Evaluation of Characters for Ascertaining Salt Stress Responses in *Lycopersicon* Species

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Abstract. Plant height; stem thickness; fresh and dry weights of leaves and stems; numbers of leaves, trusses, flowers, and fruits; and leaf concentrations of Cl, Na, N-NO₃, K, Ca, and Mg were measured in mature plants from 39 tomato accessions representing five species of *Lycopersicon* [*L. esculentum* Mill., *L. peruvianum* (L.) Mill., *L. pimpinellifolium* (Jusl.) Mill., *L. hirsutum* H. & B., *L. pennellii* (Corr.) D'Arcy] in response to various NaCl concentrations. Plants were irrigated with a nutrient solution, plus one of four levels of NaCl with electrical conductivities of 0.28, 0.63, 1.39, and 2.15 S·m⁻¹. Characters were evaluated for each genotype taking into consideration: 1) the significant differences between NaCl concentrations, 2) the experimental errors in the analyses of variance, and 3) the uniformity of response to the salt concentrations. The characters that fulfilled these criteria for all 39 genotypes were: plant height, dry weights of leaves, fresh and dry weights of stems, and leaf concentrations of Cl and Na. However, other characters, although not generally applicable to the entire data set, were good indicators of response differences within a particular species. Leaf concentrations of N-NO₃ and Mg were useful indicators in *L. pimpinellifolium* and *L. esculentum* and number of leaves and leaf concentration of Mg were useful indicators in *L. hirsutum* for responses of mature plants to salt stress.

Breeders developing crops tolerant to saline soils find it difficult to choose traits to use as markers for the reliable assessment of salt tolerance (Epstein, 1983). Ideally, characters should be simple to measure and permit the identification of salinity tolerance during seed germination or at the seedling stage. Methodology should be precise, economical, and relatively rapid. Salt tolerance of young tomato (*Lycopersicon esculentum*) plants is not well correlated with that of mature plants (Guerrier, 1984; Norlyn and Epstein, 1984; Shannon, 1979); consequently, our work was carried out with mature plants. Salt tolerance of mature plants has been measured by various indicators, such as mortality rate, root and shoot growth, plant height, number of leaves, leaf succulence and area, contents of amino acids and sugars, leaf electrical conductivity, fruit yield and quality, and the concentrations of Na, K, Mg, Cl, Ca, and N-NO₃. Results for the different characters between genotypes and/or experiments are difficult to compare. For example, there were no significant differences in plant height among tomatoes that received different salt treatments, but there were differences between plant heights of saline-treated and control tomatoes (West et al., 1979). Sacher et al. (1982) and Shannon (1984) found that smaller losses of shoot dry and fresh weights than in the controls indicate greater salinity tolerance. However, these criteria are insufficient to rank various species according to their salt tolerance (Storey and Wyn Jones, 1977). Dehan and Tal (1978), Tal (1971), and Tal and Shannon (1983) concluded that the higher the salinity tolerance of tomato plants, the higher were the Na and Cl contents, but some tolerant hybrid lines of *L.*

esculentum x *L. pennellii* had concentrations of Cl and Na similar to those of salt-sensitive lines (Sacher et al., 1983).

Previous information on the response to saline stress of characters described in the literature is confusing, possibly because studies were carried out under diverse environmental conditions and with various genotypes. The objective of the present work was to choose characters that showed the smallest uncontrolled environmental variation with the strongest change with different salinity levels. Furthermore, the changes of characters should be repeatable among genotypes. Consequently, the 16 most commonly used characters in the literature were recorded for 39 genotypes of five species cultivated with four NaCl concentrations.

