Thesis of Doctoral (Ph.D.) Dissertation

THE EFFECT OF DIFFERENT FERTILIZER DOSE AND WATER SUPPLY ON SOME ELEMENT OF SULPHUR TURNOVER IN WHEAT (*Triticum aestivum* L.) PRODUCTION

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1. Introduction and objectives

Sulphur (S), as an essential macronutrient, plays a specific role in the metabolic processes of plants sulphur is essential for the synthesis of sulphur-containing amino acids, proteins and other organic compounds.

In agricultural practice, S supplementation was not addressed for a long time. Because of the role of sulphur in plant nutrition, S supplementation may be necessary to avoid sulphur deficiency and to ensure a harmonious supply of nutrients, depending on the nutrient availability of the soil. Optimum sulphur supply promotes the vegetative growth of plants, increases the protein content and influences the amount and proportion of S-containing amino acids (methionine, cysteine).

The sulphur content of soils has been decreasing over the last 3 decades. From the 1990s onwards, the use of superphosphate fertilizer with 11% S content, which was still widely used at that time, declined in Hungary, resulting in a significant decrease in the regular replenishment of the plant available S content of soils. Stricter environmental regulations have also reduced industrial S emissions. This has also reduced the significant source of sulphur that was deposited in the soil by precipitation. Today, the decrease in the plant-available sulphur content of soils is mainly compensated by the increased use of sulphur fertilizers. Sulphur fertilization is becoming increasingly important in several European countries, where it is becoming an increasingly important element of agrotechnology.

The effect of sulphur supply was studied in a fertilization experiment on winter wheat and in pot experiments on spring wheat. Adequate sulphur supply is important for the quality of wheat because it positively influences yield, protein and gluten content, the baking value parameters, and can increase the efficiency of nitrogen fertilization and contribute to the increase of resistance to biotic and abiotic stress indicators.

The objectives of the experimental work were to investigate the effect of different NPK supply and irrigation on the variation of some elements of sulphur turnover. The research was carried out between 2017-2019, at the University of Debrecen, in a long-term fertilization experiment established in 1983 in a bicultural (winter wheat-maize) production system for winter wheat.

In the long-term fertilization experiment, phosphorus (P) was supplemented with superphosphate from 1983 to 2009. The superphosphate contains $CaSO_4$ as a by-product, so the increasing P application rates during this period also meant an increasing sulphur application rate. Between 2010 and 2018, the area was not fertilized with sulphur, as the phosphorus nutrient was then replaced by monoammonium dihydrogen phosphate (MAP).

Taking into account the presented experimental conditions, the following objectives were defined:

- How increasing rates of NPK fertilization and irrigation affect the nitrogen, sulphur, organic-S and sulphate-S, N/S ratio during the growing season and the amount of sulphur-containing amino acids (methionine, cysteine) measured in wheat grain in wheat grown without sulphur supplementation.

- How the sulphur content of the superphosphate utilized in the year of application and how the sulphur uptake of winter wheat, the incorporation of sulphur into organic compounds and the N/S changes during the growing season as a result of increasing sulphur doses.

- How increasing rates of NPK fertilization and irrigation affect the 1M KCl, 0.0M $CaCl_2$ and 0.016M KH_4PO_2 soluble sulphate-S content of the soil in years without sulphur supplementation and during repeated sulphur application.

- How increasing doses of NPK fertilization and irrigation affect the changes in the aryl-sulphatase activity of soil during periods of no sulphur supplementation and repeated sulphur application.

- To analyze the correlations between 1M KCl, 0.01M $CaCl_2$, and 0.016M KH_2PO_4 soluble sulphate values.

-To analyze the correlations between the sulphur content of the plant and the 1M KCl, 0.01M $CaCl_2$, and 0.016M KH_2PO_4 soluble sulphate content of the soil.

In addition to the measurements in the soil-plant system of the long-term fertilization experiment, the effect of different forms and rates of S fertilization and different water supplies were analyzed in pot experiments on spring wheat.

In the pot experiments, we aimed to investigate whether:

- how sulphate and thiosulphate forms of sulphur and increasing N, S ratios affect the growth, nitrogen and sulphur content of spring wheat, the incorporation of sulphur into organic compounds, the N/S of the plant during the growing season and the soluble sulphate-S content of the soil.

- how drought stress affects spring wheat yield, N and S content, and changes in organic sulphur and sulphate sulphur content.

2. Material and methods

2.1. The long-term fertilization experiment of Latókép

In one part of the research, the sulphur supply of winter wheat grown on chernozem soil was analysed in 2017, 2018 and 2019 at the Research Station of Latókép of the University of Debrecen. The plant and soil samples were collected from the control and two selected NPK fertilizer doses of irrigated and non-irrigated plots in the bicultural rotation. The selected treatments and the amount of active ingredient of the fertilizer are presented in *Table 1*.

Table 1. Fertilizer rates applied in the long-term fertilization experiment (Debrecen-Látókép 2017, 2018, 2019)

Treatments		2017-2018			Treatments		2019			
		N	P_2O_5	K_2O			N	P_2O_5	K_2O	S
		kg/ha/year					kg/ha/year			
irrigated	control	0	0	0	irrigated	control	0	0	0	0
	NPK1	100	70	80		NPKS1	100	70	80	42.4
	NPK2	200	140	160		NPKS2	200	140	160	84.8
non-irrigated	control	0	0	0	non-irrigated	control	0	0	0	0
	NPK1	100	70	80		NPKS1	100	70	80	42.4
	NPK2	200	140	160		NPKS2	200	140	160	84.8

In addition to calcium dihydrogen phosphate, calcium sulphate is also present in superphosphate, so the fertiliser also contains 10.9% sulphur. As a consequence, sulphur addition to the fertilized plots was continuous during the period 1983-2009. During this period, 42.4 kg/ha of sulphur was applied annually in the NPK1 treatment and 84.8 kg/ha in the NPK2 treatment. Between 2010 and 2018, phosphorus was replaced with monoammonium dihydrogen phosphate, so sulphur supplementation of the area was interrupted during this period. In 2019, the phosphorus was supplemented with superphosphate, so from this year onwards, there was also repeated sulphur replenishment.

In the case of irrigation, the rainfall of the growing season take into account and is only irrigated as needed. Based on this, irrigation was carried out on two occasions in 2017: on 27-28 May and 1-2 June. On both occasions, 20-20 mm of water was irrigated. In 2018, irrigation was sufficient, and in 2019, due to the favourable distribution of natural rainfall, no irrigation was necessary. Accordingly, the actual effect of irrigation was analysed in 2017, while in 2018 and 2019, the after-effect of the previous irrigation period was analysed on the irrigated plots.

2.2. Pot experiments

In the second part of the experiments, the effects of different sulphur supplies on the growth and yield parameters of spring wheat were analyzed under controlled conditions in a soil-plant system. The experiments were set up in the greenhouse of the Institute of Agricutural Chemistry and Soil Science in the spring 2018 and 2019.

Analysis of the effect of drought stress and increasing doses of sulphur fertilization (2018)

The first objective of the greenhouse pot experiment was to examine the effect of different doses of sulphur fertilization (22.4, 28, 56 kg S ha^{-1}) on the yield and nutrient content of spring wheat during the growing season under adequate water supply on chernozem soil. The second aim of this experiment was to observe how these parameters change under water stress conditions.

The experiment was set up with 10 kg of air-dry soil. The indicator plant was the Stanga variety of spring wheat. Fundamentally the moisture content of soil was set up to 60% of the established maximum water holding capacity. In this case, the pots were weighed every day and the missing water was added with ion exchange water. This condition provides an adequate water supply for the plants. In addition, each treatment had a pair with a reduced water supply. In these treatments, the plant was not irrigated before the appearance of wilting symptoms. After this, the irrigation was applied to 40% of the established maximum water-holding capacity.

Considering the nutrient requirements of the plant and the nutrient supply of the experimental soil, the following nutrient requirements were calculated assuming an average yield of 4 t ha^{-1}: 112 kg N ha^{-1}, 88 kg P_2O_5 ha^{-1}, 84 kg K_2O ha^{-1}. NPK nutrients were applied in proportion to 10 kg of soil. Increasing doses of sulphur (22.4, 28, 56 kg S ha^{-1}) were used with the same nitrogen dose (112 kg N ha^{-1}) which caused different N/S ratio background (1:0.2; 1:0.25; 1:0.5) (*Table 2.*).

Table 2. Rates of fertilizers applied in the pot experiment

	Water supply	N/S treatment	N doses kg/ha	S doses kg/ha
1.	Adequate	control	0	0
2.		1:0.2	112	22.4
3.		1:0.25	112	28
4.		1:0.5	112	56
5.	Reduced	control	0	0
6.		1:0.2	112	22.4
7.		1:0.25	112	28
8.		1:0.5	112	56

In the experiment, biomass during the growing season was determined. The total N, S content and N/S ratio of the plant was measured and the sulphate-S content of the plant tissue at flowering was determined. The KCl, $CaCl_2$ and KH_2PO_4 soluble sulphate-S content of soil was also measured.

