The contents of amino acids and sterols in maize plants growing under different nitrogen conditions

M. Pavlík[1], D. Pavlíková[2], J. Balík[2], M. Neuberg[2]

[1]*Isotope Laboratory, Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague, Czech Republic*
[2]*Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*

ABSTRACT

The effect of nitrogen nutrition on phytosterol and amino acid content in aboveground biomass of maize (*Zea mays* L.) was investigated in a pot experiment. For cultivation of maize plants nitrogen dose (2 or 4 g N/pot) was applied in the form of ammonium nitrate (AN) for control treatments or urea ammonium nitrate solution (UAN). UAN solution was applied according to the CULTAN method (Controlled Uptake Long Term Ammonium Nutrition). The content of amino acids as important nitrogen-containing compounds in plant biomass was affected by nitrogen nutrition. An increase of glutamine and asparagine levels in maize aboveground biomass was observed after UAN solution application. The results of free β-sitosterol analyses by HPLC showed its 94% increasing concentration after UAN application in contrast to AN treatments. Our results confirmed that sterol interconversions are controlled by environmental conditions and they are involved in the regulation of membrane properties in response to changing growth conditions.

Keywords: plant metabolism; ammonium nutrition; phytosterol; *Zea mays*; CULTAN

Nitrogen fertilizers based on ammonium or urea are converted to nitrate by microorganisms after their application to the soil. Transformation of ammonium to nitrate greatly enhances N mobility and thus increases the risk of N being lost through leaching from soil-plant system. To avoid this negative effect different techniques are used by farmers (Schittenhelm and Menge-Hartmann 2006). In Germany, CULTAN system (Controlled Uptake Long Term Ammonium Nutrition) is used (Sommer et al. 2002, Weber et al. 2008). Instead of top-dressing N-fertilizers in multiple split application in cereal production, CULTAN fertilization consists of injecting the entire amount of N (ammonium, urea or both) in a single dose locally to the root zone (Sommer et al. 2002). System of application to the root zone affected nitrogen uptake by plants. Plants mainly utilized nitrogen as ammonium. Ammonium is assimilated in leaf cells mainly through the plastidic isoform of glutamine synthetase and liberated mainly through the serine synthase reaction of the photorespiratory nitrogen cycle, amino acid catabolism and phenylalanine ammonia lyase activity (Britto et al. 2001, Hodges et al. 2003, Nourbakhsh and Alinejadian 2009). When nitrate and ammonium are provided to plants at similar concentrations, ammonium is generally taken up more rapidly than nitrate. This preference for ammonium over nitrate is explained by extra energy the plant must expend in reducing nitrate to ammonium before it can be incorporated into organic compounds (Howitt and Udvardi 2000). Whereas nitrate can be stored in vacuoles without detrimental effect, ammonium, and in particular ammonia, are toxic at quite low concentrations. The formation of amino acids, amides and related compounds is the main pathway of detoxification of ammonium ions. Most of ammonium has to be incorporated into organic compounds in the roots (Britto and Kronzucker 2002).

Supported by the Ministry of Agriculture of the Czech Republic, Project No. 1G58027, and by the Ministry of Education, Youth and Sports of the Czech Republic, Projects No. MSM 6046070901 and 2B06024.

The content of amino acids as important nitrogen-containing compounds in plant biomass is affected by nitrogen nutrition. Ammonium-grown plants often show a higher concentration of amino acids than nitrate-grown plants and also higher nitrogen supply caused an overall increase in amino acid contents (Loqué and von Wirén 2004, Atanasova 2008). According to Tilsner et al. (2005) under low nitrogen supply, the amino acid contents were comparable at all leaf ages and decreased slightly from young to mature leaves.

