








Influence of nitrogen, sulphur, and selenium foliar application on yield and accumulation of selenium in spring wheat grains (*Triticum aestivum* L.)

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Abstract: The study is focused on the evaluation of selenium, nitrogen and sulphur effects on yield, macro- and micro-nutrient content (N, P, K, Ca, Mg, S, Zn, Fe, Mn, Cu) and quality (Se content, starch, fibre, ash and fat) in wheat grain. Small-plot field experiments (10 m² each plot) were established on loam to clay loam mollic soil with total Se content 0.21–0.22 mg/kg in Želiezovce on the land of the Central Control and Testing Institute in Agriculture of the Slovak Republic. The effect of growing season on two sources of selenium, in the form of sodium selenite (Na₂SeO₃ · 5H₂O) and sodium selenate (Na₂SeO₄), was monitored during the growth phase BBCH 29 (the end of the tillering phase) in a two-year experiment. The experiment included six foliar treatments in four repetitions, which were differentiated as follows: T1 – 30 kg N/ha; T1 SeO₃²⁻ – 30 kg N/ha and 20 g Se/ha; T1 SeO₄²⁻ – 30 kg N/ha and 20 g Se/ha; T2 – 30 kg N/ha and 10 kg S/ha; T2 SeO₃²⁻ – 30 kg N/ha, 10 kg S/ha and 20 g Se/ha; T2 SeO₄²⁻ – 30 kg N/ha, 10 kg S/ha and 20 g Se/ha. A statistically significant difference in yield was found between the growing seasons. Statistically non-significant impact of treatments on achieved yields was found. The highest average Se content in grain, 0.90 ± 0.28 mg/kg, was achieved on treatment T2 SeO₄²⁻. The application of sodium selenite appeared to be less effective than selenate form in the evaluation of average Se content in grain, where statistically significantly higher Se contents (T1 SeO₄²⁻ 0.78 ± 0.22 mg/kg; T2 SeO₄²⁻ 0.90 ± 0.28 mg/kg) were found after selenate application. The application of two types of fertilisers and two forms of selenium did not significantly increase the content of N, P, Mg, and S in grain. The Fe content in the grain was increased by treatment T2 SeO₃²⁻. The application of sodium selenate compared to sodium selenite significantly increased the starch content (T1 SeO₄²⁻ 56.39 ± 4.44%; T2 SeO₄²⁻ 55.87 ± 4.05) in the grain of spring wheat.

Keywords: nutritional value; deficiency; biofortification; plant uptake; fertilisation

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Micronutrient fertilisation is an important tool for intensifying agricultural production. In recent years, there has been discussion about enhancing the nutritional value of crops in relation to selenium (Se), which is not an essential microelement for plants but is necessary for the nutrition of animals and humans (Tlustoš et al. 2024). In terms of complex nutrition, selenium is an essential micronutrient for humans that is important for the proper functioning of the human body. The low selenium content in crops intended for the food industry is closely related to selenium deficiency in the soil (Yeasmin et al. 2022). The Panel on Nutrition, Novel Foods and Food Allergens (NDA) of the European Food Safety Authority (EFSA 2024) concluded that the tolerable upper intake level of 255 µg Se/day is safe for adult men and women (including pregnant and lactating women). This level is lower than the upper intake level of 300 µg Se/day for adults, set by the Scientific Committee on Food (SCF) in 2000. Currently, selenium is authorised for use in all animal species with maximum contents of 0.5 mg total selenium/kg complete feed from all inorganic sources, and 0.2 mg supplemented selenium from organic sources/kg complete feed (within a maximum of 0.5 mg total selenium/kg complete feed).

