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Evaluation of the Effects of Drought Stress and Nitrogen-Sulfur Fertilization on Productivity and Yield Parameters of Spring Wheat

Evelin Kármén Juhász ^{*}, Rita Kremper, Magdolna Tállai , Áron Béni , Tibor Novák 
and Andrea Balla Kovács 

Institute of Agricultural Chemistry and Soil Science, Faculty of Agricultural, Food Sciences and Environmental Management, University of Debrecen, 4032 Debrecen, Hungary; kremper@agr.unideb.hu (R.K.); tallaim@agr.unideb.hu (M.T.); beniaron@agr.unideb.hu (Á.B.); novak.tibor@science.unideb.hu (T.N.); kovacs@agr.unideb.hu (A.B.K.)

* Correspondence: juhasz.evelin@agr.unideb.hu

Abstract: The combined effects of increasing sulfur (S) fertilization rates and drought stress on the yield and compositional parameters of spring wheat on Chernozem soil were studied. In a greenhouse pot experiment, increasing S doses (22.4, 28, 56 kg S/ha) were used with a constant nitrogen (N) dose (112 kg N/ha), resulting in different N:S ratios (1:0.2; 1:0.25; 1:0.5). Water supply treatments included optimal irrigation, maintaining 60% of field capacity, and a water stress treatment where irrigation was withheld until wilting symptoms appeared, followed by irrigation to 40% of field capacity. By measuring the dry biomass production; plant N and S%; and inorganic sulfate-S content, the N/S ratio; harvest index (HI); and organic S, N and S uptake were determined. Our findings indicate that, under water stress, S incorporation into plants is limited, as it tends to remain in an inorganic form. Furthermore, results showed an increase in the N/S ratio under drought conditions, suggesting that drought stress impedes S uptake more significantly than N uptake. In this experiment, fertilization with 112 kg N/ha and 56 kg S/ha (N:S = 1:0.5) proved to be most effective under adequate water supply. In this treatment, grain N and S% were 1.80% and 0.18%, respectively.

Keywords: water stress; N-S fertilization; S metabolism; spring wheat



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1. Introduction

Wheat is one of the most important staple crops in the world, providing a primary food source for billions of people. It is valued for its high nutritional content, including essential carbohydrates, proteins, vitamins, and minerals, which contribute to food security and human health globally. Wheat's adaptability to diverse climates, ease of storage, and versatility in products (such as bread, pasta) make it a vital crop in agriculture and trade. With growing global demand and environmental challenges, improving wheat yields and nutrient efficiency has become crucial for sustainable food production and resilience in the face of climate change [1–3].

Sulfur (S) and nitrogen (N) are significant nutrients for plant growth and development, their assimilation in plants is similar, and both are essential components in the structure of the plant proteins [4,5].

Nitrogen plays an essential role in plant productivity, being a major component of chlorophyll, proteins, and nucleic acids. Nitrogen promotes growth and water use efficiency, increases leaf size, and assists in seed development [6,7]. Sulfur has specific functions in plant development, metabolism, and enzymatic reactions of plants [8]. Sulfur is required for the synthesis of S-containing amino acids such as cysteine and methionine, and is therefore also an important factor in the bread-making quality of wheat [9]. Sulfur also increases the tolerance of plants to biotic and abiotic stresses [10,11]. In recent years, the

role of S and S-containing compounds in abiotic stress defense has also been assumed. According to Chan et al. [12], S may play an essential role in the response of plants to water stress. Stress significantly affects the uptake, transport, and assimilation of S, and thus has a significant negative effect on key pathways such as photosynthesis and stress tolerance mechanisms [13]. Sulfur plays a crucial role in enhancing the ability of plants to cope with stress, including drought. It is a component of several molecules involved in stress responses, such as glutathione, which helps to protect cells from oxidative damage [14].