NH_4^+ ion affects membrane activities, strongly decreases membrane potential. Phytosterols are primary components of cellular membranes where they regulate fluidity and water permeability. Plant cell membranes incorporate a complicated mixture of sterols. The most common representatives are sitosterol, stigmasterol and campesterol (Guo et al. 1995). β-Sitosterol has been shown to be a membrane reinforcer, which regulates acyl chain ordering and water permeability of the phospholipidic bilayers (Ness 2003, Schaller 2004). A detailed study of sterol biosynthesis in *Zea mays* has demonstrated that composition of steroids varies as a function of both plant organs and period of development (Guo et al. 1995). Sterol interconversions are controlled by phytohormone levels and environmental conditions (light, temperature, water, response to ozone stress, ions etc.), it has been postulated that they are involved in the regulation of membrane properties in response to changing growth conditions (Moreau et al. 2002).

Because urea and ammonium-based fertilizers are commonly used, the toxicity of ammonium can have important implications in the agricultural practice. For this reason the goal of this study is a better understanding of the effect of ammonium nitrogen nutrition to changes in the composition of amino acids synthesized in maize plants after treatments with different levels and forms of nitrogen fertilizers. According to our hypothesis free β-sitosterol content in maize can be also affected by period, and system of nitrogen application. This paper discusses the plant response to injected nitrogen application by CULTAN system compared to conventional technology of nitrogen split application.

MATERIAL AND METHODS

The effect of nitrogen nutrition for plant metabolism was investigated in a pot experiment. For the experiment, maize seeds (hybrid Rivaldo) were sown into plastic pods (10 l volume) containing soil mixture as specified below. The plants (10 plants per pot) were cultivated under natural light and temperature conditions at the experimental hall of the Czech University of Life Sciences in Prague, Czech Republic. The water regime was controlled and the soil moisture was kept at 60% MWHC.

For cultivation of maize plants (*Zea mays* L.), 10 kg of Chernozem soil (pH_{KCl} = 7.2, C_{ox} = 1.83%, CEC = 258 $mmol_{(+)}$/kg) was thoroughly mixed with N dose applied in the form of ammonium nitrate (AN) for control treatments or was left without nitrogen for treatments with local nitrogen application. Nitrogen in the liquid form of urea ammonium nitrate (UAN) solution was applied into top soil (100 mm depth) at two points of pot 30 days after maize sowing. Each treatment was performed in five replications (Table 1). The pot experiment was repeated in two years. The results of both experimental years were not significantly different, therefore all results of analyses were averaged (two experimental years and five replications per each year).

The aboveground biomass of maize was sampled after 3 and 13 days after nitrogen application. The changes of dry matter yield, total nitrogen levels in the above-ground biomass as well as amino acid contents and β-sitosterol concentrations in maize leaves were determined.

The dried aboveground maize biomass was used for determination of total nitrogen, ammonium and nitrate nitrogen contents. For determination of total nitrogen content the plant material was decomposed by a liquid ashing procedure in H_2SO_4 solution (1:20 w/v) and analyzed by

Table 1. Design of experiment

Treatment	N rate (g/pot)	Application time
Ammonium nitrate 1 (AN1)	2	before sowing
Urea ammonium nitrate solution 1 (UAN1)	2	30 days after maize sowing
Ammonium nitrate 2 (AN2)	4	before sowing
Urea ammonium nitrate solution 2 (UAN2)	4	30 days after maize sowing

the Kjeldahl method on a KJELTEC AUTO 1030 Analyzer (Tecator). Ammonium and nitrate nitrogen was measured in H_2O solution (1:100 w/v) after 2 h shaking using segment flow analysis with colorimetric determination in a SKALAR plus SYSTEM apparatus.

The contents of macro elements in plant biomass were determined after dry ashing procedure using ICP – OES (Varian VistaPro, Australia).

Analyses of β-sitosterol from petroleum ether (Pe) extracts were performed on a HPLC instrument (Waters: Delta 600E multisolvent delivery system, Waters 3996 PDA detector, and Empower 1 PDA software) under the following chromatographic conditions: HPLC column packed with the Ascentis C8 reverse phase (Supelco, 250 mm × 4.6 mm 5µm particle size), using a mixture of the mobile phase A (water) and the mobile phase B (MeOH) at a flow rate of 0.6 ml/min. UV detection was monitored at 210, 205, 215, 245 and 220 nm. A gradient program (initial conditions of 20% A and 80% B, linearly increasing to 100% B over 15 min, holding for 55 min, returning to the initial conditions of 20% A and 80% B over 2 min, and holding for 18 min) was employed.

