Research Article

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Stimulation Effects of Foliar Applied Glycine and Glutamine Amino Acids on Lettuce Growth

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Abstract: Amino acids have various roles in plant metabolism, and exogenous application of amino acids may have benefits and stimulation effects on plant growth and quality. In this study, the growth and nutrient uptake of Romain lettuce (*Lactuca sativa* subvar *Sahara*) were evaluated under spray of glycine or glutamine at different concentrations of 0 (as control), 250, 500 and 1000 mg.L⁻¹, as well as a treatment of 250 mg.L⁻¹ glycine+250 mg.L⁻¹ glutamine. The results showed that there was significant increase in leaf total chlorophyll content under Gly250+Glu250, Gly250 and Glu1000 mg.L⁻¹ treatments, and in leaf carotenoids content under 250 mg.L⁻¹ glutamine spray compared with the control plants. Shoot fresh and dry weights were highest under 500 mg.L⁻¹ glycine, whereas root fresh weight was highest under 250 mg.L⁻¹ glycine spray. Foliar application of glycine and glutamine had no significant increase in leaf mineral concentrations except for iron, in which 1000 mg.L⁻¹ Gly spray resulted in significantly higher leaf Fe concentration compared with the control plants. Leaf vitamin C was increased at 250 and particularly 500 mg.L⁻¹ spray of glycine and glutamine compared with the control. Nevertheless, different amino acid treatments had no significant effect on plant height, leaf SPAD value, root dry weight, and leaf concentrations of N, K, Ca, Mg and Zn. The results indicate that foliar application of glycine and glutamine amino acids can have beneficial effects on lettuce growth, as higher fresh yield, leaf chlorophyll content and vitamin C were obtained by low to moderate concentrations of glycine and/or glutamine amino acids.

Keywords: fertilization, *Lactuca sativa*, leafy vegetable, nutrient uptake, root growth, vitamin C

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1 Introduction

In biology, amino acids have vital roles in cell life. Amino acids are among the most important primary metabolites within the plant cells. However, they are frequently regarded as secondary metabolites, particularly in the case of proline, glycine and betaine amino acids. Many physiochemical characteristics of plant cells, tissues and organs are influenced by the presence of amino acids (Rai 2002; Marschner 2011). They are the building units of proteins, as the main component of living cells that have vital roles in many cell metabolic reactions (Kielland 1994; Rainbird et al. 1984; Jones and Darrah 1993). In addition, amino acids have various important biological functions in plant cells including detoxification of toxins and heavy metals (Hussain et al. 2018; Rizwan et al. 2017; Bashir et al. 2018), optimizing the nutrient uptake, translocation and metabolism, vitamin biosynthesis, growth biostimulation, creating higher tolerance to environmental stresses such as drought, salinity and cold conditions, as well as in the synthesis and production of aminochelate fertilizers (Jeppsen 1991; Sharma and Dietz 2006; Souri and Hatamian 2019).

The exogenous application of amino acids has been shown to induce growth promotion effects on many crops (Cao et al. 2010; Amin et al. 2011; Khan et al. 2012; Mohammadipour and Souri 2019a; Fahimi et al. 2016). The growth stimulation effect of exogenous amino acids is particularly higher under adverse environmental conditions such as salinity or water stress (Rai et al. 2002; Cerdán et al. 2013; Sadak et al. 2015; Souri et al. 2018). Therefore, recently various amino acids, mainly glycine, have been incorporated into nutrient fertilizer formulation in the form of chelate or a simple complex, in order to improve the plant use efficiency of applied fertilizers (Fahimi et al. 2016; Souri and Hatamian 2019).

Application of amino acids such as glycine can have beneficial effects on yield and quality of leafy vegetable crops. Glycine is a proteinogenic amino acid and the smallest biological amino acid in cells with a molar mass of 75 g mol⁻¹. It is a hydrophilic and non-polar amino acid and due to its chemical structure, it can have acidic or

basic reaction in different mediums. Glycine has a simple industrial synthesis process and therefore attracted interests to be incorporated with nutrient elements to produce chelates in order to facilitate nutrient uptake and translocation in plants (Souri and Hatamian 2019). Nowadays it plays an important role in nutritional management of many plants, particularly horticultural crops (Souri and Hatamian 2019). Glutamine also is a vital amino acid in plant physiology with key roles in many metabolic processes, including nitrogen assimilation pathways (Amin et al. 2011; Marschner 2011). Glutamine, arginine and asparagine are the main amino acids involve in many metabolic and biochemical reactions in plants including detoxification of toxins, neutralizing the produced H^+ in ammonium fed plants and higher tolerance to stress conditions (Cao et al. 2010; Amin et al. 2011; Marschner 2011).

