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SELENIUM APPLICATION EFFECTS ON QUALITY AND DISTRIBUTION OF TRACE ELEMENTS IN SINK-SOURCE ORGANS OF WILD EMMER WHEAT

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ABSTRACT

Mineral nutrient malnutrition, especially deficiency of selenium (Se) affects the health of approximately one billion people worldwide. Wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*), the progenitor of common wheat, harbors a rich genetic diversity for mineral nutrients. The study was conducted on two wild emmer wheat genotypes differing in Se tolerance (R113, Se-sensitive; R171, Se-tolerant) with 2 Se application methods and 3 Se levels (foliar rates of 0, 11.5 and 23 $mg{\cdot}L^{-1}$; fertigation rates of 0, 5 and 10 $mg{\cdot}kg^{-1}$) in 2017 having 5 replications, at an experimental farm, Sichuan Province, China. It evaluated the effects of Se application on wild emmer wheat growth, grain yield and quality, and 14 other trace elements absorption and translocation in sink-source organs (flag leaves, husks and grains). The results showed that both foliar Se and fertigated Se application methods increased Se contents in sink-source organs, wheat health benefits and yield, while the foliar application was more effective than fertigation. Moreover, two Se application methods decreased toxic trace elements (Pb, Al, As, Li and Cd) contents in wheat, indicating a possible antagonistic effect. Accordingly, this study provided useful information concerning agronomic biofortification of wheat, indicating that it is feasible to apply Se in fertilization programmes to inhibit the heavy metal elements contents and improve yield and quality in agricultural crops. The higher Se, Fe, Zn and Mo contents found in R171 suggested that its germplasm conferred higher abilities for mineral uptake and accumulation, which can be used for genetic studies of wheat nutritional value and for further improvement of domesticated cereals.

Keywords: Selenium, wild emmer wheat, genetic diversity, sink-source organs, yield and quality.

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INTRODUCTION

Selenium (Se) is essential for humans and animals due to its antioxidant properties, which form part of a series of chemical reactions (Harvey *et al*., 2020). Nowadays, Se has been recognized to be an essential component of more than 30 mammalian selenoenzymes or selenoproteins (Sonet *et al*., 2018). The recommended dietary allowance of Se for adults is 50~200 μg per day as WHO limits (Maurer, 2011). Se deficiency in humans has been closely related to a series of diseases such as cardiovascular disease, heart disease and multiple cancers (Sahebari *et al*., 2019).

Plants are the main source of Se in human diets (White, 2015). However, the Se contents of edible plants are usually insufficient to meet the nutritional needs of the human body due to low Se bioavailability in a large number of agricultural soils which result in low levels of Se in foods (Peng *et al*., 2019). It is reported that approximately one billion people worldwide have Se deficiency and many more suboptimal, considered to be the fourth most serious mineral deficiency after iron (Fe), zinc (Zn) and iodine (I) (White and Broadley, 2009). Therefore, it is very important to find strategies to improve Se levels in crops.

Studies have suggested that Se contents in plants can be increased by applying Se fertilizer, which would increase Se intake of the population (Smoleń *et al*., 2018). The Se can be applied either to the growing crop or to the soil. According to Eurola *et al*. (2010), Se fertilization significantly increased Se contents in the UK and Finland. In addition, numerous studies also showed that selenite (SeO_4^{2-}) was more effective in improving crops Se contents compared to selenite (SeO_3^{2-}) (Lidon *et al*., 2018). Currently, the main task of the research is to determine the most efficient method for improving Se contents in the edible parts of plants. Another important consideration is whether there is an "inhibitory effect" on other trace elements with the increase of Se fertilization. In order to maximize the amount of Se in crop products and the qualities of crops, it is necessary to consider "inhibitory effect", yet there is a little information in the literature on this topic.

Trace elements are importantly required for the human nutrition (Konikowska and Mandecka, 2018). These are required for the body functions in minute

quantities but their deficiency may result in several health issues such as "hidden hunger" (Titcomb and Tanumihardjo, 2019). In 1990, WHO and FAO Expert Committees had divided essential trace elements into three categories (Khouzam *et al*., 2011): these include human essential trace elements, such as I, Fe, Zn, Se, copper (Cu), molybdenum (Mo), chromium (Cr), cobalt (Co), probably human essential trace elements, such as silicon (Si), nickel (Ni) ,and potentially toxic trace elements, such as fluorine (F), lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), aluminum (Al), lithium (Li), tin (Sn), which may also have some essential functions at low levels. These trace elements, other nutrients (e.g. protein) and secondary metabolites (e.g. total flavonoids and total phenols), etc. together maintain the health of the human body.

Wheat (*Triticum* spp.) is one of the main plants of bioavailable Se, which provides about 20% of the calories consumed by humans (Jat *et al*., 2017). Thus, increasing the Se contents of wheat by Se fertilization may meet human dietary requirements. However, for programs involving Se biofortification, the targeted experimental materials must be taken into account, since modern wheat cultivars have lost genetic diversity due to the worldwide pursuit of high-yielding crop varieties alone. Wild emmer wheat harbors extensive allelic variations associated with many economically important traits of improved cultivated wheat, including grain mineral contents (Zvi *et al*., 2009). It has been shown that the wild emmer allele at *Gpc-B1* locus demonstrated continuously improving effect on trace elements contents (Distelfeld *et al*., 2010). Because of the importance of wheat and Se for human nutrition and health, this study designed to evaluate the effects of different Se application methods on wild emmer wheat growth, grain yield and quality, as well as Se and other metal trace elements absorption and translocation in sink-source organs (flag leaves, husks and grains).

MATERIALS AND METHODS

Experimental materials and growth conditions: For this study, R113 (Se-sensitive genotype) and R171 (Se-tolerant genotype) seeds of F_8 recombinant inbred lines (RILs) derived by single-seed decent from a cross between durum wheat (female) cultivar Langdon (LDN) and wild emmer wheat(male) accession G18-16 were provided by the Institute of Evolution, University of Haifa, Israel. A sand culture experiment was carried out in 2017 at an experimental farm at the Chengdu University (30°64'N, 104°19'E, 512 m above sea level), Sichuan Province, China. The soil at the experimental site was sandy clay loam with uniform. The soil characteristics selected for sowing the crop are shown in Table 1.The meteorological data (from Chengdu Bureau of Statistics, China) during the growing seasons of wheat in 2017 is presented in Fig S1.

The experiment included two methods of Se application (fertigation and foliar spray) and one Se fertilizer (selenate as Na_2SeO_4). In the fertigation treatments, the Se fertilizer was applied at rates of 5 and 10 $mg{\cdot}kg^{-1}$. In the foliage treatments, the selenate fertilizer was applied using Na_2SeO_4 solution of 11.5 and 23 $mg{\cdot}L^{-1}$, respectively. The fertigation and foliage treatments each had a control (no Se fertilizer), making a total of 6 treatments. The treatments were replicated 5 times.

Wild emmer wheat seeds were surface sterilized with 3.6% NaClO for 10 min and then rinsed with distilled water. The 1/2 strength Hoagland solution was supplemented every 3 days from 5 days after the germination. The 15-day-old seedlings were sown in tanks which were prepared using standard agronomic practices. Each of the tanks was 5 meters long and 2 meters wide. The selenate fertilizer was applied once to each treatment at heading stage. The applying was performed between 8 am and 10 am on a dry, sunny day. In the fertigation treatments, selenate fertilizer was dissolved and poured into the sand culture. In the foliage treatments, a compression sprayer of 10 L capacity was used to ensure even distribution of selenate on leaves. The tanks were 1 m apart to prevent contamination. After maturity, the wild emmer wheat plants were harvested and their sink-source organs (flag leaves, husks and grains) were separated, oven dried at 60 °C for 72 h, and then kept saved for analysis.

