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EFFECTS OF SALINITY AND CALCIUM ON SEEDLING EMERGENCE, GROWTH, AND SODIUM AND CHLORIDE CONCENTRATIONS OF CITRUS ROOTSTOCKS

MONGI ZEKRI¹

*Citrus Research and Education Center
University of Florida, IFAS
700 Experiment Station Road
Lake Alfred, FL 33850*

Abstract. Salt tolerance of several citrus rootstocks during germination, emergence, and early seedling stage was studied under greenhouse conditions. Salinity delayed and depressed seedling emergence, reduced seedling biomass, and altered mineral status of young citrus seedlings. However, magnitude of these parameters varied among rootstocks. The addition of 50 mol m⁻³ NaCl to a nutrient solution delayed seedling emergence by 3 to 5 days with the exception of Troyer citrange (TC) which emerged the soonest beginning at day 10 after sowing. Final emergence was reduced by less than 30% in Carrizo citrange (CC), TC, and Swingle citrumelo (SC), and by more than 65% in Ridge pineapple (RP), Cleopatra mandarin (CM), and rough lemon (RL). Total seedling biomass was reduced by over 50%. The addition of NaCl increased Na and Cl in the shoots and roots of all rootstocks. The addition of 5 mol m⁻³ CaSO₄ to the saline solution enhanced the emergence of the first seedling in CC, CM, and RL, improved final emergence in SO, SC, RP, CM, and RL, and improved seedling growth of SC and RL. For citrus, no uniform trend was found in the relationship between salt tolerance during emergence and during seedling growth. Salt tolerance at emergence may not be a useful indicator for rapid screening of citrus cultivars.

Citrus rootstocks are propagated mostly from seeds. Although modern techniques place an increasing emphasis on plant uniformity and the importance of seeds in the overall citrus nursery operation (Castle, 1981), there are only 2 reported studies of water stress involving the effect of polyethylene glycol (PEG) on citrus seedling emergence (Chilembwe et al., 1992; Kaufmann, 1969) and only one

study dealing with the effect of PEG, NaCl, and Na₂SO₄ on citrus germination (Mobayen and Milthorpe, 1978).

Soil salinity may reduce seed germination by reducing water uptake by seeds or by allowing seeds to absorb excessive ions that are potentially toxic (Ayers and Hayward, 1948). These physico-chemical effects may reduce germination and the emergence of seeds. Salt stress may have different effects on seed germination and young seedling growth. Some crops, such as alfalfa and sugar beets, are relatively tolerant to salinity during the later stages of growth but are sensitive to salinity during germination (Ayers and Hayward, 1948). Other crops, such as rice, are much more salt sensitive during the young-seedling stage of development than during germination (Pearson et al., 1966). In citrus, it is not known whether salt tolerance during germination or seedling emergence is related to tolerance during later growth stages.

The effect of salinity on citrus seedling emergence has not been well investigated, and information on the tolerance of citrus seedlings at early stages is lacking. Therefore, a study was initiated to evaluate the effect of salinity on seedling emergence and early stages of seedling development of various citrus rootstocks. Another objective of this work was to investigate the potential improvement in seedling emergence under saline conditions due to supplemental Ca because CaSO₄ has been found to alleviate the adverse effects of NaCl on citrus tree growth (Zekri and Parsons, 1990).

Materials and Methods

The experiment was carried out in a greenhouse in which the temperature and relative humidity ranged from 16 to 34°C and from 50 to 100%, respectively. The tested rootstocks were sour orange (*Citrus aurantium*) (SO), Volkamer lemon (*C. volkameriana*) (VL), Ridge Pineapple sweet orange (*C. sinensis*) (RP), Cleopatra mandarin (*C. reshni*) (CM), rough lemon (*C. jambhiri*) (RL), Carrizo (CC) and Troyer citranges (TC) (*C. sinensis* × *Poncirus trifoliata*), and Swingle citrumelo (*C. paradisi* × *P. trifoliata*) (SC).

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¹Present address: c/o Jack M. Berry, Inc., P. O. Box 459, LaBelle, FL 33935.

