Impact of Sulfur on Biofortification and Speciation of Selenium in Wheat Grain Grown in Selenium-Deficient Soils

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Abstract
Selenium (Se) is an essential micronutrient in humans that is required for both physical and mental well-being. Low Se content in food crops is linked to Se-deficient soils globally. The aim of this study was examined the influence of sulfur (S) on the speciation and accumulation of selenium (Se) in three wheat cultivars grown in Se-deficient soils. Plants were grown in soil under glasshouse conditions with two doses of S (0 and 14 mg kg⁻¹) as sulfate and three doses of selenium (0, 1, and 2 mg kg⁻¹) as selenate (SeVI) in a randomized factorial design. Selenium speciation was determined using liquid chromatography inductively coupled plasma mass spectroscopy after enzymatic hydrolysis. Selenocysteine (SeCys), selenomethyl-cysteine (SeMeCys), selenomethionine (SeMet), selenite (SeIV), and selenate (SeVI) were determined. The addition of SeVI increased the Se content in grain in all wheat cultivars compared to the control treatment. Selenium accumulated to the highest extent in leaf tissue while stem accumulated low amounts of Se. Speciation analysis in grain showed that most of the Se accumulated in wheat grain in the organic forms, SeCys and SeMeCys. Inorganic Se was below 10%, primarily as SeVI. Longsword, a multi-tillering variety, accumulated the highest proportion of SeMeCys (67%). Fertilization with S concurrently with Se resulted in decreased production of SeCys and SeMeCys in grain. The findings from this study provide new insights into the Se biofortification and speciation transformation processes in wheat as impacted by S supplementation in Se-deficient soils.

Keywords Selenium · Selenate · Wheat cultivars · Accumulation · Transformation · Speciation

1 Introduction
Selenium (Se) was first recognized in the late 1950s as an essential micronutrient in humans (Schwarz and Foltz 1957). The deficiency of Se plays a critical role in the central nervous system, male reproductive biology, the endocrine system, muscle function, cardiovascular system, and immunity (Roman et al. 2014; Rayman 2012). In addition, Se deficiency may also lead to depression and mental health disorders (Rayman 2000). The daily intake of Se varies globally, and the recommended dose is 55 µg per day for adults and children above 14 years (Bendich 2001) and the maximum dose of Se is 300 µg per day (Rayman 2017).

In nutrient-deficient soils, agronomic biofortification is an effective practice for increasing the nutrient content of the edible portion of cereal crops through fertilization practices (Broadley et al. 2010; Graham et al. 2007). This practice has been recognized as a reliable long-term approach to alleviating micronutrient (including Se) deficiency in the last decade because it is relatively easy, efficient, and affordable (Broadley et al. 2010). The Se content in food depends on the soil Se bioavailability and the ability of plants to take up and accumulate Se in edible tissues (Bañuelos et al. 2017). The Se content in cereal grain can be improved by applying a small amount of Se fertilizer in the form of sodium selenate (Na₂SeO₃) in soil,
as has been practiced in Finland since the 1980s (Keskinen et al. 2011). Selenium occurs mainly as inorganic compounds in soil, primarily in the form of selenate (SeO$_4^{2−}$) and selenite (SeO$_3^{2−}$). Selenate is generally more abundant and available to plants than selenite in soils. Selenium and sulfur belong to the same group in the periodic table and have similar chemical behavior in both soil and plant systems (Wang and Becker 2013). Due to the chemical similarity to sulfate, plants absorb selenate via sulfate permeases (Schiavon et al. 2015). Sulfur (S) fertilizer exerts different regulatory effects on Se uptake in crops. Liu et al. (2017) observed that the S application along with Se fertilizer could improve the quality of *Brassica napus* and also reduce Se uptake significantly. Some other studies revealed that application of S fertilizer could reduce the Se$^{IV}$ uptake in crops such as wheat (*Triticum aestivum* L.), rape-seed (*Brassica napus* L.), ryegrass (*Lolium perenne* L.), and soybean (*Glycine max* L.). Dos Santos et al. (2022) reported that the presence of sulfate in soil reduces the Se$^{VI}$ adsorption during uptake progression between sulfate and Se$^{VI}$ at the plant root–soil solution edge, due to competition for the same membrane transporters.