Determination of Se content: Subsamples (0.2 g) of each plant organ were well ground through a tissue grinder (TL2020, Dingshengyuan Technology, Inc., Beijing), transferred to a digestion tube (HNO_3:$HClO_4$=4:1) and kept overnight. The Se content in the solution was determined by graphite furnace atomic absorption spectrometer (ICE 3500, Thermo Fisher Scientific Instruments, Inc., USA) according to the method of Djanaguiraman *et al*. (2010).

Determination of trace elements contents: To determine trace elements (Fe, Zn, Cu, Co, Mo and Cr) and toxic trace elements (Pb, Hg, Al, As, Sn, Li and Cd) contents, subsamples (0.5 g) of each plant organ were mixed with 5 mL HNO_3 in PTFE high-efficiency digestion tank. The samples were on an electrothermal plate at 80 °C for 30 min till 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. Then, these trace elements contents were measured according to inductively coupled plasma-mass spectrometry (ICP-MS, NexION300, Perkinelmer, Inc., USA) by Londonio (2019).

Determination of multi-component nutrients contents: The determination of amino acid (Adlernissen, 1979), soluble protein (Bradford, 1976), phytic acid (Ainsworth and Gillespie, 2007), inorganic phosphorus (Ficco *et al*., 2009), total flavonoids (Jianming, 1999) and total phenols (Ainsworth and Gillespie, 2007) contents in each plant organ were performed using ultraviolet-visible spectrophotometer (U-T6, Yipu Instrument Manufacturing, Inc., Shanghai) at 405, 595, 500, 825, 765 and 510 nm, respectively.

Determination of the agronomic traits: Ten plants from each tank were selected randomly to record the 1000-grain weight, grains weight per spike, grains per spike, spike length and plant height. Leaf area index was calculated using the following equation 1.

Leaf area index=$L^{*}W^{*}0.75$ (1)

Where L is the entire length of flag leaf, W is the widest place of flag leaf.

Statistical analysis: Analysis of variance was performed with JMP software (version 6.0, SAS Institute), and data in each sample was analyzed separately. Means were tested by Tukey-Kramer's honestly significant difference at the $P<0.05$ level (HSD 0.05). Pearson's product-moment correlations (r values) and histograms were performed using Sigma Plot software (version 12.0). Correlation network analysis was carried out by R language statistical package together with Cytoscape software (version 2.7.0).

RESULTS

Se contents in sink-source organs: Both foliar Se and fertigated Se application methods significantly increased Se contents in sink-source organs of 2 genotypes (Fig 1). Se contents in flag leaves and grains increased significantly as Se application rates increased, while the Se contents in husks increased first and then decreased. Foliage treatments were always higher in increasing Se contents in sink-source organs of 2 genotypes as compared to fertigation treatments of equal strength. In addition, the Se contents in sink-source organs of R171 (Se-tolerant genotype) were generally twice as high as those of R113 (Se-sensitive genotype).

Although both foliar Se and fertigated Se application can cause a large accumulation of Se in plants, most of the Se (more than 50%) was accumulated in flag leaves and husks, and lower amounts in grains (Fig 2). The effects of Se addition on proportions of Se in sink-source organs were both application methods and content-dependent manners of Se. For Se applied to the soil, flag leaves and husks accumulated higher amounts of Se at the expense of grains (Fig 2b). Foliar Se application led to a greater proportion of Se in grains compared with fertigated Se application (Fig 2a). Among the two Se application methods, the proportion of Se in R113 grains decreased with the increase of Se application rates, while the proportion of Se in flag leaves + husks increased relative to plant without Se addition. The changes in R171 grains and flag leaves + husks in response to two Se application methods showed opposite trends (Fig 2).

Trace elements contents in sink-source organs: In the foliage treatments, increasing the Se application rates from 0 to 23 $mg{\cdot}L^{-1}$ led to a significant improvement in Mo contents as well as a decrease in Cr contents in all organs of R113. The Fe contents in all organs of R113 significantly increased and then decreased with Se application rates and reached the maximum (i.e., Fe contents in flag leaves, husks and grains are 372.25, 189.74, and 42.88 $mg{\cdot}kg^{-1}$, respectively) at Se of 11.5 $mg{\cdot}L^{-1}$ (Table 2). The Zn and Co contents in flag leaves as well as Cu contents in husks of R113 improved dramatically as the Se application rates increased, while Zn and Cu contents in grains and Cu contents in flag leaves showed opposite trends (Fig S2). In addition, it was observed that Co contents in husks and grains showed no response to Se application rates. However, the above-mentioned 6 trace elements in sink-source organs of R171 showed generally different trends relative to these of the R113. The Fe, Zn, Cu, and Mo contents in all organs of R171 generally significantly increased except for Cu in flag leaves and grains, and Cr contents in all organs diminished with the Se application rates increased.

In the fertigation treatments, the Cr contents in all organs (flag leaves, husks and grains) of 2 genotypes showed opposite trends relative to that of the foliage treatments (Fig S2). Namely, as the Se application rates increased from 0 to 10 $mg{\cdot}kg^{-1}$, the Cr contents dramatically increased by 114.37, 74.74 and 200% respectively, in flag leaves, husks and grains of R113 and by 13.85, 49.30 and 42.86%, respectively, in flag leaves, husks and grains of R171 (Table 2). However, Zn, Cu and Mo contents in all organs of 2 genotypes exhibited similar changes in response to foliar Se and fertigated Se application.

Among the two Se application methods, the Co contents in grains of 2 genotypes had no significant effects as Se application rates increased, which indicated that Co contents in grains were independent of application methods and content of Se. Regardless of applying different ratios of foliar Se or fertigated Se, the Fe, Co and Cr contents in different organs of 2 genotypes generally followed the order of flag leaves>husks>grains, and the Mo contents in different organs generally followed the order of flag leaves>grains>husks. However, the distributions of Zn and Cu in sink-source organs of 2 genotypes were dependent of application methods of Se. Foliar Se resulted in Zn and Cu contents in different organs in the order of husks>grains>flag leaves and flag leaves>husks>grains, respectively.

Fertigated Se resulted in Zn and Cu contents in different organs in the order of husks>flag leaves>grains.

Toxic trace elements contents in sink-source organs: Foliar Se decreased Pb, Al, As, Li and Cd contents in all sink-source organs of R113 as Se application rates increased, but had no significant effect on Pb contents in flag leaves, Al and Li contents in husks as well as Pb, Al and As contents in grains (Table 3). Hg and Sn contents in R113 organs showed different trends from Pb, Al, As, Li and Cd. In flag leaves, Hg contents significantly decreased. In husks and grains, however, Hg contents increased. Flag leaves and husks Sn contents gradually increased with the application of Se, whereas almost no Sn in grains. Similarly, the above-mentioned 7 trace elements in sink-source organs of R171 showed the same trends as these of the R113 (Fig S3).

Fertigated Se also declined Pb, Al, As, Li and Cd contents in all sink-source organs of R113 and R171 as Se application rates increased. Furthermore, application methods and contents of Se had no marked effects on Sn in grains of 2 genotypes. However, the Hg contents in husks and grains of 2 genotypes showed an opposite trends compared with foliar Se treatments.

Among the two Se application methods, the Pb, Al, As, Sn and Li contents in sink-source organs of 2 genotypes under the corresponding Se treatment conditions followed the order flag leaves>husks>grains. However, the distributions of Hg and Cd in sink-source organs of 2 genotypes depended not only on the methods of Se application but also on the genotypes. In addition, significant difference was also found between Se application methods and sink-source organs in terms of Hg and Cd contents. Foliar Se resulted in Hg contents in different organs in the order of R113 flag leaves> grains> husks and R171 husks>grains>flag leaves, respectively. However, fertigated Se led to Hg contents in different organs in the order of R113 flag leaves> husks=grains and R171 flag leaves>husks>grains, respectively. When compared with sink-source organs with Se addition, the Cd contents in R113 grains and R171 husks were greatest of 0.15 and 0.16 $mg \cdot kg^{-1}$, respectively, in the 11.5 $mg \cdot L^{-1}$ foliage treatments and R113 flag leaves and R171 husks were greatest of 0.09 and 0.13 $mg \cdot kg^{-1}$, respectively, in the 5 $mg \cdot kg^{-1}$ fertigation treatments (Table 3).