Analysis of the effect of different sulphur forms and treatments with different N, S ratio (2019)

In the greenhouse experiment, the effect of two sulphur forms was compared: sulphate (SO_4^{2-}) and thiosulphate ($S_2O_3^{2-}$). The sulphate was applied as potassium-sulphate and thiosulphate as ammonium-thiosulphate. Increasing doses of both sulphur forms (24, 60, 120 kg S ha-1) were used with the same nitrogen dose (120 kg N ha^{-1}) which caused three different N:S ratios background (1:0.2, 1:0.5, 1:1). The objective of this study was to investigate the effect of two sulphur forms (sulphate and thiosulphate) in combination with three different N:S ratio (1:0.2, 1:0.5, 1:1) on the yield of spring wheat and total N-and S-content and uptake by the aboveground biomass on chernozem and sandy soil.

To establish the experiment, 10 kg of air-dry soil was measured in the experimental pots. The indicator plant was the Stanga variety of spring wheat. The moisture content of soils was set up to 60% of the water-holding capacity of the field. Pots were weighed daily and the missing water was supplemented with ion exchange water. There were 7 treatments with three replications and the treatment plan of the experiment is shown in *Table 3*.

Table 3. Rates of fertilisers applied in the pot experimen (2019)

	Sulphur forms	N/S treatments	N doses (kg/ha)	S doses (kg/ha)
1.	-	control	0	0
2.	SO_4^{2-}	1:0.2	120	24
3.	SO_4^{2-}	1:0.5	120	60
4.	SO_4^{2-}	1:1	120	120
5.	$S_2O_3^{2-}$	1:0.2	120	24
6.	$S_2O_3^{2-}$	1:0.5	120	60
7.	$S_2O_3^{2-}$	1:1	120	120

In the experiment, biomass during the growing season was determined. The total N, S content and N/S ratio of the plant was measured and the sulphate-S content of the plant tissue at flowering was determined. The KCl, $CaCl_2$ and KH_2PO_4 soluble sulphate-S content of soil was also measured.

2.3. Plant sampling, plant sample preparation

In the case of the long-term fertilization experiment and the pot experimints, during the growing season of wheat, plant samples were collected 3 times according to the BBCH scale: at stem elongation, flowering and ripening. For all three sampling dates, the entire above-ground plant part was sampled and at ripening, the straw was separated from the grain. The plant samples were processed and analysed in the laboratories of the Institute of Agricultural Chemistry and Soil Science. The plant samples were first air-dried under a roof in the open air to air-dryness and then dried in a MEMMERT UF1060 drying oven at 60 °C for weight stability. Subsequently, the samples were ground with a FRITSCH PULVERISETTE 14 grinder and stored in paper bags until analytical analysis.

2.4. Soil sampling, soil sample preparation

In the long-term fertilization experiment, soil samples were also collected at the same time as the plant sampling from 0-30 cm soil depth in 2017, 2018 and 2019. In 2018, soil samples were collected at 0-100 cm depth every 10 cm after harvest. In the case of the pot experiments, sampling was performed once after harvest. The soil samples were transported to the laboratory of the Institute of Agricultural Chemistry and Soil Science and air-dried. The soil was crushed and sieved through a 1 mm diameter CISA 018067.10 stainless steel sieve and stored in paper bags until analysis.

2.5. Description of the plant and soil analysis methods

Determination of the N and S content of wheat

The nitrogen and sulphur contents of the wheat samples were measured by Elementar Vario EL type CNS analyser.

Determination of the sulphate content (SO_4^{2-}) of the plant samples

The inorganic sulphur content of wheat was measured by single column ion chromatography (BALLÁNÉ, 2000).

Determination of the amino acid composition of wheat grain

The determination of the methionine and cysteine content of wheat grain was carried out by ion-exchange chromatography according to standard MSZ EN ISO 13903:2005.

Measurement of soluble sulphate forms in soil

Water-soluble inorganic sulphate was extracted with 1M KCl and 0.01M $CaCl_2$ solution. The soluble plus adsorbed sulphate was extracted with 0.016M KH_2PO_4 extractant. 10g soil was shaken with 25ml extractants in all cases. Extracts were filtered and sulphate was measured by turbidimetric method (SINGH et al., 2011).

Determination of aryl sulphatase activity of soil

Soil aryl sulphatase activity was determined photometrically using p-nitrophenyl sulphate substrate (TABATABAI and BREMNER, 1970).

2.6. Statistical analysis

For statistical analysis of experimental results, IBM SPSS Statistics 22 and Microsoft Excel 2016 programs were used. The mean values of each treatment group were subjected to comparisons analysis using the Two-way ANOVA in the case of long-term fertilization experiment and the One-Way ANOVA in the case of pot experiments with post hoc comparisons using Tukey test. Pearson's linear correlation and regression analysis were used to determine the relationship between the nutrient content of the soil and plant.

3. Results and discussion

3.1. Changes in some elements of sulphur turnover in the long-term NPK fertilization experiment

3.1.1. Simplified nitrogen and sulphur balance of the long-term fertilization experiment in the period of 1983-2016

The simplified N and S balances for plots with different fertilizer treatments for the period 1983-2016 are presented in *Table 4*.

Table 4. Simplified N and S balance for the period 1983-2016 (Debrecen-Látókép)

Treatments	**Applied dose of S fertilizer between 1983 and 2016 (kg/ha)**	**S uptake by wheat and maize between 1983 and 2016 (kg/ha)**	**Simplified S balance between 1983 and 2016 (kg/ha)**
control	0	619,7	**-619,7**
NPK1	1301,7	918,0	**383,7**
NPK2	2603,4	915,5	**1687,9**
Treatments	**Applied dose of N fertilizer between 1983 and 2016 (kg/ha)**	**N uptake by wheat and maize between 1983 and 2016 (kg/ha)**	**Simplified N balance between 1983 and 2016 (kg/ha)**
control	0	5248,8	**-5248,8**
NPK1	3740	7847,2	**-4107,2**
NPK2	7480	7873,6	**-393,6**

The cumulative nitrogen balance over the 34-year was negative in the control plots and both NPK1 and NPK2 treatments, leading to long-term exhaustion of soil reserve nutrients and soil fertility degradation. In contrast, the total sulphur balance was negative only in the control treatment and became positive in NPK1 and NPK2 due to the regular application of superphosphate in the period 1983-2009, despite the absence of sulphur addition in the studied area between 2010 and 2016.

3.1.2. Soil test results

Changes in the 1M KCl; 0.01M $CaCl_2$ and 0.016M KH_2PO_4 soluble sulphate-S content of soil at the ripening stage of winter wheat

Soil sampling was carried out 3 times in the experimental area, but in the thesis booklet, only the results of the 3rd soil sampling (ripening) are presented (*Table 5*).

In 2017 and 2019, the soluble sulphate-S content increased in fertilized plots for all extractants compared to the control treatment. This effect was presumably caused by irrigation-related changes in 2017, while in 2019 it was due to the re-application of sulphur in the form of superphosphate. In 2017, irrigation resulted significantly higher

KCl, $CaCl_2$ and KH_2PO_4 soluble sulphate-S content in irrigated plots. The highest sulphate-S content was measured in the case of the KH_2PO_4 extractant.

Table 5. 1M KCl, 0.01M $CaCl_2$ and KH_2PO_4 soluble sulphate content in topsoil at ripening stage of winter wheat (Debrecen-Látókép, 2017-2019)

Treatments	KCl-SO_4^{2-}-S (mg/kg)			$CaCl_2$-SO_4^{2-}-S (mg/kg)			KH_2PO_4-SO_4^{2-}-S (mg/kg)		
	I	NI	Mean	I	NI	Mean	I	NI	Mean
2017									
control	4,79±0,44	2,67±0,62	**3,73**^{a}	2,56±0,91	0,98±0,61	**1,77**^{a}	13,76±2,49	7,05±0,94	**10,41**^{a}
NPK1	4,88±0,44	3,82±0,76	**4,35**^{ab}	2,94±1,17	1,64±0,41	**2,29**^{ab}	16,02±1,35	12,63±0,18	**14,33**^{b}
NPK2	5,13±0,44	4,54±0,86	**4,84**^{b}	2,74±0,39	2,10±0,26	**2,42**^{b}	17,18±0,27	13,30±1,24	**15,24**^{b}
Mean	**4,93**^{B}	**3,68**^{A}	*4,31*	**2,75**^{B}	**1,57**^{A}	*2,16*	**15,65**^{B}	**10,99**^{A}	*14,66*
2018									
control	1,95±0,45	0,89±0,55	**1,42**^{a}	1,14±1,02	0,46±0,36	**0,80**^{a}	8,40±1,85	8,24±1,74	**8,32**^{a}
NPK1	1,63±0,64	0,53±0,31	**1,08**^{a}	0,57±0,31	0,34±0,19	**0,45**^{a}	8,89±1,48	10,26±1,66	**9,58**^{a}
NPK2	1,49±0,64	1,56±1,52	**1,53**^{a}	0,40±0,35	0,54±0,17	**0,47**^{a}	10,14±1,36	10,01±1,29	**10,08**^{a}
Mean	**1,69**^{A}	**0,99**^{A}	*1,34*	**0,70**^{A}	**0,45**^{A}	*0,58*	**9,14**^{A}	**9,50**^{A}	*9,32*
2019									
control	4,86±0,43	5,27±0,52	**5,07**^{a}	2,53±0,97	2,98±0,74	**2,75**^{a}	9,76±0,46	8,77±0,34	**9,27**^{a}
NPKS1	5,22±0,13	5,31±0,56	**5,27**^{a}	3,30±0,57	3,44±0,28	**3,37**^{a}	14,31±0,50	13,57±0,27	**13,94**^{b}
NPKS2	6,16±0,70	6,05±0,58	**6,11**^{b}	4,87±0,66	4,33±0,41	**4,60**^{b}	15,12±0,70	13,74±1,11	**14,43**^{b}
Mean	**5,41**^{A}	**5,54**^{A}	*5,48*	**3,56**^{A}	**3,58**^{A}	*3,57*	**13,06**^{B}	**12,03**^{A}	*12,55*