Lettuce is a major leafy vegetable crop that its safe production with good quality and enhanced nutritional value is quite important. High application rates of chemical fertilizer have caused serious environmental challenges and food quality reduction (Cao et al. 2011; Fahimi et al. 2016; Souri et al. 2017). Leafy vegetables, particularly lettuce, can accumulate high amounts of nitrate over nitrogen fertilizer application (Caruso et al. 2011; Shunka et al. 2017). Although amino acids can be a good source of nitrogen for plant uptake and utilization, they can have stimulating effects on plant root and shoot growth (Garcia et al. 2011; Shehata et al. 2011; Souri and Hatamian 2019). Therefore, in this study the bio-stimulation effects of glycine and glutamine in different concentrations, via foliar application, were evaluated on the growth of lettuce plants.

2 Material and Methods

In the present study, effects of different concentrations of glycine and glutamine amino acids were evaluated on growth characteristics of Romain lettuce (*Lactuca sativa* subvar *Sahara*) under greenhouse conditions. The study was performed during the spring of 2017 and at Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. A loamy- textured soil was used in the experiment. The soil physiochemical characteristics are presented in Table 1, and the soil analysis results indicate a moderate fertility of the soil. No further fertilization was applied to the soil. Black plastic pots with a volume of 5 L were filled with the soil and then watered up to 80% soil field capacity. After three days, 10 seeds were sown to a depth of 1-2 cm in the potted soil.

After germination, plants were thinned to the three biggest plants per pot and after two weeks they were reduced to one plant per pot. The plants were irrigated (up to 80% soil field capacity) daily, in the afternoon. The greenhouse temperature was $25 \pm 5^\circ C$, with a light intensity of $200 \mu mol m^{-2} s^{-1}$ and 70–75% air humidity.

Different concentrations of glycine and glutamine amino acids including 0, 250, 500 and 1000 $mg.L^{-1}$, as well as a treatment of 250 $mg.L^{-1}$ glycine+250 $mg.L^{-1}$ glutamine were sprayed on the lettuce plants. Pots were arranged in a completely randomized design with four replications, in which each pot represented a replicate. Different concentrations of glycine or glutamine amino acids (as treatments) were sprayed, 5 times on the plants, during a two month growth period. The first spray was done two weeks after seedling emergence, and the remaining sprays were done with one-week interval. Zero concentration of amino acids was considered as the control, and distilled water was sprayed on the control plants. Spray treatments were done in the morning, at one hour after sunrise, with a portable sprayer, by which the upper and lower surfaces of leaves were sprayed. A total amount of 90-100 mL of solution (by 5 sprays of each treatment) was applied on a single plant.

At harvest, plant height was measured using a tape and SPAD (The Soil and Plant Analysis Development) value of outer lettuce leaves was measured using a portable SPAD meter (Model SPAD-502 Plus Illinois, USA) with an average of 30 readings per plant, on middle part of leaves (Hatamian and Salehi 2017). Plants were cut at soil surface and roots were carefully separated from the soil particles by washing with tap water and then drying with tissue paper. The shoot and root fresh weights were measured using a digital scale. The shoot and root materials were then dried in an oven at $65^\circ C$ for 48 h, and thereafter the plant's dry weight was measured using a digital scale. Leaf chlorophyll (*a*, *b* and total) and carotenoids were determined using acetone extraction of 0.5 g of fresh leaf tissues (Khan et al. 2012; Kałuzewicz et al. 2018).

