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Responsive gene screening and exploration of genotypes responses to salinity tolerance in tomato

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Abstract

Salinity is a major abiotic stress affecting plant growth and productivity during all plant developmental stages. Fourteen tomato genotypes including six commercial cultivars, six improved genotypes and two salt-tolerant breeding lines were used in this study to evaluate their salinity tolerance and to explore the expression of some salt-responsive genes under saline conditions. Five salinity concentrations including 0.5 (control treatment), 2.4, 4.8, 7.2 and 9.6 dS m^{-1} NaCl were applied using a drip irrigation system. Based on the evaluation of plant growth and yield component traits, two genotypes (L56 and L46) were selected to explore expression of salt-responsive genes to be utilised as biomarkers in breeding programmes. Five important salt-responsive tomato genes (NAC, JERF3, GRX1 TAS14 and NAM) were retrieved from GenBank and primers were designed for quantitative real-time PCR (qPCR). Successive increases in salinity levels, starting at 4.8 dS^{-1}, were associated with significant decreases in most vegetative, yield and quality traits. However, TSS and pH increased at high salinity levels. Tomato genotypes showed a wide range of variability in yield and fruit quality traits in response to salinity. Based on plant growth and yield component traits and according to canonical discriminate multivariate analysis, the salt tolerances of tomato genotypes were clustered into three groups: tolerant to salinity (BL 1076, BL 1239, L26, L56, Strain-B and Pakmore), moderately tolerant to salinity (L16, L66, Imperial, and Tnshet star) and susceptible to salinity (L36, L46, Queen, and Sohba). The qPCR screening showed that the salt stress tolerant tomato genotype, L56, prominently expressed the NAC, JERF3, GRX1 and TAS14 encoding genes. The expression of NAM was equally enhanced in both salt-tolerant (L56) and salt-susceptible (L46) tomato genotypes.

Keywords: *Solanum lycopersicum* L.; growth, yield; salinity stress; fruit quality; salt-responsive genes; qPCR.
Abbreviation: Quantitative real-time PCR (qPCR); total soluble solids (TSS); L16, L26, L36, L46, L56 and L66 (improved line derived from the commercial cultivars Imberial, Pakmore VF, Queen, Shohba, Strain-B and Tnshet star, respectively); BL 1076 and BL 1239 (two salt tolerance breeding line provided by Asian Vegetables Research and Development Center; AVRDC); Electrical conductivity (EC).

Introduction

Abiotic stresses are important constraints on plant growth and development. Salinity is a major abiotic stress affecting plant growth and productivity worldwide. The plant response to salt stress involves changes in morphology, physiology and metabolism (Hilal et al., 1998). A deep understanding of plant physiology, genetics, and molecular biology is important for breeding new cultivars that can be grown under saline conditions with similar crop productivity under non-saline conditions.

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops. This plant can act as a model crop for the use of saline and low-quality water because of the wealth of knowledge available regarding its physiology and genetics (Reina-Sánchez et al., 2005). It has been classified as moderately tolerant to salinity at all plant developmental stages (Lim and Ogata, 2005). Increasing the salt tolerance of crops through plant breeding could increase the sustainability of irrigation with low-quality water (Abdel Gawad et al., 2005). There are significant differences in the salt tolerance between the domesticated tomato species and related wild-type tomato species (Guerrier, 1998). Wild-type tomato species such as *S. pennellii* or *S. pimpinellifolium* are more tolerant to salt stress than the domesticated species (*S. lycopersicum*) because of a more intense and rapid genetic response to this environmental stress. Most of the gene expression profile changes in wild-type tomato in response to salinity have not yet been identified (Sun et al., 2010).

Genetic variability within a species is a valuable tool for screening and breeding for salt tolerance. In developing salt-tolerant genotypes, the emphasis is now on marker-assisted breeding and genetic transformation (Ashraf et al., 2008). Several DNA markers are currently available for assessing genetic variation (Cuartero et al., 2006). The use of genome-wide biomarkers can facilitate tomato research, especially genetic analysis and breeding to improve important traits, such as yield, fruit quality and resistance to biotic and abiotic stresses.

Plant tolerance to abiotic stress is mediated through complex networks of responsive genes, including TAS14

Table 1. Plant height (cm) for tomato genotypes as affected by different salinity levels.

Genotypes	Level of salinity (dS m^{-1})					
	0.5	2.4	4.8	7.2	9.6	Mean**
Imberial	83.6 pqr*	82.6 q-t	78.8 uvw	73.5 DEF	70.1 cd	77.7 f
PakmoreVF	95.6 a	93.8 bc	91.4 ef	87.2 jk	84.5 nop	90.5 a
Queen	91.6 ef	88.5 ij	84.1 opq	79.5 uv	78.5 u-y	84.4 d
Shohba	83.3 pqr	81.6 st	77.3 w-z	71.6 GHI	66.5 K	76.1 g
Strain-B	89.1 hi	88.4 ij	85.6 l-o	83.7 pqr	81.4 st	85.6 c
Tnshet star	91.0 efg	89.7 ghi	85.9 klm	81.2 t	77.3 w-z	85.0 cd
L 16	86.0 klm	85.7 lmn	81.5 st	77.1 xyz	74.8 CD	81.0 e
L 26	95.6 a	94.2 ab	91.2 efg	88.4 ij	86.2 klm	91.1 a
L 36	92.1 e	90.2 fgh	86.1 klm	79.4 u	77.8 v-y	85.1 cd
L 46	84.7 mno	82.5 rst	76.9 zAB	69.2 J	66.9 K	76.0 g
L 56	92.4 cd	91.6 ef	88.4 ij	86.5 kl	85.5 lmn	88.9 b
L 66	83.2 pqr	82.8 qrs	79.0 uv	74.6 CDE	71.5 HI	78.2 f
BL 1076	77.0 yz	76.9 zA	75.4 BC	73.1 FGH	71.2 HI	74.7 h
BL 1239	78.4 u-y	78.6 u-x	75.9 A-C	73.4 DEF	72.2 GH	75.7 g
Mean***	87.4 a	86.2 a	82.6 b	78.4 c	76.0 d	

*Values of the interaction between salinity levels and genotypes (five columns) followed by different letters are significantly different, using Revised LSD Test, at $P \leq 0.05$. **Means of the genotypes (last column) followed by different letters are significantly different at $P \leq 0.05$.
*** Means of the genotypes (last row) followed by different letters are significantly different at $P \leq 0.05$.

Table 2. Stem diameter (mm) for tomato genotypes as affected by different salinity levels.

Genotypes	Level of salinity (dS m^{-1})					
	0.5	2.4	4.8	7.2	9.6	Mean**
Imberial	17.1 fgh*	16.7 hij	15.3 n-q	14.7 p-u	13.8 vwx	15.5 de
PakmoreVF	15.4 m-p	15.2 n-r	14.5 q-v	14.1 tuv	13.5 wxy	14.5 f
Queen	17.8 c-f	17.2 e-h	15.4 m-p	14.6 q-v	13.5 wxy	15.7 cd
Shohba	15.1 n-s	14.8 o-u	13.1 w-z	12.4 zA	11.6 zA	13.4 g
Strain-B	18.9 abc	18.8 abc	18.1 b-e	17.6 d-g	17.1 fgh	18.1 a
Tnshet star	17.9 cde	17.4 d-g	15.9 klm	15.2 n-r	14.5 q-v	16.2 cd
L 16	17.3 d-h	17.1 fgh	16.2 jkl	15.8 k-n	14.9 q-t	16.3 c
L 26	15.8 k-n	15.6 l-o	14.8 p-u	14.4 r-v	14.0 uvw	14.9 ef
L 36	18.3 a-d	17.6 d-h	15.9 klm	15.1 n-s	14.4 r-v	16.3 c
L 46	15.3 n-q	14.7 p-u	12.6 xy	12.0 zA	11.2 BC	13.2 g
L 56	19.3 a	19.2 ab	18.4 abc	18.1 bcd	17.8 d-g	18.6 a
L 66	18.2 a-d	17.9 cde	17.2 e-h	16.8 ghi	15.9 klm	17.2 b
BL 1076	14.3 r-v	14.2 s-w	13.8 v-y	13.7 wxy	13.1 yz	13.8 g
BL 1239	16.7 hij	16.5 ijk	15.9 klm	15.4 m-p	15.1 o-s	15.9 cd
Mean***	16.9 a	16.6 a	15.5 b	14.9 c	14.3 d	

*Values of the interaction between salinity levels and genotypes (five columns) followed by different letters are significantly different, using Revised LSD Test, at $P \leq 0.05$. **Means of the genotypes (last column) followed by different letters are significantly different at $P \leq 0.05$.
*** Means of the genotypes (last row) followed by different letters are significantly different at $P \leq 0.05$.

