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Research Article

Uptake and nitrate accumulation affected by partial replacement of nitrate-N with different source of amino acids in spinach and lettuce

Mahboobeh Jalali [1*], Mohammad Hadi Ghaffarian Mogharab [2], Hossein Nazary [3] and Ali Akbar Zare [4]

[1] Department of Soil Sciences, College of Agriculture and Natural Resources, Lorestan University, Khorramabad, Iran
[2] Soil and Water Research Department, Zanjan Agricultural and Natural Resources Research Center, Zanjan, Iran
[3] Dry Land Agricultural Research Institute (DARI), Agricultural Research, Education and Extension (AREEO), Iran
[4] Department of Soil Sciences, College of Agriculture, Isfahan University of Technology, Isfahan, Iran
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Abstract

As natural plant growth stimulators, amino acids are widely used to improve the yield and quality of crops. Change in enzymes activities of N assimilation (NR, NiR and GS), residual nitrate (NO_3^-), soluble protein content, and yield of spinach and lettuce plants were investigated under replacing 20% nitrate-N in the nutrient solution by L-glycine and blood meal amino acids. Seeds of the mentioned plants were sown in soilless medium for two weeks in the growth chamber. Seedlings in double leaf stage were transferred into the pots. After 30 days after transplanting, 20% of nitrate in the nutrient solution was replaced by L-glycine and a mixture of amino acids extracted from blood meal. Compared with the full nitrate treatment, amino acids replacement caused a decrease in nitrate accumulation in the leaves of studied plants. The decrease in nitrate accumulation was accompanied by a decrease in nitrate reductase activity, an increase in glutamine synthetase and high production of amino acids and chlorophyll content. The amino acids present in the blood meal were more effective than the L-glycine treatment to reduce the nitrate concentration in spinach (1.72%) and lettuce (17.5%). Compared to the full nitrate treatment, partial replacement of blood meal amino acids increased the soluble protein content in the leaves of spinach (67.36%) and lettuce (83.82%). Supplying with amino acids significantly enhanced total nitrogen and dry matter in the studied plants, although effects of blood meal amino acids treatment were higher than L-glycine. Based on the results, partial replacement of nitrate with amino acids could cause decrease in nitrogen accumulation in spinach and lettuce plants.

Keywords: Blood meal amino acids, L-glycine, Lettuce, Nitrate accumulation, Spinach

Introduction

Nitrate (NO_3^-) has a critical role in the nutrition and productivity of plants, especially vegetables. Vegetables can be considered as the main source of human nitrate intake and consist about 80% of daily intake of nitrate (Hord *et al.*, 2009). Leafy vegetables, such as lettuce and spinach show a preference to absorb and excessively accumulate highest concentrations nitrate in product organs (Iammarino *et al.*, 2013).

Nitrates is relatively low in toxicity, but it is broken down into much more toxic nitrites within the human body that is several times more toxic than nitrates (Gangolli *et al.*, 1994). Pathological conditions including cardiovascular diseases, diabetes, hypertension, metabolic syndromes and insulin resistance are some therapeutic properties of nitrites (Ghasemi and Zahediasl, 2013; Kevil *et al.*, 2011).

Therefore, it is essential to monitor of nitrate concentration in leafy vegetables and to provide a solution for reducing the nitrate accumulation in leafy vegetables, also there is a need for finding a cost-effective source of nitrogen to replace nitrate source in vegetables fertilization.

The nitrate concentration of plants products depends on various factors, including addition of nitrogenous fertilizers, radiance, type of growing, time of harvesting, temperature, length of growth period and pedologic properties of soil and light (Chung *et al.*, 2002). Source of nitrogen is the most important feature that affects nitrate concentration in vegetables (Chen *et al.*, 2002).

Nitrate ions after absorption by plant cells are reduced to NH_4^+ by nitrate reductase (NR) and nitrite reductase (NiR) (Guerrero *et al.*, 1981). Two possible effects of amino acids on the N-assimilation process have been suggested: Direct effect on mRNA of NR and feedback inhibition on NO_3^- reduction systems (Sivasankar *et al.*, 1997). The hypothesis is that these

*Corresponding Author, Email: jalali.mah@lu.ac.ir

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two effects can collectively influence higher N assimilation in plants.

Amino acids are natural chelates that pass readily through plant tissues and transport mineral to the plants, then mineral is released from the amino acids, the leftover shell are either used by the plant directly as amino acids or further degraded into water soluble nitrogen. Several studies have shown that plants such as tomato (*Solanum lycopersicum* L.) (Ge *et al.,* 2009), boreal forest species (Persson and Nasholm, 2001; Wang *et al.,* 2007) and wheat (*Triticum aestivum* L.) (Gioseffi *et al.,* 2012) can absorb organic form of nitrogen, especially in simple forms like amino acids.

