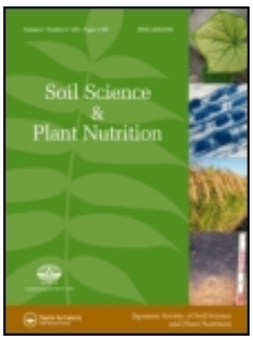

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ORIGINAL ARTICLE

Amendment of hydroponic nutrient solution with humic acid and glutamic acid in tomato (*Lycopersicon esculentum* Mill.) culture

Maryam HAGHIGHI[1] and Jaime A. TEIXEIRA DA SILVA[2]

[1]*Horticulture Department, College of Agriculture, Isfahan University of Technology, Isfahan, Iran and* [2]*Miki cho Post Office, Ikenobe 3011-2, PO Box 7, Kagawa-Ken, 761-0799, Japan*

Abstract

Can humic acid (HA) and glutamic acid (GA), when added to tomato (*Lycopersicon esculentum* Mill. cv. 'Hongyangli') nutrient solution in a hydroponic system, improve growth? Tomato seedlings were grown in six nutrient solutions: (1) control (C), (2) C + 25 mg L^{-1} HA (HA1); (3) C + 50 mg L^{-1} HA (HA2); (4) C + 100 mg L^{-1} GA; (5) HA1 + GA; (6) HA2 + GA. Various biochemical and physiological parameters were measured. HA increased photosynthesis rate and mesophyll conductance. HA did not significantly affect transpiration, stomatal conductance, titratable acidity, or antioxidant activity. In addition, GA improved protein and sugar content, mesophyll conductance and yield. The combination of HA and GA was more effective, especially with 50 mg L^{-1} HA. The activity of superoxide dismutase (SOD) and peroxidases (POD) did not change in the presence of HA or GA. Malondialdehyde (MDA) content increased by 30% in HA2 together with GA. HA has a positive effect on tomato hydroponic growth when applied with GA. This expands the use of HA and GA for horticultural commodities in hydroponic systems.

Key words: Amino acid, malondialdehyde (MDA), peroxidases (POD), superoxide dismutase (SOD), stomatal conductance.

INTRODUCTION

Humic acid (HA), which originates from different materials, has many beneficial effects on various aspects and parameters of plant growth such as yield and root growth, including that of strawberry (*Fragaria* × *ananassa* (Duchesne ex Rozier)), pepper (*Capsicum annuum* L.) (Norman *et al.* 2006), maize (*Zea mays* L.) (Eyheraguibel *et al.* 2008), lettuce (*Lactuca sativa* L.) (Haghighi *et al.* 2010), and gerbera (*Gerbera jamesonii* Bolus ex. Hook f.) (Nikbakht *et al.* 2008), among others. All HAs, regardless of their origin, nature and characteristics, generally increase plant yield and either directly or indirectly stimulate the absorption of plant nutrients, photosynthesis and respiration (Ayuso *et al.* 1996; Nardi *et al.* 2002). HAs derived from lignocellulosic raw materials showed positive effects on overall plant growth, including root, shoot and leaf biomass (Eyheraguibel *et al.* 2008). Bohme and Thi-Lua (1997) found that HA positively influenced the germination percentage of tomato (*Lycopersicon esculentum* Mill.) seeds, plant growth, length of roots and shoots and the contents of calcium (Ca) and potassium (K). Adani *et al.* (1998) found that HA from peat and leonardite affected the growth and mineral nutrition of tomato plants in hydroponic culture at 20 and 50 mg L^{-1}. Azarpour *et al.* (2012) used HA at 25 and 50 mg L^{-1} on *Solanum melongena* L. where it improved fruit number, width and length and increased yield by increasing the level of HA.

Trace amounts of dissolved organic nitrogen (N) are one of the possible sources of soil N. Accordingly, free amino acid (AA) released from organic matter can be taken up directly by roots, although the precise mechanism remains unexplained. The concentration of individual free AAs in soil solution is in the range of 0.1–10 mM and, in plant and animal cells, this range increases to 1–10 mM, with glutamic acid (GA) constituting the highest concentration of total AA in soil (Jones *et al.* 2005). AAs can be one possible source of N to plants, particularly when available at a high