Notes: Different small letters (abc) indicate significant differences between the fertilisation treatments within a column; different capital letters (ABC) indicate significant differences between irrigation treatments within a line ($P < 0.05$), I = irrigated, NI = non-irrigated

A range of 6-13 mg/kg of sulphate-S in soil has been identified as the critical limit for optimal plant growth (BROOK, 1979; HUE et al. 1984; TIWARI et al., 1985; BORNMAN, 1990).

In 2017 and 2018, when there was no S supplementation in the field for several years, the KCl and $CaCl_2$ soluble sulphate-S content did not exceed this critical range even in the NPK2 treatment with a positive cumulative S balance. The KH_2PO_4 extract removes a higher amount of sulphate from the soil, as it is assumed to dissolve the adsorbed sulphate form so that the values measured in this extract reached or exceeded the critical limits in several cases. In 2019, when superphosphate fertilizer was again applied to supplement the P nutrient, the KCl and $CaCl_2$ soluble sulphate-S content slightly increased but remained below the critical range. The amount of KH_2PO_4 soluble sulphate-S content did not change significantly.

Changes of 1M KCl soluble sulphate-S content of soil at depths of 0-100 cm

In 2018, soil samples were collected after the harvest of winter wheat, in layers 0-100 cm at 10 cm intervals. Thus, the depth variation of sulphate fractions measured in 1M KCl and 0.016M KH_2PO_4 extractants was also investigated. Only the variation of KCl soluble sulphate- S values are illustrated in the thesis (*Figure 1*).

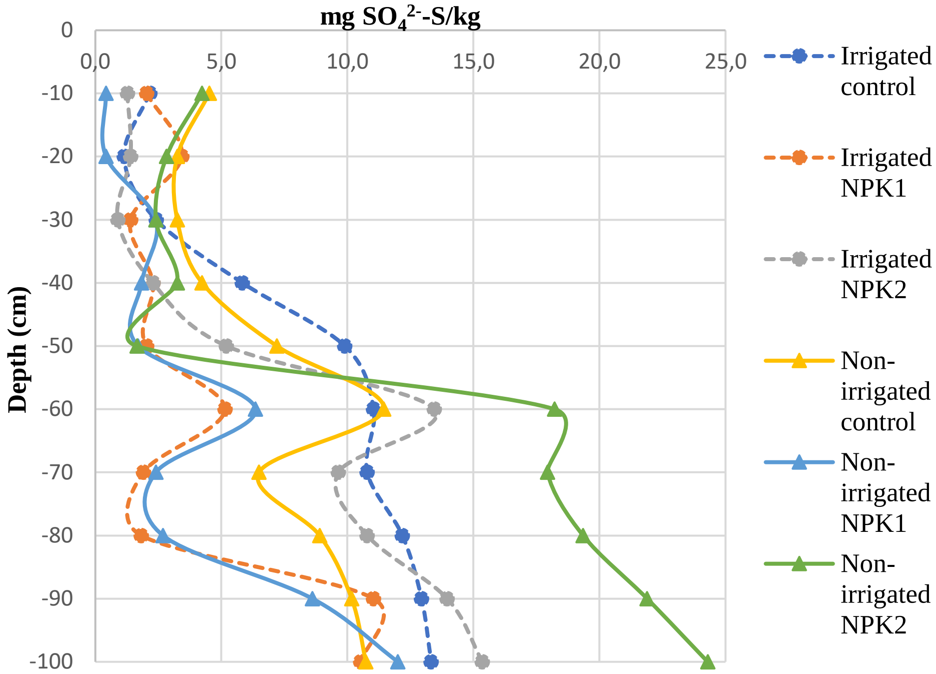


Figure 1. Vertical distribution of the 1M KCl soluble sulphate-S content (mg/kg) in the 0-100 cm soil layer of plots with different fertilizer treatments in 2018 (Debrecen-Látókép)

In summary, a sulphate accumulation zone was observed between 50 and 70 cm for KCl soluble sulphate-S content in the 0-100 cm soil profile. The annual dose of 42.4 kg S/ha applied in the NPK1 treatment from 1983 to 2009 was sufficient for the plant, so less sulphate leaching was observed in this treatment. In the NPK2 treatment, the sulphur applied at a higher dose (84.8 kg S/ha) could not be utilised by the plant and this surplus was leached out.

Relationship between 1M KCl, 0.01M $CaCl_2$ and 0.016M KH_2PO_4 soluble sulphate content

In the case of analysis of the relationship between the extractants, the measurement results of 2017-2018 and 2019 were evaluated separately. The results of regression analysis of the relationship between KCl and $CaCl_2$ soluble sulphate-S are shown in Figure 2, the results of the relationship between KH_2PO_4 and KCl soluble sulphate-S are shown in Figure 3, and the results of the relationship between KH_2PO_4 and $CaCl_2$ sulphate-S are shown in Figure 4.

The closest relationship was observed between the sulphate-S values dissolved in 1M KCl and 0.01M $CaCl_2$ extractants. These saline solutions extracted nearly similar amounts of sulphate from the soil and these dissolved sulphate-S tended to vary at similar rates during the period without S supplementation and during the period of $CaSO_4$ reapplication. The 0.016M KH_2PO_4 extractant measured the highest amount of sulphate fraction. It can be assumed that this extractant in addition to the water-soluble fraction, the sulphate fraction adsorbed on the surface of the colloids also removes and the latter fraction being less affected by the freshly applied $CaSO_4$.

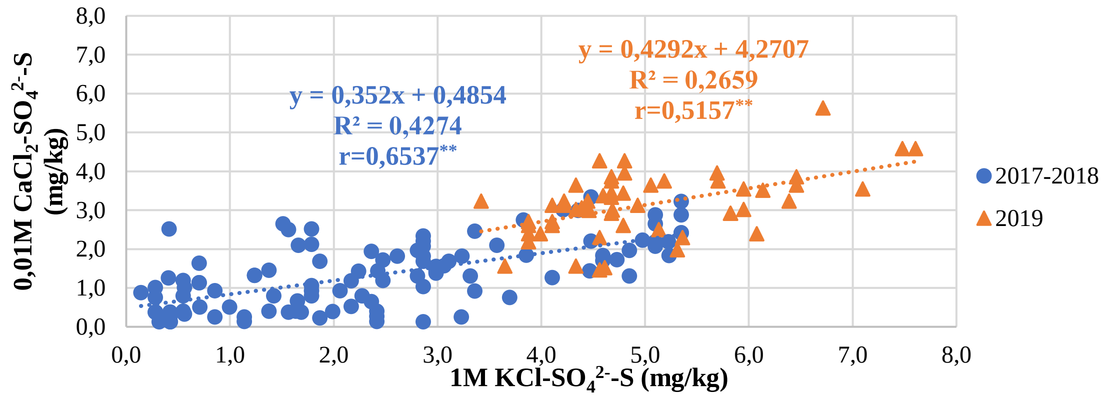


Figure 2. Changes in correlations between 1M KCl and 0.01M $CaCl_2$ soluble sulphate content of soil (2017-2018: n=288; 2019: n=144, ** significant correlation at P <0.01)

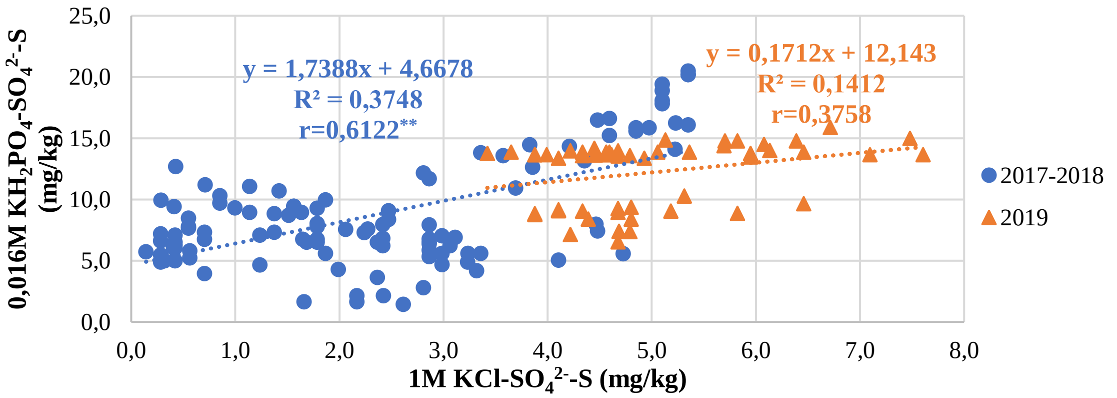


Figure 3. Changes in correlations between 1M KCl and 0.016M KH_2PO_4 soluble sulphate content of soil (2017-2018: n=288; 2019: n=144, ** significant correlation at P <0.01)

