





RESEARCH ARTICLE

# Selenium accumulation in grains of wheat cultivars grown in selenium-rich areas in China

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## Summary

Selenium (Se) is an essential micronutrient for human health, and Se concentration of wheat grain in China has no significant relationships with selenium concentration of wheat and with soil organic matter, nitrogen, phosphorus, potassium in the 0–20 cm soil layer. However, a significant indigenous positive correlation was found with soil Se concentration. Field experiments were conducted from 2018 to 2020 to clarify the differences in the Se accumulation in wheat plants grown in Se-rich areas. We used two common wheat (ZM-175, SN-20), two purple wheat (JZ-496, ZM-8555), and two black wheat (YH-161, LH-131) cultivars to investigate changes in Se build-up and transportation in plant organs. The grain Se concentration of six wheat genotypes in Se-rich areas varied between 178 and 179  $\mu\text{g Se kg}^{-1}$ , with organic Se accounting for 87 to 91%. All genotypes had more than 150  $\mu\text{g Se kg}^{-1}$ , the standard Se concentration in grains. Purple grain wheat had the highest total and organic Se concentrations. Purple wheat also exhibited significantly higher Se transfer coefficient in roots, stem and leaves, and glumes, when compared to common wheat. Moreover, purple wheat had the highest Se uptake efficiency (e.g., JZ-496 with 31%) when compared to common wheat and black wheat. Regardless of the color, wheat grains met the Se-enriched criteria (150  $\mu\text{g Se kg}^{-1}$ ) when grown in a natural Se-enriched area. Due to higher Se uptake and accumulation, purple wheat grain genotypes, such as JZ-496, are recommended for wheat breeding programs aiming for high Se functional foods.

**Keywords:** Enrichment coefficient; grain selenium concentration; organic selenium; selenium-rich area; transfer coefficient

## Introduction

Selenium (Se) is an essential micronutrient for human health, and it plays a critical role as antioxidant, anticancer, antibacterial, and antiviral (Lyons, 2018). About 1 billion people worldwide do not get sufficient dietary Se and edible plant organs can help humans to achieve their Se requirements (Combs, 2001; Tan et al., 2002). The amount of Se in food was mainly linked to the amount of Se in the environment, especially in soil and water. In 2015, the United States Geological Survey (USGS) reported that Chile, Russia, Peru, the USA, and Canada were among the nations having relatively rich Se resources on the globe (USGS, 2015). The amounts of Se in soil vary widely across the world with most soils containing between 10 and 2,000  $\mu\text{g Se kg}^{-1}$  (Kabata-Pendias, 2001). Soils containing more than 3,000  $\mu\text{g Se kg}^{-1}$  were mostly found in North

America, Western Europe, and Venezuela (Temmerman *et al.*, 2014). On the other hand, dietary Se levels are low in many regions of China, New Zealand, and Europe (Kipp *et al.*, 2015).

In fact, soils with low Se concentration are found in 72% of the total area in China (Gao *et al.*, 2011). Se concentrations in most food crops produced in China are usually less than 60 mg Se kg<sup>-1</sup> (Williams *et al.*, 2009), which causes Se deficiency and negative impacts on human health. Soils in China can be classified into four types based on total Se concentration: 200 µg Se kg<sup>-1</sup> as low Se soil; 200 to 400 µg Se kg<sup>-1</sup> as medium Se soil; 400 to 3,000 µg Se kg<sup>-1</sup> as high Se soil; and >3,000 µg Se kg<sup>-1</sup> as excessive Se soil (Tan *et al.*, 1993). The general guidelines for nutritional labeling of prepackaged foods of the National Food Safety Standard (GB28050-2011) of China indicate that foods rich in Se can be labeled when Se is greater than 150 µg Se kg<sup>-1</sup>. However, some studies have shown that Se concentration exceeding 300 µg Se kg<sup>-1</sup> can lead to food safety hazard to humans (Graham *et al.*, 2010; GB/T22499-2008).

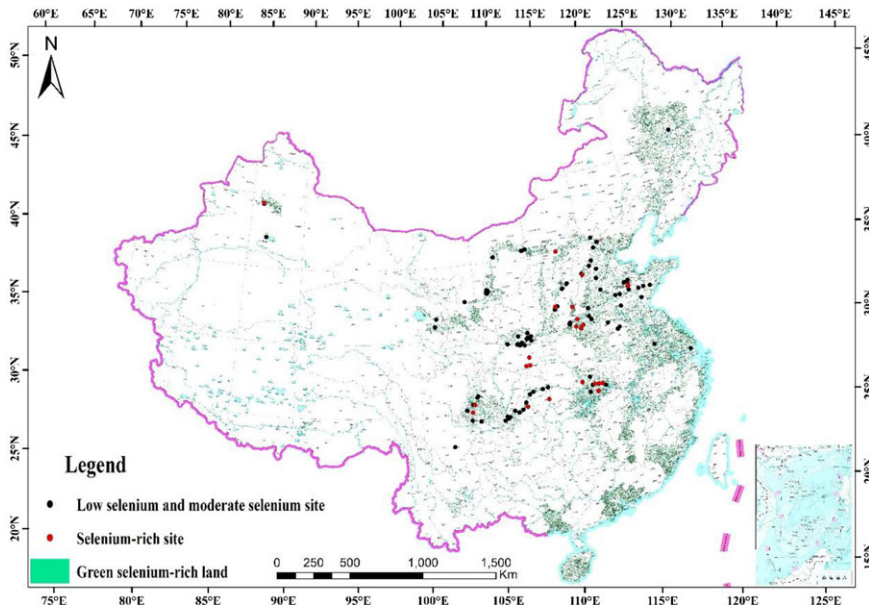
As one of the world's most important staple food crops, enriched Se wheat is an effective way to increase Se intake. According to previous reports, grain Se concentration in wheat ranges from 1 µg Se kg<sup>-1</sup> in south-west Western Australia to 30 000 µg Se kg<sup>-1</sup> in areas of South Dakota, while in most of the world, it lies between 20 and 600 µg Se kg<sup>-1</sup> (Lyons *et al.*, 2005). About 63% of wheat produced in China contains insufficient Se, with about 65 µg Se kg<sup>-1</sup> in grains (Li *et al.*, 2020). Colored wheat is a pure natural material for food and a large number of genotypes have been identified to contain high calcium, iron, zinc, Se, and other beneficial trace elements for humans. Wheat cultivars with unique hues, such as blue and purple grains, are also high in protein, amino acids, trace elements, and minerals (Xia *et al.*, 2020). Then, colored wheat is an alternative to provide high-value-added functional foods due to its high nutritional value and antioxidant pigments.