(Godoy et al., 1990), JERF3 (Wang et al., 2004) and NAC (Yang et al., 2011; Han et al., 2012). These genes regulate the response and degree of tolerance back and forth along signal transduction cascades. Such reference genes can be used to evaluate the available breeding lines and can be integrated as molecular markers in a plant breeding programmes. The complex network of tomato salt-responsive genes has been identified using suppression subtractive hybridisation (Ouyang et al., 2007) and tomato micro-array analysis (Sun et al., 2010).

This work is a part of a comprehensive breeding programme aimed to breeding the tomato cultivars with high salt tolerance. The objectives of this study were to evaluate the growth and yield traits of tomato genotypes under various salinity conditions and to explore the expression of some salt-responsive genes under these conditions.

Results and Discussion

Plant growth traits

All the plant growth traits were significantly reduced with successive increases in water salinity levels, starting at the 4.8 dS m^{-1} salinity level. However, a moderate salinity level (2.4 dS m^{-1}) had no significant effect on any trait, except for leaf area, compared to the control treatment (0.5 dS m^{-1}) (Fig.1 a-e and Tables 1-5). At the highest salinity level (9.6 dS m^{-1}), the plant growth traits were smaller than those at the control level (0.5 dS m^{-1}) by approximately 13, 11, 17, 16 and 18% for plant height, stem diameter, leaf area, leaf fresh weight and dry weight, respectively. All of the plant growth traits responded similarly to salinity.

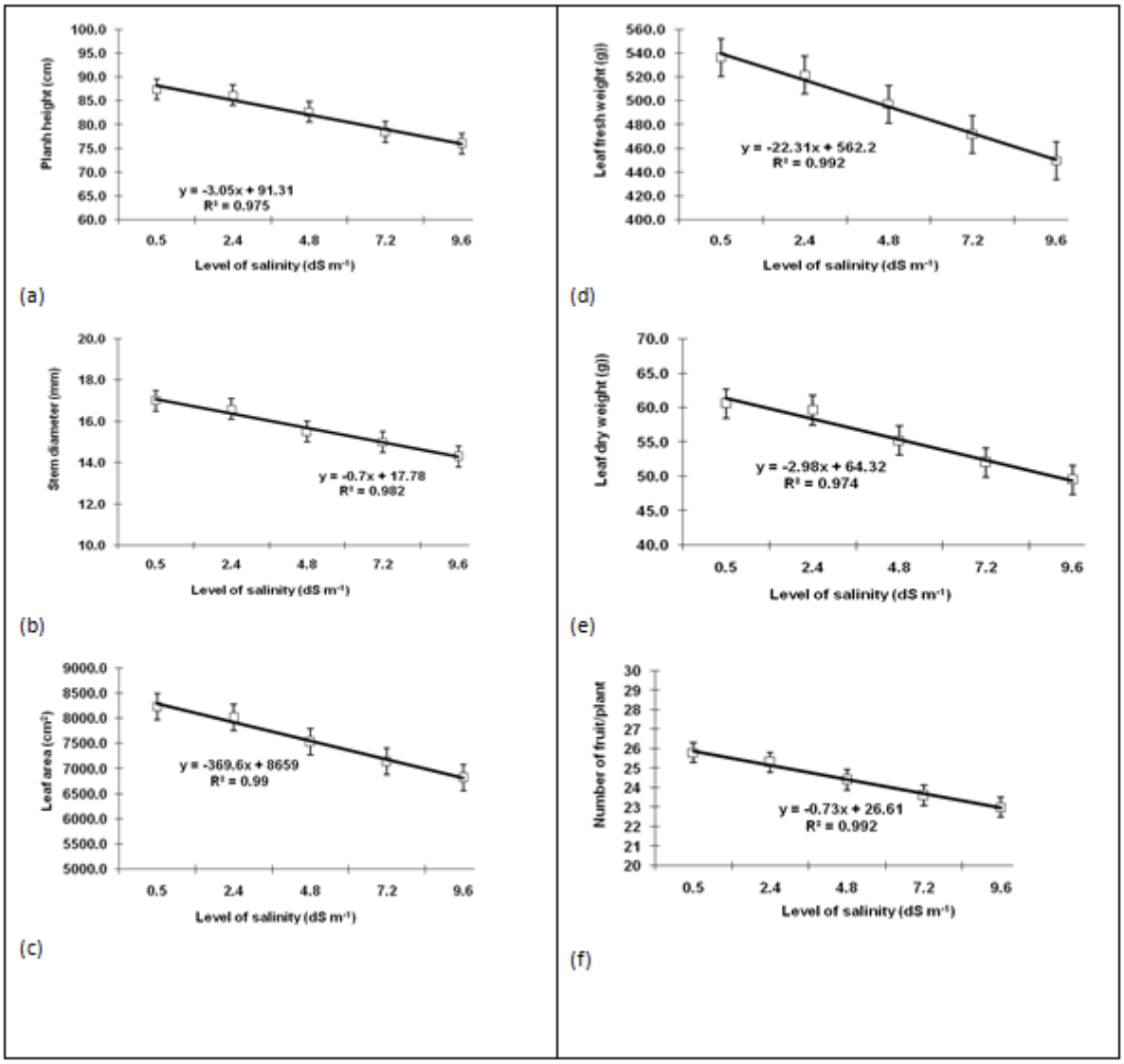

Fig 1. Influence of water salinity levels on (a) plant height, (b) stem diameter, (c) leaf area, (d) leaf fresh weight, (e) leaf dry weight and (f) number of fruit plant^{-1} for tomato genotypes. Bars represent L.S.D. value at $P \leq 0.05$ level.

However, a high salinity level (9.6 dS m^{-1}) inhibited leaf area and leaf fresh and dry weight to a greater extent than plant height and stem diameter. These results indicate that salt stress may have an effect on the allocation and distribution of photosynthetic resources within various plant organs. These results are in accordance with Olympios et al. (2003). The negative effects of higher salinity levels on the leaf mass of tomato plants have also been reported by Van-Ieperen (1996), who found that the decrease in leaf dry weight in response to salinity (especially at levels above 6 dS m^{-1}) was not caused by a reduction in the number of leaves but by a reduction in leaf area. Cruz and Cuartero, (1990) found that both the stem and leaf dry weights of tomato plants are diminished under saline conditions, but the reduction in leaf dry weight is greater than that of dry shoot weight.

The performances of different tomato genotypes under different levels of salinity demonstrated that the genotypes had a wide range of variability for all plant growth traits (Tables 1-5). Successive increases in salinity levels, starting at 4.8 dS m^{-1}, were associated with significant decreases in all traits. However, for each trait, the response varied among the genotypes. The genotypes that showed the highest values were L26 followed by Pakmore VF for plant height (Table 1), L56 followed by Strain-B for stem diameter (Table 2), L26 followed by L56 for leaf area (Table 3), and L56 followed by L26 for both leaf fresh and dry weights (Tables 4 and 5). The lowest values were found in BL1076, BL1239 and L46 for plant height (Table 1); L46, Shohba and BL1076 for stem diameter (Table 2); Queen followed by L46 for leaf area (Table 3); and L46 followed by Shohba for both leaf fresh and dry weights (Tables 4 and 5). The results indicate that salinity caused significant reductions in all traits, when comparing the responses of different genotypes under both higher (S4) and lower (S0, control treatment) salinity levels. However, the extent of that reduction varied according to the trait. The genotype that had the lowest reductions for all plant growth traits was L56 followed by L26, in addition to two salt-tolerant breeding lines BL 1076 and BL 1239. The plants with the greatest reductions were L46, Queen and Shohba.

