

Effect of L-glycine on the Growth and Selected Nutritional Elements of Butterhead Lettuce

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ABSTRACT

In agricultural practice, there is a need to change the forms of fertilizers to be friendlier to the natural environment and human health. To this end, research has been carried out on the partial replacement of fertilisers containing nitrates or ammonium compounds with amino acid nitrogen. The present studies assessed the effect of L-glycine on growth parameters and on the content of selected nutrients in the butterhead lettuce grown in an unheated greenhouse in the spring. The treatment consisted of foliar L-glycine application at the following concentrations: 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220 and 240 mg·L⁻¹, with a solution dose of 50 ml·m⁻². Foliar application of L-glycine improved the butterhead lettuce growth parameters. The most favourable values of the head weight, root system weight and the number and length of leaves were recorded for the plants fed with doses ranging from 80 to 120 mg·L⁻¹. The weight of the head and the root system was most favourable after foliar feeding with 120 mg·L⁻¹ of L-glycine. Additionally, a high value of the SPAD leaf greenness index was noted as a response to the same dose, indicating a high level of leaf nitrogen nutrition. However, foliar feeding with L-glycine had no statistically significant effect on the nutritional value of lettuce, i.e. the content of dry matter, protein, sugars and ascorbic acid.

Keywords: amino acid; L-glycine; *Lactuca sativa* L. var. *capitata*; growth parameters; nutritional value

INTRODUCTION

Plants can directly absorb a wide range of nitrogen compounds, including amino acids [Marschner, 2011]. They are a more suitable form of nitrogen taken up and assimilated by plants than its inorganic compounds [Ge et al., 2009; Garcia et al., 2011]. In recent years, numerous studies have been conducted on the use of amino acids to fertilize plants, especially under adverse environmental conditions [Sánchez et al., 2005; Garcia et al., 2011; Ma et al., 2017; Souri et al., 2017]. They are applied both in the form of non-chelated and chelated fertilizers [Mohammadipour and Souri, 2019].

Amino acids are a good source of nitrogen for plants and their foliar or soil application

affects the yield, stimulating the growth of shoots and roots and, owing to their chelating properties, improving the nutrient uptake [Liu et al., 2008; Cerdán et al., 2013; Galili and Amir, 2013; Souri et al., 2017; Souri and Hatamian, 2019]. Improved plant growth and higher yields, especially under adverse climatic conditions, may be a result of increased chlorophyll biosynthesis and a higher photosynthesis rate [Ertani et al., 2009; Garcia et al., 2011; Shams et al., 2016]. The plants supplied with amino acids generally have higher content of sugars, proteins and other nutrients [Sánchez et al., 2005; Zhou et al., 2007; Garcia et al., 2011; Cerdán et al., 2013; Galili and Amir, 2013; Souri et al., 2017]. Nitrogen applied in the form of amino acids may also reduce the content of nitrates in plant tissues [Cao et al.,

2010; Souri, 2016], which is important for human food safety. Such plants are also better protected against stress conditions, including soil salinity, drought or temperature stress [Tantawy et al., 2009; Cerdán et al., 2013; Souri, 2016]. Today, glycine plays an important role in the feeding of many plants, especially vegetables [Souri and Hatamian, 2019].

Lettuce (*Lactuca sativa* L. var. *capitata*) is one of the most important leaf vegetables grown and consumed worldwide, so it is very important that it should be produced securely to ensure a yield of good quality and high nutritional value. It is a universal vegetable that can be grown in different parts of the growing seasons and under various climatic conditions. It has been reported that amino acids, including glycine, have a beneficial effect on the yield and quality of leafy plants [Galili and Amir, 2013; Souri, 2016; Noroozlo et al., 2019].

The literature demonstrates that foliar spraying is a promising technique of applying amino acids to crops [Abdelhamid et al., 2014]. According to Wang et al. [2007], their effect on plant growth and development depends on the type of amino acids. Many studies have been conducted concerning the effects of amino acid mixtures, with much less research examining the effects of single amino acids on plants.

