AGRONOMY SCIENCE
wcześniej – formerly
Annales UMCS sectio E Agricultura

VOL. LXXIX (4) 2024

https://doi.org/10.24326/as.2024.5420

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Agrobiofortification of spring wheat with nitrogen and sulfur in terms of improving yield and grain quality

Agrobiofortyfikacja pszenicy jarej azotem i siarką w aspekcie poprawy plonu i jakości ziarna

Abstract. In order to identify the impact of nitrogen and sulfur fertilizer on the yield and grain quality of spring wheat as well as on the improvement of its chemical and health-promoting properties, a strict 3-year field experiment was carried out. The subject of the experiment was the Kandela variety of spring wheat (*Triticum aestivum* L.) fertilized with various rates of nitrogen (factor I) and sulfur (factor II). The experiment was carried out in the years 2014–2016 in a split-plot design, in a private farm in Malice near Hrubieszów (Poland), on dystrophic typical medium brown soil, made of medium-grained sandy loam and classified as a good rye soil complex. The experiment included 2 factors (in four replicates): I. nitrogen fertilization at a rate of 0, 50, 100, and 150 kg ha^{-1}; II. sulfur fertilization at a rate of 0 and 40 kg ha^{-1}. After harvesting spring wheat, grain yield (at 11% moisture content) from each plot was determined (kg) and converted into t ha^{-1}. The following grain quality characteristics were examined in the dry matter: starch content (g kg^{-1}), gluten content (g kg^{-1}), total protein (g kg^{-1}), cysteine (mg g^{-1}), methionine (mg g^{-1}), fat (g kg^{-1}), and crude fiber (g kg^{-1}). As regards the features influencing health-promoting properties, the content of flavonoids (expressed as quercetin equivalents; %) and o-dihydroxy phenols (expressed as caffeic acid equivalents; %) was determined. Based on the conducted research, it was shown that the application of nitrogen (factor I) at the rates of 100 and 150 kg ha^{-1} and sulfur fertilization (factor II) at a rate of 40 had the most beneficial effect (statistically significant difference) on spring wheat grain yield. The use of a rate of 50 kg N ha^{-1}, regardless of sulfur addition, was insufficient because it did not produce

Citation: Harasim E., Kwiatkowski C.A., Buczek J., Klikocka-Wiśniewska O., 2024. Agrobiofortification of spring wheat with nitrogen and sulfur in terms of improving yield and grain quality. Agron. Sci. 79(4), 37–58. https://doi.org/10.24326/as.2024.5420

beneficial effects. It should be stated that under negative sulfur balance in the cultivated soils of the study area, it is necessary to use sulfur fertilizers. To sum up, the obtained research results indicate that in the spring wheat production system, the variant of 150 kg N ha^{-1} combined with 40 kg S ha^{-1} should be recommended. This variant of fertilization had a significant positive impact on both the productivity and the qualitative and health-promoting characteristics of spring wheat grains.

Keywords: spring wheat, nitrogen, sulfur, agrobiofortification, yield, grain quality

INTRODUCTION

Cereals are considered strategic crops in the global economy. Cereal grain, after processing, is the most important raw material used in food production, providing many valuable nutrients and energy. The economic value of cereals results from the size of their production, which is the result of the area sown and the yield per 1 ha. According to the report of the United Nations Food and Agriculture Organization, wheat production in the world will continue to increase, and in the 2023/2024 season it reached 782 million tons [FAO 2023].

Due to its poorly developed root system, spring wheat is a cereal with high water requirements and responds with a decrease in yield in years with less rainfall, especially on lighter soils. A negative feature of spring wheat is its high sensitivity to drought in high air temperature conditions, especially during tillering, stem shooting and earing. Unfavourable conditions cause thinning of plants, drying of shoots and poorer filling and development of grain, which results in lower grain yield [Wyzińska and Grabiński 2020].

Nitrogen plays a very important role in the process of plant growth and development. It affects the distribution of assimilates between roots and assimilation organs. It determines the photosynthetic activity of leaves and the entire plant, and consequently affects the number of ears per unit area, the number of grains in an ear and the weight of a thousand grains, and thus the productivity of the crop. It is an extremely important component of proteins, chlorophyll, vitamins, hormones and participates in the process of building DNA. It is also a component of enzymes and takes part in all reactions related to them [Jamal et al. 2010].

Nitrogen fertilizers will be fully utilized by plants when they also receive sulfur in the form of sulphate SO_4^{2-}, i.e., absorbed by plants via the root system. Therefore, the most important function of sulfur is its role in nitrogen management, and its deficiency in the soil leads to a significant reduction in the use of fertilizer nitrogen by plants. It is assumed that a deficiency of 1 kg of S leads to a reduction in nitrogen uptake by the plant from 1 to 3 kg. Nitrogen not taken up by the crop is dispersed in the environment, posing a threat not only to water quality, but also to the atmosphere [Salvagiotti et al. 2009].

Empirical studies aimed to determine the impact of nitrogen and sulfur on crop yield and quality are nowadays conducted across the world. The beneficial effects of nitrogen and sulfur on the quantity and biological value of yield are particularly found in experiments with oilseed rape [Malhi et al. 2007]. The inclusion of sulfur in the basic nitrogen fertilization of cereals results in yield optimization due to the higher unit productivity and the improved quality of harvested grain [Scherer 2001, Verlinden 2002, Staugaitis et al. 2014]. Sulfur is an essential element for plant and animal life. It is an important component of amino acids (cysteine, cystine, and methionine). Sulfur activates many enzymes and participates in enzymatic and redox reactions (photosynthetic activity), thus affecting an increase in plant protein, sugar, and fat content. Appropriately balanced S and N fertilization is important due to mutual interactions during the process of uptake and assimilation of these nutrients by the plant [Pilbeam 2015]. Part of interactions between N and S metabolism originate from O-acetyl-serine, the immediate precursor for cysteine, but the concentration of this sulfur amino acid depends on nitrogen nutrition [Hesse et al. 2015]. As an important component of wheat protein, sulfur contributes to an improvement in flour quality parameters [Tea et al. 2007, Klikocka and Cybulska 2014, Dostálová et al. 2015, Klikocka et al. 2016].