Based on the response of the tomato genotypes' plant growth traits to salinity stress, the tomato genotypes used in this study can be classified into three groups: salt tolerant, moderately salt tolerant and salt susceptible. The first group consists of the two salt-tolerant breeding lines BL 1076 and BL 1239, which were provided by the AVRDC and

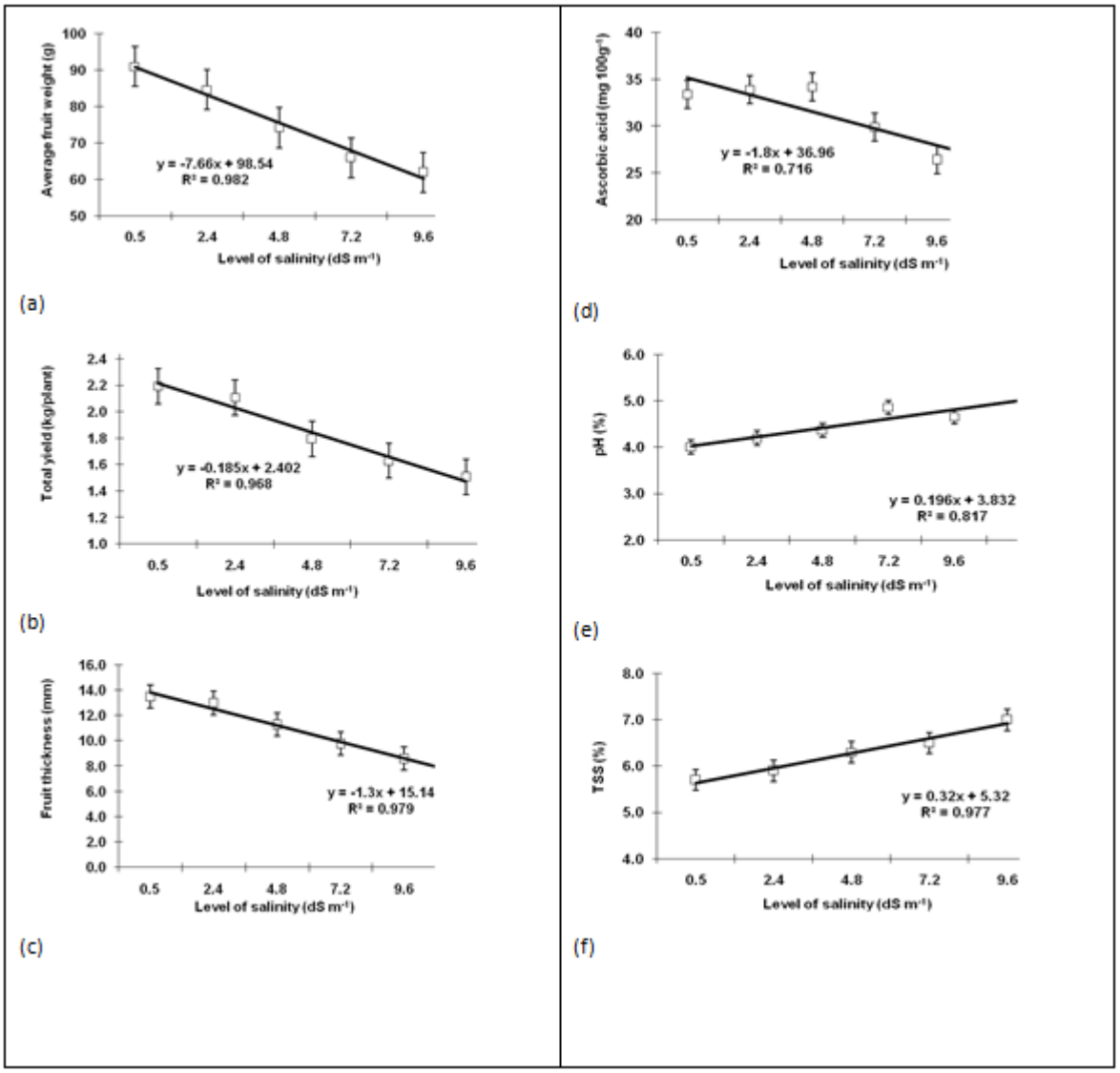

Fig 2. Influence of water salinity levels on (a) average fruit weight, (b) total yield, (c) fruit flesh thickness, (d) ascorbic acid content, (e) pH and (f) TSS for tomato genotypes. Bars represent L.S.D. value at $P \leq 0.05$ level.

previously classified as salt tolerant, in addition to the genotypes L56, L26, Strain-B and Pakmore. These genotypes had the best performance and the lowest reduction percentages for all plant growth traits at the various levels of salinity. Therefore, they could be considered as salt-tolerant genotypes. The second group includes four genotypes: L16, L66, Imperial and Tnshet star. These genotypes reflect an intermediate level of performance and reduction percentages for all traits. Therefore, they could be clustered as moderately salt tolerant. The third group contained four genotypes: L36, L46, Queen and Sohba. These genotypes had the lowest performance levels and the highest reduction percentages for all traits at the various levels of salinity. Therefore, they could be classified as salt-susceptible genotypes. Many authors have reported large variations among tomato genotypes in their responses to salinity (Alian et al., 2000, Romero-Aranda et al., 2001, Dasgan et al., 2002, Reina-Sánchez et al., 2005).

Yield component traits

Three yield component traits such as average fruit number, average fruit weight and total yield, significantly decreased with increasing salinity levels (Figs. 1f, 2a, 2b and Tables 6-8). The successive increase in salinity level from 0.5 to 2.4, 4.8, 7.2 and 9.6 dS m^{-1} caused a reduction in average fruit weight by 7, 18, 27, and 31%, respectively, compared with the control (Fig. 1f, Table 6). Although the reductions in the number of fruit per plant were 2, 5, 8 and 10% for 2.4, 4.8, 7.2 and 9.6 dS m^{-1} (Fig. 2a, Table 7), the total yield was reduced by 4, 18, 25 and 31%, respectively (Fig. 2b, Table 8). The decrease in total yield can be ascribed to the significant decrease in average fruit weight more than that to the decrease in fruit number because the reduction in fruit weight was greater than that in fruit number. The effect of salinity on yield became more marked as the harvest period progressed, initially because of a restriction of fruit size during the first four weeks of harvest and later because of a decrease in fruit number (Cuartero and Fernandez-Munnza 1999). The reduction in yield that occurred even at relatively moderate salinity levels (e.g., 2.4 dS m^{-1}) supports the suggestion of Cuartero and Fernandez-Munnza (1999) that even under normal growing conditions the electrical conductivities (EC) of the root solution is close to the threshold for yield reduction. These authors also reported that approximately 10% and 30% reductions in tomato fruit size

Table 3. Leaf area (cm^2) for tomato genotypes as affected by different salinity levels.

Genotypes	Level of salinity (dS m^{-1})					
	0.5	2.4	4.8	7.2	9.6	Mean**
Imberial	7320 k*	7070 klm	6670 nop	6235 rst	6017 tuv	6662 f
PakmoreVF	9509 def	9399 ef	8924 g	8878 g	8476 hi	9037 b
Queen	6636 n-q	6160 stu	5813 vu	5005 xy	4653 z	5653 g
Shohba	6836 lmn	6256 rst	5888 uv	5165 x	4819 yz	5793 g
Strain-B	9579 def	9499 def	8951 g	8824 g	8442 hi	9059 b
Tnshet star	8349 i	8292 i	7756 j	7350 k	6902 lmn	7730 d
L 16	8390 i	8289 i	7693 j	7129 kl	6864 lmn	7673 d
L 26	10998 a	10910 a	10348 b	10050 b	9787 cd	10419 a
L 36	6642 n-q	6492 pqr	6081 stu	5520 w	4915 xyz	5930 g
L 46	6799 mno	6317 rst	5560 w	4976 xy	4761 yz	5683 g
L 56	10356 b	10272 b	9701 de	9634 de	9534 def	9899 a
L 66	9725 d	9298 f	8748 gh	8391 i	7776 j	8787 c
BL 1076	7354 k	7291 k	6938 lmn	6776 m-p	6527 o-r	6977 e
BL 1239	6794 m-p	6680 nop	6337 qrs	6150 stu	6012 tuv	6394 f
Mean***	8234 a	8016 a	7529 b	7148 c	6820 d	

*Values of the interaction between salinity levels and genotypes (five columns) followed by different letters are significantly different, using Revised LSD Test, at $P \leq 0.05$.
**Means of the genotypes (last column) followed by different letters are significantly different at $P \leq 0.05$.
*** Means of the genotypes (last row) followed by different letters are significantly different at $P \leq 0.05$.

