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Article

Effects of Different Selenium Application Methods on Wheat (*Triticum aestivum* L.) Biofortification and Nutritional Quality

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Abstract: Mineral nutrient malnutrition, especially deficiency in selenium (Se), affects the health of approximately 1 billion people worldwide. Wheat, a staple food crop, plays an important role in producing Se-enriched foodstuffs to increase the Se intake of humans. This study aimed to evaluate the effects of different Se application methods on grain yield and nutritional quality, grain Se absorption and accumulation, as well as 14 other trace elements concentrations in wheat grains. A sand culture experiment was conducted via a completely randomized 3 × 2 × 1 factorial scheme (three Se levels × two methods of Se application, foliar or soil × one Se sources, selenite), with two wheat cultivars (Guizi No.1, Chinese Spring). The results showed that both foliar Se and soil Se application methods had effects on wheat pollination. Foliar Se application resulted in early flowering of wheat, while soil Se application caused early flowering of wheat at low Se levels (5 mg kg^{-1}) and delayed wheat flowering at high selenium levels (10 mg kg^{-1}), respectively. For trace elements, human essential trace elements (Fe, Zn, Mn, Cu, Cr, Mo, Co and Ni) concentrations in wheat grains were dependent of Se application methods and wheat cultivars. However, toxic trace elements (Cd, Pb, Hg, As, Li and Al) concentrations can be decreased by both methods, indicating a possible antagonistic effect. Moreover, both methods increased Se concentrations, and improved grain yield and nutritional quality, while the foliar application was better than soil. Accordingly, this study provided useful information concerning nutritional biofortification of wheat, indicating that it is feasible to apply Se to conduct Se biofortification, inhibit the heavy metal elements concentrations and improve yield and quality in crops, which caused human health benefits.

Keywords: Selenium; grain yield and nutritional quality; trace elements; wheat; biofortification

1 Introduction

Selenium (Se) is recognized as one of the most important trace elements required by humans, and the recommended dietary allowance of Se for adults has been set at 50 μg day^{-1}-200 μg day^{-1} by the World Health Organization (WHO) [1]. It has been considered as an essential component of more than 30 mammalian selenoenzymes or selenoproteins [2]. Se deficiency in human organism is related to a series of serious health disorders, which are caused by free radicals. Conversely, moderate Se intake in human diet confers various health benefits, including improving immunity, delaying senescence and reducing the incidence of heart disease [3]. Nowadays, it is estimated that approximately 500-1000 million people worldwide have Se deficient [4]. Thus, there is an eagerly need to take effective measures to solve this problem.

Plants are the major source of Se in human diets [5]. Se enters the food chain through the plants which take it up from soil. However, these food plants inherent (e.g., wheat and rice) in the human diets are generally insufficient to meet human Se nutritional requirements. These foods with inherently low Se concentrations could be attributed to low bioavailability of Se in most soils [6]. For instance, about 72% of the land in China is Se deficient, 30% of which is severely Se deficient, and Se intake by a Chinese adult (26 μg day^{-1}-32 μg day^{-1}) is much less than the recommended dietary allowance by WHO [7]. Therefore, Se-enriched plant foodstuffs were proposed as an effective and sustainable way to improve Se dietary intake. It is very important to find effective strategies to increase Se concentrations in the edible parts of plants.

Many studies have shown that Se concentrations in food crops could be improved with the introduction of Se fertilization in quite a few Se-deficient regions, which enables increased Se consumption by the population [8,9]. There are mainly two ways to carry out Se-biofortification: soil application and foliar application. In the soil, Se exists as elemental Se (Se^0), selenide (Se^{2-}), selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}). Among them, SeO_3^{2-} and SeO_4^{2-} are predominant inorganic Se forms available, which are soluble in the soil or sprayed on the leaves and transported to plants. Accordingly, studies on Se-biofortification mainly used SeO_3^{2-} and SeO_4^{2-} as Se resource. Furthermore, adequate results also suggested that SeO_4^{2-} was more effective than SeO_3^{2-} in improving crops Se contents [10–12]. Nowadays, the major research task is to determine the most efficient approach for improving Se concentrations in food plants. Another important consideration is whether there is an "inhibitory effect" on other nutrients with Se application levels increased. Previous results have shown cereals grown on soil with high concentrations of calcium ion were low in Fe contents [13]. Mendoza et al. [14] also reported that interaction between Zn, Fe and Ca can inhibit their respective absorption by plants. Although the content of trace elements in the human body is extremely small in quantity, they are importantly required for the human nutrition and their deficiency may lead to "hidden hungry" [15]. These trace elements work with other nutrients (e.g., amino acid) and secondary metabolites (e.g., total phenols), etc. to together maintain human health. In order to maximize the amount of Se in crop products and the qualities of crops, it is imperative to consider "inhibitory effect", yet there is a little information in the literature on this topic.

