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Sulfur Deficiency and Amino Acid Composition in Seeds and Grass

By

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Summary

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Sulfur deficiency caused decrease in the sulfur containing amino acids, methionine and cysteine (relative to the nitrogen content) in seeds of rape, grain of barley and wheat, and leaves of Italian ryegrass. Also the content of other amino acids (relative to the nitrogen content) was affected of sulfur deficiency, e.g. lysine by a decrease. For pea the effect of sulfur deficiency on the amino acid content was much less pronounced.

In the leaves of Italian ryegrass severe sulfur deficiency caused a dramatic increase in the content of aspartic acid (+ asparagine). The greater part of this content was present in the free state in form of asparagine.

Introduction

Sulfur is an important nutrient for synthesis of proteins in plants as a part of methionine and cysteine. Apart from their presence in proteins these amino acids also have other important roles. Methionine is used in initiation of the synthesis of the protein chains and cysteine is responsible for the S-S bridges between the protein chains. The latter are of importance to the tertiary structure and thus to the function of the enzyme proteins. Deficiency of sulfur must be expected to decrease the production of protein due to shortage of the sulfur amino acids, but also to slow down the whole metabolic activity in the plants due to the role of sulfur as part of several coenzymes and prosthetic groups such as ferredoxin, biotin, CoA and thiamine pyrophosphate (MARSCHNER 1986).

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Materials and Methods

Rape, pea, barley and wheat were grown in pots (0.1 m^2 surface area) in a mixture (1 : 2 : 2 by weight) of granulated mineral wool (Rockwool) free of sulfate, silica sand, and a sandy soil emptied for sulfate by leaching. Different degrees of sulfur deficiency were obtained by supplying the pots with different amounts of sulfate-S. Italian ryegrass was grown on different soils (sandy to loamy) depleted for available sulfur by leaching. From the first cut (taken before stem elongation) the plant material from four pots was selected for amino acid analysis after having been analyzed for total sulfur. Three of the samples (sulfur content 0.10, 0.15, and 0.19 % of dry matter) were from pots with symptoms of sulfur deficiency on the plants, while the fourth sample (sulfur content 0.26 % of dry matter) was from a pot with healthy plants. The plants were grown outdoors with 2 replicates except for Italian ryegrass where only one replicate was selected for analysis.

After harvest the plant material was dried and analyzed as follows: Total nitrogen was determined by a Kjeldahl-method and total sulfur by turbidimetry after wet ashing with magnesium nitrate and perchloric acid (NES 1979).

The content of protein amino acids was determined by ion chromatography after oxidation with performic acid and hydrolysis with hydrochloric acid (MASON & al. 1980). Together with the sulfur amino acids the program used for the chromatography also gave the content of the essential amino acids, lysine and threonine, and the non-essential amino acids, aspartic acid and serine. The free amino acids and amides were extracted with perchloric acid followed by derivatization with phenylisothiocyanate (PITC) (HENRIKSON & MEREDITH 1984) and determined by HPLC-chromatography on a Hewlett-Packard 1084B instrument.

Results and Discussion

Rape was grown on two levels of nitrogen and an interaction between nitrogen and sulfur was observed for the yield and nutrient content (Table 1). At the supply of nitrogen of 1.5 g per pot nitrogen was the limiting factor for yield. At this nitrogen supply no effect of sulfur could be observed, neither on yield, nor on amino acid composition (Fig. 1). At the level of 4 g nitrogen per pot there was a clear effect of sulfur. The yield of seed was increasing from an amount even lower to an amount more than twice the yield at the supply of 1.5 g of nitrogen when going from the zero to the highest supplies of sulfur. With increased sulfur supply the sulfur content was raised and the nitrogen content lowered. The concentration of the sulfur amino acids (in proportion to the nitrogen content) increased with increasing supply of sulfur, more pronounced at 4.0 g than at 1.5 g of nitrogen. This was also the fact for lysine, while the concentration of aspartic acid (includes hydrolyzed asparagine) was rather independent of the sulfur supply (Fig. 1).

For barley, wheat and pea the yield was strongly increased by increased sulfur supply indicating that severe sulfur deficiency occurred when no sulfur was given. For barley and wheat the sulfur content was nearly unchanged, while the nitrogen content was decreased. In pea both the sulfur and nitrogen content was raised by the highest application of sulfur. The discrepancy between pea and the cereals is probably for a part due to a difference in the mode of nitrogen supply. The peas had to meet their demand for nitrogen by symbiotic nitrogen fixation, while barley and wheat were fertilized with nitrate-nitrogen, and possibly sulfur is more determining for the fixation than for the assimilation of nitrogen.