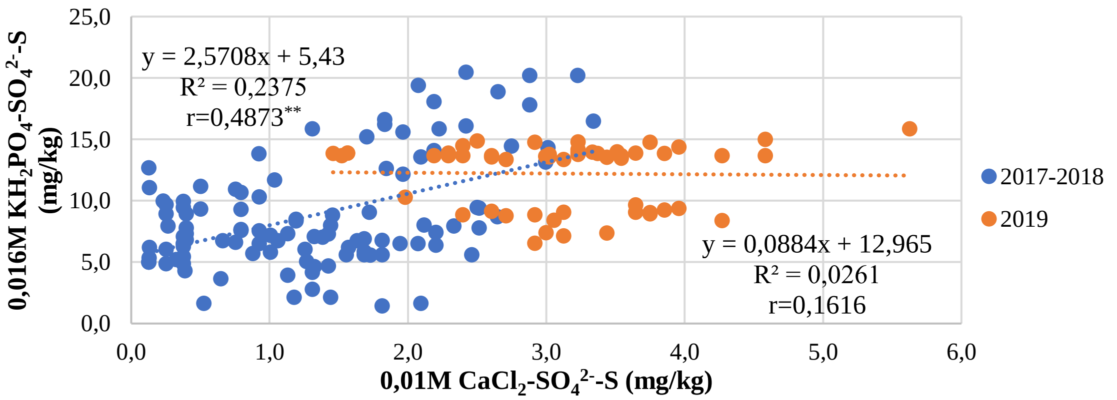


Figure 3. Changes in correlations between 0.01M $CaCl_2$ and 0.016M KH_2PO_4 soluble sulphate content of soil (2017-2018: n=288; 2019: n=144, ** significant correlation at P <0.01)

Soil aryl sulphatase activity

The changes in aryl sulphatase activity during the growing season in 2018 are shown in *Table 6* and 2019 in *Table 7*.

Table 6. Soil aryl sulphatase activity at the stage of stem elongation, flowering and ripening (Debrecen-Látókép, 2018)

Treatments	Stem elongation (µg p-nitrofenol/g/h)			Flowering (µg p-nitrofenol/g/h)			Ripening (µg p-nitrofenol/g/h)		
	I	NI	Mean	I	NI	Mean	I	NI	Mean
control	68,16±2,59	66,75±3,40	**67,45^{c}**	56,86±1,54	53,10±3,09	**54,98^{c}**	45,15±4,22	47,44±1,45	**46,30^{b}**
NPK1	62,59±2,83	61,36±1,42	**61,97^{b}**	53,43±3,19	47,06±1,55	**50,25^{b}**	52,87±2,67	43,32±1,86	**48,09^{b}**
NPK2	44,08±1,98	42,34±3,74	**43,21^{a}**	38,33±0,32	32,62±2,81	**35,47^{a}**	19,44±1,53	32,38±2,14	**25,91^{a}**
Mean	**58,27^{A}**	**56,82^{A}**	*57,55*	**49,54^{B}**	**44,26^{A}**	*46,90*	**39,15^{A}**	**41,04^{A}**	*40,10*

Notes: Different small letters (abc) indicate significant differences between the fertilisation treatments within a column; different capital letters (ABC) indicate significant differences between irrigation treatments within a line ($P < 0.05$), I = irrigated, NI = non-irrigated

Table 7. Soil aryl sulphatase activity at the stage of stem elongation, flowering and ripening (Debrecen-Látókép, 2019)

Treatments	Stem elongation (µg p-nitrofenol/g/h)			Flowering (µg p-nitrofenol/g/h)			Ripening (µg p-nitrofenol/g/h)		
	I	NI	Mean	I	NI	Mean	I	NI	Mean
control	46,74±0,99	45,75±2,15	**46,24^{c}**	13,01±0,68	13,29±0,88	**13,15^{b}**	9,84±0,37	7,92±0,84	**8,88^{ab}**
NPK1	43,16±3,67	41,52±1,62	**42,34^{b}**	12,56±2,64	12,46±0,86	**12,51^{b}**	10,15±0,59	8,47±0,87	**9,31^{b}**
NPK2	30,36±2,04	28,75±1,12	**29,56^{a}**	8,51±0,56	7,73±0,98	**8,12^{a}**	8,87±0,38	7,16±0,72	**8,02^{a}**
Mean	**40,09^{A}**	**38,67^{A}**	*39,38*	**11,36^{A}**	**11,16^{A}**	*11,26*	**9,62^{B}**	**7,85^{A}**	*8,73*

Notes: Different small letters (abc) indicate significant differences between the fertilisation treatments within a column; different capital letters (ABC) indicate significant differences between irrigation treatments within a line ($P < 0.05$), I = irrigated, NI = non-irrigated

In 2019, in the case of all sampling dates enzyme activity was lower than in 2018. It was also observed that the measured enzyme activity values decreased within one year as the growing season progressed. Lower enzyme activity was observed in the fertilized plots than in the control plot.

3.1.3. Plant test results

Changes of winter wheat yields in 2017-2019

The effect of fertilisation and irrigation on the evolution of winter wheat yield (t/ha) in the period of 2017-2019 is illustrated in *Table 8.*

Winter wheat yields ranged from 2.29-9.19 t/ha in 2017, 1.79-8.54 t/ha in 2018 and 2.92-8.76 t/ha in 2019. In all studied years, the lowest yield was obtained in the control treatment. The NPK1 treatment resulted a significant increase in yield and an additional and significant increase was observed with further increasing fertilizer dose. In 2017 the irrigation resulted significantly higher yields on the irrigated plots. In 2018 and 2019, there was no significant difference between the average yield of irrigated and non-irrigated treatments.

Table 8. The yield of winter wheat (t/ha) (Debrecen-Látókép, 2017-2019)

Treatments	**Yield (t/ha)**		
	Irrigated	**Non-irrigated**	**Mean**
2017			
control	2,38±0,56	2,29±0,28	**2,33**[a]
NPK1	6,52±0,20	6,31±0,56	**6,42**[b]
NPK2	9,19±0,15	8,09±0,36	**8,64**[c]
Mean	**6,03**[B]	**5,56**[A]	*5,80*
2018			
control	1,90±0,20	1,79±0,38	**1,85**[a]
NPK1	7,13±0,71	7,06±0,78	**7,09**[b]
NPK2	8,54±0,40	8,12±0,74	**8,33**[c]
Mean	**5,86**[A]	**5,65**[A]	*5,76*
2019			
control	2,97±0,35	2,92±0,25	**2,94**[a]
NPK1	7,47±0,76	7,38±0,26	**7,43**[b]
NPK2	8,52±0,37	8,76±0,68	**8,64**[c]
Mean	**6,32**[A]	**6,36**[A]	*6,34*

Notes: Different small letters (abc) indicate significant differences between the fertilisation treatments within a column; different capital letters (ABC) indicate significant differences between irrigation treatments within a line ($P < 0.05$).

The N-, S-content and N/S of winter wheat at the stage of ripening

The changes in the N and S content of the plant were monitored from stem elongation to ripening of wheat, but in the thesis booklet, the results measured in the wheat grain are only presented (*Table 9*).

According to RANDALL et al. (1981) and REUSSI et al. (2011), in addition to the total sulphur content of the wheat grain, the N/S ratio should also be taken into account to estimate sulphur supply. According to the authors, sulphur supply is insufficient if the S content of the grain is less than 0.12-0.15 % and the N/S ratio is greater than 13.3-17:1. Based on the literature values, in 2017 and 2018, wheat grown in the experimental area was considered sulphur deficient in all treatments, and this deficiency increased with increasing N doses. In 2019, the S content of wheat grown in fertilized plots exceeded critical values (0.12-0.15%) and the N/S ratio was also lower than the critical range of 13.3-17:1. It can be concluded that the sulphur supply of wheat was adequate when 42.4 and 84.8 kg S/ha were applied as gypsum with superphosphate.

A threshold for N content was also established, below which the wheat grain could be considered N deficient. GOOS et al. (1982) defined the critical N concentration in wheat grain as 2 %. Based on this value, N deficiency in the wheat stand was found in all years in the control and NPK1/NPKS1 treatments.