Wheat (*Triticum aestivum* L.), a staple food crop, plays an important role in producing Se-enriched foodstuffs to increase the Se intake of humans. In China, nearly 50% of Se in diet is derived from wheat [16]. In the world, with an annual harvest of more than 600 million tons produced in over 40 countries for more than 35% of the global population, wheat makes up 29%-30% of the worlds total cereal production [17]. Modern wheat cultivars are divided into two species according to their origin: hexaploid bread wheat (*Triticum aestivum*, 2n = 42, AABBDD), and tetraploid, hard or durum-type wheat (*Triticum turgidum*, 2n = 28, AABB) [18]. Currently, approximately 95% of the wheat grown worldwide is hexaploid bread wheat [19]. Thus, increasing the Se concentrations of wheat via applying exogenous Se may be beneficial to meet human dietary requirements. Considering the importance of wheat and Se to human nutrition and health, this study aimed to evaluate the effects of different Se application methods on hexaploid bread wheat grain yield and nutritional quality, grain Se absorption and accumulation, as well as the concentrations of 8 human essential trace elements (Fe, Zn, Mn, Cu, Cr, Mo, Co and Ni) and 6 toxic trace elements (Cd, Pb, Hg, As, Li and Al) in wheat grains.

2 Materials and Methods

2.1 Experimental Design and Wheat Cultivation

A sand culture experiment was conducted in 2017 in an experimental farm where has never applied fertilized with Se before at the Chengdu University (30°64N, 104°19′E at 512 m altitude), Sichuan Province, China. The experimental area experienced a temperate subtropical monsoon humid climate with an annual mean temperature of 16.2°C and annual rainfall of 881.9 mm. The soil (pH, 8.43) at the experimental site was sandy clay loam with 10.21 g kg^{-1} of organic matter, 120.12 mg kg^{-1} available nitrogen, 15.70 mg kg^{-1} available nitrogen phosphorus, 123.00 mg kg^{-1} available potassium, 0.06% of total nitrogen and 0.19 mg kg^{-1} of total soil Se. In the present study, we selected the characteristic wheat Guizi No.1 (96.1% germination rate) with high anthocyanin content and Chinese Spring (95.9% germination rate) with completed wheat genome sequencing, which are widely used in the study of wheat genetics and breeding improvement. They were provided by the Institute of Triticeae Crops, Guizhou University (Guiyang, China).

The experiment included two forms of Se application (foliar application and soil application) and a Se source (selenate as Na_2SeO_4, Analytical reagent). In the foliar Se application, the selenate fertilizer was applied using Na_2SeO_4 solution of 11.5 mg L^{-1} and 23 mg L^{-1}, respectively. In the soil Se application, the selenate fertilizer was applied at a concentration of 5 mg kg^{-1} and 10 mg kg^{-1}. The foliar and soil Se application each had a control (no Se application), making a total of six treatments with five replications.

Wheat seeds were surface sterilized with 3.6% sodium hypochlorite for 10 min to avoid fungal contamination and then rinsed with deionized water. Seeds were germinated in a growth chamber under day/night temperature of 25/20°C, 16-h photoperiod, with 60%-70% relative humidity and 300 μmol$m^{-2}s^{-1}$ of irradiance. The 1/2 strength Hoagland solution was added every 3 days. The 15-day-old seedlings were evenly sown in tanks which were 1 m apart to avoid contamination. Each of the tanks was 5 m long and 2 m wide. The Na_2SeO_4 was applied once at heading stage in each treatment. The applying was performed between 8 am and 10 am on a dry, sunny day. In the foliar Se application, a compression sprayer of 10 L capacity was used to ensure even distribution of Na_2SeO_4 on leaves. In the soil Se application, Na_2SeO_4 was dissolved and poured into the sand culture. The wheat flower was just exposed, which was considered to have been initial time of wheat pollination. The number of flowering wheat plants was recorded daily until the end of all wheat plants flowering. Plants were harvested at the end of the cycle, oven dried at 60°C for 72 h, and then kept saved for analysis.

2.2 Investigation of the Yield-Related Traits and Nutrient Content in Wheat

For yield-related traits analyses, ten plants from each tank were selected randomly to investigate the spike length, grains per spike and grains weight per spike. The 1000-grain weight, grain length and grain width were measured by Seed Analyzer (YTS-5DS, Gufeng Optoelectronics Technology, Inc., China).

Se concentrations in grains were determined according to a method described by Djanaguiraman [20]. 0.2 g subsamples of dried grains were well ground via Tissue Grinder (TL2020, Dingshengyuan Technology, Inc., China), transferred to a digestion tube (HNO_3:$HClO_4$ = 4:1) and kept overnight at room temperature (25°C). Acid digestion was subsequently conducted at 165°C in an automatic temperature-controlled furnace until the digestion solution became clear. After cooling, 2.5 mL of 6 M HCl was added into the tubes and heated at 100°C for 30 min. Then the contents were cooled and filtered and made up to 10 ml. The Se concentrations in the solution were determined with the Atomic absorption spectrometer

(ICE 3500, Thermo Fisher Scientific Instruments, Inc., USA). Essential trace elements (Fe, Zn, Mn, Cu, Cr, Mo, Co and Ni) and toxic trace elements (Cd, Pb, Hg, As, Li and Al) concentrations were analyzed according to Londonio [21]. Briefly, 0.5 g subsamples of grains were weighed into PTFE high-efficiency digestion tank, to which 5 mL HNO_3 was added. After standing overnight at room temperature, the samples were on an electrothermal plate at 80°C for 30 min until the bubbles in the digestion tank were completely released. After cooling, the acid mixture was heated at 140°C for 3 h. Next, the acid mixture was cooled again and heated at 80°C for 30 min until yellow gas (NO_2) disappeared. Standard reference material GBW07605 (tea) was used for quality control of the digestion procedure and the analysis process. The concentrations of essential and toxic trace elements were analyzed by ICP-MS (NexION300, Perkinelmer, Inc., USA).