For determination of leaf vitamin C, 5 g of fresh leaves was crushed in a porcelain mortar in 10 mL of metaphosphoric acid (6%) and centrifuged at 4000 rpm for ten min. Five mL of the supernatant was transferred into an Erlenmeyer flask, and received 20 mL of metaphosphoric acid (3%). The titration of the extract was done by di-chlorophenol indophenols until the appearance of a rosa colour. The amount of vitamin C ($mg 100^{-1} g$ FW) was calculated accordingly and based on a standard curve of L-ascorbic acid concentrations of 0, 10, 20 and 40 $mg.L^{-1}$. Nitrogen (N) concentration of leaves was determined by the Kjeldahl method, potassium (K)

using flame photometry, and magnesium (Mg), calcium (Ca), Iron (Fe) and zinc (Zn) were determined using atomic absorption spectrophotometer. Data were analysed using SPSS 16 (SPSS Inc., Chicago IL) and comparison of means was performed by Duncan multiple range test at 5% level.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

The results of analysis of variance (Table 2) showed that the treatments had a significant effect on: leaf vitamin C concentration ($P=0.01$); shoot fresh and dry weight; root fresh weight; root length; total leaf chlorophyll; carotenoids; and leaf iron concentration at $P=0.05$, whereas the treatments had no significant effect on: plant height; leaf SPAD value; leaf chl a and chl b ; root dry weight; and leaf nutrients of N, K, Mg, Ca and Zn.

Comparison of means showed that application of different concentrations of glycine and glutamine amino acids tended to increase the total chlorophyll concentration of leaves; however, foliar application of glutamine at 1000 mg.L⁻¹, and glycine at 250, 500 and 1000 mg.L⁻¹, as well as Gly250+Glu250 treatment resulted in significantly higher total chlorophyll of leaves compared

with the control plants (Table 3). Leaf carotenoids concentration was increased by foliar application of 250 mg.L⁻¹ glutamine compared with the control plants, and there was no difference among other treatments (Table 3).

An increase in leaf pigmentation, due to application of amino acids, has also been reported in other studies (Atilio and Causin 1996; Amin et al. 2011; Fahimi et al. 2016; Mohammadipour and Souri 2019b). Higher chlorophyll content of leaves can be due to the stimulating effect of amino acids on chlorophyll biosynthesis and a simultaneous decrease in chlorophyll degradation, as well (Souri et al. 2017; Fahimi et al. 2016). Amino acids have a role in protection of cell components during stress. They can probably act against oxidation, peroxidation and degradation of cell components, particularly chlorophylls, and therefore they increase the life span of cells. The bio-protection effect of amino acids has been proposed in several studies (Rai 2002; Khan et al. 2012; Souri and Hatamian 2019). Amino acids are a reduced form of nitrogen and it has been shown that application of reduced forms of nitrogen, such as ammonium (Dehnavard et al. 2017; Forde and Clarkson 1999) and glycine (Fahimi et al. 2016; Souri et al. 2017), probably via restriction of leaf area expansion, can result in higher leaf chlorophyll content. The non-linear response of these physiological traits also supports this speculation.

Table 1: Physiochemical characteristics of soil used in the experiment

Texture	EC dSm ⁻¹	pH	O.C. %	Total N mgkg ⁻¹	Available P mgkg ⁻¹	Available K mgkg ⁻¹	Fe mgkg ⁻¹	Zn mgkg ⁻¹	Lime %
Loamy-Sand	1.79	7.62	0.78	750	11.61	385	5.5	1.6	7.3

EC and OC represent electric conductivity and organic carbon, respectively.

Table 2: Analysis of variance of physiochemical traits of lettuce under foliar application of treatments

Source of variation	df	Plant height	Root length	Shoot FW	Root FW	Shoot DW	Root DW	Leaf SPAD	Chl a	Chl b	Chl total
Treatments	7	14.3ns	38.1*	245.1*	36.6*	0.880*	2.4ns	10.8ns	31.4ns	1.96ns	46.4*
Error	16	5.193	19.9	1028.20	13.38	2.51	1.31	5.41	14.75	1.55	17.33
Total	24										

**, * and ns indicate statistically significant at $P=0.01$, $P=0.05$ and not significant, respectively.

- df, FW, DW, and SPAD mean degrees of freedom, fresh weight, dry weight and the Soil and Plant Analysis Development, respectively.