Sulfur supply had different effects on the amino acid concentration (relative to the nitrogen concentration) in the different species. For pea sulfur had only a small effect even for the sulfur amino acids (Fig. 2), whereas for barley and wheat the concentration of methionine and cysteine were clearly increased. Also a change in the concentration of aspartic acid and lysine appeared, at least for barley. The concentration of threonine (not shown) followed that of lysine. The effect of sulfur deficiency on protein content in cereals, oilseeds, and legumes is often found to be variable (see for review RANDALL & WRIGLEY 1986). Changes in the amino acid composition in seeds caused by sulfur deficiency can in general be ascribed to a synthesis of proteins poor in sulfur (FULLINGTON & al. 1987, SPENCER & al. 1990).

The relative concentration of amino acids in the four samples of Italian ryegrass seems to be strongly dependent on the sulfur concentration (Fig. 3). The concentration of methionine and cysteine increased with increasing content of sulfur, which also was the case for lysine. The concentration of aspartic acid, which includes the asparagine deamidated during the hydrolysis, was extremely high at the lowest sulfur content and gradually declined for each increase in the sulfur content. A similar increase in aspartic acid at sulfur deficiency has been reported by MILLARD & al. 1985.

The content of nitrate-N neither changed noticeably (range 1.37 to 1.70 % NO_3-N of dry matter) nor in consistency with the change in sulfur content in the four samples. This is in contrast to the normally found strong increase in nitrate content at sulfur deficiency (MILLARD & al. 1985).

It was unlikely that the high content of total aspartic acid (+ asparagine) found in the grass sample with the lowest sulfur content (see Fig. 3) was present in proteins. An analysis for some of the free amino acids (Table 2) showed an extremely high content of asparagine at the lowest sulfur content. In fact nearly 100 % of the total aspartic acid was coming from asparagine in the free state at the lowest sulfur content, decreasing to about 12 % at the highest sulfur content. In contrast the content of free threonine varied only from 3 to 5 % of the total threonine, independent of sulfur supply.

The high content of asparagine in the free state indicates a severe disturbance of the protein synthesis in the leaves at sulfur deficient conditions, while the nitrogen uptake and assimilation are most likely still continued. This indicates that the uptake and assimilation of nitrogen are not regulated directly by the sulfur supply.

A few analyses for free amino acids were performed on material of rape leaves and barley grain originating from the same experiments as the material referred to in Table 1. In rape leaves, in contrast to Italian ryegrass, the greatest increase at sulfur deficient conditions was found for glutamine, while asparagine was only increased twofold (Table 2). Some amino acids, such as alanine and threonine, appeared to be independent of the sulfur content in the leaves. Grains of barley normally have a low content of free amino acids as can be seen from the figures for the sample from barley plants with no symptoms of deficiency (Table 2). For the sample from S-deficient barley asparagine was the free amino acid found in highest concentration (Table 2).

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Table 1. Effect of sulfur and nitrogen fertilization on yield and nutrient content of seed/grain of rape, barley, wheat and pea.

Species	Nutrient supply ($g\ pot^{-1}$)		Yield of seed/grain ($g\ DM\ pot^{-1}$)	Nutrient content in seed/grain (% of DM)	
	Sulfur	Nitrogen		Total S	Total N
Rape	0	1.5	16	0.23	2.79
	0.125	1.5	19	0.37	2.7
	0.25	1.5	17	0.28	2.81
	0.5	1.5	18	0.39	2.74
	0	4.0	3	0.20	4.10
	0.125	4.0	37	0.20	3.57
	0.25	4.0	46	0.24	3.19
	0.5	4.0	48	0.29	3.11
LSD (P=0.05)			4	- 1)	- 1)
Barley	0	4.0	13	0.11	3.27
	0.05	4.0	31	0.10	2.67
	0.25	4.0	110	0.10	1.51
Wheat, spring	0	4.0	23	0.12	3.23
	0.05	4.0	53	0.12	2.79
	0.25	4.0	84	0.13	2.19
Pea	0	0	43	0.13	3.10
	0.10	0	94	0.13	3.10
	0.50	0	159	0.19	3.50
LSD (P=0.05)			19	0.02	0.32

1) Single analyses on pooled samples.

Table 2. Effect of sulfur on content of some free amino acids in leaves of Italian ryegrass and rape and in barley grain.

Material	Total S	Total N*)	Status of S-def.	Content of free amino acids						
	(% of DM)			Asn	Asp	Gln	Glu	Ser	Ala	Thr
				(μmol g DM^{-1})						
Italian ryegrass, leaves	0.10	3.98	1)	789.5	34.8	39.7	15.4	59.0	37.1	3.5
	0.15	3.43	1)	168.2	20.9	18.5	14.5	26.6	45.1	2.8
	0.19	3.49	1)	67.1	14.6	11.9	14.6	18.9	35.8	3.0
	0.26	4.10	2)	6.5	16.0	2.0	10.4	5.1	45.6	3.4
Rape leaves	0.41	4.51	1)	5.2	8.9	48.9	9.8	12.7	32.1	4.3
	0.99	4.96	2)	2.6	8.0	4.0	6.0	5.5	33.9	3.9
Barley grain	0.10	3.19	1)	137.6	18.1	18.9	7.0	3.4	3.5	0.7
	0.10	1.53	2)	1.7	1.2	0.1	1.3	0.1	0.2	0.1

*) Reduced N for Italian ryegrass
1) Deficient in sulfur according to visual symptoms on the green plants (pale leaves).
2) No visual symptoms of sulfur deficiency (plants with healthy green leaves).