For secondary metabolites, phosphorus and nitrogen nutrients analyzed, grinded dry grains (50 mg) were weighed and microwave ultrasonic with 1.6 ml of 50% methanol at 65°C for 30 min and then the mixture was centrifuged at 10000 r for 10 min to get supernatant which was used to determine total flavonoids and total phenols contents by absorbance at 765 nm [22] and 510 nm [23], respectively. The above substrate was removed water by vacuum concentrator (ZLS-1, Here xi, Inc., China), and then 1.5 ml of 0.5 mol L^{-1} HCl was added. The mixture was shaken with a shaker for 30 min and centrifuged at 10000 r for 10 min to obtain supernatant which was used to determine phytic acid and inorganic phosphorus contents by absorbance at 500 nm [23] and 825 nm [24], respectively. Another 30 mg subsamples were well ground and shaken with 1.5 ml of 0.1 mol L^{-1} NaOH at 75°C for 1 h and then the mixture was centrifuged at 10000 r for 15 min to determine amino acid and soluble protein contents by absorbance at 405 nm [24] and 595 nm [25], respectively.

2.3 Statistical Analysis

Analysis of Variance (ANOVA), Spearman rank correlation and variances were calculated by using JMP® ver. 6.0 software (SAS Institute). Means were tested by Tukey-Kramer's honestly significant difference at the $p < 0.05$ level (HSD 0.05). SigmaPlot® ver. 12.0 software was used to draw histograms and line graphs, and images editing with Photoshop CS4.

3 Results and Discussion

The numbers of wheat flowers per day and Se concentrations in grains were significantly affected by the application methods and levels of Se, as well as by the interaction between these factors (Fig. 1). Foliar application of Se caused early flowering of wheat as compared to control (Fig. 1a). However, when the Se was applied to the soil, 5 and 10 mg kg^{-1} Se leaded to early wheat flowering and delayed wheat flowering, respectively, (Fig. 1b). The results indicated that Se played a role in pollination of wheat, which further confirmed Elizabeth A.H.'s conjecture that Se may influence plant pollination [26].

Both foliar Se and soil Se application methods significantly increased Se concentrations in grains of Guizi No.1 and Chinese Spring, and Se concentrations increased with Se application levels increased (Figs. 1c and 1e). The results of the present study are in line with the finding of Zhou et al. [27] in *Lentinula edodes* and Zhu et al. [7] in *Codonopsis lanceolata*. Nevertheless, Se concentrations in grains of two cultivars were always higher in the foliage treatments than in the corresponding soil treatments. This is probably because xylem transport is more difficult than phloem transport [28]. When Se is applied to foliage, Se is transported through the phloem. Contrarily, when Se is applied to soil, Se is translocated to shoots through the xylem. In addition, we also found foliar Se application caused a greater Se absorption in grains compared with soil Se application. The results of this study clearly showed that foliar Se application method is more effective in wheat biofortification with Se than soil.

It is worthy to note that the crops without Se application (control treatment) grow in soils with low natural Se concentrations (0.19 mg kg^{-1}), which was not enough to promote wheat biofortification with this element. According to the data of our study and to other researches resulting low Se and elsewhere