Cereals and their processed products provide to the modern man in the daily dietary intake about 30% of energy and protein as well as about 54% of carbohydrates. Cereal products are a source of dietary fiber, which prevents lifestyle diseases such as: diabetes, atherosclerosis, obesity, caries, myocardial ischemia, and constipation, in 65%. Amino acids, peptides, and proteins provide a proper tissue structure, regulate metabolic processes, and facilitate absorption of metabolic nutrients. Flavonoids contribute to the inhibition of the development of cancers, type 2 diabetes, and atherosclerosis [Vitaglione et al. 2008]. The above information shows how important it is to appropriately select agronomic practices (fertilization) for cereals in order to ensure the best possible grain quality parameters for the consumer.

In the context of the research problem undertaken in this article, it becomes interesting and advisable to identify the role of sulfur fertilization in determining the yield quantity and quality of spring wheat as well as the chemical composition and health-promoting properties of this cereal grain under the soil and climatic conditions of central-eastern Poland, a region with small industrial pressure, as far as this element is concerned.

The selection of the research problem was based on previously conducted field observations and an analysis of the literature regarding the status of nitrogen and sulfur in the biosphere and in crop fertilization [Salvagiotti et al. 2009, Ahmad et al. 2011, Muttucumaru et al. 2013, Tabak et al. 2020, Soofizada et al. 2022]. This allowed us to formulate a working hypothesis of the present study, which is the following: cultivated soils do not naturally meet the requirement of spring wheat for nitrogen and sulfur.

To verify the set hypothesis, a field experiment was carried out in the period 2014–2016, which was designed to determine the grain yield and quality of spring wheat (the grain chemical composition and health-promoting properties) under the application of nitrogen fertilization at rates of 0, 50, 100, and 150 kg N ha^{-1}, respectively, in combination with sulfur fertilization at a rate of 40 kg S ha^{-1}. Moreover, the study evaluated the effect of fertilization applied on the selection of the optimal combined N+S rate that would have the most beneficial influence on the productivity and quality of spring wheat grain.

MATERIALS AND METHODS

Experiment design and field management

The field experiment was conducted in the period 2014–2016, in the village of Malice near the city of Hrubieszów (50°42'N, 23°15'E), Poland on dystrophic typical medium brown soil, derived from medium-grained sandy loam [WRB IUSS 2015] (sand 68%, silt

31%, clay 1%) and classified as a good rye soil complex, with a slightly acidic pH. Immediately before the establishment of the experiment, the following soil properties were determined from the 0–25 cm of soil layer (Tab. 1).

Table 1. The soil characteristics in the field experiment – before starting the experiment

Specification	Unit	Study year		
		2014	2015	2016
pH in M $CaCl_2$	–	5.7	5.6	5.8
C – total	g kg^{-1}	9.2	8.9	8.5
N – total		0.9	0.9	0.8
N – mineral	kg ha^{-1}	70.8	69.4	67.9
P – available	mg kg^{-1}	53.5	55.5	50.3
K – available		87.6	86.2	80.6
Mg – available		35.8	34.7	35.3
S – total		98.8	89.3	82.0
S-SO_4 – available		15.4	13.6	12.3

The subject of the experiment was the Kandela variety of spring wheat (*Triticum aestivum* L.), fertilized with various rates of nitrogen (factor I) and sulfur (factor II). The field experiment was set up as a split-block design in four replicates. The objects of the experiment were blocks: A, B, C and D, in which the following were drawn: N – nitrogen fertilization (4 levels of N) and S – sulfur fertilization (at a rate of 0 and 40 kg ha^{-1}). In total, 32 plots were drawn. The size of a single plot for sowing wheat was – 30 m^2 (5 × 6 m), and the size of a single plot for harvesting was – 20 m^2 (4 × 5 m). This experimental scheme was repeated in each of the three years of research in a different part of the field (and the forecrop for spring wheat was potato each year).

The field trial included 2 factors:

1. Nitrogen fertilization at the following rates: 0 (control), 50, 100, 150 kg ha^{-1};
2. Sulfur fertilization: 0, 40 kg ha^{-1}.

Nitrogen was applied as ammonium nitrate (34%). A nitrogen rate of 50 kg N ha^{-1} was used before sowing, whereas 100 kg N ha^{-1} was applied at two times: before sowing and as top dressing at the stem elongation stage – BBCH 30-31. A rate of 150 kg N ha^{-1}, in turn, was applied at three times: before sowing, as top dressing at the stem elongation stage – BBCH 30-31, and as top dressing between the middle of heading and the end of heading – BBCH 55-59 (Tab. 2). Sulfur at a rate of 40 kg ha^{-1} was applied at two times: before sowing at a rate of 30 kg S ha^{-1} as kieserite – $MgSO_4 \times H_2O$ – and foliarly between the middle of heading and the end of heading (BBCH 55-59) at a rate of 10 kg S ha^{-1} as magnesium sulfate heptahydrate ($MgSO_4 \times 7 H_2O$) (5% solution of SO_3 per 300 dm^3 H_2O ha^{-1}).

Before sowing, phosphorus fertilizer (46% granulated triple superphosphate at a rate of 90 kg P_2O_5 ha^{-1}) and potassium fertilizer (60% potassium salt at a rate of 100 kg K_2O ha^{-1}) were applied in all treatments. The plots with different fertilization treatments were balanced by applying magnesium lime and calcium carbonate in order to equalize the soil pH. This ensured that the same input soil conditions were achieved in all experimental sites.

Table 2. The nitrogen and sulfur rate application design

Element	Rate (kg ha^{-1})	Application time		
		before sowing	BBCH 30–31	BBCH 55–59
Nitrogen (N)	0	–	–	–
	50	50	–	–
	100	50	50	–
	150	50	50	50
Sulfur (S)	0	–	–	–
	40	30	–	10

The agronomic practices for the cultivation of spring wheat were consistent with the current agronomic recommendations. The Kandela spring wheat variety, classified as class A wheat in terms of its commercial value and characterized by very good baking properties, was sown at a plant density of 500 plants per 1 m^2. Sowing was carried out between March 28 and April 5, depending on the year.

Before sowing, seeds were dressed with the seed dressing Vitavax 200 FS (a.i. carboxin) at a rate of 300 cm^3 100 kg^{-1}. A mixture of the herbicides Granstar 75 WG (tribenuron-methyl) (20 g ha^{-1}) and Puma Super 069 EW (fenoxaprop-P-ethyl) (1 dm^3 ha^{-1}) was used at the tillering stage (BBCH 28) to destroy monocotyledonous and dicotyledonous weeds. The occurrence of root rot diseases was reduced by applying Alert 375 SC (flusilazole + carbendazim) – 1.0 dm^3 ha^{-1} at the stem elongation stage (BBCH 30-32), whereas Tilt CB 37,5 (propiconazole + carbendazim) was applied at a rate 1 dm^3 ha^{-1} against leaf and ear diseases at BBCH 58-59. To control pests, Decis 2,5 EC (deltamethrin) was used at a rate of 0.25 dm^3 ha^{-1} at BBCH 58-59. The growth regulator Stabilan 750 SL (chlormequat chloride) was applied at a rate 1.8 dm^3 ha^{-1} at the stem elongation stage (BBCH 30-32) to prevent crop lodging. In each year of the study, spring wheat was harvested in the second decade of August.

