THE EFFECT OF NITROGEN AND SULPHUR FERTILIZATION ON YIELD AND QUALITY OF KOHLRABI (*Brassica oleracea*, L.)[1]

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SUMMARY

In a greenhouse pot experiment with kohlrabi, variety Luna, we explored the joint effect of N (0.6 g N per pot = 6 kg of soil) and S in the soil (25–35–45 mg kg^{-1} of S) on yields, on N, S and NO_3^- content in tubers and leaves, and on alterations in the amino acids concentration in the tubers. S fertilisation had no effect on tuber yields. The ranges of N content in tubers and leaves were narrow (between 1.42–1.48 % N and 1.21–1.35 % N, respectively) and the effect of S fertilisation was insignificant. S concentration in the tubers ranged between 0.59 and 0.64 % S. S fertilisation had a more pronounced effect on the S concentration in leaf tissues where it increased from 0.50 to 0.58 or to 0.76 % S under the applied dose. The NO_3^- content was higher in tubers than in leaves. Increasing the S level in the soil significantly reduced NO_3^- concentrations in the tubers by 42.2–53.6 % and in the leaves by 8.8–21.7 %. Increasing the S content in the soil reduced the concentration of cysteine + methionine by 16–28 %. The values of valine, tyrosine, aspartic acid and serine were constant. In the S_0, S_1, and S_2 treatments the levels of threonine, isoleucine, leucine, arginine, the sum of essential amino acids and alanine decreased from 37 to 9 %. The histidine concentration increased with increasing S fertilisation. S fertilisation of kohlrabi can be recommended to stabilize the yield and reduce the undesirable NO_3^- contained in the parts used for consumption.

Index terms: ammonium sulphate, dose, tuber, leaves, nitrates, amino acid concentrations.

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RESUMO: *EFEITO DA ADUBAGEM COM O NITROGÉNIO E ENXOFRE AO RÉDITO E À QUALIDADE DAS COUVES-RÁBANOS (*Brassica oleracea, *L.)*

*Deficiência aguda de S no solo tem sido observada na Europa desde os anos 1980. O couve-rábano é uma das plantas com maior exigência nesse nutriente e sua interação com o N é frequentemente relatada na literatura. Este trabalho foi conduzido em casa de vegetação visando testar o efeito da aplicação de S, na presença de N, na produção e qualidade de couve-rábano (*Brassica oleracea*, L., variedade Lua). As plantas foram cultivadas em vasos contendo 6 kg de solo, aos quais foram aplicados 0,6 kg de N e S para se obter os teores no solo de: 25 (teor natural), 35, ou 45 mg kg*$^{-1}$*. Foram avaliadas a produção e o teor de N, S e* NO_3^- *nas raízes e nas folhas e as alterações no teor de aminoácidos nas raízes. A produção de raízes do couve-rábano não afetada pela adubação com S. O teor de N nas raízes e nas folhas variou de 1,42–1,48 % e de 1,21–1,35 % de N, respectivamente, não sofrendo efeito significativo da adubação com S. O teor de S nas raízes oscilou entre 0,59–0,64 % de S. A aplicação de S influenciou positivamente o seu teor no tecido foliar, que apresentou estreita relação com os teores do nutriente no solo, chegando a 0,50; 0,58 e a 0,76 % de S, respectivamente, para os três teores testados. O teor de* NO_3^- *foi maior nas raízes do que nas folhas. O aumento das doses de S reduziu o teor de* NO_3^- *nas raízes, em 42,2 a 53,6 %, e nas folhas em 8,8 a 21,7 %. O aumento do teor de S no solo reduziu o teor de cisteína + metionina em valores que variaram de 16 a 28 %, mas não afetou os teores de valina, tirosina, ácido aspártico e serina. Com a variação dos teores de S entre 25 e 45 mg kg*$^{-1}$*, houve decréscimo de treonina, isoleucina, leucina, arginina, e a soma de amino ácidos esenciais e alanina na faixa de 37 a 9 %. O teor de histidina aumentou com a elevação do teor de S no solo. A adubação do couve-rábano com S é recomendada sobre tudo para a estabilização da produção e para a redução dos teores de* NO_3^-*, cuja presença em alimentos é indesejada.*

Termos de indexação: sulfato de amônio, dose, raízes, folhas; nitrato; aminoácidos.