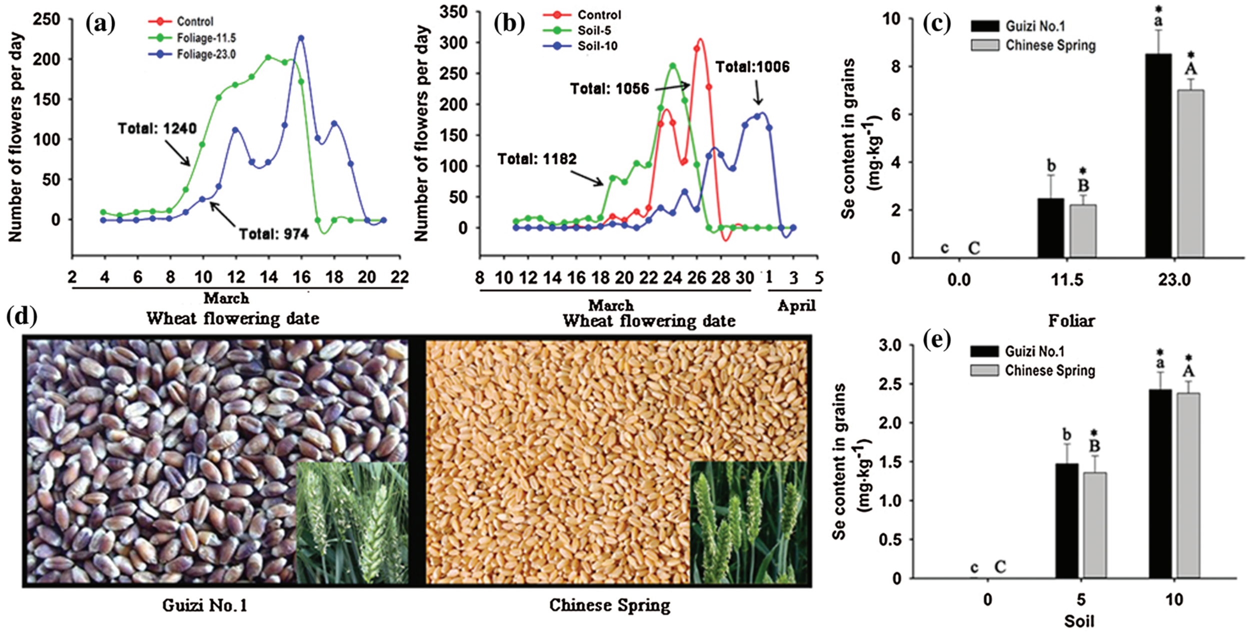

Figure 1: Effects of different Se application methods on number of wheat flowering plants per day (a, b), Se concentrations in grains (c, e) and grains phenotype (d). Error bars indicate ± SD. Different letters, comparing Se application levels within each application method are significantly different ($p < 0.05$). Asterisks (*), comparing Se application methods within each Se application level, differ between each other ($p < 0.05$)

[29,30], it could be inferred that to improve the Se concentrations of crops in Se-poor soils, it is imperative to apply Se in the fertilization programmes.

Due to the fact that wheat is the major staple food crop in many countries of the world, and that Se deficiency causes about one billion people worldwide, this crop has enormous potential to increase the Se consumption in the world's population. According to FAO [31], the average wheat flour consumption per person in the world is 200 g day^{-1}, and the recommended dietary allowance of Se for adults has been set at 50 µg day^{-1}-200 µg day^{-1}, with a maximum tolerable level of 400 µg day^{-1} [32]. Considering the data of this study, we estimated that the Se concentrations in wheat grains could contribute to the dietary Se intake with 442.72 µg day^{-1}-1703.50 µg day^{-1} via foliar application, and 271.58 µg day^{-1}-485.10 µg day^{-1} via soil application. Therefore, further researches should be conducted to assess wheat Se biofortification in order to adjust the Se application dosage to ensure food security and better improve the utilization of exogenous Se.

Six yield-related traits (spike length, grains per spike, grains weight per spike, 1000-grain weight, grain length and grain width) of Guizi No.1 and Chinese Spring exhibited similar changes in response to foliar Se and soil Se application (Tab. 1). Among the two Se application methods, results showed that Se leaded to a significant increase first and then decrease in yield-related traits with Se application levels increased. This can be related to the beneficial effects of Se at low doses. This researches are consistent with those found by Amato et al. [33] for Godina et al. [34] for tomato, which showed increased production at low Se concentrations. Furthermore, previous studies have shown beneficial effects of Se, because it increases the antioxidant activity in plants, leading to better plant yield [35]. The positive effects of foliar Se application on increasing yield was greater than that of soil Se at low Se levels (11.5 mg L^{-1} and 5 mg kg^{-1}), while the negative effects of foliar Se application on yield was more pronounced than that of soil at high Se levels (23 mg L^{-1} and 10 mg kg^{-1}). Accordingly, six negative correlations between

Table 1: Effects of different Se application methods on yield-related traits. Data are presented as mean ± SD (n = 10). Values followed by different letters within a column are significantly different (p < 0.05) among different Se application levels under the same Se application method

Methods	Genotypes	Treatments	Spike length (cm)	Grains per spike	Grains weight per spike (g)	1000-grain weight (g)	Grain length (mm)	Grain width (mm)
Foliar	Guizi No.1	0	15.62 ± 3.44ab	52.78 ± 10.56a	2.46 ± 0.37b	46.61 ± 8.86b	6.99 ± 1.05b	3.48 ± 0.52b
		11.5	15.97 ± 3.35a	53.43 ± 8.01a	2.69 ± 0.54a	50.35 ± 10.57a	7.46 ± 0.90a	3.74 ± 0.79a
		23.0	14.65 ± 2.64b	52.09 ± 8.86a	2.35 ± 0.31b	45.11 ± 7.22b	6.59 ± 1.05c	3.05 ± 0.30c
	Chinese Spring	0	8.77 ± 1.67a	49.02 ± 6.3726a	1.42 ± 0.14a	28.97 ± 5.50a	4.71 ± 0.71a	2.47 ± 0.47a
		11.5	9.18 ± 1.74a	49.56 ± 8.92a	1.52 ± 0.30b	30.67 ± 6.44ab	4.93 ± 1.03a	2.54 ± 0.54a
		23.0	7.38 ± 1.48b	49.08 ± 8.83a	1.35 ± 0.24b	27.51 ± 4.95b	4.40 ± 0.66b	2.26 ± 0.48b
Soil	Guizi No.1	0	15.62 ± 3.44a	52.78 ± 10.56a	2.46 ± 0.37ab	46.61 ± 8.86a	6.99 ± 1.05b	3.48 ± 0.52a
		5	15.71 ± 2.989a	53.01 ± 9.01a	2.57 ± 0.49a	48.48 ± 9.21a	7.34 ± 0.73a	3.67 ± 0.81a
		10	14.68 ± 2.79a	52.07 ± 7.81a	2.43 ± 0.24b	46.67 ± 7.47a	6.59 ± 1.05c	3.06 ± 0.51b
	Chinese Spring	0	8.77 ± 1.67a	49.02 ± 6.37a	1.42 ± 0.14ab	28.97 ± 5.50a	4.71 ± 0.71a	2.47 ± 0.47a
		5	9.02 ± 1.98a	49.52 ± 5.94a	1.48 ± 0.13a	29.89 ± 6.58a	4.90 ± 1.03a	2.51 ± 0.55a
		10	8.57 ± 1.117a	49.09 ± 5.40a	1.44 ± 0.32b	28.52 ± 6.56a	4.59 ± 1.06a	2.36 ± 0.07a