The proteins in blood powder are broken down to amino acids by protease enzyme. Amino acids produced by enzymatic hydrolysis are called L-amino acids and easily absorbed by plant cells. Different processes in the plant are influenced by L-amino acids, like hormone and enzymatic functions, structure building, reproduction, immune response and nutrient transport. By adding L-amino acids to hydroponic solution, plants will grow same as plants grown in organic soils. L-glycine (L-Gly) is one of the most plentiful amino acids and frequently employed as a model amino acid in plant uptake studies because of its low molecular weight (Wang *et al.,* 2013).

It has been shown that total concentration of amino acids in the symplasmic spaces regulates nitrate uptake into the roots (Sivasankar *et al.,* 1997). Muller and Touraine (1992) found that replacement of 50% nitrate in the nutrient solution by alanine, glutamine, asparagine, arginine, beta-alanine, and serine resulted in inhibition of nitrate uptake by soybean seedlings. Although the replacement of a part of nitrogen source in nutrient solution by amino acids significantly reduces accumulation of nitrate in leafy vegetables (Chen *et al.,* 2002; Zhu *et al.,* 2018), onion bulb (Mobini *et al.,* 2014) and tomato (Ti-da *et al.,* 2008), there is little information regarding the effects of a mixture of amino acids extracted from blood powder on yield and nitrate concentrations in vegetables. The objective of this research was to investigate the effects of partial replacement of nitrate with L-glycine and a mixture of amino acids extracted from blood meal on yield and nitrate accumulation of spinach and lettuce.

Materials and methods

Sampling and analysis of blood powder: Blood meal sample was collected from blood powder producer factory of Isfahan. Proteins of blood meal were extracted by using hot acid hydrolysis method (Liu *et al.,* 2009). The extraction of amino acids was performed using the method of acidic hydrolysis of proteins (Liu *et al.,* 2009). Concentration of amino acids in the purified powder was measured using Automatic Amino Acid Analyzer (Model JLC-500/V) (Table 1).

Plant materials and treatments: The experiment was carried out in the greenhouse of the Lorestan University, using the spinach (*Spinacea oleracea* L. var. Sirius) and lettuce (*Lactuca sativa* L. var. capitata). Uniform-sized, undamaged seeds of the two plants were sown in soilless medium for two weeks in the growth chamber. Seedlings in double leaf stage were transferred into the pots (inner diameter 71.4 cm × 38.9 cm × 6.4 cm) filled with 10 L of a nutrient solution. Four plants were cultivated in each pot. Plantlets were grown with 75% strength Hoagland nutrient solution containing the following nutrients (in mg/L): $Ca(NO_3)_2$ = 1122; KNO_3 = 910; KH_2PO_4 = 272; NH_4NO_3 = 40; $MgSO_4$ = 247; EDTA (ethylenediamine tetra acetic acid ferric sodium salt) = 16.80; $ZnSO_4$ = 1.20; $Na_2B_4O_7$ = 0.28; Na_2MoO_4 = 0.20; $CuSO_4$ = 0.10 and $MnSO_4$ = 0.86. An electrical conductivity (EC) of 1.5–2.0 mS cm^{-1} and pH of 6.0–6.3 were maintained for optimal plant growth, regularly.

After 30 days from transplanting, 20% of nitrate in the nutrient solution was replaced by L-glycine and a mixture of amino acids extracted from blood meal (Mobini*et al.,* 2014). An amino acid-free nutrient solution was also considered as control treatment. Nutrient solutions were replaced every four days. After 45 days from transplanting, the plants were harvested at a commercial stage of development. The plants were grown in green house with average day/night temperatures of 32/20°C, average relative humidity of 60%, and 16 hrs. photoperiod with a photosynthetic photon flux of 250 µmol m^{-2} s^{-1} (400–700 nm) at the plant level. The experiments were set up in a completely randomized factorial design with four replications. It is noteworthy that pest and disease control operations were carried out in all treatments and no specific disease was seen in treatments.

