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Effect of root temperature on plant response functions for tomato: comparison of static and dynamic salinity stress indices

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Abstract

The agronomic study of plant response to salinity uses root zone salinity as a primary treatment variable. Root zone salinity is a static index in that its value does not depend on any variable factors in the soil-plant-air continuum (SPAC) that simultaneously affect growth. In order to take into account the effect of variable parameters in the SPAC, the concept of a dynamic index was introduced and defined as the salt flux to the shoot relative to growth rate. The objective of this study was to compare the variability of these two indices as measures of salt tolerance in different environments and to ascertain which correlative variable is more directly linked to yield reduction; root zone salinity, a static index, or ion flux to the shoot, a dynamic index. This was accomplished by comparing the root temperature dependence of threshold values for each index respectively. Yield response to salinity was obtained for tomato (*Lycopersicon esculentum* Mill., cv. Heinz 1350-vf) at two root temperatures, 18 °C and 25 °C, using 14 levels of salinity, 0 to 140 mM Cl, with a 2:1 Na-Ca ratio. Based on parametric analysis, the root zone chloride concentration threshold was increased 92% over the root temperature studied. In contrast, the threshold values of the dynamic indices were not statistically different at the two root temperatures. Plant shoot chloride concentration factor, expressed as the ratio of salt concentration in the shoot to salt concentration in the nutrient solution, decreased exponentially from 8 to 2 with increasing root zone salinity and was independent of temperature. Water use exhibited a strong temperature dependence and showed a sharp decline at root zone salinities corresponding to root zone salinity threshold values. Root/shoot ratio was independent of root zone salinity at least up to 80 mM Cl, and was significantly higher at the lower root temperature. It was concluded that root zone osmotic potential is not the fundamental causative factor determining the onset of yield reduction for the two temperatures studied, and that other elements related to growth and ion transport combine to determine a threshold value. These results indicate that it is a critical value of the salt flux to the shoot in relation to shoot growth rate that determines the onset of yield reduction and therefore is related more to the average salinity in the shoot than the average root zone salinity. This is evidence that specific biochemical mechanisms responsible for yield reduction reside in the shoot.

Introduction

Root zone salinity is used as the primary treatment variable for agronomic studies of plant response to salinity. Many studies have also been directed toward finding specific salt tolerance mechanisms that are located in the shoot or root. An excellent review of some long standing views and modern concepts of plant response to salinity is given by Munns (1993) and Serrano and Gaxiola (1994). Ultimately, salt tolerance must be determined by measuring whole-plant response in saline environments. Although there are many methods for representing a plant response function to salinity, it is most often quantified by using a piecewise linear regression (threshold-slope model) of experimental data relating yields to root zone salinity Maas and Hoffman (1977). Accordingly, yield remains constant with increasing root zone salinity until a critical threshold value is reached. After reaching this

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threshold value, yield is reduced at a crop specific rate proportional to increases in root zone salinity. Using a linear switching regression technique, Feinerman et al. (1982) have tested the empirical threshold-slope model and concluded that it is an acceptable function to evaluate salt tolerance. It is often observed that yield is reduced uniformly with decreasing osmotic potential of the nutrient solution. Therefore, excluding the effects of specific ion toxicity or nutrient deficiencies, plants appear to respond more to the colligative properties of the soil water than to its chemical composition (Bernstien, 1961). In this sense it is argued that the common index of root zone salinity to which plants respond is the osmotic component of the total water potential. This concept has provided the basis for much research in attempting to understand salinity-yield interactions from a modeling point of view (Feddes et al., 1974). These concepts are also the basis for field scale salinity assessment (Rhoades and Loveday, 1990). Because osmotic potential can be easily correlated with solution electrical conductivities, root zone salinity is often reported in terms of the electrical conductivity of the nutrient solution for hydroponic studies and of saturated soil-paste extract for some field studies (Rhoades and Oster, 1986). Salt sensitive crops have threshold values as low as 1.5 dS m^{-1} 15 mM Cl), while salt tolerant crops have threshold values ranging from 6 to 10 dS m^{-1} (60-100 mM Cl) (Maas and Hoffman, 1977).

While there are many practical agronomic advantages for studying whole plant response using root zone salinity as the primary treatment variable, it is deficient in that root zone salinity, per se, does not depend on any variable climatic or soil factors in the soil-plant-air continuum that are affecting growth. Some of these parameters such as temperature, humidity, radiation, root extent, and soil water content affect both transpiration and growth (Baker et al., 1992). As a consequence, root zone salinity alone cannot be used to predict the effects of these environmental variables on plant salt tolerance. The basis of salt tolerance and salt toxicity will always be masked by the confounding affects of physical and biochemical mechanisms affecting plant yield in saline environments. This supplies the motivation for the search and development of a dynamic salinity stress index, an index with the potential to unify the physical constraints that can modulate plant salt tolerance.

In an attempt to fill this need the concept of a dynamic salinity stress index, SSI, was developed. It is defined in terms of the dynamic flux of the dominant salinizing anion to the plant shoot in relation to plant growth. Intrinsic to this approach are the biophysical properties of the root system that control salt loading to the shoot under variable environmental soil and atmospheric conditions that simultaneously affect transpiration and growth.

The basic objective of this study was to find out which index related to salt tolerance, if any, is independent of environment; root zone salinity, a static index, or ion flux to the shoot, a dynamic index. This was accomplished by comparing the temperature dependence of threshold values for each index respectively. A preliminary study (Dalton and Poss, 1989) showed that plant salt tolerance, based on the threshold values of root zone salinity, increased with root temperature up to a temperature between 25 °C and 30 °C while plant salt tolerance, based on the threshold values of the dynamic index, appeared to be invariant with root temperature. However, the results could not be statistically verified due to the small number of salt treatments. In the present study, temperature treatments were decreased from four to two and salinity treatments were increased from six to fourteen. The dependence of many plant parameters on root temperature has been studied in great detail for many years (Borochov-Neori and Shani, 1994; Bowen, 1991; Cooper, 1973; Johnson and Thornley, 1985), including recent attempts to model observations according to the Arrhenius equation (Yongsheng et al., 1990). However, there has been no definitive investigation of the dependence of the root zone salinity threshold value on root temperature. Specifically, the plan of this study was to obtain a plant response function for tomato (*Lycopersicon esculentum* Mill., ‘Heinz 1350-vf’) at two root temperatures, 18 °C and 25 °C; to precisely determine the root zone salinity threshold values for each temperature; to verify, experimentally, the existence of a plant response function based on various forms of the dynamic salinity stress index (SSI) and then to determine the dynamic index threshold values for each temperature; to compare the temperature dependence of the static and dynamic threshold values; and to use these results as a critique of the relative merits of the various forms of the SSI and the static root-zone salinity index.

