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EFFECT OF AMINO ACIDS REPLACING NITRATE ON GROWTH AND NITRATE ACCUMULATION IN LETTUCE.

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ABSTRACT

A study was carried out to determine the influence of nitrogen (N) sources on the growth and nitrate (NO_3^-) accumulation of lettuce iceberg *(Lactuca sativa*) in nutrient film technique (NFT). Plants were supplied with treatments NO_3^- and six amino acids (AA)treatments, [glutamic acid (Glu), glutamine (Gln), alanine (Ala), glycine (Gly),aspartic acid (Asp) and argnine (Arg)] , at three NO_3^--N/AA-N ratios:(1) 100:0, (2) 80:20 and (3) 60:40.The total N concentration was 12.5 mmol L^{-1} for all treatments in nutrient solution. All AAs reduced plant growth with decreasing NO_3^- -N/AA-N ratios compared to the 100% NO_3^- treatment. Fresh and dry weight of shoot and root were significantly lower in Glu treatment than in all other treatments when NO_3^--N/AA-N ratios were 80:20 and 60:40. Decreasing NO_3^- -N/AA-N ratios reduced NO_3^- concentrations in fresh shoot, regardless of AA sources. Plants grown in (Glu) treatment had lower NO_3^-concentrations in fresh shoot than grown in all other treatments when NO_3^--N/AA-N ratio was 80:20 only.

Keywords: Amino acids, nitrate, accumulation and lettuce.

INTRODUCTION

Nitrogen is the most essential mineral nutrient that promotes sufficient plant growth and consequently yield. It is absorbed by roots either as ammonium (NH_4^+) or nitrate (NO_3^-) ion and incorporated in amino acids and eventually proteins (Blom-Zandstra, 1989). The most common N source in hydroponics is nitrate (NO_3^-). Frequently farmers use excessive rates of nitrogen in vegetables to avoid N-deficiency (Porto *et al*., 2008), ignoring environmental pollution, increase in production cost as well produce quality deterioration problems(Wang *et al*., 2008 and Montemurro, 2010). Nitrate is a common chemical compound in the nature and is widely found in soils, waters, plants, and foods. Generally, nitrate in vegetables is considered to be the main source of dietary nitrate intake (Santamaria *et al*., 1998). It has been shown that 72%–94% of the NO_3^- in the human body was derived from vegetables (Dich *et al*., 1996; Shen *et al*., 1982). Some previous researches suggested that the vegetables with high nitrate in the diet could put a human into the risk of gastrointestinal cancer and methemoglobinaemia (Bartsch *et al*., 1988 and Slob *et al*. , 1995).

Therefore, there is great concern about the nitrate content in the daily diet, especially in leafy vegetables. Then, some governments have stipulated official limits for NO_3^- in certain vegetables and drinking water. So, many governments and international organizations have set maximum nitrate levels for vegetables, for example, the maximum acceptable NO_3^- concentration was 3500 mg NO_3^- kg^{-1} (fresh weight) for winter-grown crops in Germany (Anonymous, 1993) and 3000 mg NO_3^- kg^{-1} (fresh weight) for leafy vegetables in China (Anonymous, 2003) and the maximum permissible levels established by European Commission Regulation 466/2001 (2001) for lettuce produced in protected areas (4500 mg kg^{-1} FW). Abundant NO_3^- availability leads to excessive absorption by the roots in larger quantities converted into NH_4^+ via nitrate reductase (Blom-Zandstra, 1989; Wang *et al*., 2008).Resulting to NO_3^- accumulation in the vacuoles of the cells. This frequently takes place in lettuce plants to continue N uptake until harvest (Salomez and Hofman, 2009) and accumulate excessive concentrations of NO_3^- in their tissues.

Many researchers have been conducted to reduce the accumulation of NO_3^- in vegetables. The addition of amino acids (AAs) partially replace the NO_3^- in the nutrient solution may reduce the NO_3^-concentrations in vegetables (Gunes *et al*., 1994;1996; Inal and Tarakcioglu, 2001; Inal *et al*., 1995; Wang *et al*.,2004; Wang *et al*.,2008 and Tsouvaltzis *et al*.,2014)

The purpose of this study was to investigate the effects of NO_3^--N/AA-N ratios on the growth and NO_3^- accumulation in lettuce.

MATERIALS AND METHODS

The experiment was conducted in green house, at Soil Department, Faculty of Agriculture, Cairo University during 2015 winter season.

