

# Genetic variability of NaCl tolerance in tomato

A. Saeed<sup>1</sup>, M.F. Saleem<sup>3</sup>, M. Zakria<sup>2</sup>, S.A. Anjum<sup>3</sup>, A. Shakeel<sup>1</sup> and N. Saeed<sup>2</sup>

<sup>1</sup>Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan
<sup>2</sup>Department of Mathematics and Statistics, University of Agriculture, Faisalabad, Pakistan
<sup>3</sup>Department of Agronomy, University of Agriculture, Faisalabad, Pakistan

Corresponding author: A. Saeed E-mail: drasifpbg@gmail.com

Genet. Mol. Res. 10 (3): 1371-1382 (2011) Received October 22, 2010 Accepted December 17, 2010 Published July 12, 2011 DOI 10.4238/vol10-3gmr1121

ABSTRACT. Cultivation of crops in soils with high salt (NaCl) content can affect plant development. We examined the morphological and physiological mechanisms of salt tolerance in tomato. The responses of 72 accessions of tomato (Solanum lycopersicum) to salinity were compared by measuring shoot and root lengths, and fresh shoot and root weights relative to those of controls (plants grown in normal salt levels). All traits were reduced at the seedling stage when salinity levels were increased. The accession x salinity interaction was significant for all traits. Root length had higher heritability than other traits and was used as a selection criterion to identify salt-tolerant and -non-tolerant accessions. On the basis of root length, accessions LA2661, CLN2498A, CLN1621L, BL1176, 6233, and 17870 were considered to be more tolerant than accessions 17902, LO2875 and LO4360. The degree of salt tolerance was checked by analyzing K<sup>+</sup> and Na<sup>+</sup> concentrations and K<sup>+</sup>/Na<sup>+</sup> ratio in tissues of plants treated with 10 and 15 dS/m salinity levels. Tolerance of these accessions to salinity was most associated with low accumulation of Na<sup>+</sup> and higher K<sup>+</sup>/Na<sup>+</sup> ratios.

**Key words:** *Solanum lycopersicum*; NaCl; Tomato; Root length; Relative growth rate; K<sup>+</sup>/Na<sup>+</sup> ratio

Genetics and Molecular Research 10 (3): 1371-1382 (2011)

A. Saeed et al.

### **INTRODUCTION**

Salt stress is becoming a major challenge for agricultural productivity throughout the world (Rausch et al., 1996). It is important in vegetable crops due to their cash value (Shannon and Grieve, 1999). The term salt-affected soils refers to the areas that may be saline or sodic and constitute 6% of the total world land area. There are 45 million ha of irrigated areas that are salt-affected, and 32 million ha of dry land agriculture that are salt-affected (Munns, 2002).

Conventional approaches and development of cultivars for saline soils are used to address the salinity problem. Physical, chemical and hydrological approaches are used to reclaim soils. However, many of these soils cannot be reclaimed due to economic reasons or lack of fresh water. The only possibility that seems feasible is the development of cultivars for salinity tolerance (Qureshi et al., 1990; Qureshi, 1993; Hollington, 1998). Salt-tolerant plants may be developed through selection and breeding, but success depends on how much variation is present within crop species. Information has been reported on salt tolerance in tomato (Hassan et al., 1999; Shaaban et al., 2004), wheat (Mano and Takeda, 2001; Saqib et al., 2002; Sarwar et al., 2003), oats (Zhao et al., 2007), corn (Cramer et al., 1994), rice (Gain et al., 2004), and cotton (Noor et al., 2001; Bhatti and Azhar, 2002).

Variation can also be developed with mutagenic agents (Gottschalk, 1981; Forster, 2001), tissue culture techniques and somaclonal technique (Larkin and Scowcroft, 1981). By introgression, variation may also be generated with closely related species; interspecific crosses between domestic and wild relatives of tomato have overcome the limited variation of domestic tomato for salinity tolerance (Rush and Epstein, 1981; Tal and Shannon, 1983; Hassan et al., 1999). Wild species may be used as a source of genes to improve traits of existing cultivars. It is a pre-requisite for a breeding procedure to obtain information about the inheritance pattern (qualitative or quantitative), number of genes with major effects, and type of gene action that controls salinity tolerance. The genetic studies reported on citrus root stock (Furr and Ream, 1969), rice (Shannon et al., 1998), Triticeae (Farooq and Azam, 2001; Xing et al., 2002), cotton (Bhatti and Azhar, 2002), grasses (Ashraf and McNeilly, 1988), and lucerne (Al-Khatib et al., 1994) proved that salt tolerance is genetically controlled.

The objective of this study was to obtain information on the genetic mechanism that controls salinity tolerance in tomato, at early plant stages and at maturity. The plant material was analyzed for  $Na^+$  and  $K^+$  concentrations and  $K^+/Na^+$  ratio to determine the physiological mechanism affecting salinity tolerance in tomato. The information obtained here could be useful for the development of cultivars/hybrids suitable for crop cultivation under saline conditions.

### **MATERIAL AND METHODS**

The study was carried out in sand culture. Ten seeds of each accession were sown in pots containing about 10 kg river sand. The traces of salts were removed by washing it first with tap water and then deionized distilled water four times. The experiment was arranged in a completely randomized design and consisting of three NaCl treatments, three replications and 72 tomato accessions. Plants were irrigated with 0.5 strength Hoagland's solution (Hoagland and Arnon, 1950) on alternate days.

At 12 days after sowing, salt treatments were applied. The NaCl treatments (10 and 15 dS/m) were applied in 0.5 strength Hoagland's solution. The solution was applied to the sand medium until saturation, and the excess solution was allowed to drain off. Control plants were

Genetics and Molecular Research 10 (3): 1371-1382 (2011)

irrigated with 0.5 Hoagland's solution. After three weeks, root and shoot lengths and fresh root and shoot weights of six seedlings of each accession in each replication were measured.

