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Effect of Sulphur Fertilization on Amino Acid and Fraction Composition of Protein of White Mustard Seeds

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ABSTRACT

In view of the increasing sulphur deficiency in Poland's soils, in 2007-2010, a field experiment was performed with white mustard (*Sinapis alba* L.) in Haplic Luvisol with low sulphur content (mean – 9.4 mg kg^{-1}). The aim of the research was to evaluate the effect of varied sulphur application methods (foliar and pre-sowing soil application), forms (elemental and ionic), and rates (0, 20, 40, 60 kg S ha^{-1}) on the content of protein in mustard seeds, as well as on its amino acid and fraction composition. The research showed that of all the studied factors, the sulphur application rate affected the protein content the most. As compared with the control, sulphur application increased the overall sums of essential and dispensable amino acids in the mustard seeds as well as their quantitative ratio. The biological protein value indices (Chemical Score and Essential Amino Acid Index) point to a clearly positive effect of sulphur on the amino acid composition of the protein, including sulphur-containing methionine, an amino acid limiting protein biosynthesis. The sulphur rate significantly affected the content of all the protein fractions assayed, except for glutelins.

Keywords: Amino acid composition indices, Exogenous and endogenous amino acids, Protein quality.

INTRODUCTION

In 2010-2014, the global area under mustard cultivation ranged from 792,500 to 812,000 ha, and annual mustard seed production was 626,700-681,900 tons (FAOSTAT, 2015). Major producers of mustard seeds include Canada, Nepal, Myanmar, Russia, Ukraine, China and the United States. In Europe, mustard seed production in 2010-2014 ranged from 155,200 to 232,000 tons. The economic importance of mustards may increase in the near future due to the highly promising results of breeding programs established to develop mustard cultivars with canola quality, i.e. with reduced levels of glucosinolates (GLS) and erucic acid. In Europe, hopes are pinned on double low cultivars of white mustard, which are currently being tested as an alternative to spring rapeseed to be grown on drier and lighter soils (Jankowski *et al.*, 2015).

In recent years, Poland has seen increased interest in cultivation of white mustard (*Sinapis alba* L.). Compared with rapeseed, the species is less economically important in this country, but more important than brown mustard (*Brassica juncea* L.) or black mustard (*Brassica nigra* L.). Mustard seeds are a major material for mustard production, and are also used in other branches of the foodstuffs industry, as well as in home canning as a preservative agent (Filipek-Mazur and Gryzełko, 2009; Meena *et al.,* 2015; Rathore *et al.*, 2015). White mustard has recently gained importance as a plant grown in stubble catch crops for green forage. Its presence in

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the crop rotation offers the advantage of limiting the occurrence of cereal diseases and pests. Mustard seeds have a relatively high protein content of 27-32% (Barczak, 2010; Jankowski *et al.*, 2015).

White mustard is a species with high sulphur requirements, because it is part of numerous primary metabolites having essential physiological and biochemical functions in the plant (Jamal *et al.*, 2010; Jankowski *et al.*, 2015). In the last twenty years, as a result of substantial restrictions on industrial emissions, as well as a change in available fertilizers, nutrient deficiency of sulphur has been observed in the soils of Poland (Szulc, 2008; Klikocka, 2011) and other countries (Morris, 2007; Jamal *et al.*, 2010; Rathore *et al.*, 2015). In Poland, the estimated deposition of SO_2, a chemical compound which is very harmful to the environment (Ascari, *et al.*, 2016), was about 5 million tons in 1980, but by 2005, it was 5 times lower (Szulc, 2008). In these conditions, it was necessary to consider the use of sulphur in plant fertilization, especially for species with high sulphur requirements. Reports increasingly indicate that the metabolism of plants with sulphur deficiency becomes disturbed, which in turn interferes with plant growth and development, ultimately decreasing the quality of the crop (Tomar and Singh, 2007; Tripathi *et al.*, 2011; Ngezimana and Agenbag, 2013).

Due to the important role of protein in plant metabolism and the growing interest in sulphur as a fertilizer component, research was undertaken to evaluate the effect of sulphur fertilization on protein content in white mustard seeds and its amino acid and fraction composition.