The available literature suggests that the optimal levels of different amino acids, which should be determined before recommending their use, may depend on the species or even the variety [Rosati et al., 2000; Mobini et al., 2014; El-Sharabasy et al., 2015]. In those studies, usually 2-3 significantly different doses of L-glycine are used. Such a small number does not make it possible to precisely determine the optimal dose for the growth and development of lettuce. Therefore, in the present experiment, 12 gradually increasing doses of L-glycine were examined, and their effect on the growth and development of individual parts of lettuce plants was determined.

The aim of the studies was to determine the dose of L-glycine optimal for lettuce growth, development and nutritional value. As a result of the research, it will be possible to develop the recommendations for the use of L-glycine in the foliar feeding of butterhead lettuce.

MATERIALS AND METHODS

Plant material and growing conditions

The plant used in the experiment was lettuce (*Lactuca sativa* L. var. *capitata*) of the 'Justyna' variety, added to the genebank collection in 1997. It produces large, spherical heads, maintaining its consumer value for a relatively long time. It is a high-yielding variety, suitable for planting throughout the growing season, resistant to bursting into inflorescence shoots [Kapusta and Chojnowski, 2018].

The research was carried out in 2019 in an unheated greenhouse of the University of Natural Sciences and Humanities in Siedlce (52°17' N, 22°28' E) on anthropogenic soil with horticisol properties, which, as part of a horticultural farm, had long been used for horticultural purposes. The soil was pH neutral with the 40 cm deep humus layer and average organic carbon content ranging between 2.3 and 2.5%. Three weeks before planting lettuce seeds, Azofoska (INCO Group S.A., Poland) NPK (MgO+SO₃): 13.3-6.1-17.1 (4.5+21.0), a basic mineral fertilizer, was applied at a rate of 5 kg per 100 m². A week before planting the seeds, the soil samples were collected to determine the nitrogen, phosphorus, potassium, magnesium and calcium concentrations. It was as follows (mg·dm⁻³): N-NO₃ – 30.6, N-NH₄ – 52.3, P – 48.3, K – 170.8, Mg – 65.0 and Ca – 2200.0. The available phosphorus content was below satisfactory level, while the amounts of nitrogen, potassium, magnesium and calcium were optimal for lettuce. The content of phosphorus available in the soil was supplemented to the upper limit of the optimal amounts for lettuce, i.e. 70 mg·dm⁻³, provided by Sady [2014]. To this end, a phosphorus dose in the amount of 0.44 kg P per 100 m² was applied in the form of the Super Fos Dar 40[®] fertilizer (Azoty Group S.A., Poland) containing 40% P₂O₅ (the fertilizer dose was 2.5 kg per 100 m²).

The experiment was set up in a completely randomized design, with three replications. The number of combinations was 13 with 39 experimental plots, each with an area of 1.5 m². On 8th and 9th March 2019 mineral fertilizers were applied and the soil was loosened and irrigated. On March 29th, lettuce seeds were sown at a seeding rate of 0.3 g·m⁻² in rows spaced 30 cm apart. The seedlings came out evenly (BBCH 09), eight days after sowing. During the first true leaf stage (BBCH 11), seedlings were thinned to one plant

every 20 cm. The maintenance procedures consisted of loosening the surface of the soil and weeding. Lettuce was harvested on 9 May 2019 at the BBCH 48-49 stage.

L-glycine application

An aqueous solution of L-glycine was prepared immediately before its use, with the following doses: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 mg·m⁻² (with a dose of spraying liquid of 50 ml·m⁻² and concentration ranging, according to the L-glycine dose, from 20 to 240 mg·L⁻¹). The plants were sprayed on April 17th (BBCH 13-14) in the morning using a manual pressure sprayer. To avoid spraying plants on adjacent plots, polyethylene film screens were used.