All seeds were about 1 yr old with the exception of those of RL which were 2 yr old. The seeds were sown in plastic trays composed of 96 individual cells. Each cell was 4 cm in diameter at the top and 12 cm deep having a conical shape at the bottom with one hole for water drainage. Two-thirds of each cell were filled with a commercial growing medium (Terra-lite Metro-mix 500), then one intact seed per cell was placed horizontally on the growing medium and covered with 1 cm layer of coarse vermiculite. The seeds were watered to excess immediately after planting and every other day thereafter with corresponding treatment solution to prevent any build-up of salts. The initial seed water content on a dry weight basis was measured by drying 10 g of intact unsorted seeds for each rootstock in an oven at 105°C for 48 h.

The experiment consisted of 4 salinity treatments: no salt in one-tenth strength Hoagland's solution (-0.01 MPa, 100 ppm), 50 mol m⁻³ NaCl (-0.23 MPa, 3000 ppm), 100 mol m⁻³ NaCl (-0.46 MPa, 6000 ppm), and 50 mol m⁻³ NaCl plus 5 mol m⁻³ CaSO₄ (-0.24 MPa, 3500 ppm) dissolved in one-tenth strength Hoagland's solution. The experimental design was a split-plot with 4 main plots (treatments) and 8 subplots (rootstocks) and with 12 seeds as the experimental unit replicated 4 times.

The appearance of the shoot at the surface of the growing medium was considered emergence. Emerged seedlings were counted daily for 2 months after the first seedling appeared. Number of days to emergence of the first seedling, number of days between the emergence of the first and last seedling (emergence spread), and final percent emergence were calculated from the daily counts.

Ten weeks after sowing, roots were washed free of Metro-mix and were separated from the shoots. Roots and shoots were oven-dried for at least 2 d at 65°C, weighed, ground in a Wiley mill, and stored for mineral analysis. Nitrogen was determined by the micro-Kjeldahl method using Buchi digestion, control, and distillation units. Chloride was measured using a Buchler-Cotlove chloridometer after extracting the samples with a nitric-acetic acid solution. Samples were analyzed for Na after dry ashing in a muffle furnace for 5 h at 500°C and dissolving in 1N HCl solution. The concentrations of Na were determined using an inductively coupled argon plasma emission spectrometer (Perkin Elmer, Plasma 40).

Analysis of variance was used to determine significant differences among treatments and among rootstocks, and Duncan's multiple range test was used for comparison when the F test was significant at $P < 0.05$.

Results

Salinity affected emergence of the first seedling, emergence spread, time to 50% emergence, and final percent emergence of seeds of most rootstocks (Fig. 1). No emergence of any of the rootstocks occurred at 100 mol m⁻³ NaCl concentration. The initial seed water contents (% dry wt) were 60, 37, 43, 58, 59, 37, 45, and 41 for CC, SO, VL, TC, SC, RP, CM, and RL, respectively.

Emergence of first seedling varied among rootstocks even in the no salt control treatment (Fig. 1). Troyer citrange seedlings emerged the soonest beginning at day 10 after sowing. Carrizo citrange and SC seedlings also emerged relatively rapidly starting at day 16 after sowing.