Relative values of 72 accessions were computed according to the following formula (Maas, 1986):

Relative salt tolerance =  $\frac{\text{Mean value of character in NaCl}}{\text{Mean value of a character in control}} \times 100$ 

### Physiological basis of salinity tolerance

Nine tomato accessions, namely LA2661, CLN2498A, CLN1621L, BL1176, 6233, and 17870 (most tolerant) and 17902, LO2875 and LO4360 (non-tolerant) were selected on the basis of relative root length data. The differing responses of salt tolerance of these nine accessions were retested by measuring  $Na^+$  and  $K^+$  concentrations. The seedlings were grown using the method described earlier.

Leaves from seedlings having the largest roots were obtained and stored separately in micro-tubes for one week in a deep freezer. The cell sap was extracted using the standard technique of centrifugation. The concentrations of  $Na^+$  and  $K^+$  were measured with a flame photometer, and the  $K^+/Na^+$  ratio was determined. Data were evaluated by analysis of variance. Mean square values for characteristics were compared.

### Heritability (H<sup>2</sup>) estimate for salt tolerance

Heritability was calculated using the formula of Falconer and MacKay (1996) to obtain estimates of broad-sense heritability (H<sup>2</sup>): H<sup>2</sup> = V<sub>g</sub> / V<sub>p</sub>, where V<sub>g</sub> = genetic variance = [(variance between accessions - variance within accessions) / 18]; V<sub>p</sub> = phenotypic variance = [(variance between accessions - variance within accessions) / 18] + variance within accessions.

Values were subjected to analysis of variance in SPSS (1994; v. 8.0 for Windows: Advanced Statistics) to determine the genotypic differences.

### RESULTS

Analysis of variance results for relative salt tolerance (Table 1) exhibited highly significant genotypic differences for root and shoot lengths and fresh root and shoot weights. The two NaCl concentrations were also found to be significant at  $P \le 0.01$ . The accession x salinity interaction was highly significant for all the traits studied. Comparison of accessions on the basis of relative root length data (Table 2) showed the varying response of the accessions at 10 dS/m NaCl. The relative root lengths of accessions 17870, 6233, BL1176, CLN2498A, CLN1621L, and LA2661 were 73.9, 71.2, 70.6, 69.8, 64.9, and 62%, respectively, whereas it was 26.8% for 17902 and 28.1 and 31.8% for LO4360 and LO2875, respectively, at 10 dS/m. Under 15 dS/m, accessions BL1176 (58.5%) and 6233 (53.8%) appeared to be less affected than LA2661 and CLN1621L. Accessions BL1176, 17870, 6233, CLN2498A, CLN1621L, and LA2661 with tolerance indices of 64.5, 63.8, 62.5, 61.3, 56.5, and 53.6%, respectively, for root length with saline treatment, seemed to be the most tolerant accessions. On the other hand, 17902 (20.8%), LO4360 (21.7%) and LO2875 (24.4%) appeared to be the most sensitive to salinity. Other accessions showed low to moderate tolerance by producing root lengths ranging from 24.4 to 50.4% at the two salinity levels.

Genetics and Molecular Research 10 (3): 1371-1382 (2011)

Α.	Saeed	et	al.

**Table 1.** Mean squares of values for seedling traits of tomato accessions relative to the control and growth in the presence of NaCl.

Source of variation	d.f.	Root length	Shoot length	Fresh root weight	Fresh shoot weight
Accessions (A)	71	442.3**	6649.6**	204**	373**
Concentrations (C)	1	37004.6**	23075.3*	14299**	25935.4**
AxC	71	39.3**	8.2 <sup>ns</sup>	43**	109.6**
Within + residual	288	4.7	74.6	2	3.3

d.f. = degrees of freedom. \*, \*\* and ns indicate  $P \le 0.01$ ,  $P \le 0.05$  and non-significant, respectively.

**Table 2.** Relative root and shoot lengths (%) and relative fresh root and shoot weights (%) of 72 accessions grown under control conditions and at 2 salinity levels.