There are two practical methods of compensation for mineral deficiencies in plant products. The first is commercial or industrial fortification, defined as the addition of micronutrients to foods and food products. The second one is biofortification, defined as the increase in micronutrient content in the edible parts of crops. Biofortification is achieved in two ways. The first is through the application of fertilisers, which stimulate the plant to absorb the nutrients. The second is through plant breeding, which is considered the most sustainable and cost-effective approach (Balík 2024). Selenium application can be achieved in two ways: through soil application or foliar application (Wang et al. 2013). From the point of view of plant nutrition, the most important selenium forms are selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) because both anions are readily taken up by plants from the soil solution (Coppin et al. 2009). In addition, selenium in plants does not have its own transport and metabolic ways, so it competes with sulphur and phosphorus anions, which can affect selenium uptake by the plant (Schiavon and Pilon-Smits 2017, Praus et al. 2019).

The inter-element relations between selenium and micronutrients vary. The selenium application leads to a significant reduction of Fe accumulation

in lettuce (do Nascimento da Silva and Cadore 2019), inhibits Cu uptake in tomatoes (Meucci et al. 2021) or in lettuce (Do et al. 2019). Uptake of Mn depends on the concentration of applied Se. After selenium application (487 µmol/L), the Mn content in turnip (Li et al. 2018) and olive (D'Amato et al. 2018) was significantly intensified. In contrast, Se concentration can decrease Mn uptake in tomatoes when the concentration decreases under 1 µmol/L (Alves et al. 2020). A controversial interaction exists between Se and Zn, where low doses of Se inhibit Zn uptake (Feng et al. 2009). On the contrary, Chinese cabbage (Dai et al. 2019), turnip (Li et al. 2018), pak choi (Xue et al. 2020), and broccoli (Šindelářová et al. 2015) have been shown to increase the accumulation of Zn. This discrepancy may be related to differences in Se forms, concentrations, application methods, and crop species (Li et al. 2020). Based on the previously described results in the field of selenium biofortification, the goal of the experiment was to evaluate the effect of applying various forms of selenium salts and industrial fertilisers on yield, Se, and nutrient content in the grain of spring wheat during the growing seasons of 2020 and 2021.

MATERIAL AND METHODS

Small-plot field experiments were based on the lands of the Central Control and Testing Institute in Agriculture in experimental years 2020 and 2021 in Želiezovce. The soil of the experimental station is classified as clay to loam clay mollic soils according to the WRB classification (Mantel et al. 2023); the humus horizon reaches a depth of 40 to 60 cm. Oxidisable carbon (Cox) content was determined oxidometrically according to Tiurin (1966); the results are given in Table 1. Spring wheat, cv. Jariella was grown in both experimental years. The experiment was set up using the perpendicularly divided block method, with four replications, and the block size was 10 m². The characteristics of the weather conditions during the monitored years are stated in Figure 1.

The effects of two different industrial fertilisers used as sources of N and S, and two forms of selenium salts, sodium selenite ($\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$) and sodium selenate (Na_2SeO_4), were monitored. All fertilisers were applied foliarly as liquid solutions in BBCH 29 (the end of the tillering phase). The agrochemical soil analysis was conducted before sowing, with the results presented in Table 2. The fertiliser NPK

Table 1. Agrochemical characteristics of soil (to the depth of 30 cm) before trial establishment

Soil analyses	2020 (4. 3. 2020)	2021 (1. 3. 2021)
pH _{KCl}	6.45 (slightly acidic)	6.54 (slightly acidic)
N _{min} (NO ₃ ⁻ -N and NH ₄ ⁺ -N forms) (mg/kg)	7.4 (low)	6.9 (low)
P _{Mehlich 3} (mg/kg)	57.5 (suitable)	65.1 (suitable)
K _{Mehlich 3} (mg/kg)	270.2 (good)	260.3 (good)
Ca _{Mehlich 3} (mg/kg)	3 250.0 (good)	3 350.0 (high)
Mg _{Mehlich 3} (mg/kg)	434.0 (very high)	458.0 (very high)
S (CH ₃ COONH ₄) (mg/kg)	2.1 (very low)	2.4 (very low)
Se-total content (HF + HNO ₃ + HCl), (mg/kg)	0.21 (very low)	0.22 (very low)
Content of oxidisable carbon (%)	2.0	1.9

(15-15-15) in a dose of 250 kg/ha (37.5 kg N/ha, 16.65 kg P/ha, 31.13 kg K/ha) was applied in 2020 and 2021 as part of the pre-sowing fertilisation. The forecrop was corn for grain. Application of liquid fertilisers was achieved using a foliar handheld sprayer STIHL at a dose of 400 L/ha. The levels and timing of individual treatments were designed as follows:

T1 – liquid nitrogen fertiliser (30% N). One quarter of nitrogen is in ammonium form, one quarter in nitrate form

and one half in amide form. It has a density of 1 300 kg/m³ at a temperature of 25 °C, while the desalination temperature is –10 °C. Application dose 30 kg N/ha.