Correspondence: Maryam HAGHIGHI, Horticulture Department, College of Agriculture, Isfahan University of Technology, Isfahan, Iran. Tel.: 00989133170544. Fax: 00983113913412. E-mail: mhaghighi@cc.iut.ac.ir
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concentration, although this is different for different plants. For example, 1000 mg L^{-1} AA was suitable for lettuce (Haghighi *et al.* 2010). In addition, significant competition will exist between plants and microorganisms for this organic N resource (Jones *et al.* 2005). Some studies have suggested that plants have the ability to absorb and utilize AAs as an N source. For example, using three concentrations (10, 25, 100 μM), Zhou *et al.* (2007) showed that cysteine at 25 μM positively affected the growth of maize seedlings while Jämtgård *et al.* (2008) could improve barley (*Hordeum vulgare* L.) growth with 2, 5, 10 or 25 μM of serine, GA, glycine, arginine and alanine. Zhang *et al.* (2009) demonstrated that AAs, when applied exogenously, significantly increased tomato seedling growth, chlorophyll (Chl) content, micronutrient [iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn)], absorption carotenoid content, and peroxidase (POD) and superoxide dismutase (SOD) activity in leaves more than when earthworm mucus was used. In general, both earthworm mucus and AAs improved the accumulation of Zn, Fe, Mn and Cu (microelements) in tomato seedlings, and the effect of earthworm mucus (as a source of HA) was larger than that of AAs, although the exact trend of accumulation depended on the seedling organ (i.e., in roots, stems and leaves) (Zhang *et al.* 2009). Wang *et al.* (2008) compared the use of nitrate (NO_3^-) and two AAs, glutamic acid (Glu), and glutamine (Gln), at six nitrate-nitrogen (NO_3^- -N)/amino acid-nitrogen (AA-N) molar ratios (100:0, 80:20, 60:40, 40:60, 20:80, 0:100) in nutrient solutions on the growth of 15-day-old pak choi (*Brassica chinensis* L.) seedlings. They found that the composition and concentrations of AA in plants changed when N source was changed to AA from ammonium (NH_4^+) or NO_3^-, or when 80% was applied as NO_3^- -N and 20% as AA-N.

Hydroponic systems generally increase yield by different ratios depending on the plant and the hydroponic system, and diminish water supply by manipulating the nutrient solution. AAs and HA normally exist in humified organic materials (e.g., Muscolo *et al.* 2013) such as leonardites, peat and vermicompost. However, the use of these materials has rarely, if ever, been studied in hydroponic systems. In this study, we assess for the first time the use of HA and GA in a tomato hydroponic system, since tomato is a model plant.

MATERIALS AND METHODS

Plant material and experimental design

Tomato (*Lycopersicon esculentum* Mill. cv. "Hongyangli") seeds were purchased from China and were sown into a pot filled with vermiculite and perlite [2:2, volume/volume (v/v)] until they germinated. Seedlings at the 4–5 leaf stage (approx. 5 weeks old) were fertigated for 1 week with half-strength nutrient solution (NS) and then with full-strength NS, the composition of which is described in Table 1. The NS was automatically provided to plants for 10 min every 6 h through a drip irrigation system, and when plants had induced fruits, the time interval decreased to every 4 h. To prevent the accumulation of salt, the pot was washed with water every 2 weeks.

The experiment was conducted in an environmentally controlled greenhouse with an average day/night temperature of 25/17°C, 70–90% relative humidity and natural light conditions. Plants were placed in the greenhouse in a completely randomized design with six replications per treatment.

HA (composition described in Table 2) was extracted from a humified forest soil sampled from Mount Jinyun, Chongqing, China. HA was incorporated into NS at 25 mg L^{-1} (HA1) or 50 mg L^{-1} (HA2) and GA at 100 mg L^{-1} (GA) once a week.

NS was added as necessary approximately every 3 d and, to prevent the accumulation of salt in media, plantlets were irrigated with pure water every 2 weeks. Seedlings were grown on a wire trellis to prevent stem cracking, and axillary buds were removed from the stems of each plant.

Table 1 Nutrient solution* for tomato (*Lycopersicon esculentum* Mill.) planted in soilless media

Component	Dosage (g 1000 L^{-1})
$Ca(NO_3)_2{\cdot}4H_2O$	978.6
KNO_3	525
KH_2PO_4	200
$MgSO_4{\cdot}7H_2O$	250
EDTA NaFe	9.83
$MnSO_4{\cdot}H_2O$	1.99
$H_3BO_3{\cdot}H_2O$	2.43
$ZnSO_4{\cdot}7H_2O$	0.498
$CuSO_4{\cdot}5H_2O$	0.187
$Na_2MOO_4{\cdot}2H_2O$	0.09

*Recipe used by Chinese scientists in Zheijang University. $Ca(NO_3)_2{\cdot}4H_2O$, calcium nitrate; KNO_3, potassium nitrate; KH_2PO_4, monopotassium phosphate; $MgSO_4{\cdot}7H_2O$, magnesium sulphate; EDTA NaFe, sodium iron ethylenediaminetetraacetic acid; $MnSO_4{\cdot}H_2O$, manganese sulphate; $H_3BO_3{\cdot}H_2O$, boric acid; $ZnSO_4{\cdot}7H_2O$, zinc sulphate; $CuSO_4{\cdot}5H_2O$, copper sulphate; $Na_2MOO_4{\cdot}2H_2O$, sodium molybdate.