yield-related traits and foliar Se application methods, and three negative correlations between soil Se application methods were found, but not significant (Tab. 2). The results suggested that both Se application methods had stimulatory effects on wheat yield improvement, whereas wheat yield was more sensitive to foliar Se application.

Trace metal elements are widely presented in living tissue [36]. Some of them which participate in metabolic processes of enzymes, hormone vitamins and nucleic acids play vital roles in the body health [37]. Fig. 2 showed different Se application methods promoted different behaviors in terms of human essential trace elements concentrations (Fe, Zn, Mn, Cu, Cr, Mo, Co and Ni) in grains of Guizi No.1 and Chinese Spring. Foliar application of Se resulted in an increase in Fe, Mn and Mo concentrations, a decrease in Cr, Zn and Cu concentrations, and an increase and then decrease in Co and Ni concentrations in grains of two cultivars as the Se application levels increased from 0 to 23 mg L^{-1} (Figs. 2a and 2b). Similar results were obtained in rice [38] and soybean [39] plants fertilized with Se. In the soil Se

Table 2: Spearman rank correlation coefficients among different Se application methods related to essential trace elements, toxic trace elements, multi-component nutrients and yield-related traits. Asterisks (*) indicate significantly different ($p < 0.05$). n = two Se application methods × three Se application levels × two wheat cultivars × number of indicators

Se application methods	Variable	Essential trace elements		Toxic trace elements		Multi-component nutrients		Yield-related traits	
		n = 96	Spearman p	n = 72	Spearman p	n = 72	Spearman p	n = 72	Spearman p
Foliar	Se	Cr	−0.73*	Li	−0.83*	Amino acid	0.54*	Spike length	−0.08
		Mn	0.25*	Al	−0.55*	Soluble protein	0.39*	Grains per spike	−0.07
		Fe	0.57*	As	−0.59*	Total flavonoids	0.17	Grains weight per spike	−0.06
		Co	−0.19	Cd	−0.69*	Total phenols	0.17	1000-grain weight	−0.02
		Ni	−0.24	Hg	−0.66*	Phytic acid	−0.35	Grain length	−0.05
		Cu	−0.69*	Pb	−0.29	Inorganic phosphorus	−0.27	Grain width	−0.20
		Zn	−0.10*						
		Mo	0.78*						
Soil	Se	Cr	−0.67*	Li	−0.89*	Amino acid	0.26	Spike length	−0.03
		Mn	0.37*	Al	−0.65*	Soluble protein	0.12	Grains per spike	0.16
		Fe	0.54*	As	−0.56*	Total flavonoids	0.21	Grains weight per spike	0.01
		Co	−0.21	Cd	−0.58*	Total phenols	0.31	1000-grain weight	0.04
		Ni	−0.26	Hg	−0.15	Phytic acid	−0.68*	Grain length	−0.06
		Cu	−0.89*	Pb	−0.28	Inorganic phosphorus	−0.71*	Grain width	−0.10
		Zn	−0.60*						
		Mo	0.80*						

application methods, the concentrations of 8 human essential trace elements mentioned above in grains showed similar trends to foliar treatments (Figs. 2c and 2d). Moreover, Spearman rank correlation analysis also showed that foliar Se application were significantly positively correlated ($p < 0.05$) with Fe, Mn and Mo concentrations, and soil Se application were significantly positively correlated ($p < 0.05$) with Cr, Zn and Cu concentrations (Tab. 2). However, soil Se application provided higher Fe, Mn, Co and Ni concentrations in grains of Guizi No.1, and higher Fe, Mn, Co and Mo concentrations in grains of Chinese Spring, respectively, compared to foliar Se application. Moreover, significant difference was found between foliar Se and soil Se application in terms of Mn, Cu and Zn concentrations in grains of Guizi No.1, and Mn, Cu and Mo concentrations in grains of Chinese Spring, respectively. The results indicated that trace elements contents in grains were dependent of Se application methods and wheat cultivars.