Accession		Relative roo	t length		Relative shoot length			
	10 dS/m	15 dS/m	Mean for 2 salinities	10 dS/m	15 dS/m	Mean for 2 salinities		
6231	46.1	25.0	35.6	54.4	32.6	43.5		
6232	49.6	28.2	38.9	53.8	37.0	45.4		
6233	71.2	53.8	62.5	64.2	51.5	57.8		
6234	50.8	34.3	42.6	63.0	40.8	51.9		
17860	37.3	17.7	27.5	42.6	27.2	34.9		
17862	39.6	15.9	27.7	54.8	36.5	45.7		
17863	43.4	24.0	33.7	58.7	45.0	51.8		
17865	55.7	35.9	45.8	46.9	28.6	37.8		
17867	48.2	24.1	36.1	44.2	31.1	37.7		
17868	53.0	30.6	41.8	62.5	36.3	49.4		
17869	38.4	30.7	34.6	55.2	34.4	44.8		
17870	73.9	53.8	63.8	63.0	51.8	57.4		
17872	52.7	32.7	42.7	50.7	32.5	41.6		
17873	43.5	25.6	34.5	41.8	22.2	32.0		
17876	41.5	25.2	33.4	51.8	36.6	44.2		
17882	55.7	33.2	44.4	48.8	31.8	40.3		
17887	49.4	38.9	44.1	48.3	27.7	38.0		
17889	45.2	28.8	37.0	46.2	39.6	42.9		
17890	46.8	28.5	37.7	46.2	26.9	36.6		
17899	56.1	34.7	45.4	41.3	29.1	35.2		
17902	26.8	14.8	20.8	37.1	19.1	28.1		
17903	46.3	34.8	40.6	47.1	29.7	38.4		
17904	53.3	28.4	40.8	38.9	25.3	32.1		
17906	50.6	26.3	38.5	48.0	31.8	39.9		
BL1076	48.5	36.8	42.6	48.8	36.5	42.6		
BL1077	52.0	21.0	36.5	50.0	40.7	45.4		
BL1078	59.6	35.9	47 7	54.5	45.5	50.0		
BL1079	55.9	35.0	45.5	45.0	41.3	43.1		
BL1174	57.5	393	48.4	49.6	37.9	43.8		
BL1175	53.1	34.4	43.8	56.4	43.9	50.2		
BL1176	70.6	58.5	64.5	67.0	55.9	61.5		
CHICO	50.7	28.7	39.7	51.3	33.9	42.6		
CLN1621L	64.9	48.0	56.5	61.8	47.8	54.8		
CLN2001A	61.9	39.0	50.4	45.0	40.0	42.5		
CLN2366A	50.1	38.6	44.4	42.0	40.4	41.2		
CLN2418A	51.8	33.2	42.5	49.3	46.0	47.7		
CLN2443A	55.9	36.0	46.0	44.6	37.5	41.1		
CLN2498A	69.8	52.9	61.3	72.2	51.5	61.8		
LA0716	57.2	32.6	44.9	52.1	44.2	48.2		
LA1278	57.0	34.0	45.5	52.8	39.2	46.0		
LA1932	54.6	31.3	42.9	53.9	40.4	47.1		
LA2661	62.0	45.3	53.6	64.1	53.6	58.8		
LA2711	54.6	34.8	44 7	53.0	40.9	47.0		
LA3120	53.6	22.7	38.2	50.0	41.9	45.9		
LA3320	51.2	31.9	41.5	49.6	39.4	44 5		
	01.2	51.7	11.0	12.0	57.1	11.0		

Continued on next page

Genetics and Molecular Research 10 (3): 1371-1382 (2011)

©FUNPEC-RP www.funpecrp.com.br

## Table 2. Continued

Accession	R	elative root leng	ţth	R	elative shoot len	gth
	10 dS/m	15 dS/m	Mean for 2 salinities	10 dS/m	15 dS/m	Mean for 2 salinities
LA3847	56.8	27.3	42.0	54.5	37.2	45.9
LO2576	39.5	25.5	32.5	48.4	44.4	46.4
LO2692	43.4	29.7	36.5	51.5	34.0	42.7
LO2707	44.3	22.8	33.5	39.7	26.2	32.9
LO2752	47.5	31.0	39.2	41.2	26.7	34.0
LO2831	45.3	27.1	36.2	42.9	27.7	35.3
LO2840	48.7	30.6	39.7	39.8	21.9	30.8
LO2846	44.7	27.4	36.0	49.1	32.5	40.8
LO2875	31.8	16.9	24.4	36.6	17.7	27.2
LO3686	54.0	27.2	40.6	49.1	27.8	38.4
LO3708	42.9	36.4	39.6	46.5	27.6	37.0
LO4166	47.4	21.6	34.5	51.0	33.3	42.1
LO4360	28.1	15.3	21.7	32.0	19.2	25.6
LO4363	45.3	29.2	37.2	57.5	38.3	47.9
LO4379	39.1	23.4	31.3	55.8	39.8	47.8
LO4713	41.7	32.1	36.9	52.5	39.6	46.0
LYP. No. 1	53.6	40.2	46.9	56.7	43.6	50.1
MARACHIA	52.6	33.4	43.0	42.0	24.0	33.0
MONEY MAKER	48.0	29.6	38.8	47.0	35.1	41.1
PAKIT	51.6	30.8	41.2	48.9	34.7	41.8
PECDINATO	35.8	18.6	27.2	63.0	39.5	51.2
RIOGRANDE	50.0	33.9	41.9	50.8	36.0	43.4
T2-IMPROVED	46.6	24.4	35.5	55.9	34.0	45.0
TITANO	46.3	27.2	36.8	47.9	31.3	39.6
TT-21	45.0	20.0	32.5	56.8	34.2	45.5
TWL-23	51.7	32.4	42.0	55.4	42.0	48.7
TWL-29	43.7	28.4	36.0	59.1	42.0	50.6

Accession		Relative fresh	n root weight	Relative fresh shoot weight		
	10 dS/m	15 dS/m	Mean for 2 salinities	10 dS/m	15 dS/m	Mean for 2 salinities
6231	58.7	51.6	55.2	52.2	33.5	42.8
6232	59.5	42.4	50.9	58.5	38.3	48.4
6233	68.3	56.8	62.6	62.3	58.7	60.5
6234	49.2	40.2	44.7	53.1	43.9	48.5
17860	57.5	36.9	47.2	49.3	47.0	48.1
17862	52.5	36.7	44.6	43.8	35.5	39.6
17863	54.4	46.3	50.4	51.5	24.2	37.9
17865	56.1	37.6	46.9	48.9	26.7	37.8
17867	61.1	50.0	55.5	51.2	34.6	42.9
17868	57.4	49.4	53.4	49.2	27.7	38.4
17869	54.5	38.6	46.5	53.1	32.1	42.6
17870	64.1	56.2	60.1	63.4	56.7	60.1
17872	47.6	35.0	41.3	52.4	43.0	47.7
17873	52.4	37.1	44.8	54.3	37.9	46.1
17876	56.9	47.5	52.2	60.1	40.9	50.5
17882	55.3	49.7	52.5	54.0	23.7	38.8
17887	60.5	40.0	50.3	48.2	32.5	40.4
17889	46.8	37.6	42.2	41.5	28.8	35.2
17890	48.4	40.6	44.5	58.3	35.5	46.9
17899	52.5	40.8	46.6	44.2	35.8	40.0
17902	38.0	34.3	36.1	38.0	14.9	26.5
17903	49.4	39.7	44.5	47.6	31.2	39.4
17904	49.3	46.1	47.7	48.5	27.3	37.9
17906	53.2	44.9	49.1	57.8	47.0	52.4
BL1076	49.8	45.1	47.5	53.7	39.2	46.5
BL1077	54.6	40.8	47.7	59.8	48.9	54.4
BL1078	58.2	40.6	49.4	45.1	34.7	39.9
BL1079	50.1	38.5	44.3	42.2	33.9	38.0

