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Salinity Can Enhance Freeze Tolerance of Citrus Rootstock Seedlings by Modifying Growth, Water Relations, and Mineral Nutrition

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Abstract. **Seedlings of 'Pineapple' sweet orange [*Citrus sinensis* (L.) Osbeck] (Swt), Cleopatra mandarin (*C. reshni* Hort ex Tan) (Cleo)], and trifoliate orange [*Poncirus trifoliata* (L.) Raf.] (Tri) were grown from seed for 10 months in 2-liter containers of native Candler fine sand in a glasshouse, watered two times per week, and fertilized weekly with a complete nutrient solution. NaCl at 0, 15, 30, or 60 mM was added to the watering solution for 2 additional months. Increases in salinity decreased hydraulic conductivity of roots, transpiration rate, leaf water potential, and root growth. The effect of salinity on mineral composition of tissues was rootstock-dependent. High salinity leaves of Tri had the highest N, K^+, and Cl^- but the lowest Na^+, whereas Tri roots had the highest Na^+ at the highest salinity. High-salinity Cleo leaves had the lowest Cl^- and K. All seedlings survived −4°C for 6 hr in a controlled freeze test. Salinity decreased leaf loss, except in the deciduous Tri, in which 60 mM NaCl may have been excessive. Thus, moderate salinity treatment can reduce growth and modify water and mineral nutrient relations so as to increase cold hardiness of certain *Citrus* species.**

Citrus rootstocks confer different vigor, cold hardiness (Peynado and Young, 1969), mineral nutrition characteristics, drought tolerance (Wutscher, 1979), and salinity tolerance (Cooper and Gorton, 1952; Wutscher et al., 1973) to the scion. In general, trees on vigorous rootstocks confer good drought tolerance and mineral nutrient accumulation characteristics, but tend to be cold-sensitive (Wutscher, 1979); presumably, because they are more physiologically active in winter. Trees on less-vigorous rootstocks, such as Cleopatra mandarin and sour orange, which have good Cl^--excluding characteristics, tend to be relatively cold hardy (Wutscher, 1979). The deciduous trifoliate orange (Tri) does not fit this generalization, however, since trees on Tri rootstock accumulate high levels of chloride, but can be very cold hardy in cool climates.

It is difficult to separate the combined effects of drought stress and mineral nutrition on growth and cold tolerance. Citrus cold tolerance can be reduced by debilitating environmental stresses such as chronic drought (Koo, 1981; Syvertsen and Smith, 1983), mineral nutrient deficiency (Koo, 1985), and salinity stress (Peynado and Young, 1969). Moderately cold temperatures (Yelenosky et al., 1984) and moderate drought stress (Wilcox et al., 1983; Yelenosky 1979a; 1979b), however, may increase cold hardiness (Cooper and Peynado, 1959). Any increase in cold hardiness is rootstock-dependent and may be, in part, caused by changes in tissue water and osmotic relationships in health trees, which suppress new growth (Krezdorn and Martsolf, 1984; Yelenosky et al., 1984).

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Salinity treatments applied to spinach leaves have been shown to induce cold hardiness by lowering osmotic potential (Schmidt et al., 1986). In citrus, short-term treatment with moderately salinized irrigation water can reduce vegetative growth and physiological activity (El-Gazzar et al., 1979) without leaf abscission (Hartmond et al., 1987). Such responses influence mineral ion (Hartmond et al., 1987) and soluble carbohydrate accumulation and can lower leaf osmotic potential (Lloyd et al., 1987; Syvertsen et al., 1988). For this reason, a study was designed to determine the effects of various levels of salinity treatment on plant water relations, mineral nutrition, and growth as they relate to cold hardiness in citrus rootstock species.

