Effect of nitrogen sources on the nitrogenous forms and accumulation of amino acid in head cabbage

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ABSTRACT

The effect of two different nitrogen sources: ammonium nitrate and calcium nitrate, applied at increased rates, on the content of the total nitrogen, protein and non-protein nitrogen, and the content and composition of amino acids in head cabbage leaves was studied. The higher nitrogen accumulation was established at the ammonium nitrate fertilization compared to the calcium nitrate, but the application of $Ca(NO_3)_2$ resulted in a higher content of nitrate nitrogen. More protein nitrogen was also observed in plants with $Ca(NO_3)_2$ fertilization. The highest applied fertilizer rate of ammonium nitrate resulted in a significant reduction of the protein nitrogen compared to increased free amino acids. The total content of amino acids increased gradually with the increase of nitrogen rates in plants fertilized with NH_4NO_3. When $Ca(NO_3)_2$ was applied, nitrogen rates higher than 500 mg/kg soil suppressed the synthesis of amino acids. A greater increase was observed as regards the levels of arginine, proline and some essential amino acids as lysine, phenylalanine and histidine. The increase of proline and alanine could serve as an indicator for unbalanced nitrogen nutrition.

Keywords: head cabbage; total nitrogen; protein nitrogen; non-protein nitrogen; amino acids

Head cabbage is a vegetable crop with enhanced uptake of nutrients resulting in a large vegetative biomass accumulation for a relatively short period. Balanced mineral nutrition with macro- and micronutrients is a necessary precondition for the normal plant growth and high yields of good quality. Plants absorb nitrogen from the soil mainly as ammonium nitrogen and nitrate nitrogen. Cabbage is a good source of carbohydrates (mainly sugars), vitamins, minerals, amino acids and other biologically active substances (Huxsoll et al. 1989). In most non-leguminous crop plants, leafs are the dominant organ in the amino acid synthesis and distribution (Noctor et al. 2002). The leaf amino acid content increases with enhanced supply of nitrogen during growth. The content of amino acids is a good indicator for the quality of vegetables. From about 80 known amino acids only 23 are important for the human diet. Essential amino acids are those that cannot be synthesized *de novo* by the organism, and therefore must be supplied in food. Nine amino acids generally regarded as essential for humans are: isoleucine, leucine, lysine, threonine, tryptophan, methionine, valine, phenylalanine and histidine. Some essential amino acids have been observed to be deficient in different plant food products; for example lysine is deficient in wheat, tryptophan in maize, and methionine in legumes. Generally, fluctuations in the amino acid proportion in plants might reflect changes in the source of nitrogen for growth (Peoples et al. 1987).

The aim of our study was to establish changes in different nitrogenous forms and in the composition of amino acids synthesized in head cabbage leaves after treatments with different rates and sources of nitrogen fertilizers.

MATERIAL AND METHODS

White head cabbage plants (*Brassica oleracea* var. *capitata*), cv. Balkan were grown in 3 kg plastic pots under glasshouse conditions on an alluvial meadow soil from Chepinci-Sofia region until head formation stage. All treatments were carried out in four replications. Nitrogen was applied as ammonium nitrate and calcium nitrate in increased rates as follows: 250, 500, 750 and 1000 mg/kg soil (N_{250}, N_{500}, N_{750}, and N_{1000}) on the background of the control without fertilization. Potassium and phosphorus were applied at the rate of 400 mg/kg soil. Initial accessible NPK reserves were as follow: nitrogen (NH_4^+-N – 8.9 mg/kg soil, NO_3-N – 26.5 mg/kg soil), P_2O_5 – 166 mg/kg, K_2O – 255 mg/kg soil and pH (H_2O) – 6.7. Exchangeable Al was zero, i.e. no exchangeable acidity in this soil type was found. At cabbage harvest, the following parameters were measured: total nitrogen – spectrophotometrically after Kjeldahl digestion, protein nitrogen – after Bernstain. Total amino acid content (free and bound into proteins) was determined after preliminary acid hydrolysis with 6N HCl by the aminoanalytic chromatograph Hromaspect according to the method of absorption chromatography. The leaf N-NO_3^- content was determined after extraction with 1% $KAl(SO_4)_2 \cdot 12\ H_2O$ (1:5) by ion selective electrode on MIN-100 measuring microprocessor (Stoicheva et al. 2002).