Measurements and analyses: Two plants from each pot were sampled for NO_3^- -N determination on fresh material. The plants were rinsed with deionized water, separated into shoot and root, weighed and dried at 70C° for 48 hrs. in an oven. Leaf chlorophyll content was measured in acetone leaf extracts (Arnon, 1949) at stage V8. Maximal chlorophyll fluorescence (Fv/Fm) was measured after keeping the leaves in a dark place for about 40 mins. by the portable plant stress meter (Handy PEA V1.3, U.K) (Frankenberg, 2011). Nitrate-N was determined by the method as described by Singh (1988). The total nitrogen was determined by Kjeldahl's method (Saez-Plaza *et al.,* 2013). The samples were dried at 65ºC - 70ºC and then ground for analysis. In the wet digested sample extracts, P was measured calorimetrically; K and Na flamephotometrically, and Mg, Fe, Ca, Zn, Mn and Cu by atomic absorption spectrophotometer (Shimadzu AA-670-G, Japan) (Basgel and Erdemoglu, 2006). Samples were extracted with 50 mM potassium phosphate buffer (pH 7.0) and soluble protein content was measured using bovine serum albumin (BSA) as standard (Bradford, 1976).

Total amino acid concentration: Total amino acid concentration was measured using the method of Rosen (1957). The plant samples were extracted with 2 M KCl

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Table 1. Chemical characteristics and concentration of amino acids in the blood meal

Chemical characteristics			
parameter	Quantity	parameter	Quantity
pH	6.71	Total nitrogen (%)	12.8
EC (dS m^{-1})	8.97	Moisture (%)	9.8
Crude protein (%)	82.4	Mg (%)	0.09
Fe (%)	0.3	Ca (%)	0.6
Zn (%)	0.5	P (%)	0.9
Amino acid Concentration			
Amino acid	Concentration (%)	Amino acid	Concentration (%)
Arginine	17.7	Alanine	4.51
Serine	8.73	Aspartic acid	4.31
Glutamic acid	7.83	Cysteine	3.60
Lysine	6.17	Tyrosine	2.98
Glutamine	6.09	Phenylalanine	2.62
Glycine	5.51	Tyrosine	2.45
Leucine	5.46	Histidine	2.31
Threonine	5.28	Tryptophan	2.25
Proline	4.79	Isoleucine	2.01
Valine	4.71	Methionine	0.56

(1:10 plant to solution ratio) by shaking at 200 rpm for 1 hrs. The extract was filtered through Whatman 42 filter paper. Aliquots of 1 mL extracts were mixed with 0.5 mL cyanide–acetate buffer and 0.5 mL 3% ninhydrin solution in methyl cellosolve and heated for 15 mins. in a 100ºC water bath. Concentrations of amino acid were determined by comparing the optical absorbance (570 nm) of the samples relative to a standard curve prepared with leucine.

Nitrate reductase (NR): Frozen plant materials were homogenized in 4ºC and pestlewith 100 mM potassium phosphate buffer (pH 7.5) containing 5 mM cysteine, 2 mM EDTA and 0.5% (w/v) polyvinyl pyrrolidone. The homogenate was centrifuged at 20000 × g for 20 mins. at 4ºC. Nitrate reductase activity (NR) was determined according to the method as described by Debouba *et al*. (2006). The extract (0.1 mL) was incubated in a reaction mixture containing 0.1 M potassium phosphate buffer (pH 7.5), 0.14 mM NADH, and 7 M KNO_3 at 27ºC for 30 mins. Nitrate reductase (NR) was incubated with excess of 5 mM EDTA (for maximum NR determination). The reaction was stopped by of 0.5 mM zinc acetate. Nitrite ions were assayed after diazotation with 1% (w/v) sulfanilamide, 0.01% (w/v) N-naphthyl ethylenediamine-dichloride. After 20 mins. of incubation at room temperature, the absorbance was measured at 540 nm and amount of nitrite was calculated using standard calibration curve prepared for $NaNO_2$.

Glutamine synthetase (GS): Fresh bulb samples were homogenized (1:5, w/v) in the 4ºC using 50 mM Tris–HCl buffer (pH 7.6) containing 1 mM EDTA, 1 mM $MgCl_2$, 10 mM mercaptoethanol, 1 mM dithiothreitol, and 0.5% (w/v) polyvinylpyrrolidone (PVP). After centrifugation (20000 × g) for 20 mins., the supernatant was used for the enzymatic assay. Activity of glutamine synthetase (GS, EC 6.3.1.2) in the onion bulbs was determined according to Agbaria *et al*. (1998). The GS assay mixture, with a total volume of 2 mL, contained 50 mM Tris–HCl buffer (pH 7.2), 1 mM ADP, 50 mM glutamine, 20 mM $MgCl_2$, 20 mM sodium arsenate, 13 mM hydroxylamine, and enzyme extract. The reaction was initiated by the addition of hydroxylamine. After 30 mins. incubation at 30ºC, the reaction was terminated by addition of 3 mL the mixture of 0.5 M HCl, 0.2 M $FeCl_3$ and 0.24 M trichloroacetic acid. After centrifugation (3000 × g, 10 mins.), the absorbance was measured at 540 nm.