Observations and measurements

During the BBCH 46-47 stage, the leaf greenness index (SPAD) was measured (with a SPAD-502 Plus Konica Minolta®). The SPAD index closely correlates with the nitrogen nutritional status of plants [Uchino et al., 2013]. During the harvest, the yield of 1 m² was determined and measurements of the following plant characteristics were made:

- the weight of the above-ground part, i.e. head weight (g),
- the weight of the root system (g),
- the diameter of the root neck (mm)
- the number of head leaves with a main nerve length of more than 10 cm,
- the length of the outer head leaf measured along the main nerve (cm).

Laboratory analysis

Chemical analyses were carried out at the Laboratory of Structural Research and Natural Analysis of Siedlce University. The content of the following parameters was determined:

- dry matter, by drying to the constant weight at 105°C [PN-EN 12145, 2001],
- protein, with the Kjeldahl method using the 6.25 conversion factor [AOAC, 2001],
- total sugars, with the Luff-Schoorl method [EU Commission Regulation NO 152/2009, 2009],
- ascorbic acid, with the Tillman's method [PN-A-04019, 2009].

Statistical analysis

The results of the research were processed statistically using one-way analysis of variance for a completely randomized design. The significance of differences between means was verified with Tukey's HSD test at a significance level $P \leq 0.05$. Statistical calculations were performed with Excel software using the authors' own algorithm based on the mathematical model:

$$Y_{i,j} = \mu + T_i + e_{ij} \quad (1)$$

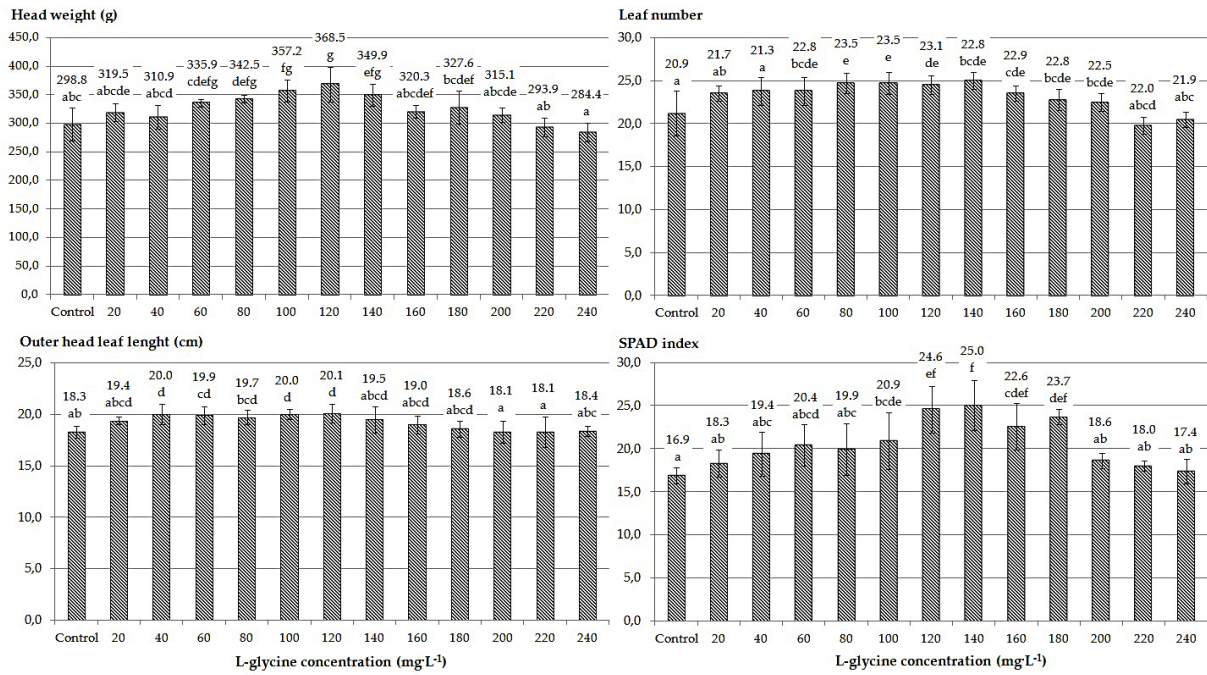
where: $Y_{i,j}$ – the value of the characteristic, μ – population mean, T_i – the effect of the i -th level of factor A (amino acid), e_{ij} – random error.