Even more researchers believe that S fertilization has a positive impact on cereal production [15–17]. This effect was caused by low initial levels of S available to plants in the soil. In general, the decline in the use of sulfate-containing fertilizers and the massive reduction in atmospheric S deposition have led to a decrease in the amount of plant-available S in the soil [18,19]. In addition, other limiting factors may affect crop production, such as water or drought stress due to climate change. Water stress can occur simultaneously with S deficiency in low fertilizer crop production systems, so it is important to study the effect of S deficiency combined with water stress. Water stress in agriculture reduces crop yield and quality by limiting essential physiological processes, plant growth, and nutrient absorption, making crops more vulnerable to pests and diseases. It also degrades soil health and increases production costs, leading to economic and environmental challenges. To sustain productivity, agriculture increasingly relies on efficient water management and resilient crop varieties, though these solutions require investment and resources [20].

The objective of our study was to compare the effects of different S doses (22.4, 28, 56 kg S/ha) on the yield and S content and S metabolism of spring wheat during the growing season under both adequate water supply and water stress conditions on S deficient Chernozem soil with 9.25 mg/kg KCl-SO₄²⁻ S content. Furthermore, our goal was to determine an optimal S dose with optimal N:S ratio background for spring wheat.

2. Results and Discussion

2.1. The Dry Biomass Production of Spring Wheat During the Growing Season

The dry biomass of spring wheat at the BBCH 30–32 stage did not change significantly due to the different water supply (Table 1). Under adequate water supply, slightly higher dry biomass production was observed in the fertilized treatments. Even the lowest dose of S (N₁₁₂S_{22.4}) led to an increase in the dry biomass of spring wheat compared to the control, but no further increase was observed with higher S doses. Increasing S doses had no significant effect on dry biomass production under reduced water supply.

Table 1. The changes of dry biomass of spring wheat at different development stages.

Treatments			BBCH 30–32	BBCH 61–65	BBCH 89	BBCH 89	BBCH 89
			g/3 Plants	g/3 Plants	Straw (g/Pot)	Grain (g/Pot)	HI
Adequate watering (60%)	N ₀ S ₀	control	4.17 ± 0.12 a	17.10 ± 0.89 ab	20.30 ± 0.82 b	8.20 ± 0.29 ab	0.288 bc
	N ₁₁₂ S _{22.4}	N:S = 1:0.2	4.83 ± 0.06 ab	21.00 ± 1.93 c	25.43 ± 1.64 d	14.76 ± 0.65 c	0.368 c
	N ₁₁₂ S ₂₈	N:S = 1:0.25	5.00 ± 0.35 b	19.23 ± 1.72 bc	23.83 ± 1.80 cd	11.25 ± 3.05 bc	0.314 bc
	N ₁₁₂ S ₅₆	N:S = 1:0.5	5.03 ± 0.25 b	20.43 ± 1.00 c	26.37 ± 1.36 d	15.84 ± 2.16 d	0.374 c
Reduced watering (40%)	N ₀ S ₀	control	4.50 ± 0.36 ab	15.50 ± 0.20 a	16.73 ± 0.40 a	4.80 ± 0.96 a	0.222 ab
	N ₁₁₂ S _{22.4}	N:S = 1:0.2	4.50 ± 0.26 ab	15.83 ± 0.72 a	20.87 ± 0.90 bc	4.37 ± 1.19 a	0.171 a
	N ₁₁₂ S ₂₈	N:S = 1:0.25	4.67 ± 0.40 ab	15.63 ± 0.60 a	21.40 ± 1.21 c	3.88 ± 1.36 a	0.151 a
	N ₁₁₂ S ₅₆	N:S = 1:0.5	4.77 ± 0.21 ab	15.83 ± 0.40 a	20.43 ± 0.51 b	4.69 ± 1.11 a	0.186 a

Note: Data marked with the same letter in the columns is not significantly different at $p \leq 0.05$, HI: harvest index.