Se-rich areas offer a distinct opportunity as they promote natural Se-rich crops (Ren *et al.*, 2016). Planting crops in natural Se-rich locations produces higher-quality Se-rich goods, with low cost effective and environment impact (Liao *et al.*, 2016). However, the mechanisms underlying the enrichment of Se in colored grain wheat genotypes need further investigation. In this context, field trials were conducted in a natural Se-enriched area in order to clarify the differences in Se enrichment ability among color grain wheat genotypes. We evaluated the Se concentration in the 0–20 cm soil layer in the major wheat growing regions in China, and also the relationships between soil and grain Se concentrations, and differences in Se uptake and use among wheat varieties. Finally, the aim was to provide the theoretical basis for the production of naturally enriched Se grains by wheat plants.

## Materials and Methods

### ***Relationship between soil Se concentration and grain Se concentration in wheat***

We conducted a literature search in Web of Science (<https://webofknowledge.com/>) and China National Knowledge Infrastructure (CNKI, <https://cnki.com.cn/>) databases with the keywords: 'Wheat' and 'selenium' and 'China'. We obtained 152 papers about soil Se concentration and wheat grain Se concentration published in China in the last 20 years. The retrieved articles were selected for inclusion by the following criteria: (1) the test site is in China; (2) plants were grown in soil media, including both field trials and pot experiments; (3) the experimental designs had no exogenous Se addition; (4) total soil Se concentration was evaluated; and (5) the basic physical and chemical properties of soil, soil Se concentration, and wheat grain Se concentration were all reported. After this initial screening, 57 articles met the requirements. The experimental sites, soil Se concentration, grain Se concentration, soil pH, soil organic matter, and total N were extracted from the selected literature and data presented in charts after digitization using GetData Graph Digitizer 2.24 (<http://www.getdata-graph-digitizer.com/>). The data were summarized to analyze the effects of soil Se concentration on wheat grain Se concentration. The distribution of test sites in China is shown in Figure 1.



**Figure 1.** Spatial distribution of survey data in China. The distribution map of green Se-rich land resources in China, provided by the Institute of Geophysical and Geochemical Exploration of the Chinese Academy of Geological Sciences. The map shows a total area of 166 993 km<sup>2</sup> of green Se-rich soil, accounting for only 10% of China's land resources. According to the 'local disease and environment atlas of the People's Republic of China' (Tan, 1989), Se-rich land has higher than 400  $\mu\text{g Se kg}^{-1}$  in topsoil. The Se-rich area is represented by red points, and the other areas are represented by black points.

### Experimental site and design

Field experiments were conducted for two seasons (2018–2019 and 2019–2020) in Hongtong County, Shanxi Province, China (36°31'N, 111°65' E). Hongtong test area is located in the west of Huang-Huai-Hai basin, and belongs to Huang-Huai winter wheat area, with a warm temperate semihumid continental monsoon climate. The site has an altitude of 460 m, frost-free period of 210 days, an annual average air temperature of 12.1°C, and annual average precipitation of 441.5 mm. Planting mode is a typical winter wheat-summer corn system, in which winter wheat is sown in mid-October and harvested in early June of the second year, while corn is sown in mid-late June and harvested in early October of the same year, with all straw crushed and returned to the field. The soil is a Se-rich loam type. Soil Se concentration and pH, organic matter, total and available N, available P, and available K in 20 cm soil layer were evaluated on 3 October 2018 and 5 October 2019 (Table 1). In both growing seasons, the soil Se concentration reached the Se enrichment standard, reaching 609 and 620  $\mu\text{g Se kg}^{-1}$  in the first and second season, respectively.

The experiment was set up as a single-factor randomized block design with six cultivars of *Triticum aestivum* L: Zhongmai-175 (ZM-175, common wheat); Shannong-20 (SN-20, common wheat); Linhei-131 (LH-131, black grain); Yunhei-161 (YH-161, black grain); Jizi-496 (JZ-496, purple grain); and Zimai-8555 (ZM-8555, purple grain). The plot area for each wheat genotype was 30 m<sup>2</sup> (3 × 10 m) and was repeated three times. Wheat seeds were cultivated in a low Se soil (102  $\mu\text{g Se kg}^{-1}$ ). We measured seed Se concentration before sowing for evaluating the difference of selenium concentration between presowing seeds and harvested seeds. The before-sowing selenium Se concentrations ( $C_0$ ) for LH-131, YH-161, JZ-496, and ZM-8555 seeds were 24, 35, 59, 51, 75, and 63  $\mu\text{g Se kg}^{-1}$ , respectively.

All corn straw was returned to the field after harvesting in the previous two experimental years. Wide-space sowing (row spacing 25 cm, seedling bandwidth 8 cm) was used. Before sowing, 126 kg N ha<sup>-1</sup>, 150 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and 90 kg K<sub>2</sub>O ha<sup>-1</sup> were applied and basic seedling was

**Table 1.** Basic soil physical and chemical properties of the experimental site

| Year      | Selenium concentration in soil ( $\mu\text{g}/\text{kg}$ ) | pH  | Organic matter ( $\text{g}/\text{kg}$ ) | Total nitrogen ( $\text{g}/\text{kg}$ ) | Available nitrogen ( $\text{mg}/\text{kg}$ ) | Available phosphorus ( $\text{mg}/\text{kg}$ ) | Available potassium ( $\text{mg}/\text{kg}$ ) |
|-----------|--|-----|---|---|--|--|---|
| 2018–2019 | 609  | 8.1 | 13.2                                    | 0.8                                     | 42.1   | 17.5   | 209.5   |
| 2019–2020 | 620  | 8.0 | 13.1                                    | 0.8                                     | 42.5   | 17.7   | 203.5   |

Five points sampling method was used to collect soil before sowing each year, and the average value of five sampling sites was calculated.