Materials and Methods

Thirty-nine genotypes of tomato consisting of 12 *L. esculentum*, 13 *L. peruvianum*, five *L. pimpinellifolium*, seven *L. hirsutum*, and two *L. pennellii* were used. Seeds were planted in rock wool (Grodan, Roermond, Holland) cubes (35 x 35 x 35 mm). When the plants had two true leaves, each of the cubes was placed in a larger cube (70 x 70 x 65 mm). When the plants developed five true leaves, they were placed on rock wool packs (100 x 15 x 7.5 cm). Three plants were placed on each pack. The composition of the aqueous fertilizer solution was: (in mol·m⁻³) K₂SO₄, 1.72; KNO₃, 5.44; Ca(NO₃)₂, 3.05; NH₄NO₃, 0.50; HNO₃, 1.35; H₃PO₄, 1.81; (in mmol·m⁻³) H₃BO₃, 16.70; ZnSO₄, 1.38; CuSO₄, 1.10; (NH₄)₆Mo₇O₂₄, 0.52; FeNa EDTA, 75.10; and MnCl₂, 16.40.

For each genotype, 84 plants were cultivated in a plastic-covered greenhouse from April to September with minima and maxima of 22 ± 1°C and 37 ± 2°C, respectively. The solar radiation in the greenhouse ranged between 225 to 760 μmol·s⁻¹·m⁻², with an average of 605. Plants were divided into four groups, one for each NaCl treatment. Each group was divided into three replications of seven plants. The seedlings

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Table 1. Percentage of the genotypes for which the saline treatments were statistically significant, percentage variance of experimental error in comparison with the total variance, and percentage of genotypes that displayed the same ranking of treatments for each character.

Character ^z	Significant NaCl treatments (%)						Variance of experimental error (%)						Ranking of treatments (%)					
							Species ^{y,x}											
	pim	per	pen	hir	esc	Global	pim	per	pen	hir	esc	Global	pim	per	pen	hir	esc	Globa
Plant ht	100	100	100	100	100	100	24	23	19	12	18	20	100	92	100	86	100	95
Leaves (no.)	80	85	50	100	83	85	42	31	55	16	34	32	100	69	50	86	67	74
Trusses (no.)	80	77	100	86	75	77	44	38	20	26	34	35	100	69	50	57	58	64
Flowers (no.)	100	69	50	100	83	77	33	33	40	36	35	34	100	62	50	29	67	62
Fruits (no.)	100	46	0	71	75	55	38	38	50	31	32	39	100	69	50	43	67	27
Stem thickness	80	85	50	14	83	80	42	35	57	33	34	37	80	92	50	86	67	80
Leaf fresh wt	80	100	100	71	100	92	29	27	24	21	14	22	80	100	100	57	50	74
Leaf dry wt	80	100	50	71	100	92	22	20	47	26	19	22	80	100	100	100	100	97
Stem fresh wt	100	100	100	100	100	100	16	14	29	14	11	14	100	100	100	100	92	97
Stem dry wt	80	100	100	100	100	97	23	14	26	16	12	16	100	100	100	100	100	100
Cl	100	100	100	100	100	100	3	10	8	8	6	8	100	100	100	100	92	97
Na	100	100	100	100	100	100	3	14	9	9	7	9	100	100	100	100	100	100
K	80	92	100	86	100	92	33	40	15	23	25	30	80	46	50	86	83	69
N-NO ₃	80	100	100	86	100	92	24	20	15	22	16	11	80	31	50	71	92	64
Ca	100	54	100	100	100	85	21	42	28	31	31	33	80	31	50	71	25	44
Mg	100	69	100	100	100	90	13	35	34	20	11	22	100	62	50	100	83	80

^zMineral contents refer to leaves.

^ypim = *Lycopersicon pimpinellifolium*, per = *L. peruvianum*, pen = *L. pennellii*, hir = *L. hirsutum*, esc = *L. esculentum*.

^xGlobal refers to the whole group of genotypes without considering species separately.

initially were irrigated with the fertilizer solution, and after their establishment on the packs, each group was fertilized simultaneously with one of the saline treatments (i.e., NaCl at 0,2,4, or 8 g-liter⁻¹) until the end of harvest. Plants were irrigated eight times daily with 0.1 liter/plant from the start of the experiment until day 19, 11 times daily with 0.1 liter/plant from day 19 to day 41, and 11 times daily with 0.14 liter/plant from day 41 to the end of the experiment (13 weeks). The small initial increments in salinity were chosen because an accumulation of salt in the rock wool substrate can be expected until equilibrium between the fertilizer solution and evapotranspiration is reached. For this reason, the four NaCl concentrations measured 60 min after irrigation gave substrate electrical conductivities of 0.28, 0.63, 1.39, and 2.15 S·m⁻¹. The pH values were 6.86, 6.84, 6.40, and 6.35, respectively.

