

## The effect of sulphur and nitrogen fertilization on grain yield and technological quality of spring wheat

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### ABSTRACT

The aim of the study was to evaluate the effect of nitrogen (N) and sulphur (S) fertilizer on grain yield of spring wheat and its technological quality. A field experiment (2009–2011) was conducted in south-eastern Poland on Cambisols. The experiment included 2 factors: N fertilization (0, 40, 80, 120 kg/ha) and S fertilization (0, 50 kg/ha). The experiment showed that spring wheat cv. Tybalt exhibited a positive reaction of N and S fertilization on grain yield, which was the highest at the application of 80 kg N/ha (5.40 t/ha), increasing by 1.30 t/ha (13.1%) with respect to the control. S fertilization increased grain yield by 3.58%. S application increased significantly the content of gluten by 3.2%, cysteine by 6.0% and methionine by 16.5%. The most beneficial effect on the content of N, S, total protein, gluten, cysteine and methionine was observed for N application a rate of 80 kg/ha and for S at a rate of 50 kg/ha. Positive correlation was found between the content of S in grain and grain yield ( $r = 0.73$ ). Significant correlations were found also between grain yield and all other quality characteristics except for N and starch content.

**Keywords:** *Triticum aestivum* L.; macronutrient; deficiency; nutrition; S-amino acids

A noticeable problem in agriculture in European countries is the worsening sulphur (S) deficiency caused by an extreme tightening of environmental standards at the end of the last century. In Poland in 2010 as many as 94% of profiles tested were classified as low in S ( $< 16.5 \text{ mg SO}_4^{2-}/\text{kg}$ , Klikocka et al. 2015). This situation raises the concern that the NPK fertilizer generally applied will not be balanced, and the S deficiency may reduce utilization of the remaining components, primarily nitrogen (N).

N and S are both important constituents of protein, and adequate supplies of both nutrients are important for optimum crop yield (Klikocka and Cybulska 2014, Dostálová et al. 2015). S and N nutrition interact at many levels, as the uptake and assimilation of  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  have much in

common, and there are many common products of N and S metabolism (Pilbeam 2015). S-deficient plants accumulate arginine and asparagine in particular, but have lower levels of the S-containing amino acids cysteine and methionine (Hesse et al. 2004). Accumulation of asparagine in wheat grains under S deficiency increases the risk of formation of acrylamide when flour products are cooked (Halford et al. 2012). Some of the interaction between N and S metabolism comes from o-acetylserine, the immediate precursor of cysteine that does not itself contain S. For assimilation of sulphate to occur, plants must contain adequate levels of this precursor, and as an amino acid its concentration is dependent on N nutrition (Hesse et al. 2004). Sulphur is an important component of protein in wheat, being a constituent of

SH-containing amino acids that enhance the quality of protein for baking (Zhao et al. 1999). Interchain disulphide bonds formed by the S-rich subunits of the gliadins and glutenins that comprise gluten influence the elasticity of dough by stabilizing the polymer network formed by gluten molecules (Shewry et al. 2002). If the S supply is low relative to the N supply, content of S-poor proteins such as  $\omega$ -gliadins increases while that of S-rich proteins such as  $\gamma$ -gliadins and low-molecular-weight subunits of glutenin decreases (Tea et al. 2007).

The aim of the study was to evaluate the effect of N and S fertilizer on grain yield of spring wheat and its chemical and technological quality.

## MATERIAL AND METHODS

A field experiment was carried out in 2009–2011 in Malice, in south-eastern Poland (50°42'N, 23°15'E), in a randomized split-plot design (with four replications) on Cambisols (WRB 2007) consisting of light silty sand (sand 68%, silt 31%, clay 1%). The soil was slightly acidic (pH = 5.6), with high available phosphorus (P) content, medium content of potassium (K) and magnesium (Mg), and low S content (Table 1).

The precipitation and air temperature during the growing season (March–August) in 2009–2011 are presented in Table 2. On the basis of meteorological data Selyaninov's hydrothermal coefficient was

calculated. The 2009 growing season was classified as rather dry, bordering on optimal (1.3), while the 2010 and 2011 seasons were optimal, bordering on rather wet (1.6).