$315 \times 10^4$  plants  $\text{ha}^{-1}$ . Flood irrigation (approximately  $60 \text{ m}^3 \text{ ha}^{-1}$  each time) was performed before wintering, at the jointing stage, and at the anthesis stage. An additional  $84 \text{ kg N ha}^{-1}$  was applied at the jointing stage of wheat. Weeds, pests, and diseases were controlled according to the field practice in Hongtong.

### **Sample collection, preparation, and yield determination**

At maturity (250 days after planting), 20 representative plants were sampled and cleaned with deionized water. The plants were dried to constant weight at  $75^\circ\text{C}$  (GFL-230, Blabotery, Tianjin, China) and separated into root, stem and leaf, glume, and grain. The dry matter of each part was weighed and crushed (Miniature plant sample mill, FZ102, Beijing Ever Bright Medical Treatment Instrument Co. Ltd., China). Also, at maturity, wheat plants were randomly harvested in  $10 \text{ m}^2$  from each plot to evaluate grain yield. Grain moisture concentration was determined using a grain moisture meter (PM-8188-A, KETT, Japan), and the actual grain yield was calculated using the national grain storage standard moisture concentration conversion (13%).

### **Total, inorganic, and organic Se concentration and related factors and efficiency**

The total Se concentration was determined in wheat samples ( $0.3 \text{ g}$ ), which were digested with a  $10 \text{ mL HNO}_3$ , using a microwave digestion apparatus (CEM-Mars One, USA). After cooling, the samples were filtered, and the volume was corrected with deionized water to  $10 \text{ mL}$ . Using hydride generation atomic fluorescence spectroscopy, the Se concentration of plant samples was measured with an atomic fluorescence photometer (AFS-9780, Beijing) according to the Chinese National Standard Methods (GB/T35876-2018). The measuring lamp used in the instrument is a hollow cathode lamp containing Se, and the instrument was set as: negative high pressure  $340 \text{ V}$ ; lamp current  $100 \text{ ma}$ ; primordial temperature  $800^\circ\text{C}$ ; furnace height  $8 \text{ mm}$ ; carrier gas flow rate  $500 \text{ mLmin}^{-1}$ ; shielding gas flow rate  $1,000 \text{ mLmin}^{-1}$ ; standard curve method of measurement; reading mode peak area; delay time  $1 \text{ s}$ ; reading time  $15 \text{ s}$ ; filling time  $8 \text{ s}$ ; and injection volume was  $2 \text{ mL}$ .

Inorganic Se concentration was evaluated in the same wheat samples, which were extracted in a water bath with hydrochloric acid ( $6 \text{ molL}^{-1}$ ). Samples were prepared according to DB36/T 1243-2020 and the same method used for total Se was applied. The difference between the total Se concentration and the inorganic Se concentration was the organic Se concentration (DB36/T 1243-2020).

The bioconcentration factor (BCF) and transfer factor (TF) of Se from soil to aboveground plant parts were estimated as follows (Salt *et al.*, 1995):

$$\text{BCF}_{\text{Se}} = C_{\text{plant}}/C_{\text{soil}} \quad (1)$$

where  $\text{BCF}_{\text{Se}}$  means BCF of Se in wheat plants;  $C_{\text{plant}}$  means Se concentration in wheat plants ( $\mu\text{gkg}^{-1}$ ); and  $C_{\text{soil}}$  means Se concentration in soil ( $\mu\text{gkg}^{-1}$ ).

$$TF_{\text{plant-grain}} = C_{\text{grain}}/C_{\text{plant}} \quad (2)$$

where  $TF_{\text{plant-grain}}$  means TF from wheat organs to grain;  $C_{\text{grain}}$  means Se concentration in wheat grains ( $\mu\text{g kg}^{-1}$ ); and  $C_{\text{plant}}$  means Se concentration in organs of wheat plants ( $\mu\text{g kg}^{-1}$ ).

The efficiency of wheat grain in uptaking soil Se (AE) was estimated as follows (Delaqua et al., 2021):

$$AE(\%) = (C_{\text{Se}} - C_0)/C_{\text{soil}} \times 100 \quad (3)$$

where  $C_{\text{se}}$  is Se concentration in wheat grains ( $\mu\text{g kg}^{-1}$ );  $C_0$  is Se concentration in wheat grain before sowing ( $\mu\text{g kg}^{-1}$ ); and  $C_{\text{soil}}$  is soil Se concentration ( $\mu\text{g kg}^{-1}$ ).

### Statistical analysis

The spatial distribution map of the survey data sites was prepared using Arc GIS10.2 software (Environmental Systems Research Institute, Inc., Red Lands CA, USA). The other data were processed using Excel-2013 software (Microsoft Corp., Redmond WA, USA) and figures were prepared with Sigma Plot 12.5 software (Syst at Software Inc., San Jose CA, USA). The data are the average value  $\pm$  standard error. SPSS-22.0 statistical analysis software (IBM Corp., Armonk NY, USA) was used, and least significant difference (LSD) method was applied to compare cultivars at  $\alpha = 0.05$ .

