Pak. J. Bot., **48(1): 67-74, 2016.**

CITRIC ACID IN THE NUTRIENT SOLUTION INCREASES THE MINERAL ABSORPTION IN POTTED TOMATO GROWN IN CALCAREOUS SOIL

FABIÁN PÉREZ-LABRADA, ADALBERTO BENAVIDES-MENDOZA*, LUIS ALONSO VALDEZ-AGUILAR AND VALENTÍN ROBLEDO-TORRES

Universidad Autónoma Agraria Antonio Narro, Departamento de Horticultura, Calzada Antonio Narro 1923, Saltillo, Coah., México, C.P. 25315
**Corresponding author's email: abenmen@gmail.com*

Abstract

Calcareous soils have low availability of mineral nutrients that are essential for crops. The aim of the study was to determine the effect of citric acid (CA) applied in a nutrient solution on the composition of the pore water of a calcareous soil and its correlation with the chemical composition and quality of the tomato plants and fruits. The experimental design was a randomized block; the treatments were different concentrations of CA added to the Steiner nutrient solution. We used CA at 10^{-2}, 10^{-4} and 10^{-6} M and a test treatment without CA. A decrease in the electrical conductivity (EC) and oxidation-reduction potential (ORP) was observed in soil pore water (SPW), as well as changes in the concentrations of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} in response to the addition of CA. The concentrations of Zn, Na, Ca, and N increased in leaves, whereas in the fruit, the concentrations of Mn, Na, Mg, and P increased. Significant correlations arise between Na^{+} and Mg^{2+} (R=0.60), Na^{+} and Ca^{2+} (R=0.68), and Mg^{2+} and Ca^{2+} (R=0.76) in the soil pore water, as well as a significant negative correlation (R=-0.73) between the concentration of K^{+} in the soil pore water and the concentration of Mg in stems and leaves. No relationship arises between the mineral content in the fruits and the mineral composition of the SPW. The addition of CA changed the chemical composition of the soil pore water and plant tissues. CA at 10^{-6} M increased the fruit production per plant significantly.

Key words: Calcareous soil, Nutrient, Citric acid, Tomato

Introduction

Calcareous soils present high levels of free $CaCO_3$, a high pH and elevated electrical conductivity, in addition to large amounts of soluble salts (Rivera *et al.*, 2008), resulting in the reduced availability of essential mineral nutrients such as Zn, Cu, Mn, Fe, B, and P that are necessary for the growth and development of crops (Arizmendi *et al.*, 2011). To ameliorate such deficiencies, different soil management techniques have been proposed to increase the solubility and availability of mineral nutrients. These techniques include the application of chelates (Xi-wen *et al.*, 2011), elemental sulfur (Sierra *et al.*, 2007), the use of strong acids (Lee *et al.*, 1998), and as an alternative, the exogenous addition of organic acids such as citric acid (CA) (Ferreyra *et al.*, 1998).

Organic acids are produced by different biosynthetic pathways in plants and are involved in various metabolic activities such as energy storage, the biosynthesis of amino acids (López *et al.*, 2000), stomatal functioning (Wang & Blatt, 2011), ion balance and the transport of compounds from the vacuole and the mitochondria to the cytoplasm (Çali□kan, 2000). These organic acids (i.e., citric, oxalic, malic) are exuded by the roots of the plant and, according to Taghipour & Jalali (2013), are present in the soil at different concentrations ranging from 10^{-3} to 10^{-5} M depending on the type of soil. The acids play a significant role in the response to nutrient deficiencies (Dakora & Phillips, 2002), mobilizing some nutrients such as Ca^{2+}, Fe^{3+}, K^{+}, Na^{+}, Mg^{2+} and PO_4^{-3} in calcareous soils (Wang *et al*., 2008). The increased availability of nutrients results both from the supply of H^{+} that acidifies the rhizosphere and soil pore water (Zhu *et al.*, 2005) and from the formation of soluble cation-organic acid complexes, which provides a direct route for absorption in addition to protecting their precipitation (Ryan *et al.*, 2001; Ström *et al.*, 2005).

Citric acid is one of the most common organic acids, and in its deprotonated form, makes complexes with elements present in the soil such as Al, Fe, Mn, and Cu (Campbell, 2010). Root cells exude citric acid; the exudation occurs in response to damage caused by Al in acid soils or under Fe or P deficiency in alkaline soils (Neumann *et al.*, 2000; Hu *et al.*, 2005; Shlizerman *et al.*, 2007). In the latter case, the element is mobilized (Wang *et al.*, 2008) due to the complexation action of CA and its ability to supply protons to the soil solution (Jones & Darrah, 1994). For Fe, the presence of citric acid induces the dissolution of insoluble ferric oxyhydroxide (Sánchez-Rodríguez *et al.*, 2014).

The exudation of CA is involved in many root processes. However, the sorption to the mineral phase and the mineralization by microorganisms cause the concentration in the rhizosphere to remain variable (Jones, 1998). For that reason, many researchers aim to raise the concentration of organic acids present in both plant tissues and the soil solution (López e*t al.*, 2000), either by increasing the photosynthetic activity and transport of photosynthates to the roots (Watt & Evans, 1999) or by the exogenous applications of these acids (Palomo *et al.*, 2006).

Some works reported the use of citric acid, i.e., Sánchez-Rodríguez *et al*. (2014) cited that a mixture of organic acids (oxalic, citric and malic acid) at concentration 10 mM decrease iron chlorosis on calcareous soils in chickpea. Likewise, Ström *et al*. (2005) report the efficiency of nutrient extraction through the application of citric acid (10 mM).