Materials and Methods

Uniform seedlings of 'Pineapple' sweet orange (Swt), Cleopatra mandarin (Cleo), and trifoliate orange (Tri) were grown in plastic 2-liter pots of previously autoclaved Candler fine sand soil in a well-ventilated glasshouse. Maximum photosynthetic photon flux was reduced by 50% shade cloth to 1000 $\mu mol \cdot s^{-1} m^{-2}$ under natural photoperiod and average air maxima/minima of 31°/20°C. Plants were watered twice per week and fertilized weekly with a complete nutrient solution totaling 200 mg N, P, and K per plant. All irrigations consisted of at least 250 ml of water, which was enough to thoroughly leach through the pot.

Salinity treatments were begun in August, when seedlings were 10 months old. Sodium chloride at 0, 15, 30, or 60 mM, with ECs of 0.01, 2.4, 4.2, and 7.1 $dS \cdot m^{-1}$, was added to the watering solutions. With added nutrients, these solutions had ECs of about 4.8, 7.5, 8.9, and 11.7 $dS \cdot m^{-1}$. In the 30- and 60-mM treatments, plants were exposed to salinity in increasing increments of 15-mM NaCl over a 10-day period to avoid osmotic shock. Salinity treatments continued for 8 weeks until plants were 1 year old. By this time, average daily air maxima/minima were 27°/15°C.

Six uniform plants of each rootstock from each salinity treatment were selected for transpiration studies. Well-watered and drained pots were weighed in the morning and again in the afternoon to determine daytime whole-plant evapotranspiration under glasshouse conditions. Average weight loss from soil evaporation was measured with six pots without plants and used as a daily correction factor. Transpiration (T) rates were calculated as water loss per leaf area over four 5- to 7-hr daytime periods. Total leaf area per plant was determined at harvest with a LI-COR leaf area meter.

Leaf osmotic potential (π) was measured with pressure/volume techniques (Lloyd et al., 1987) on a single mature leaf from four of the same plants used for measuring T. Plants were well-watered and brought into the laboratory the night before to minimize leaf water deficits. Maximum leaf water potential (ψ) was measured in the morning with a pressure chamber. Leaf proline concentration ($mg \cdot g^{-1}$ dry weight) was evaluated as an indicator of plant stress and was determined colorimetrically with acid ninhydrin (Syvertsen and Smith, 1983) on two 1-cm-diameter disks from a different leaf of the four plants from each treatment.

Shoots were removed from the same four 1-year-old plants and the hydraulic conductivity of roots (G_r) was determined on intact root systems in a pressure chamber (Syvertsen and Graham, 1985). The pressure was raised to either 0.3 MPa or 0.5 MPa (depending on salinity treatment), exudation rates were allowed to equilibrate for 15 min, and the exudate was collected over at least three 1-min periods. Total fibrous root (<2 mm in diameter) length was determined by line intercept (Tennant, 1975) and used to express G_r in $\mu g\ H_2O/m$ per s per MPa. Plants were separated into taproot, fibrous roots, stems, and leaves. After drying for at least 48 hr at 60°C, each component was weighed and ground in a Wiley mill.

Total tissue N was determined by semi-micro Kjeldahl and total chlorides by silver ion titration with a Buchler–Cotlove chloridometer. Tissue samples were digested with perchloric–nitric acid. Phosphorus was determined by using the vanadate molybdate-yellow method (Walsh, 1971); Na^+, K^+, and Ca^{++} concentrations were determined by flame emission spectrometry.

In November, when plants were ≈13 months old, four uniform seedlings of each species from each salinity treatment were subjected to a controlled freeze in facilities previously described (Yelenosky, 1978). The freeze test started at 4°C for 2 hr, followed by a 1° per hr decrease to −4° for 6 hr, and then returned to 4° at 1° per hr. These temperatures and durations were selected based on previous experience in an attempt to challenge the experimental plants with nonlethal freezing temperatures (Yelenosky, 1978). Potential differences in supercooling of leaves were avoided by spraying them with water to induce freezing when air temperature reached 0°. Plants were kept at 20° for 3 hr following the freeze test. The total number of leaves and total stem length per plant were recorded and plants were returned to the glasshouse for 2 weeks of observation of freeze injury. Sodium chloride treatments were discontinued during freeze recovery and seedlings were rated on percentage of leaves and length of stem killed.

