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The effect of partial replacement of nitrate with arginine, histidine, and a mixture of amino acids extracted from blood powder on yield and nitrate accumulation in onion bulb

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ABSTRACT

The effect of replacement of 20% nitrate-N in the nutrient solution by arginine, histidine, and blood powder amino acids on nitrate metabolism and bulb yield of two onion cultivars (*Allium cepa* L., cvs. Dorrcheh and Valenciana) was investigated. Addition of amino acids into the nutrient solution significantly reduced nitrate accumulation in onion bulb. This decrease was accompanied with lower activity of nitrate reductase (NR) and higher production of ammonium and amino acid in onion bulb. A mixture of amino acids extracted from blood powder was more effective than individual arginine and histidine treatments in decreasing bulb nitrate concentration. Onion plants supplied with amino acids accumulated higher levels of total nitrogen and amino acids in their bulbs compared with those unsupplied with amino acids. Activity of glutamine synthetase (GS) in onion bulb was also increased by amino acids. Application of amino acids significantly enhanced fresh and dry matter yield of onion bulb, although this increase was smaller by histidine in comparison with blood powder amino acid and arginine treatments. According to the results obtained, the mixture of amino acids extracted from blood powder can be used as a source of nitrogen in nutrient solution culture of onion.

1. Introduction

While nitrate is the main source of nitrogen for most plant species which have no N_2-fixing symbioses, excess accumulation of this nutrient ion in edible plants is a serious challenge for human health and increase the risk of carcinogenic diseases and methemoglobin (Zhu, 2002). Therefore, reducing nitrate level in edible plants is highly recommended for human health.

Vegetables are the main source of nitrate entrance into the human body and consist about 80% of daily intake of nitrate (Shen et al., 1982). Therefore, it is important to monitor accumulation of nitrate in vegetables and finding proper approaches to reduce nitrate concentration in leafy vegetables. Accumulation of nitrate in vegetables is dependent on external (e.g., rate and source of nitrogen, light intensity, temperature, and photoperiodism) and internal factors (i.e., plant cultivar) (Wenke et al., 2009). Source of nitrogen is the most important factor that affects concentration of nitrate in vegetables (Chen et al., 2002).

Nitrate ions (NO_3^-) after absorption by plant cells are reduced to NH_4^+ by nitrate reductase (NR) and nitrite reductase (Guerrero et al., 1981). NH_4^+ is then incorporated into amino acids by the glutamine synthetase-glutamine-2-oxoglutarate amidotransferase (GSGOGAT) enzyme system (Miflin and Lea, 1977) Both NO_3^- uptake and reduction processes are substrate inducible and are regulated by endogenous metabolites including amino acids (Guerrero et al., 1981).

Amino acids may also inhibit nitrate uptake by plant root cells (Aslam et al., 2001; Muller and Touraine, 1992; Sivasankar et al., 1997; Vidmar et al., 2000). Amino acids are end product of uptake and reduction of nitrate. Therefore, an increase in concentration of amino acids in plant tissues has inhibitory effect on nitrate absorption (King et al., 1993).

Recent studies show that replacement of a part of nitrate in nutrient solutions by amino acids significantly reduces accumulation of this anion in leafy vegetables (Chen et al., 2002). A possible reason for reduction of nitrate concentration in plant tissues is inhibitory effect of amino acids on transcription of HvNRT2, a gene considered to encode the high affinity transports of nitrate (Vidmar et al., 2000). Wenke et al. (2009) reported that exogenous amino acids increased activity of nitrate reductase (NR) and nitrite reductase and thereby reduced accumulation of nitrate in leaves of

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Table 1
Elemental analysis of blood powder.

Protein (%)	Fe	Zn	Cu	Mn	Ni
			($mg\,kg^{-1}$)		
80.5	2.6	12.8	5.5	12.4	2.2

lettuce. 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. There are several reports indicating the positive effect of exogenous amino acids on reduction of nitrate concentration in Chinese cabbage (Chen et al., 2002), onion (Güneş et al., 1996), and Pak-choi (*Brassica chinensis* L.) (Wang et al., 2007).

However, there is little information regarding the effects of a mixture of amino acids extracted from blood powder on bulb yield and nitrate concentrations in onion, which is one of the main vegetables grown in the Iran (Khodadadi and Hassanpanah, 2010). There are several reports indicating excess accumulation of nitrate in vegetables particularly onion produced in Iran (Mahmoudi, 2005; Rostamforoudi et al., 1999). In addition, there is a need for finding a cost-effective source of nitrogen replacing nitrate for vegetables.

The objective of this research was to investigate the effects of partial replacement of nitrate with two individual amino acids (histidine and arginine) and a mixture of amino acids extracted from blood powder on yield and nitrate accumulation of onion bulb.