Al-Bahrany & Al-Shabaan (1995) reported that exogenous application of CA changed the availability of mineral nutrients in calcareous soils, thus increasing the absorption of Ca^{+2}, K^{+}, Mg^{+2}, Fe^{+3} and Zn^{+2} in tomato plants, but they did not report the behavior of minerals in the soil pore water and the probable correlations that it could present with plant tissue. Hence, it is possible that the addition of CA to calcareous soil could modify the chemical characteristics of the soil pore water and its ionic composition and, consequently, induce changes in the elemental composition and concentrations in leaf tissue and fruit. Therefore, the first aim of this study was to determine the effect of the addition of CA on the chemical characteristics and the concentration of anions and cations in the soil pore water. The second aim was to verify the correlation between the concentrations of different elements in the soil solution and their contents in the plants, as well as to determine the impact of CA on fruit production.

Materials and Methods

The study was carried out at the Universidad Autónoma Agraria Antonio Narro, located in Buenavista, Saltillo, Coahuila, Mexico (25° 22' N and 101° 00' W, 1760 m). The climate of the region corresponds to the BWhw (x') (e) type: very dry, semi-cool, winter extremes, rain in summer and winter precipitation exceeding 10% of the annual total. The biological material used was tomato (*Lycopersicon esculentum* L. [Mill.]) cv. Rio Grande, cultivated in a greenhouse with 70% natural irradiance, a temperature range of 20-30ºC and 50-60% relative humidity. Transplantation was performed using 30-day old seedlings in black polyethylene containers of 35 cm diameter and 32 cm height filled with 17 kg of Calcisol soil: calcareous clay loam type, non-saline, pH = 8.7 and with an available mineral concentration of 4.29 ppm of phosphorus, 5.20 ppm of iron, 0.39 ppm of copper, 0.71 ppm of boron, 2.20 ppm of sulfur, 6.01 ppm of zinc, 7.70 ppm of manganese, 27.90 ppm of potassium, 439.25 ppm of magnesium and 3066.25 ppm of calcium (Anon., 2002). The containers were placed on leveled soil, which was covered with black polyethylene, thus preventing weed growth and the roots from outgrowing the container.

The experiment was set up in a randomized complete block design with four treatments and four replicates per treatment. The experimental unit was a 1-plant container. Plants were irrigated with Steiner's nutrient solution (Steiner, 1961) at different concentrations (25%, 50%, 75% and 100%) according to the age of the plants. Treatments consisted of Steiner's solution with the addition of food grade citric acid (CA) ($C_6H_8O_7$) (99.97 % purity) at different concentrations of 10^{-2}, 10^{-4} and 10^{-6} M (1.92, 0.0192 and 0.000192 g L^{-1}, respectively), and a test treatment consisting of Steiner's nutrient solution with no CA. Lower, intermediate and higher concentrations were selected after taking into consideration the ranges cited by Taghipour & Jalali (2013). Treatments were prepared by dissolving the CA in Steiner's nutrient solution at a pH of 6.0, resulting in a pH of 3.06, 6.35, 6.39 and 6.30 in concentrations 10^{-2}, 10^{-4}, 10^{-6} M and test, respectively. Treatment applications started immediately after transplantation through the irrigation system and continued for the duration of the experiment. The irrigation volume varied according to the age of the plants, daily dispensing ~2.4 L per plant in the stages with the highest evapotranspiration rate.

Soil pore water (SPW) was collected by suction lysimeters with a ceramic cup placed at a depth of 15 cm and using an extraction pressure of 50 kPa. Lysimeters were placed in an intermediate position after planting, between the edge of the container and the plant stem. There were six lysimeters per treatment (24 total lysimeters). SPW was collected weekly and the electrical conductivity (EC) (HI98130, Hanna Instruments), pH and oxidation-reduction potential (ORP) (pH/mV/ISE meter, HI98185, Hanna Instruments) were measured *in situ*. Additional SPW samples were obtained at 71, 83 and 119 days after transplantation (DAT) to determine the mineral concentration of the SPW (Fertilab, 2011). In this case, the six SPW samples of each treatment were mixed to form a composite sample.

The mineral content of the leaves and stems on different sets of plants (at 71, 83 and 119 DAT) and fruit (at 119 DAT) was determined for acid digested samples (perchloric acid: nitric acid, 1:3) using an atomic absorption spectrophotometer (VARIAN AA 1275) (Jones & Case, 1990). Phosphorus was assessed using a colorimetric method (Olsen e*t al*., 1954), while the total N was determined by a micro Kjeldahl's procedure (Muller, 1961).

Fruit production was evaluated as the sum of the total weight of fruits per plant of three harvests made at 119, 121 and 125 DAT. The number of fruits per plant, weight, and diameter of all harvested fruits were also recorded.

SPW data were analyzed using a repeated-measures multivariate analysis of variance (RM-MANVA) followed by multiple means comparison tests using the Fisher Least Squares Difference (LSD) with the program STATISTICA 7.0 (Hill & Lewicki, 2007). The correlations between the concentrations of minerals in the SPW sample and minerals in the leaves and fruits were calculated using Spearman's rank order correlation (Spearman's R). Plant and fruit data were analyzed using a one-way ANOVA and a multiple means comparison test using the Fisher Least Squares Difference (LSD) with the SAS software (Anon., 2002).

Results and Discussion

Chemical properties of the soil pore water: According to the RM-MANVA the chemical properties of the SPW showed highly significant differences ($p\leq0.05$) between treatments.

Electrical conductivity: According to the RM-MANVA, there were statistically significant differences ($p\leq0.05$) among treatments in the EC of the soil pore water (SPW) (Fig. 1). The electrical conductivity of the SPW was very similar in all treatments over the experiment duration. In comparison with the control treatment, a noticeable decrease in EC was observed at 39 DAT (Fig. 1); however, there was a remarkable increase starting at 73 DAT, which was associated with the beginning of the flowering stage of the plants. The change observed at 52 DAT was coincident with the rise in the concentration of the Steiner solution, which was applied to the plants.

