

# Effects of foliar application of glycine and glutamine amino acids on growth and quality of sweet basil

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All relevant data are within the paper and its Supporting Information files.

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**Abstract:** Amino acids have diverse roles in plant metabolism, and recently amino acid based fertilizers have been used largely in crop production systems. Despite most of these new fertilizers are formulated for foliar application; however, morphophysiological responses of crops to amino acids application have not yet been well documented. In the present study, foliar application of glycine or glutamine in different concentrations of 0 (distilled water), 250, 500, 1000 mg·L<sup>-1</sup>, as well as a treatment of 250 mg·L<sup>-1</sup> glycine + 250 mg·L<sup>-1</sup> glutamine were evaluated on growth of sweet basil (*Ocimum basilicum* L.) plants. The results showed that foliar application of glycine or glutamine at 1000 mg·L<sup>-1</sup> showed no improvement in comparison with control for all traits except for leaf L-ascorbic acid concentration that showed the highest value under these amino acids treatments. However, foliar application of these amino acids at 250 and particularly 500 mg·L<sup>-1</sup> showed promising effect on sweet basil growth. Plant shoot fresh and dry weight, leaf area, leaf SPAD value, and leaf chlorophyll content were improved by foliar application of 500 mg·L<sup>-1</sup> glycine or glutamine in comparison with control plants. Foliar application of amino acids increased leaf nitrogen (glutamine 250 and 500 mg·L<sup>-1</sup>), potassium (glycine 250 mg·L<sup>-1</sup>), magnesium (glutamine 250 or 500 mg·L<sup>-1</sup>, glycine 250 mg·L<sup>-1</sup>, glycine + glutamine; 250 + 250 mg·L<sup>-1</sup>), iron (glycine and glutamine at 500 mg·L<sup>-1</sup> and glutamine at 250 mg·L<sup>-1</sup>), and zinc (glycine and glutamine at 250 or 500 mg·L<sup>-1</sup>), whereas the increase in leaf nutrients caused by other treatments was not significantly different from control plants. Leaf calcium concentration was not changed by amino acid treatments. The results indicate that foliar application of moderate to low concentrations of glutamine or glycine can improve sweet basil growth.

## 1. Introduction

Basil (*Ocimum basilicum* L.) is a high marketable value vegetable, which is consumed as a fresh aromatic ingredient and included in cooked dishes. Increasing importance is being given to this produce due to its remarkable quality and nutritional features (Morano *et al.*, 2017).

Chemical fertilizers have an inevitable role in food production of agricultural crops; however, during last decades the soil fertility and quality have been adversely affected by many fertilization strategies (Souri and Hatamian, 2019). This is mainly due to the low efficiency of applied fertilizers which can even cause yield reduction upon increasing supply (Ercolano *et al.*, 2015). In some situations and particularly under adverse climatic conditions, foliar instead of soil application (Golubkina *et al.*, 2018), or chelated forms instead of simple chemical forms of fertilizers (Garcia *et al.*, 2011; Marschner, 2012; Souri and Aslani, 2018) can improve nutrient uptake and use efficiency of applied fertilizers. Each year, agriculture production needs a large amount of expensive nitrogenous fertilizers that generally have low uptake efficiency rates and result in both plant (Caruso *et al.*, 2011) and environmental pollution (Souri and Hatamian, 2019). Amino acids are being incorporated for more than two decades into fertilizer formulations to improve fertilizers use efficiency (Abdul-Qados, 2009; Souri, 2016).

Foliar application of amino acids can have beneficial effects on plant growth and production (Sadak *et al.*, 2015; Shams *et al.*, 2016; Hussain *et al.*, 2018; Souri and Aslani, 2018). It has been shown that foliar application of arginine amino acid has improved shoot growth and grain yield of wheat in saline and non saline conditions (Abdul-Qados, 2009; Rizwan *et al.*, 2017). Better growth and higher biomass production via application of various amino acid chelates of iron or zinc has been also reported on some agronomic and horticultural crops (Khan *et al.*, 2012; El Sayed *et al.*, 2014). Similarly, foliar application of a mix of amino acids at 500 and 700 ppm increased plant height, plant fresh and dry weights, leaf N concentration, yield of leaves and leaf soluble carbohydrates in celery (Shehata *et al.*, 2011).

Glycine is the simplest amino acid and is used largely for production of chelated fertilizers in form of aminochelates (Souri, 2016), whereas effect of foliar application of glutamine has not been well documented so far. In the present study, the effect of foliar application of different concentrations of glycine and glutamine has been evaluated on growth

characteristics of sweet basil plants.