There are some interactions involving the responses of crops exposed to different concentrations of Se on trace elements levels, like antagonistic or synergistic effects [40]. In this study, we found that both foliar

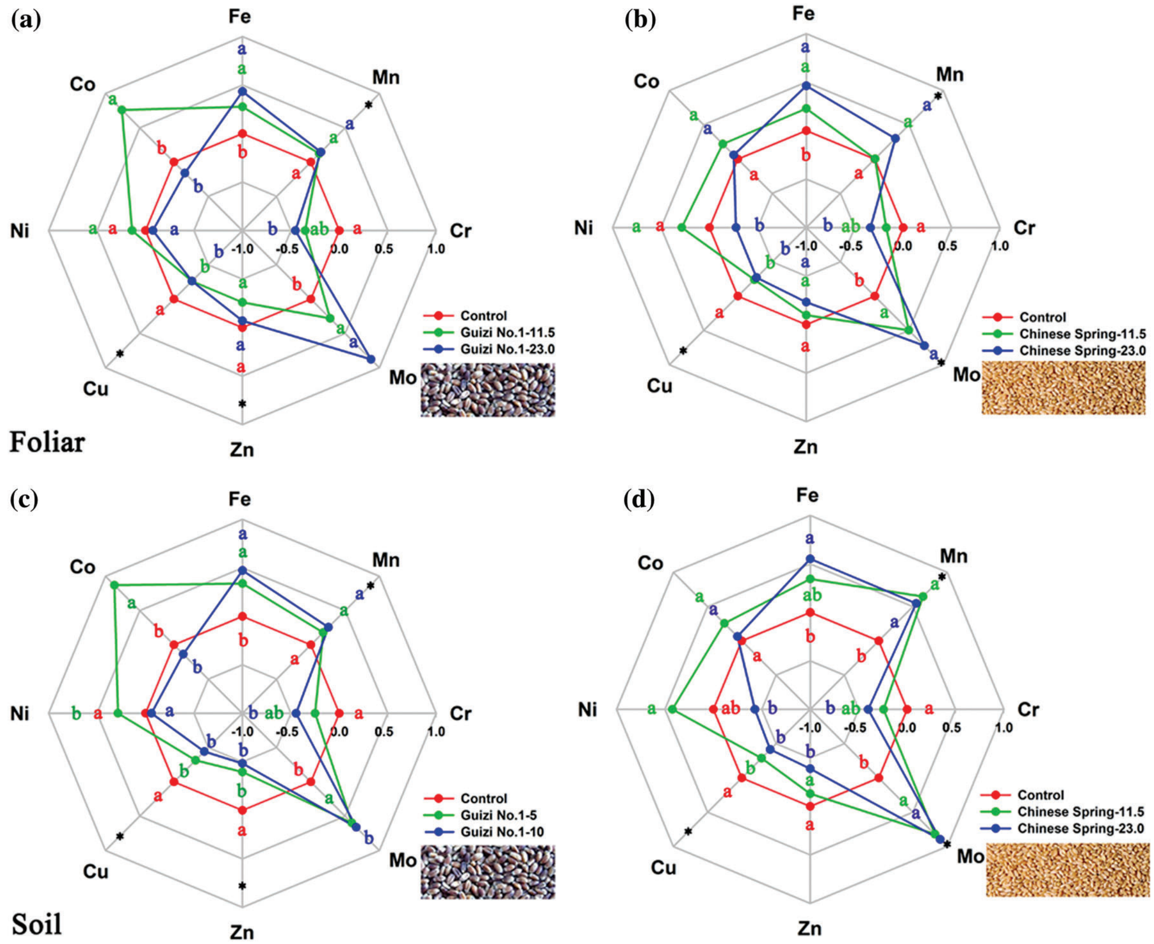

Figure 2: Effects of different Se application methods on essential trace elements concentrations in grains of two wheat cultivars. Values are means (n = 5). Different letters, comparing Se application levels within each application method are significantly different ($p < 0.05$). Asterisks (*), comparing Se application methods within each Se application level, differ between each other ($p < 0.05$)

Se and soil Se application generally significantly decreased toxic trace elements (Cd, Pb, Hg, As, Li and Al) concentrations in grains of two wheat cultivars (Fig. 3.), indicating a possible antagonistic effect. Foliar Se and soil Se application methods had significant differences in Hg and Pb concentrations in grains of Chinese Spring, while the two Se application methods only had significant differences in Pb concentrations in grains of Guizi No.1. Finding of present study corroborated the observation that Se interferes in the uptake of ions. The effect of Se on the absorption of toxic trace elements in wheat depends not only on the content and method of Se application, but also on the cultivar of wheat. Although some of them are also beneficial for human functional metabolism at low doses, excessive retention of any of toxic trace elements in the environment imposes threats to human health [41]. For example, Cd, Pb and Hg are endocrine-disrupting chemicals [42]. Zhou et al. [43] and Hu et al. [44] reported that Se application reduced Hg accumulation and Pb uptake in rice, respectively. Therefore, based on our research data and previous results, inhibiting the heavy metal elements concentrations in agricultural crops to reduce their harm to the human body, Se for fertilization programmes is feasible.