T1 SeO₃²⁻ – liquid nitrogen fertiliser described above (in dose 30 kg N/ha) + solution of 20 g Se/ha in the form of an aqueous solution of sodium selenite.

T1 SeO₄²⁻ – liquid nitrogen fertiliser described above (in dose 30 kg N/ha) + solution of 20 g Se/ha in the form of an aqueous solution of sodium selenate.

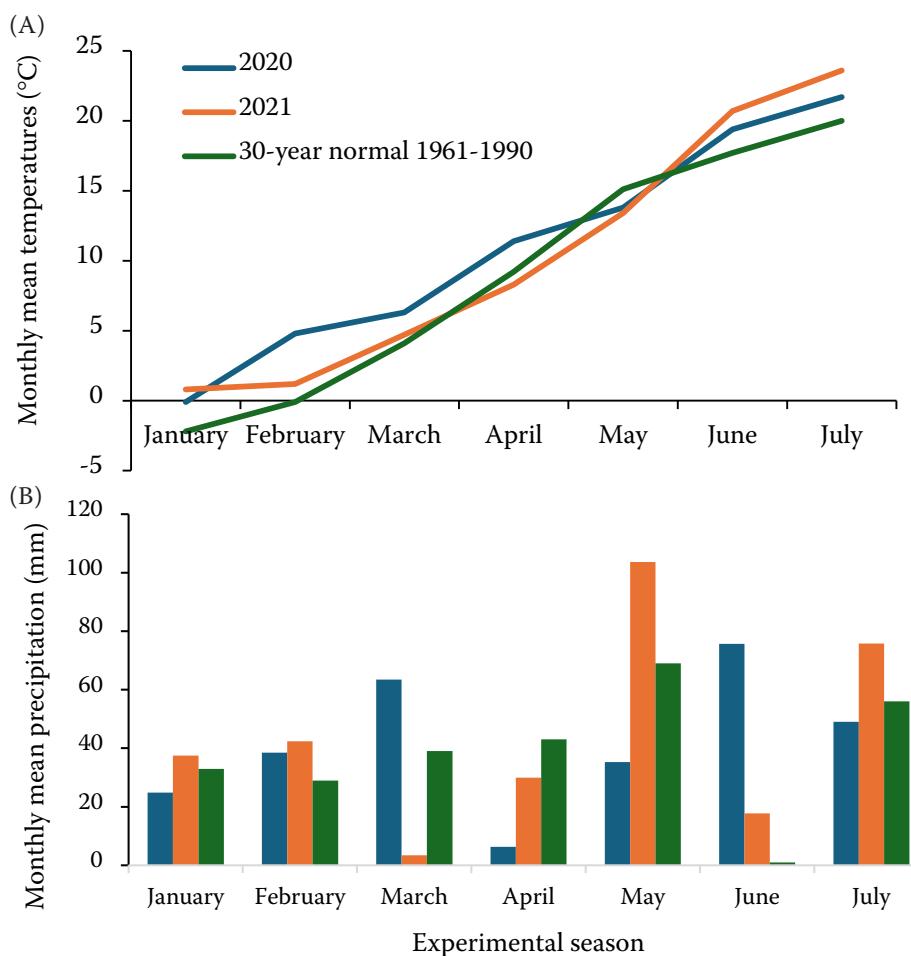


Figure 1. Monthly mean (A) temperatures and (B) precipitation compared to the 30-year normal 1961–1990

