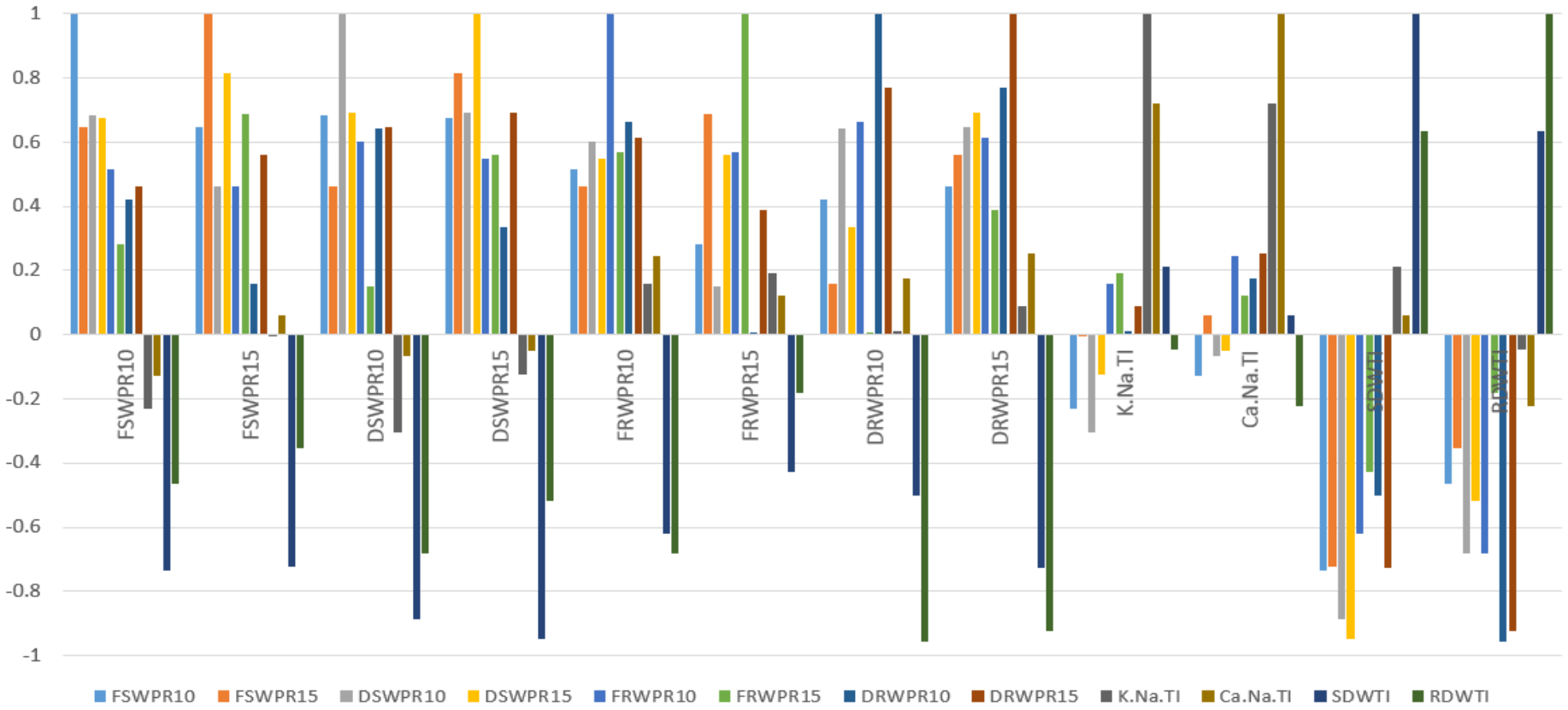

Figure 1. A graphical representation of correlation among 12 seedling salt tolerance traits.
FRWPR10; DRWPR10 and FSWPR10; DSWPR10 (Fresh/dry root and shoot weight % reduction at 10 dSm-1), FRWPR15; DRWPR15 and FSWPR15; DSWPR15 (Fresh/dry root and shoot weight % reduction at 15 dSm-1), K.Na.TI (K+/Na+ tolerance index) and Ca.Na.TI (Ca2+/Na+ tolerance index) SDWTI (Shoot dry weight tolerance index), RDWTI (Root dry weight tolerance index)

cumulative effect of all salinity levels, whatsoever, PCA distributes the overall mean data into individual PC contributive loadings. These loadings are representative of variability produced by all variables in the form of individual PC (Tables 3 and 4).