## 2. Materials and Methods

This study was conducted under greenhouse conditions and from 20 May till the end of July 2017. The soil used in experiment had a loamy texture and moderate levels of nutrients. The soil physiochemical characteristics are presented in Table 1. 4 L black plastic pots (30 cm height and 20 cm diameter) were filled with about 4 kg dry soil and watered at 80% of its soil field capacity (FC). Three days later 40 seeds of sweet basil (*Ocimum basilicum* L.) from a local population (Karaj landrace) were sown in 1 cm depth of the soil. Two weeks after germination plants were reduced to 10 uniform seedlings per pot. The plants were irrigated daily based on 80% soil field capacity. The temperature was on average  $28 \pm 5^\circ\text{C}$ , the light intensity  $200 \mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , and the air humidity about 70-75%.

Different concentrations of two amino acids of glycine and glutamine including 0, 250, 500 and 1000  $\text{mg}\cdot\text{L}^{-1}$ , as well as a treatment of 250  $\text{mg}\cdot\text{L}^{-1}$  glycine + 250  $\text{mg}\cdot\text{L}^{-1}$  glutamine were sprayed on sweet basil plants. Treatments and pots were arranged in completely randomized design and each treatment contained three replications. Each pot represented one replication and contained 10 plants. Each concentration of glycine or glutamine was sprayed on plant leaves five times during two-month growth period, and the first spray was done two weeks after seedling emergence. The remaining sprays were done in one week interval. Distilled water was sprayed on control plants. Spray treatments were done in the early morning, one hour after sunrise and with a portable sprayer by which the upper and lower surface of leaves were sprayed. A total amount of 80-90 mL solution of each treatment was sprayed on plants per pot.

Plants were harvested two months after emergence and before flowering stage. The average height of ten plants in each pot was measured by a ruler before harvest. The leaf SPAD value was measured by a portable SPAD meter (Model SPAD-502

Table 1 - Physico-chemical characteristics of soil used for the experiment

Texture	EC ( $\text{dS}\cdot\text{m}^{-1}$ )	pH	O.C. (%)	Total N ( $\text{mg}\cdot\text{kg}^{-1}$ )	Available P ( $\text{mg}\cdot\text{kg}^{-1}$ )	Available K ( $\text{mg}\cdot\text{kg}^{-1}$ )	Fe ( $\text{mg}\cdot\text{kg}^{-1}$ )	Zn ( $\text{mg}\cdot\text{kg}^{-1}$ )	Lime (%)
Loamy-Sand	1.79	7.62	0.78	750	11.61	385	5.5	1.6	7.3

Plus Illinois, USA) performing 30 readings (on middle part of leaves) per pot. The plant leaf area of three randomly selected plants per pot was measured by leaf area meter (Delta-T Devices Ltd, England). Plants were cut at soil surface and shoot fresh weight was measured by a precise digital scale. Fresh shoots of each pot were transferred to an oven at 65°C for 48 h, and thereafter plant dry weight was measured accordingly. Leaf chlorophyll and carotenoids were determined using acetone extraction of 0.5 g fresh leaves following the methods of Khan *et al.* (2012) and Kałużewicz *et al.* (2018). For determination of leaf L-ascorbic acid (vitamin C), 5 g of fresh leaf tissue were crushed in a porcelain mortar in 10 mL metaphosphoric acid 6%, and then the juice was transferred into a 25 mL tube and centrifuged at 4000 rpm for 10 min. 5 mL of the supernatant was transferred into an Erlenmeyer flask, and added with 20 mL of metaphosphoric acid 3%. The extract was then titrated using di-chloro phenol indophenol reagent until appearance of a rosa color. The amount of vitamin C (mg 100 g<sup>-1</sup> FW) was calculated according to a standard curve of L-ascorbic acid concentrations of 0, 25, 50, 2100 and 200 mg·L<sup>-1</sup> following Souri and Aslani (2018). Nitrogen concentration of leaves was determined by the Kjeldahl method, K using flame photometry, Mg, Ca, Fe and Zn by atomic absorption spectrophotometer.

Data were analyzed by analysis of variance using SPSS 16 and comparison of means was performed using LSD test at P≤0.05 probability level.