A rational basis for defining a dynamic salinity stress index

In his book on systems analysis, Casti (1989) states, “The study of natural systems begins and ends with the specification of observables belonging to such a system and a characterization of the manner in which they are linked.” In terms of this analysis, an actively transpiring plant constitutes the “system” and the specified observables for such a system have been yield and root zone salinity. The manner in which these specified observables are linked is given by the plant response function for a site specific environment. For this system, the magnitude of the value of root zone salinity is usually governed by irrigation water quality and is not linked to any dynamic variables of the soil-plant-air continuum. A few examples of dynamic variables are, water use, temperature, radiation and even dimensional aspects of the system such as root surface area. Root zone salinity is therefore a static index. In contrast, a dynamic index is related to the forces operating within the system. Such forces can be linked to the mass or energy flux into the system and have the potential of being described by the known laws of mass and energy transport. The basic requirements of an effective dynamic salinity stress index, SSI, are: 1. that its value can be experimentally determined, preferably without complicated or expensive equipment; 2. that it correlates in a useful way with plant response in saline environments, and most importantly; 3. that it be quantitatively coupled by an appropriate model to the dynamic processes occurring in the soil-plant-air continuum. In this study, the mass flux of the dominant salinizing anion into the plant relative to growth is chosen as the dynamic correlating index. Such an index has been defined previously in terms of an integrated average of salt-loading to the shoot relative to its growth rate expressed on a volume basis, (Dalton and Poss, 1989). This definition meets the first requirement of a dynamic index because the salt load to the shoot relative to growth rate can be easily measured by analyzing for the total accumulation of the dominant salinizing anion at any given growth stage. The third and most important requirement is met because the mathematical link connecting salt loading to variable environmental conditions is a previously developed theory relating water and ion transport into the shoot in terms of the bio-physical transport properties of the root system. From this theory, ion transport to the shoot depends not only on the solute concentration in the root zones, but also on transpiration rate, root surface and and the bio-physical transport properties of the root, including salt exclusion and temperature dependent metabolic ion transport, (Dalton et al., 1975; Dalton and Gardner, 1978; Fiscus,1975). Verifying that this index correlates with plant response in a useful way, the second requirement, is a partial objective of this investigation.

A dynamic index defined

The dynamic index is composed of two components. The first component is an expression for the transport of the dominant salinizing ion to the shoot. It can be expressed phenomenologically as,

$$\int A_r(t) J_s(t) dt = \text{ moles solute to shoot} \qquad (1)$$

where,
$A_r(t)$ = active root surface area development rate: (cm^2 sec^{-1}).
$J_s(t)$ = time dependent solute flux to the root: (mol sec^{-1} cm^{-2}).

Equation 1 simply states that, over the growing period, the observed total ion accumulation in the shoot can be expressed as an integrated average over time of the product of the salt flux into the root, and the area over which transport takes place, that is, the active root surface area. It is recognized that what actually constitutes the active root surface area is still a research problem, (Dalton, 1995), however the direct calculation of J_s and its dependence on osmotic potential of the soil solution, water and ion transport coefficients of the root, root surface area, and, most importantly, transpiration rate, has been previously demonstrated and shown to account adequately for the observed relationships between water flux, plant pressure potential and temperature dependent metabolic ion transport, (Dalton et al., 1975; Dalton and Gardner, 1978) (Appendix A). Since calculations of J_s are based on thermodynamics or phenomenological principles (Katchalsky and Curran, 1965), the results do not depend on actual mechanisms of ion and water transport to the shoot. In any case, for the purposes of this study, these points become mute because salt accumulation is determined experimentally and is independent of any model assumptions.

The second component of the dynamic index is a measure of shoot growth rate. As an index, the total solute flux to the shoot can only be meaningful when it is expressed relative to the growth rate of the plant. Growth rate can alternatively be expressed in terms of shoot volume, biomass or fresh weight. By choosing the growth rate as a volume production rate, the index represents an integrated average of salt concentration on a shoot volume basis without regard to partitioning and can be directly compared to root zone salinity. The expression for shoot volume is,

$$\int V_s(t)dt = \text{ shoot volume,} \tag{2}$$

where,
$V_s(t)$ = shoot growth rate based on volume production: ($cm^3 sec^{-1}$).
Under ideal environmental conditions, the unstressed value of V_s is limited only by genetic constraints and represents the maximum genetic potential for growth of the species or cultivar. The shoot volume, V_s, (cm^3), is related to biomass, MV_s (g), by the dry tissue bulk density, ρ_d (g cm^{-3});

$$\rho_d = \frac{M_s}{V_s} \tag{3}$$

and is related to the fresh weight, M_t, by the wet tissue bulk density, ρ_w,

$$\rho_w = \frac{M_t}{V_s} \tag{4}$$

In the previous study, the dynamic index was expressed only on the basis of shoot volume. In this study the properties of the dynamic index expressed on the basis of shoot volume, shoot dry weight and shoot fresh weight were measured and compared.

The analytic expression for the dynamic salinity stress index based on shoot volume becomes,

$$SSI_{sv} = \frac{\int A_r(t)J_s(t)dt}{\int V_s(t)dt} \tag{5}$$

This ratio has the units of mol L^{-1} and represents an integrated average salt concentration relative to the volume of the plant. We can also define indices which represent the integrated salt concentration on a fresh weight and dry weight basis by making use of Equations 3 and 4:

$$SSI_{\rho_w} = \frac{SSI_{sv}}{\rho_w} \tag{6}$$

and

$$SSI_{\rho_d} = \frac{SSI_{sv}}{\rho d} \tag{7}$$

This brief outline is presented to give a rational basis for the concept and definition of a dynamic salinity stress index (SSI). However, it is not the intent of this investigation to make model calculations, but as a first step, to make the necessary experimental measurements to verify the existence of a useful plant response function based on this definition. Then the variability of the temperature dependence of the static threshold value and the dynamic threshold value are compared in order assess the hypothesis that the dynamic index has the potential to provide an environmentally independent criterion for yield reduction. Because the analytic description of the salt accumulation process, Equation (1), has been independently verified, it is assumed to be valid and provides a rational basis for this experimental study. The analytic formulation provides a basis from which future simulations can be made and forms part of a continuing investigation.