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Table 2. Effect of treatments on grain yield

Treatment	Grain yield (t/ha)		
	2020	2021	2-year average
T1	5.84 ± 0.24 ^a	3.47 ± 0.08 ^a	4.66 ± 1.28 ^a
T1 SeO ₃ ²⁻	6.00 ± 0.20 ^a	3.47 ± 0.14 ^a	4.74 ± 1.36 ^a
T1 SeO ₄ ²⁻	6.10 ± 0.34 ^a	3.65 ± 0.32 ^a	4.88 ± 1.34 ^a
T2	5.81 ± 0.82 ^a	3.62 ± 0.26 ^a	4.72 ± 1.30 ^a
T2 SeO ₃ ²⁻	5.92 ± 0.24 ^a	3.72 ± 0.40 ^a	4.82 ± 1.22 ^a
T2 SeO ₄ ²⁻	6.16 ± 0.12 ^a	3.86 ± 0.27 ^a	5.01 ± 1.25 ^a
LSD _{0.05}	0.59	0.39	1.30

T1 – N-fertiliser; T1 SeO₃²⁻ – N-fertiliser and sodium selenite; T1 SeO₄²⁻ – N-fertiliser and sodium selenate; T2 – N-S fertiliser; T2 SeO₃²⁻ – N-S fertiliser and sodium selenite; T2 SeO₄²⁻ – N-S fertiliser and sodium selenate. Different lower-case letters in a column indicate a significant difference at the level of significance $P = 0.05$

T2 – nitrogen is present in three forms – nitrate form, amide form and ammonium form. Sulphur is present in sulfate and elemental form with a nitrogen content of 24% and sulphur content 8% (SO₄²⁻ 67%, elemental sulphur 33%). Application at a dose of 30 kg N/ha and 10 kg S.

T2 SeO₃²⁻ – liquid nitrogen fertiliser with sulphur (in dose 30 kg N/ha and 10 kg S) + with a solution of 20 g Se/ha in the form of an aqueous solution of sodium selenite applied.

T2 SeO₄²⁻ – liquid nitrogen fertiliser with sulphur (in dose 30 kg N/ha and 10 kg S) + with a solution of 20 g Se/ha in the form of an aqueous solution of sodium selenate applied.

Wheat was harvested by a small-plot grain combine harvester at full maturity, phase BBCH 91, for grain yield, which was subsequently analysed to determine the nitrogen, sulfur, and selenium content. Analyses of elemental composition were determined by standard methods. Nitrogen content was determined according to Kjeldahl after mineralisation with concentrated H₂SO₄, as described by Cohen. Sulphur content was determined nephelometrically after mineralisation of plant matter, following the method described by Koppová. Selenium content was determined by the mass spectrometry method ICP-MS (inductively coupled plasma mass spectrometry) (ICP-MS Agilent 7900, Tokyo, Japan). Macronutrients (P, K, Ca, Mg, S) were determined after mineralisation of plant matter, where P was spectrophotometrically as phosphomolybdenum blue and K flame spectrophotometrically according to Koppová; Mg and Ca were determined by atomic absorption spectrophotometry according to Kováčik. Microelements (Fe, Zn, Cu, Mn) by using 0.1 mol/dm³ HNO₃. Qualitative parameters (wet

gluten, starch, ash, and fat content) were determined using the following methods. The wet gluten was determined by washing the dough until excess water was removed. The wet gluten was then weighed and converted to the percentage of wet gluten in the dry matter of the flour, according to Šedivý. Starch was determined polarimetrically using the Ewers method, and fibre was measured by a non-enzymatic gravimetric method using H₂SO₄ and NaOH. The principle is based on the action of a 5% sulfuric acid solution and a 5% sodium hydroxide solution; the amount of fibre obtained is determined gravimetrically, as described by Hrstka and Somrová. The ash content is determined by burning the sample at 900 °C ± 10 °C. The ash content was determined according to the method of Becker and Nehring.