Continued on next page

Genetics and Molecular Research 10 (3): 1371-1382 (2011)

©FUNPEC-RP www.funpecrp.com.br

A. Saeed et al.

Accession		Relative fresh	root weight		Relative fresh	shoot weight
	10 dS/m	15 dS/m	Mean for 2 salinities	10 dS/m	15 dS/m	Mean for 2 salinities
BL1174	53.6	45.5	49.5	57.5	42.9	50.2
BL1175	58.3	36.9	47.6	48.1	33.5	40.8
BL1176	67.3	58.4	62.9	68.7	51.4	60.1
CHICO	52.0	43.0	47.5	58.1	49.6	53.8
CLN1621L	63.6	55.0	59.3	65.4	58.5	61.9
CLN2001A	56.7	45.8	51.3	53.3	37.2	45.3
CLN2366A	55.8	42.8	49.3	46.2	28.7	37.5
CLN2418A	59.5	57.5	58.5	46.0	41.0	43.5
CLN2443A	57.0	38.6	47.8	54.0	33.6	43.8
CLN2498A	69.6	56.8	63.2	63.0	54.1	58.5
LA0716	56.2	36.8	46.5	60.8	49.5	55.2
LA1278	54.1	42.9	48.5	61.6	43.8	52.7
LA1932	49.6	40.6	45.1	57.9	46.1	52.0
LA2661	65.5	58.5	62.0	69.8	52.6	61.2
LA2711	51.3	53.3	52.3	49.2	40.8	45.0
LA3120	59.2	49.8	54 5	47.6	39.7	43.7
LA3320	53.1	39.7	46.4	51.1	45.8	48.4
LA3847	50.6	52.7	51 75	45.7	40.0	42.9
L02576	49.3	49.8	49.6	52.8	45.3	49.1
LO2692	55.3	48.0	51.6	47.3	32.8	40.1
LO2072	48.9	45.5	47.2	46.7	21.9	34.3
LO2752	48.6	44.1	46.3	53.2	38.6	45.9
LO2732	54.0	48.2	51 5	57.5	22.5	40.0
LO2831	50.2	37.1	13.6	51.2	44.9	40.0
LO2846	52.6	13 7	49.0	15.3	43.8	40.0
LO2840	32.0	20.2	40.2	22.0	16.2	24.1
1.02686	38.0 40.4	29.3	42.0	32.0	26.2	24.1
1 02708	47.4	30.4	42.9	40.0	22.2	28.0
LO3/08	52.2	33.2	43.7	43.0	32.5	38.0
LO4100	50.0	38.8	44.4	44.7	39.0	42.1
L04300	44.5	33.3	39.8	54.7	15.7	25.2
L04303	59.0	44.8	51.9	51.7	45.5	48.0
LO4379	48.7	35.2	42.0	55.0	29.7	42.4
L04/13	60.8	36.4	48.0	56.1	22.2	39.2
LYP. NO. I	57.5	47.9	52.7	58.1	17.2	37.7
MARACHIA	55.2	35.3	45.3	52.3	17.2	34.7
MONEY MAKER	61.3	39.0	50.1	57.3	30.7	44.0
PAKII	57.5	41.4	49.4	57.4	24.2	40.8
PECDINATO	58.5	44.9	51.7	54.8	47.3	51.0
RIOGRANDE	61.1	45.6	53.4	57.6	45.2	51.4
12-IMPROVED	46.1	35.3	40.7	55.8	43.3	49.5
TITANO	57.3	49.0	53.1	57.0	33.6	45.3
TT-21	58.4	35.8	47.1	56.4	34.8	45.6
TWL-23	55.2	47.0	51.1	52.7	42.7	47.7
TWL-29	59.4	46.9	53.2	46.7	28.9	37.8

Regarding the shoot lengths (Table 2), the accession LO4360 with a tolerance index of 32% at 10 dS/m appeared to be more sensitive than other lines; in contrast, CLN2498A, BL1176, 6233, and PECDINATO had the highest indices of tolerance at 72.2, 67.0, 64.2, and 63%, respectively. Accessions 17870, CLN2498A and 6233 with a tolerance index of 51.8, 51.5 and 51.5%, respectively, showed almost the same tolerance at 15 dS/m. Based on overall assessment of the accessions, CLN2498A, BL1176, LA2661, 6233, 17870, and CLN1621L, with mean values of 61.8, 61.5, 58.8, 57.8, 57.4, and 54.8%, respectively, seemed to be the most tolerant, and LO4360, LO2875 and 17902, with a tolerance index of 25.6, 27.2 and 28.1%, respectively, appeared to be the most sensitive.