Continue of table 2

Source of variation	df	Carotenoids	Vitamin C	Leaf N	Leaf K	Leaf Mg	Leaf Ca	Leaf Fe	Leaf Zn
Treatments	7	0.520*	64.1**	0.058ns	0.061ns	0.003ns	0.065ns	49.5*	33.9ns
Error	16	0.709	12.11	0.063	0.049	0.002	0.061	37.16	34
Total	24								

**, * and ns – statistically significant at $P=0.01$, $P=0.05$ and not significant, respectively.

Table 3: Growth characteristics of lettuce plants under foliar application of different levels of glycine or glutamine

	Chl total mg g ⁻¹ FW	Carotenoid mg g ⁻¹ FW	Fe mg kg ⁻¹ DW
Control (distilled water)	18.8±2.3c	2.6±1.2b	60±5b
Gly1000	24±4.6bc	3.4±0.8ab	72±6a
Glu1000	27.1±3.1ab	3.2±0.2ab	68±5.6ab
Gly500	26.1±3.9abc	3.5±0.3ab	67.7±4.5ab
Glu500	23.2±3bc	3±0.5ab	64.7±6.4ab
Gly250	27.8±3.6ab	2.8±0.6ab	63.7±8ab
Glu250	24.3±4.5bc	3.7±0.7a	62±5.6ab
Gly250+Glu250	32.3±6.6a	2.8±0.1ab	69.7±7ab

Gly and Glu represent glycine and glutamine amino acids, respectively.

Data are average of four replications ± SD; comparison of means was done at 5% level of Duncan multiple ranges test.

Determination of leaf nutrient concentrations showed that only leaf Fe concentration was significantly affected by amino acids treatments. Leaf Fe was significantly increased under foliar application of 1000 mg L⁻¹ glycine, whereas the increase in leaf Fe by other amino acid treatments was not statistically significant compared with the control plants (Table 3). Application of amino acids, via the root system or foliar feeding, has been known to improve the uptake and concentrations of leaf nutrients

(Garcia et al. 2011; Cerdán et al. 2013; Pranckietiene et al. 2015; Mohammadipour and Souri 2019a and 2019b). Such effects have been widely reported for micronutrients, mainly iron and zinc (Jeppsen 1991; Zhou et al. 2007; Souri et al. 2018). Amino acids have affinity with nutrient elements and some amino acids can make chelates with nutrients. This characteristic has been widely used to improve the uptake and delivery of micronutrients, mainly Fe, in humans, animals and more recently in plants (Souri and Hatamian 2019). In plants, the benefits of amino acid chelated nutrients, are particularly enhanced under saline, drought or lime soil conditions (Rai 2002; Cerdán et al. 2013; Sadak et al. 2015; Pranckietiene et al. 2015).

Application of glycine in nutrient solution has resulted in higher leaf concentrations of N, K, Mg and Zn compared with the control plants (Mohammadipour and Souri 2019a). Nevertheless, in the present study, although the foliar application of amino acids showed no significant effects on other leaf nutrient concentrations, there was an increasing trend in most nutrients, under glycine or glutamine sprays (data not shown).

Lettuce shoot fresh and dry weights were increased by foliar applications of glycine and glutamine; however, plants treated with 500 mg.L⁻¹ glycine or Gly250+Glu250 had significantly higher shoot fresh weight compared with the control plants (Figure 1). Similarly, shoot dry weight was significantly improved with 500 and 1000 mg.L⁻¹ of glycine, as well as in Gly250+Glu250 treatment, compared

Figure 1: Shoot fresh and dry weight of lettuce plants under foliar application of glycine and glutamine amino acids. Data are average of four replications ± SD; comparison of means was done at 5% level of Duncan multiple ranges test

with the control plants (Figure 1). Increases in biomass and yield production of other crop plants, by foliar application of amino acids, has also been reported (Atilio and Causin 1996; Basanth and Mahesh 2018; Shaheen et al. 2010; Amin et al. 2011; Sourì et al. 2017). Such improvements can be mainly due to higher chlorophyll biosynthesis and photosynthesis rates, enhanced protein biosynthesis, and better tolerance to adverse climatic conditions (Näsholm et al. 2009; Khan et al. 2012; Sourì and Hatamian 2019). Better uptake and metabolism of nutrient elements can also play an important role in this regard, despite the leaf nutrient concentrations (except Fe) being non-significantly compared with the control plants. In celery, foliar application of a mix of amino acids, in concentrations of 500 and 700 ppm, increased plant height, shoot fresh and dry weights, leaf N concentration, leaf yield and leaf soluble carbohydrates (Shehata et al. 2011).