Data were expressed as means ± standard error where *n* = 4. Comparison of means were performed by the Fisher LSD test ($P \leq 0.05$) after performing multifactor ANOVA analysis.

RESULTS AND DISCUSSION

The percentage of total nitrogen towards total dry biomass varied between 1.22 and 3.96 in plants with $Ca(NO_3)_2$ fertilization (Table 1). A significant increase of total nitrogen was observed in the treatments with 1000 mg N in comparison with other treatments. Plants fertilized with NH_4NO_3 accumulated more total nitrogen than those fertilized with $Ca(NO_3)_2$ with the exception of the plants grown at the highest fertilizer rate. Higher values of non-protein N than the protein N values were established in the control plants. The percentage content of N-NO_3^- in the head cabbage was detected at N_{750} and N_{1000} rates after application of both nitrogen sources. Higher N-NO_3^- content was observed in the treatments with $Ca(NO_3)_2$. The content of protein N increased with the increase of nitrogen rates and maximal values were observed at 1000 mg N. In all the fertilized plants protein N predominated but the proportion between protein and non-protein nitrogen varied (Table 2).

Table 1. Percent content of nitrogen (total, protein, non-protein and nitrate) in head cabbage leaves (dry biomass)

Variants	Total N	Protein N	Non-protein N	N-NO_3^-
Nitrogen applied as $Ca(NO_3)_2$ (mg/kg soil)				
Control	1.22[a, *]	0.48[a]	0.74[c]	
N_{250}	1.39[a]	0.93[b]	0.46[a]	
N_{500}	2.00[b]	1.14[c]	0.86[d]	
N_{750}	2.09[b]	1.51[d]	0.58[b]	0.00056
N_{1000}	3.96[c]	2.12[e]	1.84[e]	0.0036
LSD($P \leq 0.05$)	0.193	0.262	0.091	
Nitrogen applied as NH_4NO_3 (mg/kg soil)				
Control	1.22[a]	0.48[a]	0.74[a, b]	
N_{250}	1.83[b]	1.11[b]	0.72[a]	
N_{500}	1.96[c]	1.14[b]	0.82[b]	
N_{750}	2.70[d]	1.56[c]	1.14[c]	0.00009
N_{1000}	3.00[e]	1.72[c]	1.28[d]	0.00065
LSD($P \leq 0.05$)	0.242	0.156	0.086	

*values are means ± SE; *n* = 4; different letters indicate significant differences assessed by the Fisher LSD test ($P \leq 0.05$) after performing ANOVA analysis

Table 2. Percentage proportion of protein N in relation to total N

Variants	$Ca(NO_3)_2$	NH_4NO_3
Control	39.34	39.34
N_{250}	66.90	60.65
N_{500}	57.00	58.16
N_{750}	72.25	67.77
N_{1000}	53.53	33.33

The proportion of protein N was almost equal at 500 mg nitrogen in both fertilizer forms. At other fertilizer rates the proportion of protein nitrogen was higher when $Ca(NO_3)_2$ was applied as a nitrogen source. At the highest supplied rate – N_{1000}, protein nitrogen percentage declined, hence the non-protein nitrogen increased, especially in plants fertilized with NH_4NO_3. The protein nitrogen proportion declined to a lesser extent in plants fertilized with $Ca(NO_3)_2$ (Table 2). As Mihov and Manuelyan (1985) suggested in head cabbage plants, the protein nitrogen was 60–67% of the total N, amino acids N were 18–21% and amides N were 7–9%. Protein nitrogen substances were mainly albuminous substances, while the non-protein N substances were mainly free amino acids including minor amino acids as lysine, arginine, histidine, thriptophan, methionine.

The total amino acids content significantly changed as a result of fertilization. When $Ca(NO_3)_2$ was applied (Figure1) the content of both total and essential amino acids was the highest at N_{500} – about two times higher than the control. Total amino acids content was suppressed at the maximal fertilizer rate – N_{1000}, while the values of essential amino acids remained unchanged in comparison with the values at N_{750}.