According to Imadi et al. [21], water stress causes metabolic changes in plants that can lead to a reduction in growth and photosynthesis. In our experiment, this decrease was first observed at the BBCH 61–65 stage, when the effect of different water supplies on wheat biomass became statistically significant. Higher biomass production was detected under

adequate water supply, where favorable biomass-increasing effects of nitrogen and sulfur fertilization were observed compared to the control. Gupta et al. [22] also investigated the effect of water stress on wheat growth and development. In their experiment, a decrease in biomass production at the flowering stage was reported under water stress conditions.

At the BBCH 89 stage, the weights of grain and straw were measured separately, and yields were significantly higher under adequate water supply. Farooq [23] and Alqudah et al. [24] also described water stress as a major yield-limiting factor in wheat production, as it reduced plant growth and lead to grain loss, thereby decreasing grain yield. Grain yield under adequate water supply ranged from 8.20 to 15.84 g/pot. In this case, the lowest dose of S ($N_{112}S_{22.4}$) resulted in a significantly higher yield compared to the control and the treatment with the highest S dose (56 kg S/ha) proved to be the most favorable.

Under reduced water supply, water stress was a limiting factor, and the beneficial effects of fertilization did not result in increased grain yield. Grain yield under water stress conditions ranged from 3.88 to 4.80 g/pot. These values were 29.6% to 42.6% lower than those from the treatments with adequate water. Henriet et al. [25] investigated the combined effect of water stress and sulfur deficiency on pea yield and reported a reduction in yield, which also led to a decrease in the harvest index. In our experiment, the smallest yields were observed with the combination of water stress and smaller sulfur doses.

Under adequate water supply, straw yield ranged from 20.30 to 26.37 g/pot. The highest straw yield was observed in the treatment with the highest S dose (56 kg S/ha). Water stress reduced straw weight, which ranged from 16.73 to 21.40 g/pot. Under water stress conditions, fertilization did not influence grain yield, however it resulted in an increase in straw yield.

Under adequate water supply, the harvest index (HI) ranged from 0.288 to 0.374, while under reduced water supply, it varied from 0.151 to 0.222. According to Gent et al. [26], the harvest index (HI) for spring wheat typically ranged from 0.31 to 0.51. In a study by Unkovich et al. [27], a wider range of HI values for wheat was reported, ranging from 0.08 to 0.56. In our experiment, the HI values decreased under water stress conditions. Varga et al. [28] investigated the drought tolerance of wheat varieties in a greenhouse experiment and consistent with our findings he also found that the HI was lower under limited water supply. In our study, N and S fertilization under drought stress resulted in a lower HI, whereas with adequate water supply, fertilization increased the HI.

2.2. The N and S Content and N/S Ratio of Spring Wheat During the Growing Season

The N content of wheat at the BBCH 30–32 stage ranged from 3.19% to 4.94%, and the impact of water stress was not yet then reflected in the N% (Table 2). The N-fertilized treatment increased the N content compared to the control, but increasing S doses did not cause any further changes in N%. The S content varied between 0.27% and 0.33%. Treatments with increasing S doses and varying water supplies did not significantly affect these values.

According to Reussi et al. [29], the N/S ratio calculated at the stem elongation stage can be used to infer the N/S ratio of the wheat grain, which helps determine the optimal fertilizer dose during the early development stage of wheat. Spencer and Freney [30] found that for winter wheat, an N/S ratio lower than 16–19:1 is appropriate at the beginning of stem elongation. Additionally, Jiang and Huang [31] highlighted that nutrient management, including optimal N/S ratios, plays a critical role in mitigating stress impacts and optimizing growth under various environmental conditions. In our experiment, under favorable water supply, the N/S ratios were within the appropriate range. However, in the fertilized pots with unfavorable water supply, the N/S ratio reached the critical range.

At the BBCH 61–65 stage, plant N content ranged from 1.11% to 2.63%. Significant differences in N content were observed and a higher N% was found under water stress conditions compared to adequate water supply. This increase was attributed to the dilution effect caused by the higher biomass resulting from favorable water supply conditions. Under both watering regimes, the lowest N content was observed in the control, and a

significant increasing effect of fertilizers was noted only in the reduced watering treatments. Increasing S doses did not impact the N content of the flowering plants.