Statistical analysis. The results were evaluated using standard statistical methods using TIBCO Statistica®, Version 14.0 (TIBCO Software Inc., Palo Alto, USA). The one-way ANOVA at the $P = 0.05$ level was used to analyse the differences between groups. Before the analysis itself, the homogeneity of variances between groups was verified by using Levene's test. Subsequently, Tukey's *HSD* (honestly significant difference) post hoc test was used and applied to detect differences between individual groups.

RESULTS

The evaluation of experimental factors confirmed a statistically highly significant effect of the growing year on grain yield (Table 3). Significantly higher yields were observed in 2020.

The analysis of the treatment effect on spring wheat grain yield did not reveal statistically significant differ-

Table 3. Effect of experimental year on the yield of grain

	Grain yield	Selenium content in grain
2020	5.97 ± 0.38 ^b	0.39 ± 0.07 ^a
2021	3.63 ± 0.28 ^a	0.45 ± 0.08 ^a
2-year average	4.80 ± 1.23	0.42 ± 0.36
<i>LSD</i> _{0.05}	0.19	0.21

Different lower-case letters in a column indicate a significant difference at the level of significance $P = 0.05$

ences (Table 2). Significantly higher selenium contents in grain were found after selenate application, with an average content of 0.78 ± 0.22 mg/kg and 0.90 ± 0.28 mg/kg (Table 4). In 2020, significantly higher selenium contents were found in variant T1 SeO_4^{2-} (0.93 ± 0.20 mg/kg) and variant T2 SeO_4^{2-} (0.76 ± 0.26 mg/kg) compared to selenite application. The difference between variants T1 SeO_4^{2-} and T2 SeO_4^{2-} was not statistically significant, with a higher selenium content in the grain in variant T1 SeO_4^{2-} (Table 5). In 2021, significantly higher Se contents were found in the grain of variants T1 SeO_4^{2-} and T2 SeO_4^{2-} , with a statistically significant difference between them (Table 5). A higher Se content was achieved in variant T2 SeO_4^{2-} 1.03 ± 0.25 mg/kg than in the variant T1 SeO_4^{2-} . Two-year average confirmed a trend of significantly higher Se accumulation rates in spring wheat grain in variants T1 SeO_4^{2-} 0.78 ± 0.22 mg/kg and T2 SeO_4^{2-} 0.90 ± 0.28 mg/kg compared to the other variants, with the highest accumulated selenium content in variant T2 SeO_4^{2-} (Table 5).

A comparison of macroelement content after the application of two types of fertilisers and two forms of selenium showed statistically significant differences in K and Ca content between the treatments in the

2-year average (Table 6). The application decreased the potassium content in grain, which was confirmed by the highest average K content of 4.46 ± 0.63 g/kg in variant T1. On the contrary, magnesium content in grain increased with selenate application, with the same value of 1.43 ± 0.15 g/kg in variants T1 SeO_4^{2-} and T2 SeO_4^{2-} , and the highest sulphur content of 1.79 ± 0.30 g/kg in variant T1 SeO_4^{2-} (Table 6).

The evaluation of the average microelement content in grains revealed a statistically significant difference in iron and manganese content, where the highest values of Fe 64.71 ± 9.02 mg/kg were found in variant T2 SeO_3^{2-} (Table 5).

On average, statistically significantly higher starch contents of $56.39 \pm 4.44\%$ and $55.87 \pm 4.05\%$ were achieved after selenate application over the 2 years. Statistical differences in the ash and fat content obtained were ambiguous (Table 7).

DISCUSSION

Weather conditions during the growing season have a clear impact on the yields of crops (Ernst et al. 2016, Pačuta et al. 2024, Zapletalová et al. 2024). The same tendency was reached in the spring wheat cultivation. Significant differences in achieved grain yield between growing seasons could have been caused by varying moisture conditions due to weather conditions during the growing season. Similar results were found by Meng et al. (2025), who recorded significant differences in black wheat grain yield and selenium content in grain after selenium application in controlled irrigated treatments. Soil moisture combined with selenium nutrition caused a 4–7 times higher increase in the Se content in grain. This finding disagrees with our