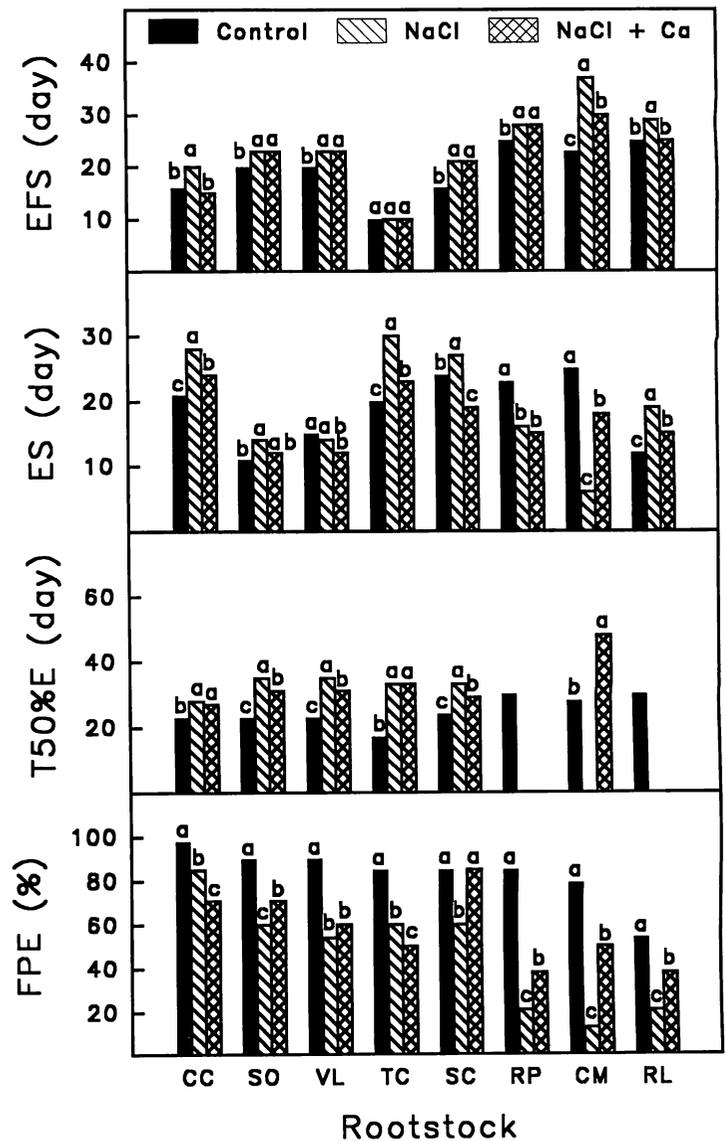


Fig. 1. Emergence of first seedling (EFS), emergence spread (ES), time to 50% emergence (T 50% E), and final percent emergence (FPE) of seeds of 8 citrus rootstocks treated with 3 treatments. Each bar is the mean of 4 replicates. Treatment means for each rootstock were separated by Duncan's multiple range test, 5% level.

Ridge pineapple, CM, and RL seedlings were the slowest to emerge, while SO and VL were similar and intermediate in terms of emergence of first seedling. With the exception of TC, the addition of 50 mol m⁻³ NaCl to the control solution delayed seedling emergence by 3 to 5 days. The addition of 5 mol m⁻³ CaSO₄ to the saline solution did not further delay the emergence of the first seedling but reduced the time to the emergence of the first seedling in CC, CM, and RL rootstocks.

For the no salt control treatment, the duration between the emergence of the first and last seedling (emergence spread) also varied among rootstocks with the least occurring in SO, VL, and RL and the most in SC, RP, and CM (Fig. 1). In the NaCl treatment, emergence spread was increased in most of the rootstocks as compared to the control treatment. However, addition of CaSO₄ to the saline solution reduced emergence spread in some of the root-

stocks as compared to the NaCl treatment. There was no clear relationship between emergence of the first seedling and emergence spread.

In the no salt control treatment, time to 50% emergence generally followed the same trend as emergence of the first seedling with the least time occurring in TC and the most in RP, CM, and RL (Fig. 1). Some of the data of time to 50% emergence for RP, CM, and RL are not available because less than 50% of the seeds emerged. Similar to emergence of the first seedling and emergence spread, time to 50% emergence was increased by NaCl treatments and reduced for some rootstocks by addition of Ca.

In the no salt control treatment, final emergence was greater than 80% for all rootstocks with the exception of CM and RL because of lower seed quality or age (Fig. 1). Final germination ranged from 98% for CC to 54% for RL. Salinity significantly reduced final germination for all rootstocks. In the 50 mol m⁻³ NaCl treatment, final germination was reduced by less than 30% in CC, TC, and SC and by more than 65% in RP, CM, and RL. The addition of 5 mol m⁻³ CaSO₄ to the saline solution improved final germination in SO, SC, RP, CM, and RL but further depressed final germination in CC and TC.

Under non-stressed conditions, shoot and root biomass of 2-month-old seedlings differed among rootstocks (Table 1). Shoot and root dry weights of SO and TC were the highest, while those of RP and CM were the lowest. The addition of 50 mol m⁻³ NaCl to the nutrient solution reduced both shoot and root biomass by over 50%. The addition of 5 mol m⁻³ CaSO₄ to the NaCl solution improved seedling growth of SC and RL rootstocks.

Addition of 50 mol m⁻³ NaCl to the nutrient solution increased Na and Cl concentrations in both shoots and roots of all rootstocks (Fig. 2). However, roots generally accumulated lower amounts of Na and Cl than shoots. The addition of 5 mol m⁻³ CaSO₄ to the saline solution reduced Na and/or Cl concentrations in shoots of SO, TC, SC, RP, and RL but did not affect Na or Cl content in roots of any of the rootstocks.