MATERIALS AND METHODS

Field Experiment

The present research was based on a field experiment carried out in 2007-2010 at the Experiment Station at Wierzchucinek (53° 26' N, 17° 79' E, Kujawsko-Pomorskie Province, northern Poland). The experiment was set up in three replications, with a randomized split plot design, on Haplic Luvisol produced from loam, with heavy loamy sand composition, representing the agronomic category of light soil. It had acidic pH_{KCl} (5.1-5.4) and average content of available forms of phosphorus (mean: 60.4 mg kg^{-1}), potassium (107.4 mg kg^{-1}) and magnesium (49.2 mg kg^{-1}). Interestingly, the content of sulphate (VI) forms $S\text{-}SO_4^{2-}$ in the soil, where the field experiment was performed, qualifies as soil of low sulphur content (9.4 mg kg^{-1}).

In the field experiment, different application methods (Factor A: sulphur applied pre-sowing to the soil and as a foliar fertilizer), forms of sulphur (Factor B: elemental sulphur in the form of Siarkol Extra 80 and an ionic form as sodium sulphate (VI)) and application rate (Factor C: rates in kg S ha^{-1}: 0, 20, 40 and 60) were used.

The experiment involved the cultivation of white mustard (*Sinapis alba* L.), representing *Brassicaceae*, *var*. Barka. The forecrop in each research year was spring barley. Homogenous pre-sowing fertilization was carried out with nitrogen, phosphorus and potassium: nitrogen was applied at a rate of 70 kg N ha^{-1} in the form of ammonium nitrate (34% N, Producer: Anvil, Poland), phosphorus at 32 kg P ha^{-1} in the form of triple superphosphate (40% P, Producer: Siarkopol Tarnobrzeg, Poland) and potassium at 63 kg K ha^{-1} as potassium salt (60% K, Producer: Luvena Lubon, Poland). A second application of nitrogen (70 kg N ha^{-1}) was provided as top fertilization at the beginning of budding.

The total number of plots was 48, the area of the one plot (replication) was 18 m^2, and the area for harvest was 15 m^2. Total area of field experiment was 720 m^2. The growing period for white mustard was about 18 weeks. Each year the plants were harvested during the fully ripe seed stage (11% moisture content), in mid-August. About 1 kg of seeds was collected from each plot for chemical analysis.

Laboratory Analysis Procedure

The following parameters were assayed in the mustard seeds:

- Content of total protein ($6.25 \times N_{total}$) by the Kjeldahl method (McDonald, 1977),
- Protein amino acid composition by High Performance Liquid Chromatography (HPLC), following hydrolysis in hydrochloric acid at a concentration of 6 mol dm^{-3}, at 105°C,
- Protein fraction composition by the Michael-Blume method (1960).

A three-step extraction protocol was used to separate the protein fractions. Fraction I (nitrogen non-protein compounds and albumins) was separated with distilled water, fraction II (globulin) with a 5% solution of K_2SO_4, and fraction III (glutelins and prolamins) – with an NaOH solution at a concentration of 0.1 mol dm^{-3} in 70% ethanol. Albumins were separated from non-protein nitrogen compounds by precipitating them with a 20% solution of trichloroacetic acid. Glutelins were separated from prolamins by decreasing the pH value to 5.5±0.1, which resulted in glutelin precipitation. Quantitative nitrogen assays in each of the protein fractions were performed by the Kjeldahl method. The seed protein amino acid composition in all the plant species was used to calculate the following indices:

- *EAAI* (Essential Amino Acid Index), defined as the geometric mean, expressed as a percentage, from the product of the ratios of the contents of respective essential amino acids in the protein investigated to their content in the reference protein:

$EAAI = (c_1/c_{01} x c_2/c_{02} x \ldots . x c_n/c_{0n} \times 100)^{1/n}$, (1)

Where, $c_1, c_2, \ldots, c_n$: The content of successive essential amino acids in the protein studied, and $c_{01}, c_{02}, \ldots, c_{0n}$: the content of successive essential amino acids in the reference protein (chicken egg protein)

- *CS* (Chemical Score)= $(c_i/c_{0i}) \times 100\%$, (2)

Where, c_i: The content of the essential amino acid in the protein studied, and c_{0i}: The content of the same amino acid in the reference protein (chicken egg protein).

The field experiment was performed in an area with a mean annual air temperature of 7.8°C and precipitation generally not exceeding 450 mm, including about 300 mm per growing period (means for 1949-2010). Field work begins in early April and the growing period generally lasts 205-230 days.