When NH_4NO_3 was used as a nitrogen source the content of total amino acids as well as the content of essential amino acids increased with the increase of nitrogen fertilizer rate until N_{1000} (Figure 2). The increase of total amino acids was about 2.5 times compared to the control. Consequently, the application of NH_4NO_3 did not suppress the level of total and essential amino acids, still protein nitrogen decreased significantly at the highest rate of NH_4NO_3 (Table 2). Therefore, the increase of total amino acids was higher compared to free amino acids. Zushi and Matsuzoe (2006) suggested that total quantity of free amino acids increased in tomato fruits under conditions of salinity stress.

Noctor et al. (2002) established a significant variability in the content of amino acids in leaves of wheat, potatoes and barley depending on the photosynthetic conditions. Six-fold changes in total amino acids content were observed in wheat and potatoes. Supplied inorganic nitrogen is assimilated in plants into amino acids glutamine, glutamate, asparagine and aspartate, which serve as important nitrogen carriers (Ta et al. 1984) and reflect changes in the nitrogen rates. At N_{1000}, glutamate increased twice over the control when nitrogen was applied as NH_4NO_3 (Table 4), but did not change when $Ca(NO_3)_2$ was used (Table 3). The level of aspartate increased 4 times compared to the control at N_{500}

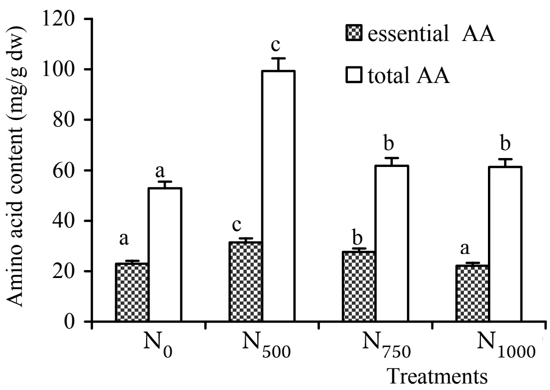

Figure 1. Content of total and essential amino acids depending on the nitrogen fertilizer, applied as $Ca(NO_3)_2$. Values are means ± SE, $n = 4$; different letters indicate significant differences assessed by the Fisher LSD test ($P \leq 0.05$) after performing ANOVA analysis

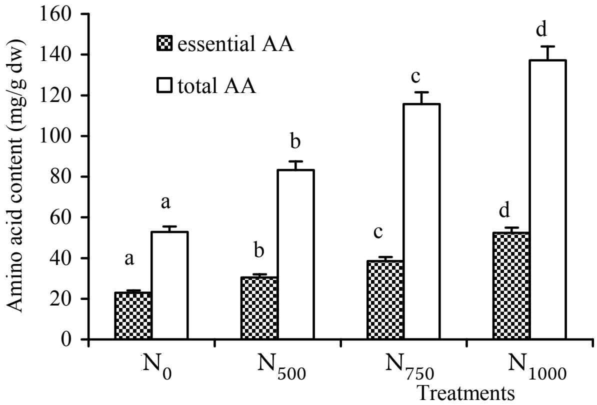

Figure 2. Content of total and essential amino acids depending on the nitrogen fertilizer, applied as NH_4NO_3. Values are means ± SE, $n = 4$; different letters indicate significant differences assessed by the Fisher LSD test ($P \leq 0.05$) after performing ANOVA analysis

Table 3. Amino acid composition of head cabbage, in dependence on nitrogen fertilizer, applied as $Ca(NO_3)_2$

Amino acids (mg/g dw)	Treatments			
	control	N_{500}	N_{750}	N_{1000}
Aspartate	3.31	12.80	5.08	4.80
Threonine	3.21	4.47	2.97	2.59
Serine	3.60	5.22	3.49	3.38
Glutamate	8.21	8.88	7.38	8.23
Glycine	3.31	4.64	2.97	2.90
Alanine	3.73	5.21	3.29	2.83
Valine	3.51	5.98	3.11	2.83
Methionine	trace	trace	trace	trace
Isoleucine	2.45	2.66	2.76	2.64
Leucine	4.97	6.55	5.19	5.27
Tyrosine	1.59	2.14	2.51	1.68
Phenylalanine	2.71	3.67	4.49	2.41
Histidine	1.66	2.76	2.77	2.11
Lysine	4.43	5.36	6.33	4.30
Arginine	1.24	4.65	5.24	11.37
Proline	4.91	24.36	4.22	3.96
Cystine	trace	trace	trace	trace
Total	52.83	99.37	61.80	61.31

$Ca(NO_3)_2$. At this nitrogen rate of calcium nitrate the content of all free amino acids significantly increased (Figure 1, Table 3). In the treatments with ammonium nitrate the highest increase of aspartate level and all the other amino acids were observed at N_{1000} (Figure 2, Table 4). The level of essential amino acids as lysine, phenylalanine and histidine also increased about 3–4 times under the same conditions of fertilization.