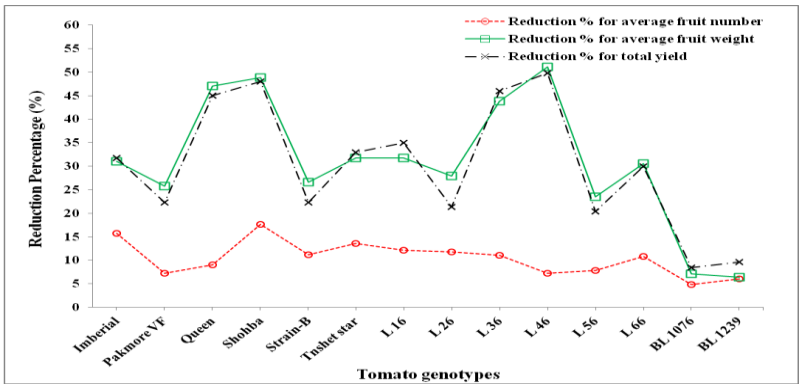

Fig. 3. Reduction percentages for yield components traits for different tomato genotypes under higher salinity level (9.6 dS $^{-1}$) as comparing with their relative control treatment (0.5 dS $^{-1}$)

occur at EC levels of 5-6 and 8 dS m^{-1}, respectively. Van-Ieperen (1996) reported a significant reduction in the average fruit weight but not in fruit number, even at low salinity levels applied for the entire experimental period.

The means of the different genotypes over all levels of salinity demonstrated a wide range of variability in average fruit weight, fruit number and total yield (Tables 6-8). The L56, followed by Strain-B, had the highest average fruit weight, fruit number and total yield. BL 1239 and BL 1076 had the lowest values for these three traits, followed by L46 for average fruit weight and by Queen for both total yield and number of fruit.

Comparing the yield component traits at a high salinity level (9.6 dS m^{-1}) with the those at the control level (0.5 dS m^{-1}), the results indicate that the two salt-tolerant breeding lines BL1076 and BL1239 have the lowest reduction percentages for all yield traits, followed by L56 and Pakmore for both average fruit weight and total yield and L46 and Pakmore for average fruit number (Fig. 3). However, the highest reduction percentages were found in L46 for both fruit weight and total yield and for Shohba for average fruit number. These results indicate that the L56 and Pakmore genotypes can be classified as salt tolerant, whereas L46 and Shohba can be classified as salt susceptible. These results confirm the classification of these genotypes based on plant growth traits as mentioned above. The results also demonstrate that the percentage reduction in average fruit weight and total yield was greater than that in fruit number for all genotypes.

Fruit quality traits

A significant variation in tomato fruit quality traits was observed when the genotypes were irrigated with saline water. High salinity levels reduced fruit flesh thickness and ascorbic acid content (Fig. 2 c-d and Tables 9-10); however, they increased pH (Fig. 2e, Table 11) and total soluble solids (TSS) (Fig. 2f, Table 12). The pH was the highest at the 7.2 dS m^{-1} level. The TSS of tomato fruit increased in response to increased salinity levels, which is in agreement with results reported by Campos et al. (2006), Tantawy et al. (2009) and Al-Yahyai et al. (2010). The increase in TSS with increasing water salinity might have been caused by the reduction in the import of water by the fruit under saline conditions (Sakamoto et al., 1999) and by active accumulation of solutes (mainly ions and organic molecules), which typically occurs in salt-stressed plants (Munns, 2002) and results in the concentration of soluble solids in the pulp. This concentration is due mainly to the secondary osmotic stress induced by the abiotic stress.

Table 4. Leaf fresh weight (g) for tomato genotypes as affected by different water salinity levels.

Genotypes	Level of salinity (dS m^{-1})					
	0.5	2.4	4.8	7.2	9.6	Mean**
Imberial	498 p-s*	481 r-u	460 t-w	430 w-B	415 x-C	456 f
PakmoreVF	608 a-d	601 a-e	578 c-j	575 c-j	549 i-m	582 a
Queen	446 u-z	414 x-C	396 B-E	341 FGH	317 H	382 gh
Shohba	448 u-y	410 z-D	391 CDE	343 FGH	320 H	382 gh
Strain-B	595 a-f	590 a-h	563 f-k	555 h-l	531 k-p	566 b
Tnshet star	588 a-h	584 a-i	554 h-m	525 l-q	493 q-t	548 cd
L 16	500 o-s	494 o-t	464 s-w	430 w-B	414 x-C	460 f
L 26	620 a	615 ab	590 a-h	573 d-j	558 g-l	591 a
L 36	444 v-z	434 w-A	412 x-C	374 DEF	333 GH	399 g
L 46	437 w-A	406 A-D	362 EFG	324 H	310 H	367 h
L 56	615 ab	610 abc	583 b-i	579 b-j	573 d-j	592 a
L 66	593 a-g	567 e-k	540 u-x	518 m-q	480 r-v	539 d
BL 1076	580 b-i	575 c-j	556 h-l	543 j-n	523 l-q	555 bc
BL 1239	535 k-o	526 l-q	507 n-r	492 q-t	481 r-u	508 e
Mean***	536 a	522 b	496 c	471 d	449 e	

*Values of the interaction between salinity levels and genotypes (five columns) followed by different letters are significantly different, using Revised LSD Test, at $P \leq 0.05$.
**Means of the genotypes (last column) followed by different letters are significantly different at $P \leq 0.05$.
*** Means of the genotypes (last row) followed by different letters are significantly different at $P \leq 0.05$.

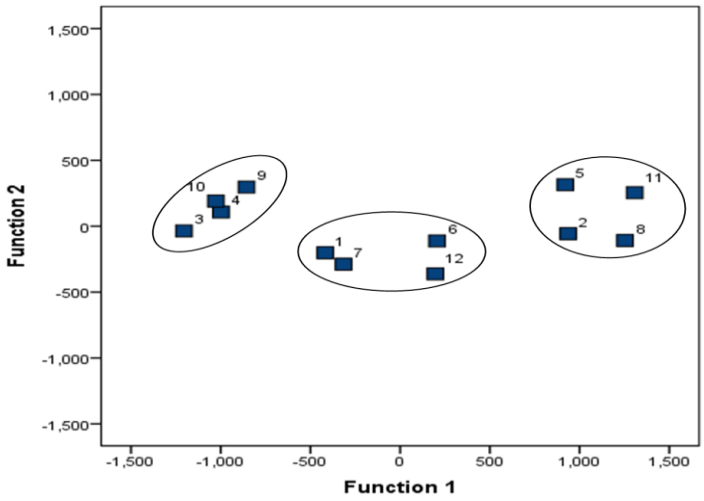

Fig 4. Clustering of twelve tomato genotypes indicated by numbers (1-12 as listed in table 15) based on Canonical discriminant analysis. The two main functions were performed on the basis of 10 agronomical characters listed in table (14).

The tomato genotypes showed a wide range of variability in fruit quality traits (fruit flesh thickness, ascorbic acid content, pH and TSS) in response to salinity (Tables 9-12). The genotypes L56 and Strain-B had the highest values for fruit flesh thickness, ascorbic acid content and pH. However, the lowest value was observed for BL1076 for fruit flesh thickness and pH traits, and the lowest value for ascorbic acid was observed for the genotype BL 1239. For TSS, the BL 1239 and L16 genotypes had the highest values, whereas Tnshet star had the lowest. These results are in agreement with those reported by Cuartero and Fernandez-Munoz (1999), who found that the TSS of two commercial tomato cultivars increased at a rate of 10.5% per 1 dS m^{-1} when the EC of the nutrient solution was increased from 2.5 to 8.0 dS m^{-1}. The TSS of tomato fruit is one of the most important variables in determining fruit flavour and quality because TSS is the most common index associated directly with the sugar and organic acid concentrations in juice (Young et al., 1993).

Cluster analysis

To cluster the twelve tomato genotypes, canonical discriminant multivariate analysis was performed based on their agronomical characters. The canonical discriminant analysis performed with the standardized canonical discriminant function coefficients showed that the first five functions accounted 100% of the total variation (92.4%, 5.6%, 1.1%, 0.7% and 0.2%, respectively) (Table 13). The first function was strongly influenced by leaf fresh weight, average fruit weight, and number of fruits/plant (Table 14). The second canonical discriminate function was found to be strongly influenced by total yield, ascorbic acid and average fruit weight (Table 14). In the third and fourth functions, leaf fresh weight was the main influencing character, while it was number of fruits in the fifth function. The upper two functions were utilized to cluster the investigated tomato genotypes (Fig. 4).