Materials and methods

The experiment consisted of measuring the vegetative yields of tomatoes grown in a green house at 14 different salinities with 2 replications in continuously aerated saline base nutrient solution (BNS), at 18 °C and 25 °C. Tomato seeds (cultivar Heinz 1350) were sown in vermiculite and kept moist until the eight-day-old seedlings were transplanted into temporary BNS pots at ambient temperature. Sixteen days after germination, root temperature and salinity treatments were imposed after transplanting one plant to each 10 litre earthenware pot. The crocks were then immersed in four temperature baths (0.8 m H × 1.3 m L × 0.9 m W). Root temperature targets were reached within 24 hours from ambient conditions. Salination was accomplished by adding equal increments of $NaCl:CaCl_2$ (2:1 molar basis) over a five day period to decrease the osmotic potentials (OP) of the BNS to approximately 0, 0.03, 0.067, 0.10, 0.13, 0.167, 0.20, 0.23, .267, 0.30, 0.33, 0.367, 0.40, and 0.50 MPa. The BNS (in mol m^{-3}) was composed of: 2.5 $Ca(NO_3)_2$, 3.00 KNO_3, 1.5 $MgSO_4$, 1.67×10^{-1} K_2PO_4, 5.00×10^{-2} Fe (as sodium ferric diethylenetriamine pentaacetate), 2.31×10^{-2} H_3BO_3, 4.8×10^{-3} $MnSO_4$, 4.00×10^{-4} $ZnSO_4$, 2.0×10^{-4} $CuSO_4$, 2.00×10^{-4} H_2MoO_4. Root bath temperatures were maintained using controller-driven alternate/simultaneous heating and refrigeration systems. Thermocouples were used in conjunction with a micro logger to monitor root bath temperatures and the time averaged temperatures were 18.1 ± 1.1 Oc. and 25.1 ± 2.1 Oc. Day time temperatures were 35.3 ± 8.6 Oc. and night time temperatures were 27.6 ± 3.8 Oc. The pH of the BNS was maintained between 5.5 and 6.5. Plant stems and leaves were harvested 44 days after the planting date of Aug. 1. In this analysis, the two components, Equation (1) and Equation (2), of the dynamic index, Equation (5), were experimentally measured. The shoot volume, Vs, was measured immediately after harvesting using Archimedes principle (Raskin, 1983). Fresh and dry weights of shoot and root were measured. Wet and dry bulk densities were determined according to Equation (3) and Equation (4). In this experiment, chloride was the dominant salinizing ion and was used as a measure of the total solute flux to the shoot. The chloride content of the shoot at harvest was measured without regard to charge-balancing cations. The total salt load to the shoot in moles was obtained by measuring shoot chloride concentration in the leaves and stem. Total chloride transport to the shoot was then calculated as the product of chloride concentration on a dry weight basis and total shoot dry weight. Chloride determinations were made on dilute acetic acid and nitric acid extracts of leaves and stems by coulometric-amperometric titration (Cotlove, 1963). The dynamic indices, Equations (5), (6) and (7), were then calculated as the ratio, millimole chloride/; shoot volume, (m mol C L^{-1}), fresh weight, (mmol Cl g^{-1}) and dry weight, (mmol Cl g^{-1}), respectively.

Data analysis

The data is presented using two methods of analysis relating yield to the independent variables-root zone salinity and dynamic indices. For visualization, a locally weighted least squares technique for non-parametric curve fitting routine, "lowess", was used (Software provided by Axum Triletrix, 444 NE Ravenna Blvd., Suite 210, Seattle, WA, 1995)[1] The "lowess" method gives a smooth curve with a scatter plot of the associated data. This method is capable of giving a more realistic fit of the data than the forced parametric regressions, (Cleveland, 1993). For statistical inference a parametric non linear least squares method is used. The parametric model is a piece wise linear function, (Maas and Hoffman, 1977)

Table 1. Static and dynamic threshold values obtained by parametric regression of plant response data at two root temperatures

Root temperature	Threshold values		
	Static Index	Dynamic indices	
	Root zone salinity ($mM\ Cl^-\ L^{-1}$)	SSI_{sv} ($mM\ Cl^-\ L^{-1}$)	$SSI_{\rho d}$ ($mM\ Cl^-\ g^{-1}$)
18 °C	33.3 ± 13.1	67.8 ± 10.7	1.100 ± .126
25 °C	64.0 ± 12.9	85.7 ± 12.7	1.192 ± .127
% Increase from 18 °C to 25 °C	96	26	8
Sig. Different at at 95% CL	Yes	No	No

SSI_{sv}: chloride concentration on shoot volume basis.
$SSI_{\rho d}$: chloride concentration on dry weight basis.

$$Y = Y_m \quad \text{for } x < xT \tag{8}$$

$$Y = Y_m - s(x - xT) \qquad \text{for } x > xT \tag{9}$$

where Y, Ym, s, x, xT are the yield, maximum yield, slope, correlating index (root zone salinity or dynamic index) and threshold value of the correlating indices respectively. The non linear least squares program, NLIN, from SAS[1] is used for parametric analysis. The NLIN program finds best estimates and corresponding confidence intervals for the threshold values of the piece wise linear model above.

Results

Plant response functions

The plant response functions resulting from this investigation are presented using both absolute and relative yield. Relative yield is normalized with respect to the maximum yield of the "no-salt" treatment and can be useful when comparing plant response functions from different environments that have different maximum yields. Figure 1a shows the absolute dry weight yield as a function of root zone chloride concentration at two root temperatures, 18 °C and 25 °C. The threshold values of root zone salinity, and 95% confidence limits, at the two root temperatures are obtained by parametric regression and are designated by the solid lines shown in Figure 1a. A non parametric regression is superimposed with the parametric regression and is

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[1] Names and products are mentioned for the benefit of the reader and do not imply endorsement or preferential treatment by the USDA.