To provide more complete characteristics of the weather conditions in the research period, for the months of the mustard growing period the Selyaninov hydrothermal coefficient was calculated:

$K = P/0.1\Sigma T$ (3)

Where, P: monthly precipitation total in mm, and T: monthly air temperature total > 0°C

Weather Conditions

The Selyaninov's coefficient calculated values (Table 1) confirm high variation in weather conditions in the research years. The greatest temperature and precipitation fluctuations occurred in the 2009 growing period, when the highest water deficits were recorded (the Selyaninov's coefficient reached a mean value of 0.43 in June and 0.35 in July). Moreover, in April of that year, the coefficient reached its highest mean value (K=3.62), which showed that conditions were extremely moist. In terms of temperature and precipitation, the year 2008 was more stable; it stood out from the other research years with low rainfall from June through August.

The results of the chemical tests were verified by analysis of variance for the three-factor experiments in the randomized block design in a mixed model (Mead, 2002). To evaluate the significance of differences of object means, the Tukey range test was used at a level of significance of $P< 0.05$.

RESULTS AND DISCUSSION

Protein Content

The mean protein content in the mustard seeds ranged from 298.0 to 319.8 g kg^{-1}, depending on the year (Table 2). However,

Table 1. Selyaninov's coefficient (K) values during the research period.[a]

Years	Months				
	IV	V	VI	VII	VIII
2007	1.43	1.56	0.90	1.05	2.50
2008	1.57	2.18	0.68	0.56	0.85
2009	3.62	1.55	0.43	0.35	2.50
2010	0.69	1.71	1.93	1.88	0.76

[a] Threshold values: 0.4< K< 0.7 – very dry, 0.7< K< 1.0 – dry, 1.0< K< 1.3 – fairly dry, 1.3< K< 1.6 – optimal, 1.6< K< 2.0 – fairly moist, 2.0< K< 2.5 – moist.

the application potential of mustard protein is limited by the presence of antifeedants in the seeds, including the products of enzymatic hydrolysis of glucosinolates, as well as fibre and phytates (Jamal *et al.*, 2010).

Of the researched factors, the sulphur application rate was the only one which significantly determined protein content. In all the research years, significant differences were demonstrated between the control and the fertilization treatments with 20 and 60 kg S ha^{-1}. Between the rates of 40 and 60 kg S ha^{-1} significant differences were found only in the years 2007 and 2008.

Many authors have reported a positive effect of sulphur application on protein synthesis for seeds representing the family *Brassicaceae* (Brodowska, 2004; Ahmad *et al.*, 2007; Tripathi *et al.*, 2011; Ray *et al.*, 2015). This dependence can be explained by the presence of sulphur in ferredoxin, the enzyme playing the key role in this process (Jamal *et al.*, 2010). The activity of this enzyme is conditioned by sulphide bonds formed by disulphide groups of sulphur amino acids. Another research indicates a diversified response of rape varieties to sulphur (Malhi and Gill, 2006). Some authors, however, signal a lack of a clear effect of sulphur fertilization on protein content, e.g. in research on winter rapeseed (Lošak *et al.*, 2000; Hassan *et al.*, 2007) and mustard (Filipek-Mazur and Gryzełko, 2009).

The hydrothermal conditions were most favourable to protein accumulation in the mustard seeds in 2009, which may have been due to the temperature in the last two thirds of June and in July that was much higher than the long-term average, as well as the low precipitation persisting over that period (Table 1). Protein content in seeds seems to depend mainly on the moisture conditions of the habitat, especially during the seed maturation period. High variation in the protein content of crop yields depending on weather factors is highlighted by Paszkiewicz-Jasińska (2005) in a study of the role of sulphur in the agro-technical

Table 2. Content of total protein (6.25×N) in white mustard seeds (g kg seeds^{-1}).

Factor			Year of study				LSD[a]
			2007	2008	2009	2010	
A	Application method	Soil fertilizer	300.6	293.8	318.8	321.5	ns
		Foliar fertilizer	295.6	296.9	320.6	312.5	
B	Form of sulphur	Elemental	299.4	296.3	320.6	310.6	ns
		Ionic	296.3	294.4	318.8	313.8	
C	Rate (kg S ha^{-1})	0	293.8	295.6	315.0	311.3	2.69
		20	297.5	296.3	319.4	310.0	
		40	298.1	294.4	321.9	313.1	
		60	302.5	300.6	323.1	315.0	

[a] Significant at $P< 0.05$.

practices for mustard. Klikocka (2004) emphasizes that the weather factor is more important than the form and the rate of sulphur application for protein content in potato.

