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The effects of amino acids fertilization incorporated to the nutrient solution on mineral composition and growth in tomato seedlings

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Abstract

The aim of this research was to determine the effects on growth variables and leaf mineral concentration of tomato plants watered with nutrient solutions containing amino acids. Two separate experiments were then carried out to achieve this goal. In the first experiment, plants were watered with seven different nutrient solutions consisting of half-strength Hoagland solution supplemented with single (Alanine, Serine, Phenylalanine, Tyrosine) or combined (Ala + Ser; Phe + Tyr) amino acids, each at 0.2 mM of concentration. The control nutrient solution did not have any amino acids added. Relative to the control, growth variables were not affected by the presence of amino acids. In general, the mixture of Ala + Ser increased the leaf Ca^{2+} concentration, and the aliphatic amino acid treatments favoured an increase in leaf K^+, Fe, Cu, and Mn concentrations. In addition, amino acids with hydroxyl groups in their structure, Ser and Tyr, increased Mg^{2+} concentration. In the second experiment, the nutrient solutions were supplemented with 0.05 mM of the same amino acids (T1). Control plants were irrigated with amino acid-free nutrient solution (T0). The concentration of Ca^{2+}, K^+, Mg^{2+}, Fe, Cu, and Mn in the leaf also increased due the amino acids treatment. Chlorophyll contents in the leaves and amino acids compositions in the xylem sap and leaf water relation were also determined. In conclusion, the data reported in the two experiments point out that the application of amino acids to the nutrient solution has a beneficial effect on the leaf mineral status and on the chlorophyll concentration of the leaves.

Additional key words: amino acid fertilization; biofertilizer; hydroponic culture; *Solanum lycopersicum* L.

Resumen

Efectos de la aplicación de amino ácidos en la solución nutritiva sobre la composición mineral y el crecimiento de plántulas de tomate

En este trabajo se estudiaron los efectos sobre el crecimiento y la concentración mineral de hojas de plantas de tomate cultivadas con soluciones nutritivas con distinta composición en aminoácidos. Para ello se plantearon dos experimentos. En el primero las plantas se regaron con siete soluciones nutritivas 1/2 Hoagland que diferían en la composición de los aminoácidos, todos ellos a una concentración total de 0,2 mM (Ala, Ser, Fen y Tir aplicados de forma individual, dos con la combinación Ala+Ser y Fen+Tir, un control sin aminoácidos). Las variables de crecimiento no mostraron diferencias entre los tratamientos; sin embargo, se observaron cambios en las concentraciones de nutrientes aunque los efectos dependieron de los aminoácidos utilizados. La mezcla de Ala + Ser aumentó la concentración de Ca^{2+}, mientras que los tratamientos de aminoácidos alifáticos favorecieron un aumento de K^+, Fe, Cu y Mn, y aquellos con grupos hidroxilo (Ser y Tyr) aumentaron el Mg^{2+}. En el segundo experimento las plantas se regaron con disolución 1/2 Hoagland (T0) o con disolución 1/2 Hoagland más una mezcla de los 4 aminoácidos (0,05 mM) ensayados (T1). En este experimento también aumentó la concentración de Ca^{2+}, K^+, Mg^{2+}, Fe, Cu, y Mn en las hojas. Asimismo se determinaron la concentración de clorofilas en las hojas, relaciones hídricas y la composición de aminoácidos del xilema. En conclusión, los datos obtenidos en los dos experimentos indican que la aplicación de aminoácidos a la solución nutritiva podría mejorar el estado nutricional de las plantas y la concentración de clorofilas en las hojas.

Palabras clave adicionales: biofertilizante; cultivo hidropónico; fertilización con aminoácidos; *Solanum lycopersicum* L.

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Introduction

Higher plants are considered autotrophic not only with respect to carbon but also to nitrogen. The absorption and assimilation of different forms of nitrogen, such as nitrate and ammonium have been studied widely (Masclaux-Daubresse *et al*., 2010). However, less attention had been paid to organic forms of nitrogen, such as amino acids. Currently, it is well known that amino acids can be taken up directly by the roots (Fischer *et al.,* 1998).