Table 4. Effect of treatments on selenium (Se) content in wheat grain

Treatment	Se content in grains (mg/kg)		
	2020	2021	2-year average
T1	0.14 ± 0.05 ^a	0.04 ± 0.01 ^a	0.09 ± 0.07 ^a
T1 SeO_3^{2-}	0.32 ± 0.06 ^a	0.24 ± 0.15 ^{ab}	0.28 ± 0.11 ^{ab}
T1 SeO_4^{2-}	0.93 ± 0.20 ^b	0.62 ± 0.06 ^b	0.78 ± 0.22 ^c
T2	0.15 ± 0.23 ^a	0.04 ± 0.01 ^a	0.09 ± 0.06 ^a
T2 SeO_3^{2-}	0.38 ± 0.04 ^a	0.40 ± 0.32 ^{ab}	0.39 ± 0.21 ^b
T2 SeO_4^{2-}	0.76 ± 0.26 ^b	1.03 ± 0.25 ^c	0.90 ± 0.28 ^c
<i>LSD</i> _{0.05}	0.20	0.38	0.19

T1 – N-fertiliser; T1 SeO_3^{2-} – N-fertiliser and sodium selenite; T1 SeO_4^{2-} – N-fertiliser and sodium selenate; T2 – N-S fertiliser; T2 SeO_3^{2-} – N-S fertiliser and sodium selenite; T2 SeO_4^{2-} – N-S fertiliser and sodium selenate. Different lower-case letters in a column indicate a significant difference at the level of significance $P = 0.05$

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Table 5. Effect of treatments on micronutrient content in wheat grain

Treatment	Micronutrient content (mg/kg) in grains in the 2-year average			
	Zn	Fe	Mn	Cu
T1	33.98 ± 3.38 ^a	39.78 ± 4.47 ^a	40.75 ± 2.47 ^{ab}	5.11 ± 1.48 ^a
T1 SeO ₃ ²⁻	35.89 ± 6.08 ^a	40.04 ± 7.55 ^a	39.93 ± 1.80 ^{ab}	5.00 ± 1.55 ^a
T1 SeO ₄ ²⁻	34.50 ± 3.65 ^a	41.90 ± 9.56 ^a	37.68 ± 3.15 ^a	4.94 ± 1.35 ^a
T2	33.86 ± 4.00 ^a	46.05 ± 12.39 ^a	38.88 ± 1.72 ^{ab}	5.16 ± 0.98 ^a
T2 SeO ₃ ²⁻	35.89 ± 5.96 ^a	64.71 ± 9.02 ^b	40.89 ± 0.72 ^b	6.14 ± 1.56 ^a
T2 SeO ₄ ²⁻	34.03 ± 3.89 ^a	45.38 ± 7.13 ^a	39.23 ± 2.87 ^{ab}	5.33 ± 1.09 ^a
LSD _{0.05}	4.66	8.78	2.29	1.36

T1 – N-fertiliser; T1 SeO₃²⁻ – N-fertiliser and sodium selenite; T1 SeO₄²⁻ – N-fertiliser and sodium selenate; T2 – N-S fertiliser; T2 SeO₃²⁻ – N-S fertiliser and sodium selenite; T2 SeO₄²⁻ – N-S fertiliser and sodium selenate. Different lower-case letters in a column indicate a significant difference at the level of significance $P = 0.05$

results, which could have been caused by the different effects of weather precipitation and controlled irrigation, resulting in opposite outcomes. Poblaciones et al. (2014) and Rodrigo et al. (2013) hypothesised that lower water availability may cause lower absorption and consequently lower Se accumulation in grain. Therefore, the irregularity of precipitation in concrete climatic conditions may cause insufficient consistency in Se uptake and accumulation in grain after fertilisation. The selenium content of spring wheat was affected not only by industrial fertilisers but also by the selenium form. Preferably in the form of sodium selenate salt, best in combination with N or N/S-based industrial fertiliser. Regarding the effect of different selenium forms on maize cultivation, the application of both selenium forms (sodium selenite and sodium selenate) significantly increased the amount of biomass reached (Płaczek and Patorczyk-Pytlik 2014). Our results showed that the selenate form significantly

