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Artigo/Article

Effects of non-uniform salinity and calcium on growth and physiology of *Citrus* seedlings

Mongi Zekri[1] & Lawrence R. Parsons[1]

SUMMARY

Soil water content and salinity levels are not uniform in citrus groves, particularly with the use of micro-irrigation systems that water only a portion of the root zone. An experiment using a split-root system of sour orange seedlings was designed to mimic non-uniform salinity under field conditions. Growth and physiological variables were evaluated when half or whole root systems were stressed with sodium chloride. Plant growth, leaf water potential, osmotic potential, stomatal conductance, and evapotranspiration decreased with increasing salt concentrations. Shoot and root dry weights and leaf water and osmotic potentials were more disturbed under uniform salinity than under non-uniform salinity. Seedlings with only half root system stressed had shoot dry weight and leaf water potential values closer to those of the non-stressed control than to those with the completely stressed root system. The non-stressed portion of the root system compensated for the decrease in growth, water uptake, and physiological processes by the stressed portion. Another experiment was set up to determine if addition of calcium to saline irrigation water would reduce salt damage to sour orange (*Citrus aurantium*) seedlings. This study demonstrated that calcium sulfate improved the ability of sour orange to tolerate salinity and that the beneficial effect of adding calcium to saline irrigation water depended on the anion accompanying the calcium. Calcium sulfate, but not calcium chloride, was found to overcome the detrimental effects of NaCl by decreasing the concentrations of both Na and Cl in citrus leaves.

Index terms: citrus, salinity, stress physiology.

Efeitos da salinidade não-uniforme e do cálcio no crescimento e fisiologia de plântulas de citros

RESUMO

O teor de água do solo e os níveis de salinidade não são uniformes nos pomares de citros, particularmente com o uso de sistemas de micro irrigação que regam apenas uma parte da zona da raiz. Um experimento usando um sistema subdividida de mudas de laranja azeda foi projetado para imitar a salinidade não uniforme em condições de campo. O crescimento e as variáveis fisiológicas foram avaliadas quando metade ou sistemas radiculares inteiros foram estressados com cloreto de sódio. O crescimento das plantas, o potencial da água foliar, o potencial osmótico, a condutância estomática e a evapotranspiração diminuíram com o aumento das concentrações de sal. Os pesos secos e de raiz seca e a água foliar e os potenciais osmóticos foram mais perturbados sob salinidade

[1] Hendry County Extension, Institute of Food and Agricultural Sciences (IFAS), University of Florida, LaBelle, Florida, USA

Corresponding author: Mongi Zekri, Hendry County Extension, Institute of Food and Agricultural Sciences (IFAS), University of Florida, P.O. Box 68, FL 33975, Florida, LaBelle, USA. E-mail: maz@ufl.edu

uniforme do que sob salinidade não uniforme. As mudas estressadas com sistema radicular subdividido tiveram valores de peso seco e valores de água foliar mais próximos dos do controle não estressado do que aquelas estressadas com o sistema radicular completo. A porção não estressada do sistema radicular compensou a diminuição do crescimento, a absorção de água e os processos fisiológicos pela porção estressada. Outro experimento foi estabelecido para determinar se a adição de água de irrigação de cálcio a salina reduziria o dano de sal nas mudas de laranja azeda (*Citrus aurantium*). Este estudo demonstrou que o sulfato de cálcio melhorou a capacidade de laranja azeda para tolerar a salinidade e que o efeito benéfico da adição de cálcio a água de irrigação salina dependia do ânion que acompanha o cálcio. Verificou-se que o sulfato de cálcio, mas não o cloreto de cálcio, superou os efeitos prejudiciais do NaCl, diminuindo as concentrações de Na e Cl nas folhas de citros.

Termos de indexação: citros, salinidade, fisiologia do estresse.

INTRODUCTION

Salts in soil and irrigation water are a serious problem for commercial citriculture, particularly in arid and semi-arid regions. However, the potential for salinity damage also exits in humid areas. In Florida, many citrus plantings are located in coastal areas where high salinity waters are being used for irrigation.