Consumption of Se-biofortified plant products containing organic Se forms may lead to a higher intake of Se in humans. Moreover, organic forms of Se such as selenomethionine, selenocysteine, methyl selenocysteine, and γ-glutamyl-methyl-selenocysteine contained in some Se-enriched plant tissues may be more readily used by enzymes for promoting antioxidant activities in humans. In this regard, Ávila et al. (2014) reported that biofortification of Se in *Brassica* sp. showed that application of 50 μM Na$_2$SeO$_4$ significantly increased synthesis of a counter-carcinogenic compound, Se-methyl selenocysteine (SeMeCys). Selenium readily substitutes for S in amino acids and proteins due to the high chemical similarity of the two elements. Selenium species, selenocysteine (SeCys), and selenomethionine (SeMet) are analogs of S-containing amino acids (e.g., cysteine, methionine) (Terry et al. 2000). Hence, speciation of Se compounds in Se-biofortified grain produced via biofortification strategies is potentially linked to the production of S-enriched proteins. In addition, there are potentially antagonistic Se-S interactions in the soil environment, both at the root plasma membrane and adsorption sites in the soil matrix (Kikkert and Berkelaar 2013). We hypothesized that S would influence the total Se uptake and the incorporation of Se into desirable organic S forms in grain, such as SeMeCys.

In this study, we investigated the ability of three different wheat cultivars to accumulate Se in Se-deficient soil with the application of variable levels of Se and S. The objective of our study was to determine the chemical species of Se in the grain of wheat cultivars after being applied to various doses of selenate and sulfate.

### 2 Materials and Methods

#### 2.1 Soil Collection, Processing, and Characterization

In this experiment, surface soil (0–15 cm) was collected from farmland located at Condobolin (NSW), Australia. After collection, debris and other unwanted materials were removed, air-dried, sieved to 4 mm, and homogenized. A portion of processed soil was used for physicochemical characterization using standard protocols (Table 1) (Lamb et al. 2016). The organic carbon, total N, and total S were analyzed using a CNS analyzer (LECO, TruMac CNS). Readily soluble S (measured as sulfate) was measured using a gravimetric approach (Rayment and Higginson 1992) with both water and salt (10 mM CaCl2) at a 1:5 solid-solution ratio. In this soil, sulfate solubility is not likely to be influenced by Ca from the salt extract (Lebedev and Kosorukov 2017). The adsorption behavior for Se$^{VI}$ was determined by following the procedure of Silva et al. (2019) with a slight modification. Briefly, 1 g soil was weighed and 20 mL of a Se$^{VI}$-enriched solution containing 0.01 M MES buffer (pH 6) and 0.01 M NaCl suspension was reacted (24 h). Two concentrations of SO$_4$ as 0 (control) and 100 μM were added to each sample as MgSO$_4$. The Se$^{VI}$ concentrations were 0 (control), 1, 5, 10, 25, 50, 100, and 200 μM as sodium selenate. Reaction vessels were shaken in a rotatory shaker at 150 rpm for 24 h at 23 °C. After shaking, the samples were centrifuged for 20 min at 5000 rpm. A 10 mL aliquot was collected from each of the samples and filtered to a 0.22 μm. The concentration of dissolved Se in each sample was obtained using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7900, Japan). The sorption isotherms were described using Langmuir’s equation using a non-linear fitting procedure in SAS (SAS, version 9.4) (Langmuir 1997).

#### 2.2 Glasshouse Experiment

The growth experiment was conducted in a glasshouse at the University of Newcastle with five replicates.