INTRODUCTION

An acute deficiency of sulphur (S) in the soil has been observed in Europe since the 1980ies. In the Czech Republic this phenomenon was intensified after 1990. Vegetables requiring a higher supply of S began to respond very sensitively. One of the most demanding vegetables is kohlrabi, which absorbs 1.5 kg S per ton yield. It is the most S–dependent of all brassicas. The typical visual S deficiency symptom in kohlrabi is inhibited leaf formation and a light colour of the youngest leaves. The reduced soil content of available S is the result of reduced applications of mineral and organic fertilisers and fungicides (Hlušek et al., 2003).

The substantial decrease in SO_2 emission to less than 10 kg ha^{-1} of S further intensified S deficiency in plants, because as much as 30 % of its total amount can be absorbed from SO_2 in the air. The S cycle and its effect on plants are often compared to N (oxidation in soil and reduction in plants). The main difference is that S from organic compounds can be re-oxidised to SO_4–S in plants (Vanìk et al., 2001).

Much like N, S is highly mobile in the soil with a limited sorption capacity. Plants take it up from the soil solution mainly in the form of sulphates (SO_4^{2-}) (Marschner, 1995). After reduction in the plant S participates in various primary and secondary compounds, such as the amino acids cysteine and methionine, vitamins B_1 and H and enzymes and coenzymes (Haneklaus et al., 1997). Other S-containing compounds are, e.g., tripeptide glutathione (antioxidant and precursor of phytochelatins, which are able to influence the detoxification of some heavy metals), ferredoxine, sulpho-lipids, glucosinolates and others (Schubert, 2002).

One of the essential amino acids, Methionine ($C_5H_{11}O_2NS$) is, together with cysteine ($C_3H_7O_2NS$), an indispensable component of proteins – their bonds play an important role in the protein structure where they often form intra or inter-chain disulphide bridges. The S content in proteins is relatively stable and in a certain proportion to N (Vaněk et al., 2001).

The balance of all macro and micro biogenic elements in the soil is crucial when growing kohlrabi, particularly in the case of N, which determines the yield level as well as the NO_3^- content. The production of one ton of kohlrabi draws 5 kg N. In terms of its use and losses it would be suitable to split the total amount of N in mineral fertilisers in doses and apply it in the soil prior to sowing (Hlušek et al., 2002). Schenk et al. (1991) reported that for kohlrabi the content of mineral N should be contained in the 0–0.3 m layer. The use of fertiliser N is inhibited in S-deficit crops. This could increase the escape of N into the environment, particularly by NO_3^- leaching into the hydrosphere or via gas losses into the atmosphere. Every missing kilogram of S, which limits plant growth, causes N losses of 4–15 kg (Schnug, 1991). Under S deficiency higher N doses increase the deficiency (Janzen & Bettany 1984), resulting in further yield reduction.

The nitrate (NO_3^-) concentration in vegetables is an important quality criterion (Schuphan, 1976; Vetter, 1988). In plant chloroplasts S is a component of the enzyme nitrite reductase which is responsible for the reduction of NO_2^- to NH_3 (Mengel & Kirkby, 1978). Paulsen (2001) pointed out the risk of increased levels of non-protein N in plants. Under S deficiency, the NO_3^- content can increase and after reduction to NO_2^-, block the ability of haemoglobin to transfer oxygen, or form the first degree of carcinogenic nitrosamines. Schnug (1990) also stated that the NO_3^- content in vegetable tissues increased under acute S deficiency.

The objective of the greenhouse pot experiment with kohlrabi was to test the effect of joint N and S fertilisation on kohlrabi yields, NO_3^- content in tubers and leaves, and changes in the amino acid concentrations.

MATERIALS AND METHODS

The greenhouse pot experiment with kohlrabi was established on 24 March 2005 in the greenhouse of the Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition at the Mendel University of Agriculture and Forestry in Brno. Mitscherlich pots were filled with 6 kg soil (45 % clay) characterised as fluvial soil. The agrochemical properties are given in Table 1.

The soil was analyzed for CH_3COOH, NH_4NO_3, NH_4F, HNO_3 and EDTA by Mehlich III extraction (Mehlich, 1984). Colorimetry was used to determine the content of available P in the extract and atomic absorption spectrophotometry (AAS) to determine available K, Mg and Ca. The micronutrients Zn and Fe were extracted using the Lindsay-Norwell procedure (Richter et al., 1999) and the AAS method was used for their determination. Extraction with de-mineralised water at a ratio of 1:5 for 16 h on a rotation shaker preceded the determination of SO_4–S in the soil. For the actual measurements we used capillary zone electrophoresis (CZE) with silica capillary. The activity of hydrogen ions was detected in a $CaCl_2$ (c = 0.01 mol dm^{-3}) soil extract by a potentiometer with a glass electrode against a reference electrode. The soil showed alkali soil reaction (pH) and the content of available P was high. The K, Ca, Mg, Zn, and Fe levels represented a good nutrient level.