Nitrite reductase: Nitrite reductase activity was assayed by following the disappearance (reduction) of NO_2^- from the assay mixture (Miflin, 1967). The assay mixture contained 40 µmol potassium phosphate (pH 7.5), 0.5 µmol KNO_2, 0.04 mg methyl viologen, and 0.1 mL extract in a total volume of 1.1 mL. The reaction was started by addition of 0.2 mL of $Na_2S_20_4$ solution (8 mg/ mL) in 0.1 M $NaHCO_3$ and was terminated after 15 mins. by vigorously mixing the content of the assay tube on a vortex mixer until the methyl viologen was completely oxidized (for 10-15 s). Residual NO_2^- in the assay tubes was determined colorimetrically.

Statistical analysis: Experiment was carried out with at least four independent repetitions in triplicate. The one-way ANOVA procedure of the statistical program SAS 9.3 for Windows software package (SAS Institute Inc., Cary, NC) was used. The significance of differences between treatments was evaluated using LSD test at level of $P \leq 0.05$ and data were expressed as the mean values ± SD.

Results

Biomass of spinach and lettuce: Fresh and dry weights of spinach and lettuce showed quick responses to different forms of N supply as described in table 2. No significant differences in fresh biomass were observed between treatments in spinach plants. For the lettuce, compared to the full nitrate treatment, the fresh weights of plants receiving the L-glycine and a mixture of amino acids replacement treatment were increased by 27.09% and 32.31%, respectively. Dry weights in both plants were significantly higher at the mixture of amino acids

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Table 2. Effect of 20% nitrate replacement with L-glycine and blood meal amino acids on fresh and dry weight of spinach and lettuce

Treatment	Fresh weight (g plant^{-1})		Dry weight (g plant^{-1})		Total chlorophyll content (mg g^{-1}FW)		Fv/Fm	
	Spinach	Lettuce	Spinach	Lettuce	Spinach	Lettuce	Spinach	Lettuce
Nitrate	185.2±18.3^{a}	176.4±14.6^{b}	9.85±0.93^{b}	10.2±1.03^{c}	0.95±0.10^{c}	0.81±0.05^{c}	0.44±0.05^{bc}	0.34±0.03^{c}
L-glycine	174.5±19.1^{a}	224.2±17.1^{a}	10.79±1.1^{b}	12.5±1.17^{b}	1.03±0.08^{bc}	0.98±0.09^{b}	0.5±0.04^{bc}	0.49±0.06^{ab}
Blood amino acids	182.8±15.8^{a}	233.4±20.03^{a}	13.61±1.16^{a}	14.9±1.26^{a}	1.14±0.12^{a}	1.1±0.09^{a}	0.63±0.07^{a}	0.56±0.04^{a}

The data in the table represent the means ± the standard error (n=4). Different letters in the same column indicate a significant difference at P≤ 0.05 by LSD multiple range test

extracted from blood meal treatments than L-glycine and nitrate treatments. Different source of amino acids influenced on chlorophyll content. Blood amino acids were the most effective treatment in chlorophyll enhancement in both plants (Table 2). There was no significant difference between l-glycine and nitrate treatment in spinach. Also, amino acid treatments increased Fv/Fm compared to nitrate treatment (Table 2). The lowest Fv/Fm was observed in the nitrate treatment in both plants.

Nitrate accumulation: Compared to the full nitrate treatment, all amino acid treatments significantly (P≤ 0.05) reduced nitrate concentration (Figure 1). The reduction was greatest in the blood meal amino acids treatment which reduced nitrate concentration in both plants compared to the nitrate treatment. There was no significant difference between L-glycine and mix amino acid of blood meal in spinach plants (Figure 1). Generally, nitrate accumulation in lettuce is less than spinach.

Total nitrogen concentration: Total nitrogen concentration was significantly affected by nitrogen source although this effect was dependent on the plant species (Figure 2). In general, total nitrogen concentration in spinach leaves is greater than lettuce. In spinach plants, there was no significant difference between the impact of L-glycine and blood meal amino acids on total N concentration while blood meal amino acids treatment significantly (P≤ 0.05) increased total N concentration in spinach by 36.87% compared to the full nitrate treatment. In lettuce, all amino acid treatments significantly (P≤ 0.05) increased total N concentration. The increase was highest in the blood meal amino acids treatment (Figure 2).