Amino Acid Composition of Protein

Sulphur fertilization, by affecting nitrogen metabolism, differentiated the amino acid composition of mustard protein, which resulted in changes in the content of individual amino acids, as well as in the quantitative relationships between essential and dispensable amino acids. Interestingly, there are a few reports on this subject, and they usually draw on pot experiments and are often limited to sulphur amino acids (Eriksen and Mortensen, 2002; Smatanova *et al.*, 2004; Ciurescu, 2009).

The sulphur rate was the only factor that significantly determined the sums of essential and dispensable amino acids in mustard seeds (Table 3). In all the research years, except for 2009, a significant increase was demonstrated in these sums following sulphur application at the rate of 40 kg S ha^{-1}, as compared with the control. There were no significant differences between the rates of 40 and 60 kg S ha^{-1}. The ratios of the sums of essential to dispensable amino acids for the non-fertilized treatment (0 kg S ha^{-1}) were lower than for the sulphur-fertilized treatments (20, 40, and 60 kg S ha^{-1}), and were 1.021:1 and 1.034:1, 1.033:1, 1.031:1, respectively. One of the evaluation criteria for the nutritive value of protein is *CS* (Chemical Score), which expresses the ratio of the amino acid content in the protein to the content of a given amino acid in chicken egg protein, used as a reference, with optimal nutritive value. The *CS* index for all the essential amino acids showed that in the mustard seed protein the first limiting amino acid was sulphur-containing methionine, which indicates that sulphur is indispensable in protein biosynthesis and in its quantity and quality. In the years 2007 and 2008, with more favourable weather conditions for mustard growth (Table 1), the higher the sulphur application rates, the higher the CS_{met} values obtained (Table 4), as previously reported by Eriksen and Mortensen (2002). In the samples from

Table 3. Sums of exogenous and endogenous amino acids in white mustard seeds (g kg $protein^{-1}$).

Factor			Years of study 2007	2008	2009	2010	LSD[a]
Sum of exogenous amino acids							
A	Application method	Soil fertilizer	407.5	411.2	418.2	441.2	ns
		Foliar fertilizer	407.9	408.3	427.8	423.6	
B	Form of sulphur	Elemental	404.4	408.6	423.5	436.0	ns
		Ionic	411.1	410.8	422.5	428.8	
C	Rate (kg S ha^{-1})	0	384.4	390.0	425.3	413.2	11.71
		20	404.4	419.1	425.9	434.1	
		40	419.8	417.8	420.5	439.0	
		60	422.3	412.0	420.3	443.3	
Sum of endogenous amino acids							
A	Application method	Soil fertilizer	414.5	407.4	411.3	393.9	ns
		Foliar fertilizer	400.3	402.4	420.6	398.2	
B	Form of sulphur	Elemental	394.6	403.1	417.2	397.3	ns
		Ionic	420.3	406.7	414.7	394.8	
C	Rate (kg S ha^{-1})	0	388.6	389.0	416.4	385.5	12.12
		20	406.4	405.2	419.9	396.6	
		40	417.4	414.9	411.2	400.0	
		60	417.2	410.6	416.4	402.1	

[a] Significant at $P< 0.05$

Table 4. Protein amino acid composition indices in white mustard seeds in each year of research.[a]

Factor			Years of study 2007	2008	2009	2010	LSD[b]
			CS_{methionine}				
A	Application method	Soil fertilizer	60.3	54.2	59.0	71.3	ns
		Foliar fertilizer	65.4	59.3	70.9	56.4	
B	Form of sulphur	Elemental	64.9	57.7	62.4	64.7	ns
		Ionic	60.8	55.7	67.4	63.0	
C	Rate (kg S ha^{-1})	0	54.6	49.5	68.4	65.8	2.13
		20	60.4	59.2	63.8	62.9	
		40	67.1	58.1	61.2	62.9	
		60	69.2	60.1	66.3	63.8	
			EAAI				
A	Application method	Soil fertilizer	77.9	77.8	79.4	78.8	ns
		Foliar fertilizer	78.4	77.7	82.2	79.6	
B	Form of sulphur	Elemental	77.7	77.5	80.7	81.2	ns
		Ionic	78.6	78.0	80.8	82.3	
C	Rate (kg S ha^{-1})	0	73.3	73.4	81.3	80.1	2.92
		20	77.4	79.6	81.3	81.0	
		40	80.8	79.4	80.1	82.6	
		60	81.2	78.6	80.3	83.4	

[a] *CS*: Chemical Score index, *EAAI*: Essential Amino Acid Index. [b] Significant at $P< 0.05$.