In all probability, the observed dynamic response of the EC depended on the concentration of the nutrient solution applied (Bosch *et al*., 2012), since, as the plants grew, they required a greater amount of irrigation water and a more concentrated nutrient solution. An alternative explanation is that, under constant irrigation, the plants exhibited an increased transpiration rate, causing an accumulation of salts in the root zone (Ferreyra *et al*., 1998). The control treatment showed a consistently higher EC compared to treatments with CA applications, probably due to the formation of complexes between CA with cations present in the SPW and the movement of anions from the SPW (Sagoe e*t al.*, 1998) towards the roots, thereby reducing the amount of ions present in the SPW.

pH: Citric acid treatments significantly ($p \leq 0.05$) affected the pH of the SPW (Fig. 2) according to the RM-MANVA. On average, a lower pH was obtained with CA at 10^{-4} M, whereas the other two concentrations were no different from the control. As with the EC, the pH showed very similar behavior among the treatments.

After transplantation, there was a steady increase in the pH of the SPW in all treatments (Fig. 2), with higher values in the control treatment. This trend changed by 52 DAT and the pH started to decrease, reaching values near to the initial baseline at 73 DAT, coincident with the onset of plant flowering. Subsequently, the pH increased, reaching a new peak at approximately 88 DAT.

The differences among treatments and the variation of pH over time are not explained by the ability of CA to yield H^+ to the SPW. Strobel (2001) and Liu *et al*. (2013) reported a relationship between the presence of organic acids in soil (under concentrations of 0-50 µM) and pH, but in the current experiment, this was not observed as CA at 10^{-4} M caused the largest decline in the pH. The same concept applies to the oscillating dynamic behavior, which is evident in Fig. 2. The values of pH observed could be the result of alterations in the relative absorption of anions versus cations, which could change the pH of the SPW through the action of root cells (Haynes, 1990; Thibaud *et al.*, 1994).

Oxidation-reduction potential: Citric acid at 10^{-2} M induced the highest values of the ORP in the lysimeter-collected SPW, followed by the concentration of 10^{-6} M, while treatment with 10^{-4} M of CA showed a very similar value to that of the control (Fig. 3). In consideration of their dynamic behavior, this ORP showed an oscillatory behavior during the growing season, ranging between +100 and +260 mV and showing the highest average values between 39 and 81 DAT (Fig. 3).

According to Liu ***et al.*** (2013), the main influence of organic acids (mainly pyruvic, oxalic and citric) on the value of the ORP is that they act on organic ligands, leading to the formation of complexes of Fe (II) and Fe (III). Another factor that could explain the fluctuation of the ORP in the lysimeter-collected SPW is the variation in the water content of the soil (Pezeshki & DeLaune, 2012), but this possibility does not seem applicable to the present case because the water content remained relatively constant in the plants due to the irrigation system used.

The Spearman correlation analysis of the variables of the SPW samples indicated that the EC, pH and ORP showed a statistically significant positive correlation (Table 1). The highest correlation coefficient was between the EC and the ORP in the test treatment (R = 0.495). However, the 10^{-2} and 10^{-6} M treatments of CA showed a higher correlation between the pH and the ORP (R = 0.356 and R = 0.306, respectively) and between the variables pH and EC (R=0.361) under the concentration 10^{-6} M of CA.

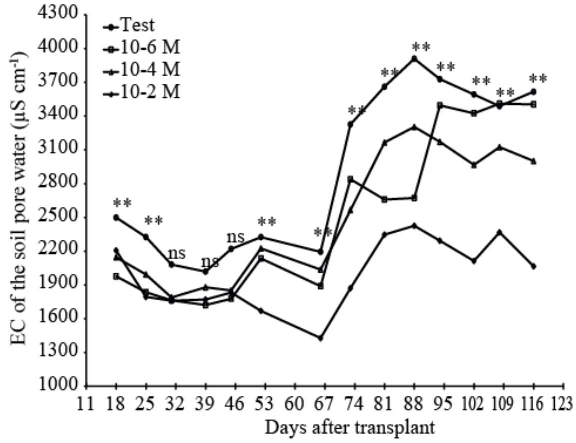

Fig. 1. Electrical conductivity dynamics determined in the samples of pore water of a calcareous soil in which different concentrations of citric acid were added to the nutrient solution. †Means with different letters in the same columns are significantly different (LSD, α ≤0.05). ns = not significant; ** = significant at p≤0.05.

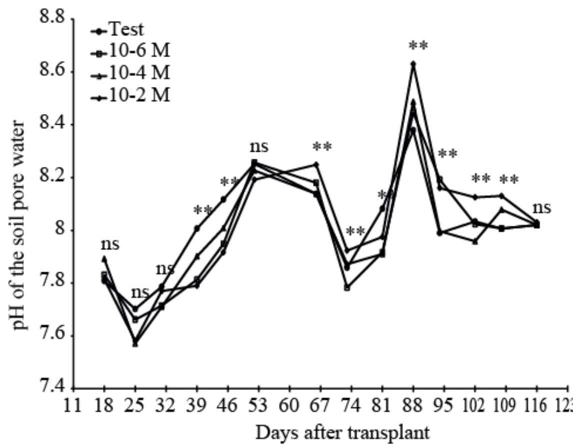

Fig. 2. Dynamics of the pH determined in the samples of pore water of a calcareous soil in which citric acid was added to a nutrient solution at different concentrations. †Means with different letters in the same columns are significantly different (LSD, α ≤ 0.05). ns = not significant; ** = significant at p≤0.05.