## Results

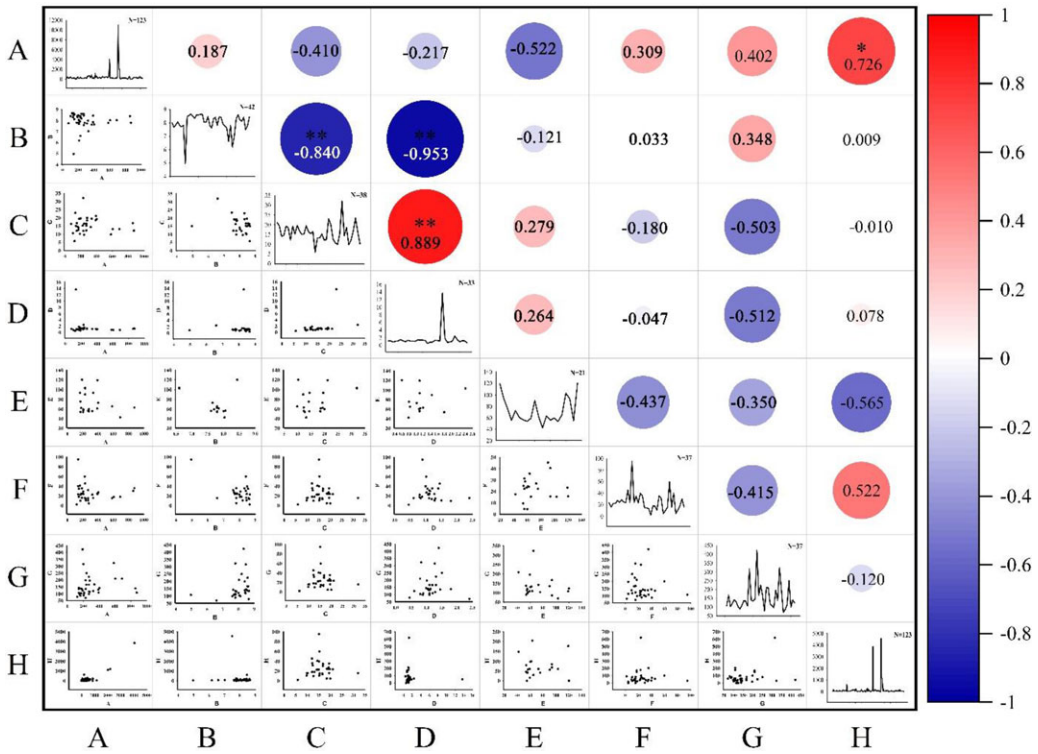
### Selenium: soil and grain

A total of 57 papers were selected for comparing Se concentrations in soil and wheat grain (Fig. 2). Grain Se concentration was positively correlated with soil Se concentration. However, nonsignificant correlations were found between wheat grain Se concentration and soil organic matter, total N, alkali-hydrolyzable N, available P, or available K in the topsoil layer. The test sites in Changzhi City, Shanxi Province, Hongtong County, Shanxi Province, and Xiantao City, Hubei Province, were found to be in Se-rich areas and to be in compliance with the Se-rich wheat grain standard (Fig. 3). Soil Se concentrations ranged from 0.5 to 11 020  $\mu\text{g Se kg}^{-1}$ , with an average of 432  $\mu\text{g Se kg}^{-1}$  and a coefficient of variation (CV) of 246%. Soil Se concentration was lower than 400  $\mu\text{g Se kg}^{-1}$  in 77.2% of samples. Moreover, 21.1% and 1.6% of the samples showed more than 400 and 3,000  $\mu\text{g Se kg}^{-1}$ , respectively. Grain Se concentrations ranged from 0 to 4,570  $\mu\text{g Se kg}^{-1}$ , with an average of 158  $\mu\text{g Se kg}^{-1}$  and CV of 346%. Grain Se concentration below 150  $\mu\text{g Se kg}^{-1}$  made up 86.2% of samples, while grain Se concentration between 150 and 300  $\mu\text{g Se kg}^{-1}$  was found in 9.8% of samples, and more than 300  $\mu\text{g Se kg}^{-1}$  in only 4.1% of all samples. The Se-rich region Hongtong County in Shanxi Province with 874  $\mu\text{g Se kg}^{-1}$  was thus selected in this experiment and the harvested wheat grain also fulfilled the Se-rich standard, with 206  $\mu\text{g Se kg}^{-1}$ .

### Wheat cultivars growing in Se-rich area

Grain Se concentration was greater than 150  $\mu\text{g Se kg}^{-1}$  with an average of 178 and 179  $\mu\text{g Se kg}^{-1}$  in 2018–2019 and 2019–2020 growing seasons, respectively (Table 2). Purple wheat genotypes (JZ-496 and ZM-8555) had the highest Se concentration, followed by black wheat (YH-61 and LH-131) and common wheat (ZM-175 and SN-20) genotypes. The highest grain Se concentration was found in JZ-496, reaching more than 190  $\mu\text{g Se kg}^{-1}$  in both seasons. In the Se-enriched area, the purple grain wheat had a greater organic Se concentration and proportion than the other grain colors (Table 2). The organic form of Se in wheat grains was the main one, which accounted for 85–95% in 2018–2019 and 83–94% in 2019–2020 when considering the total Se concentration (Table 2).





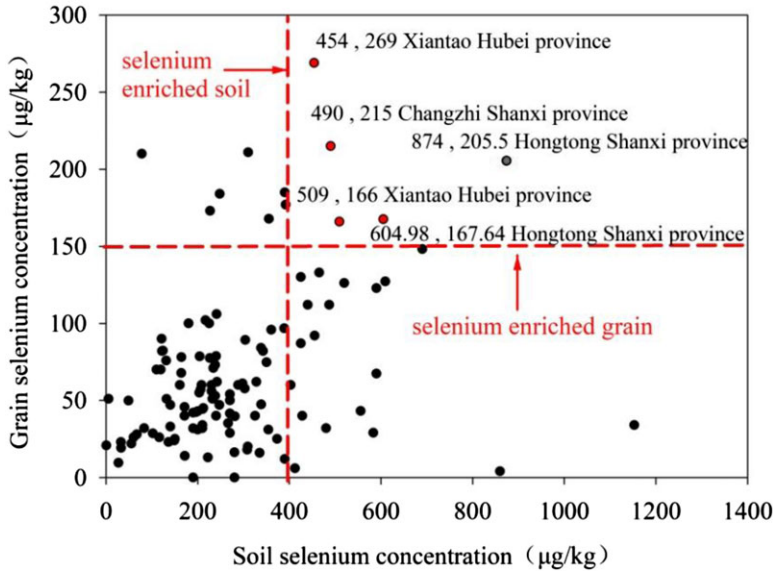
**Figure 2.** Correlation analysis between Se content in wheat grain and soil physical and chemical properties. (a) soil Se concentration; (b) soil pH; (c) soil organic matter; (d) total soil nitrogen; (e) alkali-hydrolyzable soil nitrogen; (f) available soil phosphorus; (g) available soil potassium; and (h) grain Se concentration. \*  $p < 0.05$ ; \*\* $p < 0.01$ .