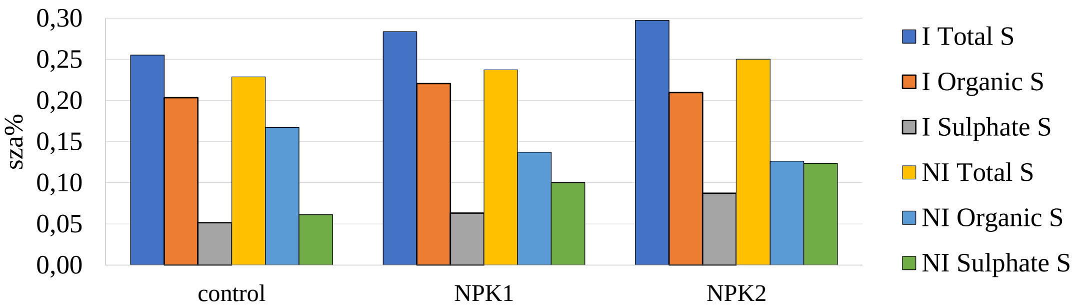
Table 9. Average N, S content and N/S at ripening stage of winter wheat grain from plots with different fertilizer treatments (Debrecen-Látókép, 2017-2019)

Treatment	N (%)			S (%)			N/S		
	I	NI	Mean	I	NI	Mean	I	NI	Mean
2017									
control	1,35±0,11	1,41±0,11	**1,38^a**	0,09±0,01	0,08±0,01	**0,09^a**	14,8±2,1	17,2±1,7	**16,0^a**
NPK1	1,86±0,16	1,72±0,23	**1,79^b**	0,09±0,01	0,09±0,01	**0,09^a**	21,5±4,9	19,4±4,0	**20,4^ab**
NPK2	2,28±0,07	2,04±0,17	**2,16^c**	0,10±0,01	0,10±0,01	**0,10^a**	22,0±3,5	21,1±2,5	**21,5^b**
Mean	**1,83^A**	**1,72^A**	*1,78*	**0,09^A**	**0,09^A**	*0,09*	**19,4^A**	**19,2^A**	*19,3*
2018									
control	1,43±0,11	1,47±0,15	**1,45^a**	0,10±0,01	0,10±0,01	**0,10^a**	13,9±1,9	15,5±2,4	**14,7^a**
NPK1	1,81±0,19	1,83±0,18	**1,82^b**	0,11±0,01	0,09±0,00	**0,10^a**	16,9±2,2	20,5±3,7	**18,7^b**
NPK2	2,47±0,13	2,31±0,11	**2,39^c**	0,11±0,01	0,11±0,00	**0,11^a**	22,8±2,8	21,8±2,9	**22,3^c**
Mean	**1,91^A**	**1,87^A**	*1,89*	**0,11^A**	**0,10^A**	*0,10*	**17,8^A**	**19,2^A**	*18,5*
2019									
control	1,51±0,15	1,53±0,04	**1,52^a**	0,14±0,01	0,15±0,00	**0,15^a**	10,1±1,1	10,2±2,2	**10,1^a**
NPKS1	1,64±0,06	2,10±0,10	**1,87^b**	0,17±0,00	0,20±0,03	**0,18^b**	9,6±0,5	10,6±1,7	**10,1^a**
NPKS2	2,33±0,10	2,63±0,06	**2,48^c**	0,20±0,02	0,23±0,02	**0,22^c**	11,7±2,0	11,4±1,6	**11,6^b**
Mean	**1,83^A**	**2,08^A**	*1,96*	**0,17^A**	**0,20^A**	*0,18*	**10,5^A**	**10,7^A**	*10,6*

Notes: Different small letters (abc) indicate significant differences between the fertilisation treatments within a column; different capital letters (ABC) indicate significant differences between irrigation treatments within a line ($P < 0.05$), I = irrigated, NI = non-irrigated

Changes of the organic and inorganic sulphur fractions of winter wheat

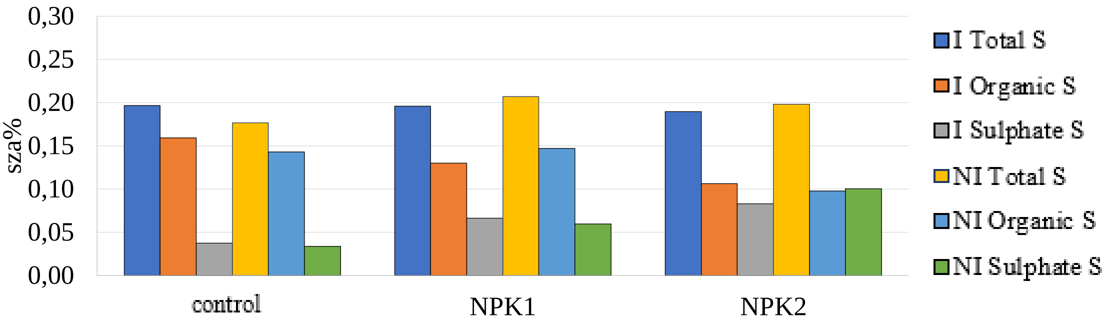
The changes of the organic and sulphate-S fractions of winter wheat are presented only at the stage of stem elongation (*Figures 5, 6, 7*).



	Összes S (%)			Szerves-S (%)			SO_4^{2-}-S (%)		
	Ö	NÖ	Átlag	Ö	NÖ	Átlag	Ö	NÖ	Átlag
kontroll	0,25±0,03	0,23±0,03	0,24^a	0,20±0,03	0,17±0,03	0,19^a	0,05±0,00	0,06±0,00	0,06^a
NPK1	0,28±0,05	0,24±0,03	0,26^a	0,22±0,05	0,14±0,03	0,18^a	0,06±0,00	0,10±0,01	0,08^b
NPK2	0,30±0,03	0,25±0,01	0,27^a	0,21±0,03	0,13±0,01	0,17^a	0,09±0,00	0,12±0,00	0,11^c
Átlag	0,28^B	0,24^A	0,26	0,21^B	0,14^A	0,18	0,07^A	0,09^B	0,08

Figure 5. Effect of different fertilization rates and irrigation on the total, organic and sulphate-S contents of winter wheat at stem elongation (Debrecen-Látókép, 2017)

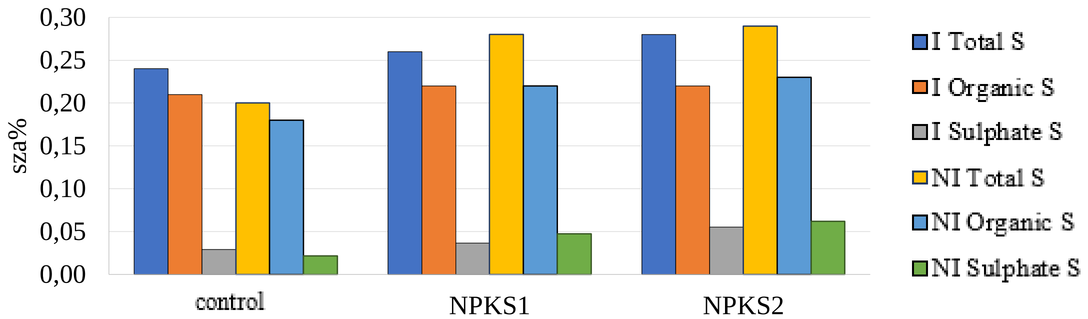
Notes: Different small letters (abc) indicate significant differences between the fertilisation treatments within a column; different capital letters (ABC) indicate significant differences between irrigation treatments within a line ($P < 0.05$), I = irrigated, NI = non-irrigated



	Összes S (%)			Szerves-S (%)			SO_4^{2-}-S (%)		
	Ö	NÖ	Átlag	Ö	NÖ	Átlag	Ö	NÖ	Átlag
kontroll	0,20±0,02	0,17±0,02	0,19^{a}	0,16±0,02	0,14±0,02	0,15^{b}	0,04±0,01	0,03±0,00	0,04^{a}
NPK1	0,20±0,03	0,21±0,03	0,20^{a}	0,13±0,03	0,15±0,03	0,14^{b}	0,07±0,01	0,06±0,01	0,06^{b}
NPK2	0,19±0,02	0,20±0,01	0,19^{a}	0,11±0,02	0,10±0,00	0,11^{a}	0,08±0,00	0,10±0,01	0,09^{c}
Átlag	0,19^{A}	0,19^{A}	0,19	0,13^{A}	0,13^{A}	0,13	0,06^{A}	0,06^{A}	0,06

Figure 6. Effect of different fertilization rates and irrigation on the total, organic and sulphate-S contents of winter wheat at stem elongation (Debrecen-Látókép, 2018)

Notes: Different small letters (abc) indicate significant differences between the fertilisation treatments within a column; different capital letters (ABC) indicate significant differences between irrigation treatments within a line ($P < 0.05$), I = irrigated, NI = non-irrigated



	Összes S (%)			Szerves-S (%)			SO_4^{2-}-S (%)		
	Ö	NÖ	Átlag	Ö	NÖ	Átlag	Ö	NÖ	Átlag
kontroll	0,24±0,04	0,20±0,01	0,22^{a}	0,21±0,03	0,18±0,01	0,20^{a}	0,03±0,00	0,02±0,00	0,03^{a}
NPKS1	0,26±0,02	0,27±0,03	0,27^{b}	0,22±0,02	0,22±0,03	0,22^{a}	0,04±0,00	0,05±0,01	0,04^{b}
NPKS2	0,28±0,03	0,29±0,07	0,28^{b}	0,22±0,01	0,23±0,06	0,23^{a}	0,06±0,01	0,06±0,01	0,06^{c}
Átlag	0,26^{A}	0,26^{A}	0,26	0,22^{A}	0,21^{A}	0,22	0,04^{A}	0,04^{A}	0,04

Figure 7. Effect of different fertilization rates and irrigation on the total, organic and sulphate-S contents of winter wheat at stem elongation (Debrecen-Látókép, 2019)

Notes: Different small letters (abc) indicate significant differences between the fertilisation treatments within a column; different capital letters (ABC) indicate significant differences between irrigation treatments within a line ($P < 0.05$), I = irrigated, NI = non-irrigated

In the years without sulphur fertilisation (2017-2018), the total S content of wheat at the stage of stem elongation did not differ between fertilised and control plots. The organic S content in 2017-2018 in the fertilized plots decreased slightly compared to the control and in these years the sulphate S content of plant tissues reached 50%.