RESULTS

Growth parameters

Analysis of variance showed that foliar application of L-glycine to lettuce had a significant effect on the head weight, leaf number, outer leaf length of the head, SPAD index, root system weight and root collar diameter (Table 1). The average weight of the lettuce head was 324 g, with the average root weight of 66.4 g (Figures 1 and 2). The smallest weight of the above-ground part (284 g) was recorded for the plants treated with the highest concentration of L-glycine of 240 mg·L⁻¹ and the smallest root system (51.3 g) was in control, without foliar feeding with L-glycine, and on the plot with the highest L-glycine dose of 240 mg·L⁻¹ (54.7 g). The highest head weight (368.5 g) was noted after the application of a 120 mg·L⁻¹ of L-glycine. Heads were only a little smaller on the plots treated with L-glycine concentrations ranging from 60 to 100 mg·L⁻¹ and at 140 mg·L⁻¹. At concentrations of 20 to 200 mg·L⁻¹, the weight of the lettuce head was greater than in the control, without foliar feeding. A statistically significant increase was recorded for a concentration ranging from 80 to 140 mg·L⁻¹. For the same concentration range, the plants also had the highest weight of the root system. With an increase in a concentration of L-glycine from 140 to 240 mg·L⁻¹, a gradual decrease in the weight of the head and the root system was recorded, with a slight increase for a concentration of 180 mg·L⁻¹.

The head of lettuce consisted on average of 22.4 leaves. Foliar L-glycine application of 60 to 200 mg·L⁻¹ contributed to a statistically significant



Mean (n = 3) followed by the same letters does not differ significantly at $P \leq 0.05$.

Figure 1. Effect of L-glycine concentration on lettuce leaves growth parameters

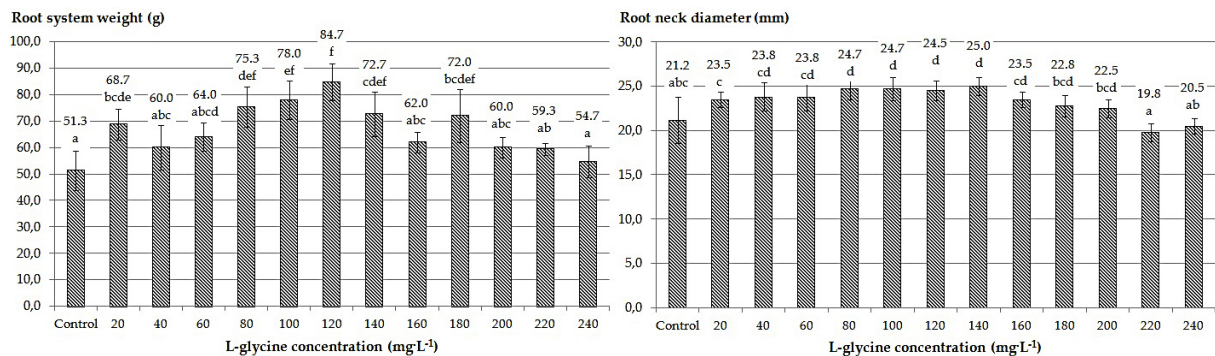
increase in the number of head leaves compared to control, without foliar L-glycine feeding (Figure 1).

The largest number of leaves was recorded on the plots with a foliar L-glycine concentration of 80 and 100 mg·L⁻¹, and significantly lower in the control, without foliar feeding with L-glycine, and after feeding at a concentration of 20 and 40 mg·L⁻¹ and 220 and 240 mg·L⁻¹.

