Article

Source-Sink Manipulation Affects Accumulation of Zinc and Other Nutrient Elements in Wheat Grains

Lan Wang [1,2,†], Haiyong Xia [1,2,3,*,†], Xiaojing Li [1,2], Yuetong Qiao [1,2], Yanhui Xue [1], Xilong Jiang [1,4], Wei Yan [5], Yumin Liu [6], Yanfang Xue [5] and Lingan Kong [1,2]

[1] Crop Research Institute, Shandong Provincial Key Laboratory of Crop Genetic Improvement, Ecology and Physiology, Shandong Academy of Agricultural Sciences, Jinan 250100, China; wl96abc@163.com (L.W.); lixiaojing4306@163.com (X.L.); qiaoyt126@163.com (Y.Q.); 1255639487@qq.com (Yanh.X.); 814456821@qq.com (X.J.); kongling-an@163.com (L.K.)
[2] College of Life Sciences, Shandong Normal University, Jinan 250014, China
[3] College of Agronomy, Qingdao Agricultural University, Qingdao 266109, China
[4] College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China
[5] Maize Research Institute, National Engineering Laboratory of Wheat and Maize, Shandong Academy of Agricultural Sciences, Jinan 250100, China; weiysdl@163.com (W.Y.); xyfang198692@163.com (Yanf.X.)
[6] Institute of Agricultural Resources and Environment, Shandong Academy of Agricultural Sciences, Jinan 250100, China; liuyumin666@126.com
* Correspondence: haiyongxia@cau.edu.cn
† These authors are equally contributed.

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Abstract: In order to better understand the source-sink flow and relationships of Zinc (Zn) and other nutrients in wheat (*Triticum aestivum* L.) plants for biofortification and improving grain nutritional quality, effects of reducing photoassimilate source (through the flag leaf removal and spike shading) or sink (through 50% spikelets removal) in the field on accumulation of Zn and other nutrients in wheat grains of two cultivars (Jimai 22 and Jimai 44) were investigated under two soil Zn application levels. The single panicle weight (SPW), kernel number per spike (KNPS), thousand kernel weight (TKW), total grain weight (TGW), concentrations and yields of various nutrient elements (Zn, Fe, Mn, Cu, N, P, K, Ca and Mg), phytate phosphorus (phytate-P), phytic acid (PA) and phytohormones (ABA: abscisic acid, and the ethylene precursor ACC: 1-aminocylopropane-1-carboxylic acid), and C/N ratios were determined. Soil Zn application significantly increased concentrations of grain Zn, N and K. Cultivars showing higher grain yields had lower grain protein and micronutrient nutritional quality. SPW, KNPS, TKW (with an exception of TKW in half spikelets removal), TGW, and nutrient yields in wheat grains were most severely reduced by half spiklets removal, secondly by spike shading, and slightly by flag leaf removal. Grain concentrations of Zn, N and Mg consistently showed negative correlations with SPW, KNPS and TGW, but positively with TKW. There were general positive correlations among grain concentrations of Zn, Fe, Mn, Cu, N and Mg, and bioavailability of Zn and Fe (estimated by molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe, or PA × Ca/Fe). Although concentrations of Zn and Fe were increased and Ca was decreased in treatments of half spikelets removal and spike shading, the simultaneously increased PA limited the increase in bioavailability of Zn and Fe. In general, different nutrient elements interact with each other and are affected to different degrees by source-sink manipulations. Elevated endogenous ABA levels and ABA/ACC ratios were associated with increased TKW and grain-filling of Zn, Mn, Ca and Mg, and inhibited K in wheat grains. However, effects of ACC were diametrically opposite. These results provide basis for wheat grain biofortification to alleviate human malnutrition.

Keywords: wheat; micronutrient; macronutrient; source-sink regulation; biofortification; phytate; bioavailability

1. Introduction

Zinc (Zn) is an essential micronutrient for the nutrition health of plants, animals and humans [1,2]. According to reports, at least one-third of the world's population is suffering from potential Zn deficiency [3-5]. Wheat (*Triticum aestivum* L.) is an important cereal crop in the world, accounting for about 30% of human daily calorie intake [6]. The Zn concentration in wheat grains is generally low, with an average value around 28-32 $mg{\cdot}kg^{-1}$ globally [7,8], which is far lower than the target value of 38-50 $mg{\cdot}kg^{-1}$ for biofortification recommended by the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO) and the HarvestPlus project from the Bill & Melinda Gates Foundation [9,10]. It is therefore of great interest to biofortify wheat grains with Zn to alleviate global malnutrition.

The source-sink relationship is the basis for grain yield and nutrient concentration formation [11]. Source-sink regulation involves the absorption/production, distribution, transport, transformation and accumulation of photoassimilates and nutrient elements, as well as the interaction between organs, and the coordination of this process is the prerequisite for high yield of crops [12]. Source-sink interactions have been intensively studied for nearly a century to improve crop yield potential, but less on grain nutritional quality [13,14]. Reducing the carbohydrate source from photosynthesis through defoliation or spike shading or reducing the grain sink size through partial spikelet removal has been commonly conducted to investigate the source-sink limitations of crop assimilates for grain development and dry matter accumulation [15,16]. These physical manipulations have been recently applied to investigate the source-sink relationship of micronutrient accumulation in wheat grains, especially for Zn [17-19]. In these experiments, results suggested that the accumulation of Zn and dry matter in wheat grains is restricted by source supply and sink capacity, but the effects of reducing source supply or sink capacity on grain Zn concentrations are inconsistent. For examples, both Zhang et al. [17] and Xia et al. [19] observed that defoliation by removing all the leaf blades from tagged culms reduced the source-to-sink ratio and decreased grain Zn concentrations accordingly, but in another study undertaken by Zhang et al. [18], defoliation increased the grain Zn concentration. Spike shading and partial spikelet removal led to increases in grain Zn concentrations in the studies of Zhang et al. [17,18], but decreases in the treatment of spike shading in Xia et al. [19]. Grain size and number are important factors determining the source-sink relationship of wheat plants. As reported by previous studies [20-26], there are inconsistent conclusions on whether the Zn concentrations depend on the size and number of wheat grains or not. No correlation between grain Zn and thousand grain weight was observed by Velu et al. [23] in adapted wheat lines, indicating no concentration effect due to the grain size. A significant negative correlation in unadapted wheat was reported by Morgounov et al. [20], but a significant positive correlation was found in our previous study [26]. Grain Zn concentrations correlated with grain numbers per m^2 [20] or per pot [25], or spike numbers [26] negatively, but not with kernel numbers per spike [26]. Such contradictory and inconsistent results are possibly resulted from the different genotypes of wheat varieties used or different environmental conditions, which need to be further verified across multiple location-years and more wheat varieties to better understand the source-sink relationships of Zn accumulation in wheat grains, especially on impacts of artificial source-sink manipulations on grain Zn accumulation and its interaction with the transport of photosynthate.

Wheat flag leaf as the main source of photoassimilate was found to contribute to more than 50% of grain filling, indicating the higher importance of the flag leaf than other leaves to source strength and sink deposition, while its defoliation generated grain yield losses of 18-30%, suggesting the role of other lower leaves to yield increased when the flag leaf was shaded or removed [27]. Similarly, the source of micronutrient (e.g. Zn and Fe) in the wheat grain depends mainly on the flag leaf and, to a lesser extent, on the lower leaves [28]. Most previous studies on how defoliation affects grain Zn accumulation of wheat are conducted through removing all leaf blades from tagged culms [17-19]. However, the effect of only the flag leaf removal on the source-sink relationship of grain Zn accumulation and the role of other lower leaves are unclear and less investigated.

Various phytohormones coordinate the source-sink relationship within the crop plant, with abscisic acid (ABA)-based chemical signaling playing important roles in promoting leaf senescence and grain-filling [13,29]. Senescence-associated mRNAs were induced by exogenous ABA application, and thus accelerated leaf senescence [30]. ABA levels seem to be elevated during senescence induced by drought or heat [31]. Yang and Zhang [13] found that elevated endogenous ABA levels and higher ratios of ABA/ethylene and ABA/gibberellins (GAs) were necessary for the efficient grain-filling of wheat. Moderate soil-drying after anthesis or applying exogenous ABA at a low concentration elevated the endogenous ABA level, which can improve the activities of the key enzymes involving carbohydrate metabolism in stem and grain, enhance the loading and unloading capacity of assimilates, and finally accelerate remobilization of assimilates to wheat grains and promote starch synthesis in grain [13]. Although hormone signaling, root and leaf growth and senescence, Zn uptake, transport and remobilization, and kernel development are intrinsically linked together during grain-filling of wheat in theory [14,32], the relationship between ABA and Zn accumulation in wheat grains has not been observed or established.

The bioavailability of Zn in wheat grains is also an important factor affecting human intake of Zn. Wheat grain is rich in anti-nutritional compounds such as phytic acid (PA) and phenolic compounds that reduce the biological availability of Zn in the human digestive tract [33], and the human body lacks phytase. Therefore, the molar ratio of PA/Zn is often used to evaluate the bioavailability of Zn in wheat-based food [34,35]. Ca^{2+} can enhance the binding ability of PA and Zn^{2+}, forming a phytic acid-calcium-zinc complex, so the molar ratio of PA × Ca/Zn can better predict the bioavailability of Zn [36]. According to the WHO [37], the critical value of the molar ratio of PA/Zn that affects Zn absorption is 15. When the value exceeds 15, the bioavailability is only 10-15%, and Zn absorption will be severely inhibited; when it is below 15, it can represent 30-35% Zn availability. Only when less than 5, it has no effect on Zn absorption. A molar ratio of PA × Ca/Zn below the critical value of 200 suggested a good Zn bioavailability [36]. Similarly, the molar ratio of PA/Fe and/or PA × Ca/Fe has also been calculated to estimate Fe bioavailability in wheat grains, and the critical value of PA/Fe molar ratio is 10 [38]. Therefore, the content of PA and the molar ratio of PA/Zn or PA/Fe in wheat grains are the key factors affecting the absorption and utilization of Zn. Reducing the PA content and the molar ratio of PA/Zn or PA/Fe is a feasible means to improve the bioavailability of Zn/Fe in wheat food. However, the effects of physical source-sink manipulations through defoliation, spike shading or spikelet removal on bioavailability of Zn and Fe in wheat grains have never been investigated.

In addition to Zn, micronutrient elements, such as Fe, manganese (Mn) and copper (Cu) also play an important role in crop yield and quality and human nutrition, more than 60% of the world's population is Fe deficient, and the deficiency of Cu is also common in developing countries [39]. As is known to all, in addition to micronutrient, crop plant has a large demand for carbon (C), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) to sustain normal growth. There have been some reports that supply of exogenous carbohydrate, N, P, K and some other nutrients (including the Irving-Williams series metals), and their status within the plant affects grain Zn accumulation of wheat [14,40,41]. At present, most studies on source-sink manipulations focus on their effects on the micronutrient accumulation in wheat grains, but less on the grain macro-elements (C, N, P, K, Ca, and Mg). Consequently, there is a lack of systematic understanding of the changes of macro- and micronutrients and the cross-talks between Zn and C, N, P, K, or other divalent cations in wheat grains.