The highest root Se concentration was found in common wheat, ZM-175 in 2018–2019 and SN-20 in 2019–2020 (Fig. 4a). The majority of root Se is organic, accounting for 76.7% of total Se in 2018–2019 and 75.4% in 2019–2020 (Fig. 4b, c). Purple (JZ-496, ZM-8555) and black (YH-161, LH-131) grain wheat cultivars had significantly greater organic Se concentration in roots than common wheat (ZM-175, SN-20).

SN-20 cultivar had the greatest Se concentration in stems and leaves in 2019–2020 with  $155 \mu\text{g Se kg}^{-1}$  (Fig. 5a). JZ-496 had the highest absolute proportion of organic Se in the stems and leaves (Fig. 5b, c). The highest Se concentration of the glume shell was found in ZM-175(2018–2019) and SN-20 (2019–2020), two common wheat cultivars (Fig. 5d). The majority of Se in wheat glume was the organic one (Fig. 5e, f).

Common wheat cultivars ZM-175 and SN-20 had the highest root Se enrichment coefficients in 2018–2019 and 2019–2020, respectively (Table 3). Overall, the highest transfer Se coefficient from root to grain was found in purple and black grain cultivars, ranging between 1.67 and 1.87 in 2018–2019 and between 1.58 and 1.67 in 2019–2020. The variety had little effect on the Se enrichment ability of wheat stem, leaf, and glume. Purple (JZ-496 and ZM-8555) and black (YH-161 and LH-131) grain cultivars had significantly greater translocation coefficients than common wheat (ZM-175 and SN-20), when considering both stem+leaves to grain and glume to grain (Table 3).

The highest grain Se absorption efficiency was noticed in JZ-496, while ZM-175 had the lowest efficiency (Fig. 6a). The grain yields of ZM-175 and SN-20 were significantly higher than those of JZ-496, ZM-8555, YH-161, and LH-131 (Fig. 6b). Taken together, our data revealed a trade-off between Se absorption efficiency and yield, with colored wheat cultivars showing higher efficiency and lower yield than common wheat cultivars.



**Figure 3.** Correlation between Se concentration in wheat grain and soil. Red dashed lines indicate grain and soil Se concentrations of 150 and 400  $\mu\text{g Se kg}^{-1}$ , respectively. Red symbols Se-enriched grains in Se-enriched soils. Black symbols represent other survey areas. The numbers indicate the corresponding soil and grain Se concentrations in a given place, respectively. According to the general rules of nutritional labeling for prepackaged food (GB28050–2001), Se concentration (DB61/T556–2012) in Se-rich food and related products should be 150 to 300  $\mu\text{g Se kg}^{-1}$ . According to the atlas of endemic illnesses and the environment of the People's Republic of China, 400  $\mu\text{g Se kg}^{-1}$  is adopted as the lower limit for Se-rich areas.

## Discussion

### **Selenium concentration in topsoil and in wheat grain**

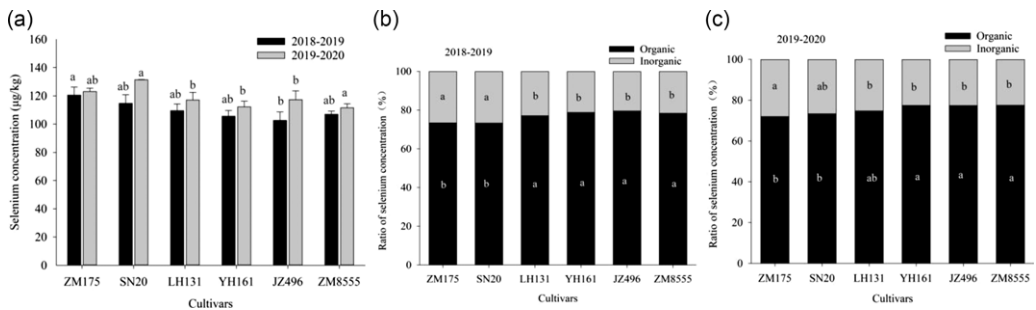
The Se concentration in wheat grains in this study ranged from 0 to 4,570  $\mu\text{g Se kg}^{-1}$  with an average of 158  $\mu\text{g Se kg}^{-1}$ , which was lower than in the USA and Canada. In fact, North America wheat grain Se levels were reported to be high, with wheat grain Se concentration ranging from 20 to 3,120  $\mu\text{g Se kg}^{-1}$  and presenting 457  $\mu\text{g Se kg}^{-1}$ , on average (Hahn et al., 1981). Hard red spring wheat grain Se levels ranged from 25 to 3,760  $\mu\text{g Se kg}^{-1}$  in western Canada, with average of 400  $\mu\text{g Se kg}^{-1}$  (Gawalko et al., 2002). Herein, 86.2% of sampled wheat grains did not meet the Se enrichment standard. As an explanation, differences in Se concentration among studies could be due to the wheat varieties used. However, the influence of soil Se content on wheat grain Se concentration is much higher than the effect induced by differences among cultivars (Lyons et al., 2005). Many major wheat-producing locations in North America have Se-rich soil (Adams et al., 2002), with higher soil Se concentration than in key wheat-producing areas in China, a likely primary cause of low grain Se concentrations.