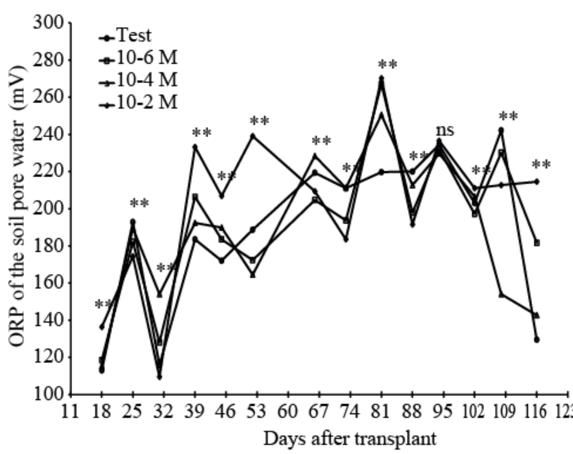

Fig. 3. Oxidation-reduction potential (ORP) dynamics determined in the samples of pore water of a calcareous soil in which citric acid was added to a nutrient solution at different concentrations. †Means with different letters in the same columns are significantly different (LSD, α ≤ 0.05). ns = not significant; ** = significant at p≤0.05.

Table 1. Spearman's matrix correlation (R) between the chemical properties of the samples of soil pore water.

Parameters	Test			CA 10^{-6} M		
	ORP	EC	pH	ORP	EC	pH
ORP	1			1		
EC	0.495**	1		0.465**	1	
pH	0.285**	0.151 ns	1	0.306**	0.316**	1
	CA 10^{-4} M			CA 10^{-2} M		
ORP	1			1		
EC	0.318**	1		0.155 ns	1	
pH	0.109	0.274**	1	0.356**	0.086 ns	1

ns = not significant, ** = significant at $p \leq 0.05$

Chemical characteristics and mineral concentrations in the soil solution: The results showed a reduction in the concentrations of Ca^{2+}, Mg^{2+}, SO_4^{2-} and Cl^- in the SPW with the application of CA. The lowest values were observed with 10^{-2} M of CA, except for Cl^-, where treatment with 10^{-4} M of CA showed the lowest values (Table 2). High values of SO_4^{2-} are possibly explained by the use of small amounts of sulfuric acid to acidify the irrigation water. The lower concentrations of the minerals in the soil pore water in response to the addition of CA may be a result of the higher solubility and availability facilitated by the exogenous application of the organic acid and by the formation of compounds of CA with the soil salts (Dakora & Phillips, 2002; Campbell, 2010; Najeeb *et al.,* 2011), both processes which result in the improvement of the absorption of nutrients by plants. The overall result was a lower concentration of certain elements in the SPW. The variability in the behavior shown by the elements could indicate a response dependent on an optimal concentration of CA, as demonstrated by Hu *et al*. (2005) and Palomo *et al*. (2006).

When comparing the concentrations of cations and anions present in the SPW of a calcareous soil with those reported by Román *et al.* (2002) in a fluvisol soil, it was observed that Ca^{2+}, Na^+, Cl^- and HCO_3^- were lower, while the concentrations of K^+ and CO_3^{2-} were above the values reported by those authors, whereas Mg^{2+} and SO_4^{2-} were very similar. Such levels will depend on the composition of each soil studied.

Table 2. Chemical characteristics and concentrations of mineral elements in the samples of pore water of a calcareous soil where citric acid was added at different concentrations to the nutrient solution. Data are the average and standard error of three samples at 77, 83 and 119 days after transplant.

Variable	Treatments			
	Test	10^{-6}M	10^{-4}M	10^{-2}M
Ca^{2+}	486.09 ± 87.40	457.45 ± 112.48	364.35 ± 55.15	211.38 ± 12.40
Mg^{2+}	144.72 ± 33.56	113.46 ± 8.27	115.41 ± 6.82	81.08 ± 2.51
Na^+	87.05 ± 0.41	88.27 ± 3.34	90.72 ± 1.78	84.19 ± 0.71
K^+	43.15 ± 1.48	45.68 ± 1.13	45.68 ± 1.57	42.96 ± 1.37
HCO_3^-	136.67 ± 9.86	155.37 ± 11.30	162.70 ± 22.93	205.77 ± 20.47
$SO_4^=$	1692.47 ± 266.16	1521.77 ± 311.34	1327.70 ± 170.83	764.43 ± 55.74
SAR	0.91 ± 0.08	0.99 ± 0.07	1.07 ± 0.05	1.25 ± 0.02
Cl^-	113.47 ± 3.55	101.65 ± 2.37	87.47 ± 8.28	92.20 ± 10.25
ES	38.44 ± 5.60	34.25 ± 6.38	29.84 ± 3.18	18.32 ± 0.60

Ca^{2+}, Mg^{2+}, Na^+, K^+, Bicarbonates (HCO_3^-), $SO_4^=$, and Cl^- in mg/L; SAR = sodium absorption ratio; ES = effective salinity (meq/L)

Table 3. The mean values of the concentrations of mineral elements in shoot tissues of tomato plants grown in calcareous soil. Citric acid was added at different concentrations to the nutrient solution used for the plants.