In fact, each variable contributes in each PC, so a complete data matrix table is formed. This data matrix was used to draw a principal component biplot (PC-biplot) which was a very handy graphical representation of variability within the germplasm (Figure 2). The PC-biplot had shown a complete relationship among observed salt tolerance traits and among genotypes, and particularly the response of individual genotype for all traits, so selection pressure can be easily applied (Figure 2). Principal component analysis was used as one of the most reliable statistical model that best expresses the genotypic performance at given saline conditions (Kaya et al., 2006; Ali et al., 2012).

First of all, PC-biplot had shown variability and association of salt tolerance traits. Each trait was allocated its demonstrative vector in the PC-biplot

Table 2. Eigen values, percent variances and cumulative percent variance for each principal component depending upon 12 seedling tolerance traits.

Principal Component	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
Eigen values	6.261	2.098	1.689	0.604	0.504	0.352	0.255	0.109	0.097	0.031	0.000	0.000
% Variance	52.172	17.484	14.075	5.036	4.197	2.931	2.127	0.910	0.812	0.255	0.000	0.000
Cumulative % var.	52.172	69.656	83.731	88.767	92.964	95.896	98.023	98.933	99.745	100.000	100.000	100.000

Table 3. Principal component loadings of 25 tomato genotypes.

S/N	Genotypes	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
1	NUTYT-701	0.0380	1.1561	1.0341	1.3290	0.2246	-0.3601	-0.3135	0.0419	-0.4084	-0.0238	0.0003	0.0001
2	ANAHU	0.0540	0.1881	-0.5588	0.3453	-1.4353	-0.0900	-0.1970	0.2269	-0.1248	0.0570	-0.0007	-0.0004
3	ZARNITZA	5.5399	2.0294	2.3663	0.7076	-1.1320	1.0247	-0.2466	0.1439	0.4966	-0.0830	0.0001	0.0000
4	LA-2821	-1.3137	-1.8764	0.5176	-0.4788	0.7667	0.5362	-0.8446	1.2227	-0.2302	-0.0217	0.0001	0.0000
5	Rio-GRANDE	0.1390	-2.2204	1.3552	-0.4044	0.5166	-0.2363	-1.3852	-0.6773	0.4978	0.2792	0.0001	-0.0001
6	LA-1021	-0.5131	0.8300	1.9295	-0.5300	-0.1472	0.4681	0.0321	-0.1451	-0.1476	0.1046	-0.0006	0.0002
7	ROMA	-1.2780	1.2395	0.3987	-0.5614	0.4717	-0.4239	0.1950	-0.0362	-0.2164	0.1358	0.0004	0.0002
8	FORME DE COEUR	3.6851	0.6069	2.1839	-1.5284	0.9501	0.0472	0.4596	-0.2062	-0.4759	-0.2914	0.0001	-0.0002
9	EARLY ANNIE	-1.7592	1.4341	0.3332	1.4287	0.9540	-0.5395	-0.1426	-0.0388	0.0446	-0.0979	-0.0002	-0.0002
10	NAGINA	0.4045	1.9915	-0.2094	-0.9073	0.5575	-0.3926	0.1530	0.1339	0.0075	0.3459	-0.0007	0.0000
11	BEAVER LODGE SLICER	7.1370	-1.5658	-2.7811	0.2743	-0.1259	-0.6067	-0.6606	-0.1035	-0.4281	-0.1171	-0.0001	0.0002
12	GLACIER	2.4020	-3.7121	1.5325	0.8316	0.0857	-0.8512	1.4182	0.2467	0.3246	0.1076	-0.0003	0.0001
13	UOVO ROSEO	1.6848	1.9397	-1.4809	0.0567	-0.2867	-0.4484	0.1103	0.2736	0.2094	0.2727	0.0004	0.0002
14	17860	0.3058	-0.3843	-0.8380	-0.7285	-0.9457	0.1443	0.2578	-0.3873	-0.4004	0.0728	0.0003	-0.0001
15	LO-2831	-1.4090	-0.5949	-0.0272	-0.1536	-0.5302	0.2396	0.2449	0.0171	-0.0918	0.0745	0.0004	-0.0002
16	BL-1079	-0.7463	0.9814	-0.6517	0.4003	0.4638	-0.2446	0.2325	-0.0539	-0.1439	0.1350	0.0000	-0.0003
17	6232	-1.4204	1.0056	-0.5403	0.1573	0.2600	-0.3002	-0.0662	0.0730	0.2174	-0.0417	0.0002	0.0002
18	17856	-0.7833	-0.9209	-1.3515	1.2149	0.7229	1.9901	0.3923	-0.3032	-0.2571	0.1570	-0.0003	0.0002
19	6233	-0.9946	-0.9159	0.0827	0.5057	0.6979	0.0167	0.0175	-0.2217	0.1803	-0.1669	0.0006	-0.0002
20	PB-017909	-1.9624	-0.0798	-0.4635	-0.0347	-0.1014	-0.2571	-0.2026	-0.1139	0.2083	-0.3292	-0.0013	0.0000
21	LO-2576	-3.0807	-0.1034	0.0226	-0.0362	-0.3835	-0.1900	-0.0109	-0.1927	-0.1021	-0.2077	-0.0002	0.0004
22	LO-2692	-2.4546	-1.5640	0.1931	-0.2575	-0.8621	0.1711	0.0653	-0.0388	-0.1840	0.0536	0.0004	0.0000
23	LO-2707	1.2578	0.3389	-2.5795	-1.1739	0.8042	0.6235	0.3921	0.0782	0.7112	-0.1518	0.0001	-0.0001
24	LO-2752	-2.7535	-0.6232	-0.0094	-0.9817	-1.0706	-0.0874	0.0421	0.0678	0.1708	-0.0704	0.0000	0.0001
25	LO-2831-23	-2.1794	0.8201	-0.4583	0.5250	-0.4553	-0.2334	0.0570	-0.0071	0.1421	-0.1931	0.0008	-0.0001