### 3. Results and Discussion

In the present study a soil with moderate levels of nutrient elements was used and no further applica-

tion of chemical fertilizers was applied. The results showed that plant height was significantly increased by foliar application of 250 mg·L<sup>-1</sup> glutamine compared to control plants (Table 2). Application of both amino acids of glycine and glutamine significantly increased the leaf concentration of chlorophyll a (at 250 or 500 mg·L<sup>-1</sup>) and chlorophyll b (at 500 mg·L<sup>-1</sup>) compared to control plants (Table 2). Total chlorophyll concentration in leaves was significantly increased than control by glutamine or glycine at 500 mg·L<sup>-1</sup> or glutamine at 250 mg·L<sup>-1</sup> (Table 2). Leaf carotenoids concentration increased and decreased upon foliar application of 1000 mg·L<sup>-1</sup> glycine and 1000 mg·L<sup>-1</sup> glutamine compared to control plants, respectively (Table 2). The plant leaf SPAD value (Fig. 1) was not significantly changed under different concentrations of glutamine or glycine compared to control plants. However, foliar application of 1000 mg·L<sup>-1</sup> glycine reduced leaf SPAD value of plants compared to some amino acid treatments (Fig. 1). This could be due to damage of high glycine concentration to basil leaves, as the same finding was also reported by

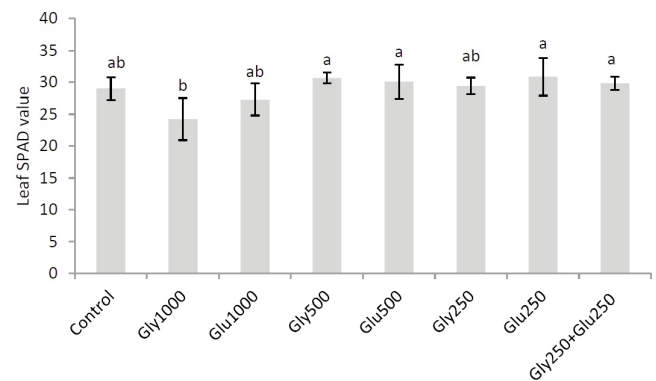


Fig. 1 - Leaf SPAD value of sweet basil plants under foliar application of glycine or glutamine amino acids. Comparison of means was performed at P≤0.05 probability level of LSD test.

Table 2 - Plant height and leaf pigments concentration of sweet basil plants under foliar application of glycine and glutamine amino acids

Treatments	Plant height (cm)	Chl a (mg.g <sup>-1</sup> FW)	Chl (mg.g <sup>-1</sup> FW)	Total Chl (mg.g <sup>-1</sup> FW)	Carotenoids (mg.g <sup>-1</sup> FW)
Control (distilled water)	46±5 bc	10.5±1.4 d	5.6±0.8 b	16.1±1.3 bc	1.7±0.3 b
Gly1000	44±4 c	11.9±3.2 cd	6.0±1 b	18.0±4.1 bc	2.3±0.1 a
Glu1000	43±10 c	10.3±1 d	5.2±0.9 b	15.5±1.4 c	1.2±0.1 c
Gly500	55±15 abc	18.3±1.3 ab	9.0±2 a	27.3±3.4 a	1.5±0.2 bc
Glu500	56±10 ab	19±2.4 a	9.2±1.3 a	28.2±3.6 a	1.4±0.1 bc
Gly250	53±11 abc	17.4±0.7 ab	7.8±0.9 ab	25.2±1.4 a	1.6±0.5 b
Glu250	62±13 a	15.2±2.7 bc	5.0±0.9 b	20.2±3.6 b	1.4±0.3 bc
Gly250+Glu250	50±30 b	13.1±0.5 c	5.2±3.7 b	18.3±3.6 bc	1.5±0.3 bc

- 250, 500 and 1000 indicates their concentration as mg·L<sup>-1</sup>.

- Comparison of means was performed at P≤0.05 probability level of LSD test.

Fahimi *et al.* (2016) on cucumber plants. The increase in plant height and leaf greenness can be due to beneficial effects of glycine and particularly glutamine on leaf pigmentation (Sadak *et al.*, 2015; Fahimi *et al.*, 2016). Amino acids can have stimulation effect on plants growth, and in this respect, it has been shown that application of amino acids can enhance chlorophyll content and leaf greenness of plants (Kielland, 1994; Souri *et al.*, 2017; Kałużewicz *et al.*, 2018), probably due to higher protein biosynthesis and reduction in chlorophyll degradation rates (Rainbird *et al.*, 1984; Souri and Hatamian, 2019). Better nutrients use efficiency and their enhanced translocation play also an important role in this regard (Marschner, 2012). Application of aminolevulinic acid (ALA), as a common precursor to tetrapyrrole compounds found in chlorophyll and hemes, improved spinach plant growth and increased the concentration of photosynthetic pigments (Smoleń *et al.*, 2010).