In this study, we reduced the source of photosynthesis through flag leaf removal and spike shading or reduced the total grain sink by 50% spikelet removal in two wheat cultivars under different soil Zn application levels, and investigated their effects on (1) grain yields and yield components (e.g. single panicle weight, kernel number per spike, thousand kernel weight); (2) grain micronutrient accumulation including Zn, Fe, Mn and Cu; (3) changes of grain macro-elements (N, P, K, Ca, Mg, C/N ratio, phytate-P) and bioavailability of Zn and Fe (evaluated by molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe and PA × Ca/Fe) in wheat grains; (4) changes of phytohormones (i.e. ABA, ACC and ABA/ACC ratio) in wheat grains at maturity; (5) relationships among the above-mentioned grain yield and nutritional traits, and phytohormones across different wheat cultivars and soil Zn application rates. The differences in various grain traits between different kinds of wheat genotypes were also evaluated. These results will provide a better systematic understanding of the source-sink interactions of Zn and other nutrient elements for wheat grain biofortification to alleviate malnutrition.

2. Results

2.1. Grain Yields and Yield Components

Soil Zn application had non-significant impacts on single panicle weights, kernel numbers per spike, thousand kernel weights and total grain weights of these two wheat cultivars (Table 1). The single panicle weight, kernel number per spike and total grain weight of Jimai 22 were significantly higher than those of Jimai 44, with an increase of 13.3%, 14.5% and 12.2%, respectively. No significant effects of cultivars on the thousand kernel weight were observed. The single panicle weight, kernel number per spike and total grain weight are highest in the control treatment, followed by flag leaf removal secondly, spike shading thirdly, and the half spikelets removal lastly. The single panicle weight, kernel number per spike and total grain weight were most significantly decreased from 2.2 g in the control to 1.3 g in the treatment of half spikelets removal by 40.9%, from 38.6 to 19.4 by 49.7%, and from 52.2 to 29.0 g by 44.4% respectively. Among different source-sink treatments, the thousand kernel weights varied from 37.5 to 49.7 g and are in the order of "half spikelets removal" > "control" > "flag leaf removal" > "spike shading", spike shading had the lowest value, which was significantly lower than the control, and half spikelets removal had the maximum thousand kernel weight, which was significantly higher than the control (Table 1). In addition, as ANOVA indicates, the interaction of cultivars × source-sink manipulations significantly affected single panicle weights, and for the kernel number per spike, a significant interaction was found between treatments of soil Zn applications and source-sink manipulations (Table S1).

Table 1. Effects of soil Zn application and source-sink manipulations on grain yields and yield components of different wheat cultivars.

Treatments	Single panicle weight (g)	Kernel number per spike	Thousand kernel weight (g)	Total grain weight (g)
Zn application rate ($kg \cdot ha^{-1}$)				
$ZnSO_4 \cdot 7H_2O$ (0)	1.6a	30.4a	42.5a	38.3a
$ZnSO_4 \cdot 7H_2O$ (30)	1.6a	30.3a	42.9a	38.5a
$LSD_{0.05}$	0.1	1.2	1.6	2.5
Cultivar (C)				
Jimai 22	1.7a	32.4a	42.4a	40.6a
Jimai 44	1.5b	28.3b	43.0a	36.2b
$LSD_{0.05}$	0.1	1.2	1.6	2.5
Source-sink treatment (SS)				
Control	2.2a	38.6a	45.1b	52.2a
Flag leaf removal	1.7b	34.2b	38.7c	39.6b
Half spikelets removal	1.3d	19.4d	49.7a	29.0d
Spike shading	1.4c	29.2c	37.5c	32.9c
$LSD_{0.05}$	0.1	1.7	2.3	3.5
ANOVA				
Zn	0.6016	0.9573	0.6063	0.8436
C	<0.0001	<0.0001	0.4486	0.0009
SS	<0.0001	<0.0001	<0.0001	<0.0001
Zn × C	0.8687	0.9866	0.3948	0.6383
Zn × SS	0.1414	0.0213	0.4815	0.1067
C × SS	0.0074	0.0649	0.1021	0.0942
Zn × C × SS	0.3890	0.3733	0.6299	0.4968

Values followed by different lowercase letters in the same column are significantly different among treatments at $p \leq 0.05$. Values under ANOVA are probabilities (p values) of the source of variation.

2.2. Grain Zn, Fe, Mn and Cu Concentrations and Yields

Grain Zn concentration increased from 41.1 to 43.2 $mg \cdot kg^{-1}$ after applying Zn to the soil, with a significant increase of 5.1% (Table 2). However, grain Cu concentration was significantly reduced from 5.6 to 5.3 $mg \cdot kg^{-1}$. Grain Zn, Fe, Mn and Cu concentrations of Jimai 22 were generally lower than those of Jimai 44, with a decrease of 7.1%, 5.7%, 5.1% and 12.1%, respectively. In the source-sink treatments, grain Zn, Fe, Mn and Cu concentrations varied dramatically from 35.8 to 53.2 $mg \cdot kg^{-1}$, from 42.1 to 52.8^{-1}, from 39.0 to 59.6 $mg \cdot kg^{-1}$, and from 4.9 to 6.0 $mg \cdot kg^{-1}$, respectively, with highest values observed in the treatment of half spikelets removal (except for Cu). Both half spikelets removal and spike shading treatments had relatively higher micronutrient values than those of control and flag leaf removal, with an exception of Mn in spike shading. Compared with the control, half spikelets removal increased grain Zn, Fe and Mn concentrations up to 48.6%, 25.4% and 31.3%, respectively, spike shading increased grain Cu concentration up to 22.4%. For Zn, Fe, Mn and Cu, the flag leaf removal only decreased grain Mn concentration significantly (Table 2). Grain Zn concentrations were significantly affected by interactions of soil Zn applications × cultivars, cultivars × source-sink manipulations, and soil Zn applications × cultivars × source-sink manipulations (Table S2). Interactions of soil Zn applications × cultivars and cultivars × source-sink manipulations on grain Cu concentrations were also significant.

There were no significant impacts of soil Zn application or cultivars on grain Zn, Fe, Mn and Cu yields (Table S3). For source-sink treatments, grain Zn, Fe, Mn and Cu yields were all decreased as compared with the control. Grain Mn yields in spike shading and Cu yields in half spikelets removal were most significantly reduced. The interaction of cultivars × source-sink manipulations significantly affected grain Zn and Mn yields (Table S3).

Table 2. Effects of soil Zn application and source-sink manipulations on concentrations of Zn, Fe, Mn and Cu in grains of different wheat cultivars.

Treatments	Zn	Fe	Mn	Cu
	(mg·kg^{-1})			
Zn application rate (kg·ha^{-1})				
$ZnSO_4 \cdot 7H_2O$ (0)	41.1b	49.8a	46.4a	5.6a
$ZnSO_4 \cdot 7H_2O$ (30)	43.2a	42.5a	46.2a	5.3b
$LSD_{0.05}$	2.0	9.2	2.2	0.2
Cultivar (C)				
Jimai 22	40.6b	44.8a	45.1b	5.1b
Jimai 44	43.7a	47.5a	47.5a	5.8a
$LSD_{0.05}$	2.0	9.2	2.2	0.2
Source-sink treatment (SS)				
Control	35.8c	42.1a	45.4b	4.9b
Flag leaf removal	35.7c	44.1a	41.2c	5.0b
Half spikelets removal	53.2a	52.8a	59.6a	5.9a
Spike shading	43.6b	45.6a	39.0c	6.0a
$LSD_{0.05}$	2.9	13.0	3.2	0.3
ANOVA				
Zn	0.0420	0.1125	0.8720	0.0007
C	0.0040	0.5612	0.0332	<0.0001
SS	<0.0001	0.3744	<0.0001	<0.0001
Zn × C	0.0417	0.3430	0.4459	0.0148
Zn × SS	0.7241	0.9119	0.3553	0.2445
C × SS	<0.0001	0.4729	0.2124	0.0469
Zn × C × SS	0.0449	0.4959	0.3544	0.1657

Values followed by different lowercase letters in the same column are significantly different among treatments at $p \leq 0.05$. Values under ANOVA are probabilities (p values) of the source of variation.

2.3. Concentrations and Yields of N, P, K, Ca, Mg and Phytate-P, C/N ratios, and Molar Ratios of PA/Zn, PA × Ca/Zn, PA/Fe and PA × Ca/Fe in Wheat Grains

Compared to zero Zn supply, 30 kg $ZnSO_4 \cdot 7H_2O$ ha^{-1} significantly increased grain N and K concentrations from 17.1 to 19.2 g·kg^{-1}, and from 3.8 to 4.3 g·kg^{-1}, respectively (Table 3). Similar results were found in grain N and K yields (Table S3). Variation in Zn supply had non-significant impacts on other nutritional traits in wheat grains. "Jimai 44", as a high-quality strong gluten wheat cultivar, had significantly higher grain N concentration, and significantly lower grain K and Ca concentrations, grain N, K, Ca, Mg and phytate-P yields and molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe and PA × Ca/Fe than those of the high-yielding "Jimai 22" (Tables 3 and S3).

Compared with the control, flag leaf removal significantly decreased the grain N concentration, but significantly increased the grain Mg concentration (Table 3). Both half spikelets removal and spike shading significantly increased grain N, P and phytate-P concentrations as compared to the control and flag leaf removal treatment, with maximum values in half spikelets removal, but significantly reduced C/N ratios and molar ratios of PA × Ca/Zn, with minimum values in spike shading. In addition, grain Ca concentrations and molar ratios of PA/Zn, PA/Fe and PA ×Ca/Fe in treatments of half spikelets removal and spike shading were all relatively lower than the control and flag leaf removal treatment. Compared with the control and flag leaf removal, grain K concentration was significantly decreased by half spikelets removal, but significantly increased by spike shading. Grain Mg concentration was significantly enhanced by half spikelets removal as compared to the control and flag leaf removal treatment, but significantly decreased by spike shading as compared to the treatment of flag leaf removal (Table 3). Differently from the results of grain N, P, K, Ca, Mg and phytate-P concentrations, the corresponding yields were all decreased by source-sink treatments as compared with the control (Table S3). Grain K and Ca yields in half spikelets removal, and Ca and Mg yields in spike shading were most significantly reduced (Table S3).

The interaction of cultivars × source-sink manipulations significantly affected grain K concentrations and yields (Tables S2 and S3). Fertilizer Zn applications × source-sink manipulations interaction as well as cultivars × source-sink manipulations interaction significantly affected grain Ca concentrations and yields (Tables S2 and S3).