Table 4. Principal component loadings of 12 seedling tolerance traits.

Seedling tolerance trait	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
$FSWPR_{10}$	-0.3000	0.2215	0.0433	-0.3089	-0.5467	-0.5450	-0.0410	-0.3338	-0.2248	-0.0644	0.0005	0.0001
$FSWPR_{15}$	-0.2934	0.0845	0.4289	-0.1739	0.1509	-0.3242	-0.2307	0.6559	0.2849	-0.0049	0.0004	0.0001
$FRWPR_{10}$	-0.3362	0.1649	-0.2099	-0.2000	-0.1782	0.4797	0.0119	0.4023	-0.4774	-0.0237	-0.3508	0.0194
$FRWPR_{15}$	-0.3457	0.1514	0.2361	-0.1727	0.2276	0.1839	0.2755	-0.4064	0.3896	0.1980	-0.5035	0.0279
$DSWPR_{10}$	-0.3157	-0.1895	0.0180	0.4527	-0.5219	0.2172	0.0050	0.0345	0.4215	-0.4008	-0.0001	0.0002
$DSWPR_{15}$	-0.2025	-0.0854	0.5544	0.5400	0.0342	0.0327	-0.1457	-0.1356	-0.4696	0.3005	0.0000	-0.0002
$DRWPR_{10}$	-0.2838	-0.1860	-0.4704	0.1836	-0.0325	-0.2125	0.0026	0.1062	0.1261	0.5665	-0.0277	-0.4845
$DRWPR_{15}$	-0.3485	-0.1707	-0.1184	0.0310	0.5036	-0.1479	-0.0399	-0.1608	-0.2183	-0.5951	-0.0194	-0.3596
SDWTI	0.0239	-0.6012	0.2140	-0.1941	-0.1106	-0.1159	0.6903	0.1730	-0.1499	0.0030	0.0005	-0.0002
RDWTI	-0.0404	-0.6000	0.0829	-0.4246	-0.0817	0.2386	-0.5802	-0.1953	0.0487	0.1025	0.0001	0.0000
K^+/Na^+ TI	0.3705	-0.1704	-0.0567	0.1987	-0.0660	-0.3317	-0.1812	0.0806	-0.0369	-0.1169	-0.7876	0.0440
Ca^{2+}/Na^+ TI	0.3304	0.1905	0.3399	-0.1258	-0.2080	0.1960	0.0162	0.0081	0.0221	-0.0763	-0.0442	-0.7955

$FRWPR_{10}$; $DRWPR_{10}$ and $FSWPR_{10}$; $DSWPR_{10}$ (Fresh/dry root and shoot weight percent reduction at 10 dSm^{-1}), $FRWPR_{15}$; $DRWPR_{15}$ & $FSWPR_{15}$; $DSWPR_{15}$ (Fresh/dry root and shoot weight percent reduction at 15 dSm^{-1}), K.Na.TI (K^+/Na^+ ratio tolerance index), Ca.Na.TI (Ca^{2+}/Na^+ ratio tolerance index). SDWTI (Shoot dry weight tolerance index), RDWTI (Root dry weight tolerance index).