Discussion

Salinity affects germination or emergence either by decreasing the osmotic potential of the soil solution to a point which will retard or prevent the intake of water or by being toxic to the embryo and seedling. The net result is a delay and a reduction in germination or emergence percentage as this has been observed in this study. Salinity depressed

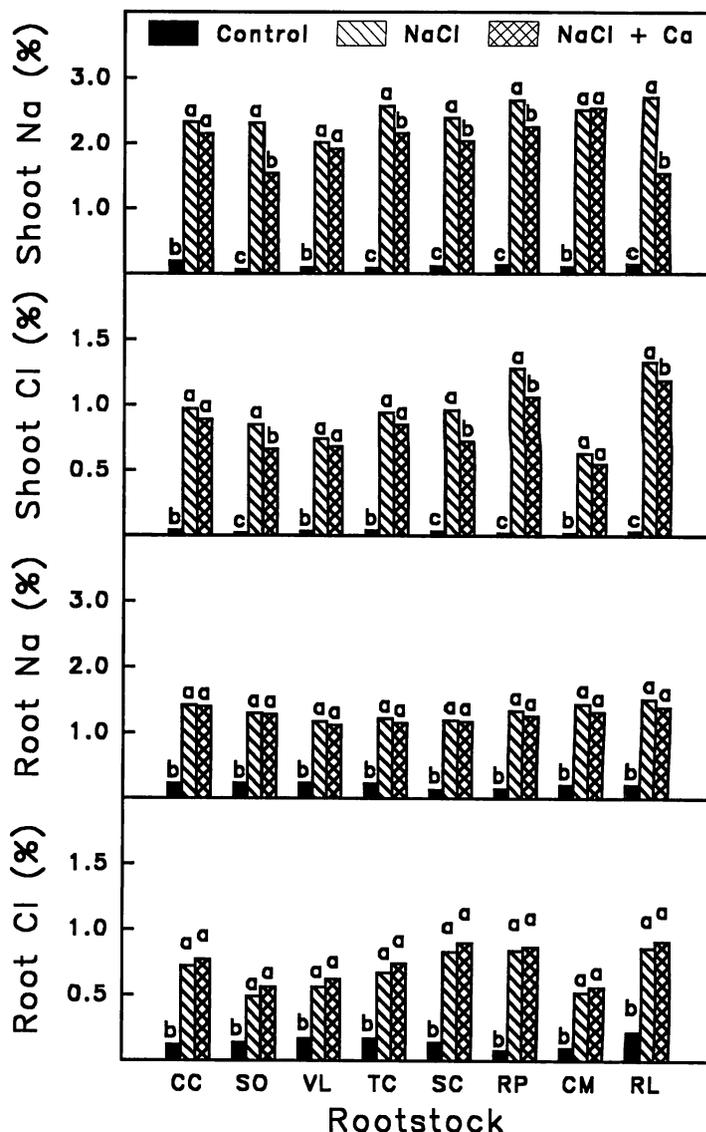


Fig. 2. Sodium and chloride concentrations (% dry wt) in shoots and roots of seedlings of 8 citrus rootstocks grown under 3 treatments. Each bar is the mean of 4 replicates. Treatment means for each rootstock were separated by Duncan's multiple range test, 5% level.

and delayed seedling emergence of most citrus rootstocks studied, but the degree of reduction and delay varied among rootstocks. Similar results were obtained by Mobayen and Milthorpe (1978) when seeds of *Poncirus*

Table 1. Shoot and root dry wt (mg) of 2-month-old seedlings of 8 citrus rootstocks grown under 3 treatments.

Growth variable	Carrizo citrange ²	Sour orange	Volkamer lemon	Troyer citrange	Swingle citrumelo	Ridge pineapple	Cleopatra mandarin	Rough lemon
Shoot dry weight								
Control	248 a ³ (0)	285 a (0)	248 a (0)	289 a (0)	262 a (0)	174 a (0)	123 a (0)	252 a (0)
NaCl	90 b (64)	141 b (51)	93 b (63)	123 b (56)	30 c (89)	85 b (51)	47 b (62)	50 c (80)
NaCl + Ca	102 b (59)	137 b (52)	82 b (67)	83 c (70)	97 b (63)	69 b (60)	55 b (55)	110 b (56)
Root dry weight								
Control	139 a (0)	146 a (0)	117 a (0)	201 a (0)	122 a (0)	71 a (0)	42 a (0)	113 a (0)
NaCl	42 b (70)	69 b (53)	40 b (66)	51 b (75)	21 c (83)	35 b (51)	18 b (57)	20 c (82)
NaCl + Ca	45 b (68)	65 b (56)	36 b (69)	30 c (85)	37 b (70)	28 b (61)	20 b (52)	37 b (67)

Numbers between parentheses are expressed as % lower than the control.