Atkins and Beevers (1990) reported the presence of all proteinogenic amino acids in the phloem fluid, and their differential absorption by the phloem tissue had been described previously by Schobert and Komor (1989). In addition, their transport through the tissues also has been studied (Gilbert *et al*., 1998; Endres and Mercier, 2003). Positive interactions between amino acids and some mineral nutrients have been observed in nature. Plants are able to increase nutrient availability in the rhizosphere (Dakora and Phillips, 2002) by exuding amino acids through the roots. Besides, nutrient translocation through the vascular system has been shown to be facilitated by the enhancement of their permeability in cell membranes (Franco *et al*., 1994). The foliar application of amino acids improved uptake efficiency of N from the soil and prevented N loss through leaching (Liu *et al*., 2008; Junxi *et al*., 2010). In the agricultural sector, many products from different origins (for instance, peat, compost, leonardite) are used commonly as fertilizers or soil amendments due to their positive influence on plant nutrition, growth and yield (Bryson and Barker, 2002; Hu and Barker, 2004). However, few studies have been made on the effect of amino acid fertilization on nutrition of cultivated plants. Moreover, existing studies determine the usefulness of using commercially available amino acids to improve macronutrient and oligoelement fertilization (Franco *et al*., 1994, 1999; Sánchez-Sánchez *et al*., 2002). To date, commercial products combine mineral elements with mixtures of free amino acids and hydrolysis-derived peptides with low molecular weight. Given the high complexity of these amino acid-based products, it is very difficult to elucidate the exact mechanisms involved in the interaction between amino acids and mineral nutrients. The aim of this research was to evaluate the effects of four amino acids, with contrasting chemical characteristics (alanine, Ala; serine, Ser; phenylalanine, Phe; and tyrosine, Tyr), on the mineral composition and growth of tomato plants. Amino acids were applied in single or combined forms, as supplements in nutrient solutions. The resulting information should enable us to identify industrial residues of vegetal origin as potential fertilizers, based on their amino acid composition.

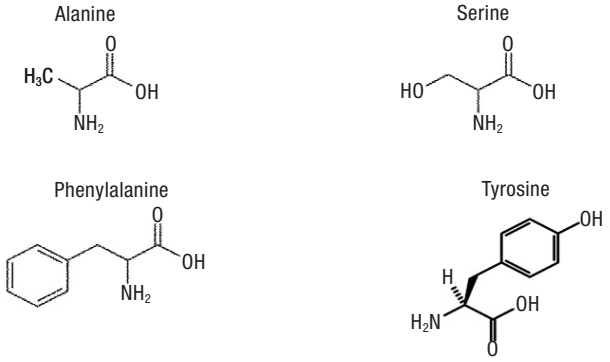

Figure 1. Chemical structure of the aliphatic (alanine and serine) and aromatic (phenylalanine and tyrosine) amino acids used in this research. Note: the OH of the carboxyl group is not considered to be a structural OH component of the molecule; hence, Ala and Phe do not have hydroxyl groups.

Ala, Ser, Phe and Tyr were selected based on remarkable structural and functional differences among them (Fig. 1). These amino acids, given their polarity, are able to establish interactions with ionic forms of elements in the nutrient solution or in the xylem. Ala and Ser are aliphatic with a very simple structure, and their activities can be contrasted with those of Phe and Tyr (aromatics). Additionally, the hydroxyl group (–OH) present in the structure of Ser and Tyr confers them a greater reactivity when compared to Ala and Phe.

Material and methods

Plant material and general growth conditions

Tomato seeds (*Solanum lycopersicum* L.) were surface sterilized with 1% NaOCl for 15 min to prevent fungal contamination. Seeds were then wrapped with synthetic cotton-made wool before placing them on discs of expanded polystyrene (polyspan). Finally, they were germinated in trays covered with perforated aluminium foil and containing a 0.5 mM $CaSO_4$. Germination took place within a forced-air oven at constant temperature (2 ± 1°C). The resulting seedlings were

Abbreviations used: Ala (alanine); chl (chlorophyll); DM (dry matter); Phe (phenylalanine); Ser (serine); Tyr (tyrosine).

acclimated for one week in a growth chamber where the mean temperature varied between 30°C (day) and 20°C (night) with a relative humidity between 60% (day) and 70% (night), a maximum photosynthetic photon flux density of 320 μmol m^{-2} s^{-1}, and a 12-h photoperiod. Plantlets with uniform sizes were then selected and transplanted into polyethylene pots (1-L) containing a 3:1 (v/v) mixture of autoclaved (120°C, 20 min) vermiculite-perlite (107 g; water holding capacity of 305%). Irrigations were performed daily with half-strength Hoagland-based solution until leakage was observed at the bottom of the pots. The modified Hoagland solution was prepared with de-ionized and sterilized water as follows: 7 mM NO_3^-, 3.5 mM K^+, 2 mM Ca^{2+}, 0.5 mM $H_2PO_4^-$, 0.50 mM SO_4^{2-}, 0.50 mM Mg^{2+}, 20 μM Fe-EDTA, 25 μM B, 2 μM Mn, 2 μM Zn, 0.5 μM Cu and 0.5 μM Mo. In all experiments, the pH was maintained between 5.5 and 6.0 by the addition of 1 M H_2SO_4 or 0.5 M KOH.