Most work on salinity has been done by exposing plant roots to nearly uniform concentrations, but exposure of all roots to a uniform salinity may differ from its situation in the field. Under field conditions, the roots of a plant grow in soil which varies in water content, salt concentration, and water potential both in space and with time.

One way to study the effects of different root environment is to use split-root systems. Treatments can be applied to one part of the root system, while the other part is untreated.

The first experiment deals with the response of sour orange seedlings to non-uniform salinity stress using a split-root system treated with equal as well as unequal concentrations of NaCl. This study was undertaken to determine changes in plant growth, stomatal behavior, water use, and water relations when half of the root system was stressed with NaCl.

Another study was conducted to investigate the effect of NaCl on sour orange seedling growth in relation to ion toxicity and nutritional imbalance, and to determine if addition of Ca to saline irrigation water would reduce salt damage to sour orange seedlings grown in sandy soil.

MATERIAL AND METHODS

Split-root experiment

The tap root of each sour orange seedling at the three-leaf stage was cut to a 1.0 cm length and all other roots were removed. The remaining portion of the tap root was dipped into a 50% ethanol solution containing 5 g dm^{-3} of IBA (indolebutyric acid). Seedlings were then placed in a mix of peat, perlite, and vermiculite (PROMIX BX), watered daily and fertilized weekly for 2 months. Seedlings with two uniform adventitious roots were selected and transplanted when 5-months-old into 2.2 dm^3 square plastic containers stapled together along one side and filled with sandy soil. Sodium chloride was added to the half-strength Hoagland's solution to achieve final osmotic potentials of -0.10, -0.20, and -0.35 MPa. The basic nutrient solution NS (no salt or half-strength Hoagland's solution) had an osmotic potential (OP) of -0.05 MPa. The experiment consisted of 7 treatments listed in Tables 1, 2, and 3. Each treatment was replicated 4 times in a randomized complete-block design. Plants were irrigated with the treatment solutions every 2 days. Solutions were added each time by bringing the soil moisture to slightly above the soil water-holding capacity to prevent drought or salt accumulation in the soil. Osmotic potentials of the treatments were determined with a vapor pressure osmometer and electrical conductivities were determined with a conductivity meter.

Components of the water balance were monitored on four successive days during the fourth month of salt treatment. Leaf water potential was measured at sunrise and at midday on fully-expanded leaves using a pressure chamber. The osmotic potential of the leaf sap was determined with a vapor pressure osmometer. Turgor potential was obtained by subtracting the osmotic potential value from the water potential value. Morning and midday stomatal conductance was measured with a steady-state porometer. Water use or evapotranspiration from each of the two sides was estimated by the amount of solution added each time to bring the soil to slightly above its water holding capacity.

Table 1. Shoot and root dry weights of split-root sour orange seedlings under NaCl stress. 0.10, 0.20, and 0.35 in parentheses under Treatment indicates osmotic potential of solution

Treatment	Average OP of solution (MPa)	Shoot dry wt. (g)		Root dry wt. (g)	
		Mean[z]	% Lower than NS/NS	Mean	% Difference than NS/NS
NS/NS	-0.05	37.1 a[y]	0	9.29/9.41 a	0/0
NS/NaCl (0.10)	-0.08	33.6 ab	9	9.64/6.12 ab	+4/-35
NaCl (0.10)/NaCl (0.10)	-0.10	20.4 d	45	5.33/5.27 cde	-43/-44
NS/NaCl (0.20)	-0.12	31.1 bc	16	9.49/4.22 bc	+2/-55
NaCl (0.20)/NaCl (0.20)	-0.20	14.9 e	60	3.85/3.98 ef	-59/-58
NS/NaCl (0.35)	-0.20	29.4 bc	21	9.74/3.21 bcd	+5/-66
NaCl (0.35)/NaCl (0.35)	-0.35	7.1 f	81	1.97/2.01 g	-79/-79

[z] Mean of four plants; [y] Mean separation within columns by Duncan's Multiple Range Test, 0.05 probability level.