All data were analyzed with a 3 × 4 factorial analysis of variance with the three species and four salinity levels as main effects. Transpiration was measured on six plants per treatment; n = 4 for all other data. Linear regression analysis was used to test for significant ($P < 0.05$) correlations between salinity treatments and growth characteristics, water relations, and mineral nutrient data.

Results

Overall, total root dry weight (DW) was decreased significantly by increased salinization, whereas shoot DW was not (Fig. 1). Cleo had the highest shoot : root (Sh:Rt) ratio of the three species, but salinity had little affect on Sh:Rt. Total length of fibrous roots per plant generally decreased in proportion to decreases in root DW; consequently, fibrous root length : DW ratio was not affected by salinity (data not shown).

Swt had the highest G_r and Tri the lowest in the absence of added salinity (Table 1). Both G_r and T were greatly reduced by salinity in all three rootstocks. Maximum ψ indicated that the 60-mM-treated plants suffered salinity-induced water deficit stress. Leaf osmotic potentials of plants in the 0-mM NaCl treatment were low and did not decrease with increasing salinity in Swt and Cleo, but did increase significantly with salinity in Tri. Leaf proline content was not correlated with salinity treatments.

Foliar Na^+ and Cl^- were positively correlated with salinity in all species (Fig. 2; Table 2). Although the Na^+ concentration of fibrous roots of 60-mM-treated Tri was high, Na^+ apparently was not transported to the leaves, as Tri maintained relatively low concentrations of foliar Na^+. Sodium concentrations in taproots and stems did not differ among rootstocks. Foliar and fibrous root Cl^- concentrations were highest in salinized Tri. Cleo leaves maintained Cl^- levels below those considered to be toxic ($7.0\ mg \cdot g^{-1}$) for bearing citrus (Smith, 1966), even at the highest salinity. There was no abcission or visible leaf burn symptoms in any treatment. Foliar K^+ levels generally remained well above the $7.0\ mg \cdot g^{-1}$ considered sufficient (Smith, 1966). Potassium concentration decreased in all tissues with increased salinity except in Tri leaves.

The concentrations of Na^+ and K^+ in each plant component (Fig. 2) were multiplied by the respective DW of each component to determine if patterns of total ion contents and distri-

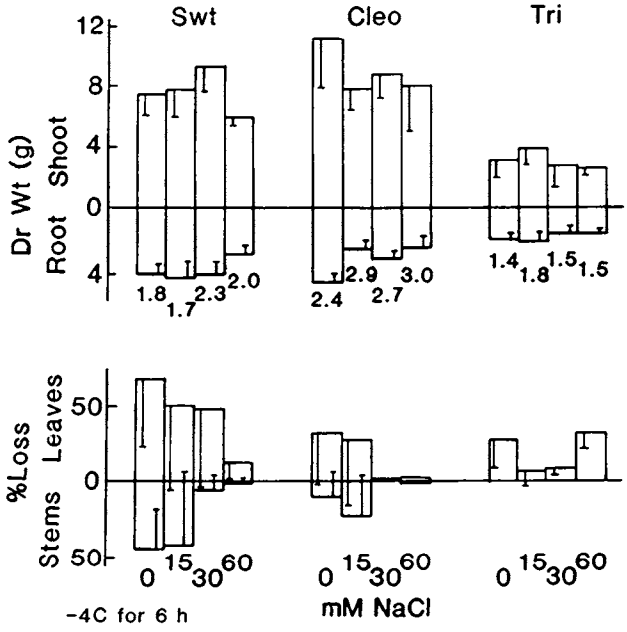

Fig. 1. Effects of added salinity (0, 15, 30, or 60 mM NaCl) on mean (n = 4 ± 1 SD) shoot and root dry weight and freeze (−4°C for 6 hr) injury of leaves and stems of 12- to 13-month-old 'Pineapple' sweet orange (Swt), Cleopatra mandarin (Cleo), and Trifoliate orange (Tri) seedlings. Shoot : root ratios are listed below the root dry weight bars.