The experiment involved three treatments, all of which were fertilised at the same level (0.6 g N per pot) in the form of ammonium nitrate (34.5 % N). Table 2 shows the experimental treatments. Treatment 1 (S_0) was not fertilised with S (natural content in the soil-25 mg kg^{-1} of SO_4–S). In treatments 2 (S_1) and 3 (S_2) the SO_4–S in the soil was increased to the required level (35 and 45 mg kg^{-1} of SO_4–S, respectively) by applying ammonium sulphate (20.5 % N and 24 % S, respectively). This is the most widespread nitrogen-mineral fertiliser with a physiological acid reaction, which is particularly suitable for neutral to alkali soils.

Kohlrabi variety Luna was sown in pots on 29 March 2005. Fertilisers were applied via irrigation solution on 9 May 2005, after previous thinning to 3 plants per pot at the 4-leaf stage. The experiment involved 3 treatments, each of which was repeated 4 times. Plants were regularly irrigated with de-mineralised water during the growing season to 60 % of the maximal capillary capacity. The pots were monitored for water content every day. The top soil layer (0.02 m) was loosened with a special garden fork five times, together with weeding. Plants were protected against aphids (*Aphis*) with Pirimor (0.05 %).

Table 2. Experimental treatments

N°	Treatment	N dose	Content of SO_4–S
		g/pot	mg kg^{-1}
1	S_0	0.6	25
2	S_1	0.6	35
3	S_2	0.6	45

Table 1. Agrochemical characteristic of the soil

pH/$CaCl_2$	P	K	Ca	Mg	SO_4–S	Zn	Fe
	mg kg^{-1} (Mehlich-3)				mg kg^{-1}		
7.5	138	226	2.784	167	25	2.4	42

The tubers were harvested at full maturity on 27 June 2005. Immediately after harvest the individual tubers without leaves were weighed. Nitrate concentration (mg kg^{-1} of NO_3^-) was determined in the fresh matter tubers and leaves by a potentiometer using ion selective electrode (ISE). The raw tubers were chopped, then lyophilised and homogenised in a fragmentation mill. The methods for laboratory feeds testing of were applied according to regulations of the Ministry of Agriculture of the Czech Republic (Ministerstvo, 2001).

Samples for amino acid determination were adjusted using acidic and oxidative acidic hydrolysis HCl (c = 6 mol dm^{-3}). The chromatographic analysis of sample hydrolysates was performed in the analyzer AAA 400 (manufacturer INGOS Prague, CR) and using Na-citrate buffers and ninhydrin detection (Official Journal…, 1978; Kráčmar et al., 1998).

All results were evaluated using the analysis of variance (ANOVA). Correlation matrices and regression functions were calculated according to Snedecor & Cochran (1967) by the statistical software package Unistat, v.5.1 - UNISTAT® Limited, London, England (Minařík & Hofbauer, 2003).

RESULTS AND DISCUSSION

A typical characteristic of kohlrabi is the high withdrawal of N from the soil (Feller & Fink, 1997) and any NO_3-N deficiency in the soil reduces yields (Steingrobe & Schenk, 1991). Sharof & Weir (1994) studied the minimum amount of N required for vegetable crops including kohlrabi in relation to components of N balance in the soil and calculated that N requirement values were invariably lower than N soil levels. Results of yields are listed in table 3.

The tuber weight in fresh matter was not significantly affected by S application with a slight variation between 106.8 and 108.5 g (Table 3). Nevertheless Prášková (2006) reported that in a pot experiment with kohlrabi the yields increased from 2.7 to 8.5 % under S doses increasing from 13.7–20–40 mg kg^{-1}. Likewise, Hlušek et al. (2003) stated that kohlrabi tuber yields increased by 10.5 % when the S soil content increased from 13.7 to 40 ppm and the N level was 0.75 g N per pot. Contrastingly, Sanderson & Carter (2002) discovered that yields of brassicas were not stimulated by S application.