Experiment 1

The first experiment was conducted with 3-week-old pot-grown seedlings. Treatments were established by watering plantlets with six nutrient solutions consisting of the modified Hoagland solution supplemented with single (Ala, Ser, Phe, Tyr) or combined (Ala + Ser; Phe + Tyr) amino acids, each at a concentration of 0.2 mM. Control plants without amino acids added were kept under normal irrigation. Six replicates were used for each treatment. Seedlings were placed randomly in a growth chamber and irrigated daily with 60 to 120 mL of nutrient solution for three weeks. In all cases, 1.92 L of nutrient solution per plantlet were applied during the length of the experiment. Plant height (cm), length of 5th leaf (cm), number of leaflets per plant, and total plant biomass (fresh matter weight, mg) were then recorded. To determine dry weights and mineral concentrations, plantlets were harvested and separated into leaves, stems, and roots. They were oven-dried in a two-step procedure: first at 65°C for 24 h, and then at 105 to 110°C until constant weight was reached.

The concentrations of N, P, Na, K, Ca, Mg, Fe, Mn, Zn, and Cu were determined in leaves. The N content was determined according to a Kjeldahl method (Nelson and Sommers, 1973) modified at a semimicro scale with 50 mg of dry leaf sample. Phosphorus was determined colorimetrically by measuring the absorbance of the phosphovanadate complex (Chapman and Pratt, 1979). The remaining nutrients were determined by atomic absorption spectrometry (Ca, Mg, Fe, Mn, Zn and Cu) or emission spectrometry (Na and K) after mineralization with a nitric-perchloric mixture of dry leaf material (Perkin-Elmer 5500, Norwalk, CT, USA; García-Sánchez *et al*., 2003). In addition, amino acid concentrations were measured in the xylem sap. For this measurement, the plants were placed in 250-mL flasks containing the respective nutrient solutions. After two hours of acclimation, the aerial part was decapitated 2 cm from the base of the root, and a silicone capillary was placed on the stem. The xylem sap was thus collected after 2 hours in Eppendorf tubes, and its volume was determined by weighing. Samples were diluted with 10% (v/v) trichloroacetic acid, and the resulting extracts were then centrifuged at 9,000 × g for 10 min followed by filtration through 0.22-μm pore size membranes. Free amino acids were quantified using an auto-analyser equipped with a fluorescence detector and a computer program for quantification (Chromospek, Rank-Hilger, Margate, UK; Martínez *et al*., 1994). Nor-leucine was used as internal standard. Results were averaged over five biological replicates per treatment.

Extracts were obtained from frozen root, stem, and leaf tissues. Osmotic potential was then measured by pressing into a plastic syringe. A micro-osmometer (Roebling mod. 13-13DR-autocal, address) was used to determine the freezing point of the solution (García *et al*., 2007). The osmotic potential was calculated according to Van't Hoff equation: PV = nRT; where P = osmotic potencial (atm), V = volume (L), n = nº moles, R = 0.082 L atm °K^{-1} mol^{-1}, T = temperature °K.

Experiment 2

Six-week-old seedlings, grown under conditions described in the first experiment, were used as plant material. Treated plants (T1) were irrigated with the modified Hoagland solution supplemented with a mixture of all amino acids used in the first experiment (Ala + Ser + Phe + Tyr), each at 0.05 mM. Control plants (T0) were kept under normal irrigation. In both cases, plants were distributed randomly in a growth chamber, and their position was changed occasionally. Seedlings from both treatments were watered daily with 150 to 300 mL of nutrient solution for three weeks, depending on plant growth rate. The following variables were then determined: chlorophyll a (chla) and chlorophyll b (chlb) content, chla/chlb ratio, and surface chlorophyll

density (total chlorophyll/area ratio), according to García and Nicolás (1998). Growth variables and leaf mineral composition were determined as indicated above. Twelve biological replicates were used for each treatment.