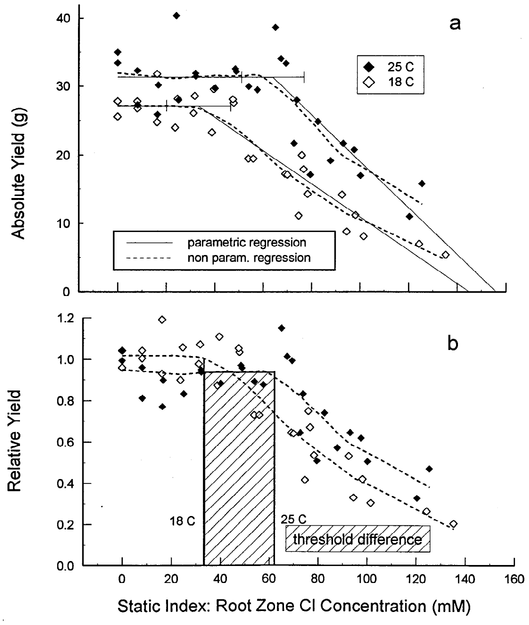

Figure 1. (**a**) Parametric (solid line) and non parametric (dashed line) regression analysis of shoot biomass yield with respect to root zone salinity at two root temperatures, 18 °C and 25 °C. Parameter estimation of root zone salinity threshold values are shown with 95% confidence limits. (**b**) Non parametric regression analysis of relative yield with respect to root zone salinity. Difference in root zone salinity threshold values at 18 °C and 25 °C are delimited by cross hatching.

shown with a dashed line. The non-parametric analysis closely approximates the parametric linear piece wise model and corroborates the conclusion of Feineman et al. (1982). The non parametric regressions show slightly different threshold values in this and the following response functions. The non parametric regression consistently show a non linear yield decline after the threshold value and could represent a better reality than the forced parametric regression. Figure 1b shows the plant response function in terms of relative yield using the nonparametric curve fitting routine. The observed threshold difference for the two temperatures is emphasized with cross hatching. The parameter estimates of threshold values for all plant response functions are also shown in Table 1.

Using the threshold value of root zone salinity as criteria, these results clearly show a significant increase in plant salt tolerance at the higher root temperature from both the nonparametric and parametric analysis. Based on the parametric analysis, the root zone chloride concentration for the threshold value at 18 °C is 33.3 ± 13.1 mM, (Table 1). The chloride concentration for the threshold value at 25 °C is increased to 64.0 ± 12.9 mM. These threshold values are significantly different at the 95% confidence level indicating that plant salt tolerance, based on the traditional concept of root zone salinity, is increased by 92% over the temperature range 18 °C to 25 °C. Assuming a linear response over the temperature range studied, the temperature dependence of the static threshold value is an astounding 13% per °C. It is a reasonable assumption that the large variation in salt tolerance exhibited by this cultivar is not due to a new genetic expression. While there are metabolic or physiological differences, these results quantitatively demonstrate the magnitude to which non-genetic forces may govern plant salt tolerance. The higher threshold value at the higher temperature cannot be attributed to a reduced osmotic stress at the higher temperature. From van't Hoffs law, π = RTC, (Barrow, 1961), we can calculate the temperature dependence of the osmotic potential of NaCl as $d\pi/dT$ = RC, where π is the osmotic potential, T is the absolute temperature, R is the universal gas constant and C is the solute concentration. The osmotic potential (MPa) changes a maximum of 1.3% per degree Kelvin over the chloride concentrations used in this experiment. Furthermore, the osmotic potential does not decrease, but increases, giving an even greater osmotic stress to the plants at the higher root temperature. Root zone salinity threshold values can obviously depend heavily on environmental conditions and at least with respect to root temperature, root zone salinity threshold values are not constant. We now proceed to test the temperature dependent properties of the dynamic indices.

Figures 2a and b show the temperature dependence of the absolute and relative yields, respectively, as a function of the dynamic salinity stress index based on shoot volume, SSI_{sv}. These plant response functions show the same threshold-slope correlations observed when using the static index of root zone salinity (Figure 1), and, therefore, meet the second requirement of a dynamic index, namely, usefulness. The parametric estimates for threshold values at 18 °C and 25 °C are 67.8 ± 10.7 mM Cl and 85.7 ± 12.7 mM Cl respectively. In sharp contrast, the difference in the dynamic threshold values at 18 °C and 25 °C (Table 1) are much smaller, 26%, and not statistically different at the 95% confidence level. An even smaller difference between threshold values is observed when the dynamic index is expressed on the basis of tissue dry weight or dry tissue bulk density, Equation (7). In this case, and as can be seen in Figure 3a and b, the threshold values

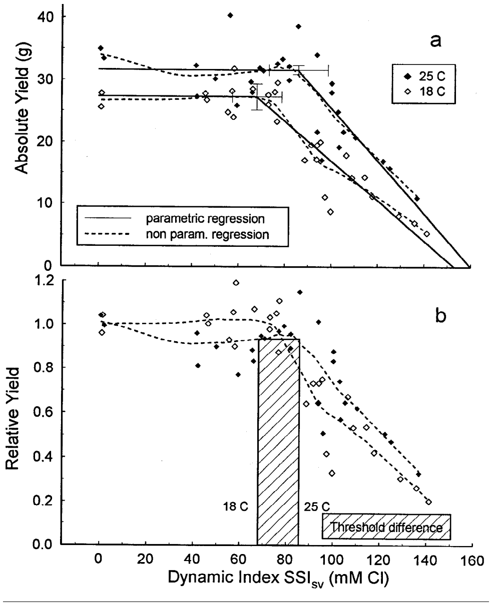

Figure 2. **a**) Parametric (solid line) and non parametric (dashed line) regression analysis of shoot biomass yield with respect to the dynamic salinity stress index (SSI$_{\rho v}$) at two root temperatures, 18 °C and 25 °C. Parameter estimation of the dynamic index threshold values are shown with 95% confidence limits. (**b**) Non parametric regression analysis of relative yield with respect to dynamic salinity stress index. Difference in dynamic index threshold values at 18 °C and 25 °C are delimited by cross hatching.