Table 5. Leaf dry weight (g) for tomato genotypes as affected by different water salinity levels.

Genotypes	Level of salinity (dS m^{-1})					
	0.5	2.4	4.8	7.2	9.6	Mean**
Imberial	54.8 qr*	52.9 s	49.7 t	46.0 wx	44.4 yz	49.6 g
PakmoreVF	71.1 bc	70.3 cd	66.5 hi	65.6 ij	62.3 lm	67.2 b
Queen	48.1 u	44.7 xy	42.0 AB	35.8 E	33.2 F	40.8 i
Shohba	47.9 uv	43.8 yz	41.1 BC	35.6 E	33.2 F	40.3 i
Strain-B	69.1 de	68.4 ef	64.2 jk	62.3 lm	60.0 no	64.8 c
Tnshet star	67.1 f-i	66.6 hi	62.1 lm	58.3 p	54.7 qr	61.8 d
L 16	55.5 pqr	54.8 qr	50.6 t	46.4 vw	44.7 xy	50.4 g
L 26	73.1 a	72.5 ab	68.4 ef	65.9 hi	64.2 jk	68.8 a
L 36	48.4 u	47.3 uvw	44.1 yz	39.6 C	35.3 E	42.9 h
L 46	46.3 w	43.1 z-C	37.6 D	33.4 F	31.2 G	38.3 j
L 56	73.2 a	71.9 ab	68.2 efg	67.2 fgh	66.5 hi	69.4 a
L 66	67.0 f-i	64.1 jk	59.9 no	52.8 s	50.0 t	60.2 e
BL 1076	66.7 ghi	66.1 hi	62.8 kl	60.8 mn	58.6 op	63.0 d
BL 1239	59.9 no	58.9 op	55.8 q	53.6 rs	52.4 s	56.1 f
Mean***	60.6 a	5839 b	55.2 c	51.4 d	49.5 e	

*Values of the interaction between salinity levels and genotypes (five columns) followed by different letters are significantly different, using Revised LSD Test, at $P \leq 0.05$.
**Means of the genotypes (last column) followed by different letters are significantly different at $P \leq 0.05$.
*** Means of the genotypes (last row) followed by different letters are significantly different at $P \leq 0.05$.

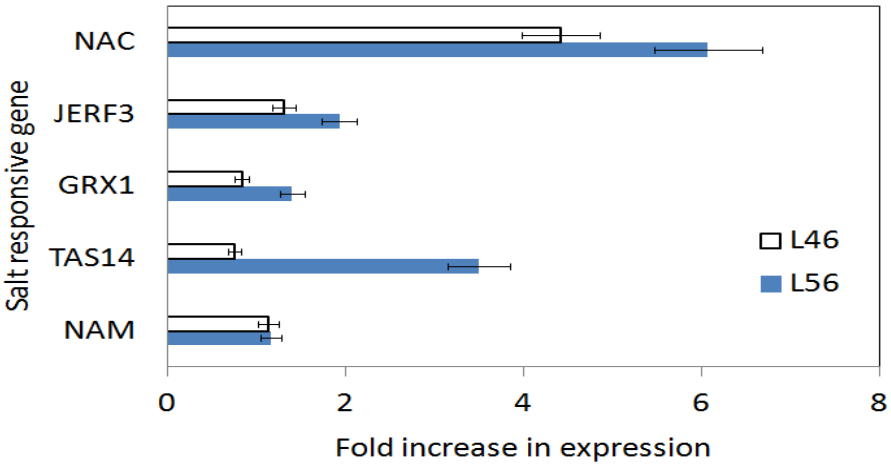

Fig 5. Fold increase in genes expression under salt stress compared to the control of two improved tomato lines. Bars represent 10% value error.

The plot revealed three clusters; the salt tolerant genotypes (2, 5, 8 and 11), the salt intermediate tolerant genotypes (1, 6, 7 and 12) and the salt susceptible genotypes (3, 4, 9 and 10).
Based on the performance of the different genotypes at different levels of salinity for all studied traits and according to canonical discriminant multivariate analysis, the tomato genotypes can be classified into three groups (salt tolerant, moderately salt tolerant and salt susceptible) as mentioned above. The L56 and L46 genotypes were selected to represent salt tolerant and salt susceptible genotypes, respectively, to explore the expression of some salt-responsive genes.

Responsive gene screening

Based on the evaluation of plant growth and yield component traits for all genotypes, two tomato genotypes were selected for this experiment: L46 (susceptible) and L56 (tolerant). The goal of this study was to investigate the correlation between agronomical evaluation and the expression of salt-tolerant genes, in order to utilise these genes as molecular markers in breeding programmes.

Gene expression was investigated using quantitative real-time PCR (qPCR) primers. The tolerant tomato genotype L56 showed a prominent increase in the expression of the NAC, JERF3, GRX1 and TAS14 genes in response to salinity compared with the salt-susceptible genotypes L46. By contrast, the expression of NAM was slightly enhanced in response to salt treatment, to an equal extent in both tolerant and susceptible tomato genotypes.
The tomato carries at least three NAC genes that have been cloned and functionally characterised (Yang et al., 2011; Han et al., 2012). Although NAC expression was increased in both tomato genotypes after salt stress treatment, its level of expression was 30% higher in L56 than in L46. Likewise, JERF3 expression was enhanced in both genotypes following salt stress treatment but its level of expression was 45% higher in L56 than in L46. JERF3 is an ethylene response factor (ERF), which isolated from the tomato and enhanced the salt tolerance of transgenic tobacco (Wang et al., 2004).
The expression levels of GRX1 and TAS14 differed in the investigated tomato genotypes. The expression levels of both genes were enhanced by salt stress in the L56 genotype and

Table 6. Number of fruits plant^{-1} for tomato genotypes as affected by different water salinity levels.

Genotypes	Level of salinity (dS m^{-1})					
	0.5	2.4	4.8	7.2	9.6	Mean**
Imberial	24.2 s-v*	23.5 u-x	21.5 z-B	20.6 B	20.4 B	22.0 f
PakmoreVF	34.7 abc	34.6 abc	33.2 cde	32.4 ef	32.2 ef	33.4 a
Queen	17.7 C	17.3 CD	16.9 CD	16.2 CD	16.1 DE	16.8 g
Shohba	27.8 i-m	27.0 k-o	26.1 n-q	25.2 p-t	22.9 v-z	25.8 d
Strain-B	29.5 g	28.7 g-j	28.4 g-k	26.5 l-p	26.2 nop	27.9 b
Tnshet star	25.8 n-r	24.6 q-u	23.6 m-p	22.6 w-z	22.3 x-A	23.8 e
L 16	23.8 t-x	23.2 u-x	22.3 x-A	21.6 y-B	20.9 AB	22.4 f
L 26	35.7 a	35.1 ab	34.1 bcd	32.7 def	31.5 f	33.8 a
L 36	28.9 ghi	28.4 g-k	26.4 l-p	26.1 n-q	25.7 o-s	27.1 c
L 46	27.9 h-l	27.2 j-o	27.8 i-m	26.3 m-p	25.9 n-r	27.0 c
L 56	29.4 gh	29.1 ghi	27.8 i-m	27.3 j-n	27.1 k-o	28.1 b
L 66	25.9 n-r	25.1 p-t	24.5 r-u	24.1 t-w	23.1 u-y	24.5 e
BL 1076	14.7 EF	14.9 EF	14.4 F	14.1 F	14.0 F	14.4 h
BL 1239	15.0 EF	14.8 EF	14.5 F	14.3 F	14.1 F	14.5 h
Mean***	25.7 a	25.2 a	24.4 b	23.5 c	23.0 c	

*Values of the interaction between salinity levels and genotypes (five columns) followed by different letters are significantly different, using Revised LSD Test, at $P \leq 0.05$. **Means of the genotypes (last column) followed by different letters are significantly different at $P \leq 0.05$.
*** Means of the genotypes (last row) followed by different letters are significantly different at $P \leq 0.05$.

Table 7. Average fruit weight (g) for tomato genotypes as affected by different salinity levels.