The differing responses of the accessions to NaCl salinity were also prominent for fresh root weight (Table 2). At 10 dS/m, accession CLN2498-A, with the highest tolerance

index of 69.6%, appeared to show the highest salt tolerance, followed by 6233, BL1176, LA2661, 17870, and CLN1621L, having 68.3, 67.3, 65.5, 64.1, and 63.6% relative fresh root weight, respectively. At 10 dS/m, accessions LO2875, 17902 and LO4360, with 38.0, 38.0 and 44.3% relative fresh root weight, respectively, showed poor tolerance to salinity. At the higher salinity level, accessions LA2661, BL1176, CLN2418A, CLN2498A, 6233, and 17870, with relative fresh root weight of 58.5, 58.4, 57.5, 56.8, 56.8, and 56.2%, respectively, appeared to be the most tolerant. Overall assessment again showed CLN2498A, BL1176, 6233, LA2661, 17870, and CLN1621L, with respective mean values of 63.2, 62.9, 62.6, 62.0, 60.1, and 59.3% salt tolerance index, as the most tolerant accessions. Accessions LO2875, 17902 and LO4360, with respective mean values of 33.7, 36.1 and 39.8%, seemed to be the most sensitive.

As far as relative fresh shoot weight is concerned (Table 2), the accession LA2661 showed the highest tolerance index of 69.8% at 10 dS/m followed by BL1176, CLN1621L, 17870, CLN2498A, and 6233, with a range of 62.3 to 68.7% relative fresh shoot weight. At 10 dS/m, accessions LO2875, LO4360 and 17902, with respective relative fresh shoot weight of 32.0, 34.7 and 38%, showed poor tolerance to salinity. At 15 dS/m, accessions LA2661, BL1176, CLN1621L, CLN2498A, 6233, and 17870, with a range of relative fresh shoot weight from 51.4 to 58.7%, seemed to be the most tolerant. Overall, the accessions CLN2498A, BL1176, 6233, LA2661, 17870, and CLN1621L, with the highest salt tolerance indices, appeared to be the most tolerant accessions. While accessions LO2875, 17902 and LO4360, with the lowest salt tolerance indices, seemed to be the most sensitive.

### Estimation of heritability (H<sup>2</sup>)

Under salinity stress of 10 and 15 dS/m,  $H^2$  for relative root length was 68, and 63, respectively, while estimates of  $H^2$  for relative shoot length were 57, and 62, respectively (Table 3). Regarding fresh root weight, the estimates of  $H^2$  were 41 and 46, respectively, while for fresh shoot weight, they were 0.55 and 0.62 with 10 and 15 dS/m, respectively (Table 4).

Table 3. Components of variance and broad-sense heritability of NaCl tolerance at seedling stage.								
Component	Relative re	oot length	Relative shoot length					
	10 dS/m	15 dS/m	10 dS/m	15 dS/m				
V <sub>o</sub>	81.1	74.5	58.3	64.3				
V "	119.1	118.5	102.3	103.3				
$H^{2}$	68	63	57	62				

Vg, Vp and H2 indicate genotypic variance, phenotypic variance and broad-sense heritability, respectively.

Table 4. Components of variance and broad-sense	heritability of NaCl tolerance at seedling stage.

Component	Relative free	sh root weight	Relative fresh shoot weight		
	10 dS/m	15 dS/m	10 dS/m	15 dS/m	
V	33.8	45.2	52.2	63.7	
V	81.8	98.2	95.5	102.7	
$H^{2}$	41	46	55	62	

 $V_{e}, V_{p}$  and  $H^{2}$  indicate genotypic variance, phenotypic variance and broad-sense heritability, respectively.

Genetics and Molecular Research 10 (3): 1371-1382 (2011)

#### A. Saeed et al.

### Physiological analysis of accessions selected on the basis of root length

After a preliminary investigation, a new experiment including the six most tolerant (LA2661, CLN2498A, CLN1621L, BL1176, 6233, and 17870) and three least tolerant (17902, LO2875 and LO4360) accessions was designed to elucidate the mechanism of tolerance to increased salinity. In this experiment, the plant material was analyzed during early plant development for Na<sup>+</sup> and K<sup>+</sup> concentrations and K<sup>+</sup>/Na<sup>+</sup> to see whether or not we may use the root length as a selection criterion.

The mean squares for root lengths, Na<sup>+</sup>, K<sup>+</sup>, and K<sup>+</sup>/Na<sup>+</sup> ratio of the nine tomato accessions are given in Table 5, which exhibited highly significant differences for root lengths, Na<sup>+</sup>, K<sup>+</sup>, and K<sup>+</sup>/Na<sup>+</sup> ratio measured between the accessions. Differences between the three salinity levels were also significant at  $P \le 0.01$ . Accession x salinity interaction was also found to be significant at  $P \le 0.01$ , suggesting that root length, K<sup>+</sup> and K<sup>+</sup>/Na<sup>+</sup> ratio were affected by the two elevated salinity levels. The interaction for Na<sup>+</sup> concentration was not significant.

conditions and at two NaCl concentrations.									
Source of variation	d.f.	Root length	Na <sup>+</sup> concentration	K <sup>+</sup> concentration	K+/Na+ ratio				
Accessions (A)	8	2288.7**	17569**	485.8**	7.1**				
Concentrations (C)	1	3596.7**	4616469**	33692.2**	471.4**				
AxC	8	14.2**	13022 <sup>ns</sup>	61.0**	3.0**				
Within + residual	36	4.5	68224	0.001	0.7**				

d.f. = degrees of freedom. \*\* and ns indicate  $P \le 0.05$  and non-significant, respectively.

-----

It was observed that BL1176, CLN2498A, 6233, 17870, CLN1621L, and LA2661, with longer roots, exhibited better salt tolerance, while LO4360, 17902 and LO2875, with shorter root length, seemed to be less tolerant (Table 6).

Table 6. Mean values of relative root length	, K <sup>+</sup> and Na <sup>+</sup> concentrations	and K <sup>+</sup> /Na <sup>+</sup>	ratio of six t	olerant and
three non-tolerant accessions.				