Sample	Treatment	Cu	Mn	Zn	Fe	K	Na	Mg	Ca	N	P
71 DAT	Test	20.00a&	388.00 a	77.50 b	115.50 ab	19.00 a	1.20 a	10.20 a	10.20 b	24.00 a	0.91 a
	10^{-6} M	17.75 a	329.00 a	87.75 ab	124.25 ab	12.90 b	1.10 a	12.10 a	14.30 b	22.50 a	0.84 a
	10^{-4} M	18.25 a	458.80 a	91.75 ab	136.75 a	15.30 ab	1.30 a	9.80 a	25.70 a	25.30 a	0.76 a
	10^{-2} M	15.50 a	532.00 a	104.75 a	106.50 b	13.40 b	1.30 a	10.90 a	23.80 a	22.10 a	0.79 a
83 DAT	Test	19.50 a	234.25 a	84.50 a	123.75 ab	20.00 a	0.30 c	17.70 a	25.50 ab	25.80 b	1.42 a
	10^{-6} M	19.75 a	251.00 a	87.00 a	99.00 b	18.40 a	0.40 bc	16.50 a	22.60 b	33.70 a	1.44 a
	10^{-4} M	19.25 a	239.50 a	108.25 a	133.50 a	19.90 a	0.90 a	17.80 a	27.30 a	35.70 a	1.56 a
	10^{-2} M	14.25 b	271.50 a	124.25 a	101.00 b	16.70 a	0.60 ab	16.00 a	25.50 ab	32.00 ab	1.01 b
119 DAT	Test	19.50 a	189.25 a	74.00 b	143.25 a	17.20 a	0.70 a	22.60 a	24.90 a	18.90 a	0.91 a
	10^{-6} M	20.75 a	176.00 a	64.75 b	145.25 a	14.60 ab	0.80 a	21.40 a	24.90 a	21.90 a	1.03 a
	10^{-4} M	19.25 a	244.50 a	77.25 b	165.25 a	14.80 ab	1.10 a	22.30 a	25.50 a	21.60 a	1.05 a
	10^{-2} M	18.75 a	228.00 a	117.25 a	143.50 a	12.20 b	1.00 a	23.70 a	28.10 a	18.50 a	0.73 a

Cu, Mn, Zn, y Fe are in mg kg^{-1}. K, Na, Mg, Ca, N y P are in g kg^{-1}. [&]Means with different letters within the same column are significantly different (LSD, $p \leq 0.05$)

Sodium absorption ratios (SAR) were higher in the SPW of the CA treatments (Table 2). The SAR is dependent on the concentrations of Na^+, Ca^{2+} and Mg^{2+}, which were modified by the presence of CA. Despite the changes observed, none of the values of SAR exceeded recommendations for tomato cultivation (Bosch *et al.*, 2012). As observed for the effective salinity (ES), SAR show decreased values in response to CA application (Table 2). The lowest values could be because the treatment with 10^{-2} M of CA generated a lower contribution of anions on cations in the SPW (Valdivia *et al.*, 2009).

Mineral contents in leaves and stems, and fruits: Tables 3 and 4 show the concentrations of mineral elements in the shoots and fruits, respectively. In the shoots, an ANOVA indicated significant differences for K, Ca and N at 71 DAT, Cu, Na, Ca, N and P at 83 DAT and Zn, K at 119 DAT. Such significant differences presented themselves when CA was at 10^{-4} M, except for Cu and Zn, which responded better to treatments of 10^{-6} and 10^{-2} M of CA, respectively. The response in the concentration of Zn, Ca and Na in shoots is similar to that reported by Al-Bahrany & Al-Shabaan (1995), who reported that the exogenous application of organic acids (citric and oxalic) in calcareous soils resulted in an increase in the absorption of minerals. The uptake of P not seems to respond in the same manner as that cited by Hu *et al*. (2005) and Palomo *et al*. (2006), who reported the increased availability and uptake of P when CA was applied to calcareous, eutric cambisol and haplic podzol soils. The increase in the concentrations of some minerals in the leaf tissue and the fruit could result from the increased availability of mineral nutrients in the SPW due to the application of CA (Baldotto e*t al.*, 2011; Najeeb *et al.,* 2011).

In the leaves, the concentrations of N, Fe and Cu were found to be within the nutrient sufficiency range (NSR), while P and K were below the NSR and Ca, Mg, Zn and Mn were above the NSR, as also reported by Mills *et al.* (1996).

In tomato fruits, the concentrations of Mn, Na, Mg and P (Table 4) exhibited significant effects from CA treatments ($p \leq 0.05$). These differences arose when the CA concentrations were 10^{-2} M and 10^{-4} M. It is noteworthy that the concentrations of the different minerals in the fruits were within the ranges reported by González-Raya *et al*. (2005) and Hernandez *et al*. (2008).

Correlation between the levels of minerals in the samples of soil pore water and mineral concentration in leaves and stems and fruits: According to the Spearman correlation analysis (Table 5a) there was a significant negative correlation between K^+ in the SPW and Mg^{2+} in leaf tissue (R = -0.73, $p \leq 0.05$). Similarly, the concentration of Na^+ in the SPW samples was correlated positively with the concentrations of Mg^{2+} and Ca^{2+} in the SPW (R = 0.60, R = 0.68, $p \leq 0.05$). Finally, the concentration of Mg^{2+} in the SPW showed a correlation with the concentration of Ca^{2+} in the SPW (R = 0.76, $p \leq 0.05$). In this respect, Gagnon *et al*. (2003) reported a positive correlation between the concentrations of Ca^{2+} and K^+ in soil with concentrations in leaf tissue of various forage species; however, the results reported in our study refer to the SPW, so it is possible that the response could be modified by the presence of CA. The changes in SPW may explain the very low values of the correlation coefficient. In contrast, no statistically significant correlations between concentrations of elements in the SPW and the fruit tissues were evident (Table 5b), possibly reflecting a lower level of translocation from leaf tissues to the fruit compared to that occurring from the roots to the stems and leaves (Chandra *et al*., 2009).

Fruit production: The ANOVA indicated statistically significant differences ($p \leq 0.05$) in response to the addition of CA (Table 6) for the weight of fruits per plant, the number of fruits per plant, and fruit dry weight. Citric acid at 10^{-6} M increased the production of fruits per plant, with the lowest dry weight per fruit. Because no significant effects were detected in fruit fresh weight, it is possible that this result was a consequence of a source-sink imbalance, i.e., the higher number of fruits per plant, the lower the partitioning of dry biomass to each fruit (Peil & Galvez, 2005).