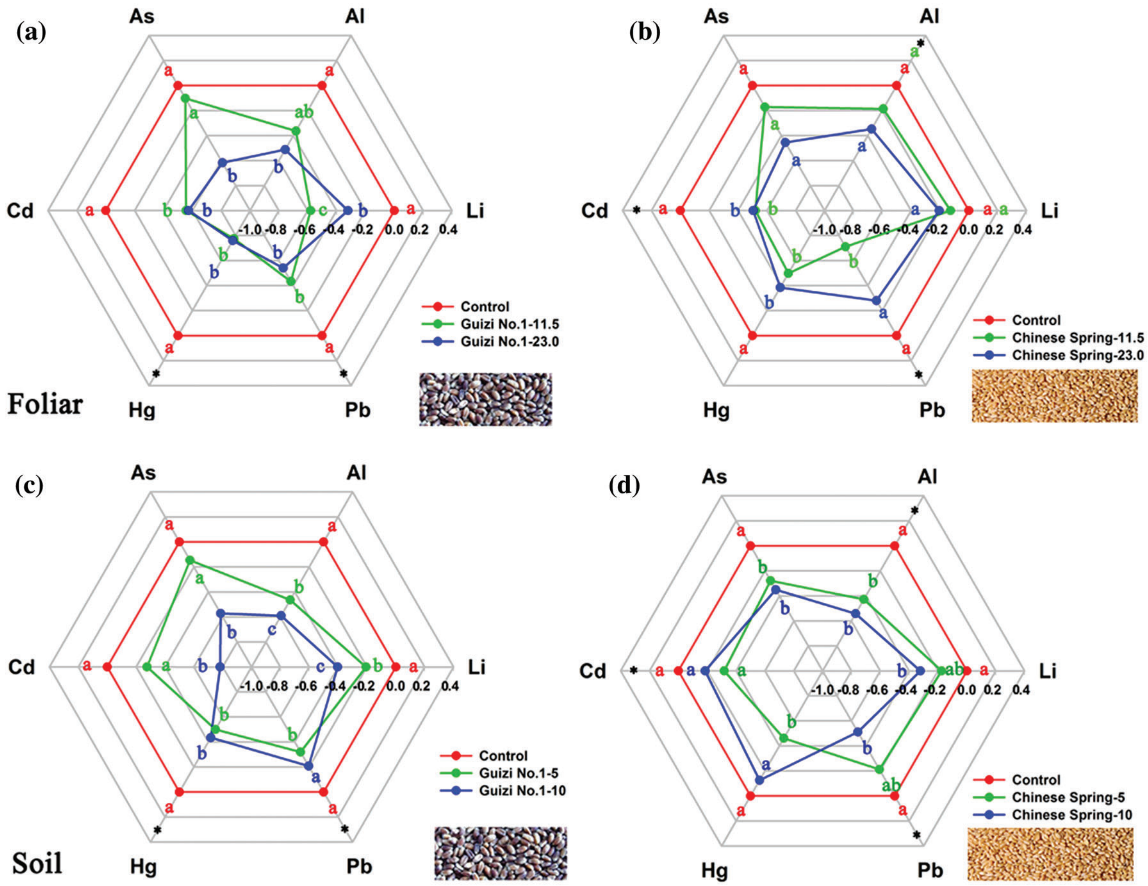

Figure 3: Effects of different Se application methods on toxic trace elements concentrations in grains of two wheat cultivars. Values are means (n = 5). Different letters, comparing Se application levels within each application method are significantly different ($p < 0.05$). Asterisks (*), comparing Se application methods within each Se application level, differ between each other ($p < 0.05$)

Nitrogen nutrients (amino acids and soluble proteins) are beneficial nutrients for human health, and also one of the important indicators for evaluating the nutritional quality of wheat. Nevertheless, once taken up by plant, Se follows the same sulfate assimilation pathway due to the chemical similarity between Se and sulfur (S), and incorporated into amino acid in the form of Selenomethionine (Se-Met) or Selenocysteine (Se-Cys) [45]. Tapiero [46] reported that Se was present as Se-Cys in proteins, Se-Cys in place of Cys participated in the synthesis of protein, which caused modification of the protein structure, consequently leading to Se toxicity. Foliar Se increased amino acids and soluble proteins concentrations in grains of two wheat cultivars as the Se application levels increased from 0 mg L^{-1} to 11.5 mg L^{-1}, while decreased in all grains as the Se application levels increased from 11.5 mg L^{-1} to 23 mg L^{-1} (Figs. 4a and 4b), indicating more Se-Cys and Se-Met participated in the synthesis of protein as Se application levels increased. The different behavior was observed for the nitrogen nutrients concentrations when Se was applied to the soil (Figs. 4c and 4d). Namely, amino acid and soluble protein concentrations increased concomitantly with an increase in Se application rates, moreover, significant differences between the application methods were observed for the amino acid and soluble protein (Fig. 4). Correlation analysis showed that amino acid (r = 0.54) and soluble protein (r = 0.39) were only found to be significantly positively correlated with foliar Se application methods (Tab. 2). These results are similar to those observed by Shalaby et al.