2. Materials and methods

2.1. Sampling and analysis of animal blood powder

Blood powder sample was collected from Blood Powder producer factory of Fasaran that is located at 45 km north Isfahan. In this factory, animal blood is converted to powder at high pressure and temperature. Blood powder samples were kept at $4 \pm 0.5\,^{\circ}C$. Concentration of Fe, Zn, Cu, and Mn was analyzed by atomic absorption spectrophotometery (AAS) (Perkin Elmer Model 3030). Protein content was measured by using Autotech (Model 300) according to Kjeldahl method (Bremmer and Mulvancey, 1982) (Table 1).

2.2. Extraction of blood powder proteins

Proteins of blood powder were extracted by using hot acid hydrolysis method as described by Liu et al. (2009). In this method, 5 g blood powder and 50 mL distilled water were mixed and shaked for 10 min and pH of the mixture was adjusted at 0.5 using 2 M HCl. The solution was transferred at the reactor of hydrolysis at 120 °C for 10 min. The samples were centrifuged at $10{,}000 \times g$ for 10 min and then, the protein solution was collected.

2.3. Extraction of amino acids from the blood powder

The extraction of amino acids was performed using the method of acidic hydrolysis of proteins. 40 mL of 6 M HCl was added to the protein solution and the mixture was kept in the hydrolysis reactor at 120 °C for 12 h, the result solution was centrifuged at $10{,}000 \times g$ for 15 min. The collected raw amino acids were purified according to Liu et al. (2009). Concentration of amino acids in the purified powder was measured using Automatic Amino Acid Analyzer (Model JLC-500/V) (Table 2). The amino acid extraction efficiency value was around 61%.

Table 2
Concentration of amino acids in the blood powder.

Amino acid	Concentration (%)	Amino acid	Concentration (%)
Alanine	4.11	Lysine	3.92
Arginine	18.6	Methionine	0.34
Aspartic acid	4.38	Phenylalanine	2.12
Cysteine	7.3	Proline	5.05
Glutamic acid	8.48	Serine	8.66
Glutamine	6.73	Threonine	5.12
Glycine	6.29	Tryptophan	1.92
Histidine	1.91	Tyrosine	2.62
Isoleucine	2.44	Valine	4.16
Leucine	5.85	Lysine	3.92

2.4. Plant cultivation

Seeds of two onion cultivars (*Allium cepa* L., cvs. Dorrcheh and Valencian), most commonly grown in Iran, were surface-sterilized in a 1% aqueous solution of Na-hypochlorite for 10 min, rinsed with distilled water and germinated on moist filter paper in an incubator at 28 °C. 'Dorrcheh' is a white onion cultivar that its origin is city of Dorrcheh in Iran. 'Valenciana' is a Spanish red cultivar. Four weeks later, uniformly sized seedlings were transferred to 2 kg polyethylene pots, which contained sterilized quartz sand. One plant was planted in each pot and irrigated daily with 100 mL Johnson nutrient solution. The nutrient solution was prepared in $18\,M\Omega\,cm^{-1}$ and conductivity of $0.055\,\mu S\,cm^{-1}$ de-ionized water. Concentration of macronutrients in the nutrient solutions used for different nitrogen treatments is presented in Table 3. The nutrient solution also contained 100 μM Fe-EDTA, 50 μM KCl, 0.25 μM H_3BO_3, 2.0 μM $MnSO_4$, 2.0 μM $ZnSO_4$, 0.5 μM $CuSO_4$, and 0.5 μM $H_2Mo_7O_4$ adjusted to pH 6 with NaOH or HCl as a buffer. One month after transfer to the pots, 20% of nitrate in the nutrient solution was replaced by histidine, arginine, or a mixture of amino acids extracted from blood powder. An amino acid-free nutrient solution was also considered as control treatment. Experiments were performed in a greenhouse under controlled conditions with a 16/8 h light period at intensity of $390\,\mu mol\,m^{-2}\,s^{-1}$, 25/20 °C day/night temperature, and 65–75% relative humidity. Each treatment was run with three replicates under the same conditions.

Plants were harvested approximately 16 weeks after seeding, when about between 80% of the plant tops had collapsed. Upon harvest, the roots and leaves were removed from the bulbs. Fresh and dry matter yields of bulb were determined for each pot. The bulbs were placed at 65 °C for 48 h and dry matter weight of them was measured.

2.5. Ammonium, nitrate, and total nitrogen concentration

The amount of NH_4-N and NO_3-N in the plant extracts was measured by steam distillation (Keeney and Nelson, 1982(. The total N concentration of onion bulb was measured using Autotech (Model 300) according to Kjeldahl method (Bremmer and Mulvancey, 1982).

Table 3
Concentration of macronutrients in the nutrient solutions used for different nitrogen treatments.

	Free amino acid treatment	With amino acid treatments (mM)
KNO_3	4	4
$Ca(NO_3)_2$	1.75	3
KH_2PO_4	2	2
NH_4NO_3	2.5	–
$CaCl_2$	1.25	–
$MgSO_4$	1	1
Amino acid	–	2.5