Genotypes	Level of salinity (dS m^{-1})					
	0.5	2.4	4.8	7.2	9.6	Mean**
Imberial	85.4 hij*	80.4 lm	71.3 p-s	64.3 w-z	58.9 C-F	72.1 e
PakmoreVF	84.2 ijk	75.4 no	70.4 q-t	64.7 w-z	62.5 y-B	71.4 ef
Queen	101 cd	90.2 efg	73.5 opq	62.1 y-C	53.4 AB	76.0 d
Shohba	88.2 fgh	84.5 ijk	72.4 o-r	49.8 I	45.2 J	68.0 f
Strain-B	111 ab	108 b	90.4 efg	85.4 hij	81.5 klm	95.4 b
Tnshet star	91.6 ef	82.5 jkl	78.5 mn	71.6 p-s	62.5 y-B	77.3 cd
L 16	87.4 ghi	79.5 lm	69.2 r-u	64.5 w-z	59.6 B-F	72.0 e
L 26	87.6 ghi	82.6 jkl	70.6 q-t	65.5 v-y	63.2 x-A	73.9 de
L 36	104 c	92.4 e	74.6 op	61.7 z-D	58.4 DEF	78.2 c
L 46	90.4 efg	81.2 klm	70.2 q-t	50.2 HI	44.2 J	67.2 f
L 56	114 a	111 ab	98.5 d	90.4 efg	87.2 gi	100 a
L 66	98.5 d	87.5 ghi	75.2 no	70.4 q-t	68.5 s-v	80.0 c
BL 1076	70.5 q-t	69.2 r-u	67.3 t-w	66.4 u-x	65.5 v-y	67.8 f
BL 1239	60.7 A-D	58.2 DEF	57.3 EF	56.5 EFG	56.8 G	57.9 g
Mean***	91.0 a	84.4 b	74.2 c	65.9 d	61.9 e	

*Values of the interaction between salinity levels and genotypes (five columns) followed by different letters are significantly different, using Revised LSD Test, at $P \leq 0.05$. **Means of the genotypes (last column) followed by different letters are significantly different at $P \leq 0.05$.
*** Means of the genotypes (last row) followed by different letters are significantly different at $P \leq 0.05$.

Table 8. Total yield (kg plant^{-1}) for tomato genotypes as affected by different salinity levels.

Genotypes	Level of salinity (dS m^{-1})					
	0.5	2.4	4.8	7.2	9.6	Mean**
Imberial	1.891 s*	1.805 stu	1.531 yzA	1.323 DEF	1.291 DEF	1.568 g
PakmoreVF	2.669 d-g	2.610 e-g	2.268 lmn	2.161 n-q	2.075 pq	2.356 c
Queen	1.645 v-y	1.513 z-B	1.202 F	1.004 GH	0.904 G-J	1.253 h
Shohba	2.304 j-m	2.131 opq	1.658 v-y	1.382 BCD	1.198 EF	1.734 f
Strain-B	2.938 ab	2.864 bc	2.585 fg	2.450 hi	2.282 k-n	2.623 b
Tnshet star	2.172 m-p	2.113 pq	1.737 tuv	1.694 uvw	1.455 z-C	1.834 e
L 16	1.924 rs	1.847 st	1.539 xyz	1.366 CDE	1.250 EF	1.585 g
L 26	2.761 cd	2.705 def	2.419 ij	2.321 i-l	2.170 m-p	2.475 c
L 36	2.787 cd	2.605 efg	2.034 qr	1.672 vwx	1.504 z-B	2.120 d
L 46	2.406 ijk	2.249 l-o	1.754 tuv	1.415 A-D	1.207 F	1.806 ef
L 56	3.070 a	3.008 a	2.732 cde	2.556 gh	2.445 hi	2.762 a
L 66	2.247 l-o	2.190 l-p	1.842 st	1.730 tuv	1.572 wxy	1.916 e
BL 1076	1.010 G	0.999 GH	0.970 GH	0.935 GHI	0.925 G-J	0.967 i
BL 1239	0.885 G-J	0.870 HIJ	0.830 IJ	0.810 IJ	0.800 J	0.839 j
Mean***	2.193 a	2.107 b	1.792 c	1.629 d	1.505 e	

*Values of the interaction between salinity levels and genotypes (five columns) followed by different letters are significantly different, using Revised LSD Test, at $P \leq 0.05$.**Means of the genotypes (last column) followed by different letters are significantly different at $P \leq 0.05$.
*** Means of the genotypes (last row) followed by different letters are significantly different at $P \leq 0.05$.

suppressed by salt stress in the L46 genotype (Fig. 5). The GRX1 is a tomato glutaredoxin gene that has been found to regulate plant responses to salt stress (Guo et al., 2010). The enhanced expression of TAS14 was prominent compared to GRX1, showing a 3.5-fold increase in L56 in response to salt stress. TAS14 was first cloned from the tomato and was found to be induced by abscisic acid and salt stress (Godoy et al., 1990; Godoy et al., 1994).

Materials and methods

Plant materials

The plant materials used in the present study consisted of fourteen tomato genotypes including six commercial cultivars, six improved genotypes and two salt-tolerant breeding lines (Table 15). The commercial cultivars were previously evaluated and selected as being good cultivars for growth under various environmental conditions (Alsadon and Wahb-Allah, 2007, Alsadon et al., 2009 and Wahb-Allah et al., 2011). The improved genotypes were produced through the tomato breeding programme at the Vegetable Improvement Unit, College of Food and Agricultural Sciences, King Saud University. They were produced from the commercial cultivars using selection and selfing for six generations. The salt-tolerant breeding lines were provided by the Asian Vegetables Research and Development Centre (AVRDC, Shanhua, Taiwan, ROC).

Seeds of the fourteen genotypes were sown in seedling trays on Sep. 1, 2010 and Sep. 3, 2011 for the 2010/2011 and 2011/2012 seasons, respectively, in a controlled environment at 27 ± 1ºC day/19 ± 1ºC night temperatures. One-month-old seedlings were transplanted into soil in a fibreglass greenhouse during the 2010/2011 and 2011/2012 seasons at the Agricultural Research and Experimental Station in Dirab, 35 km southwest of Riyadh, Saudi Arabia (24º39ˉN, 46º44ˉ E). The soil used was non-saline (EC ranged from 2.0 to 2.8 dS m^{-1}) calcareous ($CaCO_3$ ranged from 25 to 30%) and sandy in texture with a pH range from 7.3 to 7.8. The air temperature in the greenhouse was set to approximately 26 ± 1°C during the day and 20 ± 1ºC during the night, and relative humidity was 75 ± 2% for the entire growing season. Fertilisation and other cultural practices were applied as recommended for commercial tomato production (Maynard and Hochmuth, 2007).

Salt stress treatments

The electrical conductivity (EC) of the irrigation water was 0.5 (S0, control), 2.4 (S1), 4.8 (S2), 7.2 (S3) and 9.6 (S4) dS m^{-1} prior to the addition of fertiliser. The salinity was increased by adding molar concentrations of NaCl to the low salinity (0.5 dS m^{-1}) irrigation water. A drip irrigation network was designed for this study. Five water containers (1 m^3 each) were assigned to each salinity treatment. Each container was connected to two dripper lines. The salinity treatments started 5 days after transplantation and lasted until the end of the growth period, which was 150 days. The irrigation water was applied three times a day at a target rate of 100% ET_c.

Experimental layout

The experimental layout was split-plot in a randomised complete block design with three replicates. The irrigation treatments and the genotypes were randomly allocated to the main plots and sub-plots, respectively. The sub-plot area was 6 m^2 (2 x 3 m) and included 15 plants. The planting distance was 40 cm and 100 cm between plants and lines, respectively.

Data Recorded

At 50% flowering (45 days after transplantation), random samples of four plants from each sub-plot were chosen for measuring plant height, stem diameter, leaf area (with a portable area metre, LI-COR model 3000A) and leaf fresh weight. Leaves were collected, washed with distilled water and dried at 70°C in a forced-air oven until the weight became constant, after which the leaf dry weight was measured. The following traits were recorded: total yield (the total weight of all harvested fruit from each sub-plot through the entire season), average fruit weight (the total weight of all harvested fruit from each sub-plot divided by the number of fruit) and the number of fruit per plant. Random representative samples of five fruits were taken from each experimental unit at three harvesting times to determine fruit quality traits. Fruit flesh thickness was measured using a digital calliper. From the homogenised fruit juice, pH was recorded using a handheld pH metre, total soluble solids (TSS) were measured using a refractometer and ascorbic acid (mg/100 cm^3 juice) was estimated using the 2,6-dichlorophenolindophenol method.