2009, with water deficits in June and July, and from 2010, with the most precipitation in the summer, intensification of sulphur application resulted in a reduction in values for this indicator. The application method and sulphur form had no significant effect on this index.

The second criterion of the nutritive value of protein is *EAAI* (Essential Amino Acid Index), which enables a more complete characterization of the nutritive value of protein than *CS*, as its value depends on the share of all the essential amino acids. Some authors (Barczak and Nowak, 1995; Smulikowska, 2006), however, note that in the case of *EAAI*, a surplus of some amino acids can compensate for a deficiency of others, and in protein biosynthesis, and thus in animal and human nutrition, none of the essential amino acids can be absent. For the treatments with rates of 40 and 60 kg S ha^{-1}, the *EAAI* was generally higher than in the control. In all the research years, except for 2009, application of the ionic form of sulphur, as compared with its elemental form, was conducive to a higher value for this index.

The analysis of the values of both indices points to a clearly positive effect of sulphur on the protein amino acid composition, including amino acids limiting its biosynthesis. According to Smulikowska (2006), there is a significant correlation between indices calculated based on amino acid composition and the biological value of protein assayed in animals in biological research on its digestibility and availability.

Fraction Composition of Protein

Changes in the amino acid composition of mustard seed protein due to sulphur application can be accounted for by the changes in the ratios of its fractions. The average share of the structural and enzymatic proteins assayed in the mustard seed protein, i.e. the sum of albumins and globulins, was 56.4%, and that of storage proteins (the sum of prolamins and glutelins) was 11.9% (Table 5).

Table 5. Content of protein fractions in white mustard seeds in relation to sulphur fertilization – mean for 2007-2010 (g kg^{-1} of total protein).

Rate (C) kg S ha^{-1}	Application method (A)						Form of sulphur (B)		
	Soil fertilizer			Foliar fertilizer					
	Form of sulphur			Form of sulphur					
	Elemental	Ionic	Mean	Elemental	Ionic	Mean	Elemental	Ionic	Mean
Globulins									
0	452.0	376.0	414.0	399.0	398.0	398.0	426.0	387.0	406.0
20	441.0	351.0	396.0	374.0	424.0	399.0	407.0	387.0	397.0
40	422.0	430.0	426.0	363.0	457.0	410.0	393.0	443.0	418.0
60	412.0	415.0	414.0	403.0	484.0	444.0	407.0	450.0	429.0
Mean	432.0	393.0	412.0	385.0	441.0	413.0	408.0	417.0	413.0
LSD[a]: C - 30.12 A×B-20.13									
Albumins									
0	161.0	163.0	162.0	121.0	131.0	126.0	141.0	147.0	144.0
20	172.0	173.0	172.0	126.0	127.0	126.0	149.0	150.0	149.0
40	158.0	185.0	171.0	133.0	139.0	136.0	145.0	162.0	154.0
60	157.0	167.0	162.0	162.0	137.0	149.0	160.0	152.0	156.0
Mean	162.0	172.0	167.0	135.0	133.0	134.0	149.0	153.0	151.0
LSD[a]: A - 5.21 A×C-16.97									
Glutelins									
0	98.6	81.4	90.0	104.0	93.8	98.9	101.0	87.6	94.4
20	82.3	70.9	76.6	108.0	96.5	102.0	95.3	83.7	89.5
40	99.9	117.0	109.0	103.0	80.1	91.7	102.0	98.7	100.0
60	97.9	91.9	94.9	104.0	92.9	98.5	101.0	92.4	96.7
Mean	94.7	90.4	92.5	105.0	90.8	97.9	99.8	90.6	95.2
LSD[a]: B-1.54 A×C-2.03									
Prolamins									
0	27.2	32.5	29.8	18.8	22.6	20.7	23.0	27.5	25.3
20	23.2	37.6	30.4	19.7	21.4	20.6	21.4	29.5	25.5
40	31.1	20.1	25.6	18.2	18.1	18.2	24.6	19.1	21.9
60	31.4	21.7	26.6	19.9	22.8	21.4	25.7	22.3	24.0
Mean	28.2	25.5	26.8	19.1	21.2	20.2	23.7	24.6	24.1
LSD[a]: C-2.11									
N-non protein compounds									
0	193.0	223.0	208.0	145.0	152.0	148.0	169.0	187.0	178.0
20	160.0	229.0	194.0	157.0	156.0	157.0	159.0	193.0	176.0
40	150.0	145.0	148.0	142.0	159.0	150.0	146.0	152.0	149.0
60	144.0	149.0	146.0	148.0	160.0	154.0	146.0	155.0	150.0
Mean	162.0	186.0	174.0	148.0	157.0	152.0	155.0	172.0	163.0
LSD[a]: A-7.67 B-8.13 C-8.56									