The amino acids from the aqueous extracts were determined using an EZ-faast amino acid analysis procedure (Phenomenex, USA). Samples were analyzed for amino acid contents for the gas chromatography coupled with mass spectrometry detection using a HP 6890N/5975 instrument (Agilent Technologies, USA). Samples were separated on a ZB-AAA 10 m × 0.25 mm amino acid analysis GC column under these conditions: the carrier gas (He) flow was kept constant at 1.1 ml/min. The oven temperature program was as follows, initial temperature 110°C, a 30°C/min ramp to 320°C. The temperature of the injection port was 280°C. A 1.5–2 µl sample was injected in split mode (1:15, v/v). MS conditions were as follows: MS source 240°C, MS quad 180°C, auxiliary 310°C, electron energy was 70 eV, scan m/z range 45–450 and sampling rate was 3.5 scan/s.

All chemicals (Merck) used were of analytical grade (p.a.) and ultra-pure water was used for sample analyses and isolations.

RESULTS

The lower nitrogen content in soil of UAN treatments in the first period of experiment and in following nitrogen application (30 days after maize sowing) affected yield and also total nitrogen content of aboveground biomass (Table 2). Yield decrease of UAN treatments compared to AN treatments varied between 22–37% in the first two sampling periods. Difference in harvest period was only 7–9%. Total nitrogen contents of UAN treatments were not significantly lower in contrast to AN treatments in the first sampling period. In the following sampling period (13 days after UAN application) and harvest N contents of UAN treatments were higher about 5–10% (Table 2).

Application of UAN fertilizer increased significantly N-NH_4^+ contents in plant biomass. The results confirmed a decline of cation contents (Ca, Mg, K) and an increase of anion content (P) Table 3.

Table 2. The yield of dry aboveground biomass (g/pot), total content of nitrogen in dry aboveground biomass (%)

Treatment	Sampling period		
	1	2	harvest
Yield of dry aboveground biomass (g/pot)			
AN1	2.7 ± 0.3	14.7 ± 2.3	216.4 ± 10.4
UAN1	2.1 ± 0.5	10.9 ± 1.8	198.0 ± 14.5
AN2	2.8 ± 0.4	15.2 ± 2.5	220.2 ± 9.1
UAN2	2.3 ± 0.5	11.1 ± 2.3	205.7 ± 6.8
Total content of nitrogen in dry biomass (%)			
AN1	3.12 ± 0.21	2.30 ± 0.41	1.05 ± 0.12
UAN1	3.01 ± 0.30	2.44 ± 0.52	1.14 ± 0.16
AN2	3.19 ± 0.19	2.49 ± 0.36	1.28 ± 0.22
UAN2	3.00 ± 0.35	2.80 ± 0.46	1.38 ± 0.19

The values represent the means of data obtained in the both series of experiments (n = 10, i.e. two experimental years and five replication per each year; ± standard deviation of results)

Table 3. Contents of ammonium and nitrate nitrogen, calcium, magnesium, potassium and phosphorus in plant aboveground biomass (mg/kg dry matter)

Treatment	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	P	Ca	Mg	K
	(mg/kg dry matter)					
Sampling period 1						
AN1	707 ± 27	4231 ± 62	1156 ± 36	16320 ± 118	2641 ± 52	24007 ± 169
UAN1	838 ± 19	3262 ± 101	1256 ± 63	12915 ± 165	1924 ± 21	22069 ± 216
AN2	694 ± 42	4807 ± 98	1322 ± 108	14709 ± 174	2246 ± 87	23030 ± 111
UAN2	850 ± 33	4255 ± 116	1462 ± 95	12574 ± 101	1821 ± 65	22035 ± 200
Sampling period 2						
AN1	720 ± 35	4725 ± 110	1213 ± 65	15605 ± 98	2830 ± 36	21761 ± 139
UAN1	827 ± 56	3902 ± 57	1450 ± 49	12475 ± 211	2556 ± 45	20743 ± 164
AN2	719 ± 68	4901 ± 78	1234 ± 89	13761 ± 169	2720 ± 71	20762 ± 213
UAN2	851 ± 83	4655 ± 66	1352 ± 102	12254 ± 144	2437 ± 49	20020 ± 91