Table 1. The effect of added salinity (0, 15, 30, or 60 mM NaCl) on mean (±1 SD) hydraulic conductivity of roots (G_r), whole plant transpiration rate (T), maximum leaf water potential (ψ), leaf osmotic potential (π), and proline content of 1-year-old 'Pineapple' sweet orange, Cleopatra mandarin, and Trifoliate orange seedlings.

Species	NaCl (mM)	G_r (μg per m per s per MPa)	T ($mg \cdot s^{-1} \cdot m^{-2}$)	ψ (MPa)	π (MPa)	Proline ($mg \cdot g^{-1}$ dry wt)
Sweet orange (Swt)	0	33.8 ± 18.8	50.6 ± 22.8	−0.46 ± 0.17	−2.25 ± 0.16	15.3 ± 5.2
	15	10.3 ± 4.4	---	−0.40 ± 0.13	−2.33 ± 0.16	9.9 ± 3.6
	30	8.2 ± 3.3	22.9 ± 8.4	−0.44 ± 0.06	−2.32 ± 0.09	17.1 ± 3.3
	60	7.4 ± 2.3	23.5 ± 6.1	−0.65 ± 0.31	−2.43 ± 0.17	19.6 ± 3.6
r, NaCl		−[z]	−	NS	NS	+
Cleopatra mandarin (Cleo)	0	21.3 ± 5.4	26.1 ± 5.0	−0.38 ± 0.06	−2.27 ± 0.18	30.6 ± 4.3
	15	14.4 ± 4.7	---	−0.48 ± 0.17	−2.13 ± 0.13	25.8 ± 4.5
	30	7.4 ± 1.9	18.2 ± 5.8	−0.62 ± 0.21	−2.30 ± 0.09	25.4 ± 8.8
	60	6.7 ± 3.5	8.4 ± 4.7	−0.78 ± 0.32	−2.24 ± 0.48	32.5 ± 3.3
r, NaCl		−	−	−	NS	NS
Trifoliate orange (Tri)	0	10.0 ± 1.9	26.9 ± 16.2	−0.48 ± 0.07	−2.49 ± 0.08	16.9 ± 3.3
	15	5.6 ± 1.2	---	−0.50 ± 0.06	−2.05 ± 0.23	18.9 ± 6.8
	30	3.0 ± 0.9	19.2 ± 13.3	−0.56 ± 0.06	−1.85 ± 0.27	14.5 ± 4.6
	60	0.6 ± 1.3	15.4 ± 15.5	−0.73 ± 0.09	−1.84 ± 0.14	14.2 ± 3.3
r, NaCl		−	NS	−	+	NS
Main effects						
Species		**	**	NS	**	**
Salinity		**	**	**	NS	NS
Species × salinity		*	NS	NS	**	**

[z]Significant ($P < 0.05$) correlation coefficients with level of NaCl (*r*, NaCl) are indicated with their sign, + or −, or as nonsignificant (NS).

NS, *, **Nonsignificant or significant at the 5% or 1% levels, respectively.

butions differed from patterns of ion concentrations. In general, patterns of total ion content and ion concentrations were similar. For example, the highest salinity did not increase the fractional amount of Na^+ in the leaf component of Tri seedlings, despite the more than 10-fold increase in total Na^+ per plant (Fig. 3). Furthermore, there was a significant increase in the total Na^+ in fibrous root component of 60-mM NaCl-treated Tri seedlings that was similar to the increase in Na^+ expressed on a concentration basis. Total K^+ per plant was decreased by salinity in Swt and Cleo seedlings (data not shown). However, total K^+ was not significantly affected by salinity treatment in Tri seedlings, nor were there any differences the fractional contributions of K^+ in the various plant components in response to salinity (Fig. 3).