Activity of nitrate reductase (NR), nitrite reductase (NiR) and glutamine synthetase (GS): Replacement of nitrate with L-glycine or blood meal amino acids had different influences on activity of NR, NiR and GS of both lettuce and spinach (Table 3). In spinach, L-glycine and blood meal amino acids treatment significantly (P≤ 0.05) decreased activity of NR compared to the full nitrate treatment. In lettuce, there was no significant decrease in activity of NR between full nitrate and L-glycine treatments while blood meal amino acids decreased the activity of NR about 52.83% and 8% compared to full nitrate and L-glycine treatments, respectively (Table 3).

Results showed that there was no significant difference in activity of NiR between different treatments in spinach plants (Table 3). In lettuce plants, the activity of NiR was significantly decreased by blood meal amino acids treatments replacement compared with the full nitrate and L-glycine treatment. Activity of glutamine synthetase (GS) affected by nitrogen sources(Table 3). For both plants, compared with full nitrate treatment, activity of GS increased with L-glycine and blood meal amino acids replacement treatment. The highest activity of GS in both plants belonged to the blood meal amino acids treatment (Table 3).

Soluble protein content: The effect of replacement treatment on soluble protein contents was dependent on the type of N source and plant species (Figure 3). In spinach, the soluble protein content was significantly increased only by blood meal amino acids treatment replacement compared to the full nitrate and L-glycine treatments. All amino acid treatments significantly increased (P≤ 0.05) soluble protein content in lettuce compared to the full nitrate treatment (Figure 3). The increase was highest in the blood meal amino acids treatment. The mentioned treatment enhanced the soluble protein content in lettuce by 83.82% compared to the nitrate treatment.

Total amino acid concentration: There was no significant difference in total amino acid concentration in spinach between L-glycine and nitrate treatments although blood meal amino acids treatment significantly (P≤ 0.05) increased total amino acid concentration (Figure 4). Both amino acid treatments significantly (P≤ 0.05) increased total amino acid concentration in lettuce compared to the full nitrate (Figure 4). The increase was highest in the blood meal amino acids treatment in which the total amino acid concentration in plants increased by 82.36 % compared to the nitrates treatment.

Nutrient contents: Nutrient content analysis (Table 4) revealed a dynamic changes in response to all treatments. The content of essential elements varied among treatments. In both plants, the highest concentration of phosphorus, potassium and zinc was observed in blood meal amino acids treatment. There was a significant increase in the concentration of calcium and magnesium in comparison to the amino acid treatments.

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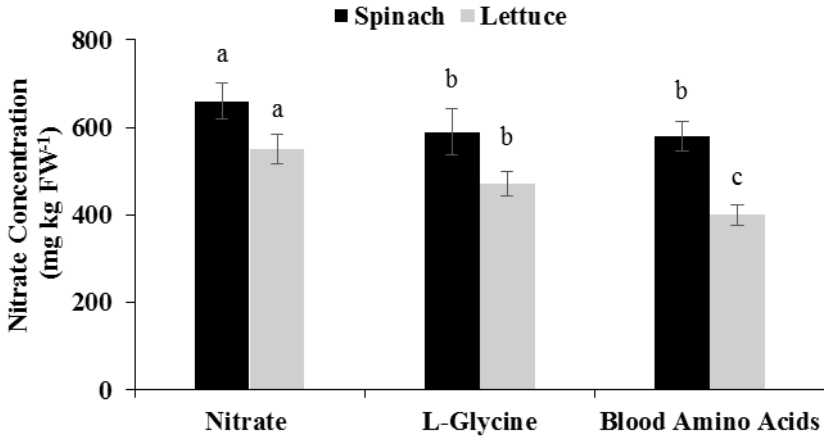

Figure 1. Effect of 20% nitrate replacement with L-glycine and blood meal amino acids on nitrate accumulation of spinach and lettuce. The data in the table represent the means ± the standard error (n=4). Different letters in the same column indicate a significant difference at P≤ 0.05 by LSD multiple range test.