Table 2. The total N, total S and N/S ratio of spring wheat at different development stages.

Treatments			BBCH 30–32	BBCH 61–65	BBCH 89	
					Straw	Grain
N%						
Adequate watering (60%)	N ₀ S ₀	Control	3.19 ± 0.24 a	1.11 ± 0.11 a	0.34 ± 0.03 a	2.05 ± 0.05 a
	N ₁₁₂ S _{22.4}	N:S = 1:0.2	4.84 ± 0.11 b	1.25 ± 0.03 a	0.36 ± 0.08 a	1.95 ± 0.07 a
	N ₁₁₂ S ₂₈	N:S = 1:0.25	4.62 ± 0.33 b	1.37 ± 0.12 ab	0.42 ± 0.16 a	2.05 ± 0.16 a
	N ₁₁₂ S ₅₆	N:S = 1:0.5	4.62 ± 0.39 b	1.25 ± 0.05 a	0.32 ± 0.06 a	1.80 ± 0.13 a
Reduced watering (40%)	N ₀ S ₀	Control	3.35 ± 0.17 a	1.67 ± 0.08 b	0.54 ± 0.12 a	2.94 ± 0.43 b
	N ₁₁₂ S _{22.4}	N:S = 1:0.2	4.94 ± 0.09 b	2.59 ± 0.30 c	0.96 ± 0.06 b	4.21 ± 0.29 c
	N ₁₁₂ S ₂₈	N:S = 1:0.25	4.81 ± 0.15 b	2.63 ± 0.10 c	0.94 ± 0.06 b	4.45 ± 0.33 c
	N ₁₁₂ S ₅₆	N:S = 1:0.5	4.69 ± 0.19 b	2.56 ± 0.08 c	0.83 ± 0.02 b	3.91 ± 0.25 c
S%						
Adequate watering (60%)	N ₀ S ₀	Control	0.28 ± 0.06 a	0.21 ± 0.03 abc	0.29 ± 0.04 a	0.20 ± 0.01 cd
	N ₁₁₂ S _{22.4}	N:S = 1:0.2	0.33 ± 0.03 a	0.17 ± 0.00 a	0.25 ± 0.02 a	0.15 ± 0.01 a
	N ₁₁₂ S ₂₈	N:S = 1:0.25	0.31 ± 0.01 a	0.17 ± 0.00 a	0.27 ± 0.03 a	0.17 ± 0.01 ab
	N ₁₁₂ S ₅₆	N:S = 1:0.5	0.32 ± 0.03 a	0.19 ± 0.01 ab	0.28 ± 0.02 a	0.18 ± 0.01 abc
Reduced watering (40%)	N ₀ S ₀	Control	0.31 ± 0.01 a	0.18 ± 0.01 a	0.24 ± 0.03 a	0.15 ± 0.01 a
	N ₁₁₂ S _{22.4}	N:S = 1:0.2	0.28 ± 0.02 a	0.25 ± 0.02 c	0.31 ± 0.02 a	0.24 ± 0.02 d
	N ₁₁₂ S ₂₈	N:S = 1:0.25	0.27 ± 0.01 a	0.24 ± 0.03 bc	0.32 ± 0.06 a	0.23 ± 0.01 d
	N ₁₁₂ S ₅₆	N:S = 1:0.5	0.29 ± 0.01 a	0.21 ± 0.01 ab	0.30 ± 0.03 a	0.19 ± 0.01 bcd
N/S						
Adequate watering (60%)	N ₀ S ₀	Control	11.68 ± 1.97 ab	5.18 ± 0.72 a	1.17 ± 0.20 a	10.08 ± 0.45 a
	N ₁₁₂ S _{22.4}	N:S = 1:0.2	14.81 ± 1.52 bc	7.32 ± 0.08 b	1.39 ± 0.18 ab	12.80 ± 0.30 a
	N ₁₁₂ S ₂₈	N:S = 1:0.25	14.87 ± 1.41 bc	8.06 ± 0.58 bc	1.54 ± 0.40 ab	12.38 ± 0.41 a
	N ₁₁₂ S ₅₆	N:S = 1:0.5	14.58 ± 1.53 bc	6.61 ± 0.42 ab	1.13 ± 0.23 a	10.20 ± 1.15 a
Reduced watering (40%)	N ₀ S ₀	Control	10.99 ± 0.13 a	9.38 ± 0.51 cd	2.27 ± 0.53 bc	19.34 ± 2.75 b
	N ₁₁₂ S _{22.4}	N:S = 1:0.2	17.58 ± 1.01 c	10.75 ± 0.35 de	3.11 ± 0.05 c	19.49 ± 1.15 b
	N ₁₁₂ S ₂₈	N:S = 1:0.25	17.54 ± 0.05 c	11.09 ± 1.13 de	2.96 ± 0.49 c	19.52 ± 0.14 b
	N ₁₁₂ S ₅₆	N:S = 1:0.5	16.33 ± 0.35 c	12.04 ± 0.76 e	2.74 ± 0.34 c	20.14 ± 0.94 b