### Table 1 Physiochemical properties of soil are determined by using standard protocol

| Parameter | Value |
|-----------|-------|
| EC (ds m.−1) | 2.2  |
| pH | 4.8 |
| Adding CaCO$_3$ | 6.5 |
| Sand % | 38.25 |
| Clay % | 24 |
| Silt % | 37.75 |
| OC (g kg.−1) | 11.80 |
| N (g kg.−1) | 1.10 |
| S (g kg.−1) | 0.08 |
| Se (µg kg.−1) | 3 |

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Glasshouse conditions were maintained at minimum–maximum temperature cycle of 16–27 °C and a 12-h photoperiod. Soils were amended with calcium carbonate (2 g kg\(^{-1}\)) to increase soil pH. The amendment rate was based on a bench top trial with increasing amendment rates of calcium carbonate (Broadhurst et al. 2015). After amending with lime, the soil was incubated for 2 weeks for correction of soil acidity. The amendment was chosen which produced a final pH of 6.5 (2 g kg\(^{-1}\)). Soils were also amended with ammonium phosphate (27 mg kg\(^{-1}\)) and potassium nitrate (14 mg kg\(^{-1}\)) and mixed thoroughly in a mixer. Each pot was filled with 3 kg mixed soil, and five seeds each of the three cultivars (Condo, Longsword, and Spitfire) were placed under the soil surface for germination. After germination, the plants were thinned to one plant after 2 weeks of sowing. The soils were maintained close to field capacity by watering the soil surface regularly with RO water. In order to investigate the uptake capacity of Se (selenate) and the interaction of S, two doses of sulfate (0 and 14 mg kg\(^{-1}\) soil) were added to the pots at 5 weeks after seeding (Both et al. 2020; Kikkert and Berkelaar 2013). After 1 week of adding sulfate, three doses of Se (control, 1, and 2 mg kg\(^{-1}\) soil) were used in this study. These concentrations were used to investigate the Se speciation in grains and based on previous reports in the literature (Jiang et al. 2015; Kikkert and Berkelaar 2013). Plants were grown to grain maturity. Upon harvesting, the stem, leaf, and spike were separated and rinsed with reverse osmosis water. Plant biomass was dried at 60 °C for 72 h and wheat grains were separated by hand. All plant parts were ground to a fine powder using a stainless steel grinder.

For total Se contents in plant tissue, approximately 0.25 g of plant sample was weighed into digestion tubes and 5 mL of H\(_2\)NO\(_3\) acid was added. Samples were cold digested overnight. The following day, the samples were heated at 70 °C for 30 min, 90 °C for 30 min, 110 °C for 30 min, and finally 140 °C (BD 50, SEAL Analytical). The digestion at 140 °C was continued until only a small residual liquid remained in each tube (approximately 1 mL) and prepared for analysis (Ming et al. 2012) by inductively coupled plasma optical emission spectrometry (ICP-OES, Avio 200, PerkinElmer Instruments, USA). Analytical accuracies of Se and S were verified using SRM 1570a (trace elements in spinach leaves) and SRM 1568a (rice flour) from NIST, USA. The total Se and S concentrations of the reference materials were within the 90–110% and 94–116% recovery of the certified values, respectively.

### 2.3 Chemical Speciation of Se in Wheat Grain

Selenium speciation in wheat grain samples was analyzed by high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) after extraction of Se species with enzymatic hydrolysis following the procedure of Hart et al. (2011) with some modifications. Sample preparation for Se speciation was followed by Godin et al. (2015) with slight modification. Briefly, 100 mg of ground grain was weighed in a polypropylene tube with 10 mg protease XIV type and dissolved in 5 mL 0.1 M TRIS–HCl buffer solution (pH 7.5). The mixture was shaken in an incubator at 37 °C for 22 h, centrifuged at 3000 g for 20 min, and filtered to 0.22 µm. The solution was collected and analyzed for selenite (Se\(^{IV}\)), selenate (Se\(^{VI}\)), selenomethionine (SeMet), selenocysteine (SeCys), and Se-methyl selenocysteine (SeMeCys). Selenium standards were purchased from Sigma Aldrich. Chromatographic separation was achieved with a Hamilton PRP-X100 anion exchange column (Hart et al. 2011). Operating parameters are detailed in Table 2.