Statistical analysis

All statistical analyses were carried out using SPSS (SPSS statistical package, Chicago, IL) Data were subjected to ANOVA procedures, and the mean values were compared by using Duncan's multiple range test at a confidence level of 0.05 (Little and Hills, 1987). The experiments were repeated twice with similar results in both cases according to ANOVA.

Results

Experiment 1

Growth variables measured during this experiment showed no significant differences among treatments (Table 1). However, the concentrations of Ca^{2+}, K^+, Mg^{2+}, Fe, Cu, and Mn varied significantly among the amino acid treatments (Fig. 2). The rest of the elements analysed (N, P, Na, and Zn) did not vary significantly with the treatments. In the case of Ca^{2+}, the influence exerted by the amino acids depended on their nature. In general the aromatics, relative to aliphatic amino acid, induced significant declines in Ca^{2+} concentration. Phe and Tyr caused decreases of 38% and 14%, respectively, if applied in single forms. In addition, their mixture (Phe + Tyr) induced a decrease of about 8% (Fig. 2a). On the contrary, aliphatic amino acids significantly increased Ca^{2+} concentration (24%) when they were combined (Ala + Ser) in the nutrient solution. Nevertheless, Ser and Ala treatments did not produce significant differences when compared to the control treatment.

The K^+ concentration was not significantly affected by neither Phe nor Tyr, or their combination with regard to untreated plants. On the other hand, aliphatic amino acids-treated plants showed significantly higher K^+ concentrations than control plants. Increases were about 8% when Ala and Ser were added in single form to the nutrient solution, whereas increases corresponding to the mixture were about 18% (Fig. 2b). The Mg^{2+} concentrations in leaves increased with aromatic or aliphatic treatments. In this case, values obtained in Phe and Ala-treated plants were similar to those recorded in control (Fig. 2c). High levels of Mg^{2+} concentration were observed in Ala + Ser, Phe + Tyr, and Tyr treatments.

The concentrations of Fe, Cu and Mn were the most affected by the amino acids treatments. Significant increases in Fe concentration (12%), relative to the other minerals, were induced by nutrient solutions containing Ala or Ser in single forms. However, the remaining treatments assayed, including the mixture of Ala+Ser, caused significant declines. In this case, the lowest value was obtained in leaves from Phe + Tyr-treated plants (21%; Fig. 2d). Results obtained with respect to Cu concentration are new evidences suggesting nature-dependent influence of the assayed amino acids on mineral composition. In this case, the aliphatic amino acids (Ser and Ala) were responsible for highly significant enhancements (in single forms or mixtures) with regard to untreated plants, whereas treatments with the aromatic ones induced no differences (Fig. 2e). Ala or Ser treatments increased leaf Cu concentration about 53% and 86%, respectively. The latter value was similar to that obtained in plants treated with the mixture (Ala + Ser). On the other hand, all aliphatic amino acids-treated seedlings showed statistically higher values of Mn concentration than control plants (Fig. 2f). Treatments with aromatic amino acids (Phe, Tyr and Phe + Tyr) tended to increase the Mn concentration in leaves but not significantly.

The concentrations of asparagines (Asn), serine, glutamine (Gln), alanine, phenylalanine and tyrosine in the xylem fluid were influenced significantly by at least two different treatments (Table 2). Treatments with

Table 1. Effect of amino acids treatments on the total plant biomass growth variables: T = fresh matter weight, H = height, L = length of the 5th leaf and N = number of leaflets per plant, of plants from the experiment 1

Treatments	Growth variables			
	T (g)	H (cm)	L (cm)	N
Control	328^{ns}	47.5^{ns}	17.3^{ns}	12^{ns}
Ala	326	47.4	17.2	13
Ser	319	46.5	17.4	13
Ala + Ser	315	48.0	17.3	11
Phe	316	47.4	19.2	13
Tyr	329	49.0	18.0	12
Phe + Tyr	311	48.1	17.6	12

For data in columns, ns indicates non-significant differences ($p = 0.05$) according to Duncan's multiple range test.