Note: Data marked with the same letter in the columns is not significantly different at $p \leq 0.05$.

The S content varied between 0.17 and 0.25% in all treatments. In the fertilized treatments, S content was higher under reduced water supply. Interestingly, as S doses increased, S content decreased.

The N/S ratio of wheat ranged from 5.18 to 12.04, and its value decreased with plant age. Nitrogen and sulfur fertilization resulted in an increased N/S ratio in both water supply conditions compared to the control.

At the flowering stage—when nutrient uptake by wheat is the most intensive—the inorganic (sulfate-S) and organic S contents were also determined (Figure 1).

Based on our results, a significant portion (74.7–98.0%) of the total S content was present in inorganic sulfate form. Abdallah et al. [32] observed similar trends in their study, where they investigated the effects of S limitation on N and S uptake and remobilization in oilseed rape during vegetative growth, concluding that sulfate-S was the major S fraction, accounting for over 86% of the total S in oilseed rape leaves. Mcgrath et al. [33] also discuss S uptake and metabolism in crops, including wheat, emphasizing the dominance of sulfate-S during critical growth stages.

In the fertilized treatments, the total S content was higher under reduced water supply. Under stress conditions, increasing S fertilization doses corresponded to a decrease in plant S%.

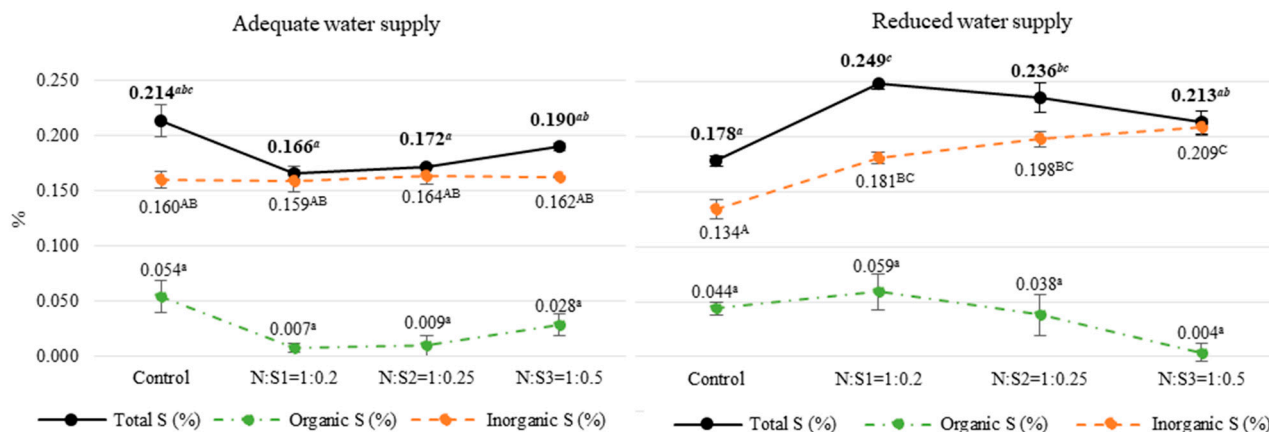


Figure 1. Changes in total S, organic S, and inorganic S ($\text{SO}_4^{2-}\text{-S}$) forms in spring wheat at the flowering stage. Note: Data marked with the same letter is not significantly different at the significant level of $p \leq 0.05$.