2.4. Concentrations and Yields/Accumulation of ABA and ACC in Wheat Grains

Soil Zn application significantly increased the ABA concentration from 25.2 to 31.0 ng g^{-1}, and the ratio of ABA/ACC from 0.7 to 1.0, but significantly decreased the ACC concentration and yield from 41.0 to 35.3 ng g^{-1} and from 1529.6 to 1322.2 ng, respectively (Tables 4 and S4). The ACC concentration and yield of "Jimai 44" was significantly higher than those of "Jimai 22", but the results of ABA yield and ABA/ACC ratio were significantly lower than "Jimai 22". Analysis of variance revealed significant effects of source-sink treatments on grain concentrations of ABA (15.4-42.6 ng g^{-1}) and ACC (30.9-52.7 ng g^{-1}), grain yields of ABA (510.8-1485.8 ng) and ACC (875.5-1741.7 ng), as well as on the ratios of ABA/ACC (0.3-1.5). Compared with the control, the grain ABA concentration and ABA/ACC ratio were significantly increased by half spikelets removal, but decreased by spike shading. Spike shading significantly increased the grain ACC concentration. Grain ABA and ACC yields were all reduced by source-sink manipulations as compared with the control, with the ABA yield in spike shading and ACC yield in half spikelets removal decreased most significantly. The interaction of cultivars × source-sink treatments significantly affected grain ABA yields. Grain ACC concentrations and yields were significantly affected by the interaction of fertilizer Zn supply × cultivars (Tables 4 and S4).

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Table 3. Effects of soil Zn application and source-sink manipulations on concentrations of N, P, K, Ca, Mg and phytate-P, C/N ratios, and molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe and PA × Ca/Fe in grains of different wheat cultivars.

Treatments	N ($g·kg^{-1}$)	P ($g·kg^{-1}$)	K ($g·kg^{-1}$)	Ca ($mg·kg^{-1}$)	Mg ($mg·kg^{-1}$)	C/N	Phytate-P ($g·kg^{-1}$)	PA/Zn	PA × Ca/Zn	PA/Fe	PA × Ca/Fe
Zn application rate ($kg·ha^{-1}$)											
$ZnSO_4·7H_2O$ (0)	17.1b	3.4a	3.8b	458.9a	1533.9a	26.3a	3.1a	30.3a	348.0a	22.7a	261.9a
$ZnSO_4·7H_2O$ (30)	19.2a	3.3a	4.3a	461.3a	1566.0a	24.7a	3.2a	29.1a	336.1a	25.6a	294.8a
$LSD_{0.05}$	0.5	0.4	0.2	10.9	54.2	1.8	0.2	2.1	25.8	3.4	41.6
Cultivar (C)											
Jimai 22	17.8b	3.2a	4.3a	474.7a	1543.3a	26.0a	3.2a	31.3a	371.2a	25.9a	307.1a
Jimai 44	18.5a	3.4a	3.8b	445.5b	1556.6a	25.0a	3.1a	28.0b	312.9b	22.4b	249.6b
$LSD_{0.05}$	0.5	0.4	0.2	10.9	54.2	1.8	0.2	2.1	25.8	3.4	41.6
Source-sink treatment (SS)											
Control	17.0c	2.6b	3.6b	495.2a	1439.2c	27.4a	2.8c	31.0a	383.4a	23.4a	289.8ab
Flag leaf removal	16.2d	2.9b	3.9b	491.7ab	1524.5b	27.6a	2.9c	32.1a	393.9a	25.6a	315.8a
Half spikelets removal	20.1a	4.0a	3.2c	477.5b	1820.7a	24.0b	3.6a	26.1b	312.8b	22.8a	274.3ab
Spike shading	19.2b	3.8a	5.5a	376.0c	1415.3c	23.0b	3.3b	29.7a	278.7b	24.9a	233.6b
$LSD_{0.05}$	0.7	0.5	0.3	15.4	76.7	2.5	0.2	3.3	39.1	4.8	58.9
ANOVA											
Zn	<0.0001	0.6780	0.0007	0.6502	0.2367	0.0751	0.3775	0.2692	0.3507	0.0917	0.1176
C	0.0157	0.1611	0.0003	<0.0001	0.6197	0.2441	0.3197	0.0032	<0.0001	0.0421	0.0085
T	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0006	<0.0001	0.0023	<0.0001	0.6304	0.0540
Zn × C	0.5808	0.0532	0.1328	0.0883	0.4575	0.2489	0.8534	0.1911	0.1263	0.5354	0.3964
Zn × SS	0.4284	0.1946	0.2364	0.0004	0.2855	0.3302	0.4835	0.5974	0.8836	0.8139	0.9642
C × SS	0.1999	0.0593	0.0084	0.0064	0.1488	0.0592	0.0837	0.2684	0.5623	0.9104	0.9586
Zn × C × SS	0.3281	0.4752	0.3643	0.0695	0.5977	0.4837	0.1155	0.0274	0.0538	0.7255	0.6029

Values followed by different lowercase letters in the same column are significantly different among treatments at $p \leq 0.05$. Values under ANOVA are probabilities (*p* values) of the source of variation.

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Table 4. Effects of soil Zn application and source-sink manipulations on concentrations of ABA and ACC and ratios of ABA/ACC in grains of different wheat cultivars.

Treatments	ABA ($ng \cdot g^{-1}$)	ACC ($ng \cdot g^{-1}$)	ABA/ACC
Zn application rate ($kg \cdot ha^{-1}$)			
$ZnSO_4 \cdot 7H_2O$ (0)	25.2b	41.0a	0.7b
$ZnSO_4 \cdot 7H_2O$ (30)	31.0a	35.3b	1.0a
$LSD_{0.05}$	6.3	5.2	0.2
Cultivar (C)			
Jimai 22	30.9a	32.6b	1.1a
Jimai 44	25.3a	43.7a	0.7b
$LSD_{0.05}$	6.3	5.2	0.2
Source-sink treatment (SS)			
Control	28.4b	34.4b	0.9b
Flag leaf removal	25.8b	34.5b	0.8b
Half spikelets removal	42.6a	30.9b	1.5a
Spike shading	15.4c	52.7a	0.3c
$LSD_{0.05}$	9.0	7.4	0.3
ANOVA			
Zn	0.0700	0.0349	0.0152
C	0.0811	0.0002	0.0029
SS	<0.0001	<0.0001	<0.0001
Zn × C	0.2616	0.0125	0.2208
Zn × SS	0.2612	0.6570	0.0634
C × SS	0.1900	0.7373	0.1347
Zn × C × SS	0.5728	0.3375	0.8401

Values followed by different lowercase letters in the same column are significantly different among treatments at $p \leq 0.05$. Values under ANOVA are probabilities (p values) of the source of variation.

2.5. Relationships among Grain Yield Traits and Nutritional Quality-related Parameters

Considering all 48 data points in this study, the SPW was positively correlated with the KNPS and TGW (Table 5). The KNPS was negatively correlated with TKW, but positively correlated with TGW. Single panicle weights, kernel numbers per spike or total grain weights were all negatively correlated with grain concentrations of Zn, Mn, Cu, N, P, Mg and phytate-P (with an exception of single panicle weight and Mn), but positively correlated with grain Ca concentration, C/N ratios, or molar ratios of PA/Zn, PA × Ca/Zn and PA × Ca/Fe (with an exception of KNPS/TGW and PA × Ca/Fe). TKW was positively correlated with grain Zn, Mn, N, Ca or Mg concentration, but negatively correlated with grain K concentration and the PA/Zn molar ratio (Table 5).

Grain concentrations of Zn, Fe, Mn, Cu, N and Mg were all positively correlated with each other, except for Fe and Cu, and Fe and N. Grain Zn and Cu concentrations were all positively correlated with grain P or phytate-P concentrations, but negatively correlated with C/N ratios. There were significant and positive correlations between grain Mn and Ca or phytate-P concentrations, and significant and negative correlations between grain Mn and K concentrations and between grain Cu and Ca concentrations. Most correlations between grain micronutrient concentrations including Zn, Fe, Mn and Cu and molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe, or PA × Ca/Fe were negative (Table 5).

For macronutrients N, P and K, only grain N concentrations were positively correlated with P, and grain N and P concentrations were all positively correlated with grain Mg and phytate-P concentrations, but negatively correlated with grain Ca concentrations, C/N ratios and molar ratios of PA × Ca/Zn. Grain N concentrations were negatively correlated with molar ratios of PA/Zn. Grain K concentrations were negatively correlated with grain Ca and Mg concentrations, and C/N ratios, but positively correlated with molar ratios of PA/Fe (Table 5).

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There were significant and positive correlations between grain Ca concentrations and grain Mg concentrations, C/N ratios, or molar ratios of PA × Ca/Zn and PA × Ca/Fe, between grain Mg and phytate-P concentrations, and between C/N ratios and molar ratios of PA/Zn or PA × Ca/Zn. There were significant and negative correlations between grain Mg concentrations and molar ratios of PA/Zn, and between C/N ratios and grain phytate-P concentrations. In addition, positive correlations were observed among molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe, and PA × Ca/Fe (Table 5).

As shown in Table S5, there were negative correlations between the TKW and grain K yield, between grain Zn yields and molar ratios of PA/Zn, and between grain Fe yields and molar ratios of PA/Fe or PA × Ca/Fe. Non-significant correlations were observed between the TKW and grain yield of Fe, Cu, N, P, Ca, or phytate-P, between grain Fe and P yield, between grain K and Zn, Fe, or Mn yield, between the C/N ratio and grain yield of Zn, Fe, Cu, N, P, K, phytate-P or TKW, between the molar ratio of PA/Zn and grain yield of Fe, Mn, Cu, N, P or Mg, and between the molar ratio of PA × Ca/Zn and grain yield of Zn, Fe, Cu, P or K. Except for negative correlations between the grain Fe yield and the molar ratio of PA/Fe or PA × Ca/Fe and positive correlations between the molar ratio of PA × Ca/Fe and grain yield of Ca or phytate-P, there were non-significant correlations between the molar ratios of PA/Fe or PA × Ca/Fe and other grain nutritional traits. The correlations for other values presented were all positive (Table S5).

2.6. Relationships between Grain Phytohormones and Grain Yield Traits or Nutritional Quality-related Parameters

Pearson correlation showed that grain ABA concentrations and ratios of ABA/ACC were positively correlated with parameters of TKW, grain concentrations of Zn, Mn, Ca, Mg, and molar ratios of PA × Ca/Fe (except for ABA and PA × Ca/Fe), but negatively correlated with grain K concentrations (Table 6). However, grain ACC concentrations were negatively correlated with SPW, TKW, grain Ca and Mg concentrations, and molar ratios of PA × Ca/Zn or PA × Ca/Fe, and positively correlated with grain Cu and K concentrations (Table 6). Grain ABA yields and ABA/ACC ratios were all positively correlated with TKW, grain Mn yields and PA × Ca/Fe molar ratios (Table S6). In addition, grain ABA yields were positively correlated with SPW, TGW, grain Zn, N, P, Ca, Mg and phytate-P yields, and molar ratios of PA × Ca/Zn. Grain ACC yields were positively correlated with SPW, KNPS, TGW, and grain Cu, N and K yields, but negatively correlated with TKW (Table S6). In terms of concentrations or yields, there were positive correlations between ABA and ABA/ACC, and negative correlations between ABA and ACC, and between ACC and ABA/ACC (Tables 6 and S6).