The subject of the experiment was the cv. Tybalt of spring wheat (*Triticum aestivum* L.) fertilized with different levels of N and S, which gave 8 combinations: 0N-0S; 40N-0S; 80N-0S; 120N-0S; 0N-50S; 40N-50S; 80N-50S and 120N-50S. Schema for rates of N and S and time of application presented Table 3. The area of each plot was 30 m<sup>2</sup> (5 m × 6 m). Before sowing, P (as 17.4% granular triple superphosphate at 39.6 kg P/ha) and K (as 49.8% potassium salt at 83 kg K/ha) fertilizers were applied. N fertilizer was applied in the form of 34% ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>). S was applied first before sowing (40 kg S/ha) in the form of kieserite – MgSO<sub>4</sub> · H<sub>2</sub>O (as 15.1% Mg, 20.0% S), followed by foliar application of 10 kg S/ha in the form of magnesium sulphate heptahydrate (MgSO<sub>4</sub> · 7 H<sub>2</sub>O) (10.2% Mg, 12.8% S or 32% SO<sub>3</sub>) (3.2% SO<sub>3</sub> solution (in 100 L of water) per 300 L water per ha). The soil in the plots was balanced depending on pH and on Mg applied in the form of kieserite and magnesium sulphate heptahydrate using Mg lime and Ca carbonate.

Spring wheat was sown in an amount ensuring a density of 500 plants per m<sup>2</sup>. Sowing was carried out between 28 March and 5 April, depending on the year. A mixture of the herbicides Granstar 75

Table 1. Chemical characteristics of soil (spring before sowing, layer 0–25 cm)

Description	Method	Unit	2009	2010	2011
pH in 0.01 mol/L CaCl <sub>2</sub>	potentiometrically using a Metrohm 605 pH-meter	–	5.6	5.7	5.8
C <sub>tot</sub>	combustion by LECO EC-12 <sup>®</sup> , model 752-100	g/kg	9.2	8.9	7.7
N <sub>tot</sub>	by the Kjeldahl's method		0.9	0.9	0.7
N <sub>min</sub>	content N-NO <sub>3</sub> + N-NH <sub>4</sub> × 1.38 (soil bulk density, mg/m <sup>3</sup> ) (PN-R-04038, 1997)	kg/ha	72.8	68.4	64.9
P <sub>avail</sub>	extracted by double lactate and determined by colorometric method – Egner Riehm DL method (PN-R-04023, 1996)		54.5	53.5	48.3
K <sub>avail</sub>	extracted: see phosphorus. Determined by the photometric method. (PN-R-04022, 1996)		88.6	85.2	79.6
Mg <sub>avail</sub>	extracted by 0.0125 m/L CaCl <sub>2</sub> and determined by AAS. (PN-R-04020, 1994)	mg/kg	34.8	33.7	35.1
S <sub>tot</sub>	by ICP-AES, mineralization with HNO <sub>3</sub> + Mg(NO <sub>3</sub> ) <sub>2</sub>		102.8	86.3	72.0
S-SO <sub>4</sub>	extracted by 0.025 mol/L KCl and determined by ion-chromatograph		14.2	12.6	10.3

doi: 10.17221/18/2016-PSE

Table 2. Sums of rainfall (mm) and mean air temperature (°C) in the years 2009–2011 and in the long-term period 1971–2005 (Zamość Research Station, Poland)

	Month (k)						Sum–mean (III–VIII)		
	III	IV	V	VI	VII	VIII	<i>k</i>	<i>p</i>	<i>t</i>
2009	5.3	0.5	2.4	2.1	0.4	0.8	1.3	349.1	2652
2010	1.8	1.1	2.0	1.1	2.1	1.3	1.6	443.4	2715
2011	1.2	1.1	0.7	1.0	2.7	2.3	1.6	414.6	2581
1971–2005	5.1	1.8	1.5	1.6	1.7	1.0	1.6	367.4	2353

*p* – precipitation (mm); *t* – temperature (°C); *k* – Selyaninov's hydrothermal coefficient [ $k = (p \times 10) / \Sigma t$ ]

WG (tribenuron-methyl) (20 g/ha) and Puma Super 069 EW (fenoxaprop-P-ethyl) (1 L/ha) was used at 28 BBCH stage. For fungi prevention Alert 375 SC (flusilazole + carbendazim – 1 L/ha) was applied at 30–32 BBCH stage and Tilt CB 37.5 (propiconazole + carbendazim – 1 L/ha) at 58–59 BBCH stage. The insecticide Decis 2.5 EC (deltamethrin) was applied at 0.25 L/ha at 58–59 BBCH stage.