In 2019, the year of sulphur replenishment, the total average S content of winter wheat biomass increased significantly due to fertilization compared to the control. In this year, the organic S content of the fertilized plots did not differ from the control, but sulphate S increased significantly with increasing doses, but the maximum was only 21%, so a significant part of the S uptake was incorporated into organic compounds.

3.1.4. Utilisation of superphosphate $CaSO_4$-S content in the year of application

The utilisation of the sulphur content of superphosphate ($CaSO_4$) in the year of application was analysed (*Table 10*).

Our results indicate that approximately one-third of the sulphur content of superphosphate was utilised by winter wheat in the year of application.

Table 10. Utilisation of gypsum in superphosphate fertiliser

Treatment	S dose kg/ha		S uptake by plant kg/ha		Utilization %	
	Irrigaton	Non-irrigation	Irrigaton	Non-irrigation	Irrigaton	Non-irrigation
control	0	0	8,8	9,1	-	-
NPKS1	42,4	42,4	20,7	27,3	28,1	42,9
NPKS2	84,8	84,8	37,1	40,3	33,4	36,8

3.2. Changes of some elements of sulphur turnover in pot experiments

3.2.1. Effect of drought stress and increasing sulphur fertilizer doses on productivity, S-, N-content and N/S of spring wheat

The effect of two different water supplies condition and fertilizer treatments with increasing N and S ratios (N/S= 1:0.2; 1:0.25; 1:0.5) on the yield and yield parameters of spring wheat were investigated in the greenhouse pot experiment.

Changes of biomass of spring wheat at different phenophases

The effect of treatments on the changes of biomass is shown in *Table 11*.

Table 11. Changes of spring wheat biomass at different development stages

Water supply	N/S treatment	Stem elongation	Flowering	Ripening		
				Straw	Grain	HI
		g/3 plant	g/3 plant	g/pot	g/pot	
Adequate	control	4,17±0,12a	17,10±0,89ab	20,30±0,82b	8,20±0,29ab	0,288bc
	N/S =1:0,2	4,83±0,06ab	21,00±1,93c	25,43±1,64d	14,76±0,65c	0,368c
	N/S =1:0,25	5,00±0,35b	19,23±1,72bc	23,83±1,80cd	11,25±3,05bc	0,314bc
	N/S =1:0,5	5,03±0,25b	20,43±1,00c	26,37±1,36d	15,84±2,16d	0,374c
Reduced	control	4,50±0,36ab	15,50±0,20a	16,73±0,40a	4,80±0,96a	0,222ab
	N/S =1:0,2	4,50±0,26ab	15,83±0,72a	20,87±0,90bc	4,37±1,19a	0,171a
	N/S =1:0,25	4,67±0,40ab	15,63±0,60a	21,40±b1,21c	3,88±1,36a	0,151a
	N/S =1:0,5	4,77±0,21ab	15,83±0,40a	20,43±0,51b	4,69±1,11a	0,186a

Note: Data marked with the same letter in the columns is not significantly different at the significant level of $p < 0.05$. HI=Harvest index

The unfavourable effect of the water stress on the yield was first observed at the stage of flowering. Water stress had an adverse effect mainly on grain yield supported by the fact that the harvest index also decreased under water stress conditions. Under adequate water supply, significantly higher yield was achieved already with the smallest sulphur dose, but under water stress conditions the beneficial effect of fertilization was not shown. In our experiment, the spring wheat fertilization with 112 kg N/ha and 56 kg S/ha (N/S=1:0.5) was the most effective.

The N- and S-content and N/S ratio of spring wheat during the growing season

Table 12. shows the nitrogen-, sulphur-concentration and N/S ratio of spring wheat at the different development stages.

Table 12. The total N, total S and N/S ratio of spring wheat during the growing season

Water supply	N/S treatment	Stem elongation	Flowering	Ripening	
				Straw	Grain
N %					
Adequate	control	3,19±0,24a	1,11±0,11a	0,34±0,03a	2,05±0,05a
	N/S =1:0,2	4,84±0,11b	1,25±0,03a	0,36±0,08a	1,95±0,07a
	N/S =1:0,25	4,62±0,33b	1,37±0,12ab	0,42±0,16a	2,05±0,16a
	N/S =1:0,5	4,62±0,39b	1,25±0,05a	0,32±0,06a	1,80±0,13a
Reduced	control	3,35±0,17a	1,67±0,08b	0,54±0,12a	2,94±0,43b
	N/S =1:0,2	4,94±0,09b	2,59±0,30c	0,96±0,06b	4,21±0,29c
	N/S =1:0,25	4,81±0,15b	2,63±0,10c	0,94±0,06b	4,45±0,33c
	N/S =1:0,5	4,69±0,19b	2,56±0,08c	0,83±0,02b	3,91±0,25c
S %					
Adequate	control	0,28±0,06a	0,21±0,03abc	0,29±0,04a	0,20±0,01cd
	N/S =1:0,2	0,33±0,03a	0,17±0,00a	0,25±0,02a	0,15±0,01a
	N/S =1:0,25	0,31±0,01a	0,17±0,00a	0,27±0,03a	0,17±0,01ab
	N/S =1:0,5	0,32±0,03a	0,19±0,01ab	0,28±0,02a	0,18±0,01abc
Reduced	control	0,31±0,01a	0,18±0,01a	0,24±0,03a	0,15±0,01a
	N/S =1:0,2	0,28±0,02a	0,25±0,02c	0,31±0,02a	0,24±0,02d
	N/S =1:0,25	0,27±0,01a	0,24±0,03bc	0,32±0,06a	0,23±0,01d
	N/S =1:0,5	0,29±0,01a	0,21±0,01ab	0,30±0,03a	0,19±0,01bcd
N/S					
Adequate	control	11,68±1,97ab	5,18±0,72a	1,17±0,20a	10,08±0,45a
	N/S =1:0,2	14,81±1,52bc	7,32±0,08b	1,39±0,18ab	12,80±0,30a
	N/S =1:0,25	14,87±1,41bc	8,06±0,58bc	1,54±0,40ab	12,38±0,41a
	N/S =1:0,5	14,58±1,53bc	6,61±0,42ab	1,13±0,23a	10,20±1,15a
Reduced	control	10,99±0,13a	9,38±0,51cd	2,27±0,53bc	19,34±2,75b
	N/S =1:0,2	17,58±1,01c	10,75±0,35de	3,11±0,05c	19,49±1,15b
	N/S =1:0,25	17,54±0,05c	11,09±1,13de	2,96±0,49c	19,52±0,14b
	N/S =1:0,5	16,33±0,35c	12,04±0,76e	2,74±0,34c	20,14±0,94b

Note: Data marked with the same letter in the columns is not significantly different at the significant level of $p < 0.05$.

The fertilization also modified the N and S content and N: S ratio of wheat, and the extent of the effects depended significantly on the water supply.

At the stage of stem elongation, the effect of fertilization was similar under both forms of water supply. The smallest sulphur dose (22.4 kg ha^{-1}) increased the N%, but the increasing sulphur doses did not modify further the values. The sulphur content and N/S ratio of the plant were not affected by fertilization.

At the flowering stage, fertilization did not affect the N % and S % of spring wheat under an adequate water supply. However, under water stress conditions, an increase in N and S content was observed as a result of the lowest sulphur dose compared to the control treatment and the N content did not change further with increasing sulphur doses but the S content decreased with increasing sulphur doses.

At this development stage, a significant portion (74.7-98.0% in all treatments) of the total sulphur content was present in the inorganic sulphate form. The sulphur fertilizer only under water stress conditions influenced the sulphate-S, higher value was observed with increasing sulphur doses. The organic sulphur did not change significantly as a result of the studied treatments (*Figure 8*).