Multi-component nutrients contents in sink-source organs: Foliar Se generally significantly increased nitrogen nutrients (amino acid and soluble protein) and secondary metabolites (total flavonoids and total phenols) contents in R113 all organs as the Se application rates increased from 0 to 11.5 $mg \cdot L^{-1}$, while decreased in all plant organs as the Se application rates increased from 11.5 to 23 $mg \cdot L^{-1}$. The effects of foliar Se application on phosphorus nutrients (phytic acid and inorganic phosphorus) contents showed different trends from them. Foliage treatments caused a notable decrease in phytic acid contents in all organs and a significant increase in inorganic phosphorus contents in flag leaves and husks. The total flavonoids contents in R171 showed similar trends to R113, but nitrogen nutrients and phytic acid contents showed different trends, when foliar Se application rates increased. As Se application rates increased, foliar Se treatments always enhanced contents of nitrogen nutrients in R171 sink-source organs and led to a significant rise in phytic acid contents in flag leaves and husks (Table 4).

The changes in nitrogen nutrients and phosphorus nutrients contents in R113 and R171 sink-source organs in response to fertigation Se treatments showed similar trends to foliar Se treatments (Fig S3). However, the secondary metabolites contents in R113 and R171 sink-source organs exhibited different changes in response to foliage treatments and fertigation treatments. Fertigated Se increased total flavonoids and total phenols contents of R113and R171 flag leaves and grains, and reduced the contents in the husks with the Se application rates increased.

Among the two Se application methods, multi-component nutrients contents in sink-source organs of 2 genotypes showed different distributions. Under the corresponding Se treatment conditions, the amino acid, soluble protein, phytic acid, inorganic phosphorus, total flavonoids and total phenols contents in R113 sink-source organs followed the order flag leaves>grains>husks, grains>husks>flag leaves, grains>flag leaves>husks, husks> grains>flag leaves, flag leaves>grains>husks, flag leaves>husks>grains, respectively, and in R171 sink-source organs followed the order flag leaves> husks>grains, grains> husks>flag leaves, grains>flag leaves>husks, husks>flag leaves>grains, flag leaves> grains>husks, flag leaves>husks>grains, respectively. Furthermore, the results also showed multi-component nutrients contents in sink-source organs of R171 were generally higher than those of R113 as compared to corresponding Se treatments.

Agronomic traits: In the foliage treatments, increasing the Se application rates from 0 to 23 $mg \cdot L^{-1}$ led to a significant increase first and then decrease in 1000-grain weight, spike length, grains weight per spike and grains per spike as well as decrease in leaf area index and plant height of R113. However, the 1000-grain weight, spike length, grains weight per spike and grains per spike always increased as well as the leaf area index and plant height of R171 increased first and then decreased with the Se application rates from 0 to 23 $mg \cdot L^{-1}$. In the fertigation treatments, the above-mentioned 6 agronomic traits of R113 and R171 showed same behaviors relative

Table 1. Basic properties of the soil used in the experiments.

Texture	pH	Organic matter (g kg^{-1})	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})	Total N (%)	Total Se (mg kg^{-1})
sandy loam	8.43	10.21	120.12	15.70	123.00	0.06	0.19

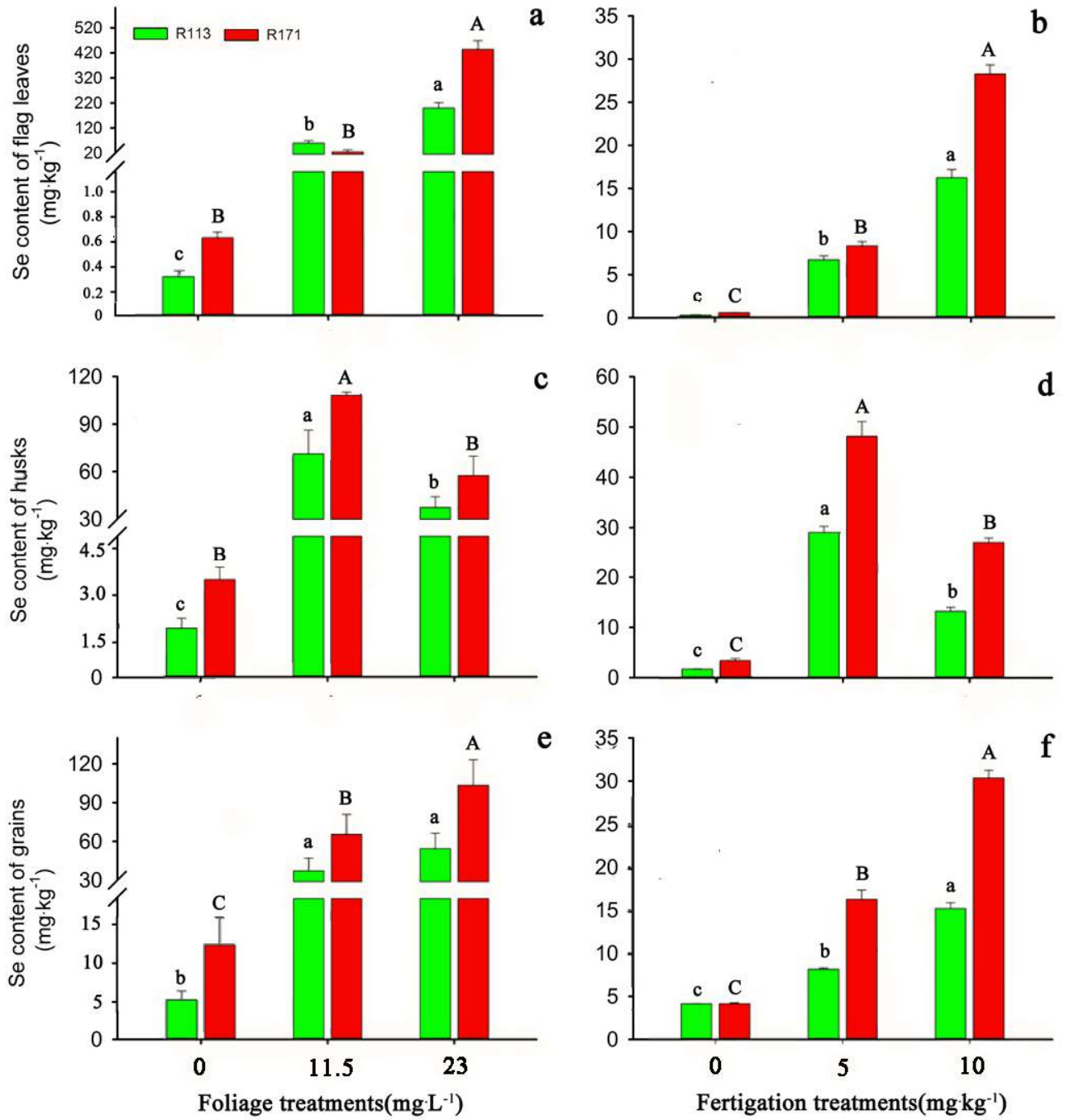

Fig 1. Se contents in sink-source organs of wild emmer wheat as affected by Se application methods. Error bars indicate ± SD. Within a genotype, bars with different letters are significantly different at P<0.05, n=5.

to those of the foliage treatments except for plant height. The results indicated that both foliage treatments and fertigation treatments had stimulatory effects on 2 genotypes yield improvement, while the positive effects of higher Se application rates (23 mg·L^{-1} and 10 mg·kg^{-1}) on these agronomic traits declined. Moreover, agronomic traits of 2 genotypes were generally higher in the foliage treatments than the corresponding fertigation treatments, which demonstrated the foliar application was more effective than fertigation on yield improvement (Table 5).