Seeds of lettuce iceberg *(Lactuca sativa*) var Sahara were sowed in peat moss blocks .Fifteen- day-old seedlings were transplanted at rate of three plants per meter in gutters of 4 meters in length, spaced 30 cm apart, and held at a slope of 5%. Lettuce plants were grown in green house from 10 January to 25 February, 2015. The nutrient solution were held in 20- L containers, each container supplying on gutters (i.e. one treatment)

Eighteen treatments were used in the experiment (Table 1). The eighteen treatments included six amino acids (AA), argnine (Arg), alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), glutamine (Gln), , glycine (Gly) and three NO3-N/AA-N molar ratios: (1) 100: 0, (2) 80:20 and (3) 60:40 . All treatments had the same total N concentration at rate of 12.5 mmol L^{-1} in nutrient solution.

Other macronutrient compositions in the nutrient solutions were kept the same for all treatments [2 mmol L^{-1} magnesium sulfate($MgSO_4 \cdot 7H_2O$), 1 mmol L^{-1} monopotassium phosphate (KH_2PO_4), 4 mmol L^{-1} calcium chloride ($CaCl_2$), 0.02 mmol L^{-1} iron-[ethylenediaminetetraacetic acid (EDTA)] . The nutrient solutions also contained the same concentrations of micronutrients for all treatments as described by Hoagland and Arnon (1950). Treatments were arranged in a randomized complete block design with three replicates.

During the experiment, pH of nutrient solutions was maintained at about 6.0 by addition of either 1 mmol L^{-1} sodium hydroxide (NaOH) or sulfuric acid (H_2SO_4) and the nutrient solutions were replaced every four days.

After 45 days from transplanting, the plants were harvested at a commercial stage of development. 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 hr in an oven. Thereafter plants weighed.

Nitrate-N was determined by colourimetric method as described by Singh (1988).

Table 1. Nitrogen compositions (mmol L^{-1}) in nutrient solutions for different treatments.

Amino acids (AA)	NO_3^--N/AA-N	NO_3^--N	Arg -N	Ala-N	Asp-N	Glu-N	Gln-N	Gly-N
	100:0	12.5	0.0	_	_	_	_	_
Arg	80:20	10.0	2.5	_	_	_	_	_
	60:40	7.5	5.0	_	_	_	_	_
	100:0	12.5	_	0.0	_	_	_	_
Ala	80:20	10.0	_	2.5	_	_	_	_
	60:40	7.5	_	5.0	_	_	_	_
	100:0	12.5	_	_	0.0	_	_	_
Asp	80:20	10.0	_	_	2.5	_	_	_
	60:40	7.5	_	_	5.0	_	_	_
	100:0	12.5	_	_	_	0.0	_	_
Glu	80:20	10.0	_	_	_	2.5	_	_
	60:40	7.5	_	_	_	5.0	_	_
	100:0	12.5	_	_	_	_	0.0	_
Gln	80:20	10.0	_	_	_	_	2.5	_
	60:40	7.5	_	_	_	_	5.0	_
	100:0	12.5	_	_	_	_	_	0.0
Gly	80:20	10.0	_	_	_	_	_	2.5
	60:40	7.5	_	_	_	_	_	5.0

RESULTS AND DISCUSSION

Growth of lettuce:

Fresh and dry weight of shoot and root were significantly affected by AA sources, NO_3^--N/AA-N ratios and AA sources × NO_3^--N/AA-N ratio interactions (Table 2,3). When 20% and 40% of NO_3^--N were replaced by different AA in nutrient solutions, fresh and dry weight of shoot and root, reduced significantly compared to the 100% NO_3^- treatment. These results are in agreement with Wang *et al*.,(2008) who reported that when 20% of nitrate-N was replaced with (Arg)compared to the full nitrate treatment, pak-choi shoot fresh and dry weight increased significantly ($P \leq 0.05$), but when 20% of nitrate-N was replaced with (Ala), (Asp), (Glu), (Gly), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), phenylalanine(Phe), methionine (Met), lysine (Lys), serine (Ser), threonine (Thr), cysteine (Cys), and tyrosine (Tyr), shoot fresh and dry weight decreased significantly ($P \leq 0.05$). After replacing 20% of nitrate-N with asparagine (Asn) and (Gln), shoot fresh and dry weights were unaffected. Fresh and dry weight of shoot and root were significantly lower in Glu treatment than in all other treatments when NO_3^--N/AA-N ratios were 80:20 and 60:40.Shoot fresh weights, root fresh and dry weights were significantly higher in (Arg) treatment than other treatments, while shoot dry weight was significantly higher only when 40% of the NO_3^--N was replaced by (Arg) than other treatments. These results indicated that the replacement of NO_3^- -N by AAs as N sources inhibited plant growth and (Glu) had stronger inhibitory effect than all other treatments. The inhibitory effect increased with increasing replacement of NO_3^- -N by AA-N. Nitrate increases production of physiologically active forms of cytokinins which stimulate leaf growth (Rahayu *et al*., 2005). Nitrate also functions as osmotica in vacuoles for cell extension (Marschner, 1995).