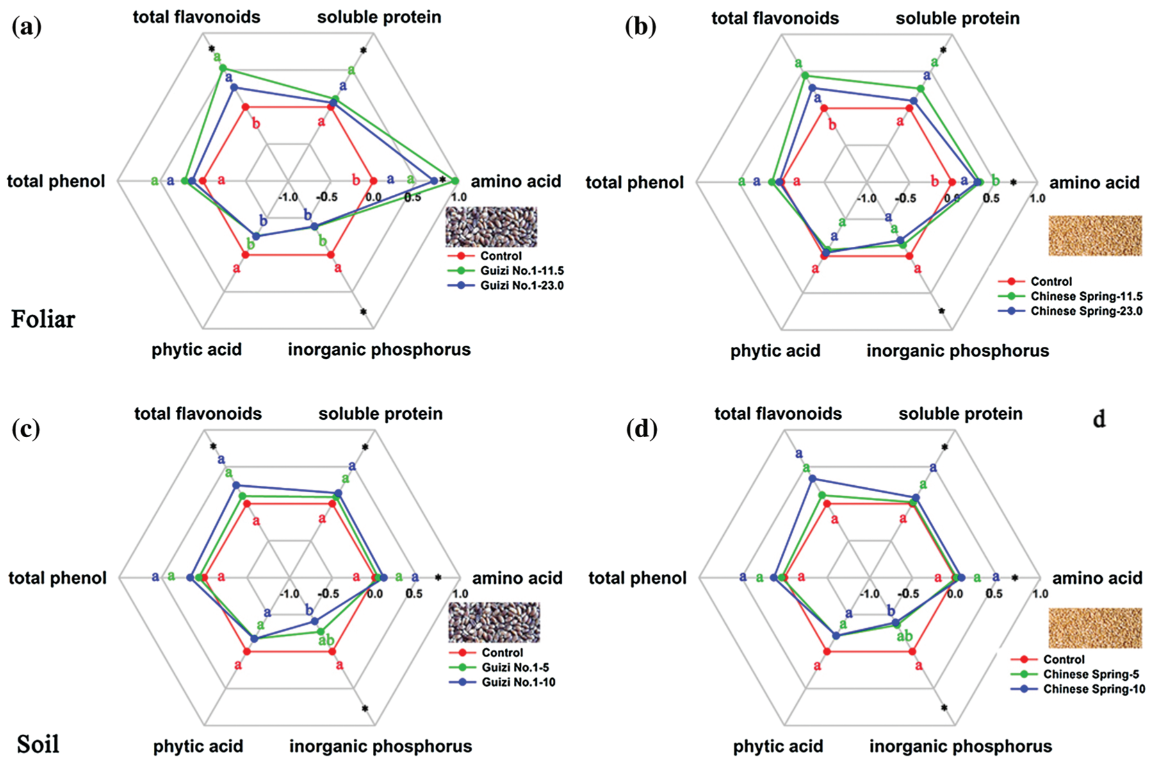

Figure 4: Effects of different Se application methods on secondary metabolites, phosphorus and nitrogen nutrients concentrations in grains of two wheat cultivars. Values are means (n = 5). Different letters, comparing Se application levels within each application method are significantly different ($p < 0.05$). Asterisks (*), comparing Se application methods within each Se application level, differ between each other ($p < 0.05$)

[35], who applied Se to leaves and soil of lettuce and also observed greater mobility in the phloem when using foliar Se application methods, which improved the nutritional quality in this culture.

A continuous decrease in phosphorus nutrients (phytic acid and inorganic phosphorus) concentrations was verified when 0 to 23 mg L^{-1} or to 10 mg kg^{-1} of Se was applied to leaves or soil (Fig. 4). The results are consistent with Hopper's [47] findings that Se has a competitive inhibitory effect on phosphorus absorption, which affects the phosphorus concentrations in plants. Furthermore, in this study, soil Se application methods resulted in a more reduction of phosphorus nutrients concentrations of two wheat cultivars as compared to foliar treatments of equal strength, and significant differences between the application methods were observed for inorganic phosphorus. Correlation analysis showed that soil Se application methods were only found to be significantly positively correlated with phytic acid (r = −0.68) and inorganic phosphorus (r = −0.71) (Tab. 2). It should be considered that Se undergoes specific adsorption onto Al and As oxides in the soil, reducing its absorption by plants, as shown in Fig. 3. Accordingly, there would be more competition with phosphate in the absorption process.

Total flavonoids and total phenols have antioxidant properties and senescence-resistance. Regardless of applying different levels of foliar Se or soil Se, Fig. 4 showed that secondary metabolites (total flavonoids and total phenols) concentrations in all grains increased compared to control, which is consistent with the reports of Maria et al. [48] in tomato and Thiruvengadam et al. [49] in turnip. Therefore, our results indicated that Se application can enhance the health benefits of wheat by improving the concentrations of secondary metabolites.

4 Conclusions

The application of Se, both foliar and soil, had effects on pollination of wheat. Foliar Se application caused early flowering of wheat as compared to control. However, when the Se was applied to the soil, 5 and 10 mg kg^{-1} Se leaded to early wheat flowering and delayed wheat flowering, respectively. Human essential trace elements (Fe, Zn, Mn, Cu, Cr, Mo, Co and Ni) concentrations in wheat grains were dependent of Se application methods and wheat cultivars. However, two Se application methods generally significantly decreased toxic trace elements (Cd, Pb, Hg, As, Li and Al) concentrations in grains, indicating a possible antagonistic effect. Both foliar Se and soil Se application increased grain yield, Se, amino acids, soluble proteins, total flavonoids and total phenols concentrations, and foliar application was the more effective procedure. Our results indicated that it is possible to promote Se-biofortification and its nutritional quality in wheat, both via foliar and soil application. And the foliar application was better than soil.

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