### 2.4 Statistical Analysis

Statistical data analysis was done by using SPSS (version 27) or SAS (SAS, version 9.4). Analysis of variance (three-way ANOVA) with post hoc Tukey’s HSD (\(p < 0.05\)) was used for multiple comparisons (Table 3).
3 Results

3.1 Soil Characterization and Sorption Study

The soil pH was initially lower than initially anticipated at pH 4.8. The addition of lime (CaCO₃) raised the pH to 6.5, making it conducive for wheat growth (Table 1) (the addition of lime rate was based on previous bench-top trial) (Broadhurst et al. 2015). The soil texture was a clay loam with the sand, clay, and silt composition as 37.5, 24, and 38.5%, respectively. The organic carbon content, total N, and total S of the soil were 11.8, 1.11, and 0.08 g/kg, respectively. Salt (10 mM CaCl₂) and water extracted 12.8 ± 1 and 19 ± 1 mg/kg, respectively, indicating that 16–24% of total S was readily soluble. The soil was also Se-deficient (3 µg kg⁻¹). The sorption study showed that an increasing amount of SeVI was adsorbed with an increasing concentration of Se in the equilibrium solution (Fig. S1, supporting information). The presence of sulfate noticeably reduced the adsorption of Se in soil.

3.2 Response of Plant Biomass to Se and Sulfate

The effect of Se and sulfate treatments on dry matter production of the stem, leaf, and grain varied substantially between species (Fig. 1). The dry biomass of stem and leaf in the Condo cultivar did not show significant differences with the increasing Se doses compared to control (Fig. 1a). However, the dry weight of grain showed significant differences (*p < 0.05) with Se doses, while the highest grain dry weight (3.5 g) was observed in Se 1 treatment and the lowest (1.4 g) was in Se 2 treatment. Longsword did not show any significant differences in terms of stem and grain dry weight with the increased Se doses, whereas the leaf dry weight was significantly reduced (**p < 0.01) with the increase of Se doses (Fig. 1b).

Sulfate addition did not show any significant differences in the dry weight of the Condo and Longsword cultivars in terms of stem, leaf, and grain dry weight. However, S enormously enhanced (***p < 0.001) (twofold) Spitfire grain production compared to control. Sulfate also significantly increased the stem (***p < 0.01) and leaf dry weight (*p < 0.05).

Low SeVI doses with S increased the dry matter content of Condo wheat. However, when the Se doses were higher than 1 mg kg⁻¹ of soil, they led to significant decreases in the dry weight of stem (***p < 0.001) and leaf (**p < 0.01). Compared with the control, the highest dry weight of stem, leaf, and grains were obtained from 1 mg kg⁻¹ Se with 14 mg kg⁻¹ S (Se 1 + S) treatment. The overall plant biomass in other treatments was significantly lower than the control except for Se 1 + S.

3.3 Total Content of Selenium in Shoots and Grain

The addition of SeVI in soil increased the Se content in all the wheat cultivars compared to the control treatment (Fig. 2). The three cultivars varied in the Se contents in stem, leaf, and grain. As expected, the accumulation of Se in the leaf was the greatest while the stem accumulated low amounts of Se. However, the accumulation of SeVI in stem, leaf, and grain in all wheat cultivars showed significant differences (***p < 0.001) with the SeVI treatments. The addition of 2 mg kg⁻¹ SeVI in soil showed the highest Se content in all the three cultivars, with the Spitfire variety showing the highest leaf Se content, about 51% of the total accumulation (Fig. 2e). Among the cultivars, Longsword accumulated the lowest Se contents in all parts (stem, leaf, and grain) (Fig. 2b). The addition of S to Se-treated soils did not show any significant difference in stem, leaf, and grain Se content for the Condo cultivar. For Longsword, only significant differences (*p < 0.05) were found between SeVI and S interactions in the grain Se contents. Finally, for Spitfire, stem and leaf Se contents showed a significant difference (**p < 0.01 and ***p < 0.001, respectively) with SeVI and S.