Table 4. Means of mineral elements in the fruits of tomato plants grown on calcareous soil in which citric acid was added in different concentrations to the nutrient solution.

Treatment	Cu	Mn	Zn	Fe	K	Na	Mg	Ca	N	P
Test	8.00 a[&]	4.50 b	27.00 ab	203.25 a	13.80 a	0.10 b	2.50 b	1.10 a	26.60 a	1.18 b
10^{-6} M CA	9.00 a	7.00 ab	29.75 a	204.50 a	11.40 a	0.20 ab	3.10 ab	0.80 a	27.60 a	1.38 ab
10^{-4} M CA	7.00 a	6.50 ab	24.25 b	125.25 a	16.10 a	0.50 ab	2.70 ab	1.10 a	26.90 a	1.45 a
10^{-2} M CA	8.50 a	9.50 a	29.00 ab	68.50 a	15.40 a	0.60 a	3.20 a	1.30 a	26.50 a	1.22 ab

Cu, Mn, Zn, y Fe are expressed in mg kg^{-1}. K, Na, Mg, Ca, N y P are in g kg^{-1}. [&]Means with different letters within the same column are significantly different (LSD, $p \leq 0.05$)

Table 5. Spearman´s matrix correlation (R) between the concentrations of minerals in the samples of soil pore water and shoot tissue (a) and between the concentrations of minerals in the samples of soil pore water and fruits (b).

a) Parameters	K^+-SPW	K^+-ST	Na^+-SPW	Na^+-ST	Mg^{2+}-SPW	Mg^{2+}-ST	Ca^{2+}-SPW	Ca^{2+}-ST
K^+-SPW	1							
K^+-ST	-0.18 ns	1						
Na^+-SPW	0.27 ns	0.47 ns	1					
Na^+-ST	0.15 ns	-0.49 ns	-0.32 ns	1				
Mg^{2+}-SPW	-0.02 ns	0.11 ns	0.60**	-0.23 ns	1			
Mg^{2+}-ST	-0.73**	0.48 ns	0.28 ns	-0.47 ns	0.28 ns	1		
Ca^{2+}-SPW	-0.12 ns	0.50 ns	0.68**	-0.49 ns	0.76**	0.41 ns	1	
Ca^{2+}-ST	-0.18 ns	0.32 ns	0.28 ns	-0.10 ns	-0.22 ns	0.43 ns	-0.10 ns	1

SPW = soil pore water; ST = shoot tissue; ns = not significant; ** = significant at p≤0.05.

b) Parameters	K^+-SPW	K^+-Fruit	Na^+-SPW	Na^+-Fruit	Mg^{2+}-SPW	Mg^{2+}-Fruit	Ca^{2+}-SPW	Ca^{2+}-Fruit
K^+-SPW	1							
K^+-Fruit	-0.32 ns	1						
Na^+-SPW	0.89 ns	-0.11 ns	1					
Na^+-Fruit	-0.11 ns	0.60 ns	-0.32 ns	1				
Mg^{2+}-SPW	-0.11 ns	0.00 ns	0.32 ns	-0.80 ns	1			
Mg^{2+}-Fruit	0.11 ns	0.00 ns	-0.32 ns	0.80 ns	-1.00 ns	1		
Ca^{2+}-SPW	0.63 ns	-0.80 ns	0.63 ns	-0.80 ns	0.40 ns	-0.40 ns	1	
Ca^{2+}-Fruit	-0.83 ns	0.63 ns	-0.83 ns	0.63 ns	-0.32 ns	0.32 ns	-0.95 ns	1

SPW = soil pore water; ns = not significant

Table 6. Means of production and characteristics of the fruits of tomato plants grown on calcareous soil in which citric acid was added in different concentrations to the nutrient solution.

Variable	Treatments			
	Test	10^{-6} M CA	10^{-4} M CA	10^{-2} M CA
Production of fruit (g plant^{-1})¥	559.5 b&	945.7 a	690.6 ab	556.6 b
Number of fruits per plant	9.5 ab	12.0 a	10.0 ab	7.0 b
Fruit length (cm)	56.63 a	57.25 a	62.10 a	60.25 a
Fruit width (cm)	44.13 a	56.25 a	47.96 a	44.38 a
Fresh weight of fruit (g)	58.84 a	78.65 a	70.66 a	71.52 a
Dry weight of fruit (g)	6.71 a	3.77 b	8.53 a	6.47 ab

¥Fruit production and number of fruits were evaluated as the sum of the total weight of fruits per plant of three harvests made at 119, 121 and 125 DAT. The weight and diameter of all harvested fruits were also recorded. &Means with different letters within the same column are significantly different (LSD, p≤0.05)

Conclusions

The addition of citric acid to the nutrient solution applied to the soil resulted in a decrease in electrical conductivity and oxidation-reduction potential in the soil pore water and induced changes in the concentrations of Ca^{2+}, Mg^{2+}, $SO_4^{=}$ and Cl^- in the soil pore water. The concentrations of Zn, Na, Ca, and N in the leaves and stems and the concentrations of Mn, Na, Mg, and P in the fruit rose when citric acid was applied in the nutrient solution. However, there was only one statistically significant correlation between the mineral concentrations of the soil pore water and that of the leaves and stems. This correlation was between K^+ in the soil pore water and the concentration of Mg in stems and leaves (R=-0.73). The addition of citric acid at 10^{-6} M to the nutrient solution raised the fruit production per plant. Applying CA to the nutrient solution is recommended as part of the nutritional management of tomato.