In the present study, plant leaf area (Fig. 2) was increased by foliar application of both glycine and glutamine at 500 mg·L<sup>-1</sup> compared to control plants. Both glycine and glutamine at either concentration of 250 or 500 mg·L<sup>-1</sup> increased shoot fresh weight (Fig. 3); whereas plant shoots dry weight was increased only under foliar application of 500 mg·L<sup>-1</sup> glycine or glutamine compared to control plants. Leaf area expansion is a function of interacting various external and internal factors that results in increased cell division or particularly cell enlargement. Various amino acids like glutamine and glycine are required components for normal cell growth and expansion (Marschner, 2012; Souri, 2016). The increase in plant biomass caused by foliar application of moderate levels of glutamine or glycine could be due to the latter stimulation effects on plant growth. The beneficial effects of foliar application of amino acids on plant

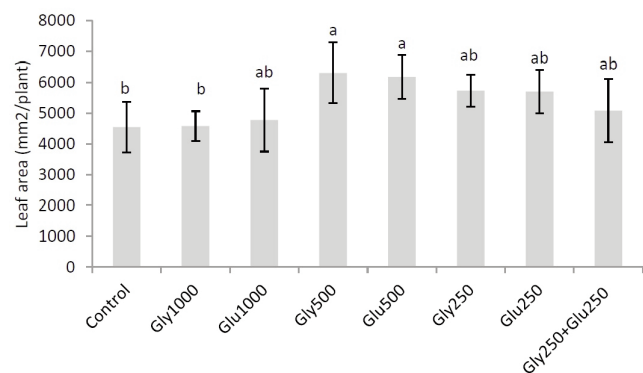


Fig. 2 - Plant leaf area of sweet basil under foliar application of glycine or glutamine amino acids. Comparison of means was performed at P≤0.05 probability level of LSD test.

growth, yield and quality of the product has been shown for some crops (Keutgen and Pawelzik, 2008; Amin *et al.*, 2011; Khan *et al.*, 2012; Sadak *et al.*, 2015). In addition, amino acids can enhance nutrient uptake and act as precursors for certain plant hormones (Marschner, 2012; Souri and Hatamian, 2019). Foliar application of different concentrations of a mix 17 different amino acids after 45 and 60 days from sowing showed promising effects on bean plant growth and productivity under salinity conditions (Sadak *et al.*, 2015). A similar effect was also obtained in bean plants under field conditions (Souri and Aslani, 2018). Foliar application of zinc-lysine significantly increased the photosynthesis, grain yield, enzyme activities and Zn contents in different wheat plant tissues (Rizwan *et al.*, 2017). Multiple sprays of a mix of amino acids (0.5 mL·L<sup>-1</sup>) at different growth stages of grapevines particularly at flowering stage, increased growth characteristics and various components of fruit yield and quality compared to control (Khan *et al.*, 2012).

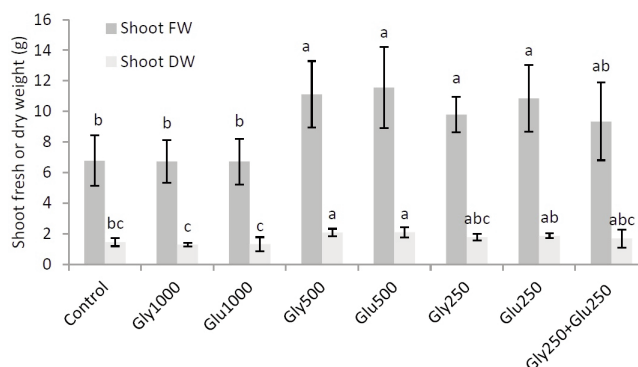


Fig. 3 - Shoot fresh and dry weights of sweet basil plants under foliar application of glycine or glutamine amino acids. Comparison of means was performed at P≤0.05 probability level of LSD test.