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Table 5. Pearson correlation coefficients among single panicle weight (SPW), kernel number per spike (KNPS), thousand kernel weight (TKW), total grain weight (TGW), concentrations of Zn, Fe, Mn, Cu, N, P, K, Ca, Mg and phytate-P, C/N ratio, and molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe and PA × Ca/Fe in wheat grains across different soil Zn applications, cultivars and source-sink treatments (*n* = 48).

Parameters	KNPS	TKW	TGW	Zn	Fe	Mn	Cu	N	P	K	Ca	Mg	C/N	Phytate-P	PA/Zn	PA × Ca/Zn	PA/Fe	PA × Ca/Fe
SPW	0.868**	ns	0.934**	-0.711**	ns	ns	-0.679**	-0.572**	-0.598**	ns	0.509**	-0.452**	0.465**	-0.598**	0.461**	0.650**	ns	0.292*
KNPS	-	-0.374**	0.884**	-0.821**	ns	-0.639**	-0.649**	-0.666**	-0.626**	ns	0.288*	-0.679**	0.412**	-0.642**	0.532**	0.572**	ns	ns
TKW	-	-	ns	0.485**	ns	0.764**	ns	0.307*	ns	-0.652**	0.437**	0.624**	ns	ns	-0.409**	ns	ns	ns
TGW	-	-	-	-0.642**	ns	-0.321*	-0.628**	-0.552**	-0.617**	ns	0.484**	-0.444**	0.395**	-0.596**	0.359*	0.557**	ns	ns
Zn	-	-	-	-	0.325*	0.607**	0.588**	0.767**	0.509**	ns	ns	0.653**	-0.614**	0.630**	-0.760**	-0.688**	ns	ns
Fe	-	-	-	-	-	0.294*	ns	ns	ns	ns	ns	0.289*	ns	ns	ns	ns	-0.789**	-0.729**
Mn	-	-	-	-	-	-	0.350*	0.421**	ns	-0.654**	0.324*	0.880**	ns	0.406**	-0.411**	ns	-0.299*	ns
Cu	-	-	-	-	-	-	-	0.514**	0.442**	ns	-0.532**	0.354*	-0.499**	0.417**	-0.441**	-0.650**	-0.304*	-0.500**
N	-	-	-	-	-	-	-	-	0.486**	ns	-0.349*	0.437**	-0.720**	0.538**	-0.537**	-0.616**	ns	ns
P	-	-	-	-	-	-	-	-	-	ns	-0.407**	0.315*	-0.341*	0.505**	ns	-0.404**	ns	ns
K	-	-	-	-	-	-	-	-	-	-	-0.641**	-0.527**	-0.289*	ns	ns	ns	0.294*	ns
Ca	-	-	-	-	-	-	-	-	-	-	-	0.321*	0.343*	ns	ns	0.660**	ns	0.527**
Mg	-	-	-	-	-	-	-	-	-	-	-	-	ns	0.472**	-0.437**	ns	ns	ns
C/N	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.473**	0.412**	0.515**	ns	ns
Phytate-P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ns	ns	ns	ns
PA/Zn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.802**	0.481**	0.446**
PA × Ca/Zn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.384*	0.646**
PA/Fe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.871**
PA × Ca/Fe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

"-" indicates no this value, "ns" indicates not significant, "*" and "**" indicate significant correlations at $p \leq 0.05$ and $p \leq 0.01$, respectively.

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Table 6. Pearson correlation coefficients between grain phytohormones (concentrations of ABA, ACC and ratios of ABA/ACC) and grain yield traits or nutritional parameters (concentrations or ratios), and among different phytohormones across different soil Zn applications, cultivars and source-sink treatments (n = 48).

Parameters	SPW	KNPS	TKW	TGW	Zn	Fe	Mn	Cu	N	P	K	Ca	Mg	C/N	Phytate-P	PA/Zn	PA × Ca/Zn	PA/Fe	PA × Ca/Fe	ABA	ACC	ABA/ACC
ABA	ns	ns	0.543**	ns	0.380*	ns	0.552**	ns	ns	ns	-0.359*	0.486**	0.455**	ns	ns	ns	ns	ns	ns	-	-0.575**	0.974**
ACC	-0.291*	ns	-0.427**	ns	ns	ns	ns	0.578**	ns	ns	0.341*	-0.652**	-0.319*	ns	ns	ns	-0.384**	ns	-0.408**	-	-	-0.687**
ABA/ACC	ns	ns	0.576**	ns	0.293*	ns	0.543**	ns	ns	ns	-0.384**	0.558**	0.482**	ns	ns	ns	ns	ns	0.310*	-	-	-

"SPW": single panicle weight, KNPS: "kernel number per spike", "TKW": thousand kernel weight, "TGW": total grain weight. "-" indicates no this value, "ns" indicates not significant, "*" and "**" indicate significant correlations at $p \leq 0.05$ and $p \leq 0.01$, respectively.

3. Discussion

3.1. Effects of Soil Zn Fertilization on Grain Yield Traits and Nutrient Accumulation of Wheat

In our study, soil Zn application did not affect yield traits of wheat (SPW, KNPS, TKW or TGW) and accumulation of most nutrient elements significantly, indicating Zn was not a growth limiting factor on the experimental site (Tables 1-3 and S1-S3). Similarly, in the study of Zhang et al. [42], grain yield, harvest index, and thousand kernel weight of winter wheat were unaffected by soil or foliar Zn applications. Zhao et al. [43,44] also found that regardless of the application method (soil or foliar alone, soil + foliar) and form ($ZnSO_4$ or Zn-EDTA), Zn fertilization had no significant effect on grain yield. These results might be firstly due to the relatively high DTPA-Zn in the soils studied and thus good plant Zn nutritional status [45]. Here, the soil DTPA-Zn was 1.6 $mg·kg^{-1}$, much higher than the reported critical value 0.46-0.75 $mg·kg^{-1}$ below which wheat responds to applied Zn [45,46], and no visible symptoms of Zn deficiency during the whole wheat growth period were observed. Therefore, Zn fertilization via soil had no significant effect on grain yield traits of wheat in the current experiment. Similar results were also reported by Zou et al. [47] on a larger scale, who investigated biofortification of wheat with Zn through soil, foliar or combined (soil + foliar) Zn fertilization at 23 experimental site-years in seven countries (China, India, Kazakhstan, Mexico, Pakistan, Turkey, and Zambia). The significant grain yield increase in response to soil Zn fertilization was found at all 6 experimental site-years only in Pakistan, whereas not at any other countries. Across all locations and cropping years, soil Zn fertilization led to yield increase only by 5.1% [47]. Secondly, no significant differences especially in the grain yield traits may be attributed to the uneven spread Zn fertilizer and insufficient sample numbers in our research, where only 30 wheat spikes of each plot were collected for analysis especially under field conditions. Differently from the results obtained under real field conditions above-mentioned, a well-controlled pot experiment showed that Zn fertilizer application to soil significantly increased grain yield and weight per grain in both wheat cultivars and both experimental years [48].

Our research showed that the application of Zn fertilizer (30 kg $ZnSO_4·7H_2O$ ha^{-1}) significantly increased grain yields/accumulation of N and K, and grain concentrations of Zn, N and K, but reduced Cu concentrations in wheat (Tables 2-3 and S2-S3). This is consistent with most previous studies on Zn [14,49,50], and consistent with Tao et al. [48] on N. However, very few findings on the relationships between soil Zn application and grain accumulation of K or Cu were reported in wheat plants, which need a further verification. The simultaneous increase in grain concentrations of Zn and N could be explained by the interaction and co-localization of both within the grain, largely in the embryo and aleurone [51]. In general, rational Zn supply is beneficial for the improvement of grain Zn and protein/N nutrition for better human dietary quality.

3.2. Cultivars Showing Higher Grain Yields Had Lower Grain Protein and Micronutrient Nutritional Quality

The contradiction between grain yield and nutritional quality in crop breeding and production has been observed in many previous studies [14,26]. Breeders usually struggle with the 8-25% yield reduction because of low phytate wheat lines used to improve grain Zn bioavailability [52,53]. Although the "Green Revolution" since the 1960s and improved soil and crop management practices have increased the average yield of wheat more than twofold [54-57], grain Zn concentrations have been considerably decreased due to the so called "dilution" effect [58-61]. Some results have demonstrated that wheat grain Zn concentrations were negatively correlated with grain yields or cultivar release years among diverse wheat cultivars and regions [24,60]. Results in this current study can also confirm the above statement. The two wheat cultivars "Jimai 22" and "Jimai 44" obviously differed in yield components (SPW, KNPS and TGW), grain concentrations of micronutrient, N, K and Ca, and bioavailability of Zn and Fe (Tables 1-3). The high-quality strong gluten wheat cultivar "Jimai 44" with relatively lower SPW, KNPS and TGW than the most commonly used high-yielding "Jimai 22", exhibited higher grain concentrations of Zn, Mn, Cu and N, and bioavailability of Zn and Fe, but lower grain concentrations of anti-nutritional compounds including K and Ca. Grain concentrations of Zn, Mn, Cu and N were negatively correlated with SPW, KNPS and/or TGW, while grain Ca concentrations and molar ratios of PA/Zn, PA × Ca/Zn and PA × Ca/Fe were positively correlated with SPW, KNPS and/or TGW (Table 5). All these results indicated that the higher the grain yields and concentrations of antinutritional compounds, the lower the grain micronutrient and N nutritional quality will be.

3.3. Effects of Physical Manipulation of Source/sink on Grain Yield Traits and Nutrient Accumulation of Wheat

3.3.1. Effects of source-sink regulation on grain yields and yield components of wheat

Many studies have reported the effects of source-sink treatments on wheat grain yields and/or yield components. Zhang et al. [17] showed that the defoliation (by removing all the leaf blades from tagged culms) and the spike shading significantly reduced the single grain weight of wheat at the mature stage, and the reduction by the defoliation was greater than the spike shading treatment. The half spikelets removal reduced the total sink capability and relatively increased the source strength, and slightly increased the single grain weight of each cultivar (0.3-7.0%) during the maturity period. Unlike Zhang et al. [17], the defoliation in our present study only removed the flag leaf, and this manipulation did not have the greatest impact on grain yield traits at the mature stage, with SPW, KNPS, TKW and TGW reduced by 22.7%, 11.4%, 14.2% and 24.1%, respectively (Table 1). Although the absence of flag leaf would weaken photosynthesis and transpiration, the production efficiency of carbohydrates to the grain would be reduced accordingly [62], the remaining leaves could still accumulate dry matter to compensate [63]. Fu et al. [64] indicated that only removing flag leaf after flowering had the least impact on grain yield composition compared to removing other leaves.