(Figure 2). Traits with longer vectors were representative of more variability (Figure 2), in which SDWTI, DRWPR15 and DSWPR15 have longest vectors depending upon values that can be seen on PC-biplot and their respective loadings (Table 4). These vectors also depicted some sort of relationship among salt tolerance traits. Vectors in same direction were positively correlated while those in opposite direction were negatively correlated. From Figure 2, it was seen that RDWTI was negatively correlated with reductions in root weight that is, FRWPR10, FRWPR15, DRWPR10 and DRWPR15.

Similarly, SDWTI was negatively associated with shoot weight reductions that is, FSWPR10, FSWPR15, DSWPR10 and DSWPR15, while both RDWTI and SDWTI were independent of K^+/Na^+ and Ca^{2+}/Na^+ ratio tolerance indices (K.Na.TI and Ca.Na.TI). All weight % reductions were positively correlated with each other, however, K^+/Na^+ and $Ca^{2+/}Na^+$ ratio tolerance indices were in a positive relation with each other.

Next from PC-biplot, the genotypes similarities were revealed with other genotypes and their response to a particular salt tolerance trait (Figure 2). Genotypes that were nearer to each other were of same group in their overall behavior in the form of observed traits for example, EARLY ANNIE, ROMA, NUTYT-701, 6232, BL-1079, LA-1021 and LO-2831-23 were very close to each other so must be place in a single group (Figure 1). Other group includes ANAHU, LO-2707, 17860, PB-017909, LO-2576, LO-2831, LO-2752, 6233 and 17856. NAGINA and UOVO ROSEO were side by side while Rio-GRANDE, LA-2821 and LO-2692 were adjacent to each other (Figure 2). Some genotypes like BEAVER LODGE SLICER, GLACIER, ZARNITZA Rio-GRANDE, NAGINA, UOVO ROSEO, FORME DE COEUR and LO-2576 had provided more diversity to the tomato germplasm, therefore, were considered a separate group (Figure 2).

Furthermore, PC-biplot depicted individual and group-wise performance of genotypes for a particular salt tolerance trait. Genotypes including GLACIER, Rio-GRANDE on or very immediate to the vectors of Ca^{2+}/K^+ and K^+/Na^+ ratio tolerance indices (Ca.Na.TI and K.Na.TI), and their projections to these vectors were longest inrespective to the origin, so, these genotypes are considered good performer (Yan, 2001) for both traits (Figure 2).

BEAVER LODGE SLICER revealed very good for SDWTI because of its longest vector considering farthest perpendicular (Yan, 2001). ZARNITZA, FORME DE COEUR, UOVO ROSEO, NAGINA and BEAVER LODGE SLICER were highly responsive for RDWTI (Figure 2). These three groups of genotypes were showing highly tolerant behavior for provided saline conditions