²Mean of 4 replicates.

³Mean separation within columns and variable by Duncan's multiple range test, 5% level.

trifoliata, Cleopatra mandarin, and Bakraie mandarin were stressed with polyethylene glycol (PEG), NaCl, or Na₂SO₄. Up to -0.50 MPa water potential, final germination for *P. trifoliata* was not affected, but the rate of germination was reduced with reduction in water potential. For Cleopatra and Bakraie mandarin, both final germination and germination rate were significantly reduced. Although reduced emergence in NaCl solutions appeared to be mostly due to osmotic effects, there was also evidence of a toxic effect of NaCl because the addition of Ca increased seedling emergence of some of the rootstocks.

In general, the delay in emergence or germination is more sensitive to salinity than the final emergence. However, this observation was not demonstrated for citrus rootstocks because the salinity levels used in this study were relatively high. For sorghum (Francois et al., 1984) and onion (Wannamaker and Pike, 1987) cultivars, within certain salt levels, no significant reduction in germination percentages occurred but germination was significantly retarded.

Emergence of the first seedling and emergence rate varied among rootstocks even in the no salt control treatment. Similar results were found earlier (Chilembwe et al., 1992). However, the ranking of the rootstocks based on speed of emergence showed little difference between the 2 studies. In this study, both SC and CC seedlings began to emerge at day 16 followed by SO at day 20 and CM at day 23. In Chilembwe et al. (1992) study, SC seedlings emerged relatively rapidly on day 10, CC and SO seedlings had a similar emergence pattern and began to emerge at day 15 and 16, respectively, and CM was the slowest to emerge on day 26. These differences in speed of emergence between the 2 studies could be attributed to temperature and relative humidity differences in the greenhouses and to differences in percent water content of the seeds.

It is known that salt tolerance is not a constant character in plants but varies with environment and plant development. Thus, relative tolerance during seed germination, seedling emergence, and later stages of plant development might differ. For citrus rootstocks, no uniform trend was found in the relationship between salt tolerance during emergence and during seedling growth. For CC, TC, and SC, seedling emergence was reduced by less than 30%, while total seedling biomass was reduced by over 65%. It can be concluded that these rootstocks are more salt-tolerant at emergence than at seedling growth. Based on the same reasoning, RP and CM are less salt-tolerant at emergence than at later growth stage. However, SO is relatively salt-tolerant, RL is salt-sensitive, and VL has intermediate salt-tolerance at both stages. The ranking of these rootstocks according to salinity tolerance is generally similar to that obtained from earlier studies conducted on 1-yr-old citrus rootstock seedlings (Zekri, 1987; Zekri and Parsons, 1989).

The addition of CaSO₄ to the saline solution was slightly beneficial to some rootstocks by reducing Na and/or Cl concentrations in the shoots and improving final emergence. In this study, the beneficial effect of CaSO₄ addition on growth or seedling biomass was not as well-defined as that in an earlier study with 8-month-old seedlings (Zekri and Parsons, 1990).

Calcium alleviation of adverse effects of salinity on germination, emergence, and early seedling growth has been also observed in *Diplachne fusca* (Myers and Morgan, 1989) and ryegrass (Marcar, 1986). It is thought that insufficient Ca in the germination medium aggravates salt effects through the membrane disruption leading to a faster accumulation of harmful salts and/or leakage of respiratory substrates (Marcar, 1986). Similar to citrus rootstocks, CaSO₄ did not improve seedling emergence of all tested triticale lines (Norlyn and Epstein, 1984). For cotton, the addition of Ca to a saline medium did not improve germination but offset the reduction in root growth caused by NaCl (Kent and Lauchli, 1985). The results of all these studies showed that the addition of Ca to NaCl solutions was not consistently beneficial to all crops, all cultivars within the same species, or all growth stages.

In conclusion, no consistent trend was found between salt tolerance at seedling emergence and salt tolerance at later growth stages of citrus rootstocks. Hence, salt tolerance at seedling emergence would not be a useful tool to select citrus rootstocks for salt tolerance.

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