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; H₂PO₄, 1.81; (in mmol·m⁻³) H₂BO₃, 16.70; ZnSO₄, 1.38; CuSO₄, 1.10; (NH₄)₂MoO₄, 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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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 rockwool 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 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 CI 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 CI and Na concentrations) revealed changes with the concentrations of NaCl independently of the genotypes used. Consequently, these char-

| Character | Significant NaCl treatments (%) | Variance of experimental error (%) | Ranking of treatments (%) |
|-----------|----------------------------------|-----------------------------------|--------------------------|
| Plant ht  | pim = Lycopersicon pimpinellifolium, per = L. peruvianum, pen = L. pennelli, hir = L. hirsutum, esc = L. esculentum. | Global refers to the whole group of genotypes without considering species separately. | |
| Leaves (no.) | 6.86, 6.84, 6.40, and 6.35, respectively. | | |
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 between treatments for most of the genotypes. 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. 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. pennellii, and L. hirsutum, with a high error variance for leaf K concentration and also significant differences between treatments in >90% of the genotypes was leaf K concentration.
nelli$, and Solarium lycopersicoides Dun. (Phillis 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 Philis 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 (Phillis et al., 1979), L. pimpinellifolium, and L. hirsutum, but not in L. peruvianum and L. pennellii (Phillis 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 2.1.5 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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