The length of the outer leaf of the head was on average 19.2 cm. After spraying the lettuce with a concentration of L-glycine ranging from 40 to 120 mg·L⁻¹, the leaf length was significantly greater than that found in control, without foliar L-glycine feeding (Figure 1). For concentration of 20 and 140 to 240 mg·L⁻¹ it was not significantly different from that in control.

The SPAD leaf greenness index averaged 20.4 and ranged from 16.9 for plants with no L-glycine feeding to 25.0 for plants treated with a 140 mg·L⁻¹ dose (Figure 1). The SPAD values similar to those found for a 140 mg·L⁻¹ concentration were also recorded in the lettuce leaves sprayed with L-glycine of 120 and 160 and 180 mg·L⁻¹. The SPAD values which were not significantly different from those found for lettuce from the control plot, without L-glycine, were also recorded at concentrations of 40-80 and 200-240 mg·L⁻¹.

The lettuce plants with the largest root neck diameter were harvested from the plots with the foliar doses of L-glycine ranging from 80 to 140 mg·L⁻¹ (Figure 2). A similar diameter of the root neck was also recorded for the plants treated with



Mean (n = 3) followed by the same letters does not differ significantly at $P \leq 0.05$.

Figure 2. Effect of L-glycine concentration on lettuce roots growth parameters

the L-glycine doses of 40 and 60 and from 160 to 200 mg·L⁻¹, while for the highest doses of L-glycine with 220 and 240 mg·L⁻¹ the parameter values was not significantly different from those found in the control.

Nutritional value

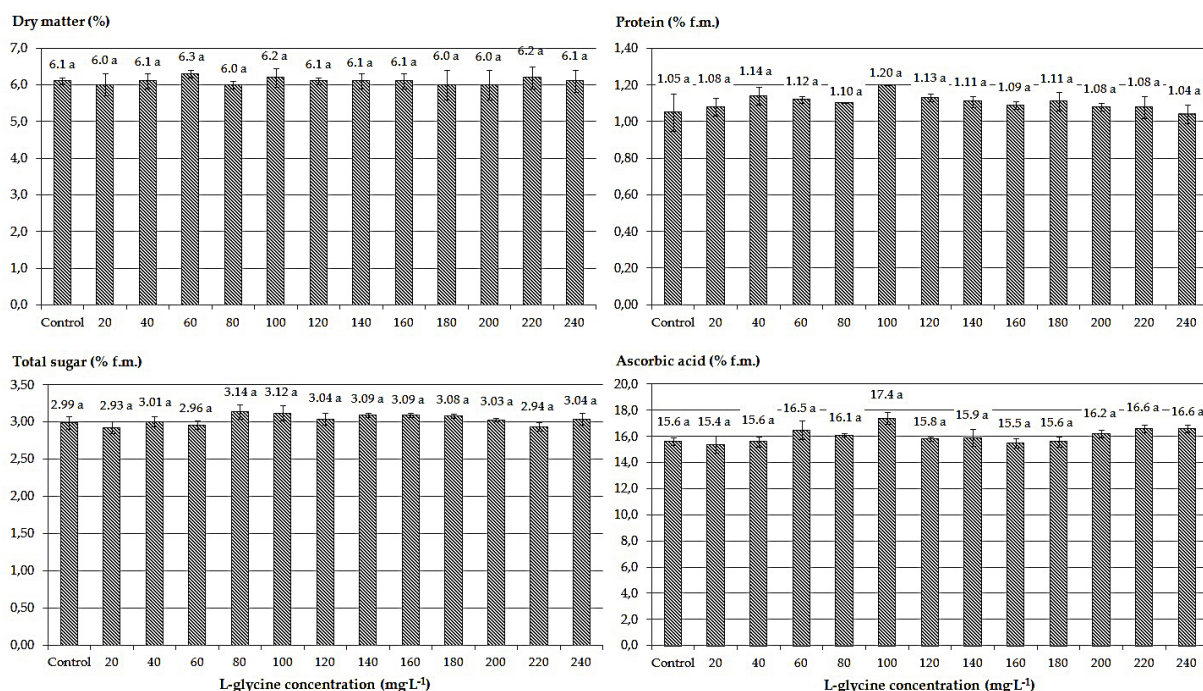
L-glycine applied to leaves had no significant effect on lettuce dry matter, protein, sugars and ascorbic acid content (Table 1). The content of lettuce nutrients after L-glycine application is presented in Figure 3.