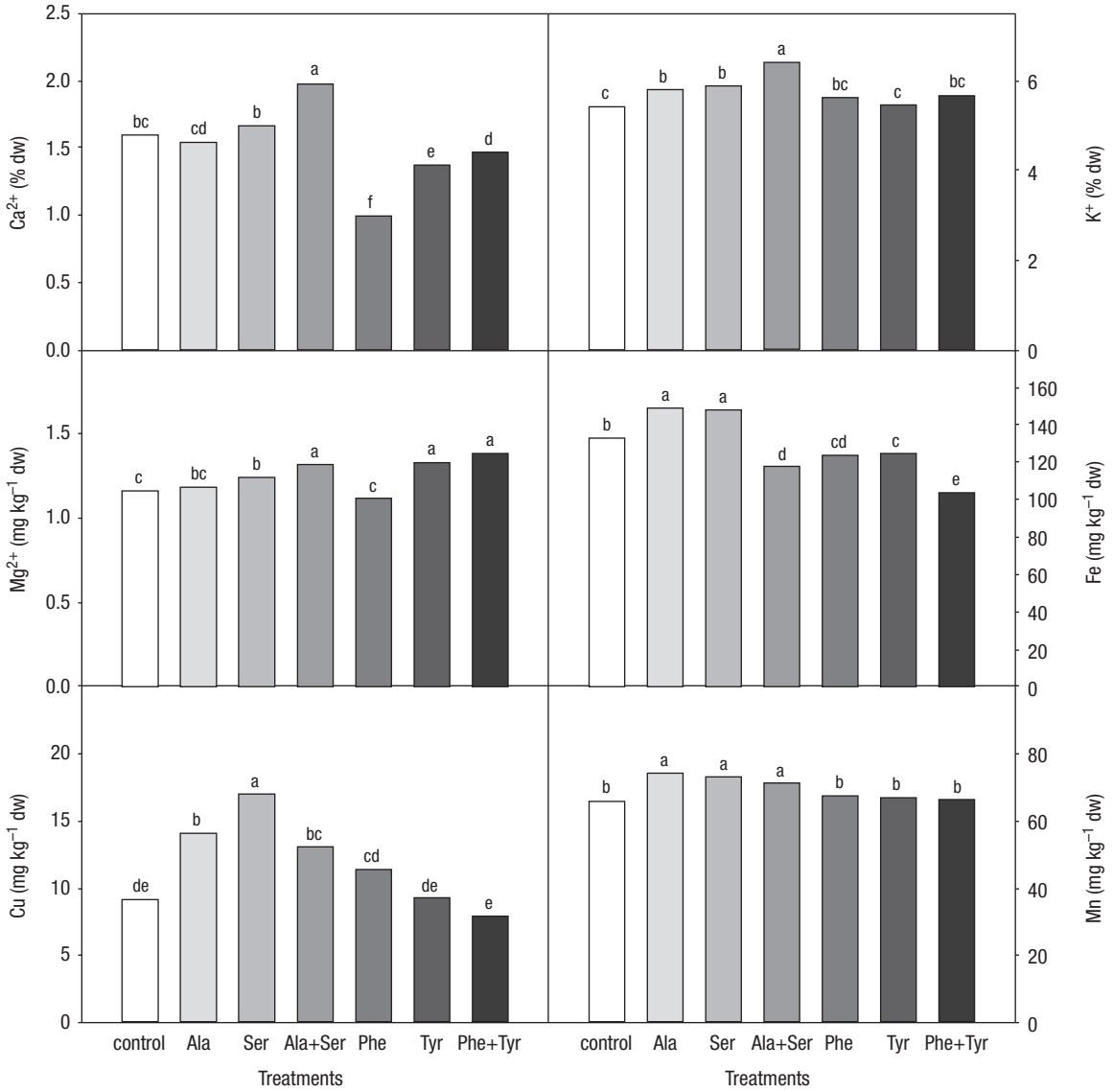

Figure 2. Effect of amino acids treatments on mineral concentrations (Ca^{2+}, K^{+}, Mg^{2+}, Fe, Cu, Mg) measured in leaves during the experiment 1. Within each figure, means of bars headed by the same letters are not significantly different at $p = 0.05$ according to Duncan's multiple range test.

Ser, Ala, Phe, or Tyr induced significant increases in the concentrations of Ser (250%), Ala (300%), Phe (450%) and Tyr (525%) compared to untreated seedlings. The levels of Ser and Ala increased by 150 and 200%, respectively, in leaves of plants treated with the aliphatic mixture (Ala + Ser). The increase induced by the mixture of aromatic (Phe + Tyr) amino acids was about 200%. Moreover, the levels of Phe and Gln were enhanced significantly by Ala or Ser treatments. The addition of single aromatic amino acids to the nutrient solution caused significant increases in the concentrations of Gln, Ala (in the case of Phe treatment), and Asn and Ser (Tyr treatment). In addition, the concentrations of Asn or Gln were significantly increased by their mixture, relative to control treatment. Asn concentration also was enhanced by the mixture of aliphatic amino acids. On the other hand, the levels of threonine, glycine, valine, isoleucine, leucine, histidine and lysine measured in the xylem fluid, were not affected statistically by the treatments.