However, Gunes *et al*., (1994, 1996) indicated that replacing 20% of the nitrate with Gly or a mixture of amino acids did not affect fresh and dry weight of onion and lettuce.

NO_3^- Concentration in lettuce Shoots:

Nitrate concentrations were significantly affected by NO_3^--N/AA-N ratios and AA source × NO_3^--N/AA-N ratio interactions (Table 4). When 20% and 40% of NO_3^--N were replaced by Arg-N, Ala-N, Asp-N, Glu-N, Gln-N and Gly-N in nutrient solutions, nitrate concentrations were reduced significantly compared to the 100% NO_3^- treatment .Decreasing NO_3^--N/AA-N ratios reduced NO_3^-concentration in fresh shoot under all AA sources except (Gln) treatment. Plants grown in (Glu) treatment had lower NO_3^-concentrations in fresh shoot than other treatments when NO_3^--N/AA-N ratio was 80:20 only, while plants grown in (Ala) treatment had lower NO_3^- concentration in fresh shoot than other treatments when NO_3^--N/AA-N ratio was 60:40. The use of hydrolyzed protein solution containing 11.3% L-amino acids (alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine), 4% total N and 25% organic matter,as alternative of inorganic supplemental fertilization is of high importance, in order to minimize nitrate content in lettuce (Tsouvaltzis *et al.* 2014) .The reasons for the inhibition were probably due to the reduction of NO_3^-concentrations in the nutrient solution after partially replacing NO_3^- by AA and NO_3^- uptake inhibition by AA. (Wang *et al.*, 2008) .The interpretation of this effect is contradictory among researchers, with other stating that amino acids are preferably absorbed by plants as reduced nitrogen source (Gunes *et al.*, 1994, 1996), while others claim that the main role of amino acids on nitrate uptake and assimilation is the regulation of many processes and metabolic pathways of plant N metabolism, such as nitrate. The results indicated that AA as a N source inhabited NO_3^-accumulation in lettuce shoots. The effect of amino acid application on NO_3^- reduction, has also been demonstrated in field grown Pak-choi (Wang *et al.*, 2007), radish (Liu *et al.*,2008 a, b), as well as lettuce (Gunes *et al.*, 1994) and onion (Gunes *et al.*, 1996) grown in NFT. The NO_3^- content of leafy radish was decreased 24-38% by applying MAA (the mixtures of alanine, β-alanine, aspartic acid, asparagine, glutamic acid, glutamine and glycine) were sprayed to plant leaf ($P < 0.001$) as compared to the control (Liu *et al.*, 2008a).The use of amino acids as alternative of mineral supplemental fertilization is of high importance, in order to minimize nitrate content in consumed vegetables particularly the nitrate content of plants was much lower than maximum permissible levels established by European Commission Regulation 466/2001 (2001) for lettuce produced in protected areas (4500 mg kg^{-1} FW).

Table2. Shoot fresh and dry weight (g. $plant^{-1}$) of lettuce grown in solutions with different NO_3^- -N/AA-N ratios and amino acid sources.

Amino acid Sources (AA) / **NO_3^- -N/AA-N Ratios(N)**	**Shoot fresh weight (g.$plant^{-1}$)**						
	(Arg)	**(Ala)**	**(Asp)**	**(Glu)**	**(Gln)**	**(Gly)**	**Mean**
80 : 20	147.49	101.84	96.04	89.55	128.17	112.37	112.58
60 : 40	140.64	98.32	90.95	86.91	131.68	108.91	109.57
Mean	144.07	100.08	93.50	88.23	129.93	110.64	111.08
100 : 0	175.58	175.58	175.58	175.58	175.58	175.58	175.58
L.S.D at 5% for	(AA) = 4.86 , (N) = 2.60 , AA × N=6.87						
	Shoot dry weight (g.$plant^{-1}$)						
Amino acid sources (AA) NO_3^--N/AA-N Ratios (N)	(Arg)	(Ala)	(Asp)	(Glu)	(Gln)	(Gly)	Mean
80 : 20	6.28	5.17	4.43	4.06	6.28	5.76	5.33
60 : 40	5.90	4.35	3.84	3.69	5.54	4.80	4.69
Mean	6.09	4.76	4.14	3.88	5.91	5.28	5.01
100 : 0	7.38	7.38	7.38	7.38	7.38	7.38	7.38
L.S.D at 5% for	(AA) = 0.39, (N) = 0.21, AA × N=0.55						

Table3. Root fresh and dry weight (g.$plant^{-1}$) of lettuce grown in solutions with different NO_3^- -N/AA-N ratios and amino acid sources.