Under adequate water supply, the inorganic $\text{SO}_4^{2-}\text{-S}$ content of wheat did not change significantly in fertilized pots compared to the control. The organic S content varied with nutrient supply similarly to total S; it decreased in N:S = 1:0.2 treatment compared to the control, but a slight increase was observed with increasing S doses. Under reduced water conditions, there was a shift in S dynamics. There was a notable increase in sulfate content with low S fertilization doses, with additional increments as S application increases. Organic S remains more stable, though a slight reduction has been observed with higher S rates. These results are consistent with Kopriva et al. [34] who emphasize the critical interaction between S and water supply, especially under drought or limited irrigation. According to Dijkshoorn et al. [35], when more sulfate is transported to the plant than can be metabolized, it accumulates as both sulfate and organic S. In our experiment, it can be stated that, under reduced water supply, only sulfate S was accumulated with increasing S doses.

At the BBCH 89 stage, the N and S contents and N/S ratios of the straw and grain are shown in Table 2.

Some significant differences in the grain N content were observed because of the different water supplies. Under drought stress, N levels (2.94–4.45%) were almost twice as high as under ideal water conditions (1.95–2.05%). Klikocka et al. [36] estimated higher values (2.54–2.88%) in their experiment with spring wheat. Similar values (2.33–2.88%) were obtained by Reussi et al. [29], who also conducted experiments with spring wheat to determine S deficiency. Under adequate water supply, the grain nitrogen content remained stable in all treatments. However, under water stress, even the lowest dose of S increased the N content, but there was no further change in the fertilized treatments.

As expected, the N content of straw was lower compared to the grain N content. Regarding the effects of the treatments, a similar trend was observed in the straw as in the wheat grain.

The grain S content varied between 0.15 and 0.24%. Under adequate water supply the S content in the fertilized pots slightly decreased compared to the control, though a smaller increase was observed with increasing S doses. However, in the water stress model, a verifiable higher S content was measured in the fertilized treatments, but this value decreased with the increasing S fertilizer application. Randall et al. [37] received a similar result that increasing S supply decreased the S content of wheat grain.

The S content of the straw was higher than that of the grain, ranging from 0.24 to 0.32%, and was not significantly affected by the different treatments.

According to Randall et al. [37], in addition to the N and S content of wheat grain, using the N/S ratio is recommended to assess S status. The N/S ratio measured in wheat grain was an order of magnitude higher than that in straw. This ratio was significantly

higher in both plant parts under treatments with reduced water supply. According to Györi [10], under favorable conditions the N/S ratio of winter wheat remains relatively constant, typically around 15:1. However, under water stress, this ratio can increase to 16–17.5:1. In our experiment, the N/S ratio in both plant parts was significantly higher under reduced water supply, though it did not change in response to increasing S doses.

2.3. Nitrogen and Sulfur Uptake by Spring Wheat Grain and Straw

Nutrient uptake varied in the case of different plant parts (Table 3). The amount of N uptake was higher in grain, while S uptake was higher in straw. Our results were in good agreement with the findings reported by Dash et al. [38] and Assefa et al. [39], which indicate that a larger portion of the sulfur absorbed by wheat from the soil is found in the straw.

Table 3. Effect of treatments on the amount of N and S uptake by grain and straw.