Plant sampling and measurement

The experiment investigated the following characteristics: grain yield (t ha^{-1}), grain starch content (g kg^{-1}), gluten content (g kg^{-1}), total protein content (g kg^{-1}), cysteine (cysteic acid) content (mg g^{-1}), methionine (methionine sulfone) content (mg g^{-1}), crude fat content (g kg^{-1}), crude dietary fiber content (g kg^{-1}), flavonoid content (expressed as quercetin equivalents; g kg^{-1}), o-dihydroxy phenol content (expressed as caffeic acid equivalents; g kg^{-1}) and ash content (g kg^{-1}).

Spring wheat grain yield was determined at the fully ripe stage (BBCH 89-92). Before weighing, the grain yield was brought to the same moisture content of 11%.

Wet gluten was obtained according to the Polish Standard [PN-A-74041 1977]. Gluten samples (2 mm thick) were placed on a Petri dish floating on the surface of an ultrasonic scrubber. The samples were sonicated for 300 s in a Sonic-0.5 ultrasonic scrubber (Polsonic Palczyński sp.j., Warsaw, Poland) using 40 kHz ultrasound at 80 W.

Grain total protein content was calculated as the product of the grain N content × 5.7. Determination of N content was carried out by the Kjeldahl method (ISO 5983-1, Animal feeding stuffs, Determination of nitrogen con-tent and calculation of crude protein content, Part 1: Kjeldahl method) [Barbano et al. 1991].

Starch content was measured as glucose using an enzymatic-colorimetric assay, after initial gelatinization in an autoclave, followed by enzymatic hydrolysis [Sindt et al. 2000].

Cysteine (cysteic acid) content (mg g^{-1}) and methionine (methionine sulfone) content (mg g^{-1}) were determined by the CLA/PLC/34/2011 method [Maćkowiak-Dryka et al. 2020].

Ash content (g kg^{-1}) was determined by the CLB/PSO/5/2019 method [Scope of accreditation… 2023].

Crude fat content (g kg^{-1}) was determined by the Soxhlet method (ON-A-74039:1964), [Salimon et al. 2014].

Total dietary fiber content (in g kg^{-1}) was determined by the enzymatic gravimetric method using a Fibertec 2010 system (FOSS, Hillerød, Denmark). The sample was subjected to digestion with the following enzymes: thermostable alpha-amylase, pepsin, and pancreatin; the weight of the undigested residue was determined, and the soluble dietary fiber supernatant was precipitated from the solution and its weight was determined.

Determination of flavonoid content was carried out using Christ–Müller's method [Polish Pharmacopoeia IX 2011]. Flavonoid content was determined spectrophotometrically, after extraction of flavonoids from the raw material, and expressed as quercetin equivalents (QE). This method involves acid hydrolysis of flavonol glycosides, followed by the formation of colored complexes of these flavonoid compounds with $AlCl_3$. Absorbance was measured at $\lambda = 425.0$ nm with a Cintra 20 UV–VIS spectrometer (GBC).

Total dihydroxy phenol content was measured spectrophotometrically at a wavelength of $\lambda = 725$ nm (Shimadzu 1800 spectrophotometer, Shimadzu Corp. Kyoto, Japan) and expressed as caffeic acid equivalents. To make the measurement on the spectrophotometer, 50–500 μL of the extract (depending on the expected value of absorption of the tested sample) was transferred into a volumetric flask. A total of 2.0 cm^3 methanol, 10 cm^3 H_2O, 2 cm^3 Folin reagent, and 1.0 cm^3 of a 10% solution of Na_2CO_3 were added. The samples were put aside for 0.5 h, while subsequently, they were made up with deionized water up to the mark and measured on the spectrophotometer at a wavelength of $\lambda = 725$ nm in relation to the reference sample [Singleton and Rossi 1965].

Statistical analysis

The Statistica PL 13.3 program was used for the analysis of variance (ANOVA), and the Tukey's test was used to determine the Honestly Significant Difference (HSD) value at $p < 0.05$. Due to the statistical insignificance of most interactions (double and triple) between the main factors (N rate; S rate) and years of research (Y), Tables 3 and 4 (wczesniej 5) present average research results from 3 years for main effects and results in individual years of research for a given feature resulting. Tables 3 and 4 also present research results regarding the interaction between the rates of N fertilization and S fertilization.

Table 3. The effect of nitrogen and sulfur fertilization on the yield and quality characteristics of spring wheat grain

Fertilization		Characteristic						
		grain yield ($t ha^{-1}$)	starch ($g kg^{-1}$)	gluten ($g kg^{-1}$)	total protein ($g kg^{-1}$)	cysteine ($mg g^{-1}$)	methionine ($mg g^{-1}$)	fat ($g kg^{-1}$)
Mean over the years of study for N fertilization rates	0 N	5.61 [d]	633.5 [a]	275.5 [d]	149.7 [d]	4.05 [b]	3.06 [cd]	7.3 [c]
	50 N	5.77 [c]	634.0 [a]	290.2 [c]	158.3 [c]	4.36 [ab]	3.18 [c]	9.3 [b]
	100 N	6.69 [b]	632.8 [a]	316.4 [b]	162.9 [b]	4.49 [a]	3.35 [b]	9.9 [b]
	150 N	6.94 [a]	635.8 [a]	361.0 [a]	168.4 [a]	4.65 [a]	3.50 [a]	12.3 [a]
Mean over the years of study for S fertilization rates	0 S	6.16 [b]	632.4 [b]	304.2 [b]	158.4 [a]	4.26 [b]	3.03 [b]	8.8 [b]
	40 S	6.34 [a]	635.7 [a]	317.4 [a]	161.3 [a]	4.51 [a]	3.51 [a]	10.6 [a]
Mean over the years of study for interaction: S rate × varied N rates	40 S + 0 N	5.65 [b]	635.3 [a]	284.3 [b]	151.0 [b]	4.33 [b]	3.29 [a]	7.7 [b]
	40 S + 50 N	5.87 [b]	636.0 [a]	298.7 [b]	160.6 [a]	4.44 [b]	3.45 [a]	9.9 [b]
	40 S + 100 N	6.80 [a]	633.7 [a]	324.6 [b]	163.3 [a]	4.52 [b]	3.57 [a]	10.6 [b]
	40 S + 150 N	7.05 [a]	637.7 [a]	362.0 [a]	170.1 [a]	4.76 [a]	3.73 [a]	14.1 a
In the years of research – regardless of S and N rates	2014	6.207 [b]	631.4 [bc]	303.7 [a]	165.1 [a]	4.49 [a]	3.30 [a]	10.4 [a]
	2015	6.131 [b]	633.5 [b]	315.0 [a]	162.3 [b]	4.49 [a]	3.25 [a]	9.8 [a]
	2016	6.435 [a]	637.3 [a]	313.6 [a]	152.2 [c]	4.18 [a]	3.26 [a]	8.8 [b]