The different doses of SeVI and S influenced the stem, leaf, and grain S contents. In the case of Condo, there was no significant difference in stem and grain S contents

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**Table 3** Quality control for Se and S based on SRM standards

| SRM          | Certified values (mg kg⁻¹) | Observed values (mg kg⁻¹) | Certified values (mg kg⁻¹) | Observed values (mg kg⁻¹) |
|--------------|----------------------------|---------------------------|----------------------------|---------------------------|
| Se           | 0.1152 ± 0.0043            | 0.088 ± 0.0040            | 0.5                         | 0.9                        |
| Trace elements in Spinach leaves | 0.365 ± 0.029 | 0.414 ± 0.022 | 1200 ± 10 | 1335 ± 11 |

*Information only*
between all treatments (Fig. 2d). However, the interaction of Se and S increased the leaf and grain S content significantly (*p < 0.05 and **p < 0.01, respectively). On the other hand, there were no significant differences in the stem and grain S contents for the Longsword variety, while there were substantial increases (**p < 0.001) in leaf S contents between Se only and Se+S treatments. Spitfire did not show any significant difference in grain sulfur content with any Se or S doses. The result showed that most of the Se accumulated in leaves in all of the wheat cultivars. On the other hand, Condo accumulated more S in the grain.

In terms of the mass of Se in grain, all of the three cultivars of wheat showed a significant difference between the treatments compared to the control (Fig. 3). The mass of Se in grain increased significantly (**p < 0.01) with increasing of Se doses compared to the control in all cultivars. The addition of S to all Se treatments in Condo significantly increased the mass of Se in grain (**p < 0.01). Indeed, even in the S-control treatment, the Se mass in grain was significantly increased in both Condo and Spitfire (*p < 0.05 and ***p < 0.001, respectively). On the other hand, Longsword did not show any significant differences in the mass of Se in grain. The mass of Se in the grain of the Spitfire cultivar increased significantly (**p < 0.001) with the increasing of Se and S addition. The highest mass of Se in grain was observed in the Se 2+S treatment for all three cultivars (0.6, 0.2, and 0.9 g, respectively).

3.4 Selenium Speciation in Wheat Grain

The percentage of each Se species in grain after enzymatic digestion of wheat grains is shown in Fig. 4. Three species were quantifiable, including SeCys, SeMeCys, and SeVI. Two unidentified large peaks were found at 12 to 14 min retention time under all treatments (Fig. 4b and c).

The percentage of Se species in grain showed significant differences (**p ≤ 0.001) with Se and S amendment compared to control treatments (Fig. 5). In terms of the Condo variety, the highest percentage (79.8%) of selenocysteine (SeCys) was found in control and S only treatments. However, it was slightly decreased in the Se 1 treatment and
further increased with the increased doses of Se and S. Selenate accounted for only 1 to 6% and the highest percentage (6%) of selenate was observed in the Se 1 treatments. The monomethylated form, SeMeCys, was found around 20 to 39.5% in Condo; the highest percentage (39.5%) was observed in the Se 1 treatment. The control and highest dose of Se + S showed a lower proportion of SeMeCys.

The greatest quantities of SeMeCys were present in Longsword. Exposure to S modified the Se species, specifically SeMeCys in the grain. The interaction of S and Se significantly (**p < 0.001) increased the SeMeCys content in grain (Fig. 5b). The increased presence of S reduced the total amount of SeMeCys, but also the conversion of SeVI to SeMeCys. The ratio SeVI/SeMeCys expresses the conversion.