increased Se content in grains. The same positive stimulating effect of Se in selenate form on yield and quality has also been recorded in various other crops, such as potatoes (Turakainen et al. 2004), winter wheat (Grant et al. 2007, Curtin et al. 2008, Broadley et al. 2010, Ducsay et al. 2016) or perennial ryegrass (Hartikainen et al. 2000). The observed disproportion in the results of the impact of fertiliser and selenium application may be due to varietal differences or the type of crop grown, as the positive effect of Se nutrition was observed in winter wheat. Similarly, the primary findings of Yan et al. (2024) revealed that the effect of Se fertilisation on yield was more significant in winter wheat than in spring wheat. Overall, this study shows that selenium fertilisation can increase the Se content in grain under suitable conditions without compromising yield. In this regard, the authors recommend applying 10–30 g/ha of selenium fertiliser in the form of selenate during the flowering

Table 6. Effect of treatments on macronutrient content in wheat grain

Treatment	Macronutrient content (g/kg) in grains in the 2-year average					
	N	P	K	Ca	Mg	S
T1	27.24 ± 2.61 ^a	4.20 ± 0.58 ^a	4.46 ± 0.63 ^d	0.31 ± 0.06 ^b	1.42 ± 0.08 ^a	1.63 ± 0.18 ^a
T1 SeO ₃ ²⁻	27.35 ± 1.85 ^a	3.99 ± 0.59 ^a	4.18 ± 0.13 ^{bc}	0.32 ± 0.10 ^b	1.39 ± 0.09 ^a	1.61 ± 0.52 ^a
T1 SeO ₄ ²⁻	26.78 ± 1.74 ^a	4.00 ± 0.94 ^a	4.37 ± 0.21 ^{cd}	0.31 ± 0.08 ^b	1.43 ± 0.15 ^a	1.53 ± 0.38 ^a
T2	25.81 ± 1.02 ^a	3.97 ± 0.60 ^a	4.28 ± 0.18 ^{ab}	0.30 ± 0.06 ^{ab}	1.40 ± 0.13 ^a	1.55 ± 0.24 ^a
T2 SeO ₃ ²⁻	25.88 ± 1.46 ^a	4.13 ± 0.80 ^a	4.07 ± 0.21 ^{ad}	0.30 ± 0.07 ^{ab}	1.40 ± 0.13 ^a	1.60 ± 0.14 ^a
T2 SeO ₄ ²⁻	26.62 ± 1.59 ^a	4.00 ± 0.53 ^a	3.90 ± 0.25 ^a	0.27 ± 0.04 ^a	1.43 ± 0.15 ^a	1.79 ± 0.30 ^a
LSD _{0.05}	1.79	0.69	0.24	0.03	0.13	0.32

T1 – N-fertiliser; T1 SeO₃²⁻ – N-fertiliser and sodium selenite; T1 SeO₄²⁻ – N-fertiliser and sodium selenate; T2 – N-S fertiliser; T2 SeO₃²⁻ – N-S fertiliser and sodium selenite; T2 SeO₄²⁻ – N-S fertiliser and sodium selenate. Different lower-case letters in a column indicate a significant difference at the level of significance $P = 0.05$

Table 7. Effect of treatments on grain quality

Treatment	Qualitative parameters (%) in grains in the 2-year average			
	starch	fiber	ash	fat
T1	52.29 ± 1.97 ^a	4.03 ± 1.54 ^a	4.28 ± 1.41 ^c	1.83 ± 0.26 ^{bc}
T1 SeO ₃ ²⁻	53.09 ± 1.32 ^a	3.87 ± 2.04 ^a	3.85 ± 0.30 ^{abc}	1.47 ± 0.11 ^a
T1 SeO ₄ ²⁻	56.39 ± 4.44 ^b	3.96 ± 1.70 ^a	3.13 ± 0.43 ^a	1.99 ± 0.34 ^c
T2	52.97 ± 1.74 ^a	3.82 ± 1.44 ^a	3.70 ± 0.56 ^{abc}	1.78 ± 0.44 ^{bc}
T2 SeO ₃ ²⁻	53.21 ± 1.52 ^a	3.70 ± 1.77 ^a	4.00 ± 0.47 ^{bc}	1.68 ± 0.39 ^b
T2 SeO ₄ ²⁻	55.87 ± 4.05 ^b	3.69 ± 1.25 ^a	3.33 ± 0.83 ^{ab}	1.79 ± 0.15 ^{bc}
LSD _{0.05}	2.82	1.66	0.77	0.20