Correlation network analysis: A total of 180 correlations were showed in foliage treatments, with values ranging from 0.985 for Al and Pb to -0.834 for soluble protein and total phenols (Table S1). In the fertigation treatments, correlation values ranged from 0.976 for Al and Pb to -0.778 for Cu and soluble protein (Table S2). Further screening in the trace elements-toxic trace elements, trace elements-multi-component nutrients, trace elements-agronomic traits, toxic trace elements-multi-component nutrients and multi-component nutrients-agronomic traits caused identification of 174 significant correlations (P<0.05) in both Se application methods (Fig 3). Of these correlations, 58 positive correlations and 37 negative correlations were found in the foliage treatments. However, the fertigation treatments had fewer positive correlations (46) and relatively more negative correlations (33). This indicated that both foliage treatments and fertigation treatments induced concerted metabolic changes that resulted in improving grain development, but the foliar application was more effective than fertigation. For example, the correlation of Se and amino acids and soluble proteins were 0.62 and 0.587, respectively, in the foliage treatments but decreased to 0.426 and 0.396, respectively, in the fertigation treatments. The results suggested that these amino acids may accelerate the synthesis of storage proteins, thus benefitting flour quality.

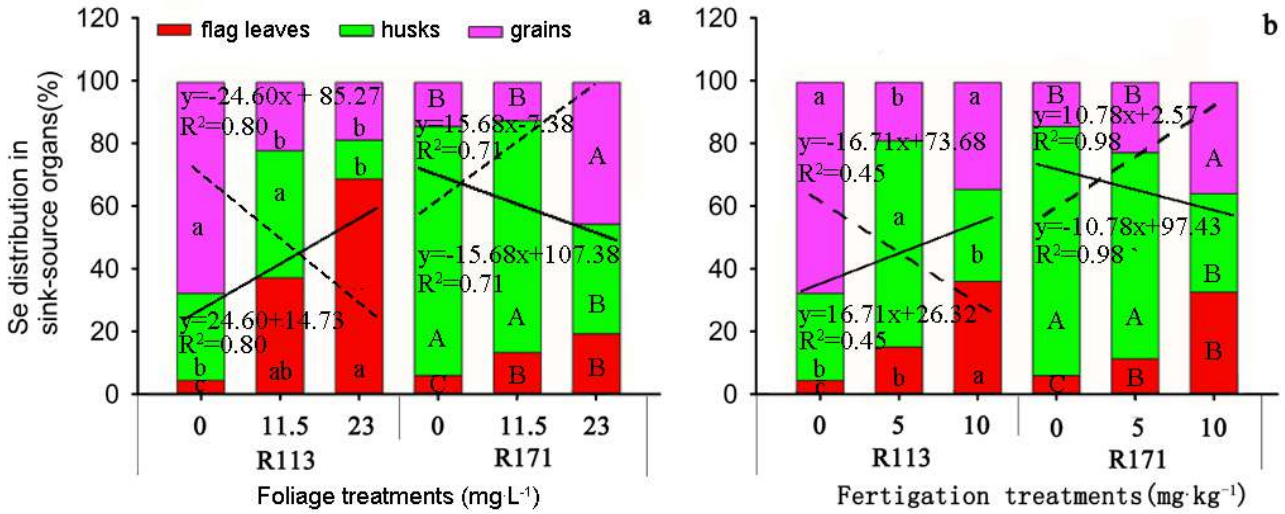

Fig 2. Se distributions in sink-source organs of wild emmer wheat as affected by Se application methods Error bars indicate ± SD. The solid and dotted lines represent the trend lines for the proportion of Se in grains and flag leaves + husks and grains of R113 and R171, respectively. Within a genotype, bars with different letters are significantly different at P<0.05, n=5.

Table 2. Trace elements contents and distributions in sink-source organs as affected by Se application methods.

Methods	Genotypes	Organs	Treatments	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Co (mg kg^{-1})	Mo (mg kg^{-1})	Cr (mg kg^{-1})
Foliage	R113	flag leaves	0	342.75±43.42b	20.34±3.04c	6.75±0.75a	0.11±0.02a	2.51±0.34c	5.01±0.60a
			11.5	372.25±38.60a	23.50±3.56bc	6.36±0.36a	0.12±0.02a	5.28±0.28b	2.95±0.50b
			23	340.03±25.99b	35.47±6.12b	5.90±1.90b	0.14±0.02a	8.61±0.99a	3.05±0.60b
		husks	0	116.47±19.55cd	31.38±4.67bc	4.71±0.73c	0.05±0.02b	1.06±0.13d	0.95±0.24c
			11.5	189.74±15.23c	77.71±10.36a	5.22±1.00c	0.05±0.00b	2.03±0.50c	0.56±0.06c
			23	108.16±20.38d	38.97±6.65b	5.80±0.80b	0.05±0.01b	1.84±0.22cd	0.61±0.10c
		grains	0	41.67±8.26e	74.08±4.08a	5.68±0.60b	0.01±0.01c	1.82±0.20cd	0.02±0.01d
			11.5	42.88±9.12de	67.19±6.95a	5.07±0.68bc	0.01±0.01c	1.97±0.40cd	0.01±0.00d
			23	33.96±5.12e	37.62±2.00b	4.45±0.45c	0.01±0.00c	2.28±0.32c	0.01±0.01d
	R171	flag leaves	0	514.12±65.12c	30.51±4.56d	10.12±1.12a	0.16±0.03c	3.76±0.50c	7.51±0.89a
			11.5	691.03±59.32b	29.41±4.00d	8.34±2.00c	0.18±0.01b	4.74±0.74b	6.17±0.95a
			23	877.45±102.35a	36.32±4.98c	9.28±3.11b	0.23±0.03a	6.27±0.27a	6.82±1.00a
		husks	0	174.71±29.32de	47.07±7.00c	7.07±1.09c	0.08±0.02d	1.59±0.20d	1.42±0.35b
			11.5	194.54±15.65cd	60.55±5.00b	9.50±0.50b	0.05±0.01de	1.78±0.22d	0.56±0.09c
			23	288.60±33.40d	82.73±9.97a	11.36±2.03a	0.10±0.02cd	1.80±0.03c	0.69±0.26c
		grains	0	28.06±2.59f	36.38±3.02c	6.85±0.47d	0.01±0.00e	1.39±0.09e	0.07±0.01d
			11.5	35.88±5.12f	36.61±6.61c	4.40±0.60e	0.01±0.01e	1.67±0.12d	0.05±0.02d
			23	52.93±6.66e	79.78±9.28a	3.47±0.69e	0.02±0.01e	1.85±0.21d	0.03±0.04d
Fertigation	R113	flag leaves	0	342.75±43.42c	20.34±3.04e	6.75±0.75a	0.11±0.02d	2.51±0.34b	5.01±0.60b
			5	680.15±50.65b	39.76±3.25bc	4.97±1.97c	0.24±0.01b	2.52±0.06b	5.14±0.36b
			10	896.63±150.36a	26.04±3.69de	5.70±1.30b	0.54±0.04a	2.86±0.20a	10.74±1.16a
		husks	0	116.47±19.55cd	31.38±4.67d	4.71±0.73c	0.05±0.02e	1.06±0.13d	0.95±0.24d
			5	171.35±25.00cd	42.59±4.44b	6.04±0.80b	0.08±0.01e	1.63±0.03cd	1.21±0.11cd
			10	212.71±22.12cd	60.00±8.00a	6.85±0.45a	0.16±0.02c	1.71±0.05c	1.66±0.20c
		grains	0	41.67±8.26de	74.08±4.08a	5.68±0.60bc	0.01±0.01f	1.82±0.20c	0.02±0.01f
			5	46.36±5.12de	28.46±3.26cde	4.17±0.17d	0.01±0.00f	2.48±0.04b	0.04±0.04e
			10	50.96±3.69d	36.22±4.98bcd	4.35±0.35c	0.01±0.00f	2.55±0.05a	0.06±0.07e
	R171	flag leaves	0	514.12±65.12c	30.51±4.56d	10.12±1.12a	0.16±0.03b	3.76±0.50b	7.51±0.89b
			5	700.28±34.00b	33.92±3.65cd	8.70±1.70b	0.18±0.02a	3.66±0.25b	8.05±0.50a
			10	855.95±68.00a	38.95±2.89cd	8.18±0.59b	0.19±0.04a	4.85±0.36a	8.55±0.61a
		husks	0	174.71±29.32d	47.07±7.00c	7.07±1.09c	0.08±0.02c	1.59±0.20d	1.42±0.35d
			5	196.55±15.00cd	59.09±5.00b	9.81±0.69a	0.09±0.02c	1.74±0.04c	2.18±0.18c
			10	208.42±15.36cd	71.03±5.00a	8.05±0.50b	0.10±0.01c	1.72±0.00c	2.12±0.08c
		grains	0	28.06±2.59e	36.38±3.02cd	3.85±0.47d	0.01±0.00d	1.39±0.09d	0.07±0.01f
			5	47.05±3.35e	37.31±4.01cd	3.73±0.55d	0.01±0.00d	1.82±0.04c	0.15±0.02e
			10	52.57±4.89e	44.07±6.00c	2.90±0.44e	0.01±0.00d	1.90±0.06bc	0.10±0.02e
Between methods				**	***	ns	***	***	ns
Between sink-source organs				***	***	***	***	***	***
Methods x sink-source organs				***	***	***	***	***	***