Some studies have proved that the ability of plants to uptake and accumulate Se is closely related to soil Se concentration (Thavarajah et al., 2011; Williams et al., 2009; Zhu et al., 2009), which was further supported by a substantial positive association between Se concentration in wheat grains, and Se concentration in topsoil of wheat-producing areas in China. The distribution of Se in soil is very uneven in various regions of China, especially in the topsoil, which plays a key role in crop growth (König et al., 2012). In this study, we found that the soil Se concentration in 0–20 cm soil layer of each experimental site in wheat region of China presented a large variation, from 0.5 to 11 020  $\mu\text{g Se kg}^{-1}$ , averaging 432  $\mu\text{g Se kg}^{-1}$ . Importantly, the investigation scope of this paper is relatively small and relevant for the topsoil layer in wheat areas of China.

**Table 2.** Differences in grain selenium concentration, selenium speciation concentration, and its proportion of wheat varieties with different grain colors

| Year      | Cultivars | Grain Se concentration (µg/kg) | Organic selenium          |                               | Inorganic selenium       |                               |
|-----------|-----------|--------------------------------|---------------------------|-------------------------------|--------------------------|-------------------------------|
|           |           |                                | Se concentration (µg/kg)  | Ratio of Se concentration (%) | Se concentration (µg/kg) | Ratio of Se concentration (%) |
| 2018–2019 | ZM175     | 163.9 ± 0.9 <sup>c</sup>       | 138.7 ± 3.7 <sup>c</sup>  | 84.6 ± 1.8 <sup>bc</sup>      | 25.2 ± 2.9 <sup>a</sup>  | 15.4 ± 1.8 <sup>a</sup>       |
|           | SN20      | 165.7 ± 1.7 <sup>c</sup>       | 144.3 ± 1.3 <sup>c</sup>  | 87.1 ± 1.6 <sup>bc</sup>      | 21.4 ± 2.8 <sup>ab</sup> | 12.9 ± 1.6 <sup>ab</sup>      |
|           | LH131     | 181.8 ± 0.2 <sup>b</sup>       | 165.8 ± 0.4 <sup>b</sup>  | 91.2 ± 0.2 <sup>ab</sup>      | 16.0 ± 0.4 <sup>bc</sup> | 8.8 ± 0.2 <sup>bc</sup>       |
|           | YH161     | 181.4 ± 0.7 <sup>b</sup>       | 165.5 ± 1.9 <sup>b</sup>  | 91.2 ± 1.0 <sup>ab</sup>      | 15.9 ± 1.8 <sup>bc</sup> | 8.8 ± 1.0 <sup>bc</sup>       |
|           | JZ496     | 190.6 ± 0.5 <sup>a</sup>       | 181.0 ± 3.7 <sup>a</sup>  | 95.0 ± 2.0 <sup>a</sup>       | 9.6 ± 3.8 <sup>c</sup>   | 5.0 ± 2.0 <sup>c</sup>        |
|           | ZM8555    | 183.7 ± 0.6 <sup>b</sup>       | 173.5 ± 2.5 <sup>ab</sup> | 94.5 ± 1.5 <sup>a</sup>       | 10.2 ± 2.7 <sup>c</sup>  | 5.53 ± 1.5 <sup>c</sup>       |
| 2019–2020 | ZM175     | 163.9 ± 1.9 <sup>d</sup>       | 137.9 ± 0.8 <sup>d</sup>  | 83.6 ± 0.1 <sup>c</sup>       | 27.0 ± 0.1 <sup>a</sup>  | 16.4 ± 0.1 <sup>a</sup>       |
|           | SN20      | 166.6 ± 1.7 <sup>d</sup>       | 138.3 ± 2.0 <sup>d</sup>  | 83.0 ± 0.4 <sup>c</sup>       | 28.4 ± 0.6 <sup>a</sup>  | 17.0 ± 0.4 <sup>a</sup>       |
|           | LH131     | 183.6 ± 0.2 <sup>bc</sup>      | 165.1 ± 2.0 <sup>c</sup>  | 89.9 ± 1.1 <sup>b</sup>       | 18.5 ± 2.0 <sup>b</sup>  | 10.1 ± 1.1 <sup>b</sup>       |
|           | YH161     | 184.4 ± 1.0 <sup>c</sup>       | 161.7 ± 1.7 <sup>c</sup>  | 89.1 ± 0.6 <sup>b</sup>       | 19.8 ± 1.0 <sup>b</sup>  | 10.9 ± 0.6 <sup>b</sup>       |
|           | JZ496     | 192.4 ± 0.3 <sup>a</sup>       | 178.5 ± 1.2 <sup>a</sup>  | 92.8 ± 0.5 <sup>a</sup>       | 13.8 ± 0.9 <sup>c</sup>  | 7.2 ± 0.5 <sup>c</sup>        |
|           | ZM8555    | 185.8 ± 0.3 <sup>b</sup>       | 173.7 ± 0.7 <sup>b</sup>  | 93.5 ± 0.1 <sup>a</sup>       | 12.0 ± 0.1 <sup>c</sup>  | 6.5 ± 0.1 <sup>c</sup>        |

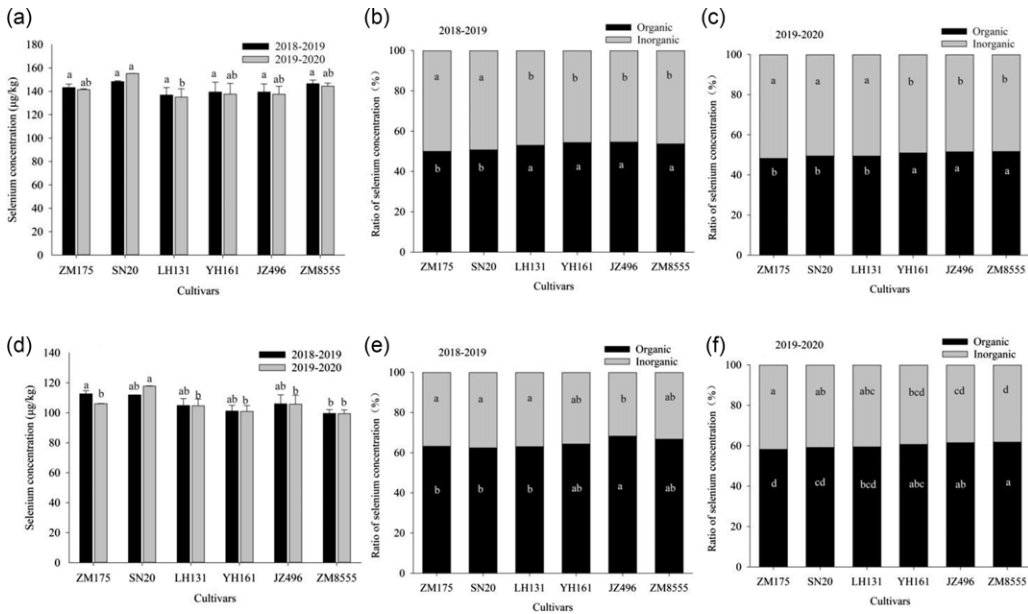
Data in the table are mean ± standard error. Different lower-case letters in the same column and same year indicate significant differences in the 95% level among different cultivars ( $p < 0.05$ ).