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Fig. 2  Se and S content of wheat influenced by different doses of Se and S. a, b, and c (left) represent the Se content, and d, e, and f (right) represent the S content of three different cultivars of wheat are Condo, Longsword, and Spitfire, respectively. Control, Se 1, and Se 2 denote the no treatment, 1 mg kg−1, and 2 mg kg−1 SeVI, respectively. S means 14 mg kg−1 sulfur. Error bar represents the standard error (n = 5).
of SeVI to SeMeCys in the grain. The ratio was not related to Se concentration. In Longsword, the presence of S reduced the conversion of Se to SeMeCys (***p < 0.0001).

The highest percentage of SeCys (74%) was found in the control of Longsword, followed by Se 2 and Se 2 + S treatments and the lowest percentage was observed in Se 1 + S treatment. The SeCys concentration was dependent on Se and S concentrations in addition to cultivar. In the highest Se application, S significantly reduced SeCys across the three varieties. Spitfire produced significantly higher SeCys in grain (**p < 0.0001). In this variety, SeCys represented up to 97% in the Se 2 treatment. In Spitfire, SeMeCys and SeVI were 4.5% and 7%, respectively (Fig. 5c). Only around 7% selenate was found in Se 2 + S treatment. Selenocysteine was the dominant species in all grains. Total Se was very strongly correlated to SeCys (r = 0.99, ***p < 0.0001, n = 72). The SeCys/SeVI conversion ratio was not related to cultivar but was significantly impacted by Se and S. At Se 2, there was significant enhancement in the production of SeCys relative to SeVI. The application S significantly reduced the SeVI/SeCys ratio (**p = 0.0077), similarly to SeMeCys, indicating S not only reduced the accumulation of Se in grain, but also inhibited the conversion to beneficial selenoproteins. Indeed, sorption indicated that application of S enhanced solubility in the soil environment via competition adsorption for adsorption sites. Thus, despite the enhanced solubility of SeVI, sulfate significantly reduced the conversion of SeVI to organic forms of Se in grains.

4 Discussion

Biofortification of food crops with Se is a potential method to supply adequate Se nutrition to humans (Pyrzynska 2009). In this study, we found an increasing concentration of Se in plant parts after Se fertilization in soil. Our findings from the sorption study are consistent with the previous results that demonstrated notable suppression of adsorption, thus increasing SeVI solubility in soil solution (Dhillon and Dhillon 2000). Our findings suggested that lower doses of Se (< 2 mg kg⁻¹) decrease plant biomass, which is consistent with previous reports (Guerrero et al. 2014). Similar results have been found with a number of plant species such as wheat (Boldrin et al. 2016), lettuce (Ramos et al. 2011), rice (Boldrin et al. 2013), and ryegrass (Hartikainen et al. 2000). The addition of Se and S increased the grain biomass up to 30% compared to control in the Spitfire cultivar; this has likely occurred due to the presence of S. Boldrin et al. (2013) reported that S increased the protein content of wheat grain. Feng et al. (2013) and Kaur et al. (2014) observed that low concentrations of Se can act as an antioxidant, abiotic stress modulator, anti-senescent, and defensive molecule against pathogens, thereby promoting plant growth. In addition, Se also increased root growth and cell elongation in the root (Silva et al. 2020). Some researchers also observed increases in shoot biomass after Se supplementation in wheat crops (Muhammad et al. 2018).