Plant overall root length (Figure 2) was not changed by foliar application of different concentrations of glycine or glutamine compared with the control plants; however, the longest plant root was in plants sprayed with the Gly250+Glu250 treatment, which showed significantly longer roots compared with those plants that were treated with 1000 or 500 mg.L⁻¹ glutamine. Root fresh weight (Figure 3) was increased only in plants treated with foliar application of 250 mg.L⁻¹ glycine compared with the control. Plants sprayed with 250 mg.L⁻¹ of glutamine or Gly250+Glu250 showed higher root fresh weight

compared with those plants that were sprayed with 500 mg.L⁻¹ of glutamine. Root growth may have been affected differently by amino acid treatments than shoot growth (Sourì and Hatamian 2019). There is no consensus on the similarity of root and shoot responses to a given treatment. Determination of root length and root fresh weight revealed that foliar application of 500 and 1000 mg.L⁻¹ of glutamine was less effective than its 250 mg.L⁻¹ or when 250 mg.L⁻¹ of glutamine was used with 250 mg.L⁻¹ of glycine. The mechanism remains unclear; however, reduced biosynthesis or translocation of one or more growth factors may be involved in these root growth responses (Marschner 2011). Reduced nitrate uptake due to the application of amino acids may induce restricted root growth. It has been shown that the supply of different amino acids to the roots of 5-day-old soybean seedlings, via nutrient solution or by immersion of the tip-cut cotyledons in a concentrated amino acid solution, inhibited the nitrate uptake, particularly by asparagine, glutamine and some other amino acids (Muller and Touraine 1992). Free amino acids could down regulate nitrate uptake and nitrate content in plants, as exogenously supplied amino acids and amides could decrease the uptake of nitrate by many plants (Muller and Touraine 1992). Changes in the biosynthesis of phytohormones and their translocation may also play a role in this regard (Amin et al. 2011; Sourì and Hatamian 2019).

In the present study, the most pronounced effect of foliar application of glycine and glutamine amino acids

Figure 2: Overall root length of lettuce under foliar application of glycine and glutamine amino acids. Data are average of four replications $\pm$ SD; comparison of means was done at 5% level of Duncan multiple ranges test

was the leaf vitamin C concentration (Figure 4). Foliar application of glycine and glutamine, at concentrations of 250 and 500 mg.L⁻¹, significantly increased leaf vitamin C content compared with the control plants. Nevertheless, the highest concentration of leaf vitamin C was in those plants treated with 500 mg.L⁻¹ of glycine or glutamine, and the lowest concentration was in the control plants and in those plants that were treated with 1000 mg.L⁻¹ of glycine (Figure 4).

L-ascorbic acid (or vitamin C) is one of the most important quality factors in leafy vegetable crops. This vitamin is a water-soluble compound and its content represents the overall quality of leafy vegetable products, as there is always a good correlation between its content and product quality (Souri et al. 2017; Souri and Hatamian 2019). It is well known that the amount of vitamin C, as a secondary metabolite in agricultural products, significantly decreases with restricted growth conditions.

Figure 3: Root fresh weight of lettuce plants under foliar application of glycine and glutamine amino acids. Data are average of four replications $\pm$ SD; comparison of means was done at 5% level of Duncan multiple ranges test

Figure 4: Leaf vitamin C concentration of lettuce plants under foliar application of glycine and glutamine amino acids. Data are average of four replications $\pm$ SD; comparison of means was done at 5% level of Duncan multiple ranges test

Its content varies greatly (from less than 10 mg to more than 230 mg/100 g FW) with genotype and environmental conditions in vegetable crops (Lintas 1992). The literature also varies greatly regarding the vitamin C content of lettuce, from about 3 mg (Szeto et al. 2002) to more than 60 mg/100 g FW (Lintas 1992). Drought, salinity and many other stresses can significantly reduce the content of this vitamin in plant tissues (Souri and Hatamian 2019). On the other hand, an increase in the quality (Junxi et al. 2010) and/or the leaf vitamin C content, by application of amino acids, has been also reported (Amin et al. 2011; Souri et al. 2017). However, Junxi et al. (2010) reported that in cabbage, foliar application of alanine and glycine reduced leaf vitamin C content. In the present study, promotion and optimization of vitamin C biosynthesis, as well as a reduction in its inactivation/degradation, are probably the mechanisms involved in its higher content under foliar application of glycine and glutamine amino acids. Enhanced chlorophyll concentration and photosynthesis are highly correlated to vitamin C content of plant tissues (Souri and Hatamian 2019), and such effects were also observed in the present study.