Correlation between the lettuce growth parameters and nutritional value parameters

The weight of the lettuce head was correlated with the number and length of leaves, the weight

of the root system, the diameter of the root neck and the SPAD greenness index, which was confirmed in a statistically significant way by correlation coefficients (Table 2). Their positive values indicated that the weight of the lettuce head increased significantly along with growth parameters and the SPAD index.

There was no significant relationship between the dry matter content of the above-ground part and the value of the growth parameters tested in the experiment, as well as the SPAD value. The total protein and sugar content of lettuce leaves was significantly positively correlated with all growth parameters, while the content of reducing sugars was correlated with the head weight, leaf length, root neck diameter and the SPAD index value. In the case of total and reducing sugars, the correlation coefficient with the SPAD greenness index, which is related to the state of nutrition of



Mean (n = 3) followed by the same letters does not differ significantly at P ≤ 0.05.

Figure 3. Effect of L-glycine concentration on selected elements of the nutritional value of lettuce

Table 1. Results of analyses of variance using a completely random model

Source of variation	Head weight	Leaves number	Outer head leaf length	SPAD	Root system weight	Root neck diameter	Dry matter	Protein	Total sugars	Ascorbic acid
df	12	12	12	12	12	12	12	12	12	12
P	0.032	0.013	0.048	0.013	0.013	0.032	>0.05	>0.05	>0.05	>0.05
F-value	3.73	4.12	2.24	4.92	4.64	3.21	0.21	0.84	0.65	1.36
HSD _{0.05}	37.7**	1.2**	1.6*	3.8**	13.1**	2.7**	ns	ns	ns	ns

* Significant at P ≤ 0.05; ** significant at P ≤ 0.01; ns – not significant.

Table 2. Correlation coefficients between lettuce growth parameters and head weight and nutritional value parameters

Growth parameters	Head weight (g)	Dry matter (%)	Protein (% f.m.)	Total sugars (% f.m.)	Ascorbic acid (mg %)
Head weight (g)	-	0.1154	0.5514**	0.4918**	0.3454*
Leaf number	0.5317**	0.2009	0.3138*	0.4610**	0.2966
Leaf length (cm)	0.7350**	0.2250	0.3989**	0.4610**	0.3754*
SPAD	0.7299**	0.1370	0.3624*	0.5277**	0.2196
Root system weight (g)	0.7704**	-0.1202	0.4502**	0.3647*	0.2333
Root neck diameter (mm)	0.8709**	0.0545	0.5951**	0.4923**	0.2627

Significant at: $P \leq 0.05^*$ (0.3041), $P \leq 0.01^{**}$ (0.3928).

plants with nitrogen and the content of photosynthetic pigments in the leaves, was of the highest value. The vitamin C content was significantly positively correlated with the weight of the lettuce head and the length of the leaf (Table 2).