After the spike shading, the reduction of SPW (36.4%), KNPS (24.4%), TKW (16.9%) and TGW (37.0%) was greater than those of removing the flag leaf, which proved that the source of photosynthesis in the spike may be more important for grain filling and carbon assimilation accumulation than the effect of flag leaf. Research showed that spike photosynthesis contributed 9.8-39.0% to wheat grain yield, with an average of 20.1% [65]; the contribution of green leaves to grains was about 40%, and flag leaf accounted for about 19% [65,66]. Experiments have proved that after flowering, the panicle organs were in a favorable photosynthetic position, which was more conducive to intercepting light and CO_2, especially in the process of leaves gradually losing photosynthetic capacity at the later stage of plant growth [67,68], indicating the importance of spike photosynthesis.

In our experiment, the SPW, KNPS and TGW were most severely reduced by 40.9%, 49.7% and 44.4%, respectively, while the TKW was significantly increased by 10.2%, after the removal of all spikelets from one side of the spike (i.e half spikelets removal). Although appropriately reducing the sink capacity made the source-sink more coordinated, which was conducive to the increase of the single grain weight, this compensation effect still cannot make up the loss caused by excessive reduction of the KNPS [69].

3.3.2. Effects of source-sink regulation on Zn and other nutrient concentrations in wheat grains

Previous studies have reported that micronutrient concentrations in wheat grains were significantly reduced after defoliation [70], grain Zn, Fe, Mn and Cu concentrations increased to varying degrees after spikelet removal [18], and the concentration of trace elements also increased after spike shading [71]. In our study, we found that removing the flag leaf and spike shading decreased the grain Mn concentration, but the concentrations of Zn and Cu in wheat grains increased after spike shading (Table 2), suggesting that different elements were affected by the source to different degrees. Reducing the source of photosynthesis in the flag leaf and in non-leaf organs would reduce the supply of Mn and reduce the efficiency of transport to the grain. However, Zn, Cu and even Fe

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concentrations in the wheat grains in our experiment were less restricted by the source of photosynthesis. It may be due to the reduction of wheat photosynthesis production efficiency and changes in the accumulation of the chemical components of photosynthetic products, resulting in changes in the ratio of mineral metal elements [71], and ultimately increasing the concentration of most mineral metal elements in wheat grains. Consistent with previous studies [17,72], the grain Zn, Fe, Mn and Cu concentrations all increased after half spikelets removal. This may be due to the relative increase in the translocation amounts of nutrient elements received by the remaining grains [73]. Although the spikelet removal reduced the total sink, it relatively increased the source-sink ratio and supply of elements [17], and the carbohydrate supply level of the roots was also improved accordingly [74], which was beneficial to mineral nutrient absorption.

He et al. [75] investigated 8 wheat cultivars and showed that the N concentration in the remaining wheat grains increased due to partial (1/4 or 50%) spikelets removal. On the other hand, removal of the flag leaf or the upper two leaves reduced the uptake of N and P as well as the grain N concentration. Flag leaf removal would reduce the source-sink ratio, the transpiration surface area and root carbohydrate levels decreased accordingly [75]. Therefore, the grain N concentration of the two cultivars also decreased after the flag leaf removal in our study (Table 3). The source-sink ratio decreased after the spike shading, and the source of photosynthesis also weakened, but the grain N and P concentrations increased significantly. This may be due to the fact that the supply of N and P had a weaker restriction on the concentrations of N and P in grains than that of carbohydrates on the starch content of grain [76-78]. On the other hand, the half spikelets removal relatively increased the source-sink ratio and improved the carbohydrate supply level of the root system [79], which was beneficial to the increase of N and P concentrations in wheat grains in the current experiment (Table 3). Grain C/N ratios decreased accordingly in both treatments of spike shading and half spikelets removal.

The previous studies were mainly focus on the effects of altered source-sink ratios on micronutrient, N and P of wheat, but rarely on the grain K, Ca and Mg. In our current study, we observed that grain Mg concentrations were increased by the flag leaf removal and half spikelets removal, but decreased by spike shading. Grain K concentration was increased by spike shading, but decreased by half spikelets removal. Grain Ca concentrations all decreased after spike shading and half spikelets removal (Table 3). The different responses of these three elements and corresponding underlying mechanisms need a further verification and investigation.

Although concentrations of some nutrient elements increased in wheat grains, grain yields/accumulation of all nutrients (Zn, Fe, Mn, Cu, N, P, K, Ca and Mg) investigated in this study decreased to different extents due to the negative effects of source-sink regulation on grain yields and yield components (Table S3). In general, concentrations or yields of different elements were affected by the source-sink manipulation to different degrees.

3.3.3. A better understanding of the “dilution effect” caused by yield increase

Concentrations and yields of most nutrient elements in wheat grains were positively or negatively correlated with grain yield traits, indicating the strong inter-link between them (Tables 5 and S5). There may be a "dilution effect" between grain yield and grain micronutrient concentration, but previous research results were controversy, contradictory or inconsistent in terms of grain size, number or total grain yield. Grain Zn concentrations correlated with thousand kernel weight negatively [20], positively [26], or not [23]. Grain Zn concentrations correlated with grain numbers per m^2 [20] or per pot [25], or spike numbers [26] negatively, but not with kernel numbers per spike [26]. Feil and Fossati [80] showed that there was a negative correlation between grain trace element concentration and grain yield. Calderini and Ortiz-Monasterio [81] reported that there was a positive correlation between grain mineral concentration and grain weight. In the present study, grain size, number and total grain yield were greatly affected by different source-sink manipulations, SPW, KNPS and TGW consistently showed negative correlations with grain concentrations of Zn, N and Mg, while the impact of TKW was absolutely opposite (Table 5). The current results indicated that the "dilution effect" occurred due to the increase of grain number and total grain yield, and the "enrichment effect" here we proposed for the first time occurred due to the increase of grain size or single kernel/grain weight. Therefore, the weight/size and nutrient accumulation per grain/kernel could increase synchronously, i.e. "enrichment effect", which can be used to distinguish from the "dilution effect" to avoid controversy for better understanding of the relationship between nutrient accumulation and grain yield traits.

3.3.4. Effects of source-sink regulation on the bioavailability of Zn and Fe in wheat grains

In addition to the concentration of Zn and Fe in grains, its bioavailability (estimated by molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe and PA × Ca/Fe) was also crucial for increasing the human body's daily Zn intake [26]. A molar ratio of PA/Zn and PA × Ca/Zn lower than the critical ratio of 15 and 200, respectively, indicated a better Zn bioavailability [36,37], but the molar ratio of PA/Zn in most cereal products ranged from 25 to 34 [82]. The critical value of PA/Fe molar ratio is 10 [38]. In the present study, we observed for the first time that the concentration and especially bioavailability of Zn or Fe in wheat grains could be simultaneously improved by the half spikelets removal and spike shading, but not by the flag leaf removal (Tables 2-3). However, the resulting molar ratios were still much higher than their critical values, indicating the bioavailability of Zn and Fe was not better enough. This should be related to the simultaneous increase of phytate-P and Zn/Fe after half spikelets removal and spike shading, because PA was closely bound to Zn/Fe to form spherical crystals with a poorly soluble protein structure [83], which had strong binding force to Zn/Fe, thus reducing the dissolution rate of Zn/Fe. In our previous study, foliar Zn spraying significantly improved wheat grain Zn concentration, but the anti-nutritional compound phytate-P concentration was less affected, so the molar ratios of PA/Zn below 15.0 and PA × Ca/Zn below 200 occurred, suggesting higher Zn bioavailability [26]. Although the concentrations of Zn and Fe were much increased and Ca was also decreased to some extent, the simultaneously increased PA limited the increase in bioavailability of Zn and Fe in the current study (Tables 2-3). Therefore, to achieve the target level of biofortification, the most critical thing is not only to significantly increase micronutrient concentrations in wheat grains, but also aim to regulate the concentration of PA appropriately (e.g. breeding the low phytate wheat cultivars), to finally improve the bioavailability of Zn and Fe in food [84-85].

3.4. Phytohormones (ABA and ACC) Involved in Nutrient and Biomass Accumulation in Wheat Grains

Although the relationship between phytohormones and homeostasis of various nutrient elements in crop plants has been very less studied, there are still several indications that phytohormones may participate in the source-sink interaction of elemental nutrition (e.g. Zn) in wheat plants. For examples, when wheat leaves were exposed to Nano-ZnO stress, the photosynthetic carbon assimilation and antioxidant capacity were improved by melatonin [86]. The manipulation of cytokinin dehydrogenase (CKX, the enzyme that inactivates cytokinin) clearly impacts yield, root growth and orientation, and grain Zn nutrition in cereals [87]. Higher ABA levels and ABA/ethylene and ABA/GAs ratios were required for the efficient grain-filling of wheat in the report of Yang and Zhang [13]. In the current study, it seems that the elevated endogenous ABA levels and ABA/ACC ratios promoted the TKW and the grain-filling of Zn, Mn, Ca and Mg, but inhibited K in wheat grains (Table 6). There were positive correlations between grain ABA concentrations or ABA/ACC ratios and TKW, grain concentrations of Zn, Mn, Ca or Mg. However, the effects of ACC were diametrically opposite. To our knowledge, this is the first report on such phenomena, which need further verification and investigation in depth, especially to provide direct experimental evidence between ABA or ACC and homeostasis genes/binding proteins or transcripts associated with efficient transport and accumulation of nutrient elements in wheat plants, and to ascertain whether the exogenous ABA can be applied to improve wheat grain nutritional quality, which would shed new light on biofortification.

4. Materials and Methods

4.1. Study Site

The field experiment was conducted during the 2018–2019 growing season at Jinan Licheng Experimental Station (36°42′39″ N, 117°4′39″ E), Crop Research Institute, Shandong Academy of Agricultural Sciences, China. The area has a typical continental and warm climate, with an annual mean temperature of 14.7℃ and a long-term mean annual rainfall of 671.1 mm. The soil at the site was classified as clay loam, with a pH of 7.8. The top 20 cm of the soil contained 21 $g{\cdot}kg^{-1}$ organic matter, 79 $mg{\cdot}kg^{-1}$ water-hydrolysable N, 27 $mg{\cdot}kg^{-1}$ Olsen-P, 178 $mg{\cdot}kg^{-1}$ exchangeable K and 1.6 $mg{\cdot}kg^{-1}$ DTPA-extractable Zn.

4.2. Experimental Design

The experiment was a split-split-plot design with three factors consisting of four source-sink treatments (split-split plot), two wheat cultivars (subplot), and two soil Zn application levels (main plot) in three replicates. Two application levels of Zn fertilizer to soil were set: (1) no Zn application (zero); (2) 30 $kg{\cdot}ha^{-1}$ $ZnSO_4{\cdot}7H_2O$. The two winter wheat (*Triticum aestivum* L.) cultivars were "Jimai 22" and "Jimai 44", respectively. "Jimai 22" is a high-yielding wheat cultivar and is sown over the largest area in contemporary China. "Jimai 44" is a high-quality strong gluten wheat cultivar, suitable for making bread. The four source-sink treatments included: (1) no treatment as a control (CK); (2) flag leaf removal, removing the flag leaf blade from a tagged culm [88]; (3) half spikelets removal, all spikelets were removed from one side of the marked spike [89]; and (4) spike shading, wrapping the marked spike with aluminum foil paper (there are several micro-holes less than 1 mm^2 in the aluminum foil paper to facilitate the exchange of internal and external gas) [17]. The area of the main plot was 25 m × 100 m = 2500 m^2, that of the subplot was 25 m × 22 m = 550 m^2, and that of the split-split plot was 2 m × 2.5 m = 5.0 m^2.