Amino acid sources(AA) / **NO_3^- -N/AA-N Ratios(N)**	**Root fresh weight (g.$plant^{-1}$)**						
	(Arg)	**(Ala)**	**(Asp)**	**(Glu)**	**(Gln)**	**(Gly)**	**Mean**
80 : 20	18.59	13.18	12.27	11.32	16.17	14.22	14.29
60 : 40	17.95	12.50	11.46	11.11	16.85	13.86	13.96
Mean	18.27	12.84	11.87	11.22	16.51	14.04	14.13
100 : 0	22.00	22.00	22.00	22.00	22.00	22.00	22.00
L.S.D at 5% for	(AA) = 0.43, (N) = 0.23,AA × N=0.61						
	Root dry weight (g.$plant^{-1}$)						
Amino acid sources (AA) NO_3^--N/AA-N Ratios (N)	(Arg)	(Ala)	(Asp)	(Glu)	(Gln)	(Gly)	Mean
80 : 20	0.93	0.81	0.69	0.64	0.92	0.89	0.81
60 : 40	0.87	0.73	0.58	0.55	0.85	0.82	0.73
Mean	0.90	0.77	0.64	0.60	0.89	0.86	0.78
100 : 0	1.06	1.06	1.06	1.06	1.06	1.06	1.06
L.S.D at 5% for	(AA) = 0.05 , (N)= 0.03,AA × N=0.07						

Table 4. Nitrate concentrations (mg kg^{-1} f.w) in lettuce fresh shoots grown in solutions with different NO_3^- -N/AA-N ratios and amino acid sources.

Amino acid sources(AA) / NO_3^--N/AA-N Ratios(N)	NO$_3$ in lettuce fresh shoots (mg kg^{-1} f.w)						
	(Arg)	(Ala)	(Asp)	(Glu)	(Gln)	(Gly)	Mean
80 : 20	4327.01	3160.20	3460.30	2981.60	3060.00	4250.50	3539.94
60 : 40	3449.03	2638.05	3014.00	2708.00	3160.00	4060.00	3171.51
Mean	3888.02	2899.13	3237.15	2844.80	3110.00	4155.25	3355.73
100 : 0	4910.00	4910.00	4910.00	4910.00	4910.00	4910.00	4910.00
L.S.D at 5% for	(AA) =145.8, (N) =77.9, AA × N=206.2						

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تأثير إحلال النترات بالأحماض الأمينيه على النمو وتراكم النترات فى نبات الخس.

يوسف على عبد العال ، سيد طه أبو زيد ، أمل لطفى عبد اللطيف و شيماء حافظ نصر الصباغ
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يهدف هذا البحث الى دراسه تأثير مصادر النيتروجين على النمو و تراكم النترات فى نبات الخس (ايس برج) وذلك باستخدام مزارع الأغشيه المغذيه . تم استخدام ٦ أحماض أمينيه هى (الأرجنين ، الالانين، اسبارتيك ،الجلوتاميك، الجلوتامين، الجليسين) لكلا منها ٣ نسب من النترات-ن/ الاحماض الأمينيه –ن هى ١٠٠:٠ ، ٨٠:٢٠ ، ٦٠:٤٠ وكان تركيز النيتروجين فى المحلول المغذى ثابت فى كل المعاملات وهو ١٢,٥ ملليمول / لتر. أشارت النتائج الى نقص فى النمو فى كل معاملات الاحماض الامينيه مقارنه بمعامله الكنترول ١٠٠:٠ . حدث نقص معنوي فى الوزن الطازج والجاف للمجموع الخضرى والجذرى لمعامله الجلوتاميك مقارنه بالمعاملات الأخرى لكلا من نسبتى الاضافه ٨٠:٢٠ ، ٦٠:٤٠ .وكذلك حدث نقص فى تركيز النترات فى المجموع الخضرى فى معاملات الأحماض الأمينه مقارنه بالكنترول . حققت معامله الجلوتاميك بنسبه ٨٠:٢٠ أقل تراكم للنترات مقارنه ببقيه المعاملات تحت نفس النسبه.