The values represent the means of data obtained in the both series of experiments (n = 10, i.e. two experimental years and five replication per each year; ± standard deviation of results)

Glutamine (Gln) is not only the major amino acid used for nitrogen transport, but also a key metabolite that acts as an amino donor to other free amino acids. Our results confirmed an increase of glutamine level in maize biomass three days after UAN application (1st sampling period). Glutamine level of UAN2 treatment was more than three times higher in contrast to AN treatments (Figure 1). The results obtained in the 2nd period (13 days after UAN application) showed a significant decrease of glutamine levels of all maize treatments.

Significant increases of asparagine (Asn) levels were observed till in the 2nd sampling period (Figure 1). The concentrations of this amino acid in both UAN treatments were practically two times higher in contrast to AN treatments. The accumulation of Gln and Asn could be associated with the remobilization of assimilated nitrogen as proteins and other substances.

The results showed a significant increase of β-alanine concentration in maize biomass after UAN application in contrast to both AN treatments (Figure 1). The differences between UAN treatments were not observed.

The results did not confirm proline increases in our treatments. The proline concentrations varied between 19.9–20.9 μmol/kg DM in all treatments and sampling periods. L-Serine is involved in protein biosynthesis and serves as a precursor in a variety of important biosynthetic pathways, including phospholipids synthesis. The increase of its concentration on AUN treatments was not significant in contrast to AN treatments.

The results of free β-sitosterol analyses showed its increasing concentration after injected UAN application in contrast to AN treatments (Table 4). The concentrations of free β-sitosterol in plants growing on UAN treatments increased more than 100% during 13 days after fertilizer application. The β-sitosterol concentration in UAN1 plants corresponds to its concentration in AN1 plants in the 2nd sampling period. The β-sitosterol concentration in UAN2 plants was higher, about 94% in contrast to AN2 treatment in this period. Increasing concentration of this sterol in plants was affected by changing growth conditions after fertilizer application – change of soil pH and nitrogen content. The activity of the plasma membrane H^+-ATPase appears to be very sensitive to its sterol environment. The changes of its activity,

Table 4. Concentration of free β-sitosterol in petroleum ether fraction (Pe) (μg/mg fraction)

Treatment	Concentration of free β-sitosterol (μg/mg Pe fraction)	
	sampling period	
	1.	2.
AN1	15.5	29.8
UAN1	10.9	29.2
AN2	30.5	22.5
UAN2	18.4	45.4

The values represent the means of data obtained in the both series of experiments (n = 10, i.e. two experimental years and five replication per each year)