Grain yield (at 11% moisture content) was calculated after the harvest (92 BBCH) from each plot. In dry matter (DM) of grain the content of total N and total S (g/kg DM) was determined with a LECO CNS-2000 analyser (Leco Corp., USA). Content of S was determined by infrared spectrometry (DIN ISO 15178, 2001) and N in a differential thermal conductivity detector (DIN ISO 13878, 1998). Content of S-containing protein amino acids (cysteine and methionine) was determined by the HPLC method in an INGOS AAA 400 amino acid analyser (LECO Corp., USA). Gluten and starch content in the grain were determined by near-infrared spectroscopy with a Perten Instruments Inframatic 9200 grain analyser (LECO Corp., USA). Total protein content in the grain was calculated as  $5.7 \times \text{N content}$  (Zhao et al. 1999).

Table 3. Schema for rates of nitrogen and sulphur

Element	Rate (kg/ha)	Time of application		
		before sowing	30–31 BBCH	55–59 BBCH
Nitrogen	0	–	–	–
	40	40	–	–
	80	40	40	–
	120	40	40	40
Sulphur	50	40	–	10

Analysis of variance was performed by the Snedecor's *F*-test. Significance of differences was calculated using the Tukey's test ( $P = 0.05$ ) followed by post-hoc analysis. The statistical software Excel 7.0 (Microsoft Office 2007 PL) and Statistica (StatSoft Polska'97) were used for the analysis.

## RESULTS AND DISCUSSION

Analysis of the results showed a significant beneficial effect of N and S fertilizer on the grain yield of spring wheat and on most of the quality characteristics of the grain. For grain yield and studied characteristics no interaction was found between increasing application rates of N and S. However, the addition of sulphur to each dose of nitrogen, independently increased yield and a number of other features. This type of yield-increasing factor, in this case fertilizer, signals the additive effect of S. This type of reaction is manifested in conditions in which the deficiency of the nutrient is small, which is consistent with the law of diminishing returns, also known as Mitscherlich's law (Grzebisz 2009). In general the additive interaction of the nutrients is manifested when there is a constant increase in weight (or yield) as a result of application of the second factor.

Grain yield was the highest following the application of 80 kg N/ha (5.40 t/ha), increasing by 1.30 t/ha (13.1%) with respect to the control, as well as in the case of 120 kg N/ha, although this was not a statistically significant increase. The 1000-grain weight increased proportionally as the N application rate increased. The content of N and S in the spring wheat grain significantly increased in direct proportion to the increase in

Table 4. Yield of grain of spring wheat, thousand-grain weight and nitrogen and sulphur content