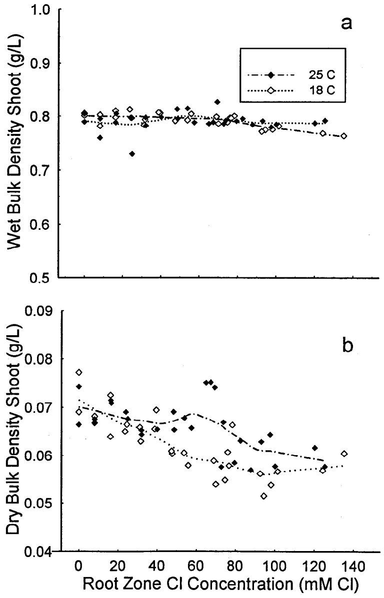

Figure 3. (**a**) Parametric (solid line) and non parametric (dashed line) regression analysis of shoot biomass yield with respect to the dynamic salinity stress index (SSI$_{\rho d}$) at two root temperatures, 18 °C and 25 °C. Parameter estimation of the dynamic index threshold values are shown with 95% confidence limits. (**b**) Non parametric regression analysis of relative yield with respect to dynamic salinity stress index. Difference in dynamic index threshold values at 18 °C and 25 °C are delimited by cross hatching.

of SSI$_{\rho d}$ for the two temperatures are almost identical. The threshold values at 18 °C and 25 °C are 1.100 ± .126 m mol Cl g^{-1} and 1.197 ± .127 m mol C g^{-1} respectively and are not statistically different. When the dynamic index is expressed on the basis of tissue fresh weight or wet tissue bulk density, SSI$_{\rho w}$, Equation (6), the plant response functions (not shown) are similar to the plant response function based on SSI$_{sv}$, Figures 2a and b.

We are left with the result that while in all cases there is no statistical temperature dependence of the dynamic index thresholds, the dynamic index based on tissue dry weight is the preferred index for representing temperature invariance.

Normalizing the SSI$_{sv}$ with respect to wet and dry bulk densities give the dynamic indices SSI$_{\rho w}$ and SSI$_{\rho d}$ (Equations (6) and (7)) and these indices are the same as those obtained directly by expressing the measured shoot chloride on a fresh weight or dry weight basis. It is therefore instructive to look at differences in the temperature dependence of wet and dry tissue bulk densities. Figures 4a and b show the wet and dry bulk density of the shoot tissue respectively. The non parametric regressions for the wet bulk density show a relatively uniform response with root zone salinity at the two root zone temperatures. Then, as would be expected with a relatively constant wet bulk density, the plant response function based on SSI$_{\rho w}$ is similar to the plant response function based on SSI$_{sv}$. In contrast, the dry bulk densities at the two root temperatures are decidedly different. Both curves decrease with increasing salinity but there is a marked peak in bulk density at or near the root zone salinity threshold value for the 25 °C treatment.

Plant shoot chloride concentration factor

Because the dynamic indices are related to the time integrated averages of the salt flux to the shoot, it is instructive to examine the temperature dependent relation between the ratio of the final salt concentration in the shoot (on a volume basis) to the salt concentration in the root zone (on a volume basis). To do this, we can take the ratio of the average chloride concentration in the plant shoot, SSI$_{sv}$ to the chloride concentration in the root zone water [Cl^{-}], to give a concentration fac-

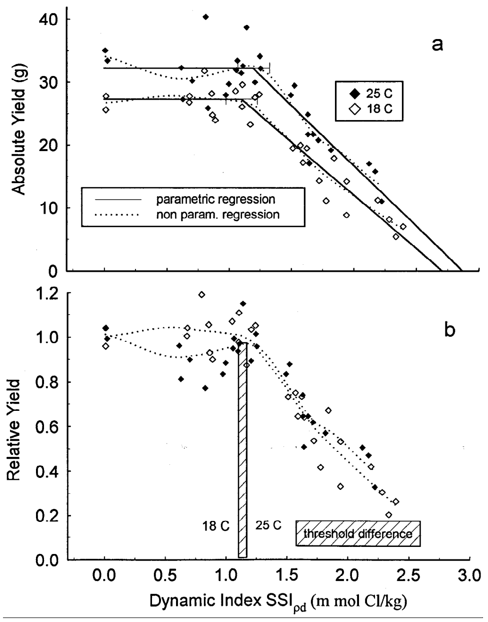

Figure 4. Bulks densities of shoot tissue as a function of root zone salinity for two root temperatures, 18 and 25 °C: (**a**) wet bulk density and (**b**) dry bulk density.

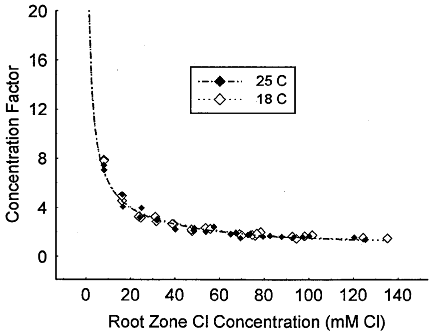

Figure 5. Concentration factor, CF, for tomato expressed as the ratio of salt concentration in the shoot to salt concentration in the nutrient solution, as a function of root zone salinity at two root temperatures 18 °C and 25 °C.

tor, CF, equal to $SSI_{SV}/[Cl^-]$. Figure 5 shows the experimental data for the concentration factor (CF) plotted as a function of the root zone salinity. The concentration factor is seen to be independent of temperature and decreases asymptotically to a value of about 8 for the first chloride concentration encountered in this experiment (8.1 mM Cl) and decreases exponentially to a factor of about 1.5 at high root zone salinities (> 130 mM Cl). Even though temperature has a large effect on growth rate, Figure 1a, temperature has no effect on the resultant plant concentration factor, Figure 5.

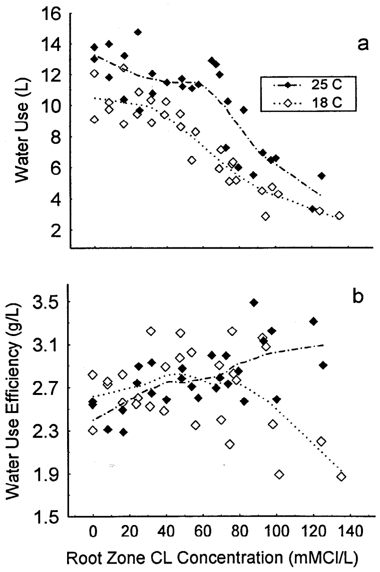

Figure 6. (**a**) Non parametric regression of total water use as a function of root zone salinity at two root temperatures 18 °C and 25 °C. (**b**) Non parametric regression of water use efficiency as a function of root zone salinity at two root temperatures 18 °C and 25 °C.

The average salt concentration in the plant is always significantly higher than the concentration of the salt in the root zone; and at low to moderate salinities the salt concentration in the plant can be many times higher than that in the root zone. The plant is in all cases concentrating salt relative to its root zone environment. These results reveal a very important property of the plant that regulates salt uptake relative to growth rate.