It seems that the growth stimulation of these amino acids is concentration dependent. Such effects were also obtained by Fahimi et al. (2016) who showed in cucumber plants, that foliar application of Biomin (an amino acid-chelated macro-micro-nutrients), at 1000 ppm, resulted in severe leaf chlorosis and necrosis symptoms, but foliar application of 500 ppm showed beneficial effects. Similarly, application of glycine at concentration of 40 mg.L⁻¹ in nutrient solution reduced many growth characteristics of coriander plants, whereas lower concentrations of 5 and 10 mg.L⁻¹ increased plant growth (Mohammadipour and Souri 2019a).

The bio-stimulating effect of amino acids on plant growth has been a matter of interest in various studies, and their protection against stress could be mainly by this mechanism (Pranckietiene et al. 2015; Rai 2002; Souri and Hatamian 2019). Foliar application of putrescine (in concentration of 25, 50 and 100 mg L⁻¹) and glutamine (at concentrations of 50, 100 and 200 mg.L⁻¹), alone or in combination, on onion (*Allium cepa* L. 'Giza 20') plants, significantly increased plant growth characteristics, including bulb yield and quality (Amin et al. 2011). Similarly, in garlic, three foliar applications of a commercial product of "Amino Total", containing a range of amino acids, significantly increased plant height, number of leaves per plant and marketable bulb yield (Shalaby and El-Ramady 2014).

Foliar spray of amino acids, or their chelated forms with nutrients (as aminochelate), is a modern

approach to improving plant growth, yield and many quality parameters, including their antioxidant capacity particularly under adverse climatic conditions (Souri and Hatamian 2019; Sadak et al. 2015). On the other hand, foliar application of amino acids can significantly increase the concentration of amino acids and proteins in plant tissues (Ashmead 1986; Jeppsen 1991; Amin et al. 2011). In plant cells, free amino acids may have several functions and benefits, including; semi-hormonal effects, osmoregulation and bioprotection, membrane stability, and frequently as an antioxidant for various vulnerable cell components including membranes (Rai 2002; Marschner 2011).

Application of different concentrations of glycine (0, 5, 10, 20 or 40 mg.L⁻¹) to coriander plants (*Coriandrum sativum* L.), via Hoagland nutrient solution, showed that leaf SPAD value, fresh and dry weights of shoots and roots were significantly increased by 10 mg.L⁻¹ glycine compared with the control plants. Application of glycine at 20 and particularly 40 mg.L⁻¹, in a nutrient solution, reduced many plant growth parameters, whereas leaf proline concentration was increased (Mohammadipour and Souri 2019a). In another study by these authors, it has been shown that sweet basil growth, including plant height, leaf SPAD value, shoot and root fresh weights, leaf concentrations of N, Ca, K, P, Fe and Zn but not Mg and Mn, as well as soluble solids (TSS) and vitamin C of plant leaf extract, were significantly increased and the number of flowering plants was decreased by soil application of glycine compared with the unfertilized control plants (Mohammadipour and Souri 2019b).

Our study showed that foliar application of moderate concentrations of glycine and/or glutamine can enhance lettuce growth, particularly leaf greenness, fresh weight and leaf vitamin C content. This achievement can highlight a no fertilizer approach to the production of leafy vegetable crops, which are healthy for people and society. This in turn can avoid many damages and negative effects of chemical fertilizers on soil physiochemical characteristics and its fertility. In addition, exogenous application of amino acids can increase the amino acids content of plant tissues and improve their food quality and nutritional value (Souri and Hatamian 2019).

4 Conclusion

In the present study, foliar application of glycine or glutamine in some concentrations showed stimulating effects on lettuce growth. The overall response of lettuce to foliar application of glycine was better than those of

glutamine for many traits. The beneficial effects of these amino acids on root growth were in low concentration of 250 mg.L⁻¹ treatments. Beneficial effects of glycine or glutamine spray on leaf mineral content was also observed; however, only leaf Fe was significantly increased by 1000 mg.L⁻¹ glycine spray compared with the control. Improvement in root and shoot growth, and leaf pigmentation and quality (vitamin C content) were mainly observed in low to moderate levels of these two amino acids. The results indicate that the application of these amino acids, as growth stimulants, has practical implications in the production of leafy vegetable crops such as lettuce and that their safe production is very important.

Conflict of interest: Authors declare no conflict of interest.

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