DISCUSSION

It was observed that the values of lettuce growth parameters increased after foliar L-glycine application. Those values were highest for the L-glycine doses ranging from 80 to 120 $\text{mg}\cdot\text{L}^{-1}$. This may be due to the stimulating effect and the role of this amino acid in plant metabolism [Marschner, 2011; Shams et al., 2016; Suori, 2016]. After L-glycine application, the rate of chlorophyll biosynthesis and photosynthesis improved, whereas the rate of protein biosynthesis and tolerance to adverse climatic conditions increased [Näsholm et al., 2009; Khan et al., 2012; Souri and Hatamian, 2019]. Similarly, using the coriander treated by means of Hoagland's nutrient solution with an addition of L-glycine (5, 10, 20 or 40 $\text{mg}\cdot\text{L}^{-1}$), Mohammadipour and Souri [2019] found that all doses of the amino acid, except for the highest (40 $\text{mg}\cdot\text{L}^{-1}$), increased the plant height, stem diameter, leaf greenness index (SPAD) and fresh and dry root weight. The five-week continuous application of the highest dose of glycine, along with the solution of 200-400 ml per day, had a negative effect on the growth of coriander, causing a decrease in plant height by more than 25%, in a stem diameter by almost 29% and in the SPAD leaf greenness index by almost 28%, compared to glycine-free control. In the present experiment, the L-glycine solution at a concentration of $> 200 \text{ mg}\cdot\text{L}^{-1}$ also had a negative effect on the growth of lettuce, causing a decrease in the weight of the head and in the diameter of the root collar. After L-glycine application

to the roots at a concentration of 210 $\text{mg}\cdot\text{L}^{-1}$, Khan et al. [2019] found a decrease in the leaf length, width and surface area by 13.8%, 18.3% and 29.7%, respectively, compared to control, without the amino acid. The only parameter increased in response to L-glycine was the number of leaves. Nitrogen is in its reduced form in glycine, which in high concentrations can be toxic to plants, causing similar effects to those resulting from ammonium over-fertilization [Souri and Römheld, 2009; Marschner, 2011].

At doses above 140 $\text{mg}\cdot\text{L}^{-1}$, a decrease in the mass of the root system was recorded. Root growth may have been affected by amino acid treatments in a different way than shoot growth [Souri and Hatamian, 2019]. Due to the application of amino acids, reduced nitrate uptake may induce restricted root growth [Noroozlo et al., 2019]. According to Amin et al. [2011], Noroozlo et al. [2019] and Souri and Hatamian [2019], the changes in the biosynthesis of phytohormones and their translocation may also play a role in this regard.

As important stimulators of chlorophyll production and photosynthesis regulators, amino acids determine the production and yield of plant biomass [Näsholm et al., 2009; Khan et al., 2019; Souri and Hatamian, 2019]. In the present studies, the largest SPAD leaf greenness index, allowing the assessment of the chlorophyll content in leaves, was found in the plants treated with an L-glycine concentration ranging from 120 to 180 $\text{mg}\cdot\text{L}^{-1}$. Both smaller and greater doses significantly reduced the SPAD index. Mohammadipour and Souri [2019] found that the SPAD index in lettuce leaves was significantly higher than in the control plants after the use of 10 $\text{mg}\cdot\text{L}^{-1}$ of glycine in the substrate solution, while the use of the highest concentration of glycine (40 $\text{mg}\cdot\text{L}^{-1}$) resulted in a significant decrease in SPAD. In the studies of Khan et al. [2019], the SPAD value in lettuce leaves after L-glycine application to the roots at a

dose of $210 \text{ mg}\cdot\text{L}^{-1}$ was slightly higher than in the control, without the amino acid, but the difference was not statistically proven. The value was comparable to that recorded in the present experiment after the foliar use of L-glycine at doses of $<120 \text{ mg}\cdot\text{L}^{-1}$ and $>180 \text{ mg}\cdot\text{L}^{-1}$. An increase in leaf pigmentation due to the use of amino acids has also been reported in the studies of Amin et al. [2011] and Fahimi et al. [2016]. According to Fahimi et al. [2016] and Souri et al. [2017], higher content of chlorophyll in leaves may be due to a reduction of its degradation and to the stimulating effect of amino acids on its biosynthesis. Amino acids play a role in protecting cell components during stress. They can probably counteract oxidation, peroxidation and degradation of cellular components, especially chlorophylls, thus prolonging the life of cells. The effect of this amino acid protection has been presented in several studies [Rai, 2002; Khan et al., 2012; Souri and Hatamian, 2019]. However, according to Noroozlo et al. [2019], L-glycine treatments at doses of 250, 500 and $1000 \text{ mg}\cdot\text{L}^{-1}$ did not have a significant effect on the SPAD value in Romaine lettuce leaves.