Fertilization		Grain yield (t/ha)	Mass of 1000 grains (g)	Nitrogen (g/kg DM)	Sulphur (g/kg DM)	N:S ratio
Sulphur (S)	nitrogen (N)					
0S (N × S)	0	4.03 <sup>a</sup>	33.78 <sup>a</sup>	25.40 <sup>a</sup>	1.24 <sup>a</sup>	20.51 <sup>a</sup>
	40	5.15 <sup>a</sup>	34.46 <sup>a</sup>	26.88 <sup>a</sup>	1.24 <sup>a</sup>	21.70 <sup>a</sup>
	80	5.36 <sup>a</sup>	35.40 <sup>a</sup>	27.69 <sup>a</sup>	1.33 <sup>a</sup>	20.86 <sup>a</sup>
	120	5.47 <sup>a</sup>	37.43 <sup>a</sup>	28.40 <sup>a</sup>	1.37 <sup>a</sup>	20.88 <sup>a</sup>
50S (N × S)	0	4.13 <sup>a</sup>	34.36 <sup>a</sup>	25.61 <sup>a</sup>	1.25 <sup>a</sup>	20.50 <sup>a</sup>
	40	4.38 <sup>a</sup>	34.82 <sup>a</sup>	27.58 <sup>a</sup>	1.28 <sup>a</sup>	21.73 <sup>a</sup>
	80	5.43 <sup>a</sup>	35.53 <sup>a</sup>	27.81 <sup>a</sup>	1.34 <sup>a</sup>	20.79 <sup>a</sup>
	120	4.72 <sup>a</sup>	36.31 <sup>a</sup>	28.78 <sup>a</sup>	1.40 <sup>a</sup>	20.70 <sup>a</sup>
Mean (S)	0	4.75 <sup>B</sup>	35.27 <sup>A</sup>	37.09 <sup>B</sup>	1.30 <sup>B</sup>	20.99 <sup>A</sup>
	50	4.92 <sup>A</sup>	35.26 <sup>A</sup>	37.44 <sup>A</sup>	1.32 <sup>A</sup>	20.93 <sup>A</sup>
Mean (N)	0	4.08 <sup>B</sup>	34.07 <sup>AB</sup>	25.50 <sup>C</sup>	1.25 <sup>C</sup>	20.50 <sup>B</sup>
	40	4.27 <sup>AB</sup>	34.64 <sup>AB</sup>	27.23 <sup>B</sup>	1.26 <sup>C</sup>	21.72 <sup>A</sup>
	80	5.40 <sup>A</sup>	35.46 <sup>A</sup>	27.75 <sup>B</sup>	1.34 <sup>B</sup>	20.83 <sup>B</sup>
	120	5.59 <sup>A</sup>	36.87 <sup>A</sup>	28.59 <sup>A</sup>	1.39 <sup>A</sup>	20.79 <sup>B</sup>
Mean	2009	4.74 <sup>B</sup>	38.15 <sup>A</sup>	28.52 <sup>A</sup>	1.25 <sup>C</sup>	22.83 <sup>A</sup>
	2010	4.68 <sup>B</sup>	31.38 <sup>C</sup>	27.94 <sup>B</sup>	1.29 <sup>B</sup>	21.64 <sup>B</sup>
	2011	5.09 <sup>A</sup>	36.25 <sup>B</sup>	25.34 <sup>C</sup>	1.38 <sup>A</sup>	18.40 <sup>C</sup>

Values with different letters in the column differ significantly ( $P < 0.05$ ) between: a – N x S; A – 0S – 50S, A – 0N-40N-80-N-120N; A – 2009-2010-2011; DM – dry matter

the N application rate and was the highest following the application of 120 kg N/ha (N – 28.59 and S – 1.39 g/kg DM). As the N application rate increased, the N:S ratio was similar in the case of the control and 80 kg N/ha and 120 kg N/ha, but at 40 kg N/ha it increased from an average of 20.71 to 21.72 (Table 4).

Gluten content increased in direct proportion as the N application rate increased, and was highest following the application of 120 kg N/ha (34.15%). A similar response was noted for the content of total protein, with the greatest increases observed following the application of 80 kg N/ha and 120 kg N/ha (15.83% and 16.28%). Cysteine content in the grain increased significantly following the application of N at a rate of 80 kg/ha, and cysteine content following the application at the rate of 40 kg/ha, and they continued to increase proportionally up to the highest rate of N application. N fertilization did not affect starch content in grain (Table 5).

The literature devotes much attention to the issue of N fertilization of quality wheat and its effect on grain yield and technological value (Pilbeam 2015). Much less emphasis is placed on fertilization with other mineral nutrients, including S (Zhao et al. 1999). Grain S concentrations lower than 1.2 mg/g and grain N:S ratio higher than 17:1 appear to be critical values for S deficiency with regard to yield (Randall et al. 1981).

Fertilization with S improved the grain yield-increasing effect of NPK by 3.58%. In the case of 1000-grain weight, no effect was noted (Table 4). In a study by Podleśna (2013), S fertilization of winter wheat at a rate of 60 kg S/ha led to an increase in grain yield of 11%, while wheat plants with limited of S-SO<sub>4</sub> in the soil showed a reduction in 1000-grain weight.