Plant water use

It has been shown that, for the two root temperatures studied, plant response is related more to factors associated with the dynamic process of salt accumulation than it is with salinity or osmotic potential of the root zone. Salt accumulation is a process which is dynamically linked to transpiration rates and root and shoot growth. From Equation (1), Appendix A, the solute flux, J_S is shown to be dependent on the transpiration flux, J_V. The transpiration rate, in turn, is known to be most sensitive to the pressure potential developed in the xylem tissue in response to the evaporative demand experienced by the plant canopy, and to the temperature dependent active ion transport system in the root (Dalton and Gardner, 1978). Measurement of water use can, therefore, be appreciated as an important variable

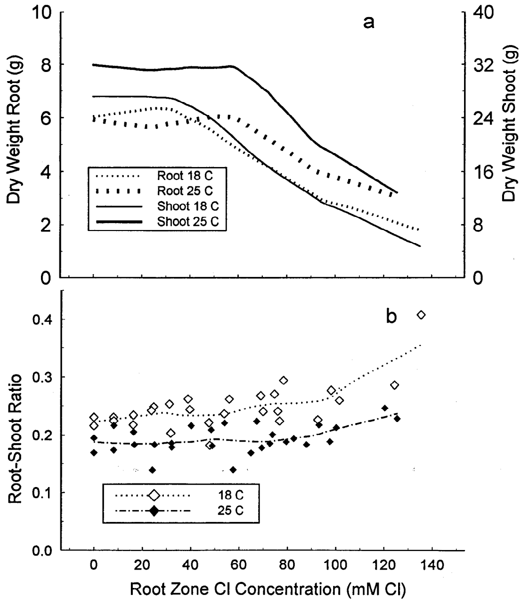

Figure 7. (**a**) Non parametric regression of root and shoot biomass as a function of root zone salinity at two root temperatures 18 °C and 25 °C. (**b**) Non parametric regression of root/shoot ratio as a function of root zone salinity at two root temperatures 18 °C and 25 °C.

for plant salt tolerance studies. In the past, neither transpiration or root temperature have been considered a significant experimental variable. The total plant water use as a function of root zone salinity is shown in Figure 6a. Water use follows a similar bi-phasic (threshold-slope) form as does the plant response function. Water use is highly temperature dependent and is seen to gradually decrease with increasing salinity up to a root zone salinity corresponding to the root zone salinity threshold values for the two root temperatures studied (33/64 mM Cl, Figure 1). Past these points, water use declines at a much greater rate. Water use efficiency for the two root temperatures is shown in Figure 6b. There is an appreciable scatter of the data, but with the lowess non parametric analysis, it can at least be concluded that, at the high root temperature, water use efficiency continues to increase past its threshold value while, at the low root temperature, water use efficiency begins to decline at or near its threshold value. Incorporating the interrelation between salt loading, pressure potential, stomata control of water use and photosynthesis are important adjuncts to these types of studies.

Root-shoot ratios

Figure 7a shows the non parametric response functions for both root and shoot at root temperatures of 18 °C and 25 °C. Data points have been omitted so that the general trends revealed by the regression analysis are more easily visualized. The threshold values of the root mirrors those for the shoot. However, unlike the shoots, at root zone salinities less than the threshold value, root production on a dry weight basis at 18 °C is equal to if not greater than root production at 25 °C. The relatively high dry matter production at 18 °C suggests that the plant has the ability to partition carbon into root biomass in spite of the less favorable temperature environment for root growth.

Figure 7b shows the root-shoot ratio as a function of root zone salinity for the two root temperatures, 18 °C and 25 °C. The lowess regression used for the curve fitting shows that the root-shoot ratio is consistently higher at the lower temperature. While there is a strong dependence of absolute yield for both root and shoot on root zone salinity, Figure 7b shows that the root/shoot ratio is relatively constant and that root zone salinity has little if any affect on the root-shoot ratio at root zone chloride concentrations less than about 60 mM.

Discussion

Root temperature is shown to have a substantial effect on the maximum allowable root zone salinity which does not reduce yield. The large temperature dependence in root zone salinity threshold values between 18 °C and 25 °C, 13% per °C, can have important practical implications for resource management in regions where opportunities exist for artificially moderating low soil temperature (Willis et al., 1957) or for multiple plantings in different seasons (Borochov-Neori and Shani, 1995) or for controlling aqua culture temperature for optimum salt tolerance. Root temperature is seldom if ever reported in salt tolerance studies and could, in part, account for large discrepancies often observed when comparing field and green house measurements of plant salt tolerance.

Of equal or possibly greater significance is the demonstrated increase in the apparent plant salt tolerance of a given cultivar obtained by a simple change in root temperature. It is well known that root and shoot growth rates have a strong temperature dependence (Bowen, 1991). However, it is not well known which, if any, cellular mechanisms are responsible for increased salt tolerance with increased root temperature. A large temperature dependence of the threshold value of root zone salinity presents an ideal system for investigating biochemical mechanisms affecting plant salt tolerance. By using identical genotypes one can create various degrees of plant salt tolerance by a simple manipulation of root temperature.

As pointed out by Serrano and Gaxiola (1994), it is not even known if the primary mechanisms responsible for plant salt tolerance reside in the root or the shoot. Because the dynamic salinity stress index(SSI) threshold value is independent of temperature, there is strong evidence that the roots, in conjunction with variable environmental parameters of the shoot, control the salt load to the shoot, but that the basic salt sensitive mechanisms causing a reduction in growth reside in the shoot. These results also provide a useful working hypothesis for the investigation and separation of the biochemical and physical factors affecting plant salt tolerance. For example, physical factors controlling the magnitude of the salt flux to the shoot, J_s, in addition to root zone salinity, are transpiration rate as influenced by leaf area index, micro-climate, root surface area and distribution, and temperature dependent water and ion transport properties of the root. An example of biochemical factors controlling the onset of yield reduction would be the salt concentration dependent mechanisms of the plant shoot cells that partition or otherwise counter the negative impact of toxic or osmotic effects due to salt loading. Unfortunately, these physical and physiological components have combined to confound our attempts to identify useful selection criteria for increased salt tolerance.