Fertilization		Characteristic						
		grain yield (t ha^{-1})	starch (g kg^{-1})	gluten (g kg^{-1})	total protein (g kg^{-1})	cysteine (mg g^{-1})	methionine (mg g^{-1})	fat (g kg^{-1})
CV%	N	9.19	0.18	104.6	4.27	5.06	5.05	18.42
	S	1.43	0.26	2.13	0.88	2.88	7.33	9.04
	Y	2.06	0.38	1.62	3.44	3.30	0.67	6.90
	N × S	9.31	0.32	10.73	4.39	6.19	8.92	21.26
	S × Y	2.53	0.66	2.73	3.62	4.58	8.83	11.72
	N × Y	9.60	0.47	10.80	5.65	6.14	5.80	20.84
	N × S ×Y	8.52	0.45	9.61	4.45	5.18	6.50	15.25
p-value	N	0.0007	0.4162	0.0000	0.0000	0.0179	0.0020	0.0003
	S	0.0000	0.0375	0.0203	0.0176	0.0346	0.0000	0.0021
	Y	0.0003	0.0208	0.1371	0.0000	0.0555	0.6403	00215
	N × S	0.0047	0.0627	0.0041	0.0039	0.0045	0.0635	0.0038
	S × Y	0.3506	0.0082	0.7419	0.1287	0.4806	0.0012	0.3365
	N × Y	0.0046	0.6569	0.2697	0.0591	0.9723	0.1244	0.1372
	N × S ×Y	0.0032	0.5637	0.3948	0.4239	0.8875	0.1356	0.1478

Values followed by different letters within a column are statistically different ($p < 0.05$); *CV%* – coefficient of variation
Variables: N – nitrogen rate; S – sulfur rate; Y – year; N × S – nitrogen rate × sulfur rate; S ×Y – sulfur rate × year); N × Y – nitrogen rate × year; N × S × Y – nitrogen rate × sulfur rate × year

Table 4. The effect of nitrogen and sulfur fertilization on the quality and health-promoting characteristics of spring wheat grain

Fertilization		Crude fiber content (g kg^{-1})	Flavonoids content (g kg^{-1})	O-dihydroxy phenols content (g kg^{-1})	Ash content (g kg^{-1})
Mean over the years of study for N fertilization rates	0 N	34.0 ^{c}	0.18 ^{d}	0.74 ^{c}	18.2 ^{d}
	50 N	37.7 ^{bc}	0.21 ^{c}	0.78 ^{c}	18.9 ^{c}
	100 N	38.9 ^{b}	0.24 ^{b}	0.81 ^{b}	19.9 ^{b}
	150 N	41.9 ^{a}	0.27 ^{a}	0.91^{a}	21.1 ^{a}
Mean over the years of study for S fertilization rates	0 S	36.6 ^{b}	0.19 ^{b}	0.77 ^{b}	18.9 ^{b}
	40 S	39.6 ^{a}	0.26 ^{a}	0.85 ^{a}	20.2 ^{a}
Mean over the years of study for interaction: S rate × varied N rates	40 S + 0 N	34.9 ^{c}	0.21 ^{b}	0.79 ^{b}	18.8 ^{c}
	40 S + 50 N	39.0 ^{b}	0.24 ^{b}	0.83 ^{b}	19.7 ^{bc}
	40 S + 100 N	40.6 ^{b}	0.28 ^{b}	0.86 ^{b}	20.5 ^{b}
	40 S + 150 N	43.8 ^{a}	0.33 ^{a}	0.94 ^{a}	21.8 ^{a}
In the years of research – regardless of S and N rates	2014	36.1 ^{b}	0.18 ^{c}	0.65 ^{c}	19.2 ^{b}
	2015	42.5 ^{a}	0.24 ^{b}	0.81 ^{b}	20.0 ^{a}
	2016	35.8 ^{b}	0.27 ^{a}	0.98 ^{a}	19.4 ^{b}

Fertilization		Crude fiber content (g kg^{-1})	Flavonoids content (g kg^{-1})	O-dihydroxy phenols content (g kg^{-1})	Ash content (g kg^{-1})
CV%	N	7.39	14.91	7.59	5.56
	S	3.86	16.50	5.41	3.44
	L	8.15	16.38	26.35	1.68
	N × S	8.40	22.68	9.34	6.55
	S × L	9.10	26.29	17.39	3.91
	N × L	11.60	22.36	18.37	6.05
	N × S ×Y	11.78	23.45	18.41	6.25
p-value	N	0.0001	0.0024	0.0087	0.0001
	S	0.0006	0.0002	0.0073	0.0001
	Y	0.0000	0.0007	0.0001	0.0170
	N × S	0.0025	0.0009	0.0008	0.0022
	S × Y	0.1778	0.0031	0.2730	0.2366
	N × Y	0.0199	0.8792	0.4486	0.1075
	N × S ×Y	0.1623	0.7985	0.4564	0.1268

Values followed by different letters within a column are statistically different ($p < 0.05$); *CV%* – coefficient of variation
Variables: N – nitrogen rate; S – sulfur rate; Y – year; N × S – nitrogen rate × sulfur rate; S ×Y – sulfur rate × year); N × Y – nitrogen rate × year; N × S × Y – nitrogen rate × sulfur rate × year.

Moreover, the coefficient of variation (CV%), which is a measure of the scatter of results, was calculated as the quotient of the standard deviation and mean. Tables 5 and 6 present correlation coefficients (r) at $p < 0.05$ between spring wheat grain yield and the examined grain quality traits and significant correlations are expressed.