Table 2. Midday leaf water, osmotic, and turgor potentials (MPa) of split-root sour orange seedlings under NaCl stress

Treatment	Water potential		Osmotic potential		Turgor potential	
	Mean[z]	% Lower than NS/NS	Mean	% Lower than NS/NS	Mean	% Higher than NS/NS
NS/NS	-1.23 a[y]	0	-1.73 a	0	0.50 a	0
NS/NaCl (0.10)	-1.22 a	-1	-1.74 a	1	0.52 a	4
NaCl (0.10)/NaCl (0.10)	-1.32 ab	7	-1.89 bc	9	0.57 a	14
NS/NaCl (0.20)	-1.26 ab	2	-1.81 ab	5	0.55 a	10
NaCl (0.20)/NaCl (0.20)	-1.48 abc	20	-2.08 d	20	0.60 a	20
NS/NaCl (0.35)	-1.32 ab	7	-1.89 bc	9	0.57 a	14
NaCl (0.35)/NaCl (0.35)	-1.60 c	30	-2.26 e	31	0.66 a	32

[z] Mean of four plants; [y] Mean separation within columns by Duncan's Multiple Range Test, 0.05 probability level.

Table 3. Midday stomatal conductance and daily evapotranspiration of split-root sour orange seedlings under NaCl stress

Treatment	Stomatal conductance (cm s^{-1})		Water use (cm^3 d^{-1})	
	Mean[z]	% Lower than NS/NS	Mean	% Lower than NS/NS
NS/NS	0.26 a[y]	0	145/155	0/0
NS/NaCl (0.10)	0.22 ab	15	181/67	+25/-57
NaCl (0.10)/NaCl (0.10)	0.20 ab	23	75/78	-48/-50
NS/NaCl (0.20)	021 ab	19	181/30	+25/-81
NaCl (0.20)/NaCl (0.20)	0.15 abc	42	37/40	-75/-74
NS/NaCl (0.35)	0.21 ab	19	183/19	+26/-88
NaCl (0.35)/NaCl (0.35)	0.13 bc	50	24/26	-83/-83

[z] Mean of four plants; [y] Mean separation within columns by Duncan's Multiple Range Test, 0.05 probability level.

After 4 months of NaCl treatment, the seedlings were harvested, and shoot and root dry weights were determined after oven-drying for 3 days at 60°C. Analysis of variance (F-test) was used to determine significant differences and Duncan's multiple range test was employed for mean comparison at $P < 0.05$.

Calcium experiment

Uniform, 3-month-old sour orange seedlings were transplanted into 19-cm-tall black plastic pots containing 5.5 liters of fine sand (Candler fine sand) taken from the top 30 cm of a citrus orchard soil in Florida. The seedlings were placed in a greenhouse and irrigated every 2 to 3 days with a modified half-strength Hoagland's solution for one month before salt treatments were started. Salt treatments were initiated by adding 40 mM NaCl to the half-strength Hoagland's solution and by adding $CaSO_4$ or $CaCl_2$ to selected treatments. The experiment consisted of 7 treatments listed in Tables 4 and 5. Each treatment was replicated 8 times in a randomized complete-block design. Seedlings were irrigated with the various solutions every 2-3 days for 4 months. The amount of solution added with each irrigation was determined by bringing the soil in the containers to slightly above water-holding capacity to prevent salt accumulation in the growth medium and to prevent plants from being drought stressed.

Seedlings were harvested after 4 months of experimental treatments. Dry weights of leaves, stems, and roots were measured after 3 days of drying at 60 °C. The dried leaves, which had been mature and fully expanded, were ground and their mineral concentration was measured. Analysis of variance was used to determine significant differences, and Duncan's multiple range test was used for mean comparison when the F test was significant at $P < 0.05$.