Data are presented as mean ± SD (n=5). Values followed by different letters within a column are significantly different among different Se content at $P<0.05$ under the same Se method. * And ** are significant at $P<0.05$ and $P<0.01$, respectively. ns is not significance.

Table 3. Toxic trace elements contents and distributions in sink-source organs as affected by Se application methods.

Methods	Genotypes	Organs	Treatments	Pb (mg kg^{-1})	Hg (mg kg^{-1})	Al(mg kg^{-1})	As (mg kg^{-1})	Sn (mg kg^{-1})	Li (mg kg^{-1})	Cd (mg kg^{-1})
Foliage	R113	flag leaves	0	0.57±0.12a	2.23±0.15a	169.63±14.35a	1.86±0.27a	0.37±0.03b	0.67±0.07a	0.11±0.02b
			11.5	0.45±0.05a	1.65±0.15b	104.84±11.97b	1.08±0.20a	0.77±0.07a	0.57±0.07a	0.08±0.02c
			23	0.52±0.10a	0.03±0.00d	113.79±10.00b	0.61±0.08b	0.86±0.10a	0.35±0.05b	0.06±0.02d
		husks	0	0.13±0.01b	0.04±0.01d	21.94±3.50c	0.21±0.01b	0.15±0.03d	0.06±0.01c	0.11±0.05b
			11.5	0.10±0.00b	0.07±0.01c	12.64±1.65c	0.11±0.01b	0.18±0.01d	0.05±0.00c	0.06±0.02d
			23	0.02±0.02c	0.06±0.10c	10.47±1.00c	0.06±0.00c	0.24±0.01c	0.03±0.01c	0.08±0.01c
		grains	0	0.03±0.00c	0.04±0.00d	1.58±0.32d	0.07±0.01c	-	-	0.25±0.04a
			11.5	0.02±0.01c	0.09±0.02c	1.49±2.00d	0.05±0.01c	-	-	0.15±0.01b
			23	0.01±0.01c	0.09±0.02c	1.27±0.07d	0.02±0.00c	-	-	0.09±0.00c
	R117	flag leaves	0	0.75±0.05a	0.13±0.02ab	187.26±29.36a	0.86±0.00a	0.48±0.08b	0.59±0.07a	0.15±0.02ab
			11.5	0.63±0.05b	0.08±0.01c	139.17±14.00b	0.80±0.10a	0.46±0.06b	0.43±0.03b	0.11±0.00c
			23	0.54±0.14b	0.04±0.00d	126.65±26.00b	0.71±0.10a	1.13±0.10a	0.51±0.13ab	0.10±0.03bc
		husks	0	0.18±0.02c	0.12±0.02b	24.10±5.00c	0.15±0.10b	0.16±0.04c	0.11±0.03c	0.20±0.02a
			11.5	0.09±0.04d	0.15±0.00a	10.52±2.00d	0.08±0.03c	0.15±0.00d	0.02±0.00c	0.16±0.01b
			23	0.02±0.04d	0.16±0.02a	9.22±5.00d	0.07±0.00c	0.19±0.04c	0.08±0.01c	0.13±0.03bc
		grains	0	-	0.05±0.01d	1.95±0.20e	0.05±0.03c	-	-	0.11±0.01c
			11.5	-	0.09±0.00c	1.66±0.20e	0.04±0.01d	-	-	0.08±0.01d
			23	-	0.08±0.01c	1.25±0.25e	0.03±0.00d	-	-	0.08±0.02d
Fertigation	R113	flag leaves	0	0.57±0.12a	2.23±0.15a	169.63±14.35a	1.86±0.27a	0.37±0.03c	0.67±0.07a	0.11±0.02b
			5	0.49±0.20b	1.25±0.01b	99.94±25.98b	0.86±0.04b	1.34±0.10a	0.41±0.04b	0.08±0.02c
			10	0.38±0.50b	0.54±0.00c	68.25±55.39c	0.60±0.05b	1.18±0.08b	0.30±0.10c	0.09±0.01bc
		husks	0	0.13±0.01c	0.04±0.01d	21.94±3.50cd	0.21±0.01c	0.15±0.03d	0.06±0.01d	0.11±0.05b
			5	0.12±0.01c	0.03±0.02de	11.40±5.89d	0.16±0.01cd	0.18±0.02d	0.04±0.01d	0.06±0.01c
			10	0.09±0.04d	0.02±0.02e	8.19±9.69d	0.11±0.02d	0.21±0.02c	0.02±0.02d	0.03±0.01d
		grains	0	0.03±0.00d	0.04±0.00d	1.58±0.32e	0.07±0.01de	-	-	0.25±0.04a
			5	0.02±0.01d	0.03±0.00de	1.32±0.10e	0.03±0.00e	-	-	0.12±0.01b
			10	0.01±0.01d	0.02±0.00e	0.98±0.08f	0.01±0.00e	-	-	0.02±0.00d
	R117	flag leaves	0	0.75±0.05a	0.15±0.02a	187.26±29.36a	0.86±0.00a	0.48±0.08b	0.59±0.07a	0.15±0.02b
			5	0.60±0.10b	0.14±0.03a	126.31±21.22b	0.43±0.03b	0.73±0.10a	0.22±0.02c	0.09±0.01c
			10	0.54±0.10c	0.13±0.00b	106.89±29.68c	0.23±0.03b	0.83±0.10a	0.31±0.04b	0.06±0.02d
		husks	0	0.18±0.02d	0.12±0.02b	24.10±5.00d	0.15±0.10b	0.16±0.04d	0.11±0.03d	0.20±0.02a
			5	0.08±0.03de	0.07±0.00c	11.02±4.00d	0.08±0.00c	0.17±0.01d	0.06±0.00d	0.13±0.00bc
			10	0.06±0.08e	0.06±0.06c	8.66±6.54de	0.05±0.01c	0.24±0.01c	0.07±0.01d	0.08±0.00d
		grains	0	-	0.05±0.01c	1.95±0.20e	0.05±0.03c	-	-	0.11±0.01c
			5	-	0.01±0.00d	0.85±0.45e	-	-	-	0.08±0.00c
			10	-	0.02±0.01d	0.65±0.89e	-	-	-	0.04±0.01d
Between methods				***	***	***	***	Ns	ns	***
Between sink-source organs				***	***	***	***	***	***	ns
Methods x sink-source organs				***	***	***	***	***	***	***

Data are presented as mean ± SD (n=5). Values followed by different letters within a column are significantly different among different Se content at $P<0.05$ under the same Se method. * And ** are significant at $P<0.05$ and $P<0.01$, respectively. ns is not significance. "-" represents the value cannot be measured below the detection limit.