The composition of amino acids was genetically determined, but the content varied depending on several factors as fertilization and different soil-climatic conditions (Shmatko et al. 1986). According to Alehina (1992) significant changes in amino acid composition were observed as a result of sources and rates of nitrogen fertilization.

Our results showed that proline and alanine levels markedly increased at N_{500} $Ca(NO_3)_2$ (Table 3). A further increase in the nitrogen rates did not result in significant changes of these amino acids. When ammonium nitrate was applied, proline content increased 6 times in comparison with the control at N_{750}. Therefore, the increase of proline and alanine may serve as indicative plant reactions for an unbalanced mineral nutrition.

Marked increase of free proline occurs in many plants during moderate or severe water and salt stress; this accumulation, mainly as a result of increased proline biosynthesis, is usually the most outstanding change of free amino acids (Fougére et al. 1991). Mayer and Boyer (1981) reported that the content of proline under water stress conditions may increase from 10 to 100 times and reach to 1% of leaf dry biomass in several plant species. As the synthesis of alanine and proline may occur at the expense of glutamate and aspartate, Zushi and Matsuzoe (2006) established an increase of proline level and reduction of the levels of asparagine and glutamine under water stress conditions.

The level of arginine also strongly increased at N_{1000} compared to the control, irrespective of the source of nitrogen, about 9 times at $Ca(NO_3)_2$ and 11 times at NH_4NO_3 (Tables 3 and 4). According to Alipieva (1986) arginine is a predominating amino acid in the leaves of white head cabbage plants – 280 mg/kg fresh weight. Under the con-

Table 4. Amino acid composition of head cabbage, in dependence on nitrogen fertilizer, applied as NH_4NO_3

Amino acids (mg/g dw)	Treatments			
	control	N_{500}	N_{750}	N_{1000}
Aspartate	3.31	4.48	7.87	8.20
Threonine	3.21	4.09	6.06	6.22
Serine	3.60	4.78	6.83	7.59
Glutamate	8.21	7.44	11.98	17.92
Glycine	3.31	4.06	4.54	6.58
Alanine	3.73	4.25	5.40	7.24
Valine	3.51	4.77	5.98	7.11
Methionine	trace	trace	trace	trace
Isoleucine	2.45	3.03	4.26	5.64
Leucine	4.97	6.04	7.16	9.51
Tyrosine	1.59	2.78	3.32	4.34
Phenylalanine	2.71	3.53	4.15	6.19
Histidine	1.66	2.88	4.30	6.55
Lysine	4.43	6.11	6.61	11.12
Arginine	1.24	4.85	6.97	14.14
Proline	4.91	20.18	30.22	18.80
Cystine	trace	trace	trace	trace
Total	52.83	83.29	115.67	137.16

ditions of our experiment, traces of cystine and methionine were found.

The present study demonstrated that head cabbage plants accumulated more nitrogen when ammonium nitrate was applied as a nitrogen source compared to the $Ca(NO_3)_2$ fertilized plants; on the other hand, application of $Ca(NO_3)_2$ resulted in higher content of nitrate nitrogen. The source of nitrogen strongly affected protein synthesis. More protein nitrogen was found in plants with $Ca(NO_3)_2$ fertilization. The highest applied fertilizer rate of ammonium nitrate resulted in a significant reduction of protein nitrogen compared to increased free amino acids. Amino acid content gradually increased with the increase of nitrogen rates at NH_4NO_3 application. A greater increase was observed in the case of arginine, proline and some essential amino acids as lysine, phenylalanine and histidine. When $Ca(NO_3)_2$ nitrogen was applied at rates higher than 500 mg/kg soil it suppressed the synthesis of amino acids. The increase of proline and alanine can serve as an indicator for unbalanced nitrogen nutrition.

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