Sampling started 10 weeks after the beginning of saline treatments and consisted of randomly selecting three plants of each of the 39 genotypes from each replication in every treatment group. The following characteristics were measured: height of the main stem plant height), number of leaves, number of trusses, total number of flowers formed in each truss (number of flowers), stem thickness (beneath the fifth leaf), fresh weight of leaves (FWL), fresh weight of stem (FWS), dry weight of leaves (DWL), and dry weight of stem (DWS).

The leaves and stem of each plant were weighed separately, washed in distilled water, and dried at 65°C. After 3 days, the tissues were weighed again to determine DWL and DWS. The dried leaf material of each plant was ground to pass through a 0.5-mm mesh screen and used to determine the leaf concentrations of Cl, N-NO₃, Na, K, Ca, and Mg. The leaf concentration of Cl was determined in the aqueous extract by potentiometric measurements with AgNO₃ following the method of Johnson et al. (1958). Nitrogen-nitrate was determined in the aqueous extract by spectrophotometry at 210 and 270 nm. For the determinations of cations, dried leaf material was digested in a 2 HNO₃: 1 HClO₄ mixture (v/v); Na and K were measured by emission spectrophotometry, and Ca and Mg by atomic absorption spectrophotometry.

Fruits from the four remaining plants in each replication were harvested three times to determine the average number of fruits per plant. Because *L. peruvianum* and *L. hirsutum* lines are not self-fertilizing, manual pollinations were made to obtain fruits from these accessions.

Analyses of variance of the factorial experiments were carried out for each one of the 16 characters in each genotype (624 analyses); the four saline concentrations and the three replications were the fixed main factors. The model for the analyses of variance was: $X_{ij} = m + T_i + R_j + TR_{ij} + E$, where m = mean, T = saline treatment ($i = 1$ to 4), R = replication ($j = 1$ to 3), and E = error.

To evaluate the appropriateness of each character for salinity tolerance measurement, we took into account the following: 1) There were significant differences between salt concentrations in the analyses of variance of the 39 genotypes. 2) There was a minimum experimental error in the analyses of variance. To compare the error variances of the 624 analyses, their percentages of the total variance were calculated and those characters that exhibited the smallest percentages were considered more appropriate to evaluate salinity tolerance. 3) The rankings of the mean values of the characters in the Newman-Keuls tests were in a logical sequence with increase in the saline concentrations of the four treatments. Thus, the mean values for each character in the four treatments could be expected to either decrease or increase with increase of salt concentration. However, we decided that lack of significance between two consecutive values would not contravene this criterion.

Results

Significant differences were revealed among the salt treatments for plant height, FWS, and leaf Cl and Na concentrations for each of the 39 genotypes (Table 1). Because the genotypes were a sample from five species of *Lycopersicon*, the four characteristics (plant height, FWS, and leaf Cl and Na concentrations) revealed changes with the concentrations of NaCl independently of the genotypes used. Consequently, these char-

Table 2. Effect of salinity on plant height, leaf dry weight (DWL), and fresh (FWS) and dry weights (DWS) of stem relative to control plants and on leaf Cl and Na concentrations for 39 genotypes.