[a] Significant at $P< 0.05$.

The sulphur application method had no significant effect on the fraction composition of mustard seed protein, and the form of sulphur showed a significant impact only on the content of glutelins. By following application of elemental sulphur, an average of 9.2% more glutelins was produced than in the case of application of the ionic form.

The sulphur rate had a significant effect on the content of all the fractions assayed, except for glutelins. The application of 40 and 60 kg ha^{-1} primarily increased the content of albumins (the differences, as compared with the control, were 6.9 and 8.3%, respectively), as well as globulins (2.9 and 5.5%). These are fractions rich in essential amino acids, especially lysine, methionine, leucine, valine, and arginine (Barczak and Nowak, 1995). Albumins influence rheological properties and enzymatic activity of wheat flour dough (Tomić *et al*., 2015). In comparison with the control, sulphur fertilization decreased the content of prolamins, fractions poor in essential amino acids, especially lysine and methionine, on average by 13.4 and 5.1% following application rates of, respectively, 40 and 60 kg ha^{-1}. Sulphur application increased the ratio between the content of structural proteins (albumins and globulins) and storage proteins (prolamins and glutelins). The quantitative ratio between these proteins for the sulphur rates was as follows: 4.59:1 (control), 4.75:1 (20 kg S ha^{-1}), 4.69:1 (40 kg S ha^{-1}), and 4.84:1 (60 kg S ha^{-1}). Considering the amino acid composition of the respective fractions and their role in mustard metabolism, we can conclude that the direction of changes in this ratio due to sulphur fertilization points to favourable changes in the nutritive value of the seed protein in this species.

Fraction analysis not only involved isolation of protein fractions, but also enabled extraction of non-protein nitrogen compounds, which include free amino acids, amides and mineral nitrogen forms ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$). The quantitative ratio between the protein and non-protein nitrogen compounds in grain is determined, apart from weather factors, by the supply to the plants of the nutrients indispensable for protein biosynthesis, most importantly nitrogen and sulphur. Many reports confirm their interaction in determining the protein content of plants representing the family *Brassicaceae* (Kachroo and Kumar, 1999; Ahmad *et al.*, 2007; Tomar and Singh, 2007; Mansoori, 2012; Ngezimana and Agenbag, 2013). In plants insufficiently supplied with sulphur, in which protein synthesis is limited as a result of decreased content of sulphur-containing amino acids, mainly cysteine and methionine, non-protein compounds accumulate (Sahota, 2006; Jamal *et al.*, 2010). This is confirmed by the present study, which demonstrated a decrease in the content of the non-protein fraction due to the application of sulphur. The mean difference between the treatment involving fertilization at the rate of 40 kg ha^{-1} and the control was 16.3%. No significant differences were noted between the rates of 40 and 60 kg ha^{-1}. The accumulation of mineral nitrogen forms is a result of a decrease in the intensity of reduction of nitrates (V) due to disturbed functioning of nitrogenase and nitrate reductase, enzymes including Fe-S bond. In sulphur deficiency conditions, the use of nitrogen for synthesis of amino acids is limited and an excess of NH_4^+ ions is bonded in the form of glutamine and asparagine (Sahota, 2006). The amide nitrogen accumulated in the cells, however, cannot be used for protein biosynthesis because sulphur deficiency disturbs photosynthesis due to an insufficient amount of ferredoxin, a biological carrier of electrons (Jamal *et al.*, 2010). The disturbed photosynthesis and the resulting decreased protein content have an unfavourable effect on the fodder value of the crop as well as its nutritive and technological value.