The root-shoot ratio for the two temperatures studied are shown in Figure 7b. The root-shoot ratio, at 25 °C is significantly lower than the root-shoot ratio at 18 °C. The lower value of root-shoot ratio is associated with the higher salt tolerance. That is, the plant can tolerate a higher root zone salt concentration with out yield reduction at the lower root-shoot ratio. It is reasonable to assume that root-shoot adjustments are processes for optimizing growth potential for different environments. Root-shoot ratios based on dry weight can be useful for studying carbon partitioning but cannot provide information on root function with respect to water and ion transport. Cumbus and Nye (1982) observed that, while dry matter production for rape (*Brassica*) was almost independent of root temperature, root length increased markedly with increase in root temperature. Dalton (1995) points out that a measure of root dry weight may not be as good a measure of root functionality as will a measure of root surface area and that root surface area may be intimately related to root functionality with respect to both water and ion transport to the shoot. Klepper (1991), in a review of the relation between root and shoot growth, emphasized the dynamic rather than static interdependence for defining functional relationships. Genetic and environmental forces can affect the partitioning of growth between root and shoot, and the plant appears to have some capability for reducing stress by adjusting the root-shoot ratio. From the conceptual basis of the dynamic index, it can be deduced that the plant would be able to withstand an increase in root zone salinity before reaching its threshold value, if the magnitude of salt accumulation with respect to growth rate were reduced. The optimum relation between evaporative surface area of the shoot, which is proportional to the rate of water use, and the root surface area across which the salt and water must travel, is not quantitatively understood. Environmental influences on root-shoot ratios and genetic differences between species and cultivars are well documented (Bowen, 1991; Klepper, 1991; Russell, 1977). The root-shoot ratio can be influenced by the plant's need to reduce both water stress and salt accumulation in the shoot. The salt load can be reduced, relative to the growth rate of the shoot, by decreasing root size. The water stress can be reduced for any given transpiration demand by increasing the size of the root system. These two stress reduction strategies are opposed to each other with regard to root size requirements. When root temperature is a factor, the problem is confounded because hydraulic conductance of the root system is affected by temperature dependent transport properties of the root and will be significantly reduced at lower temperatures (Dalton and Gardner, 1978). The higher root-shoot ratio at 18 °C may be the result of an adjustment of the root system in order to compensate for lower hydraulic conductance of roots at the lower temperature. The larger root shoot-ratio will also accommodate a higher salt flux and result in the dynamic threshold value being reached at a lower root zone salinity. The potential for any given species or cultivar to adapt an optimal root-shoot ratio in response to environmental stress, especially salt stress, is not well understood. It remains a question as to what extent the plant can manipulate its root-shoot ratio so as to reduce water stress and salt loading according to the temperature conditions of the root. It is of interest to note that under saline conditions in a sandy medium, Faden and Kirkham (1982) found that a drought resistant wheat cultivar grew better than a drought-sensitive wheat cultivar. Kirkham (1984) also found that a drought-resistant wheat cultivar was more salt tolerant than a drought sensitive wheat cultivar. These are an important factors to be considered for both drought stress and salinity stress.

From Appendix A, Equation (1) it is seen that the value for the SSI depends on the rate of root development, $A_r(t)$ with respect to the transpiration flux Jv. Transpiration rate, in turn, depends on the rate of shoot or canopy development. Total root surface area, and the fraction of the root surface in contact with soil water (Dalton, 1995; Herkelrath et al., 1977), affects transpiration rate and, therefore, salt loading to the shoot. These physical variables can be important factors related to observed variations in plant salt tolerance.

The temperature dependence of the threshold value of root zone salinity observed in these experiments can be tentatively explained on a physical basis using the functional concepts of the dynamic stress index. On this basis, in order for the plant to reach a critical value of salt concentration in the shoot (e.g. SSI threshold value) at the higher root temperature, it is necessary to increase the ion flux to the shoot commensurate with the temperature induced increase in shoot growth, V_s. This can be accomplished by increasing the salt concentration in the root zone. That is, all other factors being equal, an increase in root temperature increases the rate of shoot growth more than the rate of salt accumulation in the shoot. The higher threshold value of root zone salinity that is observed at the higher temperature is a possible manifestation of these phenomena. The computational procedures to simulate these phenomena are beyond the scope of this paper but form the basis of further investigation. The dynamic index proposed here, that is, salt loading to the shoot relative to its growth rate, integrates all variable parameters in the soil-plant-air continuum to give a quantity that usefully correlates with yield.

Summary and conclusions

In order to understand the basis of salt tolerance and salt toxicity at the whole plant level it is necessary to be able to separate out any physical and biochemical mechanisms that may be confounding our observations of plant yield in saline environments. This work was carried out in search of a dynamic salinity stress index (SSI) that would integrate variable physical parameters in the soil-plant-air continuum and provide an environmentally invariant measure of plant salt tolerance. Experiments were designed to ascertain which correlative variable is more fundamentally linked to yield reduction; root zone salinity, a static index, or ion flux to the shoot, a dynamic index. This was accomplished by comparing the temperature dependence of threshold values for each index respectively. The most significant finding of this investigation was that, for the two root temperatures studied, 18 °C and 25 °C, the threshold value calculated on the basis of root zone salinity increased by 92%, with the result that the root zone salt concentration could almost be doubled without reducing vegetative growth. This order of magnitude of change is striking and illustrates the importance of environmental factors that can affect plant salt tolerance. In contrast, the dynamic indices unify the effect of root temperature on plant salt tolerance. When the average chloride concentration of the shoot is expressed relative to the dry bulk density of the tissue, the plant response function is invariant to root temperature. Thus it has been ascertained that root zone osmotic potential is not the fundamental causative factor determining the onset of yield reduction for the two temperatures studied, and that other elements related to growth and ion transport combine to determine a threshold value. In fact, these results indicate that it is a critical value of the magnitude of the salt load to the shoot in relation to growth rate of the shoot that initiates the onset of yield reduction. This in no way negates the controlling influence that salt exclusion and hydraulic transport properties of the root has on salt loading to the shoot. However, as noted in Appendix A, Equation (1), salt loading to the shoot depends not only on root zone salinity, but also on water use, temperature dependent metabolic ion transport, hydraulic properties of the root and root surface area.

As stated previously the dynamic index represents a time integrated average of salt concentration in the shoot and because the threshold value of the dynamic index is invariant to root temperature, it can be deduced that the onset of yield reduction is associated more with the average salinity in the shoot than the average salinity in root zone. This is evidence that the specific biochemical mechanisms responsible for yield reduction reside in the shoot. It can also be argued that since the temperature variation of the dynamic index expressed relative to tissue biomass, SSI_{pd} is much less than the variation of the dynamic index expressed relative to the tissue volume or tissue fresh weight, SSI_{sv}, then yield reduction is caused by toxic affects that have been obscured by correlations of yield with osmotic potential measurements.