Entry	Species ^z	Plant ht (%)	DWL (%)	FWS (%)	DWS (%)	Leaf	
						Cl ($\mu\text{mol}\cdot\text{kg}^{-1}$ dry wt)	Na
1	pim	79	104	67	74	2160	2010
2	pim	72	48	45	39	1700	1610
3	pim	80	50	50	50	1160	850
4	pim	70	41	47	49	1630	1500
5	pim	78	48	44	47	1230	1160
6	per	71	60	53	50	1020	290
7	per	69	38	61	54	1600	920
8	per	66	19	61	48	1570	1030
9	per	72	43	56	50	1280	270
10	per	70	44	43	44	1210	620
11	per	70	44	39	38	1190	270
12	per	63	43	35	38	1450	450
13	per	71	46	49	47	1250	620
14	per	64	52	66	60	1030	220
15	per	72	32	74	51	1700	440
16	per	64	31	38	36	1520	490
17	per	62	48	39	37	1710	1490
18	per	69	44	42	40	1830	1570
19	pen	76	72	41	41	1540	1610
20	pen	80	46	48	46	1380	1310
21	hir	65	81	40	43	1490	1350
22	hir	62	40	38	44	1120	1120
23	hir	58	53	46	41	1140	680
24	hir	60	56	48	41	1260	1180
25	hir	65	28	32	29	1250	1040
26	hir	61	91	78	69	1120	980
27	hir	61	48	76	66	1260	1180
28	esc	85	66	73	68	3160	3160
29	esc	81	80	68	70	2840	2730
30	esc	74	65	63	64	2070	1670
31	esc	74	81	66	63	2400	2340
32	esc	65	75	54	54	2480	2670
33	esc	65	58	43	36	2460	2680
34	esc	67	62	67	56	2100	1510
35	esc	70	53	49	53	2130	2350
36	esc	82	73	51	46	2200	2280
37	esc	85	71	108	57	2590	2630
38	esc	71	56	45	44	2260	2050
39	esc	77	46	42	48	2000	1890
LSD ($P = 0.01$)		9	32	28	21	480	540

^zpim = *Lycopersicon pimpinellifolium*, per = *L. peruvianum*, pen = *L. pennellii*, hir = *L. hirsutum*, esc = *L. esculentum*.

acters should be useful for future studies of saline tolerance of mature tomato plants.

The FWL, DWL, DWS, and leaf K, N-NO₃, and Mg concentration characters were not as affected by changes in salinity as plant height, FWS, and leaf concentrations of Cl and Na. In 90% of cases, all six characters revealed significant differences and, therefore, would be useful for some salinity tolerance studies.

There were no significant differences between saline treatments for number of leaves, trusses, flowers, and fruits, stem thickness, and leaf Ca concentration (Table 1). Hence, these traits are not useful for measuring salinity tolerance of mature plants in experiments involving several species. However, they might be useful for comparative studies within a species.

In terms of the percentage of error variance in the analyses of variance, all the characters that showed little differences be-

tween salt treatments (salt treatments significant for <90% of the genotypes) also had percentage errors close to, or higher than, one-third of the total variance (Table 1). The only character presenting a high error and also significant differences between treatments in > 90% of the genotypes was leaf K concentration.

The third factor to be considered when assessing the suitability of a character for the evaluation of salinity tolerance is its uniformity of response to the saline treatments for most of the genotypes. When the mean values of treatments for each genotype were compared, the ranking of the four treatments was the same for leaf Na concentration and DWS in all 39 genotypes. For leaf Cl concentration, FWS, and DWL, it was the same in 38 genotypes. For plant height, the ranking was the same in 37 genotypes (Table 1). The character expressions for DWS, FWS, DWL, and plant height became less as salt concentration was increased, but the leaf concentrations of Na and Cl increased with salt concentration.

The saline treatments were found to be ranked similarly for number of trusses, flowers, and fruits, and for leaf Ca concentration in *L. pimpinellifolium* (Table 1). Treatment rankings were the same for stem thickness in *L. pimpinellifolium*, *L. peruvianum*, and *L. hirsutum*, and for FWL in *L. pimpinellifolium*, *L. peruvianum*, and *L. pennellii*. Rankings were the same for leaf K concentration in *L. hirsutum*, *L. pimpinellifolium*, and *L. esculentum*. However, because of their large error variance percentages, all these characters are unacceptable as predictors of tolerance.