The wheat sowing date was October 22, 2018 and harvest date was June 7, 2019. Thirty spikes of wheat plants that flowered at the same day were tagged for later treatments in each plot. Source-sink manipulations were conducted 5 days after flowering. At maturity, all wheat spikes treated/tagged in each plot were removed. Grains from two sampled spikes were then immediately ground into fine powder in liquid nitrogen for measurements of ABA and the ethylene precursor 1-aminocylopropane-1-carboxylic acid (ACC). The other wheat spikes were used for detailed investigation on the grain yield (TGW: total grain weight) and yield component (SPW: single panicle weight, KNPS: kernel number per spike and TKW: thousand kernel weight), and for nutrient analysis. All grains were manually separated from the husks.

A 750 $kg{\cdot}ha^{-1}$ of the compound fertilizer (N: P_2O_5: K_2O = 15: 15: 15) was evenly distributed and incorporated into the upper 20 cm of the soil prior to wheat planting. The other 112.5 kg of N ha^{-1} (supplied as urea) was top-dressed with irrigation at the jointing stage. All plots were adequately irrigated at stages of pre-wintering, stem elongation and flowering, and weeded manually. There were no fungicides applied during the growth period. At the booting stage, omethoate (2-dimethoxyphosphinoylthio-N-methylacetamide) (Dazhou Xinglong Chemical Co., Ltd., Dazhou, China) was sprayed to control aphids.

4.3. Quantification of ABA and ACC

The extraction and quantification of phytohormones for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis were described previously

[90-92]. The determination of ABA and ACC in this study was carried out at Shanghai Applied Protein Technology Company (Shanghai, China).

4.4. Nutrient Analysis

After the wheat was threshed, grain samples were quickly rinsed with deionized water, dried in an oven at 60-65℃ for 72 h, and then ground with a stainless-steel grinder (RT-02B, Chinese Taipei). Ground samples were digested with HNO_3-H_2O_2 in a closed microwave digester (CEM, Matthews, North Carolina, USA). The concentrations of nutrients (Zn, Fe, Mn, Cu, Ca, Mg, P, and K) in the digests were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, OPTIMA 3300 DV, PerkinElmer, Waltham, Massachusetts, USA). Two blanks and a standard grain sample Henan wheat GBW 10046 (GSB-24) were included in each batch to ensure analytical quality. The N concentration of grain was determined by H_2SO_4-H_2O_2 digestion-Kjeldahl method. The ratio of C/N was determined using Multi N/C 3100 (Analytik Jena AG, Germany). Phytate-P concentration was analyzed according to the method of Haug and Lantzsch [93]. Phytate-P was converted to PA by dividing by 0.282 to calculate the molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe and PA × Ca/Fe, which were used to predict the bioavailability of Zn and Fe in wheat grains.

4.5. Statistical Analysis

Data were subjected to ANOVA using SAS software (SAS 8.0, SAS Institute, Cary, North Carolina, USA) and means were compared by Fisher's protected least significant difference (LSD) at $P \leq 0.05$ or 0.01. SPSS software (18.0) was used for calculating Pearson correlation coefficients.

5. Conclusions

After soil application of Zn, there were no significant changes in grain yield components of wheat, but grain Zn and N concentration increased simultaneously. In general, rational Zn supply is beneficial for the improvement of grain Zn and protein/N nutrition for better human dietary quality. Cultivars showing higher grain yields had lower grain protein and micronutrient nutritional quality. The SPW, KNPS, TGW, and concentrations of grain anti-nutritional compounds (K and Ca) of the most commonly used high-yielding cultivar "Jimai 22" were significantly higher than those of the high-quality strong gluten wheat cultivar "Jimai 44", while the grain concentrations of Zn, Mn and Cu, and bioavailability of Zn and Fe of Jimai 22 were significantly lower than Jimai 44. Grain size, number, total grain yield and nutrient accumulation were greatly affected by different source-sink manipulations. SPW, KNPS, TKW (with an exception of TKW in half spikelets removal), TGW, and nutrient accumulation in wheat grains were most severely reduced by half spiklets removal, secondly by spike shading, and the flag leaf removal had a generally least impact. Grain concentrations of Zn, N and Mg consistently showed negative correlations with SPW, KNPS and TGW, but positively with TKW. There were general positive correlations among grain concentrations of Zn, Fe, Mn, Cu, N and Mg, and most correlations between these nutrient concentrations and molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe, or PA × Ca/Fe were negative. Although the concentrations of Zn and Fe were much increased and Ca was also decreased to some extent in treatments of half spikelets removal and spike shading, the simultaneously increased PA limited the increase in bioavailability of Zn and Fe. There are also some positive or negative correlations among the other nutrient elements in wheat grains. In general, different nutrient elements interact with each other and are affected to different degrees by source-sink manipulations. Phytohormones (ABA and ACC) involved in nutrient and biomass accumulation in wheat Grains. It seems that the elevated endogenous ABA levels and ABA/ACC ratios promoted the TKW and the grain-filling of Zn, Mn, Ca and Mg, but inhibited K in wheat grains. These results provide better understanding of source-sink relationships of Zn and other nutrient elements for wheat grain biofortification to alleviate human malnutrition. The underlying molecular and physiological regulatory mechanisms under different source-sink manipulations need to be further studied.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Effects of soil Zn application and source-sink manipulations on single panicle weights and kernel numbers per spike of different wheat cultivars, Table S2: Effects of soil Zn application and source-sink manipulations on concentrations of Zn, Cu, Ca and K in grains of different wheat cultivars, Table S3: Effects of soil Zn application and source-sink manipulations on yields of Zn, Fe, Mn, Cu, N, P, K, Ca, Mg and phytate-P in grains of different wheat cultivars, Table S4: Effects of soil Zn application and source-sink manipulations on yields of ABA and ACC in grains of different wheat cultivars, Table S5: Pearson correlation coefficients among single panicle weight (SPW), kernel number per spike (KNPS), thousand kernel weight (TKW), total grain weight (TGW), yields/accumulation of Zn, Fe, Mn, Cu, N, P, K, Ca, Mg and phytate-P, C/N ratio, and molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe and PA × Ca/Fe in wheat grains as affected by soil Zn applications, cultivars and source-sink treatments (n = 48), Table S6: Pearson correlation coefficients between grain phytohormones (yields of ABA, ACC and ratios of ABA/ACC) and grain yield traits or nutritional parameters (yields or ratios), and among different phytohormones across different soil Zn applications, cultivars and source-sink treatments (n = 48).

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References

1. Marschner P. *Marschner's Mineral Nutrition of Higher Plants*. Academic Press, Elsevier: San Diego, CA, USA, 2012.
2. Krężel, A.; Maret, W. The biological inorganic chemistry of zinc ions. *Arch Biochem Biophys*. **2016**, *611*, 3-19. doi: 10.1016/j.abb.2016.04.010
3. Prasad, A.S. Discovery of human zinc deficiency: Its impact on human health and disease. *Adv Nutr*. **2013**, *4(2)*, 176-190. doi: 10.3945/an.112.003210
4. Das, S.; Green, A. Importance of zinc in crops and human health. *J SAT Agric Res*. **2013**, *11*, 1-7. doi: http://ejournal.icrisat.org/Volume11/Agroecosystems/Importance_SDas.pdf
5. Ota, E.; Mori, R.; Middleton, P.; Tobe-Gai, R.; Mahomed, K.; Miyazaki, C.; et al. Zinc supplementation for improving pregnancy and infant outcome. *Cochrane Db Syst Rev*. **2015**, *(2)*, CD000230. doi: 10.1002/14651858.CD000230.pub5
6. Cakmak, I. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant Soil*. **2008**, *302(1-2)*, 1-17. doi: 10.1007/s11104-007-9466-3
7. Chen, X.P.; Zhang, Y.Q.; Tong, Y.P.; Xue, Y.F.; Liu, D.Y.; Zhang, W.; et al. Harvesting more grain zinc of wheat for human health. *Sci Rep*. **2017**, *7*, 7016. doi: 10.1038/s41598-017-07484-2
8. Wang, M.; Kong, F.; Liu, R.; Fan, Q.; Zhang, X. Zinc in wheat grain, processing, and food. *Front Nutr*. **2020**, *7*, 124. doi: 10.3389/fnut.2020.00124
9. Wang, J.W.; Mao, H.; Zhao, H.B.; Huang, D.L.; Wang, Z.H. Different increases in maize and wheat grain zinc concentrations caused by soil and foliar applications of zinc in Loess Plateau, China. *Field Crop Res*. **2012**, *135*, 89-96. doi: 10.1016/j.fcr.2012.07.010
10. Hao, Y.F.; Zhang, Y.; He, Z.H. Progress in zinc biofortification of crops. *Chin Bull Life Sci*. **2015**, *27(8)*, 1047–1054. doi: 10.13376/j.cbls/2015144
11. Mason, T.; Maskell, E. Studies of the transport of carbohydrate in the cotton plant: II. The factors determining the rate and the direction of movement of sugars. *Ann Bot-London*. **1928**, *os-42(3)*, 571-636. doi: 10.1093/oxfordjournals.aob.a090131
12. Liu, D.; Zhang, W.; Liu, Y.; Chen, X.; Zou, C. Soil application of zinc fertilizer increases maize yield by enhancing the kerner number and kernel weight of inferior grains. *Front Plant Sci*. **2020**, *11*, 188. doi: 10.3389/fpls.2020.00188
13. Yang, J.; Zhang, J. Approach and mechanism in enhancing the remobilization of assimilates and grain-filling in rice and wheat. *Chinese Sci Bull*. **2018**, *63(28-29)*, 2932-2943. doi: 10.1360/N972018-00577