Foliar N and P concentrations were high relative to optimum levels for bearing trees (22 and 0.9 $mg \cdot g^{-1}$, respectively) (Smith, 1966), and Tri leaves had the highest N concentration (Fig. 4). Salinity decreased foliar N in Cleo (Table 2), while tending to increase N in fibrous and taproot tissues (Fig. 4). There was little difference in P concentration across rootstocks, tissues, or salinities. Foliar Ca^{++} concentrations were below those considered sufficient for bearing trees (15 $mg \cdot g^{-1}$, Smith, 1966) and Ca^{++} decreased further with salinity in Cleo leaves (Table 2).

All rootstocks tested survived −4°C for 6 hr regardless of salinity treatment. Although plant-to-plant variation was high, the percentage of leaves killed by the freeze test on Cleo was decreased by salinization (Fig. 1). Tri showed no pattern of leaf loss with salinity, but had virtually no stem loss after the freeze. Stem loss from freezing was decreased by salinity in Swt. When data from Swt and Cleo were pooled (n = 32), there was a significant ($P < 0.05$) decrease in percentage leaf ($r = -0.42$) and stem ($r = -0.45$) loss with increased salinity.

Discussion

We chose levels of salinity and duration of treatment that reduced growth while avoiding excessive stress. Salinity stress reduced growth of new root mass, but apparently did not affect root thickness. In Cleo and Tri, significant increases in leaf dry wt : leaf area with salinity may have partially compensated for decreases in total leaf area (data not shown), thereby resulting in small changes in total shoot DW.

Overall, high foliar N, P, and K^+ contents were a function of the high level of fertilization that was used to avoid potential nutrient deficiencies under salinity stress. Although ψ decreased with salinity, π did not, despite increases in foliar Na^+ and Cl^-. Relative to previous studies, π was low (Lloyd et al., 1987; Yelenosky, 1978) and leaf proline concentrations were high in the nonsalinized control plants, even in comparison with cold-hardened citrus seedlings (Yelenosky 1979a). Thus, we may have subjected the control plants to some osmotic stress from the weekly nutrient applications even though each application was followed by a water irrigation. Such an osmotic stress of the nonsalinized plants would account for the lack of correlations of shoot dry weight, π, and proline with salinity.

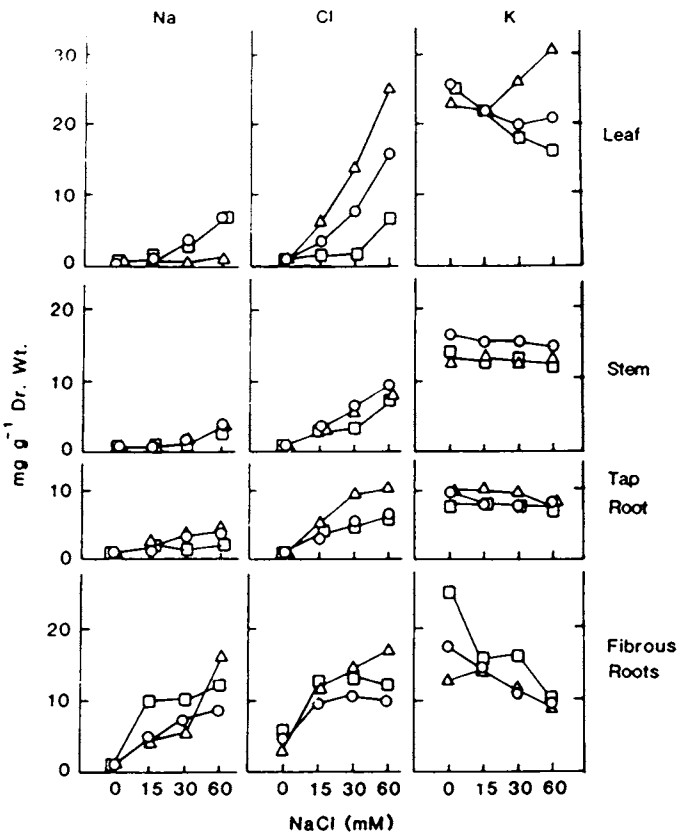

Fig. 2. Effects of added salinity (0, 15, 30, or 60 mM NaCl) on Na, Cl, and K concentration of leaf, stem, tap root, and fibrous root tissues of 1-year-old 'Pineapple' sweet orange (-○-), Cleopatra mandarin (- □ -), and Trifoliate orange (-△-) rootstock seedlings. Each symbol represents the mean of four replicate samples; SDs were generally <10% of the mean. Linear correlation coefficients for foliar values are listed in Table 2.