Statistical analysis

Data were statistically analysed using Statistical Analysis System (SAS) version 8.1 (SAS Institute, 2008). The treatment means were compared using a revised Least Significant Difference (LSD) test at the 0.05 level of significance according to Steel and Torrie (1980). The presented data are the average of the data from the two seasons (2010/2011 and 2011/2012). To cluster the twelve tomato genotypes, Canonical discriminant multivariate analysis was performed based on their agronomical characters using SPSS 20 (SPSS, 2011).

Responsive gene screening

Salt-susceptible (L46) and salt-tolerant (L56) tomato genotypes were assessed for the expression of salt-responsive genes. Leaf samples from plants grown under control (0.5 dS m^{-1}) and high salinity (9.6 dS m^{-1}) conditions were collected in an ice box, frozen in liquid nitrogen and kept at -80°C before the isolation of total RNA. Samples were ground under liquid nitrogen, after which 1 ml TRIzol® (Invitrogen, USA) was added to each 50-mg tissue sample. Samples were then incubated for 5 min at room temperature. Samples were centrifuged at maximum speed at 4°C for 10 min, after which 0.2 ml chloroform was added. Samples were then shaken vigorously for 15 sec and incubated at room temperature for 3 min. They were then centrifuged at maximum speed at 4°C for 15 min. The aqueous phase was transferred into a new tube with 0.5 ml isopropanol and centrifuged at maximum speed at 4°C for 10 min. The pellet was washed with 70% EtOH, dried in a dry bath at 60°C, re-suspended in 100 µl RNase/DNase-free water and stored at -80°C.

Tomato genes were retrieved from the GenBank (NCBI, 2011), as reference markers for salt stress. Full gene sequences, including introns, were downloaded from the Solanaceae Genomic Network (SGN, 2011). Primers for qPCR were designed to be located on two consecutive exons spanning an intron, where possible (Table 16). This was

Table 9. Fruit flesh thickness (cm) for tomato genotypes as affected by different salinity levels.

Genotypes	Level of salinity (dS m^{-1})					
	0.5	2.4	4.8	7.2	9.6	Mean**
Imberial	1.31 f-m*	1.19 k-r	1.10 p-x	0.90 z-D	0.80 B-F	1.06 fg
PakmoreVF	1.44 b-g	1.34 e-l	1.20 j-q	1.13 n-v	0.99 t-z	1.22 cd
Queen	1.15 m-u	1.10 p-x	0.98 u-z	0.90 z-D	0.67 F	0.96 gh
Shohba	1.19 k-r	1.11 o-x	0.99 t-z	0.83 z-F	0.78 C-F	0.98 g
Strain-B	1.60 ab	1.52 a-d	1.35 d-k	1.20 j-q	1.13 n-v	1.36 ab
Tnshet star	1.36 d-k	1.31 f-l	1.16 m-r	1.00 s-x	0.92 y-D	1.15 ef
L 16	1.39 c-i	1.37 d-j	1.20 k-q	1.02 r-x	0.92 y-D	1.18 de
L 26	1.49 a-d	1.47 a-e	1.28 g-n	1.12 o-u	0.94 v-z	1.26 bcd
L 36	1.22 i-p	1.17 l-r	0.98 s-z	0.87 z-E	0.76 DEF	1.00 gh
L 46	1.30 f-l	1.26 h-m	1.08 q-v	0.88 wxy	0.68 z	1.04 fg
L 56	1.64 a	1.59 ab	1.40 c-h	1.31 f-x	1.16 m-s	1.42 a
L 66	1.56 abc	1.50 a-e	1.31 f-l	1.15 m-t	0.98 u-z	1.30 bc
BL 1076	1.14 m-u	1.11 q-w	0.95 w-z	0.80 F	0.70 E	0.94 h
BL 1239	1.16 m-s	1.13 q-t	0.97 u-y	0.84 z-D	0.70 F	0.96 gh
Mean**	1.35 a	1.29 a	1.14 b	0.99 c	0.86 d	

*Values of the interaction between salinity levels and genotypes (five columns) followed by different letters are significantly different, using Revised LSD Test, at $P \leq 0.05$. **Means of the genotypes (last column) followed by different letters are significantly different at $P \leq 0.05$.
*** Means of the genotypes (last row) followed by different letters are significantly different at $P \leq 0.05$.

Table 10. Ascorbic acid (mg $100g^{-1}$) for tomato genotypes as affected by different salinity levels.

Genotypes	Level of salinity (dS m^{-1})					
	0.5	2.4	4.8	7.2	9.6	Mean**
Imberial	28.45 t-y*	28.91 s-x	29.24 r-w	23.60 B-E	20.70 E	26.18 g
PakmoreVF	33.45 h-n	33.97 f-l	34.05 f-k	29.70 q-w	26.38 w-z	31.51 cde
Queen	31.45 m-t	31.98 k-r	32.15 j-d	28.54 t-x	22.98 z-C	29.42 ef
Shohba	33.12 i-o	33.45 h-n	34.50 e-l	30.25 p-u	24.48 xyz	31.16 def
Strain-B	36.82 a-f	37.05 a-e	37.45 a-d	32.91 i-o	31.62 l-s	35.17 ab
Tnshet star	34.10 f-l	34.52 e-l	34.65 d-l	31.50 l-s	27.23 wxy	32.40 cd
L 16	31.45 m-t	31.68 k-r	31.98 k-r	28.10 u-x	23.19 yz	29.28 f
L 26	35.01 c-i	35.19 b-h	35.56 a-g	31.80 k-q	30.44 o-t	33.60 bc
L 36	32.74 i-p	32.95 i-0	33.15 i-o	28.64 s-w	23.17 xyz	30.13 ef
L 46	34.64 d-k	34.89 c-j	35.01 c-i	31.25 n-s	27.01 u-y	32.56 bc
L 56	37.52 a-e	37.95 a-d	38.43 abc	34.50 e-l	32.70 i-p	36.22 a
L 66	25.18 z-B	26.01 yzA	26.45 x-B	22.89 DE	20.32 E	24.17 g
BL 1076	38.16 abc	38.69 ab	39.01 a	33.50 g-m	31.44 m-t	36.16 a
BL 1239	36.21 a-g	37.10 a-d	37.45 a-d	32.60 j-p	27.94 v-y	34.26 ab
Mean***	33.45 a	33.88 a	34.22 a	29.98 b	26.40 c	

*Values of the interaction between salinity levels and genotypes (five columns) followed by different letters are significantly different, using Revised LSD Test, at $P \leq 0.05$. **Means of the genotypes (last column) followed by different letters are significantly different at $P \leq 0.05$.
*** Means of the genotypes (last row) followed by different letters are significantly different at $P \leq 0.05$.

Table 11. pH (%) for tomato genotypes as affected by different salinity levels.

Genotypes	Level of salinity (dS m^{-1})					
	0.5	2.4	4.8	7.2	9.6	Mean**
Imberial	4.05 zA*	4.25 vwx	4.40 p-u	4.65 g-k	4.50 l-q	4.37 cd
PakmoreVF	4.15 xy	4.37 q-u	4.50 l-q	4.88 abc	4.70 d-i	4.52 b
Queen	3.82 AB	4.05 zA	4.19 xy	4.89 abc	4.55 k-o	4.30 de
Shohba	3.78 F	3.99 zA	4.25 vwx	4.75 c-h	4.58 j-m	4.27 e
Strain-B	4.20 xy	4.44 n-s	4.60 i-m	5.05 a	4.81 b-e	4.62 a
Tnshet star	3.95 zA	4.22 wxy	4.42 o-t	4.85 b-e	4.66 g-k	4.42 c
L 16	3.92 zA	4.10 z	4.21 xy	4.88 a-d	4.69 e-i	4.36 cd
L 26	4.15 yz	4.36 r-v	4.49 m-r	4.99 ab	4.86 bcd	4.57 a
L 36	3.92 zA	4.15 yz	4.33 s-x	4.87 a-d	4.68 f-j	4.39 bc
L 46	3.80 AB	4.04 zA	4.28 wxy	4.80 c-f	4.58 j-n	4.30 de
L 56	4.20 xy	4.31 t-w	4.62 h-l	5.05 a	4.77 c-g	4.59 a
L 66	3.98 zA	4.20 xy	4.34 s-w	4.85 b-e	4.53 k-o	4.38 c
BL 1076	4.04 zA	4.20 xy	4.31 t-w	4.75 c-h	4.65 g-k	4.39 c
BL 1239	4.04 zA	4.20 xy	4.30 uvw	4.86 bcd	4.70 d-i	4.42 c
Mean***	4.00 e	4.20 d	4.37 c	4.86 a	4.66 b	

*Values of the interaction between salinity levels and genotypes (five columns) followed by different letters are significantly different, using Revised LSD Test, at $P \leq 0.05$. **Means of the genotypes (last column) followed by different letters are significantly different at $P \leq 0.05$.
*** Means of the genotypes (last row) followed by different letters are significantly different at $P \leq 0.05$.