Accession	Root	length	K <sup>+</sup> conce	K <sup>+</sup> concentration		Na <sup>+</sup> concentration		K+/Na+ ratio	
	10 dS/m	15 dS/m	10 dS/m	15 dS/m	10 dS/m	15 dS/m	10 dS/m	15 dS/m	
Tolerant									
LA2661	60.0	40.0	79.2	28.1	839.2	1556.7	9.4	1.8	
CLN2498A	74.7	58.3	80.7	31.3	897.7	1550.0	8.9	2.0	
CLN1621L	65.0	46.9	81.4	23.0	900.3	1571.7	9.0	1.4	
BL1176	86.3	69.7	83.9	30.1	986.6	1558.3	8.5	1.9	
6233	71.3	53.0	77.6	26.2	1009.8	1441.3	7.6	1.8	
17870	73.4	52.6	78.5	24.9	975.6	1524.3	8.0	1.6	
Non-tolerant									
17902	28.1	17.3	51.8	10.6	1037.2	1643.0	5.0	0.6	
LO2875	24.6	13.6	57.4	16.3	1055.4	1478.9	5.4	1.1	
LO4360	26.2	16.0	57.8	13.0	1049.7	1593.6	5.5	0.8	

The comparison of accessions on the basis of Na<sup>+</sup> concentration showed that accessions LA2661, CLN2498A, CLN1621L, BL1176, 17870, and 6233 had the lowest levels, while LO4360, LO2875 and 17902 accumulated more Na<sup>+</sup>. Regarding potassium levels, the accessions BL1176, CLN1621L, CLN2498A, 6233, 17870, and LA2661 had the highest K<sup>+</sup> concentrations. In contrast, 17902, LO2875 and LO4360 had the lowest levels. With regard

Genetics and Molecular Research 10 (3): 1371-1382 (2011)

to K<sup>+</sup>/Na<sup>+</sup> ratio, the accessions BL1176, CLN1621L, CLN2498A, 6233, 17870, and LA2661 accumulated more K<sup>+</sup> than Na<sup>+</sup>. The K<sup>+</sup>/Na<sup>+</sup> ratio in these accessions was higher as compared to the accessions 17902, LO2875 and LO4360. On the basis of relative root length, accessions LA2661, CLN2498A, CLN1621L, BL1176, 17870, and 6233 appeared to be more tolerant, whereas 17902, LO2875 and LO4360 with the smallest root lengths may be considered non-tolerant.

### DISCUSSION

The experiment was conducted to measure the genetic variation for salt tolerance among tomato accessions on the basis of root and shoot lengths and fresh root and shoot weights. There are many scientists who have used these traits to assess response to salinity (Leim et al., 1985; Noori and McNeilly, 1999, 2000; Akinci et al., 2004), and the sand culture technique employed in this study has also been used by many researchers to study salinity tolerance in tomato (Vespasiani et al., 1995), wheat (Qureshi et al., 1990; Khan et al., 2003) and rice (Aslam et al., 1993).

Relative values (Maas, 1986) were used to compare the responses of different tomato accessions. The results showed a decrease in growth parameters of tomato plants with increase in salinity level. It was also observed that the tomato accessions showed varying responses to different levels of salinity. The results reported by Aknci et al. (2004), Muralia and Sastry (1994), Salam et al. (1999), Sastry and Sharma (2000), and Khan et al. (2003) are in agreement with the present studies.

The data (Table 2) indicated that among the four traits studied root length was affected the most by salt stress. Similar results have been reported by Levitt (1980), Okusanya and Ungar (1984), Noor et al. (2001), and Bottger (1978). They observed that under severe stress the production of cytokinins ceases, which ultimately affects root growth. This is why many scientists use root length as the selection criterion for salt tolerance, for example sorghum (Azhar and McNeilly, 1989, 2001) and wheat (Ashraf and McNeilly, 1988).

It was noted that the root of BL1176 was short in control growth with a mean tolerance index of 64%, while the accessions PECDINATO and LO2752 with longer root lengths in control growth exhibited a tolerance index of 27 and 39%, respectively. The accession 17860 was also a slow-growing accession in non-saline conditions, but it showed a tolerance index of 37 and 17% under low and high salinity levels, respectively. This behavior of the lines shows that there is no clear relationship between plant growth without salt stress and plant vigor under salinity, and these results are not in agreement with the results reported by Shannon and McCreight, (1984), but the results were supported by Rosielle and Hamblin (1981).

Based on root length data, six accessions, namely LA2661, CLN2498A, CLN1621L, BL1176, 6233, and 17870, were considered to be tolerant and three others, namely 17902, LO2875 and LO4360, to be non-tolerant to salinity. These nine accessions were used for further studies. The estimates of H<sup>2</sup> showed that root length exhibited the highest broad-sense heritability of all the traits studied. Similar study had been reported in cotton (Azhar and Ahmad, 2000; Noor et al., 2001) and wheat (Ali et al., 2002). Overall, salinity stress reduces all the growth parameters, and this reduction was greater at the higher salinity level. Salinity stress may also reduce plant growth due to water shortage, ion toxicity, ion imbalance, or a combination of any of these factors. McNeilly (1990), Flowers and Yeo (1995), Munns et al. (1995), and Rodriguez et al. (1997) have reported similar results.

The nine accessions exhibited significant differences for root length, and also for Na<sup>+</sup> and K<sup>+</sup> levels and K<sup>+</sup>/Na<sup>+</sup>. The tolerant accessions, LA2661, CLN2498A, CLN1621L,

Genetics and Molecular Research 10 (3): 1371-1382 (2011)

BL1176, 6233, and 17870, were found to exclude more Na<sup>+</sup> as compared to the non-tolerant accessions, namely 17902, LO2875 and LO4360. It is a property of glycophytic species, and there is ample evidence to support these results.