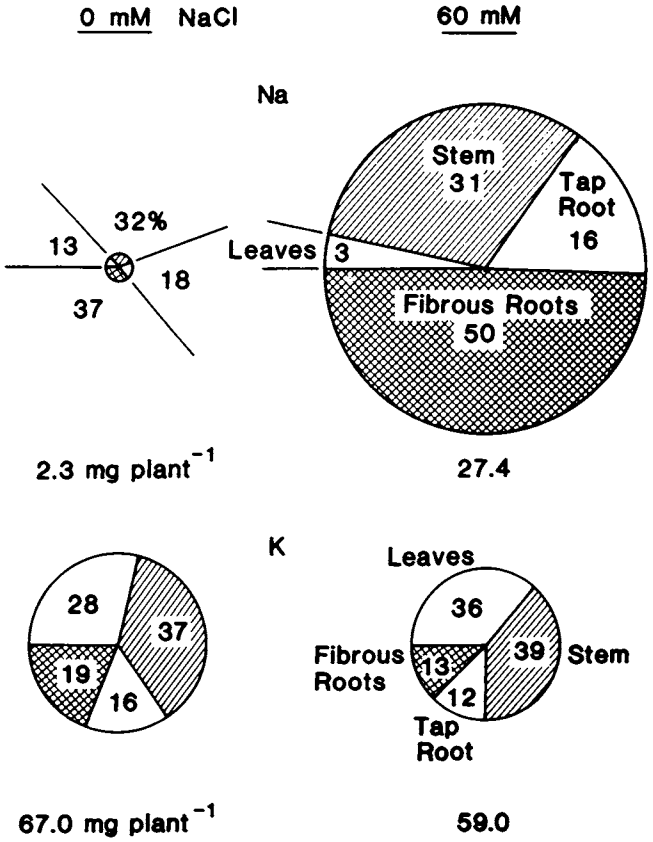

Fig. 3. Mean (n = 4) total Na^+ and K^+ content (mg/plant) of 1-year-old Trifoliate orange seedlings treated with 0 or 60 mM NaCl. Within an ion, total ion content is proportional to the area of the circles. Numbers within pie segments (%) refer to the fractional contribution of each plant component to the total ion content.

Table 2. Linear correlation coefficients of salinity level vs. mineral element content of leaves of 'Pineapple' sweet orange (Swt), Cleopatra mandarin (Cleo), and Trifoliate orange (Tri) seedlings.

	Rootstock		
Element	Swt	Cleo	Tri
Na	0.96**	0.91**	0.70**
Cl	0.98**	0.88**	0.94**
N	-0.23	-0.53*	-0.29
P	-0.44	-0.55*	0.14
K	-0.60*	-0.87**	0.53*
Ca	0.06	-0.51*	-0.14

*, **Significantly different from zero at the 5% and 1% levels, respectively.

Nonetheless, salinized plants did have lower G_r and T than nonsalinized plants. Freeze survival data of Swt and Cleo, therefore, support the hypothesis that reduced water loss enhanced cold tolerance. An older average leaf age with less physiological activity in the slower-growing salinized plants may have also contributed to their overall enhanced cold hardiness.

Salinity-induced reductions in transpiration and root conductivity probably reduced uptake and transport of N to the leaves (Syvertsen and Graham, 1985). Leaf Ca^{++} concentrations were quite low and may have affected salinity responses via high Na:Ca ratio affecting membrane permeability in leaves and fibrous roots (Greenway and Munns, 1980). Results of Na^+ and Cl^- accumulation in leaves of these species, however, support previous work describing the Cl^- exclusion ability of Cleo (Lloyd et al., 1987; Walker, 1986; Walker et al., 1983) and Cl accumulation/Na^+ exclusion ability of Tri (Walker, 1986). It is interesting to note that the Na^+ exclusion ability of Tri roots was exceeded by 60 mM of NaCl over the 2-month treatment period, but high concentrations of Na^+ were not transported to leaves. Similar to the results of Grieve and Walker (1983), this observation implies Tri has the ability to retain high Na^+ concentrations in root tissues under salinity stress.