Acknowledgements

Author Pérez-Labrada thanks CONACYT for the financial support.

References

Al-Bahrany, A.M. and A.M. Al-Shabaan. 1995. The effect of oxalic and citric acids on the plant absorption of phosphate and other nutrients from fertilizer added to calcareous soil. *Ann. Agric. Sci. Cairo*, 40: 943-951.

Anonymous. 2002. (SAS Institute). *PROC Users Manual*, version 9.0.0. 380, SAS Institute Inc., Cary, North Carolina, USA.

Anonymous. 2002. Norma Oficial Mexicana NOM-021-SEMARNAT-2002. Que establece las especificaciones de fertilidad, salinidad y clasificación de suelos. Estudios, muestreo y análisis. Diario Oficial de la Federación 31 de diciembre del 2002, México DF, México, 2002.

Arizmendi, G.N., O.P. Rivera, S.F. De la Cruz, M.B. Castro and R.F. De la Garza. 2011. Leaching of chelated iron in calcareous soils. *Terra Latinoamericana*, 29(3): 231-237.

Baldotto, M.A., V.B. Giro, L.E.B. Baldotto, L.P. Canellas and A.C.X. Velloso. 2011. Initial performance of pineapple and utilization of rock phosphate applied in combination with organic compounds to leaf axils. *Rev. Ceres, Viçosa,* 58(3): 393-401.

Bosch, M.M., J.L. Costa, F.N. Cabría and V.C. Aparicio. 2012. Relación entre la variabilidad espacial de la conductividad eléctrica y el contenido de sodio del suelo. *Ciencia del Suelo*, 30(2): 95-105.

Çali□kan, M. 2000. The metabolism of oxalic acid. *Turk. J. Zool.*, 24: 103-106.

Campbell, B. 2010. Organic matter application can reduce copper toxicity in tomato plants. *JNRLSE*, 39(1): 45-48.

Chandra, R., R.N. Bharagava, S. Yadav and D. Mohan. 2009. Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris* L.) irrigated with distillery and tannery effluents. *J. Hazard. Mat.*, 162(2-3): 1514-1521.

Dakora, F.D. and D.A. Phillips. 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant and Soil*, 245(1): 35-47.

Ferreyra, E.R., A.J. Peralta, R.A. Sadzawka, B.J. Valenzuela and S.C. Muñoz. 1998. Effect of acid application on some chemical characteristics of a calcareous soil. *Agricultura Técnica* (*Chile)*, 58(2): 163-170.

Fertilab. 2011. Manual de muestreo de Suelo, Planta y Agua. 2ª Edición, Celaya, Guanajuato, México.

Gagnon, B., G. Bélanger, M.C. Nolin and R.R. Simard. 2003. Relationships between soil cations and plant characteristics based on spatial variability in a forage field. *Can. J. Plant Sci.*, 83(2): 343-350.

González-Raya, E., A. Benavides-Mendoza, H. Ramírez, V. Robledo-Torres, R. Maiti, A. Reyes-López, A.F. Aguilera-Carbó, L.O. Fuentes-Lara and R.E.M. Hernández-Valencia. 2005. Crecimiento de jitomate y calidad de frutos con diferentes concentraciones de nitrato. *Terra Latinoamericana*, 23(1): 105-111.

Haynes, R.J. 1990. Active ion uptake and maintenance of cation-anion balance: A critical examination of their role in regulating rhizosphere pH. *Plant and Soil*, 126(2): 247-264.

Hernández, M.I., J.M. Salgado, M. Chailloux and L. Nasarova. 2008. Evaluación agronómica de fertilizantes líquidos cubanos en el cultivo protegido del tomate (*Solanum lycopersicum* L.) híbrido HA 3019. *Cultivos Tropicales*, 29(1): 73-81.

Hill, T. and P. Lewicki. 2007. *STATISTICS: Methods and Applications*. StatSoft Inc., Tulsa, OK.

Hu, H., C. Tang and Z. Rengel. 2005. Role of phenolics and organic acids in phosphorus mobilization in calcareous and acidic soils. *J. Plant Nutr.*, 28(8): 1427-1439.

Jones Jr., J. and V. Case. 1990. Sampling, handling and analyzing plant tissue samples. In: *Soil Testing and Plant Analysis*, SSSA Book Series N° 3, 3rd Edition, Westerman. Madison, Wisconsin, USA.

Jones, D.L. 1998. Organic acids in the rhizosphere–a critical review. *Plant and Soil*, 205(1): 25-44.

Jones, D.L. and P.R. Darrah. 1994. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant and Soil*, 166(2): 247-257.

Lee, R.V., F.M. Beltrán, M.J. Lerma and T.L. Licón. 1998. Sulphuric acid application to the irrigation water corrects iron-chlorosis on calcareous soils. *Terra Latinoamericana*, 16(2): 149-161.

Liu, Y., F.B. Li, W. Xia, J.M. Xu and X.S. Yu. 2013. Association between ferrous iron accumulation and pentachlorophenol degradation at the paddy soil–water interface in the presence of exogenous low-molecular-weight dissolved organic carbon. *Chemosphere*, 91(11): 1547-1555.

López, B.J., J.M.F. Nieto, R.V. Ramírez and E.L. Herrera. 2000. Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. *Plant Sci.*, 160(1): 1-13.

Mills, H.A., J.B. Jones Jr. and B. Wolf. 1996. Plant Analysis Handbook II: A practical sampling, preparation, analysis, and interpretation guide. Athens (GA): Micro-Macro Publishing.

Muller, L. 1961. Un aparato micro-Kjeldahl simple para análisis rutinarios de materiales vegetales. *Turrialba*, 11(1): 17-25.