The content of N and S in the grain following application of sulphur at a rate of 50 kg/ha increased by 1.3% and 1.5% in comparison with

doi: 10.17221/18/2016-PSE

Table 5. The quality characteristic of grain of spring wheat

Fertilization		Starch	Gluten	Total protein	Cystine	Methionine
Sulphur (S)	nitrogen (N)		(%)		(mg/g)	
0S (N × S)	0	62.2 <sup>a</sup>	25.23 <sup>a</sup>	14.47 <sup>a</sup>	3.65 <sup>a</sup>	2.70 <sup>a</sup>
	40	62.2 <sup>a</sup>	27.03 <sup>a</sup>	15.30 <sup>a</sup>	4.16 <sup>a</sup>	2.78 <sup>a</sup>
	80	62.2 <sup>a</sup>	29.70 <sup>a</sup>	15.80 <sup>a</sup>	4.33 <sup>a</sup>	3.00 <sup>a</sup>
	120	62.4 <sup>a</sup>	34.63 <sup>a</sup>	16.17 <sup>a</sup>	4.43 <sup>a</sup>	3.13 <sup>a</sup>
50S (N × S)	0	62.5 <sup>a</sup>	26.93 <sup>a</sup>	14.60 <sup>a</sup>	4.21 <sup>a</sup>	3.16 <sup>a</sup>
	40	62.6 <sup>a</sup>	28.70 <sup>a</sup>	15.70 <sup>a</sup>	4.29 <sup>a</sup>	3.32 <sup>a</sup>
	80	62.4 <sup>a</sup>	31.03 <sup>a</sup>	15.87 <sup>a</sup>	4.40 <sup>a</sup>	3.44 <sup>a</sup>
	120	62.4 <sup>a</sup>	33.67 <sup>a</sup>	16.40 <sup>a</sup>	4.64 <sup>a</sup>	3.60 <sup>a</sup>
Mean (S)	0	62.3 <sup>A</sup>	29.15 <sup>B</sup>	15.43 <sup>A</sup>	4.14 <sup>B</sup>	2.90 <sup>B</sup>
	50	62.5 <sup>A</sup>	30.08 <sup>A</sup>	15.64 <sup>A</sup>	4.39 <sup>A</sup>	3.38 <sup>A</sup>
Mean (N)	0	62.3 <sup>A</sup>	26.08 <sup>C</sup>	14.53 <sup>D</sup>	3.93 <sup>C</sup>	2.93 <sup>BC</sup>
	40	62.4 <sup>A</sup>	27.87 <sup>B</sup>	15.50 <sup>C</sup>	4.23 <sup>AB</sup>	3.05 <sup>B</sup>
	80	62.3 <sup>A</sup>	30.37 <sup>A</sup>	15.83 <sup>B</sup>	4.37 <sup>A</sup>	3.22 <sup>A</sup>
	120	62.4 <sup>A</sup>	34.15 <sup>A</sup>	16.28 <sup>A</sup>	4.53 <sup>A</sup>	3.37 <sup>A</sup>
Mean	2009	62.1 <sup>AB</sup>	28.52 <sup>B</sup>	16.25 <sup>A</sup>	4.37 <sup>A</sup>	3.17 <sup>A</sup>
	2010	62.3 <sup>B</sup>	30.14 <sup>A</sup>	15.91 <sup>B</sup>	4.37 <sup>A</sup>	3.12 <sup>A</sup>
	2011	62.6 <sup>A</sup>	30.19 <sup>A</sup>	14.45 <sup>C</sup>	4.05 <sup>B</sup>	3.13 <sup>A</sup>

Values with different letters in the column differ significantly ( $P < 0.05$ ) between: a – N x S; A – 0S – 50S; A – 0N-40N-80-N-120N; A – 2009-2010-2011; DM – dry matter

the control, respectively. S fertilization did not significantly affect the N:S ratios calculated for the grain, but they were decreased by the application of S (Table 4). The grain quality in the present

Table 6. Correlation coefficients between grain yield and grain quality characteristics