Treatments			N Uptake (mg/Pot)		S Uptake (mg/pot)	
			Grain	Straw	Grain	Straw
Adequate watering (60%)	N ₀ S ₀	Control	168.3 ± 1.4 a	69.8 ± 4.3 a	16.69 ± 0.68 abc	59.32 ± 3.83 ab
	N ₁₁₂ S _{22.4}	N:S = 1:0.2	288.7 ± 9.9 b	88.5 ± 12.8 a	22.56 ± 2.05 cd	63.19 ± 5.02 ab
	N ₁₁₂ S ₂₈	N:S = 1:0.25	227.5 ± 33.9 ab	100.2 ± 19.2 a	18.50 ± 6.01 bc	65.24 ± 4.83 ab
	N ₁₁₂ S ₅₆	N:S = 1:0.5	286.7 ± 27.5 b	83.4 ± 5.9 a	28.13 ± 5.52 d	74.42 ± 4.09 b
Reduced watering (40%)	N ₀ S ₀	Control	138.6 ± 5.7 a	90.0 ± 8.9 a	7.30 ± 1.48 a	39.83 ± 3.03 a
	N ₁₁₂ S _{22.4}	N:S = 1:0.2	181.6 ± 19.6 ab	199.9 ± 9.8 b	9.36 ± 2.38 ab	64.32 ± 3.69 ab
	N ₁₁₂ S ₂₈	N:S = 1:0.25	169.7 ± 25.1 a	201.6 ± 5.3 b	8.71 ± 2.61 a	68.27 ± 6.68 ab
	N ₁₁₂ S ₅₆	N:S = 1:0.5	182.2 ± 19.3 ab	169.9 ± 4.1 b	9.11 ± 2.26 ab	62.09 ± 3.92 ab

Note: Data marked with the same letter in the columns is not significantly different at $p \leq 0.05$.

The water supply also influenced the nutrient uptake by different plant parts. Under adequate water conditions, both N and S uptake by the grain increased, whereas N uptake by the straw was higher under reduced water conditions, with no significant difference in S uptake. In treatments with favorable water supply, the smallest S dose already increased N and S uptake by wheat grain, but further increasing S doses led to a significant additional increase only in S uptake.

3. Materials and Methods

3.1. Experimental Setup, Materials and Procedure

The pot experiment was carried out on Chernozem soil with loamy texture (according to WRB) in the greenhouse of the Institute of Agricultural Chemistry and Soil Science, Faculty of Agricultural, Food Sciences and Environmental Management at the University of Debrecen. Some of the main parameters of the experimental soil were as follows: $\text{pH}_{(\text{KCl})} = 5.16$; $\text{C}_{\text{org}} = 1.75\%$; $\text{AL-P} = 45 \text{ mg/kg}$; $\text{AL-K} = 139 \text{ mg/kg}$; $\text{KCl-SO}_4^{2-}\text{-S} = 9.25 \text{ mg/kg}$. The NPK supply of the studied soil was determined based on the Hungarian fertilizer advisory system [40]. The soil was moderately supplied with nitrogen, poorly supplied with phosphorus, and very poorly supplied with potassium. According to Balanagaudar et al. [41] and Bankole et al. [42], 10 mg/kg (measured in KCl, CaCl₂ solutions) is described as the critical limit of plant-available S in soil. Based on this value, the experimental soil was classified as low S fertility.

The pots (diameter 30 cm, height 21 cm) were filled with 10 kg of air-dried soil. The indicator plant was the spring wheat variety Stanga. Light and temperature conditions during the plant development were natural, while soil moisture was controlled through irrigation with ion-exchanged water. Basically, the soil moisture content was set at 60% of the field capacity. To achieve this, the pots were weighed daily, and any missing water was replaced with ion-exchanged water. This setting ensures an adequate water supply for the plants. In addition, each treatment included a pair with reduced watering. In these

treatments, plants were not watered until wilting symptoms appeared. Irrigation was then applied to restore the soil moisture to 40% of the field capacity.