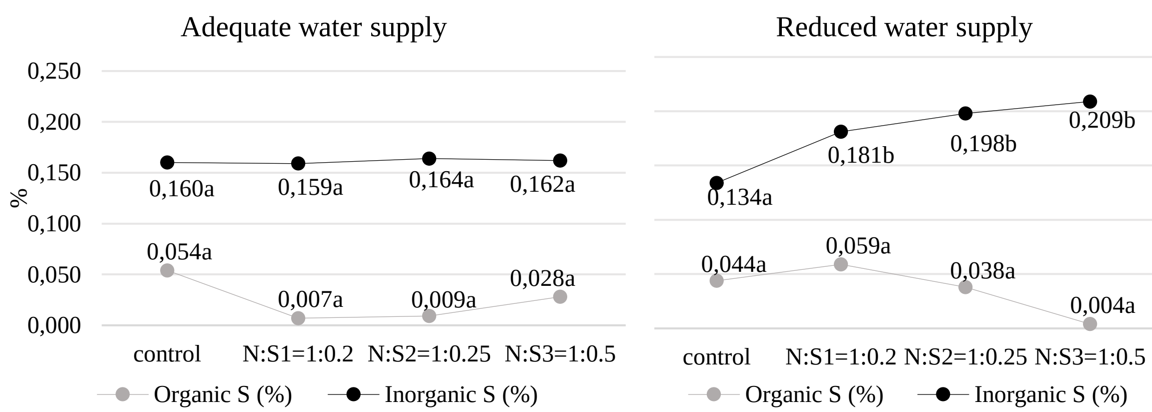


Figure 8. The changes of organic and inorganic sulphur content of spring wheat at the stage of flowering

Data marked with the same letter is not significantly different at the significant level of $p < 0.05$.

The N-content of grain under adequate water supply did not deviate from the control in the fertilization treatment, but the S % became lower. As a result of the increasing sulphur doses, the N %, S% and N/S ratio did not change significantly. Under water stress conditions, the smallest sulphur dose increased the N- and S content, but the increasing sulphur doses no longer caused a significant change in the studied parameters.

3.2.2. Effect of different sources and doses of sulphur on yield, nutrient content and uptake by spring wheat

In the experiment, the effects of two sulphur forms were compared: the sulphate (SO_4^{2-}) and the thiosulphate ($S_2O_3^{2-}$). Increasing doses of both sulphur forms (24, 60, 120 kg S/ha) were used with the same N dose (120 kg N/ha) which caused three different N:S ratios background (1:0.2, 1:0.5, 1:1). The greenhouse pot experiment was conducted on two different soils: calcareous chernozem soil of Debrecen-Látókép and sandy soil of Pallag.

The dry biomass of spring wheat at different development stages

On chernozem, there was no significant effect of different sulphur forms and different N:S ratios on dry biomass (*Table 13*) at the stage of stem elongation and flowering. Straw and grain weights were slightly higher in the sulphate treatments, but the difference was not statistically significant.

Table 13. The changes of dry biomass at different development stages of spring wheat

S forms	N/S treatment	Stem elongation g/2 plants	Flowering g /2 plants	Ripening Straw (g/pot)	Ripening Grain (g/pot)
Chernozem					
-	**control**	0,24±0,00a	2,57±0,55a	5,87±0,38a	6,31±0,77a
SO_4^{2-}	**N/S=1:0,2**	0,30±0,06a	4,03±0,86a	9,86±1,26b	12,04±1,23b
	N/S=1:0,5	0,25±0,01a	3,63±1,15a	10,82±1,78b	12,13±1,26b
	N/S=1:1	0,26±0,02a	2,47±0,64a	9,81±1,60b	10,53±1,25ab
$S_2O_3^{2-}$	**N/S=1:0,2**	0,34±0,08a	3,03±0,21a	8,81±0,63ab	10,03±1,70ab
	N/S=1:0,5	0,29±0,06a	3,87±0,47a	9,63±0,80b	10,53±1,61ab
	N/S=1:1	0,28±0,07a	3,53±1,24a	8,92±0,87ab	10,01±0,81ab
Sandy soil					
-	**control**	0,17±0,02a	1,27±0,68a	3,16±0,04a	2,53±0,11a
SO_4^{2-}	**N/S=1:0,2**	0,54±0,12c	2,73±0,67b	7,70±1,10b	6,10±0,94b
	N/S=1:0,5	0,51±0,10bc	3,00±0,61b	8,04±1,16b	6,24±1,91b
	N/S=1:1	0,40±0,16abc	2,87±0,50b	6,76±0,17b	5,37±1,38ab
$S_2O_3^{2-}$	**N/S=1:0,2**	0,35±0,01abc	3,30±0,46b	7,36±0,55b	6,45±0,88b
	N/S=1:0,5	0,28±0,01ab	2,80±0,35b	7,23±0,79b	6,62±0,68b
	N/S=1:1	0,22±0,02a	2,70±0,61b	8,54±0,72b	6,31±0,64b

Note: Data marked with the same letter in the columns is not significantly different at the significant level of p <0.05.

On sandy soils, the N/S=1:0.2 treatment significantly increased the dry biomass already at the initial development stage of wheat. Further increasing S doses did not change the biomass. Typically, slightly higher biomass production was measured when the sulphate form was applied. I observed a significant increase in biomass at flowering, as well as in wheat grain and wheat straw, compared to the control, already with the

N/S=1:0.2 treatment, but further increasing S doses did not modify the weight further for either S form.

The changes of total N, total S content and N/S ratio of spring wheat at different development stages

The evolution of N-, S-content and N/S ratio of wheat in response to different sulphur forms and treatments with different N/S ratios are shown in *Table 14*.

Table 14. Total N, total S content, and N/S ratio of spring wheat at different development stage

S forms	N/S treatment	Stem elongation			Flowering			Wheat grain		
		N %	S %	N/S	N %	S %	N/S	N %	S %	N/S
Chernozem										
-	control	4,21± 0,38b	0,54± 0,06d	7,79± 1,47a	1,70± 0,00a	0,37± 0,01cd	4,55± 0,16a	1,82± 0,05ab	0,15± 0,02a	11,94± 1,09a
SO_4^{2-}	N/S=1:0,2	4,05± 0,07ab	0,45± 0,02bc	9,03± 0,13ab	2,17± 0,19b	0,30± 0,02bc	7,59± 0,21b	1,73± 0,10a	0,16± 0,03a	10,96± 2,66a
	N/S=1:0,5	4,33± 0,10b	0,49± 0,02cd	8,94± 0,58ab	2,26± 0,09b	0,44± 0,07de	5,39± 0,60a	1,89± 0,01b	0,16± 0,02a	11,46± 1,18a
	N/S=1:1	4,40± 0,03b	0,49± 0,01cd	8,89± 0,30ab	2,22± 0,00b	0,46± 0,01e	4,90± 0,19a	2,02± 0,02c	0,17± 0,02a	12,20± 1,30a
$S_2O_3^{2-}$	N/S=1:0,2	3,61± 0,05a	0,36± 0,01a	10,13± 0,38b	2,30± 0,05b	0,20± 0,01a	11,61± 0,53c	1,92± 0,00bc	0,17± 0,01a	11,47± 0,39a
	N/S=1:0,5	3,95± 0,02ab	0,38± 0,00ab	10,52± 0,15b	2,37± 0,14b	0,22± 0,01ab	10,84± 0,25c	1,92± 0,00bc	0,18± 0,01a	10,75± 0,34a
	N/S=1:1	4,01± 0,24ab	0,39± 0,01ab	10,14± 0,14b	2,40± 0,01b	0,30± 0,00bc	8,25± 0,24b	1,94± 0,05bc	0,18± 0,01a	11,12± 0,47a
Sandy soil										
-	control	2,14± 0,04a	0,48± 0,00d	4,45± 0,05a	1,21± 0,11a	0,21± 0,01ab	5,74± 0,38ab	2,23± 0,14a	0,20± 0,01ab	10,76± 0,44a
SO_4^{2-}	N/S=1:0,2	3,43± 0,01c	0,36± 0,00a	9,52± 0,19b	1,89± 0,14c	0,25± 0,01b	7,87± 0,24bc	2,28± 0,21ab	0,22± 0,00abc	10,20± 1,14a
	N/S=1:0,5	4,08± 0,06d	0,44± 0,02c	9,21± 0,51b	1,68± 0,04bc	0,36± 0,01c	4,74± 0,02a	2,71± 0,17c	0,24± 0,00d	11,15± 0,49a
	N/S=1:1	4,22± 0,02e	0,45± 0,01cd	9,31± 0,08b	1,64± 0,16bc	0,40± 0,02c	4,06± 0,12a	2,65± 0,16bc	0,23± 0,01cd	11,53± 0,37a
$S_2O_3^{2-}$	N/S=1:0,2	4,02± 0,01cd	0,34± 0,01a	11,61± 0,18c	1,57± 0,10b	0,18± 0,00a	8,81± 0,37cd	2,13± 0,14a	0,19± 0,02a	10,60± 0,60a
	N/S=1:0,5	3,99± 0,00c	0,37± 0,03a	10,92± 0,04c	1,61± 0,05bc	0,19± 0,03a	10,07± 1,67d	2,21± 0,08a	0,21± 0,00abc	10,34± 0,18a
	N/S=1:1	4,00± 0,01cd	0,39± 0,04b	10,22± 0,15c	1,63± 0,10bc	0,22± 0,03ab	7,71± 1,03bc	2,46± 0,07abc	0,23± 0,01cd	10,71± 0,64a

Note: Data marked with the same letter in the columns is not significantly different at the significant level of $p < 0.05$.