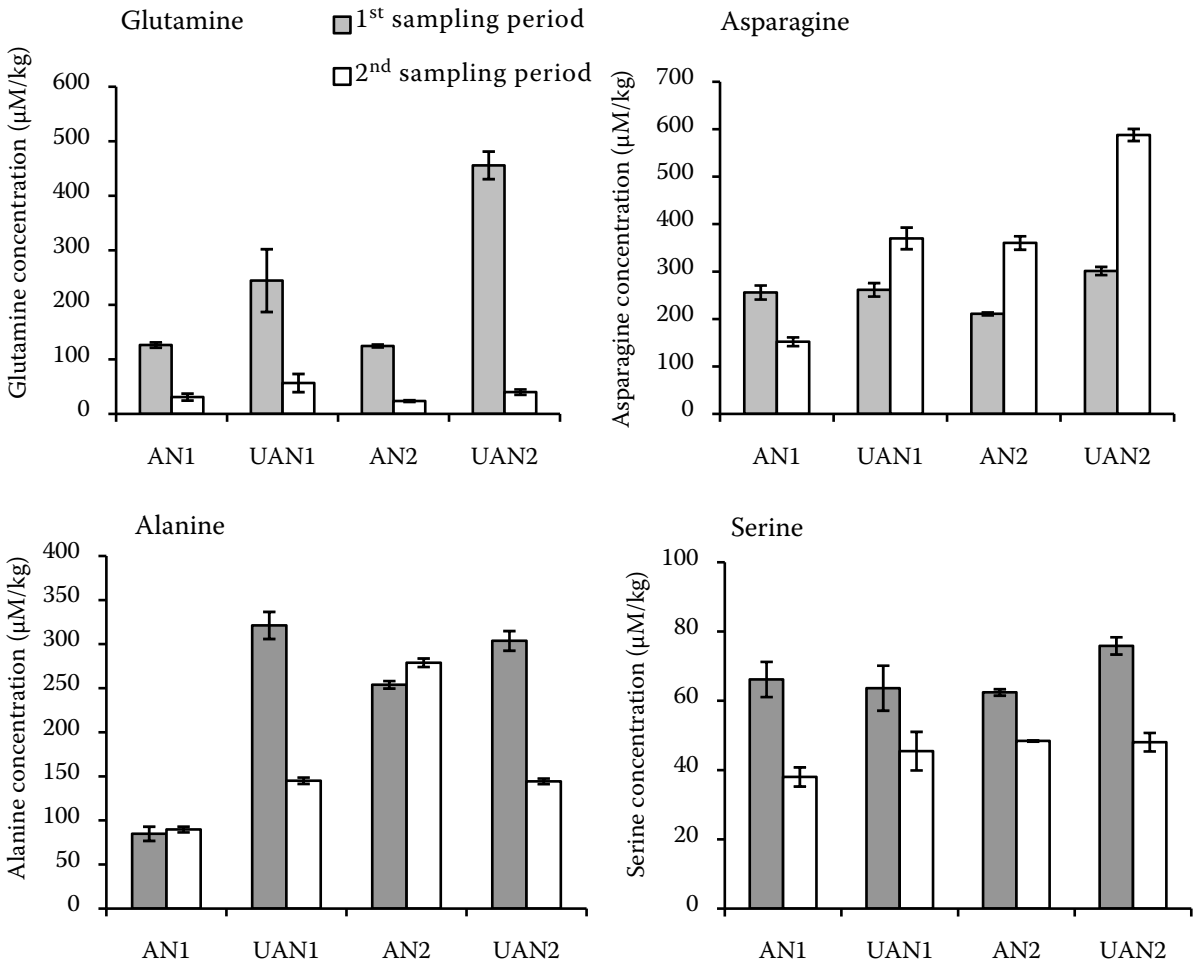

Figure 1. The effect of nitrogen application on amino acids concentration in above ground biomass of maize. The values represent the means of data obtained in the both series of experiments (n = 10, i.e. two experimental years and five replication per each year)

influenced by free sterols broke the balance of ion fluctuation across membrane.

The increase of a content of amino acids and also of acids of citrate cycle strongly affected concentration of β-sitosterol in petroleum ether fraction. Acetyl-CoA originated in amino acids catabolism is the most central intermediate in cellular metabolism, providing a link between many substances. Acetyl-CoA is a starting material for biosynthesis of fatty acids, several amino acids, sterols including their glucosides and ecdysteroids. For this reason, determination coefficients of the effect of amino acids on β-sitosterol biosynthesis are high (Table 5). Plants preferred mainly asparagine catabolism derivation to acetyl-CoA (R^2 = 0.961) in contrast to glutamine catabolism (R^2 = 0.487). The high coefficients of determination for plants growing on AN1 and UAN1 treatments (lower nitrogen rate) confirmed that catabolic derivations of amino acids to acetyl-CoA are common conversions of plant metabolism. On the other hand the coefficient of determination for serine is very low (R^2 = 0.417 and 0.029).