RESULTS

Split root experiment

Uniform salinity was significantly more damaging to sour orange seedlings than non-uniform salinity. Shoot dry weight was reduced by 9% to 21% when half of the root system was irrigated with saline solutions ranging from -0.10 to -0.35 MPa (Table 1). When both halves of the root system were irrigated with saline solutions, shoot dry weight was reduced by 45% to 81% (Table 1). With root dry weight, a similar trend was seen; stressing one-half of the root system reduced the weight by 16% to 31% while stressing both halves reduced it by 43% to 79% (Table 1).

Table 4. Shoot and root dry weights of sour orange seedlings after 4 months of treatment with salt solution.[z] (NaCl concentration was 40 mM.)

Treatment	Shoot dry wt.		Root dry wt.	
	Mean actual (g)	Relative (%) to Control	Mean actual (g)	Relative (%) to Control
Control (no NaCl)	36.6 a[y]	100	9.4 a	100
NaCl	25.9 d	71	6.8 a	72
NaCl + 1 mM $CaSO_4$	32.2 ab	88	8.2 a	87
NaCl + 5 mM $CaSO_4$	37.0 a	101	9.3 a	99
NaCl + 7.5 mM $CaSO_4$	36.3 a	99	9.2 a	97
NaCl + 5 mM $CaCl_2$	29.1 bcd	80	7.4 a	79
NaCl + 7.5 mM $CaCl_2$	28.4 cd	78	7.5 a	80

[z] Mean of eight plants; [y] Mean separation within columns by Duncan's Multiple Range Test, 0.05 probability level.

Table 5. Leaf mineral concentration (percent leaf dry weight) of sour orange seedlings after 4 months of treatment with salt solutions.[z] (NaCl concentration was 40 mM.)

Treatment	Mineral content (%)				
	Ca	Mg	Na	Cl	K
Control (no NaCl)	2.1 b[y]	0.30 a	0.02 c	0.02 d	2.8 a
NaCl	1.7 c	0.21 b	0.47 a	0.97 b	2.0 b
NaCl + 1 mM $CaSO_4$	1.7 c	0.22 b	0.43 a	0.48 c	2.1 b
NaCl + 5 mM $CaSO_4$	2.4 ab	0.21 b	0.27 b	0.41 c	1.9 b
NaCl + 7.5 mM $CaSO_4$	2.7 a	0.20 b	0.24 b	0.43 c	1.9 b
NaCl + 5 mM $CaCl_2$	2.7 a	0.21 b	0.26 b	1.28 a	2.0 b
NaCl + 7.5 mM $CaCl_2$	2.8 a	0.20 b	0.25 b	1.36 a	2.0 b

[z] Mean of eight plants; [y] Mean separation within columns by Duncan's Multiple Range Test, 0.05 probability level.

In the split-root test, shoot growth did not correlate well with the average salt stress of the total root system. The average osmotic potential of the NS/NaCl (0.20) treatment was -0.12 MPa. Even though this was a slightly greater stress than the average osmotic potential of the NaCl (0.10)/NaCl (0.10) treatment, shoot dry weight was 34% (10.7 g) less in the uniform NaCl (0.10)/NaCl (0.10) treatment (Table 1). Similarly, shoot dry weight in the NaCl (0.20)/NaCl (0.20) treatment was 49% (14.5 g) less than that in the NS/NaCl (0.35) treatment, even though both of these treatments had the same average NaCl stress (-0.20 MPa).

Similar to growth, water relations were also significantly more disturbed under uniform than under non-uniform salinity. Leaf water and osmotic potentials, stomatal conductance, and evapotranspiration decreased with increasing NaCl concentrations (Tables 1 & 2). Turgor potential increased with NaCl treatments (Table 2). Turgor tended to increase with increasing salt concentration or when both root halves were salinized. When both root halves were uniformly treated with NaCl at -0.10 MPa and -0.20 MPa, water use or evapotranspiration was reduced by approximately 50% and 75%, respectively (Table 3). Water use from the non-stressed half increased by about 25% when the other half was exposed to salinity.