The osmotic potentials (Table 3) measured in stems and leaves were similar among all treatments. In

Table 2. Effect of amino acids treatments on the concentration of amino acids compounds (μmol mL^{-1}) in the xylem fluid in plants from the experiment 1

Amino acids detected	Treatments, amino acids added						
	Control	Ala	Ser	Ala + Ser	Phe	Tyr	Phe + Thyr
Asparagine	0.021[a]	0.018[a]	0.020[a]	0.027[b]	0.017[a]	0.027[b]	0.029[b]
Threonine	0.020[a]	0.023[a]	0.026[a]	0.018[a]	0.019[a]	0.018[a]	0.018[a]
Serine	0.017[a]	0.015[a]	0.045[c]	0.027[b]	0.018[a]	0.027[a]	0.020[a]
Glutamine	0.019[a]	0.022[a]	0.026[b]	0.022[a]	0.028[b]	0.022[a]	0.026[b]
Glycine	0.007[a]	0.005[a]	0.008[a]	0.006[a]	0.005[a]	0.006[a]	0.009[a]
Alanine	0.013[a]	0.039[c]	0.017[a]	0.026[b]	0.024[b]	0.026[a]	0.014[a]
Valine	0.005[a]	0.004[a]	0.007[a]	0.006[a]	0.006[a]	0.006[a]	0.004[a]
Isoleucine	0.004[a]	0.003[a]	0.006[a]	0.004[a]	0.005[a]	0.004[a]	0.005[a]
Isoleucine	0.003[a]	0.003[a]	0.004[a]	0.005[a]	0.003[a]	0.004[a]	0.004[a]
Phenylalanine	0.004[a]	0.010[b]	0.003[a]	0.004[a]	0.018[c]	0.005[a]	0.012[b]
Histidine	0.010[a]	0.008[a]	0.011[a]	0.011[a]	0.009[a]	0.008[a]	0.010[a]
Lysine	0.014[a]	0.012[a]	0.013[a]	0.014[a]	0.012[a]	0.012[a]	0.012[a]
Tyrosine	0.003[a]	0.003[a]	0.004[a]	0.005[a]	0.016[a]	0.016[c]	0.009[b]
Total	0.140[a]	0.165[b]	0.190[c]	0.175[bc]	0.180[c]	0.181[c]	0.172[bc]

Within each row, means followed by the same letters are not significantly different at $p = 0.05$ according to Duncan's multiple range test.

contrast, root osmotic potential was significantly higher with the amino acid treatments than with control plants. Ala-treated seedlings showed the highest value (30%) whereas a similar increase in root osmotic potential (20%) was obtained with Ser, Tyr, or Al + Ser treatments. The least pronounced increase was observed in Phe-treated plants.

Experiment 2

Growth variables (plant height, length of 5th leaf, number of leaflets per plant, and total plant biomass) measured in this experiment were not affected by treatments (data not shown). With regard to mineral elements in the leaves, N and P showed similar concentrations in either treated or control plants (Table 4). K^+, Ca^{2+} and Mg^{2+} significantly increased 25, 35 and 55%, respectively, in treated plants. The oligoelements Fe, Cu, and Mn showed significantly higher concentrations in treated than in control plants. Fe, Cu, and Mn increased 26 %, 72% and 16%, respectively. Neither Zn nor Na showed different concentrations.

Amino acid treatments caused significant enhancements in the concentrations of chla and chlb (Table 4) by 37% and 30%, respectively, with regard to control plants, but chla/chlb ratio was not affected. The surface chlorophyll density was significantly higher in treated than in control plants.

Table 3. Effect of amino acids treatment on the osmotic potential (MPa) in different organs of plants from experiment 1

Treatments	Root	Stem	Leaf
Control	–1.15[d]	–1.20[ns]	–1.25[ns]
Ala	–0.81[a]	–1.15	–1.20
Ser	–0.93[b]	–1.18	–1.27
Ala + Ser	–0.92[b]	–1.16	–1.15
Phe	–1.02[c]	–1.17	–1.15
Tyr	–0.94[b]	–1.15	–1.16
Phe + Tyr	–1.02[c]	–1.18	–1.17

ns indicates non-significant differences ($p = 0.05$) among the treatments. Within each column, means followed by the same letters are not significantly different at $p = 0.05$ according to Duncan's multiple range test.