DISCUSSION

After application of urea ammonium nitrate (UAN) solution urea is converted into N-NH_4^+ by urease in the soil. N-NH_4^+ ion affected plant growth and metabolism at low concentration levels. Cruz et al. (1993) showed that ammonium inhibited the growth of 55% of a wide range of species in relation to nitrate. Cramer and Lewis (1993) found lower biomass accumulation with ammonium than with nitrate nutrition in wheat and maize. The results confirmed a decline of cation contents (Ca, Mg, K) and an increase of anion content (P). According to Britto and Kronzucker (2002) and others, a decline of essential cations such as potassium, calcium and magnesium is accompanied by an increase in

Table 5. The effect of biosynthesis of free amino acids on β-sitosterol contents (using polynomial function of the 2nd degree)

Amino acid	The effect of amino acid on β-sitosterol	
	1st sampling period	2nd sampling period
Ala	0.997	0.531
Gln	0.487	0.875
Asn	0.961	0.929
Ser	0.417	0.029

The effect is characterised by coefficient of determination R^2 – in the both sampling periods

tissue levels of inorganic anions such as chloride, sulfate and phosphate.

Glutamine is not only the major amino acid used for nitrogen transport, but also a key metabolite that acts as an amino donor to other free amino acids, primarily catalyzed by Glu synthase. This pathway interacts with carbohydrate metabolism or the energy status of the organ (Saneyuki and Wanyi 2008). Our results confirmed an increase of glutamine level in maize biomass three days after the UAN application (1st sampling period). The results obtained in the 2nd period (13 days after UAN application) showed a significant decrease of glutamine levels of all maize treatments. According to Ueda et al. (2008), ammonium supply strongly affected the content and synthesis of the amino acids in the plants. The supply of ammonium increased considerably the concentrations of the primary amino acids, and asparagine was the most predominant acid, followed by glutamine.

The significant increases of asparagine levels were observed till in the 2nd sampling period. Weber et al. (2008) reported that application of different N fertilizers affected the concentration of free asparagine in plants. According to Martínek et al. (2009), asparagine content in wheat was generally increasing at higher nitrogen doses, and nitrogen dose increase from 0 to 180 kg/ha increased the asparagine content to about 250%. The accumulation of Gln and Asn could be associated with the remobilization of assimilated nitrogen as proteins and other substance. The accumulation of greater Asn in plant biomass supplied with high N indicates that asparagine synthetase activity is almost certainly stimulated by increased N or by a decrease in C to N ratio than previously observed (Chevalier et al. 1996).

The results showed a significant increase of alanine concentration in maize biomass after UAN application in contrast to both AN treatments. The amino acid alanine accumulates markedly in response to stress in plants and it especially discussed in relation to intracellular pH regulation. Accumulation of free proline in plants has often been reported as a consequence of a wide range of environmental stresses (Pavlíková et al. 2008). According to Atanasova (2008) the increase of proline and alanine could serve as an indicator for unbalanced nitrogen nutrition.

Increasing concentration of β-sitosterol in maize plants in pot experiment was affected by changing growth conditions after UAN fertilizer application. Lindsey et al. (2003) confirmed that sterol interconversions are controlled by phytohormone levels and environmental conditions and they are involved in the regulation of membrane properties in response to changing growth conditions. According to Schubert and Yan (1997) differences in cell proton balance depend on nitrogen form of plant nutrition. Because of the large capacity for proton excretion the plasma membrane H^+-ATPase of cell plays an essential role during ammonium nutrition. H^+-ATPase is adjusted both quantitatively and qualitatively.