Table 5. The correlation coefficients (r) between the tested quality characteristics of spring wheat grain

Characteristic studied	Grain yield (t ha^{-1})	Starch content (g kg^{-1})	Gluten content (g kg^{-1})	Total protein content (g kg^{-1})	Cysteine content (mg g^{-1})	Methionine content (mg g^{-1})	Fat content (g kg^{-1})
Grain yield (t ha^{-1})	1	–	–	–	–	–	–
Starch content (g kg^{-1})	0.211	1	–	–	–	–	–
Gluten content (g kg^{-1})	0.859*	0.238	1	–	–	–	–
Total protein content (g kg^{-1})	0.534*	–0.061	0.62*	1	–	–	–
Cysteine content (mg g^{-1})	0.503*	0.051	0.637*	0.816*	1	–	–
Methionine content (mg g^{-1})	0.564*	0.511*	0.538*	0.460*	0.623*	1	–
Fat content (g kg^{-1})	0.677*	0.061	0.716*	0.623*	0.715*	0.587*	1

Significant at $p = 0.05$: $r = 0.406$; * statistically significant correlation

Table 6. The correlation coefficients between the tested technological characteristics of spring wheat grain

Characteristic studied	Grain yield (t ha^{-1})	Crude fiber content (g kg^{-1})	Flavonoids content (g kg^{-1})	O-dihydroxy phenols content (g kg^{-1})	Ash content (g kg^{-1})
Grain yield (t ha^{-1})	1	–	–	–	–
Crude fiber content (g kg^{-1})	0.455*	1	–	–	–
Flavonoid content (g kg^{-1})	0.576*	0.523*	1	–	–
O-dihydroxy phenol content (g kg^{-1})	0.487*	0.249	0.77*	1	–
Ash content (g kg^{-1})	0.738*	0.785*	0.681*	0.476*	1

Significant at $p = 0.05$: $r = 0.406$; * statistically significant correlation.

Weather conditions

The total rainfall during the growing season of spring wheat (III–VIII) in 2014 was 519.7 mm and it was higher by 152.2 mm than the long-term mean (1971–2011: 367.5 mm). In the 2015 growing season, the total rainfall was 266.4 mm and hence it was lower than the long-term mean by 101.1 mm. During the 2016 growing season, in turn, the amount of rainfall was higher than the long-term mean by 84.2 mm, standing at 451.7 mm. The sums of air temperatures in the growing seasons analyzed (III–VIII) were higher than the long-term sum (1971–2011: 2392°C). Thus, it was higher by 254°C in the 2014 season, in the 2015 season – by 320°C, while in the 2016 season – by 247°C. The mean air temperature generally exceeded the long-term mean temperature in each month of the years analyzed (Fig. 1).

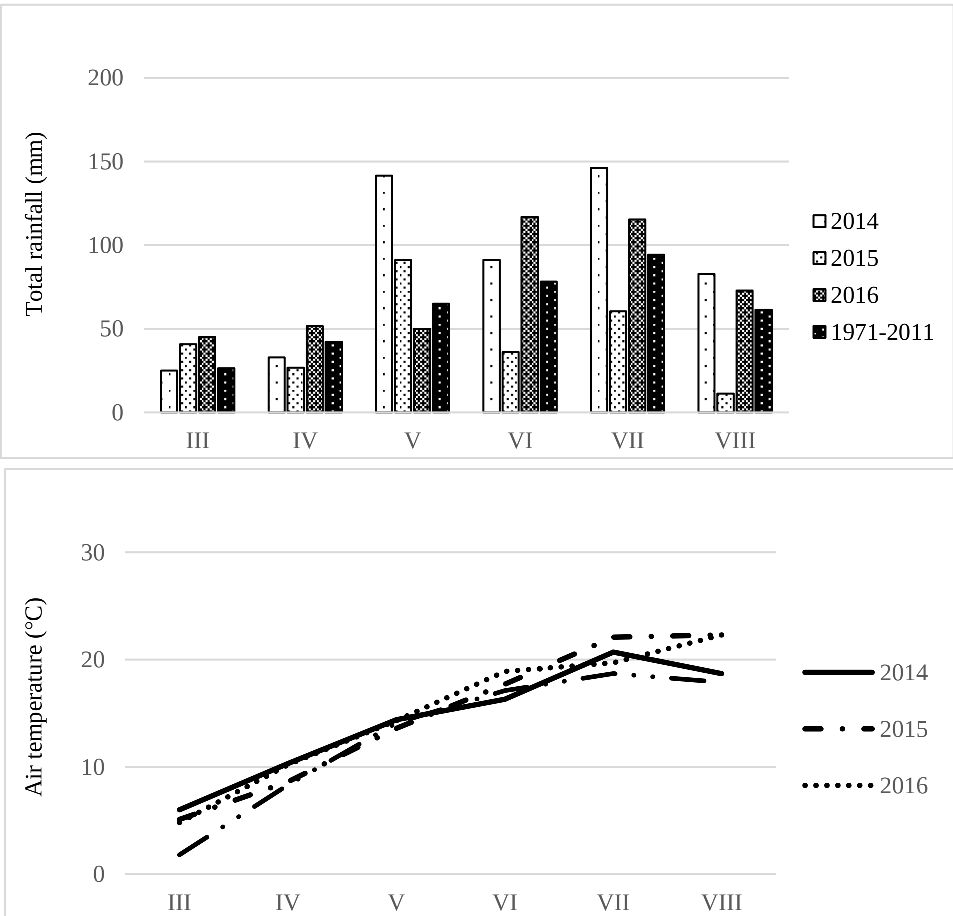

Fig. 1. The total rainfall (mm) and the sum of air temperature (°C) in the years 2014–2016 as well as the long-term means (1971–2011)

Source: Meteorological Research Station in Zamość

Based on the meteorological data, Selyaninov' s hydrothermal coefficient was calculated [Bac et al. 1993] according to the following formula:

$$k = \frac{p \times 10}{\sum t}$$

where: p – total rainfall (mm); $\sum t$ – the sum of mean daily temperatures for a given month (°C).

The values of the hydrothermal coefficients were calculated for the spring wheat growing season (III–VIII). The individual growing seasons were determined to be as follows: 2014 – rather wet (1.96); 2015 – dry, close to rather dry (0.98); and 2016 – rather wet, close to optimal (1.71).