Discussion

According to the data presented here, particularly the increases observed in leaf Ca^{2+}, K^+, Mg^{2+}, Fe, Cu, and Mn concentrations, the mineral status of leaves from tomato hydroponic grown-plants could be improved by adding amino acids to the nutrient solution. Alterations in the levels of these nutrients appear to be associated with the nature of amino acids rather than to concentration effects as a result of growth since growth variables showed no significant differences between control and treated plants. Therefore, the latter process

Table 4. Effect of amino acids treatments (T0: nutrient solution alone; T1: nutrient solution with a mixture of the four amino acids, 0.05 mM each) on the leaf mineral concentration, chlorophyll a and chlorophyll b concentration, chlorophyll a/chlorophyll b ratio, and surface chlorophyll density in plants of experiment 2

	T0	T1
Macronutrients (% DW)		
N	3.48^{ns}	3.32
P	0.55^{ns}	0.50
K^+	2.79*	3.45
Ca^{2+}	1.70*	2.26
Mg^{2+}	0.40*	0.62
Micronutrients (mg kg^{-1} DW)		
Fe	102*	129
Mn	68*	79
Zn	30^{ns}	32
Cu	11*	19
Na^+	218^{ns}	216
Chlorophyll		
Chla (µg chorophyll g^{-1} DW)	0.83*	1.37
Chlb (µg chorophyll g^{-1} DW)	0.29*	0.37
Chla/Chlb (dimensionless)	2.67^{ns}	2.75
Chlorophyll density (chl/area, µg cm^{-2})	2.33*	2.58

For data in rows, ns indicates non-significant differences ($p = 0.05$) and * indicate significant differences at $p < 0.05$ according to Duncan's multiple range test.

seems to be mediated by chemical-induced mechanistic changes in the uptake and transport of these nutrients.

Results obtained here showed that: i) the mixture of aliphatic amino acids favoured Ca^{2+} translocation from roots and stems to the leaves. These observations agree with those previously reported by McLaughlin and Wimmer (1999). The opposite effect exerted by the aromatic amino acids (Phe and Tyr) in this process should be pointed out. In this case, differences between the influence of aliphatic and aromatic amino acids on Ca^{2+} uptake and transport were not due to changes in Ca^{2+} activity in the nutrient solution since it was, as measured by a Ca^{2+} selective electrode, similar in all treatments. ii) Aliphatic amino acids increased the leaf K^+ concentration which could reflect the importance of this nutrient to charge compensation caused by absorption of additional aliphatic amino acids from the nutrient solution (Mengel and Kirkby, 2001). iii) Amino acids with hydroxyl groups as Ser and Tyr increased the leaf Mg^{2+} concentration. Thus, the presence of aliphatic amino acids in the nutrient solution could have increased the channel membrane permeability of nutrients favouring their absorption by the roots, while amino acids with hydroxyl groups, such as Ser and Tyr, could have affected the magnesium transporter activity. On the other hand, enhancements observed in Ca^{2+} and Mg^{2+} concentrations when measured in treated plants do not agree with observations previously reported by Franco *et al*. (1994), where Ca^{2+} levels determined in field-grown plants remained constant, while those regarding Mg^{2+} decreased.

In the case of micronutrients, Fe, Cu and Mn levels showed, in general, a positive response to treatments with aliphatic amino acids. It is well known that soluble Fe sources available to plants in the rhizosphere are mainly a mixture of complexes between this micronutrient and organic ligands coming from plant roots or microorganisms (siderophores and phytosiderophores; Bityutskii *et al*., 2004). In our experiment, the increase observed in leaf Fe concentration from aliphatic amino acid-treated plants might have favoured the formation of Fe-phytosiderophore complexes and enhanced the xylem transport and phloem translocation of Fe (Robin *et al*., 2008) rather than induced changes in the pH of the nutrient solution, since it remained constant during the experimental period (5.5-6.5). Tomasi *et al*. (2009) observed that Fe acquisition through roots in tomato plants depends on the nature of Fe-complexes, whereby Fe complexed to a water-extractable humic substances fraction has higher rates of reduction and uptake than Fe complexed with siderophores. Aliphatic amino acid-induced also enhancements in leaf Mn and Cu concentrations. In plants, the recent advantages in understanding the mechanism of metal uptake by the roots have reported that organic molecules are involved in metal ion homeostasis as metal ion ligands that facilitate uptake and transport. Ligands identified in Cu and Mn uptake and transport include mugineic acid, nicotianamine, organic acids (citrate and malate), and the histidine amino acid (Haydon and Cobbett, 2007). Thus, it is possible that Cu and Mn could have formed complexes with aliphatic amino acid favouring their uptake by the roots. Copper in the soil solution as well as in roots and in the xylem sap is presented in complexed form since this element has a high affinity for carboxylic and phenolic groups (Marschner, 1995).