Table 2 The composition of humic acid (HA) extracted from a humified forest soil (measured per 100 g)

Composition	Dosage
N% (dry matter)	0.571
P% (dry matter)	0.034
Water content (%)	16.043
pH	4.5 ± 0.5

N, nitrogen; P, phosphorus.

Plant fresh weight (FW) was measured by an analytical balance. Individual plant parts were dried in an oven at 70°C for 48 h, after which dry weight was determined.

Photosynthetic rate [μmol carbon dioxide (CO_2 m^{-2} s^{-1}], stomatal conductance (mmol CO_2 m^{-2} s^{-1}) and transpiration (mmol m^{-2} s^{-1}) were determined with a portable unit (Li-Cor, Li-3000, USA). They were determined twice during tomato growth on five fully expanded leaves, first at the vegetative stage (photosynthesis 1) then at the reproductive stage (photosynthesis 2). The vegetative stage began at transplanting and ended at anthesis of the first raceme (from 26–32 d after transplanting or when five compound leaves formed), and growth thereafter was considered to be the reproductive stage. The fruits were harvested three times when they turned red and were weighed to determine yield, which was recorded in g $plant^{-1}$.

Mesophyll conductance was calculated by dividing photosynthetic rate by sub-stomatal CO_2 concentration (Ahmadi and Siosemardeh 2005). Photosynthetic water use efficiency (PWUE) (μmol CO_2 m^{-2} s^{-1}) was calculated by dividing photosynthesis rate by stomatal conductance (Ahmadi and Siosemardeh 2005). Chl content (SPAD value) was measured by using a nondestructive dual-wavelength Chl meter (SPAD-502, Minolta Corp., Ramsey, NJ, USA).

Biochemical parameters assessed

Protein content (μg g^{-1}) was determined by the method of Bradford (1976) using bovine serum albumin (Sigma) as a standard.

Titratable acidity (TA; %) was determined by titration of 10 mL juice from the fruit with 0.01 M sodium hydroxide (NaOH) according to the method of the Association of Official Agricultural Chemists (AOAC 1980).

Sample preparation for enzymes assay: The material for the SOD (Enzyme Commission (EC) 1.15.1.1) and POD (EC 1.11.1.x) activities and the malondialdehyde (MDA) content was prepared according to Haghighi *et al.* (2010). After thoroughly washing fully expanded leaves with deionized water, these were homogenized with a mortar and pestle under chilled conditions in 50 mM phosphate buffer. The homogenate was centrifuged at 12,000 rpm for 10 min at 4°C, and the supernatants were used for the enzyme assays.

SOD activity (U mg^{-1} FW) was assayed according to Beauchamp and Fridovich (1971) with a slight modification. The assay mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 9.9 mM L-methionine, 57 μM nitro tetrazolium blue chloride (NBT), 0.025% [weight/volume (w/v)] Triton X-100 and 0.0044% (w/v) riboflavin. The photo-reduction of NBT (the formation of purple formazan) was measured at 560 nm. One unit of SOD activity was defined as the volume of extract that caused the inhibition of the photo-reduction of NBT by 50%.

POD activity (U g^{-1} FW min^{-1}) was measured using the method of Chandlee and Scandalios (1984) with a modification. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 6.1), 1% guaiacol (w/v), 0.4% hydrogen peroxide (H_2O_2, v/v) and 1 mL enzyme extract. The increase in absorbance due to the oxidation of guaiacol at 470 nm was measured. The enzyme activity was calculated as the μM of guaiacol oxidized min^{-1} (g fresh weight) at 25 ± 2°C.

MDA content (nmol g^{-1}) was assayed by the method described by Dhindsa *et al.* (1981) with a modification. Extracted supernatant (2 mL) was mixed with 2 mL thiobarbituric acid (0.6%) and heated at 100°C for 10 min. The mixture was subsequently cooled and centrifuged at 10,000 × *g* for 10 min, and the absorbance of the supernatant was measured at 532, 600 and 450 nm. The MDA concentration was calculated according to the following formula:

$$\text{MDA concentration} = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450} \quad (1)$$

NO_3 concentration (nmol NO_3 g^{-1} FW min^{-1}) in potassium chloride (KCl) extract was determined by the micro-Kjeldahl method (Bremner 1996).

Sugar content (°Brix) was assessed with a hand refractometer (Carl Zeiss, Jena, Germany) from the juice (2–3 drops) of ripe fruits, which was prepared by cutting fruits, blending them in a mixer and then using several drops of the juice in the assay.

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) with Statistix 8 (Tallahassee FL, USA), and the means with standard deviation were compared by least significant difference (LSD) with $P < 0.05$ indicating significantly different treatments.