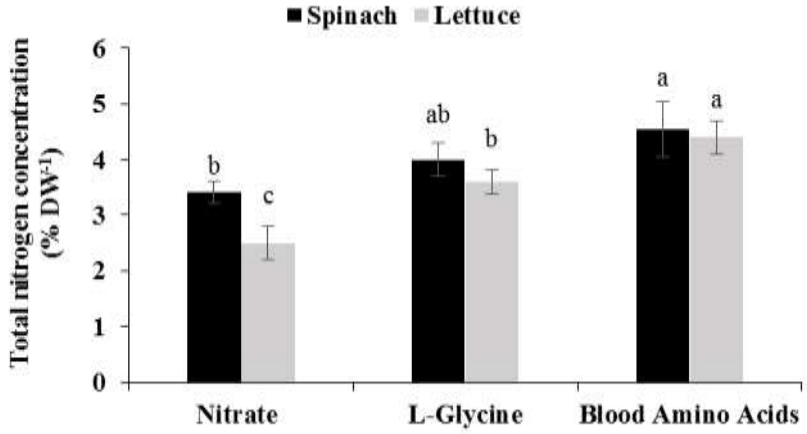

Figure 2. Effect of 20% nitrate replacement with L-glycine and blood meal amino acids on total nitrogen concentration of spinach and lettuce. The data in the table represent the means ± the standard error (n=4). Different letters in the same column indicate a significant difference at P≤ 0.05 by LSD multiple range test.

Table 3. Effect of 20% nitrate replacement with L-glycine and blood meal amino acids on activity of nitrate reductase (NR), nitrite reductase (NiR) and glutamine synthetase (GS) of spinach and lettuce

Treatment	NR (µmol NO_2^{-1} g^{-1} FW h^{-1})		NiR (µmol NO_2^{-1} g^{-1} FW h^{-1})		GS (U ml^{-1})	
	Spinach	Lettuce	Spinach	Lettuce	Spinach	Lettuce
Nitrate	0.64±0.05 [a]	0.81±0.09 [a]	12.81±1.1 [a]	12.64±0.9 [a]	1.32±0.19 [c]	1.33±0.13 [c]
L-glycine	0.44±0.02 [b]	0.75±0.05 [a]	11.74±0.9 [ab]	12.23±1.1 [a]	1.65±0.25 [b]	1.64±0.21 [b]
Blood amino acids	0.50±0.06 [b]	0.53±0.05 [b]	13.33±0.8 [a]	10.05±1.4 [b]	2.1±0.21 [a]	1.97±0.10 [a]

The data in the table represent the means ± the standard error (n=4). Different letters in the same column indicate a significant difference at P≤ 0.05 by LSD multiple range test

Discussion

The application of amino acids is a common practice for crops, the majority of treatments using biostimulants with a mixture of amino acids (Colla *et al.*, 2015). Energetically, the absorption of amino acids by plants is more beneficial compared to the absorption of NO_3^-; NH_4^+ or biological fixation, because the plant does not need energy to assimilate the absorbed nitrogen and then incorporate it into amino acids (Jones and Kielland, 2002).

In our study, we investigated replacement of 20% of inorganic N (nitrate) by organic (L-glycine or amino acids extracted from blood powder) source in the nutrient medium for spinach and lettuce. The new plants nutrition program could change uptake, metabolism and accumulation of nitrogen in spinach and lettuce, such as

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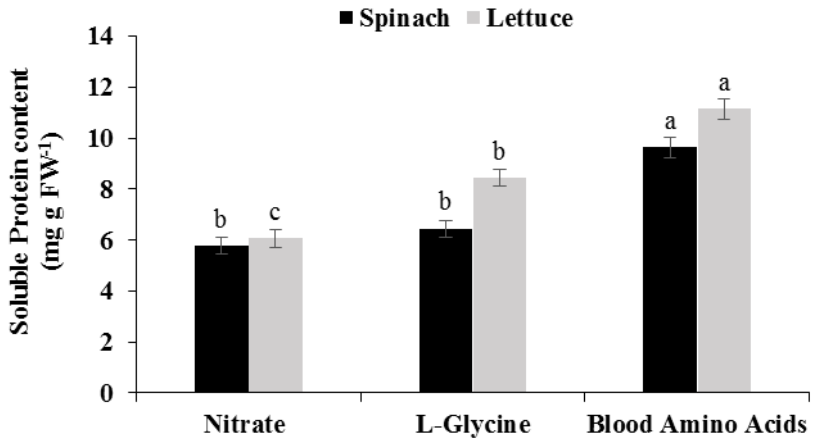

Figure 3. Effect of 20% nitrate replacement with L-glycine and blood meal amino acids on content of soluble protein of spinach and lettuce. The data in the table represent the means ± the standard error (n=4). Different letters in the same column indicate a significant difference at P≤ 0.05 by LSD multiple range test