Features ( $n = 24$ )	No	2	3	4	5	6	7	8	9	10
Grain yield	1	0.328	0.331	<b>0.730</b>	-0.257	0.007	<b>0.813</b>	<b>0.449</b>	<b>0.478</b>	<b>0.497</b>
1000-grain weight	2	–	0.152	0.128	0.017	-0.035	0.096	0.039	0.100	0.068
Nitrogen	3	–	–	-0.085	<b>0.727</b>	-0.315	<b>0.414</b>	<b>1.000</b>	<b>0.767</b>	0.359
Sulphur	4	–	–	–	<b>-0.707</b>	0.379	<b>0.764</b>	0.123	0.211	0.371
N:S ratio	5	–	–	–	–	<b>-0.463</b>	-0.205	<b>0.763</b>	0.401	-0.005
Starch	6	–	–	–	–	–	0.106	-0.341	-0.085	0.402
Gluten	7	–	–	–	–	–	–	<b>0.414</b>	<b>0.589</b>	<b>0.428</b>
Total protein	8	–	–	–	–	–	–	–	<b>0.766</b>	0.358
Cysteine	9	–	–	–	–	–	–	–	–	<b>0.609</b>
Methionine	10	–	–	–	–	–	–	–	–	–

Bold letters are significantly value. \* $P = 0.05$ –0.406; \*\* $P = 0.01$ –0.517

study was improved by the application of S in the fertilizer. Only starch and total protein content were not dependent on S application, although there was a positive response. Following application of S after NPK fertilizer the content of gluten increased significantly by 3.2%, cysteine by 6.0% and methionine by as much as 16.5% (Table 5). In a study by Podleśna et al. (2003), S fertilization of winter wheat at a rate of 60 kg S/ha led to an increase in the content of protein (by 1%) and of gluten in the grain (by 2%). However, Randall et al. (1981) reported that an increase in applied S may increase, decrease or have no effect on protein content, depending on the grain S status and the N supply.

Analysis of the effect of S with increasing application rates of N revealed that despite the lack of statistically confirmed interactions, the most favourable values for the qualitative grain characteristics studied were found in the case of the combination of N applied at 80 and 120 kg N/ha with S applied at 50 kg S/ha. It should also be noted that these increases following N application at 120 kg N/ha with respect to lower application rates were less favourable than in the case of 80 kg N/ha with respect to 40 kg N/ha. This phenomenon can be explained by the law of diminishing returns (Mitscherlich's law) (Grzebisz 2009).

The grain yield and grain quality characteristics were modified by the weather. In the case of grain yield and content of S, gluten, starch and methionine, the weather conditions in 2011 (rather wet) were the most favourable. In 2009 (rather dry) the meteorological conditions during the growing period of the spring wheat had a significant beneficial effect on 1000-grain weight, accumulation of N, total protein and cysteine. The relationships described were confirmed in a study by Woźniak and Staniszewski (2007), which showed that lower values for the hydrothermal index (lower precipitation sum and higher air temperature sum) were conducive to higher content of protein and gluten in the wheat grain.

Significant positive correlations were found between the grain yield of spring wheat and all other quality characteristics of the grain, with the exception of starch content. The highest correlation coefficient was obtained between grain yield and content of gluten, S, total protein, cysteine and methionine. More significant positive correlations were found between the quality characteristics of the grain (Table 6).

According to Inal et al. (2003), positive correlations between the S application rate and its content in the plant indicate only slight redistribution of this nutrient from the soil to the plant, as sulphates in the soil may be unavailable. In the present study a correlation was found between the content of S in the spring wheat grain and grain yield. From this it can be concluded that the S was not subject to the dilution effect and its content was at the optimal level up to the end of the vegetation of the plants.

Because Polish and European agriculture is transitioning to an integrated production system, if it is taken into account that mineral fertilization should be applied below the uptake level, the treatment of 80 kg N/ha with 50 kg S/ha should be recommended in practice. The proposed N application rate and the high degree of N availability in mineral form in the soil analysed (average 68.7 kg/ha), as well as the anticipated supply from the atmosphere, will meet the demand of spring wheat for nitrogen.

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Received on January 9, 2016

Accepted on March 31, 2016

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