Considering the nutrient requirements of the plant and the nutrient supply of the experimental soil, the following nutrient requirements were calculated based on an assumed average yield of 4 t/ha: 112 kg N/ha, 38 kg P/ha, 70 kg K/ha. NPK nutrients were applied in proportion to 10 kg of soil. Increasing S doses (22.4, 28, 56 kg S/ha) were used with a constant nitrogen dose (112 kg N/ha), resulting in different N:S ratios (1:0.2; 1:0.25; 1:0.5) (Table 4).

Table 4. Treatments in the pot experiment.

Water Supply	Field Water Capacity	N:S Ratio	N Doses (kg/ha)	N Doses (g/10 kg)	S Doses (kg/ha)	S Doses (g/10 kg)
Adequate	60%	-	0	0	0	0
		1:0.2	112	0.3733	22.4	0.0746
		1:0.25	112	0.3733	28	0.0933
		1:0.5	112	0.3733	56	0.1866
Reduced	40%	-	0	0	0	0
		1:0.2	112	0.3733	22.4	0.0746
		1:0.25	112	0.3733	28	0.0933
		1:0.5	112	0.3733	56	0.1866

Nitrogen and sulfur were supplied as ammonium nitrate (NH_4NO_3) and ammonium sulfate [$(\text{NH}_4)_2\text{SO}_4$]. Phosphorus and potassium requirements for wheat were supplied as KH_2PO_4 and KCl, respectively. The solid-phase fertilizers were applied simultaneously with sowing. There were 8 treatments, each with three replications.

Wheat samples were taken at different growth stages according to the BBCH scale: BBCH 30–32 (stem elongation), BBCH 61–65 (flowering), and BBCH 89 (ripening). At stem elongation and flowering stages, the entire above-ground biomass was sampled (3 plants from each pot), whereas at ripening, the straw and grain were sampled separately. After harvesting, the plants were dried, and the dry weight of the wheat was measured.

3.2. Methods for Analysis

The total S and total N content of the plants were determined using an ELEMENTAR VARIO EL CNS analyzer and were expressed as N% and S% on a dry matter (DM). At the BBCH 61–65 stage, the inorganic S content of spring wheat was measured by single-column ion chromatography. The organic S content was calculated as the difference of the total and inorganic S content. The N/S ratio and the harvest index (HI) value of spring wheat were determined. The HI was calculated by dividing the grain yield by the aboveground biomass [43]. The nutrients uptake by the grain and straw was estimated from the wheat yield and the plant N and S content.

3.3. Statistical Methods

Microsoft Excel 2016 and IBM SPSS Statistics 22 were used for statistical analysis of the results. The values of each treatment group were subjected to comparative analysis using a one-way ANOVA (significance level of $p < 0.05$), with post hoc comparisons using the Tukey test.

4. Conclusions

Water stress, observed from the BBCH 61–65 stage, negatively impacted wheat yield, especially grain yield, as indicated by a reduced HI under these conditions.

Our findings indicate that under water-deficient conditions, S incorporation into plants is limited, as it tends to remain in an inorganic $\text{SO}_4^{2-}\text{-S}$ form (74.7–98.0% of total S), contributing to a reduced HI. Furthermore, the results indicate an increased N/S ratio under

drought conditions, suggesting that drought stress impedes S uptake more significantly than N uptake.

Overall, S does not alleviate the adverse effects of drought; instead, drought reduces S uptake, adversely affecting both its assimilation into plant tissues and the HI.

Based on our results, under adequate water supply, fertilization with 112 kg N/ha and 56 kg S/ha (N:S = 1:0.5) is recommended for Chernozem soil with low S fertility. This treatment achieved the highest grain and straw yields, with grain N and S contents of 1.80% and 0.18%, respectively, and straw N and S contents of 0.32% and 0.28%, respectively, ensuring an adequate nutrient supply. No yield-enhancing effects of N and S fertilization were observed under water stress. However other factors, such as soil type, nutrient availability (e.g., phosphorus and potassium), and crop variety, could also significantly influence spring wheat productivity and yield. Therefore, in future research, we plan to investigate these factors alongside fertilization strategies under varying water conditions.

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