**Figure 4.** Total Se concentration (a) and its organic and inorganic forms (b, c) in wheat roots of several cultivars in 2018–2019 (b) and 2019–2020 (c) growing seasons. Different letters indicate statistical differences ( $p < 0.05$ ) among cultivars for a given season.

Wheat grain Se concentration is high in Se-rich areas and wheat outperforms other crops in terms of soil Se tolerance, uptake, and transfer efficiency (Eiche *et al.*, 2015). A recent study revealed that soil pH, organic matter, and other chemical characteristics alter Se bioavailability in rhizosphere, affecting Se uptake by winter wheat (Li *et al.*, 2020). However, there was no evidence of a link between soil physical and chemical features, nutritional indicators, and wheat grain Se concentration. According to our survey, the Se-rich areas in Shanxi Province are Changzhi, Hongtong, and Xiantao, and the wheat grain meets the Se-rich standard (DB61/T556-2012; GB28050-2011). Hubei Province is one of the most important Chinese grain-producing regions, accounting for 4.3% of the country total grain output in 2010 (Wang *et al.*, 2012). Hubei Province’s most important crop is rice, accounting for around 78% of the province’s total grain output (Wang *et al.*, 2012). Rice, rather than wheat, is the Se-rich prospective crop in Hubei Province. The northern winter wheat area includes Changzhi City in Shanxi Province, but the area of winter wheat in Changzhi City dropped significantly between 2000 and 2014 (Mi *et al.*, 2018). Therefore, Hongtong soil with  $600 \mu\text{g Se kg}^{-1}$  is a typical Se-rich soil in China wheat region, which is ideal for growing wheat with Se biofortification and offering great opportunities for wheat farmers improving wheat grain quality.





**Figure 5.** Total Se concentration (a, d) and its organic and inorganic forms (b, c, e, f) in wheat stem and leaves (a–c) and glume (d–f) of several cultivars in 2018–2019 (b) and 2019–2020 (c) growing seasons. Different letters indicate statistical differences ( $p < 0.05$ ) among cultivars for a given season.

**Genotypic variation in grain Se concentration**

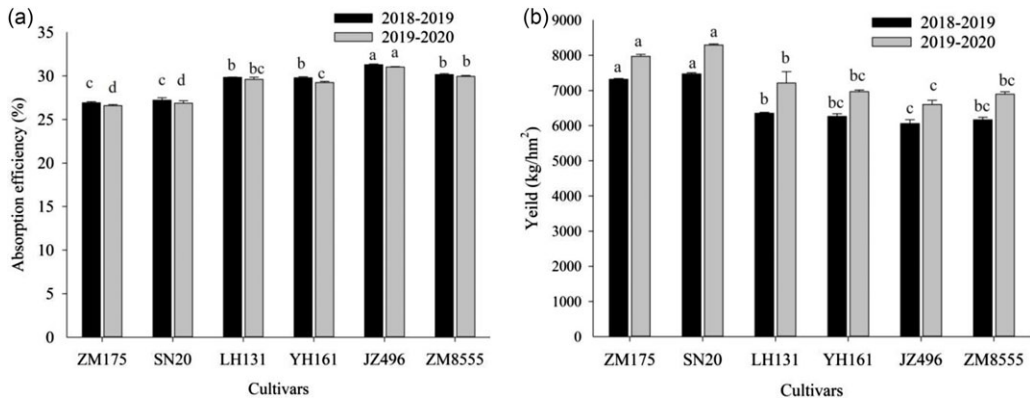
Our findings demonstrated that when wheat varieties with varying grain color were grown in Se-rich soil, the Se level in the grains of these genotypes was much greater than before sowing, reaching the Se-rich standard. Purple grain wheat has higher Se concentration than common wheat, which supports the conclusion that the Se concentration in colored grain wheat is higher (Xia et al., 2020). Many studies have been carried out about the Se concentration of wheat grain, but they are limited to exogenous Se supplying. Exogenous Se can be added to agricultural goods through soil application, seed soaking, and foliar spray (Ducsay et al., 2009). In this study, the average Se concentration of wheat grains with various colors in Se-rich areas was about  $180 \mu\text{g Se kg}^{-1}$ , which was higher than in bread-making wheat at Canterbury region of New Zealand ( $31 \mu\text{g Se kg}^{-1}$ ) and at the Cambridgeshire, Kent, Leicestershire, Lincolnshire, and Nottinghamshire region of UK ( $24 \mu\text{g Se kg}^{-1}$ ), as reported by Curtin et al. (2006) and Broadley et al. (2010). However, grain Se concentrations reported here are lower than ones found in North America (Gawalko et al., 2002; Hahn et al., 1981). The main reason of such differences is likely related to the native soil Se concentration in those regions.