In the present study, it was observed that high concentrations of salts adversely affected tomato seedlings and also that Na<sup>+</sup> concentration increased with salinity level. Two similar observations were made by Munns et al. (1995) and these caused salt injury to plants (Serrano et al., 1998). Although the study was based on seedlings, the literature (Ashraf and McNeilly, 1988; Maiti et al., 1996; Salam et al., 1999) supports the finding of a positive correlation between seedling performance and adult performance. Thus, it may be concluded that screening at seedling stage is beneficial and genetically controlled and can be exploited in a breeding program aimed at salt tolerance.

### REFERENCES

- Akinci S, Yilmaz K and Akinci IE (2004). Response of tomato (*Lycopersicon esculentum* Mill.) to salinity in the early growth stages for agricultural cultivation in saline environments. J. Environ. Biol. 25: 351-357.
- Al-Khatib M, McNeilly T and Collins JC (1994). The genetic basis of salt tolerance in lucerne (*Medicago sativa* L.). J. Genet. Breed. 48: 169-174.
- Ali Z, Khan AS and Asad MA (2002). Salt tolerance in bread wheat: genetic variation and heritability for growth and ion relation. Asian J. Plant Sci. 1: 420-422.

Ashraf M and McNeilly T (1988). Variability in salt tolerance of nine spring wheat cultivars. J. Agron. Crop Sci. 160: 14-21. Aslam M, Qureshi RH and Ahmad N (1993). A rapid screening technique for salt tolerance in rice (Oryza sativa L.). Plant

Soil 150: 99-107.

- Azhar FM and McNeilly T (1989). The response of four sorghum accessions/cultivars to salinity during plant development. *J. Agron. Crop Sci.* 163: 33-43.
- Azhar FM and Ahmad R (2000). Variation and heritability of salinity tolerance in upland cotton at early stage of plant development. *Pak. J. Biol. Sci.* 3: 1991-1993.
- Azhar FM and McNeilly T (2001). Compartmentation of Na<sup>+</sup> and Cl<sup>-</sup> ions in different parts of *Sorghum bicolor* (L.) moench during plant development. *Pak. J. Bot.* 33: 101-107.
- Bhatti MA and Azhar FM (2002). Salt tolerance of nine *Gossypium hirsutum* L. varieties to NaCl salinity at early stage of plant development. *Int. J. Agric. Biol.* 4: 544-546.
- Bottger M (1978). Levels of endogenous indole-3-acetic acid and abscisic acid during the course of formation of roots. Z. *Pflanzenphysiol* 86: 283-286.
- Cramer GR, Alberico GJ and Schmidt C (1994). Salt tolerance is not associated with the sodium accumulation of two maize hybrids. *Aust. J. Plant Physiol.* 21: 675-692.
- Falconer DS and MacKay TFC (1996). Introduction to Quantitative Genetics. Chapman and Hall, London.
- Farooq S and Azam F (2001). Co-existence of salt and drought tolerance in Triticeae. Hereditas 135: 205-210.
- Flowers TJ and Yeo AR (1995). Breeding for salinity resistance in crop plants: where next? *Aust. J. Plant Physiol.* 22: 875-884.
- Forster BP (2001). Mutation genetics of salt tolerance in barley: an assessment of Golden Promise and other semi-dwarf mutants. *Euphytica* 120: 317-328.
- Furr JR and Ream CL (1969). Breeding Citrus Rootstocks for Salt Tolerance. In: Proceedings of the First International Citrus Symposium (Chapman HD, ed.). University of California, Riverside, 373-380.
- Gain P, Mannan MA, Pal PS, Hossain MM, et al. (2004). Effect of salinity on some yield attributes of rice. *Pak. J. Biol. Sci.* 7: 760-762.

Gottschalk W (1981). Mutation: higher plants. Prog. Bot. 43: 139-152.

- Hassan AA, Nassar HH, Barkat MA and Tolba MS (1999). Tomato breeding for salinity tolerance. III. Genetics of tolerance. *Egyptian J. Hort.* 26: 391-403.
- Hoagland DR and Arnon DI (1950). The Water-Culture Method for Growing Plants Without Soil. Circular, University of California, College of Agriculture, Agricultural Experiment Station, California, 347.
- Hollington PA (1998). Technological Breakthroughs in Screening/Breeding Wheat Varieties for Salt Tolerance. In: National Conference on "Salinity Management in Agriculture". CSSRI, Karnal.

Genetics and Molecular Research 10 (3): 1371-1382 (2011)

- Khan AS, Asad MA and Ali Z (2003). Assessment of genetic variability for NaCl tolerance in wheat. *Pak. J. Agric. Sci.* 40: 33-36.
- Larkin PJ and Scowcroft WR (1981). Somaclonal variation a novel source of variability from cell cultures for plant improvement. *Theor. Appl. Genet.* 60: 197-214.
- Leim ASN, Hendriks A, Kraal H and Loenen M (1985). Effect of deicing salt on roadside grasses and herbs. *Plant Soil* 84: 299-310.
- Levitt J (1980). Responses of Plants to Environmental Stresses, Water, Radiation, Salt and other Stresses. Academic Press Inc., New York.

Maas EV (1986). Salt tolerance of plants. Appl. Agric. Res. 1: 12-26.