Table 4. Multi-component nutrients contents and distributions in sink-source organs as affected by Se application methods.

Methods	Genotypes	Organs	Treatments	Amino acid (mg g^{-1})	Soluble protein (%)	Phytic acid (mg kg^{-1})	Inorganic phosphorus (mg kg^{-1})	Total flavonoids (mg g^{-1})	Total phenols (mg g^{-1})
Foliage	R113	flag leaves	0	2.24±0.07b	7.84±0.76d	53.96±2.03c	2.13±0.16e	2.35±0.30a	19.48±5.41c
			11.5	2.85±0.21a	8.64±0.53b	45.94±4.16d	3.42±0.19d	2.70±0.24a	23.69±3.84a
			23	2.80±0.23a	8.47±0.53bc	43.32±2.03e	3.31±0.21d	2.46±0.15a	20.43±5.85ab
		husks	0	1.37±0.10c	8.15±1.18c	47.01±3.94e	6.35±0.44b	1.09±0.14c	12.79±0.94de
			11.5	1.79±0.26bc	8.90±1.46b	33.50±1.97c	7.18±0.22a	1.31±0.17b	15.66±0.78d
			23	1.63±0.13c	8.44±0.95c	29.84±3.99f	8.11±0.09a	1.30±0.19b	12.91±1.64de
		grains	0	1.63±0.24c	14.57±2.62a	108.21±4.61a	4.69±0.56c	1.01±0.10c	7.05±0.26e
			11.5	1.68±0.15c	15.85±1.71a	98.28±7.18ab	4.64±0.67c	1.42±0.16b	8.24±0.99e
			23	1.64±0.27c	14.93±2.63a	93.11±4.64b	4.21±0.62cd	1.33±0.15b	7.09±0.67e
	R117	flag leaves	0	2.95±0.27c	7.08±0.31c	74.03±2.48c	7.04±0.24de	3.08±0.19b	23.94±6.15b
			11.5	3.42±0.12b	7.25±0.36b	66.23±3.01c	9.76±0.06d	4.09±0.11a	25.34±6.26a
			23	3.87±0.25a	7.46±0.86b	53.96±2.03d	15.13±0.60b	3.20±0.28a	24.51±6.42a
		husks	0	2.11±0.08d	7.02±0.48c	39.84±5.09e	11.35±0.49c	1.64±0.22c	17.11±0.35c
			11.5	2.55±0.11cd	7.61±0.56b	41.05±1.07de	15.73±0.06b	1.61±0.13c	17.52±0.25c
			23	2.59±0.19cd	7.76±0.25b	54.81±5.43d	22.97±0.41a	1.66±0.12c	17.45±0.30c
		grains	0	1.96±0.16e	16.02±1.95a	114.31±18.20a	5.77±0.37e	0.59±0.24d	7.59±2.48d
			11.5	1.99±0.13e	16.27±2.11a	115.30±12.63b	5.70±0.50e	0.84±0.13d	9.22±1.45cd
			23	2.37±0.31cd	18.11±2.67a	153.39±12.07b	5.26±0.73e	0.74±0.09d	8.94±0.57cd
Fertigation	R113	flag leaves	0	2.24±0.07b	7.84±0.76d	53.96±2.03b	2.13±0.16d	2.35±0.3ab	19.48±5.41b
			5	2.30±0.07a	10.29±0.74b	45.94±4.16c	2.78±0.30d	2.84±0.25a	22.18±0.46ab
			10	2.29±0.15a	9.75±0.51b	43.32±2.03c	4.01±0.61c	2.95±0.20a	26.42±0.62a
		husks	0	1.37±0.10e	8.15±1.18c	47.01±3.94c	6.35±0.44a	1.09±0.14d	12.79±0.94d
			5	2.08±0.12a	12.58±1.14ab	22.68±1.29d	5.98±0.30a	1.46±0.11b	14.22±0.93c
			10	1.43±0.24c	9.05±1.08c	33.82±1.71cd	4.45±0.54b	1.18±0.20d	13.53±1.36cd
		grains	0	1.63±0.24de	14.57±2.62b	108.21±4.61a	4.69±0.56b	1.01±0.10d	7.05±0.26e
			5	1.78±0.07d	15.54±3.06a	86.23±9.98b	3.26±0.27cd	1.22±0.04cd	7.98±0.53e
			10	1.67±0.07de	14.86±1.54b	92.96±9.16ab	2.72±0.04d	1.27±0.01c	8.68±0.90de
	R117	flag leaves	0	2.95±0.27b	7.08±0.31d	74.03±2.48b	7.04±0.24b	3.08±0.19b	23.94±6.15c
			5	3.93±0.11ab	7.92±0.67d	61.81±0.27c	8.31±0.22b	5.09±0.28a	29.89±3.68b
			10	5.12±0.22a	8.89±0.19c	51.15±0.22c	10.15±0.03a	4.90±0.80a	31.58±5.37a
		husks	0	2.11±0.08bc	7.02±0.48d	39.84±5.09d	11.35±0.49a	1.64±0.22b	17.11±0.35cd
			5	2.92±0.16b	7.80±0.60d	50.20±2.13c	8.37±0.07b	1.65±0.14b	19.24±3.54c
			10	4.75±0.46a	11.15±1.82b	62.89±0.93c	8.32±0.60b	1.41±0.10bc	17.99±4.17c
		grains	0	1.96±0.16c	16.02±1.95a	114.31±18.20b	5.77±0.37c	0.59±0.24c	7.59±2.48e
			5	2.48±0.21b	17.77±2.56a	122.72±0.60a	5.16±0.66cd	0.86±0.04c	8.37±0.88d

	10	2.67±0.25b	18.54±2.75a	134.35±0.60a	4.23±0.34d	0.98±0.01c	9.30±1.18d
Between methods		***	**	***	***	**	**
Between sink-source organs		***	***	***	***	***	***
Methods x sink-source organs		***	***	***	***	***	***

Data are presented as mean ± SD (n=5). Values followed by different letters within a column are significantly different among different Se content at $P<0.05$ under the same Se method. * And ** are significant at $P<0.05$ and $P<0.01$, respectively. ns is not significance. "-" represents the value cannot be measured below the detection limit.

Table 5. Agronomic traits of wild emmer wheat as affected by Se application methods.