Najeeb, U., G. Jilani, S. Ali, M. Sarwar, L. Xu and W. Zhou. 2011. Insights into cadmium induced physiological and ultra-structural disorders in *Juncus effusus* L. and its remediation through exogenous citric acid. *J. Hazard. Mat.*, 186(1): 565-574.

Neumann, G., A. Massonneau, N. Langlade, B. Dinkelaker, C. Hengeler, V. Römheld and E. Martinoia. 2000. Physiological aspects of cluster root function and development in phosphorus-deficient white lupin (*Lupinus albus* L.). *Ann. Bot.*, 85(6): 909-919.

Olsen, S.R., C.V. Cole, F.S. Watanabe and L.A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circular 939 United States Department of Agriculture, Washington, D.C., USA, 1954.

Palomo, L., N. Claassen and D.L. Jones. 2006. Differential mobilization of P in the maize rhizosphere by citric acid and potassium citrate. *Soil Biol. Biochem.*, 38(4): 683-692.

Peil, R.M. and J.L. Galvez. 2005. Reparto de materia seca como factor determinante de la producción de las hortalizas de fruto cultivadas en invernadero. *R. Bras. Agrociência*, 11(1): 05-11.

Pezeshki, S.R. and R.D. DeLaune. 2012. Soil oxidation-reduction in wetlands and its impact on plant functioning. *Biology*, 1(2): 196-221.

Rivera, O.P., B.J. Etchevers, M.C. Hidalgo, M.B. Castro, R.F. De la Garza, A.J. Rodríguez and G.A. Martínez. 2008. Acid-iron fertigation of citrus established in calcareous soils. *Terra Latinoamericana,* 26(2): 119-125.

Román, R., R. Caballero and A. Bustos. 2002. Variability of soil solution ions in fallowland fields in central Spain. *Edafologia,* 9(2): 161-172.

Ryan, P.R., E., Delhaize and D.L. Jones. 2001. Function and mechanism of organic anion exudation from plant roots. *Annu. Rev. Plant Phyisiol. Plant Mol. Biol.* 52: 527-560.

Sagoe, C.I., T. Ando, K. Kouno and T. Nagaoka. 1998. Effects of organic-acid treatment of phosphate rock on the phosphorus availability to Italian ryegrass. *Soil Sci. Plant Nut.*, 43: 1067-1072.

Sánchez-Rodríguez, A.R., M.C. del Campillo, J. Torrent and D.L. Jones. 2014. Organic acids alleviate iron chlorosis in chickpea grown on two p-fertilized soils. *J. Soil Sci. & Plant Nutr.*, 14(2): 292-303.

Shlizerman, L., K. Marsh, E. Blumwald and A. Sadk. 2007. Iron-shortage-induced increase in citric acid content and reduction of cytosolic aconitase activity in Citrus fruit vesicles and calli. *Physiol. Plant.*, 131(1): 72-79.

Sierra, B.C., M.A. Lancelloti and P.I. Vidal. 2007. Azufre elemental como corrector del pH y la fertilidad de algunos suelos de la iii y iv región de Chile. *Agricultura Técnica (Chile)*, 67(2): 173-181.

Steiner, A.A. 1961. A universal method for preparing nutrient solutions of a certain desired composition. *Plant and Soil*, 15(2): 134-154.

Strobel, B.W. 2001. Influence of vegetation on low-molecular-weight carboxylic acids in soil solution - a review. *Geoderma*, 99(3): 169-198.

Ström, L., A.G. Owen, D.L., Godbold and D.L. Jones. 2005. Organic acid behaviour in a calcareous soil implications for rhizosphere nutrient cycling. *Soil Bio. & Biochem.*, 37: 2046-2054.

Taghipour, M and M. Jalali. 2013. Effect of low-molecular-weight organic acids on kinetics release and fractionation of phosphorus in some calcareous soils of western Iran. *Environ. Monit. Assess*., 185: 5471-5482.

Thibaud, J.B, A. Sentenac and C. Grignon. 1994. The role of root apoplast acidification by the H^+ pump in phosphorus mineral nutrition of terrestrial plants. In: *Biochemistry of metal micronutrients in the rhizosphere*. (Eds.): J.A. Manthey; D.E. Crowley and D.G. Luster. Lewis Publishers, Boca Raton. FL, pp. 309-323.

Valdivia, Z.B., M.M., Ormachea and M.E. García. 2009. Estudio de la calidad de los recursos hídricos en la localidad de Apolo, Departamento de La Paz. *Rev. Bol. Quim.*, 26(2): 57-62.

Wang, Y. and R.M. Blatt. 2011. Anion channel sensitivity to cytosolic organic acids implicates a central role for oxaloacetate in integrating ion flux with metabolism in stomatal guard cells. *Biochem. J.*, 439(1): 161-170.

Wang, Y., Y. He, H. Zhang, J. Schroder, C. Li and D. Zhou. 2008. Phosphate mobilization by citric, tartaric, and oxalic acids in a clay loam Ultisol. *SSSAJ*, 72(5): 1263-1268.

Watt, M. and J.R. Evans. 1999. Proteoid roots. Physiology and development. *Plant Physiol.*, 121(2): 317-323.

Xi-wen, Y., T. Xiao-hong, L. Xin-chun, G. William and C. Yu-xian. 2011. Foliar zinc fertilization improves the zinc nutritional value of wheat (*Triticum aestivum* L.) grain. *Afr. J. Biotechnol.*, 10(66): 14778-14785.

Zhu, Y., F. Yan, C. Zörb and S.A. Schubert. 2005. A link between citrate and proton release by proteoid roots of white lupin (*Lupinus albus* L.) grown under phosphorus-deficient conditions? *Plant Cell Physiol.*, 46(6): 892-901.

(Received for publication 21 January 2015)