- Maiti RK, Amaya LED, Cardona SI, Dimas AMO, et al. (1996). Genotypic variability in maize cultivars (Zea mays L.) for resistance to drought and salinity. J. Plant Physiol. 148: 741-744.
- Mano Y and Takeda K (2001). Genetic resources of salt tolerance at germination and the seedling stage in wheat. *Jpn. J. Crop Sci.* 70: 215-220.
- McNeilly T (1990). Selection and breeding for salinity tolerance in crop species: a case for optimism? *Acta Ecol.* 11: 595-610.
- Munns R (2002). The impact of salinity stress. Available at [http://www.plantstress.com/articles/salinity\_i/salinity\_ihtm]. Accessed November 17, 2004.
- Munns R, Schachtman DP and Condon AG (1995). The significance of a two-phase growth response to salinity in wheat and barley. Aust. J. Plant Physiol. 22: 561-569.
- Muralia S and Sastry EVD (1994). Stability analysis in wheat (*Triticum aestivum*) for seedling emergence and establishment characters at different salinity levels. *Indian J. Genet. Plant Breed.* 54: 351-356.
- Noor E, Azhar FM and Khan AA (2001). Differences in responses of *Gossypium hirsutum* L. varieties to NaCl salinity at seedling stage. *Int. J. Agric. Biol.* 3: 345-347.
- Noori SAS and McNeilly T (1999). Assessment of variability in salt tolerance in diploid *Aegilops* ssp. J. Genet. Breed. 53: 183-188.
- Noori SAS and McNeilly T (2000). Assessment of variability in salt tolerance based on seedling growth in *Triticum durum* Desf. *Genet. Res. Crop Evol.* 47: 285-291.

Okusanya OT and Ungar IA (1984). The growth and mineral composition of three species of *Spergularia* as affected by salinity and nutrients at high salinity. *Am. J. Bot.* 71: 439-447.

- Qureshi RH (1993). Alternative Strategies for Tackling the Soil Salinity Problem. Department of Soil Science, University of Agricultutre, Faisalabad, 117.
- Qureshi RH, Aslam M, Nawaz S and Mehmood T (1990). Saline Agriculture Research in Pakistan. Proceedings Indo-Pak Workshop on Soil Salinity and Water Management. PARC, Islamabad.
- Rausch T, Kirsch M, Low R, Lehr A, et al. (1996). Salt stress responses of higher plants: the role of proton pumps and Na<sup>+</sup>/ H<sup>+</sup> antiporters. *J. Plant Physiol.* 148: 425-433.
- Rodriguez HG, Roberts J, Jordan WR and Drew MC (1997). Growth, water relations, and accumulation of organic and inorganic solutes in roots of maize seedlings during salt stress. *Plant Physiol.* 113: 881-893.
- Rosielle AA and Hamblin J (1981). Theoretical aspects of selection for yield in stress and non-stress environments. *Crop Sci.* 21: 943-946.
- Rush DW and Epstein E (1981). Comparative studies on the sodium, potassium and chloride relations of a wild halophytic and a domestic salt sensitive tomato species. *Plant Physiol.* 68: 1308-1313.
- Salam A, Hollington PA, Gorham J, Wyn Jones RG, et al. (1999). Physiological genetics of salt tolerance in (*Triticum aestivum* L): performance of wheat varieties, inbred lines and reciprocal F<sub>1</sub> hybrids under saline conditions. J. Agron. Crop Sci. 183: 145-156.
- Saqib M, Akhtar J, Qureshi RH and Aslam M (2002). Performance of two wheat (*Triticum aestivum*) genotypes in response to waterlogging at different growth stages under non-saline and saline soil conditions. *Pak. J. Agric. Sci.* 39: 171-176.
- Sarwar G, Ashraf MY and Naeem M (2003). Genetic variability of some primitive bread wheat varieties to salt tolerance. *Pak. J. Bot.* 35: 771-777.
- Sastry EVD and Sharma H (2000). Effect of temperature and salinity on the germination seedling growth in wheat (*Triticum aestivum* L.). *Indian J. Agri. Sci.* 70: 117-118.
- Serrano R, Culianz-Macia FA and Moreno V (1998). Genetic engineering of salt and drought tolerance with yeast regulatory genes. *Sci. Hortic.* 78: 261-269.
- Shaaban MM, El-Fouly MM, El-Zanaty and El-Nour AAA (2004). Halophytes and foliar fertilization as a useful technique for growing processing tomatoes in the saline affected soils. *Pak. J. Biol. Sci.* 7: 504-507.

Shannon MC and McCreight C (1984). Salt tolerance of lettuce introductions. HortScience 19: 673-675.

Shannon MC and Grieve CM (1999). Tolerance of vegetable crops to salinity. Sci. Hortic. 78: 5-38.

©FUNPEC-RP www.funpecrp.com.br

Genetics and Molecular Research 10 (3): 1371-1382 (2011)

- Shannon MC, Rhoades JD, Draper JH, Scardaci SC, et al. (1998). Assessment of salt tolerance in rice cultivars in response to salinity problems in California. Crop Sci. 38: 394-398.
- SPSS (1994). Repeated Analysis of Variance. In: SPSS Advanced Statistics Release, 107-143.
- Tal M and Shannon MC (1983). Salt tolerance in the wild relatives of the cultivated tomato: responses of *Lycopersicon* esculentum, *L. cheesmanii*, *L. peruvianum*, *Solanum pennellii* and F<sub>1</sub> hybrids to high salinity. *Aust. J. Plant Physiol.* 10: 109-117.
- Vespasiani C, Arias C and Taleisnik E (1995). Effect of salinity in the early stages of tomato fruit growth. *Acta Phytopathol. Entomol. Hung.* 30: 21-25.
- Xing X, Zheng G, Deng Z, Xu Z, et al. (2002). Comparative study of drought and salt resistance of different Tricacae genotypes. *Acta Bot. Boreali-Occidentalia Sin.* 22: 1122-1135.
- Zhao GQ, Ma BL and Ren CZ (2007). Growth, gas exchange, chlorophyll fluorescence and ion content of naked oat in response to salinity. *Crop Sci.* 47: 123-131.