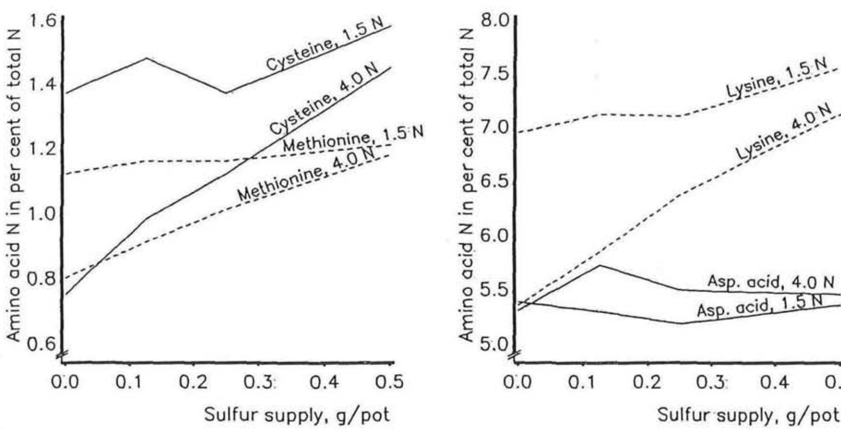

Fig. 1. Change in concentration of some amino acids in hydrolyzed samples of rape seed at increasing supply of sulfur at two levels of nitrogen (ref. Table 1). Single analyses of pooled samples.

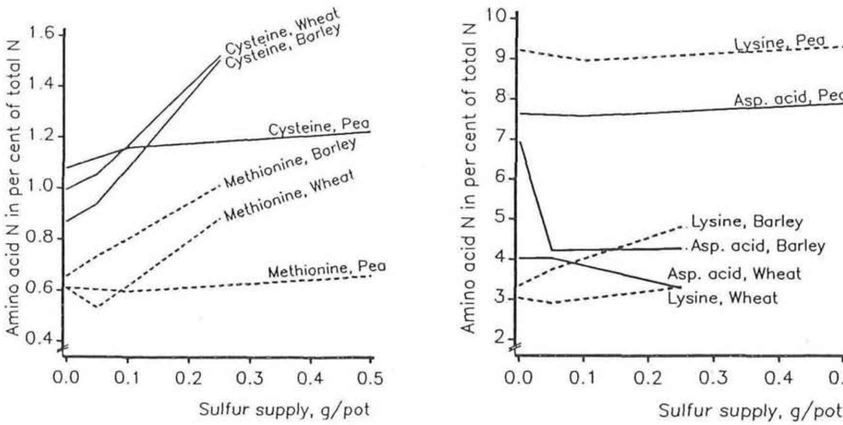

Fig. 2. Change in concentration of some amino acids in hydrolyzed samples of pea, barley and wheat grain at increasing supply of sulfur (ref. Table 1). LSD-values (P=0.05, 2 replicates) were: Cysteine 0.10, methionine 0.20, lysine 0.43, aspartic acid 1.13.

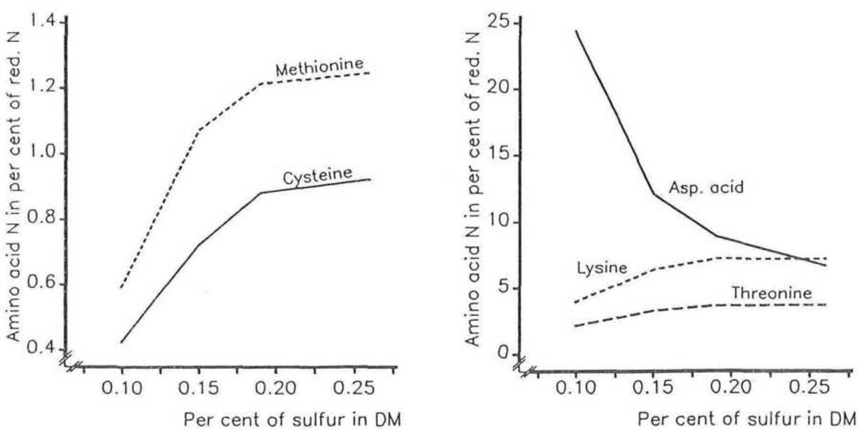

Fig. 3. Change in concentration of some amino acids in hydrolyzed samples of leaves of Italian ryegrass with different content of sulfur. One replicate analyzed.