The use of a dynamic index has the potential to distinguish quantitatively between plant properties and processes regulating plant salt tolerance that are under genetic control and those that are under physical control. Any environmental condition or plant property that is able to reduce the value of the SSI will provide improved conditions for plant growth in saline environments. It follows from this study that selecting for vigor in conjunction with increased water use efficiency is a good selection criterion for improved salt tolerance. Finally, it should be emphasized that the usefulness of this index is not diminished because explicit mechanisms for yield reduction are not specified. It can be an effective phenomenological function in the sense that, since its value is well correlated with plant response, the components affecting the index will also correlate with plant response and provide new insights for separating physical and genetic factors contributing to the phenomena of plant salt tolerance.

Appendix A

The solute flux in Equation (1) is a function of the transpiration flux, J_v ($cm^3\ cm^{-2} sec^{-1}$), nutrient solution concentration, C_1 ($M\ cm^{-3}$), and osmotic potential, π_1, (bars), root membrane transport parameters that include; the salt reflection coefficient, σ (unitless), the osmotic permeability coefficient, ω ($M\ cm^{-2} sec^{-1} bar^{-1}$), and the metabolic transport coefficient, k ($M\ cm^{-2} sec^{-1}$) (Dalton et al., 1975).

$$J_s = (1 - S)C_1 J_v \qquad (1)$$

where the selectivity coefficient, S (unitless), is a water flux, J_v, dependent function.

$$S = \frac{\sigma - nRTk(T)/\pi_1}{1 + nRT\omega/J_v} \qquad (2)$$

where n is the sum of the cation and anion valencies of the salinizing salt, R is the universal gas constant and T is the absolute temperature.

References

Baker J M, Wraith J M and Dalton F N 1992 Root function in water transport. Advances in Soil on Science. ASA. Eds. J L Hatfield and B A Stewart. Springer-Verlag, Berlin. 152 p.

Barrow G M 1961 Physical Chemistry. McGraw Hill Book Co., Inc., New York. 694 p.

Bernstein L 1961 Osmotic adjustment of plants to saline media. I. Steady state. Am. J. Bot. 48, 909–918.

Borochov-Neori H and Shani U 1994 Response of melon plants to salt: 2. Modulation by root growth temperature - the role of root membrane properties. J. Plant Physiol. 145, 545–550.

Bowen G D Soil temperature, root growth, and plant function. *In* Plant Roots the Hidden Half. Eds. Y Waisel, A Eshel and U Kafkafi. p 948. Marcel Dekker, Inc. New York.

Casti J L 1989 Alternate Realities: Mathematical Models of Nature and Man. John Wiley and Sons, New York, 493 p.

Cleveland W S 1993 Visualizing Data. Hobart Press, Summit, NJ. 360 p.

Cooper A J 1973 Root temperature and plant growth: A Review. Res/ Rev. 4. Commonwealth Bur. Hort. and Plantation Crops, Commonwealth Agric. Bur., East Malling, Maidstone, Kent 73. 72 p.

Cotlove E 1963 Determination of true chloride content of biological fluids and tissues. Analysis by simple nonisotopic methods. Anal. Chem. 35, 101.

Cumbus I P and Nye P H 1982 Root zone temperature effects on growth and nitrate absorption in rape. J. Exp. Bot. 33, 1138–1446.

Dalton F N 1995 In-situ root extent measurements by electrical capacitance methods. Plant Soil 173, 157–165.

Dalton F N and Poss J A 1989 Water transport and salt loading: a unified concept of plant response to salinity. Acta Hortic. 278, 187–193.

Dalton F N, Raats P A C and Gardner W R 1975 Simultaneous uptake of water and solutes by plant roots. Agron. J. 67, 334–339.

Dalton F N and Gardner W R 1978 Temperature dependence of water uptake by plants. Agron. J. 20, 404–406.

Faden A O and Kirkham M B 1982 Salt tolerance of drought-sensitive and drought-resistant wheat. *In* Biosaline Research. Ed. A San Pietro. pp 467–472. Plenum Press, New York.

Feddes R E, Bresler E and Neuman A P 1974 Field test of a modified numerical model for water uptake by root systems. Water Resour. Res. 9, 1199–1206.

Feinerman E, Yaron D and Bielorai H 1982 Linear crop response functions to soil salinity with a threshold salinity level. Water Resour. Res. 18 101–106.

Fiscus E L 1975 The interaction between osmotic and pressure induced water flow in plant roots. Plant Physiol. 55, 917–922.

Herkelrath W N, Miller E E and Gardner W R 1977 Water uptake by plants. II. The root contact model. Soil Sci. Soc. Am. J. 41, 1039–1043.

Johnson I R and Thornley J H M 1985 Temperature dependence of plant and crop processes. Ann. Bot. 55, 1–24.

Katchalsky A and Curran P F 1965 Nonequilibrium thermodynamics in biophysics. Harvard Univ. Press, Cambridge, Mass.

Kirkham MB 1984 Water relations of drought-resistant and drought-sensitive wheat cultivars sprinkled with saline water. Irrig. Sci. 5, 137–146.

Klepper B 1991 Root shoot relationships. *In* Plant Roots the hidden Half. Eds. Y Waisel, A Eshel and U Kafkafi. Marcel Dekker, Inc., New York. 948 p.

Maas E V and Hoffman G J 1977 Crop salt tolerance-current assessment. J. Irrig. Div. ASCE 103, 115–134.

Munns R 1993 Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ. 16, 15–24.

Raskin I 1983 A method for measuring leaf volume, density, thickness, and internal gas volume. HortScience 18, 689–699.

Rhoades J D and Loveday J 1990 Salinity in irrigated agriculture. *In* Irrigation of Agricultural Crops. Eds. B A Stewart and D R

Nielson. Agron. Monogr. No. 30, pp 1089–1142. Soil Sci. Soc. of Am., Madison, WI.

Rhoades J D and Oster J D 1986 Solute content. *In* Methods of Soil Analysis. Ed. A Klute. I, 2nd ed. Agronomy 9, 985–1006.

Russell S R 1977 Plant Root Systems: Their Function and Interaction with the Soil. McGraw-Hill Book Company, London. 298 p.

Serrano R and Gaxiola R 1994 Microbial models and salt stress tolerance in plants. Crit. Rev. Plant Sci. 13, 121–138.

Willis W O, Larson W E and Kirkham D 1957 Corn growth as affected by soil temperature and mulch. Agron. J. 49, 323–327.

Yongsheng F X L and Boersma L 1990 The arrhenius equation as a model for explaining plant responses to temperature and water stresses. Ann. Bot. 66, 237–244.

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