RESULTS

Effect of GA and HA application on some qualitative and quantitative characteristics of tomato

HA2 improved protein content and tomato yield although the results were not significant. While there was no significant effect of HA on yield, NO_3, sugar and TA content, GA enhanced sugar by 47% and protein content (7-fold higher) significantly compared to the control. The simultaneous application of GA and HA improved sugar and protein content and yield of tomato significantly compared to the control, especially at a high concentration (50 mg L^{-1}) (Table 3).

Table 3 Effect of glutamic acid (GA) and humic acid (HA) on protein, nitrate (NO_3), sugar content and titratable acidity of tomato (*Lycopersicon esculentum* Mill.)

Treatments	Yield (g plant^{-1})	Protein (μg g^{-1})	NO_3 (nmol NO_3 g^{-1} FW min^{-1})	Sugar content (°Brix)	Titratable acidity (%)
NS	2019.12 ± 56.05 c	$1.37 \times 10^{-3} \pm 4 \times 10^{-4}$ c	2.98 ± 0.11 a	2.36 ± 0.50 b	0.049 ± 0.001 a
HA1	2013.64 ± 8.78 c	$1.52 \times 10^{-3} \pm 1 \times 10^{-4}$ bc	2.94 ± 0.90 a	2.53 ± 0.04 b	0.050 ± 0.017 a
HA2	2412.48 ± 39.11 bc	$1.77 \times 10^{-3} \pm 4 \times 10^{-4}$ bc	2.02 ± 0.10 a	2.68 ± 0.50 b	0.048 ± 0.017 a
GA	2522.81 ± 28.31 bc	$7.15 \times 10^{-3} \pm 4 \times 10^{-3}$ a	2.99 ± 0.10 a	3.48 ± 0.10 a	0.042 ± 0.002 a
HA1 + GA	2939.69 ± 84.90 ab	$6.77 \times 10^{-3} \pm 5 \times 10^{-3}$ ab	2.03 ± 0.90 a	3.40 ± 0.20 a	0.044 ± 0.006 a
HA2 + GA	3311.08 ± 40.47 a	$9.26 \times 10^{-3} \pm 4 \times 10^{-3}$ a	2.06 ± 0.10 a	3.53 ± 0.10 a	0.051 ± 0.005 a

Within each column, means ± standard deviation followed by the same letter are not significantly different at $P < 0.05$ according to the Least Significant Difference test. NS, nutrient solution.

Effect of GA and HA application on antioxidant activity of tomato

SOD and POD activity was not affected by HA1/HA2 and the incorporation of GA into NS. Even though MDA content increased at high concentrations of HA2 combined with GA, the increment was not significant (Table 4).

Effect of GA and HA application on photosynthetic activity of tomato

The photosynthetic rate increased following the application of HA1/HA2 and GA. HA improved the photosynthetic rate more than GA (3-fold higher from 2.29 in NS to 8.15 in HA1) while HA2 was even more effective (4-fold higher from 2.29 in NS to 1183 in HA2). The combination of HA1/HA2 and GA improved the photosynthetic rate more effectively (Table 5). HA1/HA2 and GA, when applied separately, did not change the Chl content, although the simultaneous use of both enhanced Chl content (Table 5).

GA and HA did not significantly affect stomatal conductance, transpiration or PWUE of tomato (Table 6). Mesophyll conductance improved when a high concentration of HA (i.e., HA2) was applied together with GA by increasing all four photosynthetic parameters (Table 6).

DISCUSSION

Zhang *et al*. (2009) tested the effect of earthworm mucus and AA (undefined) on tomato. They reported that earthworm mucus was more effective due to increased chlorophyll content, antioxidative enzyme activities, and essential microelement uptake and transport in tomato seedlings. They reported that earthworm mucus was more effective than AA on growth, due to higher levels of nutritional elements and IAA-like materials in earthworm mucus. Our results similarly illustrated that HA1/HA2 and GA could improve the growth of tomato, more so when used simultaneously. AA increases the absorption of essential elements and results in plant growth (Nardi *et al*. 2002; Rai 2002). HA extracted from cattle manure, food waste and paper waste vermicomposts positively affected the growth, number of flowers and fruits of pepper (Norman *et al*. 2006), HA from leonardite affected the number of harvested flowers per plant and vase life of gerbera (Nikbakht *et al*. 2008), HA from vermicompost increased shoot and root biomass as well as nutrient content of pineapple (*Ananas comosus* (L.) Merr.) (Borges Baldotto *et al*. 2010) while HA from vermicompost improved plant length, fruit weight and yield of strawberry (Shehata *et al*. 2011). Shehata *et al*. (2011) also showed that a foliar application of HA and 20 AAs increased plant length and fruit weight but did not have any effect on fruit diameter (Shehata *et al*. 2011). GA enhanced sugar content although the simultaneous application of GA and HA1/HA2 also improved sugar content (Table 3). Similarly, HA or/and AA increased the sugar content of strawberry (Azarmi *et al*. 2009; Shehata *et al*. 2011) and, as a consequence of the enhanced growth of plants, fruit quality also improved

Table 4 Effect of glutamic acid (GA) and humic acid (HA) on superoxide dismutase (SOD) and peroxidases (POD) activity and amount of malondialdehyde (MDA) in tomato (*Lycopersicon esculentum* Mill.)