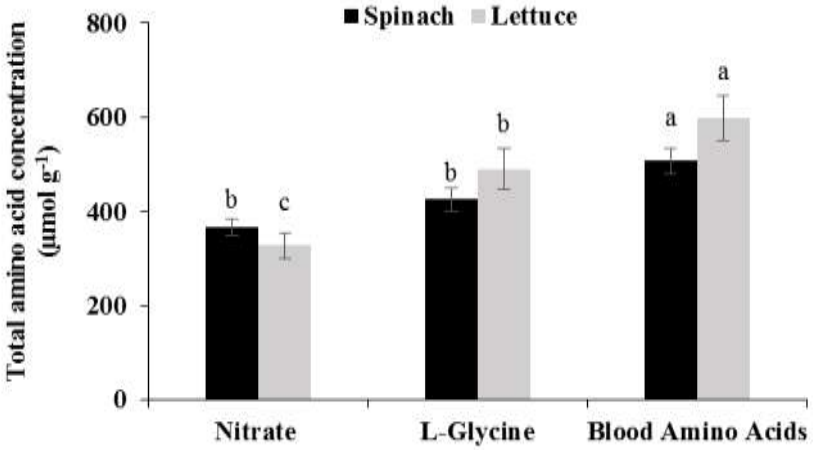

Figure 4. Effect of 20% nitrate replacement with L-glycine and blood meal amino acids on total amino acid concentration of spinach and lettuce. The data in the table represent the means ± the standard error (n=4). Different letters in the same column indicate a significant difference at P≤ 0.05 by LSD multiple range test

Table 4. Effect of 20% nitrate replacement with L-glycine and blood meal amino acids on essential elements of spinach and lettuce

Treatments	Nitrate	L-glycine	Blood amino acids	Nitrate	L-glycine	Blood amino acids
	Spinach			Lettuce		
P (% DW)	0.52±0.03 ^{c}	0.65±0.05 ^{b}	0.75±0.08 ^{a}	0.61±0.05 ^{b}	0.61±0.07 ^{b}	0.79±0.07 ^{a}
K (% DW)	1.98±0.09 ^{bc}	2.21±0.17 ^{b}	2.49±0.21 ^{a}	2.13±0.11 ^{b}	2.15±0.16 ^{b}	2.39±0.27 ^{a}
Ca (mg g^{-1} DW)	79.2±6.57 ^{a}	50.3±5.46 ^{b}	41.1±3.17 ^{b}	91.4±8.36 ^{a}	74.8±6.23 ^{b}	49.6±4.42 ^{c}
Mg (mg g^{-1} DW)	21.2±1.36 ^{a}	16.8±1.23 ^{b}	12.6±1.42 ^{c}	19.6±2.07 ^{a}	14.3±1.53 ^{b}	10.1±1.13 ^{c}
Fe (mg kg^{-1} DW)	103.1±11.32 ^{b}	126.6±10.53^{a}	112.4±9.65^{b}	98.6±8.53 ^{b}	119.8±10.9^{a}	125.6±12.65 ^{a}
Zn (mg kg^{-1} DW)	76.9±5. 73 ^{c}	92.4±7.36 ^{b}	113.7±9.22 ^{a}	80.3±6. 12 ^{c}	98.2±8.14 ^{b}	116.6±10.05 ^{a}
Cu (mg g^{-1} DW)	0.13±0.01 ^{b}	0.21±0.02 ^{a}	0.24±0.02 ^{a}	0.09±0.01 ^{b}	0.17±0.01 ^{a}	0.19±0.03 ^{a}

The data in the table represent the means ± the standard error (n=4). Different letters in the same column indicate a significant difference at P≤ 0.05 by LSD multiple range test

nitrate and protein content and the balance between them. Based on the results, compared with the full nitrate treatment, using amino acids accelerated biomass accumulation in the studied plants. These results were consistent with those for Chinese kale (*Brassica oleracea*) (Zhu *et al.,* 2018). They showed that glycine replacement could improve both the yield and qualities of Chinese kale. Also, Hassan *et al.* (2020) reported that Glutamine (Glu) is a regulator to promote nitrate uptake and root biomass accumulation in maize.

Based on the results, replacing 20% of nitrate by amino acids, regardless of amino acid treatment type,

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reduced accumulation of nitrate in spinach and lettuce plants. In lettuce, this reduction was highest in the blood meal treatment. The above mentioned agrees with the interpretation that amino acid can negatively regulate nitrate content in higher plants. Several explanations are possible for this phenomenon, such antagonism with end product repression and inhibition of NR activity (Khan *et al.,* 2019; Zhu *et al.,* 2018; Chen *et al.,* 2002; Mobini *et al.,* 2014). Also, concentration of amino acids in both plants treated with mix amino acid was correlated with activity of GS. In lettuce, increase in activity of GS at the presence of mix amino acid was accompanied with higher concentration of amino acids.