Recent evidence suggests that plant sterols are able to modulate the activity of the plasma membrane H^+-ATPases (Schaller 2003). According to Grandmougin-Ferjani et al. (1997) sitosterol and 24-methylcholesterol were found to inhibit H^+ pumping, with the percentage of inhibition increasing with sterol concentration. The sterol modulation of the plasma membrane H^+-ATPase activity was shown to be dependent both on the sterol concentration and the sterol molecular species. Thus, the activity of the plasma membrane H^+-ATPase appears to be very sensitive to its sterol environment. Phosphohydrolase activity was found to be much less sensitive to both sterol content and structure. The H^+-ATPase activity generates the proton motive force across the plasma membrane that is necessary to activity of most of the ion and metabolite transport. The changes of its activity, influenced by free sterols, broke the balance of ion fluctuation across membrane. H^+-ATPase

activity is also important for regulation of cytoplasmatic pH and for control of cell turgor, which drives organ movement, stomatal opening, and cell growth. The lower yield of aboveground biomass of maize in both UAN treatments compared to AN treatments confirmed the effect of a change of H^+-ATPase activity (cell growth).

Acetyl-CoA is starting material for the biosynthesis of fatty acids, several amino acids, sterols, including their glucosides, and ecdysteroids, and provides a link between these substances. For this reason the increase of content of amino acids and also of acids of citrate cycle strongly affected concentration of β-sitosterol in petroleum ether fraction. The importance of acetyl-CoA was described by many authors. Graham and Eastmond (2002) described degradation of amino acids on acetyl-CoA and its importance for fatty acids. Lange and Ghassemian (2003) focused their research on the isoprenoid biosynthetic pathway providing intermediates for the synthesis of a multitude of natural products which serve numerous biochemical functions in plants, for example sterols, and discussed the importance of acetyl-CoA in this pathway. Coefficient of determination for serine is very low. Serine is precursor in phospholipids synthesis. *O*-Phosphatidyl-L-serine synthesis in most plant tissues uses serine and is catalyzed by phosphatidylserine synthase.

REFERENCES

Atanasova E. (2008): Effect of nitrogen sources on the nitrogenous forms and accumulation of amino acid in head cabbage. Plant, Soil and Environment, *54*: 66–71.

Britto D.T., Kronzucker H.J. (2002): NH_4^+ toxicity in higher plants: a critical review. Journal of Plant Physiology, *159*: 567–584.

Britto D.T., Siddiqi M.Y., Glass A.D.M., Kronzucker H.J. (2001): Futile transmembrane NH_4^+ cycling: a cellular hypothesis to explain ammonium toxicity in plants. Proceedings the National Academy of Sciences of the United States of America, *98*: 4255–4258.

Chevalier C., Bourgeois E., Just D., Raymond P. (1996): Metabolic regulation of asparagine synthetase gene expression in maize (*Zea mays* L.) root tips. Plant Journal, *9*: 1–11.

Cramer M.D., Lewis O.A.M. (1993): The influence of nitrate and ammonium nutrition on the growth of wheat (*Triticum aestivum*) and maize (*Zea mays*) plants. Annals of Botany, *72*: 359–365.

Cruz C., Lips S.H., Martin-Louçäo M.A. (1993): Interactions between nitrate and ammonium during uptake by carob seedlings and the effect and the form of earlier nitrogen nutrition. Physiologia Plantarum, *89*: 544–551.

Graham I.A., Eastmond P.J. (2002): Pathways of straight and branched chain fatty acid catabolism in higher plants. Progress in Lipid Research, *41*: 156–181.

Grandmougin-Ferjani A., Schuler-Muller I., Hartmann M.-A. (1997): Sterol modulation of the plasma membrane H^+-ATPase activity from corn roots reconstituted into soybean lipids. Plant Physiology, *113*: 163–174.

Guo D.A., Venkatramesh M., Ness W.D. (1995): Developmental regulation of sterol biosynthesis in *Zea mays*. Lipids, *30*: 203–219.

Hodges M., Flesch V., Gálvez S., Bismuth E. (2003): Higher plant $NADP^+$-dependent isocitrate dehydrogenases, ammonium assimilation a NADPH production. Plant Physiology and Biochemistry, *41*: 577–585.

Howitt S.M., Udvardi M.K. (2000): Structure, function and regulation of ammonium transporters in plants. Biochimica et Biophysica Acta, *1465*: 152–170.