Other characters, because they are different for each species, are not generally applicable, but they still have validity in certain cases. For example, number of leaves could be used because it is a character that changes with salt concentrations in *L. hirsutum*. Similarly, leaf N-NO₃ concentration in both *L. pimpinellifolium* and *L. esculentum* could be used. Leaf Mg concentration could be used to evaluate *L. pimpinellifolium*, *L. hirsutum*, and *L. esculentum* tolerance. The above three characters revealed significant differences between treatments for most of the genotypes, the mean values of their responses to saline treatments were ranked similarly, and their variance errors were low (Table 1).

Analysis of the data of the six characters that exhibited significant changes in all 39 genotypes showed that NaCl-treated plants of *L. esculentum* accessions had the fewest decreases compared with the controls for the four vegetative characters of plant height, DWL, FWS, and DWS. Entries of *L. esculentum* also had the most Cl and Na in the leaves (Table 2). In each species, except *L. peruvianum*, leaf Cl concentrations were similar or slightly higher than leaf Na concentrations (Table 2; LSD 0.01). In *L. peruvianum*, the levels of Cl in the leaf were more than double those of Na. Five genotypes (1, 28, 29, 31, and 37) revealed smaller decreases of their vegetative characters when compared with control plants than the other 34. All five showed high leaf concentrations of both Cl and Na. Generally, those genotypes with smaller decreases of their vegetative characters than control plants had higher leaf concentrations of Cl and Na. However, some genotypes belonging to wild species (3, 6, and 26) showed small decreases of their vegetative characters and medium to low leaf concentrations of Cl and Na.

Discussion

Changes in leaf concentrations of Na caused by changes in NaCl concentrations of the nutrient solution have been reported for *L. esculentum*, *L. cheesmanii* Riley, *L. peruvianum*, *L. pen-*

nellii, and *Solarium lycopersicoides* Dun. (Phills et al., 1979; Sacher et al., 1983; Shannon et al., 1987). Our results are similar and they include two more species, *L. pimpinellifolium* and *L. hirsutum*. There appears to be no direct relationship between leaf Cl concentration and plant dry weight (Sacher et al., 1983). Our results are similar to those of Phills et al. (1979) because leaf Cl levels changed significantly in response to changes in the NaCl concentrations for all the species tested. The leaf concentration of Mg changes with NaCl concentration of the nutrient solution in several species, namely *L. esculentum* (Phills et al., 1979), *L. pimpinellifolium*, and *L. hirsutum*, but not in *L. peruvianum* and *L. pennellii* (Phills et al., 1979).

In the present work, plants usually were shorter at the higher salt concentrations, but some authors say that plant growth may increase slightly following treatment with low concentrations of NaCl (Nukaya et al., 1979; Shannon et al., 1987).

The smaller number of leaves and flowers observed when growing plants on saline substrate is a typical stress response to temperature, humidity, or salinity (Wrona, 1983). The results of our study show that only in 62% of the cases (in which errors were greater than one-third of total variance) did the number of flowers and trusses decrease with an increase in NaCl concentration. This result suggests that both number of leaves and number of flowers were affected by factors independent of NaCl treatments.

Accessions of *L. pimpinellifolium*, *L. pennellii*, and *L. esculentum* are self-fertilizing, but, unexpectedly, showed no significant differences between number of fruits at low salt concentrations (0.28 and 0.63 S·m⁻¹). At higher salt concentrations (1.39 and 2.15 S·m⁻¹), the number of fruits were significantly reduced. Small changes in number of fruits at low salt concentrations are reported (Rush and Epstein, 1981; Shalhevet and Yaron, 1973).

Stem thickness, a measurement of plant vigor, showed little change with NaCl concentration in our study. Hall (1983) also reported that tomato stem thickness is not affected by salinity.

The three growth characters FWS, DWL, and DWS were good indicators of salinity tolerance. Hassan and Desouki (1982), Shannon (1979), and Shannon et al. (1987) used dry weight of the whole treated plants, expressed as a percentage of the dry weight of the whole control plants, as an index of salinity tolerance for *L. esculentum*, *L. pennellii*, and several hybrids of these species. Our results showed that the measurements of FWL and DWL gave higher error variances than FWS and DWS, possibly because some leaves may have abscised during plant development; consequently, the shoot and leaf weight characters should be interpreted with caution when evaluating adult plants.

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