Methods	Genotypes	Treatments	1000-grain weight (g)	Spike length (cm)	Grains weight per spike (g)	Grains per spike	Leaf area index (cm^2)	Plant height (cm)
Foliage	R113	0	37.37±1.35b	9.50±1.49b	0.75±0.21b	20.00±1.41c	23.60±3.58a	104.88±16.15a
		11.5	40.85±1.51a	10.10±0.26a	1.02±0.20a	25.00±1.15a	18.17±1.86b	101.30±15.91a
		23	38.08±0.79b	8.85±0.90c	0.85±0.10b	22.25±0.50b	19.26±1.67ab	99.49±9.21a
	R171	0	35.75±1.34c	10.35±0.60b	0.61±0.20c	17.00±0.82c	12.16±3.01b	100.43±8.17a
		11.5	39.01±0.96b	10.70±0.47a	0.86±0.21b	22.00±0.82b	16.75±2.29a	102.03±10.59a
		23	42.31±0.75a	10.73a±0.40a	1.26±0.12a	29.75±0.96a	15.21±3.25a	98.55±7.09a
Fertigation	R113	0	37.37±1.35b	9.50±1.49a	0.75±0.21b	20.00±1.41b	23.60±3.58a	104.88±16.15a
		5	38.60±2.17a	9.71±0.55a	0.96±0.03a	24.75±1.50a	15.70±1.41ab	95.45±3.09b
		10	36.79±0.81b	8.75±0.06b	0.81±0.10ab	22.00±0.82b	11.08±2.14b	97.43±2.69b
	R117	0	35.75±1.34b	10.35±0.60a	0.61±0.20c	17.00±0.82c	12.16±3.01b	100.43±8.17a
		5	38.52±0.87ab	10.44±0.55a	0.80±0.10b	20.75±0.45b	14.11±4.86a	97.00±5.63a
		10	40.96±0.37a	10.49±0.27a	1.12±0.05a	27.25±0.55a	13.91±3.12ab	102.48±10.82a
Between methods			**	ns	ns	ns	***	ns

Data are presented as mean ± SD (n=10). Values followed by different letters within a column are significantly different among different Se content at $P<0.05$ under the same Se method. * And ** are significant at $P<0.05$ and $P<0.01$, respectively. ns is not significance.

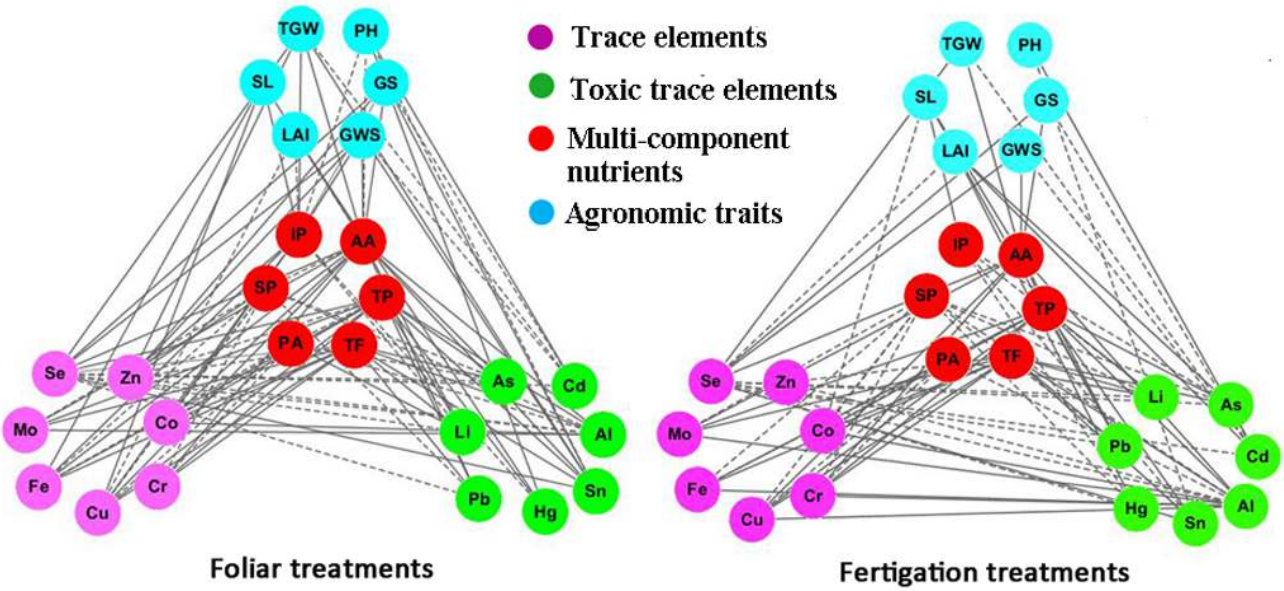

Fig 3: Comparison of nutrients-agronomic traits correlations between foliage treatments and fertigation treatments in sink-source organs of 2 genotypes. Different color nodes indicate different function categories. The edges between nodes indicate positive and negative correlations by solid and dashed lines, respectively. All the correlations reach significant levels (P<0.05). Abbreviations: AA, amino acid; SP, soluble protein; PA, phytic acid; IP, inorganic phosphorus; TF, total flavonoids; TP, total phenols; TGW, 1000-grain weight; SL, spike length; GWS, grains weight per spike; GS, grains per spike; LAI, leaf area index; PH, plant height.

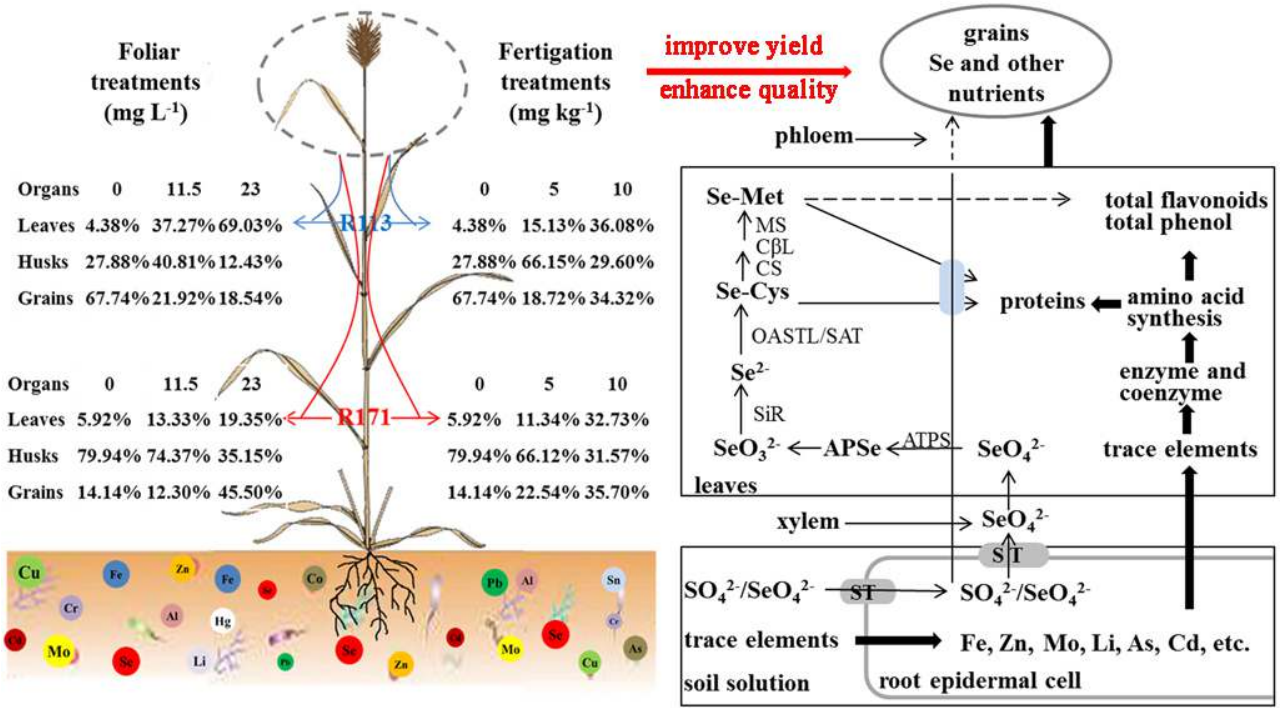

Fig 4: Schematic representation of wild emmer wheat showing the Se amount (%) in flag leaves, husks and grains treated with foliage or fertigation, and interaction of Se assimilation with other metabolic pathways. Abbreviations: ST, sulfate transporters; APSe, adenosine phospho selenite; SiR, sulphite reductase; OASTL, thiollyase; SAT, serineacetyl transferase; Blue box represents unknown transport for organic Se. Dashed arrows indicate that the process is not yet confirmed.