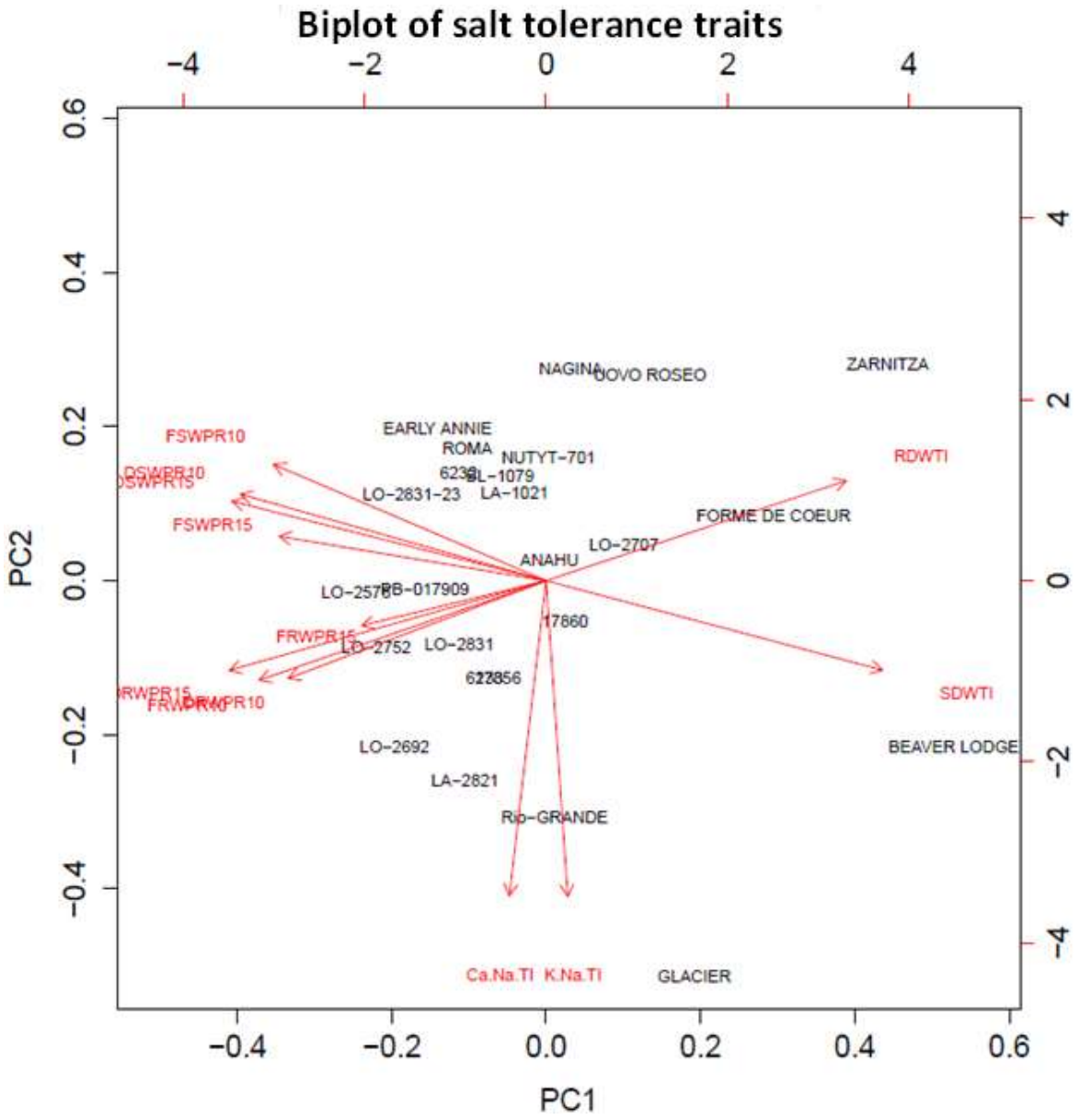

Figure 2. Principle component biplot for salt tolerance traits.
FRWPR10; DRWPR10 and FSWPR10; DSWPR10 (Fresh/dry root and shoot weight % reduction at 10 dSm^{-1}), FRWPR15; DRWPR15 and FSWPR15; DSWPR15 (Fresh/dry root and shoot weight % reduction at 15 dSm^{-1}), K.Na.TI (K^+/Na^+ tolerance index) and Ca.Na.TI (Ca^{2+}/Na^+ tolerance index) SDWTI (Shoot dry weight tolerance index), RDWTI (Root dry weight tolerance index)

Virtually, a group of 7 genotypes consisting of EARLY ANNIE, ROMA, NUTYT-701, 6232, BL-1079, LA-1021 and LO-2831-23 was found with highest shoot weight % reductions at both S_{10} and S_{15} levels of salinity, and were irrespective in their response to all roots weight % reductions (Figure 2).

On the other hand, a different group of seven genotypes including PB-017909, LO-2576, LO-2831, LO-2752, 6233, 17856 and LO-2692 were seen with a highly positive response for root weight % reductions at both S_{10} and S_{15} levels (Figure 2). This group apparently, did not seem to be effectively engaged with shoot weight % reductions. Three genotypes % (ANAHU, LO-2707 and 17860) were found with a slight involvement towards all tolerance indices but highly negative behavior for all weight % reductions, as these were closer to the origin. This group could be said to be a moderately tolerating group to the investigated salinity levels (Figure 2).

Genotypes having higher values for tolerance indices (GLACIER, Rio-GRANDE, LA-2821) and lowest values of

weight % reductions (BEAVER LODGE SLICER, ZARNITZA, FORME DE COEUR) were established as more tolerant than remaining. These genotypes can be allotted to a wide spectrum of soils that constitute harsh saline environment to bred ideotypes having a suitable combination of both traits.

A group of 6 genotypes including ANAHU, LO-2707, 17860, UOVO ROSEO, NAGINA and LA-2821 based on their little positive performances for tolerance indices but significantly negative behavior against weight % reductions were detained under moderately salt tolerant group and could be used for further breeding programs as well to retain diverse genetic base. While genotypes using greater capability for either roots weight % reductions or shoots weight % reductions were actually producing very less biomass, therefore, considered as salt susceptible and cannot be regarded as good choice for future breeding programs (Figure 2).

The differences in behavior of these groups are a result of underlying genes behind individual response. Gene mining approaches could be further applied to identify the mechanism of such genes and that when stimulated themselves produce specific combinations of salt tolerance traits, which result in higher yields.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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