The NO_3^- concentration in plants is affected primarily by the vegetable species, level of N fertilisation, the respective plant organ, growth stage and S concentration in the tissues. A negative linear correlation was discovered between NO_3^- in the tissues and the S concentration in plants (Schnug, 1990). Our results proved that the NO_3^- content was highest in the control treatment without S fertilisation, both in tubers and leaves (Table 3). More NO_3^- was stored in the tubers than in leaves and with an increasing soil S level the concentrations dropped significantly by 53.6 % in the tubers and by 21.7 % in the leaves (Treatment S_1). Together with an increasing dose of S the tuber/leaf ratio in the NO_3^- content narrowed down from 1.76 (Treatment S_0) to 1.12 (Treatment S_2). In a kohlrabi experiment Hlušek et al. (2003) also discovered that the NO_3^- content in tubers decreased by 14.2 % at a level of 40 ppm S in the soil and 0.75 g N per pot. Lošák (2005) reported a drop in NO_3^- contents in onion by 13.1 % related with an increase in the SO_4–S level in the soil to 40 ppm.

The S doses did not affect the N concentration in kohlrabi tubers, which varied in a narrow range between 1.42 and 1.48 % N (Table 4). For the S contents the differences among treatments were not significant either (0.59–0.64 % S) and the N/S ratio was also narrow, i.e. 2.22–2.50. In contrast, Smatanová et al. (2004) reported in increase in the S content in peppers from 0.08 % S to 0.24 % S associated with an increased S content in the soil from 7.8 to 30.6 ppm.

Table 4. Chemical analyses of kohlrabi tuber (% in dry matter)

Treatment	% N	% S	N/S
S_0	1.45	0.59	2.46
S_1	1.42	0.64	2.22
S_2	1.48	0.59	2.50

Table 3. Yield results of kohlrabi experiment

Treatment	Weight of 1 tuber without leaves (n = 12)		NO_3^- in tuber (n = 12)		NO_3^- in leaves (n = 12)		Ratio NO_3^- tuber/leaf
	g	rel.%	Ppm	rel.%	Ppm	rel.%	
S_0	108.5^a	100	46.5^a	100	26.3^a	100	1.76
S_1	106.8^a	98.4	21.6^b	46.4	20.6^b	78.3	1.05
S_2	107.8^a	99.4	26.9^c	57.8	24.0^c	91.2	1.12

The N content in kohlrabi (Table 5) was lower in leaves than in tubers and increased to 1.25–1.35 % N at higher S doses. Higher S soil doses were reflected in an increase of leaf concentration from 0.50 % S (Treatment S_0) to 0.76 % S (Treatment S_2) while simultaneously the N/S ratio decreased from 2.42 to 1.77; see Table 5. S deficiency symptoms on the youngest leaves were only observed towards the end of the growing season – treatment S_0. Rosen et al. (2005) stated that the N dose has no effect on S concentration in cabbage tissues, which depended only on the applied dose of 110 kg ha^{-1} of S. Sanderson & Carter (2002) also discovered that the S content in brassica tissues increased after S fertilisation in the form of gypsum. Hlušek et al. (2003) detected that the S content increased in Chinese cabbage after S application to 0.89 % S compared to 0.71 % S in the control treatment without S fertilisation, and simultaneously a lower N/S ratio, decreasing from 4.84 to 3.40. Likewise Sanderson & Ivany (1996) observed that after the application of potassium sulphate and gypsum the S concentration in cabbage leaves increased to 0.70 % and 1.45 % S respectively.

Table 5. Chemical analyses of kohlrabi leaves (% in dry matter)

Treatment	% N	% S	N/S
S_0	1.21	0.50	2.42
S_1	1.25	0.58	2.15
S_2	1.35	0.76	1.77

The effect of N and S fertilisation on the spectrum of amino acids in kohlrabi tubers is presented in Table 6. shows that with the given level of N nutrition and increasing S level in the soil (S_0, S_1 and S_2), the percentage sum (g/16 g N) of the content of sulphurous amino acids Σ cysteine + methionine decreased by 16–28 % Table 6. Cysteine (S_0:S_1 = by 23 %; S_0 : S_2 = 26 %) and methionine deceased in the same order by 9 and 30 %, resp., and glycine also decreased by 6–29 %. These results correspond to the findings of Smatanová et al. (2004) who also stated that the content of methionine in spinach decreased from 0.76 to 0.66 g kg^{-1} when the levels of S in the soil increased from 7.8 to 30.6 mg kg^{-1} of SO_4–S, simultaneously reducing the cysteine content from 0.58 to 0.45 g kg^{-1} as a result of the increasing S level in the soil from 20.6 to 30.6 ppm. Lošák & Ducsay (2005) also observed a statistically significant decrease in the methionine concentration in onion tubers by 6.8 % at the highest level of S in the soil (60 mg kg^{-1} of SO_4–S). On the contrary, Eppendorfer & Eggum (1995) reported that in plant tissue under S deficiency the cysteine concentrations (g/16 g N) declined more than methionine.