All plant species studied, including plants from all major mycorrhizal types and non-mycorrhizal species, possess the capacity to take up amino acids (Näsholm *et al*., 2009). In the present experiments, we observed that the total amino acid levels in the xylem fluids can

be increased by adding either single or combined amino acids to the nutrient solution. Moreover, our results indicate that the supply of Ser, Phe and Tyr amino acids into the solution has a higher influence on the increase in the xylem fluid total amino acid concentration than the supply of Ala amino acid. Plant amino acid transporters are functionally defined by distinct spatial and temporal expression patterns and substrate specificities (Liu and Bush, 2006). By applying amino acids treatments of Gly and Glu to bromeliad Endres and Mercier (2003) found a higher concentration of Gly than of Gln in the plants. Our results indicate that tomato plants could have selective amino acid absorption mechanisms whose specificity could be greater for Ser, Phe, and Tyr than for Ala. In addition, our results also show that the amino acids absorbed by the root can be transformed to the other amino acids, as demonstrated in the Ala treatment, where significant differences in the Phe concentration was observed with regard to the control treatment (Table 2). Metabolism and allocation of amino acids, such as Ala and Ser, have not been studied in plants, but it has been observed that a high fraction of absorbed Gly is transformed to L-Ser (Näsholm *et al*., 2009).

Although nitrogen supply was greater in the amino acids treatments than in the control, differences in leaf N concentration or in growth variables were not observed. It is well known that the amino acids added to the hydroponic medium can inhibit inorganic nitrogen uptake as a nitrate source by the roots (Stoelken *et al.*, 2010). Nitrate uptake is regulated by several feedback inhibitors such as nitrate itself (King *et al.,* 1993) and products of ammonium assimilation, in particular free soluble amino compounds (Collier *et al.,* 2003). Our finding agrees with previous amino acid fertilization experiments of plants reported by Guidi *et al*. (1998) and Santa-Cruz *et al*. (2000). The latter authors indicated that this could be due to the result of alterations in the uptake and transport of water to increase the osmotic potential of the nutrient solution by adding organic solutes. In our experiment, however, we ruled out osmotic stress as a possible cause of this lack of increased growth because the leaf water potential was not altered in the amino acid-treated plants, although the osmotic potential of the nutrient solution was slightly higher in the amino acid treatments than in the control.

Data from experiment 2 confirm the results obtained in experiment 1, where plant growth was not affected, and leaf concentrations of Ca^{2+}, K^{+}, Mg^{2+}, Fe, Cu, and Mn were increased in the treatments with amino acids (Table 4). This finding suggests that amino acid-based products having aliphatic amino acids compounds could counter the negative impact of the aromatic compounds on Ca^{2+} and Fe concentration in leaves of tomato plants as observed in experiment 1. In the second experiment, the chlorophyll content was measured as an indicator of the photosynthetic system (Fernández-García *et al*., 2002). Both chla and chlb were increased by 20% with the amino acid treatment meaning that the beneficial effects of improving the leaf mineral status also led to an improvement in the photosynthetic machinery. Other authors have described how adding amino acids to the nutrient solution of tomato seedling can increase chlorophyll content and also antioxidative enzyme activities in the plants (Zhang *et al*., 2009).

In conclusion, the beneficial effects of adding amino acids to the nutrient solution on the leaf mineral status and on the photosynthetic machinery of the plants was demonstrated since leaf concentrations of Ca^{2+}, K^{+}, Mg^{2+}, Fe, Cu, Mn, and chla and chlb increased. Also, the economic cost of adding amino acids to the nutrient solution will be negligent since a great number of fertilizer companies are offering these biofertilizer products at low cost since they come from vegetal industrial residues.

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