In the present study, higher effect of blood meal on nitrate reduction may be due to higher concentration of total amino acids added to the root media and variety of amine acids in blood powder. It has been recommended that plants preferred amino acids as sources of reduced nitrogen probably, and nitrate uptake was inhibited by amino acids (Wang *et al.,* 2007). The reduction of nitrate concentrations in plant tissues after addition of amino acids may result from reduction in nitrate uptake in plant roots (Sivasankar *et al.,* 1997; Aslam *et al.,* 2001; Zanin *et al.,* 2015). The results showed that the application of amino acids increased the concentration of phosphorus, potassium, zinc and copper in both plants. Previous studies have shown that partial replacement of nitrate with other N forms affects nutritional qualities (Chen *et al.,* 2002; Song *et al.,* 2011). Moreover, it can be suggested that amino acids function was to increase the nutrient uptake by plants through supplies nutrients (N, S, K) and improve nutrient availability and transportation (cheleated micronutrient) from the environment (Calvo*et al.,* 2014; du Jardin, 2015).

We can assume that amino acids not only make nutrients available to plants but also act as signal transducing molecules as small doses are sufficient for plant development response, whereas these molecules can act as signals of several useful plant physiological processes (Teixeira *et al.,* 2017). Interpreting the effect of amino acids on nitrate accumulation in vegetables is different among researchers. Some researchers have reported that amino acids in the form of reduced nitrogen are preferable to nitrates in the absorption of plants (Gunes*et al.,* 1996). Others have claimed amino acids can influence effective enzymes in nitrate metabolism (Liu *et al.,* 2014). The nitrate in the cytosol of the cells is converted to nitrite via the nitrate reductase enzyme and it is the first step of metabolic pathway of nitrate assimilation in plants which is done with the help of the enzyme.

The next step in NO_3^- assimilation was the conversion of the NO_2^- to NH_4 by the action of NiR. There was no significant difference between treatments in NiR activity. The response of GS to treatments was on the contrary to that of the NiR. The synthesis of NiR is induced by nitrate but although its activity is known to be repressed by ambient ammonium, there is evidence that this enzyme can be regulated by some amino acids (Oaks *et al.,* 2011).

Also, the results showed that, the highest activity of NR was obtained in full nitrate treatment in both plants, and no correlation emerged between inhibition of nitrate reductase and amino acid specifications. The result is in agreement with the other researches, which shows that nitrate and other nitrogen sources have a direct, but species-dependent effect on nitrate reductase activity in plant cells (Ohlund and Nasholm, 2004; Wallenda and Read, 1999; Kielland, 1994; Nordin, 2001). Some previous studies have shown that adding amino acid into medium has no significant effects on nitrate uptake (Gamborg, 1970; Behrend and Mateles, 1975). But Breteler and Arnozis (1985) reported that amino acids were caused a direct inhibition of nitrate transport into plant. The discrepancies from different researches may be related to the different plant species used in the experiments. Leafy vegetable have very important role in human diet. Healthy and nutritive production of spinach and lettuce, due to their fresh consumption of leaves, is unavoidable. According this result we could improve quality and quantity of spinach and lettuce with partial replacement of nitrate with amino acids.

Conclusion

The results of the present study suggest that, the different source of amino acids improved quantitative and qualitative characteristics of spinach and lettuce in comparison with nitrate. Replacing 20% nitrate-N in the nutrient solution by L-glycine and blood meal amino acids can affect the activities of three enzymes of N assimilation (NR, NiR and GS) and enhancement of dry matter, total chlorophyll content and Fv/Fm and reduction of nitrate accumulation. Furthermore, partial replacement of blood meal amino acids increase soluble protein content in the leaves of spinach and lettuce. Direct uptake of amino acids and increase of GS activity are two possible reasons for enhancement of endogenous amino acid concentration and thus reduction of nitrate accumulation in both plants. In both plants, the highest concentration of zinc, phosphorus and potassium was observed in blood meal amino acids treatment. There was a significant increase in the concentration of calcium and magnesium in comparison to amino acid treatments. According to the results, amino acids extracted from blood powder can effectively replace a part of nitrate in the nutrient solution of spinach and lettuce.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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