DISCUSSION

Application of Se fertilizer can increase the Se content in edible parts of plants. Moreover, Se contents generally improved with the increase of Se application rates (Zhu *et al*., 2017). In this experiment, both foliar Se and fertigated Se application methods significantly improved Se contents in sink-source organs (flag leaves, husks and grains) of 2 genotypes (Fig 1). However, foliage treatments were always higher in increasing Se contents in sink-source organs of 2 genotypes as compared to fertigation treatments of equal strength. This is mainly because xylem transport is more difficult than phloem transport (Marwa *et al*., 2019) (Fig 4). 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, it was also found that foliar Se application resulted in a greater proportion of Se in grains compared with fertigated Se application (Fig 2, 4). This fact allows inferring that foliar Se application method is more effective in wheat Se biofortification than fertilization.

Trace metal elements are trace minerals present in living tissue (Geng *et al*., 2018). Some of them play important effects on the body health, such as participating in oxidation-reduction reactions in energy metabolism, acting as catalysts in enzyme systems; or involvement with oxygen transport (Konikowska and Mandecka, 2018) (Fig 4). In this study, it was found that different Se application methods promoted different behaviors in terms of essential trace elements contents (Fe, Zn, Cu, Co, Mo and Cr) in sink-source organs of 2 genotypes (Table 2). Foliage treatments caused an increase in Mo contents, a decrease in Cr contents, and an increase and then decrease in Fe contents in all organs of R113 as the Se application rates improved. The Zn and Co contents in flag leaves and Zn and Cu contents in husks of R113 increased dramatically as the Se application rates improved, while Zn and Cu contents in grains and Cu contents in flag leaves showed opposite trends (Fig S2). Similar results were observed in alfalfa (Guo *et al*., 2009) and rice (Boldrin *et al*., 2013) plants fertilized with Se. Nevertheless, the Fe, Zn and Mo contents in all organs of R171 generally significantly increased with the Se application rates increased from 0 to 23 $mg \cdot L^{-1}$. The results demonstrated Se-tolerant genotype R171 also had strong enrichment ability for other trace elements. This fact was consistent with Lavu's findings that Se-enriched plants may be beneficial as fortified food with enhanced nutritional quality (Lavu *et al*., 2016) (Fig 4). In the fertigation treatments, the Cr contents in all organs of 2 genotypes showed opposite trends relative to those of the foliage treatments (Fig S2). However, Zn, Cu and Mo contents in all organs of 2 genotypes exhibited similar changes in response to foliar Se and fertigated Se application. The Co contents in grains of had no significant effects in two Se application methods as Se application rates increased, which indicated that Co contents in grains were independent of application methods and contents of Se. Moreover, regardless of applying different ratios of foliar Se or fertigated Se, the results showed Fe, Zn, Cu, Co and Cr contents in sink-source organs of R171 were generally higher than those of R113 as compared to corresponding Se treatments. In the previous studies, Gomez-Becerra *et al*. (2010) testing 19 wild emmer wheat genotypes under five different environments in two different countries revealed some outstanding accessions in terms of grain trace elements contents (i.e., Fe, Zn) and environmental stability. Accordingly, Se-tolerant genotype R171 may be used as a potential donor to improve grain trace elements contents in cultivated wheat.

There are different interactions involving the responses of crops exposed to different Se levels on toxic trace elements contents, like antagonistic or synergistic effects (Drahoňovský *et al*., 2016). The results of this study indicated that both foliar Se and fertigated Se declined Pb, Al, As, Li and Cd contents in all sink-source organs of R113 and R171 as Se application rates improved (Table 3). Among the two Se application methods, the Pb, Al, As, Sn and Li contents in sink-source organs of 2 genotypes under the corresponding Se treatment conditions followed the order flag leaves>husks>grains. Meanwhile, correlation network analysis showed that Se was found to be negatively correlated with in Pb, Al, As, Li and Cd (Fig 3). The results demonstrated that Se application decreased the contents of Pb, Al, As, Li and Cd in all sink-source organs of wild emmer wheat, indicating a possible antagonistic effect. Although toxic trace elements also have some essential functions for human body, excessive retention of either kind of toxic trace elements in the environment imposes health risk to human (Huang *et al*., 2008). For instance, Pb Hg, As and Cd are endocrine-disrupting chemicals (Li and Ji, 2017). Drahoňovský (2016) and Xu (2019) reported that Se application reduced Cd accumulation and Hg uptake in rice, respectively. Hence, according to the data of present study and previous results, it could be inferred that to inhibit the heavy metal elements contents in agricultural crops, it is feasible to apply Se in fertilization programmes.

Once absorbed by plant, Se has the same sulfate assimilation pathway due to the similar chemical structures between sulfur and Se, and incorporated into amino acid (e.g. Selenomethionine, Se-Met or Selenocysteine, Se-Cys) (Kolbert *et al*., 2018) (Fig 4). In humans, amino acid and soluble protein are beneficial nutritional effects. Therefore, they are beneficial for improving the nutritional quality of wheat. Rayman (2009) reported that Se was present as amino acid (e.g. Se-Cys) in proteins, Se-Cys was involved in protein

synthesis in place of Cys, which led to changes in protein structure, consequently causing Se toxicity (Fig 4). When Se application rates increased from 0 to 11.5 $mg \cdot L^{-1}$ or 0 to 5 $mg \cdot kg^{-1}$, amino acid and soluble protein contents in Se-treated R113 were generally significantly increased, When Se application rates were 23 $mg \cdot L^{-1}$ or 10 $mg \cdot kg^{-1}$, amino acid and soluble protein contents decreased, indicating some amino acids (e.g. Se-Cys and Se-Met) were involved in the protein synthesis (Fig 4). Nevertheless, amino acid and soluble protein contents in all sink-source organs of R171 increased concomitantly with an increase in Se application rates in two Se-treated methods. This fact allows inferring that 11.5 $mg \cdot L^{-1}$ foliar or 5 $mg \cdot kg^{-1}$ soil Se level was the toxic threshold of R113, and R171 can tolerate higher Se levels.

Total flavonoids and total phenols have antioxidant properties and senescence-resistance (Nadeem *et al*., 2018). Secondary metabolites contents in grains of 2 genotypes generally increased with Se application rates increased (Table 4) and in accordance with the report of Thiruvengadam (2015) in *Brassica rapa ssp. rapa* and Manuela (2016) in *Solanum lycopersicum*. Correlation network analysis showed that Se was found to be positively correlated with total flavonoids and total phenols in the foliage treatments (Fig 3a), and only be positively correlated with total flavonoids in the fertigation treatments (Fig 3b). Furthermore, the results also showed total flavonoids and total phenols contents in grains of R171 were generally higher than those of R113 as compared to corresponding Se treatments. Therefore, Se application can improve the health benefits of wheat by enhancing the contents of total flavonoids and total phenols. However, compared with fertigation Se treatments, foliar Se application method is more effective in health benefits of wheat.

Among the two Se application methods, researches showed that Se resulted in a marked increase first and then decrease in 1000-grain weight, spike length, grains weight per spike and grains per spike of R113 with Se application rates increased. This may be associated with the positive effects of low doses of Se. These findings are in line with those results by Godina *et al*. (2018) for tomato and Ei *et al*. (2020) for rice, which indicated improved production at low Se contents. Nevertheless, the above-mentioned agronomic traits of R171 increased with the Se application rates increased. This indicated that R171 can tolerate higher Se levels. Previous studies have shown beneficial effects of Se, as it improves the antioxidant activity in plants, resulting in increased plant yield (Hernández *et al*., 2019) (Fig 4). Correlation network analysis showed that Se was found to be positively correlated with 1000-grain weight, grains weight per spike and grains per spike in two Se application methods (Fig 3). Furthermore, it was also observed that agronomic traits of 2 genotypes were generally higher in the foliage treatments than in the corresponding fertigation treatments. The results indicated that both foliage treatments and fertigation treatments had stimulatory effects on 2 genotypes yield improvement, while foliar application was more effective than fertigation on yield improvement.

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