In other essential amino acids at different levels of S fertilisation virtually the same values of amino acids valine and tyrosine and of the non-essential amino acids aspartic acid and serine were observed. No substantial differences between treatments S_0 and S_1

Table 6. Percentage content of amino acids (AA) in kohlrabi tubers at fertilisation level S_0, S_1 and S_2 (g/16 g N)

AA	S_0			S_1			S_2			Total (n = 12)		
	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.
Cysteine	1.77	±	0.409	1.35	±	0.232	1.31	±	0.091	1.48	±	0.330
Methionine	1.76	±	0.155	1.60	±	0.163	1.22	±	0.271	1.53	±	0.301
ΣCys+Met	3.53	±	0.532	2.95	±	0.302	2.53	±	0.258	3.01	±	0.549
Threonine	5.24	±	0.437	5.24	±	0.573	4.21	±	0.210	4.89	±	0.642
Valine	6.56	±	0.186	6.37	±	0.231	6.06	±	0.377	6.33	±	0.330
Isoleucine	4.16	±	0.301	4.17	±	0.168	3.65	±	0.258	3.99	±	0.340
Leucine	8.20	±	1.274	8.50	±	0.256	5.90	±	0.203	7.53	±	1.396
Tyrosine	4.01	±	0.258	3.90	±	0.493	3.97	±	0.456	3.96	±	0.379
Phenylalanine	0.07	±	0.034	0.39	±	0.199	0.18	±	0.222	0.21	±	0.209
Histidine	1.86	±	0.062	2.25	±	0.436	2.68	±	0.136	2.26	±	0.426
Lysine	5.25	±	1.061	6.70	±	0.168	3.32	±	0.175	5.09	±	1.551
Arginine	3.88	±	1.060	3.93	±	0.386	2.47	±	0.684	3.43	±	0.986
ΣEAA	39.23	±	2.925	41.45	±	0.483	32.44	±	1.728	37.69	±	4.652
Aspartic acid	10.82	±	1.523	9.70	±	0.222	9.38	±	0.584	9.97	±	1.074
Serine	5.74	±	0.417	5.96	±	0.103	5.12	±	0.577	5.61	±	0.530
Glutamic acid	11.85	±	0.584	10.85	±	0.974	28.33	±	1.621	17.01	±	8.432
Proline	4.95	±	0.489	5.47	±	1.472	3.65	±	1.521	4.69	±	1.388
Glycine	10.51	±	0.616	9.85	±	0.276	7.50	±	0.317	9.29	±	1.402
Alanine	10.75	±	0.688	10.07	±	0.786	7.90	±	0.911	9.58	±	1.464
ΣNEAA	54.62	±	3.198	51.90	±	2.282	61.88	±	0.375	56.15	±	4.853

were found in threonine, isoleucine, leucine, arginine, ∑EAA and alanine, however between treatments S_0, S_1 and S_2 the content dropped in a range of 37–9 % in all cases. The content of amino acid lysine increased between treatments S_0 and S_1 by 28 %, that of proline by 10 %; on the contrary, from S_0 to S_2 lysine decreased by 37 % and proline by 26 %, and between treatments $S_1$and S_2 lysine dropped 50 % and proline 33 %. The only essential amino acid content that increased with increasing S doses was histidine. The increase observed on S_1 and S_2 treatments as compared with S_0 was 21 % and 44 %, respectively; from S_1 to S_2 the in increase was 9 %. In treatment S_2 the glutamic acid content increased extremely, by more than 150 %, compared to the S_0 and S_1 treatments. The high content of ∑NEAA under treatment S_2 was probably affected by the high concentration of glutamic acid. The phenylalanine concentrations ranged between 0.07 and 0.39 %. Eppendorfer & Eggum (1994) also explored the effect of different levels of N, P, K and S on the amino acid content in potato tubers. They stated that increased S doses had a negative effect on cysteine, methionine, lysine and leucine concentrations, but raised the glutamic acid concentration.

CONCLUSIONS

The result of a one-year greenhouse pot experiment was that S fertilisation contributed to stabilise kohlrabi tuber yields of while simultaneously reducing NO_3^- contents both in the consumable parts and leaves considerably. The concentrations of amino acids, with exception of cysteine, methionine, ∑ cysteine + methionine and glycine, did not change. It would also be appropriate to apply S-containing mineral fertilisers in doses of 45–90 kg ha^{-1} of S for kohlrabi cultivation.

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