Treatments	SOD activity (U/mg FW)	MDA content (nmol/g)	POD activity (U/g FW/min)
NS	137.40 ± 14.9 a	$8.36 \times 10^{-4} \pm 1.5 \times 10^{-4}$ ab	0.37 ± 0.3 a
HA1	119.02 ± 34.26 a	$7.76 \times 10^{-4} \pm 1.3 \times 10^{-4}$ ab	0.34 ± 0.8 a
HA2	137.23 ± 26.29 a	$6.55 \times 10^{-4} \pm 4.6 \times 10^{-4}$ ab	0.41 ± 0.4 a
GA	129.22 ± 42.80 a	$3.85 \times 10^{-4} \pm 4.8 \times 10^{-4}$ b	0.46 ± 0.4 a
HA1 + GA	103.29 ± 23.94 a	$5.97 \times 10^{-4} \pm 1.8 \times 10^{-4}$ ab	0.40 ± 0.5 a
HA2 + GA	128.41 ± 24.90 a	$1.08 \times 10^{-4} \pm 4.0 \times 10^{-4}$ a	0.51 ± 0.5 a

Within each column, means ± standard deviation followed by the same letter are not significantly different at $P < 0.05$ according to the Least Significant Difference test. NS, nutrient solution; FW, fresh weight.

Table 5 Effect of glutamic acid (GA) and humic acid (HA) on photosynthesis rate and chlorophyll content of tomato (*Lycopersicon esculentum* Mill.)

Treatments	Photosynthesis 1* (μmol CO_2 m^{-2} s^{-1})	Photosynthesis 2** (μmol CO_2 m^{-2} s^{-1})	Chlorophyll (SPAD value)
NS	2.2 ± 1.15 c	9.9 ± 0.86 c	36.3 ± 2.23 c
HA1	8.1 ± 1.02 b	11.6 ± 1.0 b	36.4 ± 2.45 c
HA2	11.8 ± 0.93 ab	13.3 ± 1.13 ab	37.9 ± 1.98 c
GA	9.6 ± 2.01 b	11.0 ± 0.7 bc	35.5 ± 2.00 c
HA1 + GA	12.2 ± 1.02 a	11.9 ± 0.65 b	49.1± 1.81 b
HA2 + GA	12.7 ± 1.10 a	14.6 ± 1.06 a	64.1 ± 2.08 a

Within each column, means ± standard deviation followed by the same letter are not significantly different at $P < 0.05$ according to the Least Significant Difference test. *Photosynthesis 1 = photosynthesis during the vegetative stage. **Photosynthesis 2 = photosynthesis during the reproductive stage. NS, nutrient solution; CO_2, carbon dioxide.

(Rajbir *et al.* 2008). On the other hand, tomato yield did not change significantly in response to GA. The reports on the effect of AA on tomato yield are conflicting: Neeraja and Reddy (2005) showed an increase in yield while Cerdán *et al.* (2006) indicated no significant effect. Our result indicates a positive effect of GA, which is an AA, on tomato yield (Table 3). HA2 increased protein content significantly (7-fold). No previous studies exist on how HA can cause such a high increase in protein content, this study being the first report. One possible reason could be the positive effect that HA has on growth and photosynthesis, directly resulting, through increased biomass, in increased protein content.

Although HA increased NO_3 uptake in oat seedling roots a few days after application, HA did not significantly affect NO_3 absorption (Nardi *et al.* 1991), possibly because of the molecular size of HA, the pH of nutrient solution, HA concentration and the location of NO_3 accumulation. The influence of HA on NO_3 absorption depends on the origin and structure of the HA: HA with low molecular weight fractions has gibberellin-like activity and can increase NO_3 uptake (Nardi *et al.* 2002). HA, by decreasing the pH at the surface of the plasma membranes in root cells, can facilitate NO_3 uptake (Nardi *et al.* 2002) although in the present research, the pH of NS was fixed at 5.8–6. Zhang *et al.* (2009) concluded that a high concentration of HA was most effective, so it is possible that the concentrations used for tomato in our study were not high enough to cause a significant effect on NO_3 uptake. Finally, Nardi *et al.* (1991) suggested that NO_3 taken up by roots was significantly exposed to low-molecular weight HA and subsequently transported to target organs of the plant. In the present research, NO_3 of roots and the molecular size of HA were not part of our experimental objectives and therefore were not measured. The mechanism thus remains to be clearly elucidated.

GA increased the fruit sugar content, but the effect of AA on sugar production in fruits is still not clear.