Lange B.M., Ghassemian M. (2003): Genome organization in *Arabidopsis thaliana*: a survey for genes involved in isoprenoid and chlorophyll metabolism. Plant Molecular Biology, *51*: 925–948.

Lindsey K., Pullen M.L., Topping J.F. (2003): Importance of plant sterols in pattern formation and hormone signalling. Trends in Plant Sciences, *8*: 521–525.

Loqué D., von Wirén N. (2004): Regulatory levels fort he transport of ammonium in plant roots. Journal of Experimental Botany, *55*: 1293–1305.

Martínek P., Klem K., Váňová M., Bartáčková V., Večerková L., Bucher P., Hajšlová J. (2009): Effects of nitrogen nutrition, fungicide treatment and wheat genotype on free asparagine and reducing sugars content as precursors of acrylamide formation in bread. Plant, Soil and Environment, *55*: 187–195.

Moreau R.A., Whitaker B.D., Hicks K.B. (2002): Phytosterols, phytostanols, and their conjugates in food: structural diversity, quantitative analysis, and health-promoting uses. Progress in Lipid Research, *41*: 457–500.

Ness W.D. (2003): Enzyme mechanisms for sterol *C*-methylations. Phytochemistry, *64*: 75–95.

Nourbakhsh F., Alinejadian A. (2009): Arginine ammonification and L-glutaminase assays as rapid indices of corn nitrogen availability. Journal of Plant Nutrition and Soil Science, *172*: 127–133.

Pavlíková D., Pavlík M., Staszková L., Motyka V., Száková J., Tlustoš P., Balík J. (2008): Glutamate kinase as a potential biomarker of heavy metal stress in plants. Ecotoxicology and Environmental Safety, *70*: 223–230.

Saneyuki K., Wanyi C. (2008): Analysis of nitrogen and amino acid contents in cut and potted flowers of *Eustoma grandiflorum*. Journal of the Japanese Society for Horticultural Science, *77*: 192–198.

Schaller H. (2003): The role of sterols in plant growth and development. Progress in Lipid Research, *42*: 163–175.

Schaller H. (2004): New aspects of sterol biosynthesis in grown and development of higher plants. Plant Physiology and Biochemistry, *42*: 465–476.

Schittenhelm S., Menge-Hartmann U. (2006): Yield formation and plant metabolism of spring barley in response to locally

injected ammonium. Journal of Agronomy and Crop Science, *186*: 223–228.

Schubert S., Yan F. (1997): Nitrate and ammonium nutrition of plants. Effects on acid/base balance and adaptation of root cell plasmalemma H^+-ATPase. Zeitschrift für Pflanzenernaehrung und Bodenkunde, *160*: 275–281.

Sommer K., Scherer H.W., Kunert A. (2002): CULTAN-Verfahren bei Mais. Mais, 20–23.

Tilsner J., Kassner N., Struck C., Lohaus G. (2005): Amino acid contents and transport in oilseed rape (*Brassica napus* L.) under different nitrogen conditions. Planta, *221*: 328–338.

Ueda S., Ikeda M., Yamakawa T. (2008): Provision of carbon skeletons for amide synthesis in non-nodulated soybean and pea roots in response to the source of nitrogen supply. Soil Science and Plant Nutrition, *54*: 732–737.

Weber E.A., Koller W.D., Graeff S., Hermann W., Merkt N., Claupein W. (2008): Impact of different nitrogen fertilizers and an additional sulfur supply on grain yield, quality, and the potential of acrylamide formation on winter wheat. Journal of Plant Nutrition and Soil Science, *171*: 643–655.

Received on October 27, 2009

Corresponding author:

Ing. Milan Pavlík, CSc., Academy of Sciences of the Czech Republic, Institute of Experimental Botany, Isotope Laboratory, Vídeňská 1083, 142 20 Prague 4, Czech Republic
phone: + 420 241 062 476, fax: + 420 241 062 150, e-mail: pavlik@biomed.cas.cz