T1 – N-fertiliser; T1 SeO₃²⁻ – N-fertiliser and sodium selenite; T1 SeO₄²⁻ – N-fertiliser and sodium selenate; T2 – N-S fertiliser; T2 SeO₃²⁻ – N-S fertiliser and sodium selenite; T2 SeO₄²⁻ – N-S fertiliser and sodium selenate. Different lower-case letters in a column indicate a significant difference at the level of significance $P = 0.05$

to filling phase, when it effectively increases wheat yield and Se content in grain, which is a later growth stage than the growth stage in our study.

Another important finding is the relationship between N, S and Se and their effect on crop yield and Se accumulation in grain. Since sulphates and selenates are chemically similar, plants use sulphate permeases (membranes) to absorb selenates. Various studies have shown that applying sulphur (60 kg/ha) and selenium (60 g/ha) can improve the qualitative characteristics of rapeseed, while also reducing Se uptake (Liu et al. 2017). Some studies have also shown that sulphur fertilisers can reduce selenium uptake in the selenite form. It was observed in crops such as rapeseed, barley, wheat and soybeans. This is explained by competitive struggle within the passage through sulphate permeases (Dos Santos et al. 2022). Our results revealed significant differences in Se content in grain between treatments with selenate and selenite. Non-significant differences were found between the variants fertilised with nitrogen-based fertilisers and those fertilised with nitrogen-sulphur fertilisers, which may mean that the added sulphur did not negatively affect the transfer of selenate selenium into the plant and later into the grain. This may mean that the passage through the sulphate membrane was uncomplicated in the case of selenate. A study evaluating the potential of combined Se and N fertiliser application to promote growth, yield, and Se content in potato tubers reported positive effects on Se content in potato tubers (Li et al. 2023). In the study of Klikocka et al. (2017), the application of nitrogen fertiliser (at doses of 40 kg/ha and 80 kg/ha) on Se content in grain of spring wheat

was evaluated, resulting in an increase of Se content by 19.1% and 36.8%, respectively. The uptake was 24.4% and 84.7% higher than in the control. The positive effects of nitrogen fertilisation on the rise of Se content were confirmed by the results of our study.

The effects of Se on micronutrients vary. Drahoňovský et al. (2016) noted a decrease in Cu content after the application of selenate (25 and 50 g/ha), whereas, conversely, a dose of 50 g/ha of selenate increased the Mn content. A study evaluating the accumulation of macro- and microelements in wheat grains after the application of selenate in a nutrient solution with a concentration of 5 and 15 µmol found a non-significant effect of Se on the accumulation of P, K, Ca, and S. The application of Se caused a significant decrease of Mn, Mo, and Zn content (Tobiasz et al. 2014). Ismail et al. (2024) point out that Se foliar treatment (selenate form 100 mg/L) had a highly significant correlation between Se application rates and the concentration of macronutrients (iron, zinc, calcium, and potassium). On the contrary, the content of manganese and iron was promoted by the application of N/S fertiliser and selenite form. The cause of ambiguous results could be resolved by subsequent observation at the physiological level.

In a study by Beshah et al. (2025), Se application did not affect grain yield in any of the wheat cultivars; however, it had a significant effect on quality traits, depending on the cultivar. However, the results also suggest that high doses of Se may have a negative impact on protein and starch content. Our application dose of 20 g Se/ha in the form of sodium selenate per hectare demonstrably increased the starch in spring wheat grain.

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