Zhang *et al.* (2009) revealed that exogenously applied AAs significantly increased the SOD and POD activities in tomato seedlings leaves, and found that AAs increased the SOD activity more efficiently than earthworm mucus. There are no reports supporting a change in MDA content by either GA or HA.

Pant *et al.* (2009) reported that pak choi treated with vermicompost containing HA had a lower antioxidant activity than the control; they suggested that poor growth and low N concentration caused a high level of antioxidant activity in this leafy vegetable. In this study, no N deficiencies in tomato were observed while growth was promoted by HA and GA; no increase in SOD and POD was recorded in the present study.

Zhang *et al.* (2009) found that earthworm mucus and AAs increased the Chl content of tomato seedlings' leaves and improved plant growth. Nardi *et al.* (2002) indicated some possible reasons why HA is able to increase the photosynthetic rate: firstly, HA improved the photosynthetic rate by enhancing the Chl content in tomato, although this is not supported by our data. Secondly, they revealed that HA increased the soluble sugar content in tomato, although in this study it was not significant (Table 3). Thirdly, HA interferes with the enzyme activity involved in carbohydrate metabolism, and is later used in photosynthesis. Finally, HA appears to increase the photosynthetic rate by stimulating enzyme activities related to the

Table 6 Effect of glutamic acid (GA) and humic acid (HA) on stomatal and mesophyll conductance, transpiration and photosynthetic water use efficiency (PWUE) of tomato (*Lycopersicon esculentum* Mill.)

Treatments	Stomatal conductance (mmol CO_2 m^{-2} s^{-1})	Transpiration (mmol m^{-2} s^{-1})	Mesophyll conductance (mmol CO_2 m^{-2} s^{-1})	PWUE (μmol CO_2 m^{-2} s^{-1})
NS	0.56 ± 0.13 a	31.62 ± 0.71 a	$25 \times 10^{-3} \pm 1.0 \times 10^{-4}$ c	18.24 ± 4.32 a
HA1	0.71 ± 0.30 a	31.67 ± 0.61 a	$29 \times 10^{-3} \pm 2.0 \times 10^{-3}$ b	17.72 ± 3.68 a
HA2	0.64 ± 0.29 a	31.73 ± 0.56 a	$28 \times 10^{-3} \pm 1.0 \times 10^{-4}$ bc	18.68 ± 4.02 a
GA	0.58 ± 0.13 a	31.77 ± 0.68 a	$28 \times 10^{-3} \pm 1.0 \times 10^{-4}$ bc	18.92 ± 3.97 a
HA1 + GA	0.58 ± 0.19 a	31.82 ± 0.58 a	$30 \times 10^{-3} \pm 1.0 \times 10^{-4}$ b	18.21 ± 3.75 a
HA2 + GA	0.70 ± 0.12 a	31.87 ± 0.60 a	$37 \times 10^{-3} \pm 1.0 \times 10^{-4}$ a	19.71 ± 3.78 a

Within each column, means ± standard deviation followed by the same letter are not significantly different at $P < 0.05$ according to the Least Significant Difference test. NS, nutrient solution; CO_2, carbon dioxide.

photosynthetic sulphate reduction pathway. It has been suggested that HA entering the plant cell and the functional groups of HA can serve as supplementary sources of respiratory catalysts to regulate oxidation and/or reduction (Patil *et al*. 2011), or to increase cell membrane permeability, respiration and photosynthesis (Gulser *et al*. 2010). HA has also been shown to positively affect antioxidant activity and photosynthesis of lettuce (Haghighi *et al*. 2012; Haghighi 2013). The effect of HA on plant transpiration has not been intensively studied (Nardi *et al*. 2002) although the few studies conducted on tomato and beet (Nardi *et al*. 2002) show that HA stimulated transpiration by increasing O_2 consumption and POD activity, unlike the findings of Haghighi (2013) in which there were no significant changes in transpiration of tomato following the application of HA.

As Nardi *et al*. (2002) revealed in their review, the effect of HA on plant respiration has not been intensively studied. The few studies conducted on tomato and beet (*Beta vulgaris* L.) (Nardi *et al*. 2002) show that HA stimulated transpiration by increasing O_2 consumption and POD activity, unlike the findings of our study in which there were no significant changes in transpiration or POD activity (Table 4 and 6).

Delfine *et al*. (2005) indicated that HA did not affect photosynthesis and stomatal conductance in durum wheat (*Triticum durum* L.). Similarly, in our study, HA1/HA2 did not change the stomatal conductance of tomato (Table 6).