Besides the sulphur rate, the factors significantly affecting the content of the non-protein fraction were the application methods and their form (Table 5). Higher accumulation of the non-protein fraction was shown to be enhanced by the soil application of sulphur: on average for the four research years, the difference was 22 g kg^{-1} (13.6%) as compared with the foliar application. The use of elemental sulphur produced on average 17 g kg^{-1} (11.0%) more non-protein compounds than application of the ionic form of sulphur. Sulphur in the elemental form requires biological oxidation by bacteria of the genus *Thiobacillus* to the sulphate (VI) form, which is available to plants (Grant *et al.*, 2012). Its effect is slower because this biological transformation depends on many factors, mainly temperature and moisture conditions.

CONCLUSIONS

Of all the factors studied, the sulphur rate was the only one that significantly determined the content of protein in white mustard seeds. Significant differences in all the research years were demonstrated between the control and the treatments with 20 and 60 kg S ha^{-1}. In all the research years, except for 2006, a significant increase was shown in the sum of essential and dispensable amino acids in mustard seeds following sulphur application at a rate of 40 kg S ha^{-1} as compared with the control. The ratios of these sums for the treatment without sulphur application (0 kg S ha^{-1}) were lower than for the sulphur treatments (20, 40 and 60 kg S ha^{-1}). Analysis of the value of *CS* (Chemical Score) for the mustard seed protein showed that the amino acid remaining with the lowest content was sulphur-containing methionine. As the sulphur rates increased, the CS_{met} values and the Essential Amino Acid Index (EAAI) generally increased as well. The sulphur rate significantly affected the content of all the protein fractions assayed, except for glutelins. The application of 40 and 60 kg S ha^{-1}, as compared with the treatment without sulphur application, increased the content of albumins and globulins and decreased the content of the non-protein fraction. Sulphur application enhances the quantitative relationship between structural proteins (albumins and globulins) and storage proteins (prolamins and glutelins). The sulphur application method generally affected neither the protein content of the mustard seeds nor its amino acid or fraction composition. The form of the applied element significantly affected only the content of glutelins and the non-protein fraction.

The results of the study on the effect of sulphur application on the protein content in white mustard seeds and the amino acid and fractional composition of the protein indicates that its use increases the nutritional value of the seeds. Given the symptoms of sulphur deficiency in many regions of the world in numerous plant species, changes in the level of its available forms should be monitored. Where low or very low levels are recorded, supplementation is required, in the form of sulphur fertilizers or NPK fertilizer supplemented with sulphur.

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اثر کود دهی گوگرد برترکیب آمینو اسید درپروتئین بذر خردل سفید

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چکیده

با در نظر داشت کمبود روزافزون گوگرد در خاک های لهستان، در سالهای ۱۰-۲۰۰۷، آزمایشی در مزرعه با گیاه خردل سفید (*Sinapis alba* L.) روی خاک هاپلیک لوویسول(Haplic Luvisol) با محتوای گوگرد کم (میانگین ۹/۴ میلی گرم در کیلو گرم) اجرا شد. هدف پژوهش ارزیابی اثر روشهای مختلف کاربرد گوگرد(با برگپاشی یا افزودن به خاک قبل از کاشت)، شکل های متفاوت شیمیایی (عنصری یا یونی)، و مقدار مصرف گوگرد (۰، ۲۰، ۴۰، و ۶۰ کیلوگرم گوگرد درهکتار) روی مقدار پروتئین بذر خردل و نیز روی آمینو اسیدها و ترکیب پروتئین بود. نتایج پژوهش نشان داد که در میان همه عوامل مطالعه شده، مقدار مصرف گوگرد بیشترین اثر را روی محتوای پروتئین بذر داشت. در مقایسه با شاهد، مصرف گوگرد سبب افزایش جمع کلی آمینواسید های ضروری و غیر ضروری در بذر خردل و نیز افزایش نسبت کمّی ان ها شد. شاخص های ارزش بیولوژیکی پروتئین (امتیاز شیمیایی و شاخص آمینواسید ضروری) به روشنی به اثر مثبت گوگرد روی ترکیب آمینو اسید پروتئین، شامل متایونین گوگرد دار (که آمینو اسیدی است که بیوسنتز پروتئین را محدود می کند) اشاره داشت. مقدار مصرف گوگرد بر محتوای همه قطعات آزمون شده پروتئین (به جز گلوتئین) به طور معنی داری اثر گذاشت.