Table 12. TSS (%) for tomato genotypes as affected by different salinity levels.

Genotypes	Level of salinity (dS m^{-1})					
	0.5	2.4	4.8	7.2	9.6	Mean**
Imberial	6.11 o-r*	6.40 l-n	7.10 d-f	7.40 b-d	7.59 ab	6.92 bc
PakmoreVF	6.04 o-s	6.38 l-n	6.95 gh	7.15 de	7.48 abc	6.80 cd
Queen	4.75 z	5.12 yz	5.32 xyz	5.80 s-v	6.01 p-s	5.40 h
Shohba	4.81 z	5.18 yz	5.51 v-y	5.72 t-w	5.93 q-t	5.43 h
Strain-B	5.45 wxy	5.72 t-m	6.10 o-r	6.32 l-p	6.61 ijk	6.04 f
Tnshet star	4.63 z	4.92 yz	5.12 w-z	5.33 yz	5.65 uvw	5.13 j
L 16	6.34 l-o	6.65 ijk	7.12 def	7.41 bcd	7.68 ab	7.04 ab
L 26	6.19 m-q	6.54 jkl	7.01 efg	7.24 cde	7.52 abc	6.90 bc
L 36	5.22 z	5.54 v-y	5.80 s-v	5.91 r-u	6.38 lmn	5.77 g
L 46	6.12 n-r	6.34 l-o	6.45 klm	6.84 ghi	7.40 bcd	6.63 d
L 56	5.80 s-v	5.92 q-t	6.21 m-p	6.42 k-n	7.25 cde	6.32 e
L 66	4.82 z	5.15 yz	5.38 xy	5.58 vwx	5.87 stu	5.36 h
BL 1076	6.34 l-o	6.54 jkl	6.98 fgh	7.15 def	7.44 abc	6.89 bc
BL 1239	6.55 jkl	6.71 hij	7.20 cde	7.40 bcd	7.84 a	7.14 a
Mean***	5.65 e	5.93 d	6.30 c	6.54 b	6.90 a	

*Values of the interaction between salinity levels and genotypes (five columns) followed by different letters are significantly different, using Revised LSD Test, at $P \leq 0.05$. **Means of the genotypes (last column) followed by different letters are significantly different at $P \leq 0.05$.
*** Means of the genotypes (last row) followed by different letters are significantly different at $P \leq 0.05$.

Table 13. Eigen values and percent of variability explained by each canonical discriminant function for the twelve tomato genotypes based on agronomical characters.

Function	Eigen value	% of Variance	Cumulative %
1	1199385.571	92.4	92.4
2	72886.540	5.6	98.0
3	14765.943	1.1	99.1
4	8824.893	0.7	99.8
5	2006.756	0.2	100.0

Table 14. Standardized canonical discriminate function coefficients for the twelve tomato genotypes based on agronomical characters.

	Function				
Character*	1	2	3	4	5
Plant height	-0.368	0.918	-0.011	-0.230	1.331
Stem diameter	-0.085	0.445	0.393	0.292	0.425
Leaf area	0.600	-0.731	0.918	-0.297	-0.065
Leaf fresh weight	4.208	-1.056	-3.121	1.998	-0.287
Leaf dry weight	0.727	0.116	-0.396	-0.016	0.723
Average fruit weight	-2.239	1.315	2.949	-1.033	1.596
Number of fruits/plant	-1.699	-0.945	1.188	0.156	-1.878
Total yield (kg/plant)	0.978	1.404	-0.819	-0.643	-0.084
Fruit flesh thickness (cm)	-1.263	0.640	0.530	0.578	-0.243
Ascorbic acid	0.500	1.362	1.030	1.212	-0.066

* Both pH and TSS% failed the minimum tolerance test of 0.001.

Table 15. Source of the tomato genotypes used in this study.

No.	Genotype	Type	Source
1	Imberial	Commercial Cultivar*	Atlas Seed Co., USA
2	Pakmore VF	Commercial Cultivar*	Top Harvest Co., Netherland
3	Queen	Commercial Cultivar*	Top Harvest Co., Netherland
4	Shohba	Commercial Cultivar*	Atlas Seed Co., USA
5	Strain-B	Commercial Cultivar*	Atlas Seed Co., USA
6	Tnshet star	Commercial Cultivar*	Genetics International Inc. USA
7	L16	Improved line**	Derived from Imberial cultivar
8	L26	Improved line**	Derived from Pakmore VF cultivar
9	L36	Improved line**	Derived from Queen cultivar
10	L46	Improved line**	Derived from Shohba cultivar
11	L56	Improved line**	Derived from Strain-B cultivar
12	L66	Improved line**	Derived from Tnshet star cultivar
13	BL 1076	Salt tolerance breeding line	Provided by Asian Vegetables Research and Development
14	BL 1239	Salt tolerance breeding line	Center, Shanhua, Taiwan, ROC

* The commercial cultivars were previously evaluated and selected by the authors as being good cultivars under different environmental conditions
** The improved lines were produced by the authors through tomato breeding program.

Table 16. Designed qPCR primers of tomato salt responsive genes as reference markers for salt stress.

No.	Gene	NBCI number	SGN number	Primer pair sequence (5' to 3')	Tm	bp
1	NAC	EU670750	SGN-U583008	CAAATTGGATTATGCACGAGTACCGC	61.4	256
				AAGTAGTCGTTTGCTGGTGTCGATCG	61.4	
2	JERF3	AY383630	SGN-U578247	GACCTGTGGTCCTTTGATGATGTTCC	60.6	196
				ATTCTTCTTCCACCACCAGACACACC	60.3	
3	GRX1	FN646220	SGN-U583365	TTCCCGAAGGAATCTGGTGTATATGC	60.3	189
				TGATTTCCAAGATTCAGTTAAGGCGG	60.5	
4	TAS14	X51904	SGN-U581493	CTGGTGGAGAATATGGAACTCAAGGC	60.1	173
				CTTCATGTTGTCCAGGCATCTTCTCC	61.0	
5	NAM	GU256056	SGN-U582483	AGCAATTGCAAAGCAACTAATGGAGG	60.6	180
				TCATTCTGCTGGTAGACCGACTTTCG	61.5	
6	Actin	U60481	SGN-U579208	GGACTCTGGTGATGGTGTTAG	54.8	160
				CCGTTCAGCAGTAGTGGTG	55.7	

applied to all genes to avoid unspecific amplification from any traces of genomic DNA. The tomato actin gene was used as the internal standard.

First strand DNA (cDNA) was synthesised from total RNA using a reverse transcriptase kit (Promega, USA). The gene expression was amplified with a SYBR Green mix (Qiagen, USA). Real-time amplification data were collected with an Applied Biosystems 7500 thermal cycler (ABI, USA). The fold change in gene expression relative to actin expression was determined from the C_T values using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Conclusions

The effects of salinity on tomato growth, yield and fruit quality varied for each trait. The genotypes used in this study showed a wide range of variability in their responses to salt stress. Based on the evaluation of plant growth and yield traits, in response to salinity, the different genotypes were classified into three groups according to their salt tolerance as follows: salt tolerant (BL 1076, BL 1239, L26, L56, Strain-B and Pakmore), moderately salt tolerant (L16, L66, Imperial and Tnshet star) and salt susceptible (L36, 46, Queen and Sohba). The data revealed correlation between agronomical traits and the expression of some salt-responsive genes as investigated in L56 (salt tolerant) and L46 (salt susceptible) genotypes. The conclusions were drawn from repeated, independent experiments based on real-time PCR (qPCR analysis). The salt-tolerant breeding genotype L56 is genetically robust, as it shows enhanced expression of salt-responsive genes in response to saline conditions. By contrast, the salt-susceptible genotype L46 showed a potential genetic background, although it does not cover a wide spectrum of salt-responsive genes as L56. The results presented in this report highlight the potential of integrating known salt-responsive genes into plant breeding programmes as molecular markers (biomarkers).

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