RESULTS

The grain yield of spring wheat increased proportionately after the application of each nitrogen rate (Fig. 2), but it was the largest after the application of the highest nitrogen rate used in this study (150 kg ha^{-1}), standing at 6.947 t ha^{-1}. It was higher by 1.335 t ha^{-1} (19.2%) relative to the yield obtained from the control treatment (without nitrogen fertilization application) (Tab. 3). Sulfur fertilization (factor II) showed a beneficial effect compared to the control, which was expressed by an increase in spring wheat grain yield (by 2.8%). In the case of nitrogen doses of 100 and 150 kg ha^{-1}, sulfur supplementation (factor II) at a dose of 40 kg ha^{-1} resulted in a significant increase in grain yield (Tab. 3).

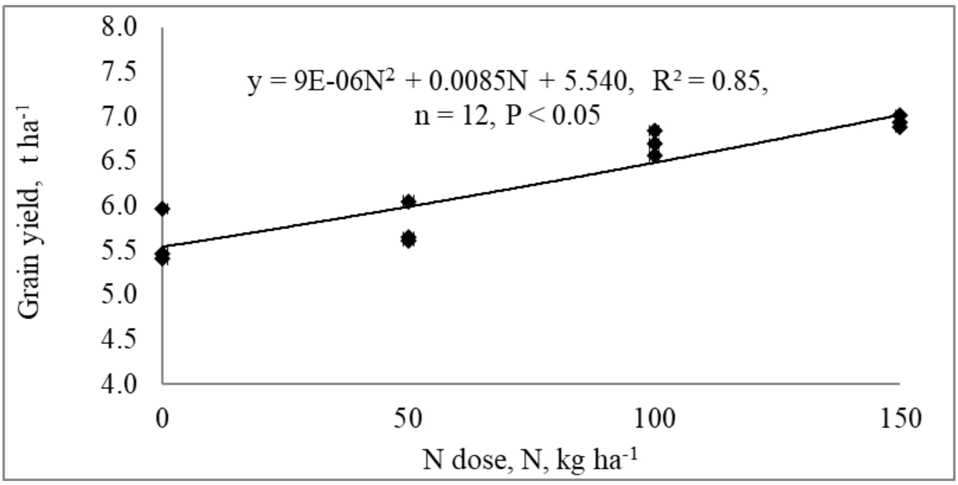

Fig. 2. The effect of nitrogen rate on the grain yield of spring wheat (t ha^{-1})

The weather conditions, i.e. the rainfall and air temperature during the spring wheat growing season in 2016 (rather wet, close to optimal – 1.71), were significantly most favorable in terms of the value of the grain yield obtained relative to 2014 (rather wet – 1.96) and compared to 2015 (dry, close to rather dry – 0.96).

The grain yield of spring wheat showed variation as affected by nitrogen fertilization (*CV%* = 9.19), sulfur fertilization (*CV%* = 1.43), and the interaction of these factors (*CV%* = 9.31). The weather factor caused low variation (*CV%* = 2.06), but its interaction with nitrogen fertilization was high (*CV%* = 9.31), whereas it was lower in the case of sulfur fertilization (*CV%* = 2.53) – as in Table 3.

The analysis of the study results revealed a significantly beneficial effect of nitrogen fertilization (factor I) on all the tested quality characteristics of spring wheat grain, except for starch content. There was a significant increase in the grain content of gluten, total protein and methionine with increasing nitrogen rate, with this content being the highest after the application of a rate of 150 kg N ha^{-1} (respectively 31.0%, 12.5%, and 14.4%).

As far as cysteine content is concerned, already the nitrogen rate, 50 kg N ha^{-1}, caused a significant increase in amino acid content (by 7.1%) relative to that found for the control treatment, without nitrogen. A further increase in nitrogen rate did not have a significant effect on increasing the cysteine content. The fat content in spring wheat grain was the significant highest after the application of nitrogen fertilization at a rate of 150 kg N ha^{-1} (68.5%). The application of the rates of 50 and 100 kg N ha^{-1} had a similar effect on fat content, which was significantly higher (by 27.4–35.6%) than in the case of grain harvested from the control treatments, where no nitrogen fertilization was used (Tab. 3).

As far as sulfur fertilization (factor II) is concerned, the analysis of the study results showed sulfur to have a beneficial effect on increasing the content of gluten (by 4.3%), cysteine (by 5.8%), methionine (by 15.8%) and fat (by 20.4%) compared to their values in the treatments without S fertilization (Tab. 3).

The influence of the interaction between the dose of nitrogen 150 kg ha^{-1} and sulfur 40 kg ha^{-1} was statistically significant in the case of gluten, cysteine, fat (Tab. 3) and crude fiber, flavonoids, o-dihydroxy phenols and ash content (Tab. 4).

The weather conditions, the rainfall and temperature during the spring wheat growing season in the years 2014–2016, did not cause significant differences in the content of gluten, cysteine and methionine in spring wheat grain. The other grain quality characteristics were modified under the influence of weather. The weather pattern in 2014 (rather wet – hydrothermal coefficient 1.96) favored the significantly highest accumulation of total protein (165.1 g kg^{-1}) and fat (10.4 g kg^{-1}). The 2015 growing season (dry, close to rather dry – 0.98) beneficially affected the total protein and fat content in wheat grain relative to the 2016 season. The 2016 growing season (rather wet, close to optimal – 1.71) promoted the significantly highest starch content (63.73%), but it significantly reduced the total protein and fat content in wheat grain (Tab. 3).

The studied quality characteristics of spring wheat grain exhibited greater variation as affected by nitrogen fertilization than under the influence of sulfur amendment. The weather factor only slightly affected the variation of the characteristics studied (from *CV%* = 0.67 for methionine content to *CV%* = 26.5 for o-dihydroxy phenol content) – as in Table 3, Table 4.

Significant positive correlations were obtained between wheat grain yield and all the other grain quality characteristics, except for starch content (r = 0.211). The highest correlation coefficient was found between grain yield and gluten content (r = 0.859) – as in Table 5.

The content of crude fiber (dietary fiber), flavonoids (expressed as quercetin equivalents), o-dihydroxy phenols (expressed as caffeic acid equivalents) and ash increased significantly with increasing nitrogen rate, being the significantly highest after the application of an N rate of 150 kg ha^{-1} (respectively 41.9 g kg^{-1}, 0.27 g kg^{-1}, 0.91 g kg^{-1}, and 21.1 g kg^{-1}). Nonetheless, as regards crude fiber content and o-dihydroxy phenol content, the application of the nitrogen rate of 50 kg ha^{-1} did not result in a significant increase in these components relative to the control. It was only a rate of 100 kg ha^{-1} and its increase to 150 kg ha^{-1} that produced significant increases in the grain content of crude fiber and o-dihydroxy phenols (Tab. 4).