Calcium experiment

Shoot dry weight was reduced (by nearly 30%) when 40 mM NaCl was added to the nutrient solution (Table 4). Although not significant, the percentage reduction in root dry weight for each treatment was nearly the same as for shoots (Table 4). We attribute this lack of significance to variation in root dry weight among replications. The addition of 1, 5, or 7.5 mM $CaSO_4$ to the saline solution significantly decreased the adverse effect of NaCl on shoot and root growth. Addition of $CaCl_2$ to the saline solution did not significantly improve shoot growth relative to NaCl alone (Table 4).

Addition of NaCl alone to the nutrient solution significantly increased leaf Na and Cl, but decreased Ca, Mg, and K (Table 5). Leaf Na and Cl accumulations above 0.4% and 0.5%, respectively, usually reduced citrus seedlings growth. Addition of 5 or 7.5 mM $CaSO_4$ to the saline solution reduced Na and Cl concentrations (Table 5). Addition of $CaCl_2$ reduced Na but increased Cl to the toxicity level (> 0.5%) in the leaves (Table 5). No significant differences in P, Fe, Mn, Zn, and Cu were found among the treatments (data not shown).

DISCUSSION

Split root experiment

The split root system experiment showed that the non-stressed roots could partially compensate for the decrease in water uptake by the stressed roots because water use by the non-stressed half of the root system increased by about 25% when the other half was exposed to salinity. Sour orange seedlings with half their root systems in NaCl had dry weights, leaf and osmotic potential values closer to those of the non-stressed control than to those of completely stressed root systems. Stomatal conductance was reduced with an increase in NaCl concentrations (Table 3). Closure of stomata might not be caused entirely by salinity-induced water stress. This possibility was based on the data presented in Table 2, which showed no turgor loss in salt-treated plants.

The present study carried out under greenhouse conditions provided several useful observations that are relevant to field conditions. Citrus is a deep and densely rooted crop which tolerates certain levels of salinity as long as a portion of the root system remains in a relatively non-saline soil. This may explain why citrus trees can be grown in some areas with salty irrigation water. A portion of the root system exposed to better quality water helps dilute the salt effect in the irrigated zone.

Calcium experiment

Growth was reduced significantly without any visible leaf symptoms of salt damage. Although root and shoot dry weights were reduced by nearly 30% in some treatments after 4 months of salinity stress, none of these treatments induced visible burn or other damage symptoms on leaves. Under saline conditions, the addition of Ca to irrigation waters altered sour orange seedling response. This study showed that the beneficial effect of Ca depended on the anion associated with the Ca. Calcium sulfate was significantly more effective than $CaCl_2$ in reducing the deleterious effect of NaCl on shoot growth (Table 4). Failure in the effectiveness of $CaCl_2$ in our work might have been due to the Cl accompanying the Ca and to the sensitivity of citrus to Cl.

In our study, NaCl reduced shoot growth due partially to excess accumulation of Na and Cl in the leaves. The improvement of shoot growth by addition of $CaSO_4$ likely was not totally due to the effect of Ca in maintaining the selective permeability of membranes, because shoot growth was not improved by addition of $CaCl_2$. We believe that the competitive interaction between Ca and Na and between SO_4 and Cl contributed to offsetting the deleterious effect of NaCl on citrus growth. Furthermore, the addition of $CaSO_4$ to the saline solution decreased both the Na:Ca and Cl:SO_4 ratios in the medium. Hence, there would be less Na and Cl uptake and accumulation in the leaves, where these ions can disturb metabolic processes.

CONCLUSION

In conclusion, this study demonstrated that $CaSO_4$ improved the ability of sour orange seedlings to tolerate salt, and that the beneficial effect of adding Ca to saline irrigation water depended on the anion accompanying the Ca. Calcium sulfate, but not $CaCl_2$, was found to overcome the detrimental effects of NaCl by decreasing the concentrations of both Na and Cl in citrus leaves.

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