Leaf L-ascorbic acid (vitamin C) was not significantly affected by foliar application of glycine or glutamine compared to control plants; however, the highest leaf ascorbic acid content was recorded in plants treated with 1000 mg·L<sup>-1</sup> glycine or glutamine (Fig. 4). Foliar application of amino acids significantly increased leaf nutrient concentration (except calcium) under some specific concentrations of glycine or glutamine (Table 3). Leaf nitrogen was significantly increased by glutamine spray either at 250 or 500 mg·L<sup>-1</sup>, whereas leaf potassium concentration was significantly increased by glycine at 250 mg·L<sup>-1</sup> compared to control plants. Foliar application of glutamine either at 250 or 500 mg·L<sup>-1</sup>, and foliar application of glycine at 250 mg·L<sup>-1</sup> significantly increased

leaf magnesium concentration compared to control plants. Leaf iron concentration was significantly increased by foliar application of glutamine either at 250 or 500 mg·L<sup>-1</sup>, or by foliar application of glycine at 500 mg·L<sup>-1</sup> compared to control plants. Similarly, leaf zinc concentration was significantly increased by foliar application of glycine or glutamine either at 250 or 500 mg·L<sup>-1</sup> compared to control plants (Table 3).

Foliar spray of 250-500 mg·L<sup>-1</sup> of both amino acids significantly increased leaf nutrient concentrations. The most significant effect of amino acids on plants is reportedly the increased plant nutrient uptake (Koksal et al., 1999; Abdul-Qados, 2009; Garcia et al., 2011). Various amino acids can act as ligand for nutrients particularly micronutrient metal ions, so they can protect metal nutrients from harmful reactions (Souri and Hatamian, 2019). The structure of amino acids allows the molecule to act as acid or base, depending on medium pH, thus helping to improve the fertility management of soils particularly under

adverse climatic conditions. Moreover, amino acids or their fertilizer products can be considered as “semi-intelligent fertilizers” (Souri, 2016). They are natural ligands with a friendlier effect on environmental issues and best candidates to partially replace fertilizer application, and to relief chemical fertilizers pressure in many cropping systems.

Amino acids are also a preferential source of nitrogen for plant nutrition. It has been shown that partial replacement of nitrate by amino acid application can have beneficial effects on plant growth and production (Marschner, 2012; Sadak et al., 2015; Souri et al., 2017). Amino acids are the intermediate compounds in nitrogen assimilation and play key roles in plant cell metabolism, as they are the main form of nitrogen translocation through phloem to growing parts (Marschner, 2012; Kolota et al., 2013). Nitrate in the soil is very vulnerable to leaching and gaseous emissions (Kolota et al., 2013); whereas the mandatory application of reduced forms of nitrogen under organic farming (Caruso et al., 2012) or the advisable supply of ammonium and amino acids can prevent these effects and reduce nitrate accumulation in plant tissues (Cao et al., 2010; Souri et al., 2017). Total concentration of amino acids and leaf protein concentration significantly increase by foliar application of single or a mix of amino acids (Marschner, 2012; Sadak et al., 2015). Moreover, it has been shown that foliar or soil application of amino acids or their chelated nutrients can improve quality parameters of plants (Koksal et al., 1999; Keutgen and Pawelzik, 2008; Shaheen et al., 2010; Smoleń et al., 2010; El Sayed et al., 2014; Souri et al., 2017). Application of glutamic acid enhanced some quality parameters of Chinese chive plants (Cao et al., 2010). Similarly, foliar spray of proline and tryptophan enhanced growth, yield and fruit quality and

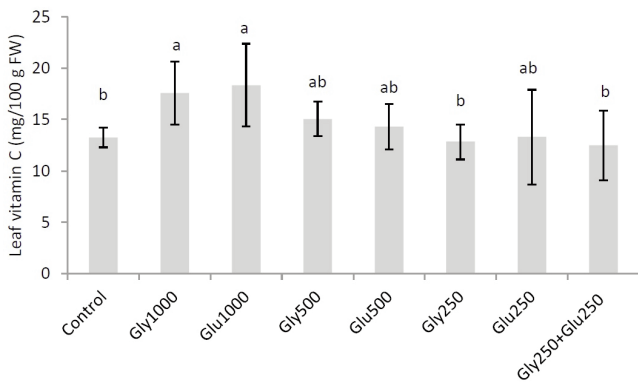


Fig. 4 - Leaf L-ascorbic acid (vitamin C) concentration of sweet basil plants under foliar application of glycine or glutamine amino acids. Comparison of means was performed at P≤0.05 probability level of LSD test.