In the case of sulfur fertilization (factor II), sulfur was found to have a significant beneficial effect on the increase in the content of crude fiber (dietary fiber; by 8.1%), flavonoids (by 36.8%), o-dihydroxy phenols (by 10.4%) and ash (by 6.8%) compared to that found in the treatments without S fertilization (Tab. 4).

The rainfall and temperature during the spring wheat growing season in the period 2014–2016 caused significant differences in the content of crude fiber, flavonoids, o-dihydroxy phenols and ash in spring wheat grain. Among all the years studied, the weather conditions in the year 2014 (rather wet) were the most unfavorable (statistically proven significance of differences) to the distribution of the results for the characteristics analyzed. The 2015 growing season (dry, close to rather dry) favored a significant increase in the grain content of crude fiber and ash. The weather pattern in 2016 (rater wet, close to optimal), on the other hand, caused a significant increase in the grain content of flavonoids and o-dihydroxy phenols (Tab. 4).

Significant positive correlations were obtained between wheat grain yield and all the quality and health-promoting characteristics. The highest correlation coefficient was found between grain yield and ash content (r = 0.738). The tested characteristics were significantly positively correlated with one another. This relationship was not statistically significant only between crude fiber content and o-dihydroxy phenols content (r = 0.249) – as in Table 6.

DISCUSSION

This study demonstrated a significantly beneficial effect of nitrogen fertilization on spring wheat grain yield. The grain yield had the most favorable characteristics after the application of the medium and highest nitrogen rates, i.e. 100 and 150 kg ha^{-1}, compared to the control treatment (without nitrogen). The increase was 16.3% and 23.7%, respectively, for the medium and highest rates. Nitrogen is considered to be one of the most important yield-forming elements and its positive influence on yields of cereals and other crops has been proven by many authors [Ladha et al. 2016, Litke et al. 2018, Boulelouah et al. 2022, Ghafoor et al. 2022, van Grinsven et al. 2022, Kamdi et al. 2024]

The thesis on the beneficial effects of sulfur on spring wheat yield has been confirmed by studies of many authors [Järvan et al. 2012, Skwierawska et al. 2016, Hemesh 2020, Yu et al. 2021]. According to Podleśna et al. [2008] as well as Klikocka and Cybulska [2014], the cultivation of wheat under sulfur deficiency conditions led to plant growth and development inhibition as well as to changes in the chemical composition of vegetative organs and grain. Plants with limited access to sulfate sulfur in the soil showed a radical reduction in the grain yield from the main stem and branches, with a simultaneous decrease in thousand grain weight. The above described observations are also reflected in the present study, in which the addition of sulfur to N fertilization (100 and 150 kg N ha^{-1}) at an amount of 40 kg S ha^{-1} had a significant effect on the increase in grain yield by 2.8%. The beneficial impact of the addition of sulfur to nitrogen fertilization on wheat yield has also been confirmed by Salvagiotti et al. [2009], Klikocka et al. [2016], and Shivay et al. [2016].

Tabak et al. [2020] proved on the example of winter wheat that the significantly highest agronomic and physiological effectiveness of nitrogen fertilization as well as the highest apparent nitrogen recovery were obtained after fertilization with 150 kg N ha^{-1} (similarly to our study on spring wheat). The incorporation of higher rates (200 kg and 250 kg N ha^{-1}), on the other hand, proved to be irrational economically and production-wise. Moreover, these authors emphasize that sulfur fertilization increased nitrogen recovery compared to nitrogen fertilization without sulfur, which is also stressed in our study results presented in this paper.

Shivay et al. [2016] prove that an even 5% addition of sulfur to nitrogen fertilizer (urea) is sufficient to cover 50% of the sulfur requirement of wheat crops and to increase nitrogen recovery efficiency by 60%. Furthermore, in the long term this will bring environmental benefits in the form of reduction in emissions and nitrogen losses because the addition of sulfur increases nitrogen recovery efficiency.

Based on the present study, it can be generally stated that sulfur fertilization increased the effects of nitrogen on the yield of spring wheat as well as on the grain quality characteristics. After the incorporation of sulfur, the values of the studied characteristics increased compared to each N rate level. This type of action of a yield-increasing factor, in this case the fertilizer factor, highlights the additive effect of sulfur. It is manifested under the action of a deficiency factor to a relatively weak degree, in accordance with the rules defined by *law of diminishing returns*, known as the Mitscherlich law [Gupta and Schnug 2001, Grzebisz 2009]. Generally, the additive interaction of nutrients manifests itself when there is a constant increase in yield weight as a consequence of the use of a second factor. Soofizada et al. [2022] found that S fertilization increases grain yield without decreasing grain protein content, whereas N fertilization effectively increases grain protein content and protein yield per hectare.

A very important element of the cultivation of quality wheat is its suitability for baking purposes. Naeem and MacRitchie [2003] noted that agro-biofortification with sulfur, combined with nitrogen fertilization, had a positive effect on most of the wheat quality parameters. Järwan et al. [2008] found that the application of sulfur in an experiment with winter wheat did not have a clear influence on grain protein and wet gluten content, but in some other experiments the gluten index increased and the protein quality improved. Johansson et al. [2004], in turn, claim that the protein and gluten content in wheat grain predominantly depends on an appropriately selected rate of nitrogen fertilization. Luo et al. [2000], on the other hand, obtained an improvement in the parameters related to grain gluten content as affected by the addition of sulfur to N fertilization. Tea et al. [2005] also reports that fertilization of wheat with sulfur affects positively the content of gluten and its rheological properties. Sulfur affected an increase in amino acid content and all baking quality parameters in a study by Podleśna [2005]. The present research also revealed a beneficial effect on sulfur amino acids: cysteine and methionine, with the latter one determining flour quality. Wang et al. [2023] note that cysteine in cereal grains optimizes the balance of amino acids, regulating the ratio of other amino acids and in this way improving the nutritional quality of grain, at the same time ensuring a constant increase in protein concentration. Overall, S applied at a rate of 60–90 kg ha^{-1} in a study conducted by these authors synergistically improved both the yield and nutritional quality of maize, meeting the requirements for sustainable development in maize production. Järvan et al. [2008] report that the first organic product of sulfur in the plant is cysteine, an exogenous amino acid that determines the quality of plant protein and its value for animals and humans. Plants well fed with sulfur increase the content of not only chlorophyll, but also protein. This dependence underlies a strong functional relationship, occurring already at the molecular level, between sulfur and nitrogen [Hesse et al. 2015]. Klikocka and Marks [2018] noted an improvement in the health-promoting properties of spring wheat grain (in particular the grain content of micronutrients) under the influence of supplementation of nitrogen fertilization with sulfur addition.