Plant growth is widely associated with biosynthesis of protein in leaves and its content in the plant [Marschner, 2011; Souri, 2016]. Glycine is one of the main amino acids and precursors necessary for the biosynthesis of proteins in plant cells [Ge et al., 2009; Ma et al., 2017]. In the present studies, the effect of L-glycine on lettuce protein content was not statistically significant, but Amin et al. [2011] claim that foliar use of amino acids, especially proteinogenic ones such as glycine, can significantly increase the concentration of amino acids and proteins in plant tissues. According to Mohammadipour and Souri [2019], moderate levels of glycine (10 or $20 \text{ mg}\cdot\text{L}^{-1}$) increased the protein concentrations in coriander leaves, which, according to the authors, was a result of more effective protein biosynthesis or a decrease in its degradation. There are also speculations that glycine, acting as a stress signal, stimulates greater protein biosynthesis [Souri, 2016]. Some studies have also shown that foliar use of amino acids increases the amount of cytokinin-like compounds in plant tissues, which may intensify protein biosynthesis [Marschner, 2011; Souri 2016].

The present studies did not report a significant effect of various doses of L-glycine on the content of dry matter and sugars in lettuce. Shehata et al. [2011] found that foliar application of an amino acid mixture to celery at concentrations

of 500 and 700 ppm increased the fresh and dry weight of the above-ground part and the content of soluble sugars in leaves.

L-ascorbic acid is one of the most important qualitative factors in assessing the nutritional value of leaf vegetables. In the present studies, there was no significant effect of various doses of L-glycine on the vitamin C content in lettuce. Other authors conducting research on the effect of L-glycine on the content of ascorbic acid in the edible parts of vegetables recorded inconclusive results. Applying L-glycine at a total dose of $210 \text{ mg}\cdot\text{L}^{-1}$ to lettuce, Khan et al. [2019] also found no significant differences in the content of vitamin C and dry matter in leaves compared to control, without the amino acid. In the studies of Noroozlo et al. [2019], the foliar application of glycine in concentrations of 250 and $500 \text{ mg}\cdot\text{L}^{-1}$ significantly increased the vitamin C content of Romaine lettuce leaves compared to the control plants. Amin et al. [2011] and Souri et al. [2017] reported that amino acids increased the vitamin C content of onion, while Junxi et al. [2010] reported that the foliar application of glycine to Chinese cabbage reduced the vitamin C content of its leaves. According to Souri and Hatamian [2019], elevated chlorophyll concentrations and the effectiveness of photosynthesis are strongly correlated with the vitamin C content in plant tissues. In the present research, however, no significant correlation between the amounts of ascorbic acid and the value of the SPAD index was observed.

CONCLUSIONS

L-glycine used for foliar feeding can significantly affect the growth and yield of leaf vegetables. The most favourable values of the head weight, root system weight and the number and length of leaves were recorded for the plants treated with foliar doses ranging from 80 to $120 \text{ mg}\cdot\text{L}^{-1}$. This range of foliar doses also increased the root-neck diameter, with a strong correlation between the latter and the weight of the root system and the head. This correlation indicates that a very important factor determining the growth of the above-ground part of lettuce is, above all, the uniform development of the whole plant and the effective transfer of water and nutrients from roots to leaves and photosynthesis products from leaves to roots. The most favourable for the weight of the above-ground part (the head weight) and the root

system was a foliar L-glycine dose of 120 mg·L⁻¹. At this dose, the plants were also characterized by a high value of the SPAD leaf greenness index, which was closely related to the supply of leaves with nitrogen. Foliar feeding with L-glycine had no statistically significant effect on the nutritional value of the lettuce tested in the experiment. However, its nutritional value did not decrease compared to the control plants, without foliar feeding.

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