Table 3 - Leaf nutrient concentrations of sweet basil plants under foliar application of glycine and glutamine amino acids

Treatments	N (% DW)	K (% DW)	Mg (% DW)	Ca (% DW)	Fe (mg.kg <sup>-1</sup> DW)	Zn (mg.kg <sup>-1</sup> DW)
Control (distilled water)	2.1±0.2 b	1.5±0.2 b	0.21±0.04 d	1.5±0.2 ab	49±6 c	33±3 c
Gly1000	2.13±0.4 b	1.6±0.1 ab	0.19±0.03 d	1.3±0.4 b	45±7 c	33±2 c
Glu1000	2.4±0.3 ab	1.5±0.3 b	0.23±0.04 cd	1.5±0.2 ab	52±10 bc	35±2 bc
Gly500	2.3±0.4 ab	1.7±0.2 ab	0.27±0.04 bcd	1.6±0.2 ab	61±10 ab	40±4 ab
Glu500	2.7±0.5 a	1.8±0.4 ab	0.36±0.06 a	1.9±0.5 a	66±8 a	42±6 a
Gly250	2.5±0.2 ab	1.9±0.3 a	0.29±0.05 abc	1.7±0.2 ab	53±6 abc	41±4 ab
Glu250	2.6±0.3 a	1.8±0.3 ab	0.33±0.04 ab	1.7±0.3 ab	62±5 ab	42±5 a
Gly250+Glu250	2.5±0.3 ab	1.7±0.1 ab	0.30±0.06 abc	1.8±0.1 a	57±9 abc	38±6 abc

- 250, 500 and 1000 indicates their concentration as mg·L<sup>-1</sup>.

- Comparison of means was performed at P≤0.05 probability level of LSD test.

minimized cracked fruit percentage of pomegranates (El Sayed *et al.*, 2014). It has also been shown that under heavy metal pollution of soil, application of amino acids can reduce the heavy metal content of plant tissues (Bashir *et al.*, 2018).

In the present study, foliar application of glutamine resulted in better growth traits than glycine spraying. This effect can be mainly due to the well-known role of glutamine in nitrogen assimilation and plant metabolism (Marschner, 2012). Enhancement of glutamine synthetase has been shown to reduce the severity of ammonium toxicity in tomato plants (Forde and Clarkson, 1999; Marschner, 2012). In addition to the amino and carboxyl groups, amino acids have a side chain or R group attached to the  $\alpha$ -carbon. Each amino acid has unique characteristics arising from the size, shape, solubility, and ionization properties of its R group. As a result, the side chains of amino acids exert a profound effect on the structure and biological activity of proteins (Souri, 2016; Souri and Hatamian, 2019). Among various amino acids used as foliar spray on bean plants, it was found that glutamic acids and arginine were the most effective on plant growth biostimulation (Sadak *et al.*, 2015).

It has been revealed that amino acids are effective components in nutrient use efficiency as well as in detoxification of toxins and heavy metals in plants that can significantly increase their antioxidant capacity and tolerance to stressful conditions. For many decades, soil fertility and quality was adversely affected by continuous application of chemical fertilizers and adverse climatic conditions. Fertilization strategies have actively contributed in enhancing soil salinity that nowadays is a global challenge in many countries. Biostimulation effect of amino acids on nutrient uptake and plant growth can reduce fertilizer application rates and detrimental effects of fertilization on environment and food quality. However, in the present study low to moderate levels of glycine or glutamine (250-500 mg·L<sup>-1</sup>) had beneficial effects on sweet basil growth, while both amino acids at 1000 mg·L<sup>-1</sup> were less or not effective on plant growth characteristics.

#### 4. Conclusions

In the present study, the growth of sweet basil plants was improved by foliar application of 250 or 500 mg·L<sup>-1</sup> of glycine or glutamine amino acids, whereas application of 1000 mg·L<sup>-1</sup> of these two

amino acids resulted in no difference from control plants. There were significant better effect on plant height, leaf SPAD value, leaf area, shoot fresh and dry weights, and nutrient uptake with foliar application of glycine and particularly glutamine at 500 mg·L<sup>-1</sup>. Leaf vitamin C was highest in plants treated with 1000 mg·L<sup>-1</sup> amino acid spray. Foliar application of glutamine was more effective than glycine on many growth traits and nutrients uptake. Our results suggest that amino acids are effective to improve the nutrient uptake and accordingly the plant growth, yield and quality under the view of a modern and sustainable agriculture.

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