To the best of our knowledge, there are no other reports on the effect of HA and AA on PWUE and mesophyll conductance. However, to better try to explain the possible mechanism, CO_2 is transferred from the atmosphere to the sub-stomatal internal cavities during photosynthesis, and then to the chloroplast stroma located in the leaf mesophyll. Thereafter, CO_2 diffuses into the mesophyll in a process called mesophyll conductance, a diffusion process that includes three stages: conductance through intercellular air spaces, conductance through cell walls and conductance through the liquid phase inside cells. A decrease in mesophyll conductance, by reducing the CO_2 concentration in chloroplasts, can limit photosynthesis. Mesophyll conductance is not constant and depends on species and changes in response to environmental factors (Flexas *et al*. 2008; Duan *et al*. 2011). With the application of HA1/HA2 and GA, mesophyll conductance was vastly improved (Table 6). Although the physiological mechanism of these changes is not clear yet, it can be concluded that as mesophyll conductance increased, so too did photosynthesis, showing a direct link between the two.

As stomatal conductance increases, PWUE decreases (Ahmadi and Siosemardeh 2005). The importance of mesophyll conductance and PWUE is still neglected in most studies, with only a few studies focusing on this phenomenon (Duan *et al*. 2011). In our study, we found that HA and GA did not affect the PWUE in tomato (Table 6). No other reference studies exist in the literature on the effect of substrate additives on PWUE.

CONCLUSION

HA and GA increased Chl content and improved photosynthesis, thus enhancing growth and yield. The simultaneous effect of HA and GA, especially at a high level of HA, suggests that a greater concentration (up to 50 mg L^{-1}) of either could be more effective than the individual use of either. Therefore, further studies using higher concentrations of HA and GA in nutrient solution are required. HA1/HA2 and GA did not improve photosynthesis through stomatal parameters like stomatal conductance, transpiration and PWUE. Rather, plant growth was improved by increasing Chl content and probably through enzyme activity in the cycle of photosynthesis in chloroplasts.

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REFERENCES

Adani F, Genevini P, Zacheo P, Zocchi G 1998: The effect of commercial humic acid on tomato plant growth and mineral nutrition. *J. Plant Nutr*., **21**, 561–575.

Ahmadi A, Siosemardeh A 2005: Investigation on the physiological basis of grain yield and drought resistance in wheat: leaf photosynthetic rate, stomatal conductance, and non-stomatal limitations. *Int. J. Agric. Biol*., **7**, 807–811.

AOAC (Association of Official Agricultural Chemists) 1980: Official methods of analysis, 13th ed. Australian Government Publishing Service, Washington, DC.

Ayuso M, Hernández T, García C, Pascual JA 1996: A comparative study of the effect on barley growth of humic substances extracted from municipal wastes and from traditional organic materials. *J. Sci. Food Agric*., **72**, 493–500.

Azarmi R, Giglou MT, Hajieghrari B 2009: The effect of sheep-manure vermicompost on quantitative and qualitative properties of cucumber (*Cucumis sativus* L.) grown in the greenhouse. *Afr. J. Biotechnol*., **8**, 4953–4957.

Azarpour E, Motamed MK, Moraditochaee M, Bozorgi HR 2012: Effects of bio, mineral nitrogen fertilizer management, under humic acid foliar spraying on fruit yield and several traits of eggplant (*Solanum melongena* L.). *African J. Agric. Res*., **7**, 1104–1109.

Beauchamp C, Fridovich I 1971: Superoxide dismutase; improved assays and an assay applicable to acrylamide gels. *Anal. Biochem*., **44**, 276–287.

Bohme M, Thi-Lua H 1997: Influence of mineral and organic treatments in the rhizosphere on the growth of tomato plants. *Acta Hortic*., **450**, 161–168.

Borges Baldotto LE, Altoé Baldotto M, Canellas LP, Smith RB, Olivares FL 2010: Growth promotion of pineapple "Vitória" by humic acids and *Burkholderia* spp. during acclimatization. *R. Bras. Ciências Solo*., **34**, 1593–1600.

Bradford MM 1976: A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., **72**, 248–254.

Bremner JM 1996: Nitrogen total. *In* Methods of Soil Analysis, Part 3: Chemical Methods. Ed. Sparks DL, pp. 1085–1121. Soil Science Society of America Book Series No. 5, American Society of Agronomy, Madison, WI.

Cerdán M, Sánchez SA, Oliver M, Juárez M, Sánchez AJJ 2006: Effect of foliar and root application of amino acid on iron uptake by tomato plants. *Acta Hortic*., **830**, 481–488.

Chandlee JM, Scandalios JG 1984: Analysis of variants affecting the catalase development program in maize scutellum. *Theor. Appl. Genet*., **69**, 71–77.

Delfine S, Tognetti R, Desiderio EO, Alvino A 2005: Effect of foliar application of N and humic acids on growth and yield of durum wheat. *Agron. Sustainable Dev*., **25**, 183–191.

Dhindsa RS, Plumb-Dhindsa P, Thorpe TA 1981: Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot*., **32**, 93–101.