14. Xia, H.; Wang, L.; Qiao, Y.; Kong, W.; Xue, Y.; Wang, Z.; et al. Elucidating the source-sink relationships of zinc biofortification in wheat grains: a review. *Food Energy Sec*. **2020**, *9(4)*, e243. doi: 10.1002/fes3.243
15. Austin, R.B.; Edrich, J. Effects of ear removal on phytosynthesis, carbohydrate accumulation and on the distribution of assimilated ^{14}C in wheat. *Ann Bot-London*. **1975**, *39(2)*, 141-152. doi: 10.1093/oxfordjournals.aob.a084922
16. Chang, T.G.; Zhu, X.G. Source-sink interaction: a century old concept under the light of modern molecular systems biology. *J Exp Bot*. **2017**, *68(16)*, 4417-4431. doi: 10.1093/jxb/erx002
17. Zhang, Y.H.; Zhou, S.L.; Zhang, K.; Wang, Z.M. Effects of source and sink reductions on micronutrient and protein contents of grain in wheat. *Acta Agron Sin*. **2008**, *34(9)*, 1629-1636. doi: 10.3724/SPJ.1006.2008.01629
18. Zhang, Y.; Zhang, Y.; Liu, N.; Su, D.; Xue, Q.; Stewart, B.A.; et al. Effect of source-sink manipulation on accumulation of micronutrients and protein in wheat grains. *J Plant Nutr Soil Sci*. **2012**, *175*, 622-629. doi: 10.1002/jpln.201100224
19. Xia, H.; Xue, Y.; Kong, W.; Tang, Y.; Li, J.; Li, D. Effects of source/sink manipulation on grain zinc accumulation by winter wheat genotypes. *Chilean J Agric Res*. **2018**, *78(1)*, 117-125. doi: 10.4067/S0718-58392018000100117
20. Morgounov, A.; Gómez-Becerra, H.F.; Abugalieva, A.; Dzhunusova, M.; Yessimbekova, M.; Muminjanov, H.; et al. Iron and zinc grain density in common wheat grown in Central Asia. *Euphytica*. **2007**, *155*, 193-203. doi: 10.1007/s10681-006-9321-2
21. Nowack, B.; Schwyzer, I.; Schulin, R. Uptake of Zn and Fe by wheat (*Triticum aestivum* var. Greina) and transfer to the grains in the presence of chelating agents (Ethylenediaminedisuccinic acid and Ethylenediaminetetraacetic acid). *J Agric Food Chem*. **2008**, *56(12)*, 4643-4649. doi: 10.1021/jf800041b
22. Velu, G.; Ortiz-Monasterio, I.; Singh, R.P.; Payne, T. Variation for grain micronutrients concentration in wheat core-collection accessions of diverse origin. *Asian J Crop Sci*. **2011**, *3(1)*, 43-48. doi: 10.3923/ajcs.2011.43.48
23. Velu, G.; Singh, R.P.; Huerta-Espino, J; Peña, R.J.; Arun, B.; Mahendru-Singh, A.; et al. Performance of biofortified spring wheat genotypes in target environments for grain zinc and iron concentrations. *Field Crop Res*. **2012**, *137*, 261-267. doi: 10.1016/j.fcr.2012.07.018
24. Velu, G.; Ortiz-Monasterio, I.; Cakmak, I.; Hao, Y.; Singh, R.P. Biofortification strategies to increase grain zinc and iron concentrations in wheat. *J Cereal Sci*. **2014**, *59(3)*, 365-372. doi: 10.1016/j.jcs.2013.09.001
25. Singh, B.R.; Timsina, Y.N.; Lind, O.C.; Cagno, S.; Janssens, K. Zinc and iron concentration as affected by nitrogen fertilization and their localization in wheat grain. *Front Plant Sci*. **2018**, *9*, 307. doi: 10.3389/fpls.2018.00307
26. Xia, H.; Xue, Y.; Liu, D.; Kong, W.; Xue, Y.; Tang, Y.; et al. Rational application of fertilizer nitrogen to soil in combination with foliar Zn spraying improved Zn nutritional quality of wheat grains. *Front Plant Sci*. **2018**, *9*, 677. doi: 10.3389/fpls.2018.00677
27. Wazzike, H.E.; Yousfi, B.E.; Serghat, S. Contributions of three upper leaves of wheat, either healthy or inoculated by Bipolaris sorokiniana, to yield and yield components. *Aust J Crop Sci*. **2015**, *9(7)*, 629-637.
28. Waters, B.M.; Uauy, C.; Dubcovsky, J.; Grusak, M.A. Wheat (*Triticum aestivum*) NAM proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. *J Exp Bot*. **2009**, *60(15)*, 4263-4274. doi: 10.1093/jxb/erp257
29. Woo, H.R.; Kim, H.J.; Lim, P.O.; Nam, H.G. Leaf senescence: Systems and dynamics aspects. *Annu Rev Plant Biol*. **2019**, *70*, 347-376. doi: 10.1146/annurev-arplant-050718-095859
30. Lee, I.C.; Hong, S.W.; Whang, S.S.; Lim, P.O.; Nam, H.G.; Koo, J.C. Age-dependent action of an ABA-inducible receptor kinase, RPK1, as a positive regulator of senescence in Arabidopsis leaves. *Plant Cell Physiol*. **2011**, *52(4)*, 651-662. doi: 10.1093/pcp/pcr026
31. Sah, S.; Reddy, K.; Li, J. Abscisic acid and abiotic stress tolerance in crop. *Front Plant Sci*. **2016**, *7*, 571. doi: 10.3389/fpls.2016.00571
32. Gupta, O.P.; Pandey, V.; Saini, R.; Narwal, S.; Malik, V.K.; Khandale, T.; et al. Identifying transcripts associated with efficient transport and accumulation of Fe and Zn in hexaploid wheat (T. aestivum L.). *J Biotechnol*. **2020**, *316*, 46-55. doi: 10.1016/j.jbiotec.2020.03.015
33. Cakmak, I.; Pfeiffer, W.H.; McClafferty, B. Biofortification of durum wheat with zinc and iron. *Cereal Chem*. **2010**, *87*, 10-20. doi: 10.1094/CCHEM-87-1-0010
34. Ryan, M.H.; Mclnerney, J.K.; Record, I.R.; Angus, J.F. Zinc bioavailability in wheat grain in relation to phosphorus fertiliser, crop sequence and mycorrhizal fungi. *J Sci Food Agric*. **2008**, *88(7)*, 1208-1216. doi: 10.1002/jsfa.3200
35. Liu, D.Y.; Liu, Y.M.; Zhang, W.; Chen, X.P.; Zou, C.Q. Agronomic approach of zinc biofortification can increase zinc bioavailability in wheat flour and thereby reduce zinc deficiency in humans. *Nutrients*. **2017**, *9(5)*, 465. doi: 10.3390/ nu9050465
36. Kwun, I.S.; Kwon, C.S. Dietary molar ratios of phytate: zinc and millimolar ratios of phytate × calcium: zinc in South Koreans. *Biol Trace Elem Res*. **2000**, *75(1-3)*, 29-41. doi: 0163–4984/00/7501–3–0029
37. World Health Organization (WHO). *Trace Elements in Human Nutrition and Health*. WHO: Geneva, Switzerland, 1996.
38. Glahn, R.P.; Wortley, G.M.; South, P.K.; Miller, D.D. Inhibition of iron uptake by phytic acid, tannic acid, and $ZnCl_2$: studies using an in vitro digestion/Caco-2 cell model. *J Agric Food Chem*. **2002**, *50(2)*, 390-395. doi: 10.1021/jf011046u
39. White, P.J.; Broadley, M.R. Biofortification of crops with seven mineral elements often lacking in human diets - iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytol*. **2009**, *182(1)*, 49-84. doi: 10.1111/j.1469-8137.2008.02738.x
40. Gupta, N.; Ram, H.; Kumar, B. Mechanism of zinc absorption in plants: uptake, transport, translocation and accumulation. *Rev Environ Sci Biotechnol*. **2016**, *15*, 89-109. doi: 10.1007/s1115 7-016-9390-1
41. Singh, D.; Prasanna, R. Potential of microbes in the biofortification of Zn and Fe in dietary food grains. A review. *Agron Sustain Dev*. **2020**, *40*, 15. doi: 10.1007/s13593-020-00619-2