Plants uptake selenate and selenite via active transport and convert them to organic Se (Eiche et al., 2015; Liu et al., 2017; White, 2018). Organic Se is dominant in wheat, whereas inorganic Se is the main form in soils (Wang et al., 2018). Inorganic Se forms IV and VI are absorbed by wheat plants and participate in plant metabolism, with most of Se being found as SeCys and SeMet (Zhu et al. 2009). This way, plants transform inorganic Se into beneficial and safer organic forms for humans (Hatfield et al., 2014; Pilon-Smits et al., 2009). Here, organic Se was the main form found in roots, stems, leaves, and seeds of plants under natural Se-rich soil cultivation, which was consistent with previous reports (Wang et al., 2018). The Se concentration in plant organs varied and was higher in grains, being followed by stems, leaves, roots, and glumes. Such pattern of

**Table 3.** Differences of selenium enrichment coefficients in root, stem+leaves, and glumes of wheat varieties with different grain colors and their transfer coefficients to grains

| Year      | Cultivars | BCF                 |                          |                      | TF                       |                               |                           |
|-----------|-----------|---------------------|--------------------------|----------------------|--------------------------|-------------------------------|---------------------------|
|           |           | BCF <sub>root</sub> | BCF <sub>Stem+Leaf</sub> | BCF <sub>Glume</sub> | TF <sub>root-grain</sub> | TF <sub>Stem+Leaf-Grain</sub> | TF <sub>Glume-grain</sub> |
| 2018–2019 | ZM175     | 0.20 <sup>a</sup>   | 0.24 <sup>a</sup>        | 0.18 <sup>a</sup>    | 1.37 <sup>c</sup>        | 1.15 <sup>bc</sup>            | 1.46 <sup>b</sup>         |
|           | SN20      | 0.19 <sup>ab</sup>  | 0.24 <sup>a</sup>        | 0.18 <sup>ab</sup>   | 1.45 <sup>bc</sup>       | 1.12 <sup>c</sup>             | 1.48 <sup>b</sup>         |
|           | LH131     | 0.18 <sup>ab</sup>  | 0.22 <sup>a</sup>        | 0.17 <sup>ab</sup>   | 1.67 <sup>ab</sup>       | 1.33 <sup>a</sup>             | 1.74 <sup>a</sup>         |
|           | YH161     | 0.17 <sup>ab</sup>  | 0.23 <sup>a</sup>        | 0.17 <sup>ab</sup>   | 1.72 <sup>a</sup>        | 1.31 <sup>a</sup>             | 1.80 <sup>a</sup>         |
|           | JZ496     | 0.17 <sup>ab</sup>  | 0.23 <sup>a</sup>        | 0.17 <sup>ab</sup>   | 1.87 <sup>a</sup>        | 1.37 <sup>a</sup>             | 1.81 <sup>a</sup>         |
|           | ZM8555    | 0.18 <sup>b</sup>   | 0.24 <sup>a</sup>        | 0.16 <sup>b</sup>    | 1.72 <sup>a</sup>        | 1.26 <sup>ab</sup>            | 1.85 <sup>a</sup>         |
| 2019–2020 | ZM175     | 0.20 <sup>ab</sup>  | 0.23 <sup>ab</sup>       | 0.17 <sup>b</sup>    | 1.34 <sup>b</sup>        | 1.17 <sup>c</sup>             | 1.55 <sup>b</sup>         |
|           | SN20      | 0.21 <sup>a</sup>   | 0.25 <sup>a</sup>        | 0.19 <sup>a</sup>    | 1.27 <sup>b</sup>        | 1.07 <sup>bc</sup>            | 1.41 <sup>b</sup>         |
|           | LH131     | 0.19 <sup>b</sup>   | 0.22 <sup>b</sup>        | 0.17 <sup>b</sup>    | 1.58 <sup>a</sup>        | 1.37 <sup>a</sup>             | 1.76 <sup>a</sup>         |
|           | YH161     | 0.18 <sup>b</sup>   | 0.22 <sup>ab</sup>       | 0.16 <sup>b</sup>    | 1.62 <sup>a</sup>        | 1.33 <sup>ab</sup>            | 1.80 <sup>a</sup>         |
|           | JZ496     | 0.19 <sup>b</sup>   | 0.22 <sup>ab</sup>       | 0.17 <sup>b</sup>    | 1.65 <sup>a</sup>        | 1.41 <sup>a</sup>             | 1.83 <sup>a</sup>         |
|           | ZM8555    | 0.18 <sup>b</sup>   | 0.23 <sup>ab</sup>       | 0.16 <sup>b</sup>    | 1.67 <sup>a</sup>        | 1.29 <sup>ab</sup>            | 1.87 <sup>a</sup>         |

Different lower case letters in the same column indicate significant differences in the 95% level among different cultivars ( $p < 0.05$ ).



**Figure 6.** Grain Se absorption efficiency (a) and grain yield (b) of several wheat cultivars in 2018–2019 and 2019–2020 growing seasons. Different letters indicate statistical differences ( $p < 0.05$ ) among cultivars for a given season.

accumulation is likely due to differences in Se absorption and transfer coefficients among plants organs. The concentration and proportion of organic Se in purple wheat grains were the highest and there was an obvious increasing trend from roots to leaves.

**Conclusion**

A positive correlation was found between soil Se concentration in the plow layer and wheat grain Se concentration. Hongtong County, Linfen City, Shanxi Province, China has  $600 \mu\text{g Se kg}^{-1}$  in soil, which is a typical Se-rich area. In this region, the average Se concentration in wheat grains with varying color was greater than  $150 \mu\text{g Se kg}^{-1}$ , meeting the Se-rich standard, and the Se form in various plant organs was primarily organic. Se concentration of colored grains was significantly higher than those of common wheat, with the purple grain wheat JZ-496 showing the highest concentrations. Purple grain wheat showed higher absorption and accumulation of Se due to its higher transfer coefficient of Se to the grain and higher absorption efficiency than other varieties. From a practical perspective, production of purple grain wheat genotypes such as JZ-496 in

Se-enriched regions in Northern China would be beneficial to wheat farmers to obtain higher grain Se concentration and to consumers for better health with Se-enriched food.

**Availability of data and materials.** The data used and analyzed in the current study are available from the corresponding author on reasonable request.

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