In general, on chernozem soil, the increasing doses of sulphur with the same N dose increased the N-content of spring wheat at all development stages and in the wheat grain. The treatment with different sulphur sources did not cause further changes in the N-content.

At flowering, in the sulphate form treatment with the lowest dose, most of the S taken up was incorporated into the organic compounds. With further increasing sulphur doses, the incorporation of S into organic compounds increased, but in the treatment with the highest S dose, sulphate accumulation was observed. This phenomenon was also observed in the thiosulphate treatments, but to a lesser extent compared to the sulphate treatments (*Figure 9*).

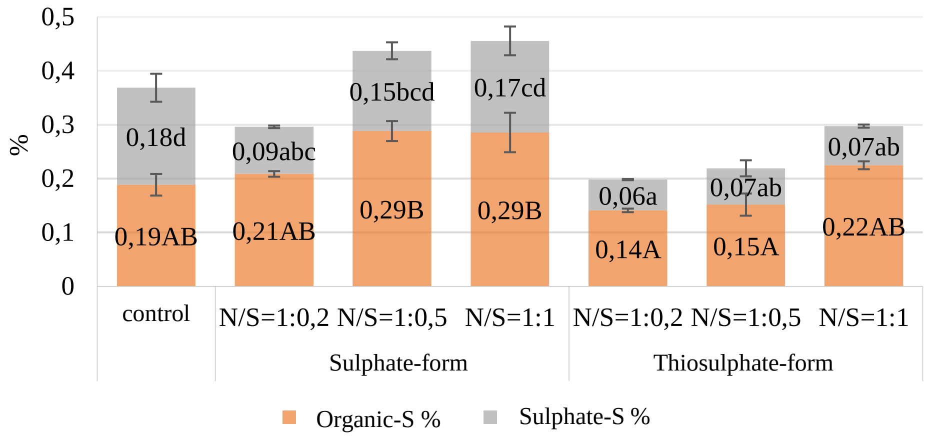


Figure 9. Changes in the organic-S and sulphate-S forms of spring wheat at flowering stage in chernozem soil

Note: Lower case refers to the change in the sulphate-S fraction and upper case refers to the change in the organic-S fraction. Values marked with the same lower case letter (abc) and the same upper case letter (ABC) are not significantly different at the p <0.05 significance level.

Treatment effects were more significant on sandy soils with poorer nutrient supply. The improved sulphur supply also increased the sulphur content of the plants at stage of stem elongation, flowering and in the wheat grain, which effect was more pronounced when the sulphate form was applied. The improved sulphur supply in the sulphate form also increased the N content at stem elongation stage, whereas the N content at flowering tended to decrease. The improved sulphur supply also increased the N content of the wheat grain, but only when the sulphate form was applied.

At flowering, the lowest dose of S in the form of sulphate enhanced the incorporation of uptaken S into organic compounds compared to the control, but further increasing doses of S resulted more intense sulphate accumulation in plant tissues. The lowest dose of thiosulphate treatment also showed a favourable change in the ratio of organic to sulphate S compared to the control, which means that the uptake of S was incorporated mainly into organic compounds. However, the improved sulphur supply did not change the ratio of organic-S to sulphate-S (*Figure 10*).

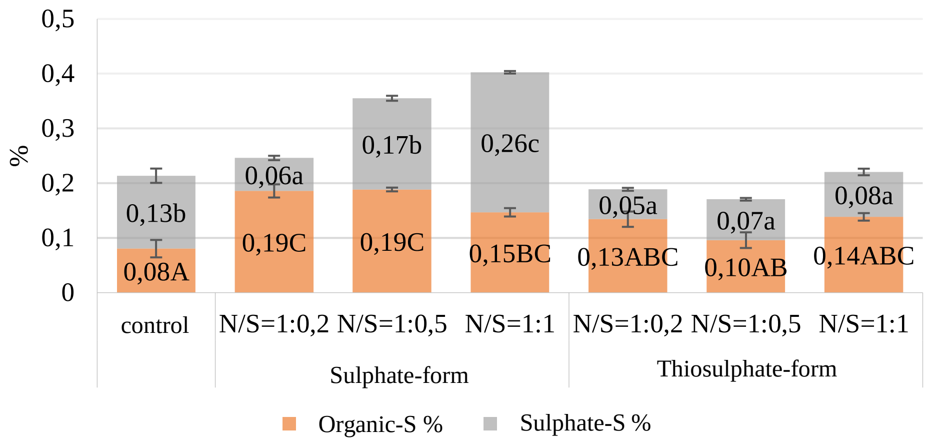


Figure 10. Changes in the organic-S and sulphate-S forms of spring wheat at flowering stage in sandy soil

Note: Lower case refers to the change in the sulphate-S fraction and upper case refers to the change in the organic-S fraction. Values marked with the same lower case letter (abc) and the same upper case letter (ABC) are not significantly different at the $p < 0.05$ significance level.

4. New scientific results

1. In the long-term fertilization experiment, winter wheat grown under $N_{200}P_{140}K_{160}$ treatment with positive cumulative S balance (+1688 kg S/ha) from 1983 to 2016 was S-deficient in 2017 according to the thresholds in the literature (S content: 0.12-0.15%; N/S: 13-17:1). S deficiency was confirmed for wheat grain with 0.10% S content and 21.5 N/S.
2. The results support that the S deficiency of winter wheat is further increased by N fertilization. In the $N_{100}P_{70}K_{80}$ treatment, the S content of wheat grain was 0.09% and the N/S was 20.4, whereas, in the $N_{200}P_{140}K_{160}$ treatment, the S content did not change significantly (0.10%), but the N/S became higher (21.5).
3. The results showed that the S content of superphosphate applied at the rate of 823.5 kg/ha (84.8 kg/ha) was utilized by winter wheat in about 35% in the year of application. At that time, the S content of the grain increased by almost 50% (0.22%) compared to the control (0.15%).
4. It was found that the amount of sulphate-S extracted by 1M KCl; 0.01M $CaCl_2$ and 0.016M KH_2PO_4 in the plots of the long-term fertilization experiment showed the following order: $CaCl_2$ <KCl <KH_2PO_4. The 1M KCl and 0.01M $CaCl_2$ extracted nearly similar amount of sulphate from the soil (KCl: 0.33-6.16 mg/kg, $CaCl_2$: 0.29-4.87 mg/kg). The amount of sulphate extracted by 0.016M KH_2PO_4 was higher (4.33-17.18 mg/kg), indicating a higher presence of adsorbed sulphate in the calcareous chernozem soil.
5. The results of Pearson correlation analysis proved that the S uptake of winter wheat grown on chernozem soil showed the strongest relationship with 0.016M KH_2PO_4-SO_4^{2-}-S (r=0,734**).
6. In the soil of the $N_{200}P_{140}K_{160}$ treatment of the long-term fertilization experiment, a significantly increased KCl-SO_4^{2-}-S content was observed at a depth of 50-70 cm as a consequence of the regular application of 823.5 kg/ha superphosphate during the period 1983-2009. At that time, KCl-SO_4^{2-}-S measured in the 0-10 cm soil layer increased from 1.27-4.24 mg/kg to 13.45-18.22 mg/kg at 50-70 cm depth and was higher in non-irrigated plots.
7. In a pot experiment with spring wheat on medium N- and poor S-supplied chernozem soil, it was proved that the most favourable ratio of N and S application was 1:0.5 (112 kg N/ha and 56 kg S/ha).

5. Practical usability of the results

1. On chernozem soil with poor S supply, the utilization of S content of superphosphate applied at a rate of 411.8 kg/ha by winter wheat varied between 28.1 and 42.9%, while the utilization of 823.5 kg/ha changed from 33.4 to 36.8% in the year of application. The application of superphosphate at a rate of 411.8 kg/ha is sufficient to achieve adequate sulphur supply of winter wheat.

2. The results of Pearson correlation analysis showed that the S uptake of winter wheat grown on chernozem soil showed the strongest relationship with 0.016M KH_2PO_4-SO_4^{2-}-S (r=0,734**), followed closely by the relationship between KCl-SO_4^{2-}-S and plant sulphur uptake (r=0.643**). Both extractants are suitable for the measurement of the plant-available sulphur content of calcareous chernozem soils.

3. In a pot experiment with spring wheat on medium N- and poor S-supplied chernozem soil, it was proved that the most favourable ratio of N and S application was 1:0.5 (112 kg N/ha and 56 kg S/ha).

4. The sulphur supplying capacity of sulphate-S and thiosulphate-S fertilizers on humic sandy soils and calcareous chernozem soils can differ significantly. The sulphur supply capacity of thiosulphate is less intense in sandy soil.

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7. Publication list

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Doctoral School: Kálmán Kerpely Doctoral School
MTMT ID: 10064266

List of publications related to the dissertation

Hungarian book chapters (1)

1. Balláné Kovács, A., Béni, Á., **Juhász, E. K.**: Eltérő arányú nitrogén- és kéntrágyázás, valamint az eltérő vízellátás hatása a tavaszi búza termésére és tápelemtartalmára.
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