42. Zhang, Y.Q.; Sun, Y.X.; Ye, Y.L.; Karim, M.R.; Xue, Y.F.; Yan, P. et al. Zinc biofortification of wheat through fertilizer applications in different locations of China. *Field Crop Res*. **2012**, *125*, 1-7. doi: 10.1016/j.fcr.2011.08.003
43. Zhao, A.Q.; Tian, X.H.; Cao, Y.X.; Lu, X.C.; Liu, T. Comparison of soil and foliar zinc application for enhancing grain zinc content of wheat when grown on potentially zinc-deficient calcareous soils. *J Sci Food Agric*. **2014**, *94(10)*, 2016-2022. doi: 10.1002/jsfa.6518
44. Zhao, A.; Wang, B.; Tian, X.; Yang, X. Combined soil and foliar $ZnSO_4$ application improves wheat grain Zn concentration and Zn fractions in a calcareous soil. *Eur J Soil Sci*. **2020**, *71(4)*, 681-694. doi: 10.1111/ejss.12903
45. Bansal, R.L.; Takkar, P.N.; Bhandari, A.L.; Rana, D.S. Critical level of DTPA extractable Zn for wheat in alkaline soils of semi-arid region of Punjab, India. *Ferti Res*. **1990**, *21(3)*, 163-166. doi: 10.1007/BF01087426
46. Wang, Z.M.; Liu, Q.; Pan, F.; Yuan, L.X.; Yin, X.B. Effects of increasing rates of zinc fertilization on phytic acid and phytic acid/zinc molar ratio in zinc bio-fortified wheat. *Field Crop Res*. **2015**, *184*, 58–64. doi: 10.1016/j.fc.2015.09.007
47. Zou, C.; Zhang, Y.; Rashid, A.; Ram, H.; Savasli, E.; Arisoy, R.; et al. Biofortification of wheat with zinc through zinc fertilization in seven countries. *Plant Soil*. **2012**, *361(1-2)*, 119-130. doi: 10.1007/s11104-012-1369-2
48. Tao, Z.Q.; Wang, D.M.; Chang, X.H.; Wang, Y.J.; Yang, Y.S.; Zhao, G.C. Effects of zinc fertilizer and short-term high temperature stress on wheat grain production and wheat flour proteins. *J Integr Agr*. **2018**, *17(9)*, 1979-1990. doi: 10.1016/S2095-3119(18)61911-2
49. Sharma, P.; Aggarwal, P.; Kaur, A. Biofortification: A new approach to eradicate hidden hunger. *Food Rev Int*. **2017**, *33(1)*, 1-21. doi: 10.1080/87559129.2015.1137309
50. Cakmak, I.; Kutman, U.B. Agronomic biofortification of cereals with zinc: a review. *Eur J Soil Sci*. **2018**, *69(1)*, 172-180. doi: https://doi.org/10.1111/ejss.12437
51. Kutman, U.B.; Yildiz, B.; Ozturk, L.; Cakmak, I. Biofortification of durum wheat with zinc through soil and foliar applications of nitrogen. *Cereal Chem*. **2010**, *87(1)*, 1-9. doi: 10.1094/CCHEM-87-1-0001
52. Guttieri, M.; Bowen, D.; Dorsch, J.A.; Raboy, V.; Souza, E. Identification and characterization of a low phytic acid wheat. *Crop Sci*. **2004**, *44(2)*, 418-424. doi: 10.2135/cropsci2004.4180
53. Guttieri, M.J.; Peterson, K.M.; Souza, E.J. Agronomic performance of low phytic acid wheat. *Crop Sci*. **2006**, *46(6)*, 2623-2629. doi: 10.2135/cropsci2006.01.0008
54. Tilman, D.; Cassman, K.G.; Matson, P.A.; Naylor, R.; Polasky, S. Agricultural sustainability and intensive production practices. *Nature*. **2002**, *418(6898)*, 671-677. doi: 10.1038/nature01014
55. Davis, D.R. Declining fruit and vegetable nutrient composition: what is the evidence? *HortScience*. **2009**, *44(1)*, 15-19. doi: 10.21273/HORTSCI.44.1.15
56. Grassini, P.; Eskridge, K.M.; Cassman, K.G. Distinguishing between yield advances and yield plateaus in historical crop production trends. *Nat Commun*. **2013**, *4*, 2918. doi: 10.1038/ncomms3918
57. Curtis, T.; Halford, N.G. Food security: the challenge of increasing wheat yield and the importance of not compromising food safety. *Ann Appl Biol*. **2014**, *164(3)*, 354-372. doi: 10.1111/aab.12108
58. Garvin, D.F.; Welch, R.M.; Finley, J.W. Historical shifts in the seed mineral micronutrient concentration of US hard red winter wheat germplasm. *J Sci Food Agric*. **2006**, *86(13)*, 2213-2220. doi: 10.1002/jsfa.2601
59. Fan, M.S.; Zhao, F.J.; Fairweather-Tait, S.J.; Poulton, P.R.; Sunham, S.J.; McGrath, S.P. Evidence of decreasing mineral density in wheat grain over the last 160 years. *J Trace Elem Med Bio*. **2008**, *22(4)*, 315-324. doi: 10.1016/j.jtemb.2008.07.002
60. Zhao, F.J.; Su, Y.H.; Dunham, S.J.; Rakszegi, M.; Bedo, Z.; McGrath, S.P.; et al. Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. *J Cereal Sci*. **2009**, *49(2)*, 290-295. doi: 10.1016/j.jcs.2008.11.007
61. Shewry, P.R.; Pellny, T.K.; Lovegrove, A. Is modern wheat bad for health? *Nat Plants*. **2016**, *2(7)*, 1-3. doi: 10.1038/nplants.2016.97
62. Liu, Y.; Zhang, P.; Li, M.; Chang, L.; Cheng, H.; Chai, S.; et al. Dynamic responses of accumulation and remobilization of water soluble carbohydrates in wheat stem to drought stress. *Plant Physiol Bioch*. **2020**, *155*, 262-270. doi: 10.1016/j.plaphy.2020.07.024
63. Makary, T.; Schulz, R.; Müller, T.; Pekrun, C. Simplified N fertilization strategies for winter wheat. Part 1: plants: compensation capacity of modern wheat varieties. *Arch Agron Soil Sci*. **2020**, *66(6)*, 847-857. doi: 10.1080/03650340.2019.1641697
64. Fu, X.; Shi, Z.; Ma, C.; Shan, Z.; Liu, Z.; Zhao, Y.; et al. Effect of N fertilizer and removing leaves after flowering on 1000-grain weight. *Chinese Agric Sci Bull*. **2015**, *31(6)*, 31-34.
65. Zhang, M.; Gao, Y.; Zhang, Y.; Fischer, T.; Zhao, Z.; Zhou, X.; et al. The contribution of spike photosynthesis to wheat yield needs to be considered in process-based crop models. *Field Crop Res*. **2020**, *257*, 107931. doi: 10.1016/j.fcr.2020.107931
66. Toyota, M.; Tsutsui, I.; Kusutani, A.; Asanuma, K.I. Initiation and development of spikelets and florets in wheat as influenced by shading and nitrogen supply at the spikelet phase. *Plant Prod Sci*. **2001**, *4(4)*, 283-290. doi: 10.1626/pps.4.283
67. Wang, Z.M.; Zhang, Y.H.; Zhang, Y.P.; Wu, Y.C. Review on photosynthetic performance of ear organs in *Triticeae* crops. *J Triticeae Crops*. **2004**, *24(4)*, 136-139.
68. Tian, J.C.; Wang, Y.X.; Tang, S.L. The relationship between different photosynthetic organs and grain yield in different genotype super wheats. *Shandong Agric Sci*. **2005**, *4*, 12-14. doi: 10.14083/j.issn.1001 -4942.2005.04.004
69. Shen, X.L. Effects of Source-Sink Change of Winter Wheat on the Yield of Winter Wheat and Its Yield Structure. PhD Thesis, Northwest A&F University, Yangling, China, 2005.

70. Kutman, U.B.; Yildiz, B.; Cakmak, I. Effect of nitrogen on uptake, remobilization and partitioning of zinc and iron throughout the development of durum wheat. *Plant Soil*. **2011**, *342*, 149-164. doi: 10.1007/s11104-010-0679-5
71. Zhang, D.; Luo, X.S.; Zhao, Z.; Hu, Z.H.; Suo, C.; Chen, Y.; et al. Effect of reduced solar radiation (shading) on wheat yield and mineral metal element content. *Jiangsu Agric Sci*. **2019**, *47(10)*, 75-78. doi: 10.15889/j.issn.1002-1302.2019.10.017
72. Liu, N.; Yu, P.; Wang, C.; Xi, W.X.; Wang, Z.M.; Zhang, Y.H. Effect of leaf and spikelet removal on grain micronutrient and protein concentration in wheat. *J China Agric Univ*. **2013**, *18(6)*, 42-53. doi: 10.11841/j.issn.1007-4333.2013.06.06
73. Loughman, B.C. The application of in vivo techniques in the study of metabolic aspects of ion absorption in crop plants. *Plant Soil*. **1987**, *99*, 63-74. doi: 10.1007/BF02370154
74. Sabrina, B.; Mohammed-Réda, D.; Rachid, R.; Kamel, R.; Houria, B. Correlation between changes in biochemical roots of wheat (*Triticum durum desf*) and stress induced by some regimes fertilizer NPK. *American-Eurasian J Toxicol Sci*. **2011**, *3(1)*, 47-51.
75. He, M.; Cao. H.; Wang, Z.; Chen, M. Uptake accumulation and utilization efficiency of nitrogen and phosphorus in winter wheat with altered source-sink ratios. *Acta Bot Boreal-Occident Sin*. **1996**, *16(4)*, 361-367.
76. Zhang, Y.H.; Sun, N.N.; Hong, J.P.; Zhang, Q.; Wang, C.; Xue, Q.W.; et al. Effect of source-sink manipulation on photosynthetic characteristics of flag leaf and the remobilization of dry mass and nitrogen in vegetative organs of wheat. *J Integr Agr*. **2014**, *13(8)*, 1680-1690. doi: 10.1016/S2095-3119(13)60665-6
77. Smith, M.R.; Rao, I.M.; Merchant, A. Source-sink relationships in crop plants and their influence on yield development and nutritional quality. *Front Plant Sci*. **2018**, *9*, 1889. doi: 10.3389/fpls.2018.01889
78. Yadav, S.; Kanwar, R.S.; Patil, J.A.; Tomar, D. Effects of *Heterodera avenae* on the absorption and translocation of N, P, K, and Zn from the soil in wheat. *J Plant Nutr*. **2020**, *43(17)*, 2549-2556. doi: 10.1080/01904167.2020.1783296
79. You, C.; Zhu, H.; Xu, B.; Huang, W.; Wang, S.; Ding, Y.; et al. Effect of removing superior spikelets on grain filling of inferior spikelets in rice. *Front Plant Sci*. **2016**, *7*, 1161. doi: 10.3389/fpls.2016.01161
80. Feil, B.; Fossati, D. Mineral composition of triticale grains as related to grain yield and grain protein. *Crop Sci*. **1995**, *35(5)*, 1426-1431. doi: 10.2135/cropsci1995.0011183X003500050028x
81. Calderini, D.F.; Ortiz-Monasterio, I. Grain position affects grain macronutrient and micronutrient concentrations in wheat. *Crop Sci*. **2003**, *43(1)*, 141-151. doi: 10.2135/cropsci2003.1410
82. Welch, R.M.; Graham, R.D. Breeding crops for enhanced micronutrient content. *Plant Soil*. **2002**, *245(1)*, 205–214. doi: 10.1023/A:1020668100330
83. Brinch-Pedersen, H.; Borg, S.; Tauris, B.; Holm, P.B. Molecular genetic approaches to increasing mineral availability and vitamin content of cereals. *J Cereal Sci*. **2007**, *46(3)*, 308-326. doi: 10.1016/j.jcs.2007.02.004
84. Hurrell, R.F.; Reddy, M.B.; Juillerat, M.; Cook, J.D. Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. *Am J Clin Nutr*. **2003**, *77(5)*, 1213-1219. doi: 10.1093/ajcn/77.5.1213
85. Egli, I.; Davidsson, L.; Zeder, C.; Walczyk, T.; Hurrell, R. Dephytinization of a complementary food based on wheat and soy increases zinc, but not copper, apparent absorption in adults. *J Nutr*. **2004**, *134(5)*, 1077-1080. doi: 10.1093/jn/134.5.1077
86. Zuo, Z.; Sun, L.; Wang, T.; Miao, P.; Zhu, X.; Liu, S.; et al. Melatonin improves the photosynthetic carbon assimilation and antioxidant capacity in wheat exposed to Nano-ZnO stress. *Molecules*. **2017**, *22*, 1727. doi: 10.3390/molecules22101727
87. Chen, L.; Zhao, J.; Song, J.; Jameson, P.E. Cytokinin dehydrogenase: a genetic target for yield improvement in wheat. *Plant Biotech J*. **2020**, *18(3)*, 614-630. doi: 10.1111/pbi.13305
88. Rivera-Amado, C.; Molero, G.; Trujillo-Negrellos, E.; Reynolds, M.; Foulkes, J. Estimating organ contribution to grain filling and potential for source upregulation in wheat cultivars with a contrasting source–sink balance. *Agronomy*. **2020**, *10(10)*, 1527. doi: 10.3390/agronomy10101527
89. Xie, Z.; Zhang, X.; Zhang, M.; Miao, F.; Ren, P. Effect of source-sink manipulation on grain material accumulation of cold-type and warm-type wheat. *Acta Agric Boreal-Occident Sin*. **2010**, *19(7)*, 53-56.
90. Glauser, G.; Wolfender, J.L. A non-targeted approach for extended liquid chromatography-mass spectrometry profiling of free and esterified jasmonates after wounding. *Methods Mol Biol*. **2013**, *1011*, 123-134.
91. Yan, C.; Fan, M.; Yang, M.; Zhao, J.; Zhang, W.; Su, Y.; et al. Injury activates Ca^{2+}/calmodulin-dependent phosphorylation of JAV1-JAZ8-WRKY51 complex for jasmonate biosynthesis. *Mol Cell*. **2018**, *70(1)*, 136-149. doi: 10.1016/j.molcel.2018.03.013
92. Shao, Y,; Zhou, H.; Wu, Y.; Zhang, H.; Lin, J.; Jiang, X.; et al. OsSPL3, an SBP-domain protein, regulates crown root development in rice. *Plant Cell*. **2019**, *31(6)*, 1257-1275. doi: 10.1105/tpc.19.00038
93. Haug, W.; Lantzsch, H.J. Sensitive method for the rapid determination of phytate in cereals and cereal products. *J Sci Food Agric*. **1983**, *34(12)*, 1423–1426. doi: 10.1002/jsfa.2740341217