SeVI application in wheat increased the Se concentration in stem, leaf, and grain (Fig. 2). The interaction of S and SeVI in wheat showed varied results upon the cultivars. The reduction of Se accumulation in the stem and leaf of Condo and Spitfire due to the antagonistic behavior of S most likely occurred at the plasma membrane. Sulfate and SeVI are not expected to interact with cell walls of plant roots, and given the shared S transporter systems, would compete at the transport across the membrane. The applied S in soil predominantly increased the S content in leaf of Longsword and Spitfire. Boldrin et al. (2018) reported that the rise in leaf S content to the improved expression of the genes related to transport proteins present in the plant roots. However, Liu et al. (2017) observed that the application of S significantly increased the available S content of soil treated with Se species and S fertilizer can influence the uptake of Se in crops through different regulatory effects. Previous research from hydroponic, pot, and field-based studies have shown contrasting results in terms of the influence of S on Se uptake in different crops (Li et al. 2008; Liu et al. 2015; Cartes et al. 2006; Golob et al. 2016; Stroud et al. 2010). Field experiments by Stroud et al. (2010) on wheat showed that S (in the form of sulfate) increased the uptake of Se in grain by 62%.
Seleno-cysteine was the dominant Se species in all cultivars, particularly the Spitfire cultivar, which disagrees with past reports (Hart et al. 2011; Cubadda et al. 2010). Seleno-cysteine was strongly correlated to total Se ($r = 0.997, n = 72$), representing the dominant chemical species in wheat grain. Wheat grown in India was dominated by SeMet, accounting for 72–85% of Se (Cubadda et al. 2010). Hart et al. (2011) reported that the SeMet accounted for 65–87% of total extractable Se species in wheat bread and flour. In our study, the proportion of SeCys decreased with the exposure of S. Selenium substitutes within the S position of amino acids of proteins. The fertilization of S appeared to reduce Se incorporation into selenoproteins, reducing the efficiency of selenate conversion to SeCys, which was also observed in SeMeCys (Cubadda et al. 2010; Duncan et al. 2017).

Methyl-Se-cysteine was of principal interest due to its association as an anticarcinogenic agent in humans and animals (Ip and Ganther 1992). The mixture of organic Se compounds that we have found in the wheat grain may be of added nutritional value compared to other Se-enriched food and feed supplements, mainly containing SeMeCys. The presence of SeMeCys in the grain suggests that human food products such as wheat flour or other food products and animal feed made with this Se-enriched wheat grain might have added health benefits such as cancer prevention. Literature showed two anticarcinogenic forms of Se are S-methyl cysteine.
SeMeCys is reportedly one of the least toxic forms of Se and one of the two most operative anticarcinogenic forms of Se (Ip and Ganther 1992; Zayed et al. 2000). Our study also found inorganic Se species selenate (SeVI) below 10%, which agrees with the previous findings (Hart et al. 2011). These results suggest that wheat plants grown with Se and Se-enriched soils are a good source for producing SeMeCys, an effective anticarcinogenic form of Se, for use in Se-enriched biofortified foods. Sulfate fertilization showed in this study caused a significant reduction in SeMeCys production in wheat grain, particularly the multi-tillered variety. As a result of the decrease in SeMeCys and SeCys with S fertilization, caution should be taken with S fertilization during Se fertilization. There was a great impact of sulfate at higher Se fertilization rates. In soils not fertilized with SeVI, sulfate fertilization did cause any antagonistic reductions in SeMeCys and SeCys in grain.

5 Conclusion

The present study examined the influence of sulfate fertilization on the biofortification of Se in three wheat cultivars. The results showed significant differences between cultivars both in terms of Se accumulation in grain and its speciation. Similarly, sulfate fertilization influenced the quantity and speciation of Se in wheat grains. In the Condo cultivar, S uptake was highest in the grain, followed by leaf and stem. Selenium in grain, mostly present in organic forms, Spitfire cultivar grain contained 97% SeCys and Longsword cultivar grain accumulated...
70% SeMeCys. Sulfate fertilization had an antagonistic impact on the conversion of SeVI to desirable organic forms, including SeCys, and importantly, SeMeCys. The results of the current study suggest that increasing the S supply negatively influences the bioavailability of Se in various plant tissues and alters the chemical species of Se in wheat grain.

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Declarations

Competing Interests The authors declare no competing interests.

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