The relatively low rates of G_r and T of nonsalinized Tri are contrary to rates measured in earlier seedling studies (Syvertsen and Graham, 1985) and also in studies of mature trees of T in the field (Sinclair and Allen, 1982), where trees on Tri rootstock had higher rates than other rootstocks. Furthermore, foliar K^+ concentration (Walker and Douglas, 1983) and π (Lloyd et al., 1987), which are typically reduced by increased levels of NaCl, were increased in Tri leaves by salinization. The increase K^+ concentration, however, must have been a function of reduced growth rates in salinized Tri since there was no change in total K^+ per plant (Fig. 3). An accumulation of organic solutes (Binzel et al., 1987) may have contributed to changes in osmotic potential. It is possible that the deciduous Tri was becoming quiescent during the end of these studies (November), which may account for its anomolous π responses to salinity.

The idea of using a moderate environmental stress to reduce growth and induce tolerance to a subsequent stress is not new

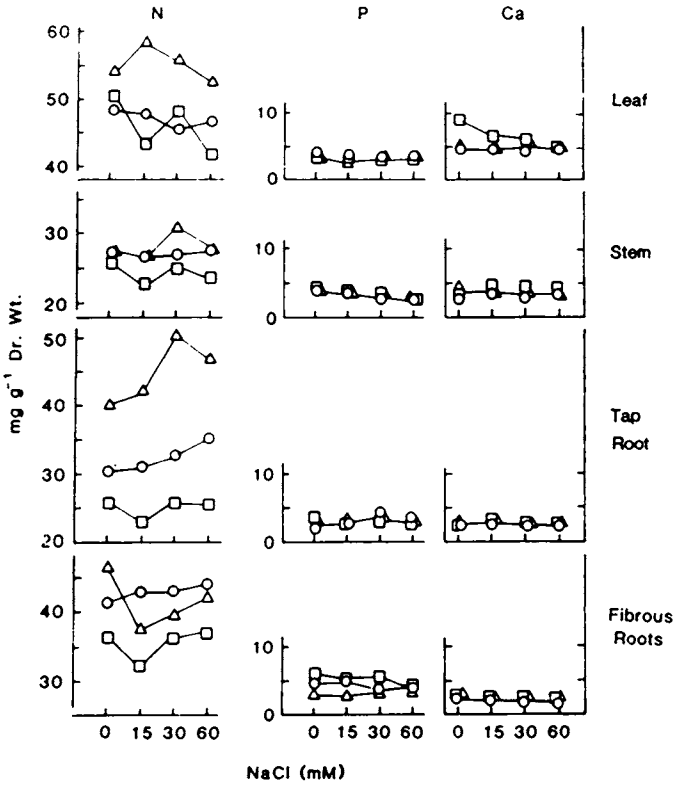

Fig. 4. The effect of added salinity (0, 15, 30, or 60 mM NaCl) on N, P, and Ca concentration of leaf, stem, tap root, and fibrous root tissues of 1-year-old 'Pineapple' sweet orange (-○-), Cleopatra mandarin (- □ -) and Trifoliate orange (-△-) rootstock seedlings. Each symbol represents the mean of four replicate samples; SDs were generally <10% of the mean. Linear correlation coefficients for foliar values are listed in Table 2.

(Yelenosky, 1978; Yelenosky, 1979a, 1979b). It appears that about 30 to 60 mM of NaCl over a 2-month period not only reduced growth and affects water relations and mineral nutrition characteristics, but also can enhance cold hardiness in some species. A greater understanding of the relationships between cold hardiness and stress-induced reductions in growth and water use could lead to practical methods of enhancing cold hardiness in citrus.

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