Duan B, Ran F, Zhang X, Zhang Y, Korpelainen H, Li CH 2011: Long-term acclimation of mesophyll conductance, carbon isotope discrimination and growth in two contrasting *Picea asperata* populations exposed to drought and enhanced UV-B radiation for three years. *Agric. Forest Meteorol*. **151**, 116–126.

Eyheraguibel B, Silvestre J, Morard P 2008: Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize. *Bioresource Technol*., **99**, 4206–4212.

Flexas J, Ribas-Carbo M, Diaz-Espejo A, Galmes J, Medrano H 2008: Mesophyll conductance to CO_2: Current knowledge and future prospects. *Plant Cell Environ*., **31**, 602–662.

Gulser F, Sonmez F, Boysan S 2010: Effects of calcium nitrate and humic acid on pepper seedling growth under saline condition. *J. Environl. Biol*., **31**, 873–876.

Haghighi M 2013: The effect of humic and glutamic acids in nutrient solution on the N metabolism in lettuce. *J. Sci. Food Agr*., **92**(15), 3023–3028.

Haghighi M, Kafi M, Fang P 2012: Photosynthetic activity and N metabolism of lettuce as affected by humic acid. *Int. J. Vegetable Sci*., **18**, 182–189.

Haghighi M, Kafi M, Fang P, Luo GX 2010: Humic acid decreased hazardous of cadmium toxicity on lettuce (*Lactuca sativa* L.). *Veg. Crops Res. Bull*. **72**, 49–61.

Jämtgård S, Näsholm T, Huss-Danell K 2008: Characteristics of amino acid uptake in barley. *Plant Soil*, **302**, 221–231.

Jones DL, Shannon D, Junvee-Fortune T, Farrar JF 2005: Plant capture of free amino acids is maximized under high soil amino acid concentrations. *Soil Biol. Biochem*., **37**, 179–181.

Muscolo A, Teixeira da Silva JA, Sidari M 2013: Screening of the biological effects of water soluble soil phenols and soil humic extracts. *Acta Physiol. Plant*., **35**, 309–320.

Nardi S, Concherig G, Dell'Agnola G, Scrimin P 1991: Nitrate uptake and ATPase activity in oat seedlings in the presence of two humic fractions. *Soil Biol. Biochem*., **23**, 833–836.

Nardi S, Pizzeghello D, Muscolo A, Vianello A 2002: Physiological effects of humic substances on higher plants. *Soil Biol. Biochem*., **34**, 1527–1536.

Neeraja GIP, Reddy BG 2005: Effect of growth promoters on growth and yield of tomato cv. Marutham. *J. Res. Angrau*., **33**(3), 68–70.

Nikbakht A, Kafi M, Babalar M, Xia Y.P 2008: Effect of humic acid on plant growth, nutrients uptake and postharvest life of gerbera. *J. Plant Nutr*., **31**, 2155–2167.

Norman Q, Arancon C, Edwards A, Lee S, Byrne R 2006: Effects of humic acids from vermicomposts on plant growth. *Eur. J. Soil Biol*., **42**, 865–869.

Pant AP, Radovich TJK, Hue NV, Talcott ST, Krenek KA 2009: Vermicompost extracts influence growth, mineral nutrients, phytonutrients and antioxidant activity in pak choi (*Brassica rapa* cv. Bonsai, Chinensis group) grown under vermicompost and chemical fertilizer. *J. Sci. Food Agric*., **89**, 2383–2392.

Patil RB, More AD, Kalyankarm M, Wadje S 2011: Effect of potassium humate on nutrients uptake of *Glycine max, Phaseolus mungo* and *Triticum aestivum*. *Plant Sci. Feed*, **1**(10), 174–178.

Rai VK 2002: Role of amino acids in plant responses to stresses. *Biol. Plant*., **45**, 481–487.

Rajbir S, Sharma RR, Satyendra K, Gupta RK, Patil RT 2008: Vermicompost substitution influences growth, physiological disorders, fruit yield and quality of strawberry (*Fragaria* × *ananassa* Duch.). *Bioresour. Technol*., **99**, 8507–8511.

Shehata SA, Gharib AA, El-Mogy MM, Gawad AKF, Shalaby EA 2011: Influence of compost, amino and humic acids on the growth, yield and chemical parameters of strawberries. *J. Med. Plants Res*., **5**, 2304–2308.

Wang H, Wu L, Zhu Y, Tao Q 2008: Growth, nitrate accumulation, and macronutrient concentration of pakchoi as affected by external nitrate-N: amino acid-N ratio. *J. Plant Nutr*., **31**, 1789–1799.

Zhang S, Hu F, Li H, Li X 2009: Influence of earthworm mucus and amino acids on tomato seedling growth and cadmium accumulation. *Environ. Pollut*., **157**, 2737–2742.

Zhou ZG, Zhou JM, Li RY, Wang HY, Wang JF 2007: Effect of exogenous amino acids on Cu uptake and translocation in maize seedlings. *Plant Soil*, **292**, 105–117.