Protein is produced in the plant at the expense of starch contained in it, which is a consequence of the process of consumption of carbon compounds during the protein synthesis process. These negative correlations were also noticed in the present study because nitrogen fertilization affected an increase in the content of protein and sulfur amino acids: cysteine and methionine, at the expense of the reduced starch content. Järvan et al. [2008] also claim that the result of an increased content of proper proteins in the plant after sulfur application is significant plant growth, while in the leaves an increase in chlorophyll, which is manifested in more intense leaf color.

Goźliński [1970] found that sulfur application in the cultivation of spring oats and barley contributed to an increased use of absorbed nitrogen for yield formation and protein synthesis. Under sulfur deficiency, the absorbed nitrogen accumulated mainly in straw and occurred in non-protein form in a large amount, due to which this part of nitrogen was not used in grain yield. A similar relationship was also observed in the study by Klikocka and Cybulska [2016].

The yield-forming role of sulfur is manifested in an increase in grain yield through better use of nitrogen fertilizer. In agricultural practice, this task consists in transformation of the nitrogen fertilizer used into protein yield. Therefore, proper nutrition of wheat with sulfur improves the grain quality characteristics [Wilson et al. 2020, Ghafoor et al. 2022, Soofizada et al. 2022]. The present study also demonstrated that the differences in the content of gluten, total protein, cysteine, methionine, fat, crude fiber, flavonoids, o-dihydroxy phenols and ash were statistically significant depending on the nitrogen rate applied (factor I) and the addition of sulfur to fertilization (factor II). The grain content of gluten, total protein and methionine increased significantly and directly proportionally with increasing nitrogen rate, being the highest after the application of a rate of 150 kg N ha^{-1} (respectively 41.9 g kg^{-1}, 0.27 g kg^{-1}, 0.91 g kg^{-1}, and 21.1 g kg^{-1}).

The fat content in spring wheat grain was the highest after the application of nitrogen fertilization at a rate of 150 kg N ha^{-1} (12.3 g kg^{-1}). The application of the rates of 50 and 100 kg N ha^{-1} had a similar effect on the fat content, which was significantly higher than in the case of grain harvested from the control plots where no nitrogen was applied. The grain content of crude fiber (dietary fiber), flavonoids (expressed as quercetin equivalents), o-dihydroxy phenols (expressed as caffeic acid equivalents) and ash increased significantly with increasing nitrogen rate and it was the highest after the application of N at an amount of 150 kg ha^{-1} (respectively 4.19%, 0.027%, 0.091%, and 2.11%). Nitrogen is considered to be one of the most important yield-forming elements, undoubtedly also having the greatest effect on the quality parameters of grain and flour, among others protein and wet gluten content, gluten weakening, falling number, Zeleny sedimentation value, flour water absorption, or bread volume [Moss et al. 1991, Dostálová et al. 2015]. In the case of sulfur fertilization (factor II), the analysis of the study results revealed that sulfur has a beneficial effect on increasing the content of gluten, cysteine, methionine and fat as well as of dietary fiber, flavonoids, o-dihydroxy phenols and ash relative to the treatments without S fertilization. In the studies by Podleśna et al. [2008], Pompa et al. [2009], and Kurmanbayeva et al. [2021], fertilization of winter wheat with sulfur at a rate of 60 kg S ha^{-1} led to an increase in the grain content of protein (by 0.8–1.2%) and gluten (by 1.5–2%). Klikocka et al. [2016], on the other hand, additionally demonstrated a beneficial effect of sulfur fertilization on increasing the grain content of cysteine and methionine.

Raffan et al. [2020] draw attention to the enormous importance of sulfur in fertilization of cereals. These authors claim that the concentration of free (soluble, non-protein) aspargine may increase in wheat grain many times in response to sulfur deficiency. This aggravates a serious problem related to food safety and regulatory compliance in the food industry because free aspargine may be converted into a carcinogenic contaminant, acrylamide, during baking and processing. Wilson et al. [2020] also proved the positive effect of sulfur addition to nitrogen fertilization on reducing the concentration of deleterious asparagine in winter wheat grain.

CONCLUSIONS

The present study revealed that the Kandela variety of spring wheat, grown on dystrophic typical brown soil, showed a positive response to nitrogen and sulfur fertilization, as expressed by yield quantity and grain quality.

The values of the studied characteristics of spring wheat grain yield and quality were determined more strongly by nitrogen fertilization and its interaction with weather conditions than by sulfur fertilization.

The application of nitrogen at the rates of 100 and 150 kg ha^{-1}, combined with sulfur fertilization at a rate of 40 kg ha^{-1}, had a significant effect on the grain yield of spring wheat, compared to the control and the nitrogen dose of 50 kg ha^{-1}.

The values of the studied quality and health-promoting characteristics of spring wheat grain, were favorable and proportional to increasing nitrogen rate and the addition of sulfur at a rate of 40 kg ha^{-1}.

Significantly the highest contents of gluten, cysteine cysteic acid), fat, crude fiber, flavonoids (expressed as quercetin equivalents), o-dihydroxy phenols (expressed as caffeic acid equivalents) and ash were found in the fertilization variant of 40 kg S ha^{-1} + 150 kg N kg ha^{-1}.

Starch content was a constant trait and was only dependent on sulfur fertilization. The application of a nitrogen rate of 50 kg ha^{-1} was insufficient, regardless of sulfur amendment.

Most of the grain quality and health-promoting characteristics tested, the grain chemical composition, and the accumulation of nutrients in grain dry matter were generally positively correlated with grain yield.

To sum up, it should be stated that under the conditions of negative sulfur balance in cultivated soils and nitrogen fertilization application, it is necessary to use sulfur in the cultivation of crops. Because cereal processed products are the basis of the food pyramid (alongside vegetables and fruit), their quality is very important for the functioning of the organism. As shown by this study, spring wheat grain is a valuable source of nutrients being a component of many diet supplements, improving human condition and health. This research has demonstrated that the addition of sulfur to NPK fertilization is an efficient method for supplementation (enrichment) of the components of spring wheat grain.

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The source of funding: The work was financed from funds for statutory activities carried out at the University of Life Sciences in Lublin under funds from the Ministry of Science and Higher Education.

Received: 22.08.2024
Accepted: 2.01.2025
Published: 18.03.2025