

Using agronomic biofortification to boost zinc, selenium, and iodine concentrations of food crops grown on the loess plateau in China

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Abstract

Micronutrient malnutrition among humans is typically caused by micronutrient deficiency in soils and then staple food crops grown on these soils. In this study, field trials were conducted to investigate the biofortification of micronutrients in the edible parts of winter wheat, maize, soybean, potato, canola, and cabbage. Fertilizers of Se, Zn and I were applied to soil independently or together, while Se and Zn were sprayed as solution on winter wheat in another part of the trials. Selenium, when applied to the soil in the form of sodium selenate, whether alone or combined with Zn and/or I, was effective in increasing Se to around target levels in all of the tested crops. Selenium as sodium selenite was effective as a foliar application to winter wheat, increasing it from 25 to 312 $\mu\text{g kg}^{-1}$ in wheat grain with 60 g Se ha^{-1} . For Zn, soil-applied zinc sulphate was only found to be effective for increasing the Zn concentration in cabbage leaf and canola seed, with 35 and 61 mg kg^{-1} , respectively, while foliar zinc sulphate application was effective in biofortifying winter wheat, increasing grain Zn from 20 to 30 mg kg^{-1} . While for I, soil-applied potassium iodate was only effective in increasing I concentration in cabbage leaf, and biofortification of the other crops was not possible. The enhancements of Se, Zn, and I concentration resulting from either the single or combined application of microelement fertilizers were similar. Therefore, agronomic biofortification of edible parts of various food crops with Zn, Se, and I can be an effective way to increase micronutrient concentrations, and the effectiveness depends on crop species, fertilizer forms and application methods.

Keywords: Biofortification, selenium, zinc, iodine, loess soil

1. Introduction

About half of the world's population suffers from micronutrient malnutrition, including Se (selenium), Zn (zinc), and I (iodine), which is mainly associated with low dietary intake of micronutrients in diets with less diversity of food (Brown *et al.*, 2001; Mayer *et al.*, 2008). Biofortification of staple food crops with micronutrients through the use of agricultural tools is a cost-effective and sustainable approach to address this problem (Cakmak, 2008; White and Broadley, 2005). However, plant breeding, the most powerful agricultural approach, may not effectively work in regions where soils have very low plant-available pools of micronutrients due to very adverse soil chemical and physical conditions (Cakmak, 2008). Besides, finding sufficient and promising genotypic variation and maintaining the stability of targeted micronutrient traits across diverse types of environments may also be difficult (Lyons *et al.*, 2005a). Under such circumstances, agronomic biofortification, including the use of micronutrient fertilizers, is an important complementary solution (White and Broadley, 2009).

Soils deficient in micronutrients including Zn, Se, and I, are widespread in China and a vast amount of soils have multi-microelement deficiency (Liu, 1994; Tan, 2004). Soils with low plant-available Zn ($<1 \text{ mg kg}^{-1}$ DTPA-extractable Zn) occupy 40% of China's territory (Liu, 1994) and affects crop yields as well as human and animal health by reducing growth rates, causing stunting, and increasing susceptibility to diseases (Prasad, 2009). Iodine deficiency is also common, with endemic goiter occurring in 28 provinces and autonomous regions in China (Tan, 2004). In severely I-deficient areas with simultaneous soil Se deficiency, a severe cretinism problem marked by irreversible mental and growth retardation occurs at prevalence rates exceeding 1% (Yang *et al.*, 2007). Selenium deficiency mainly occurs in a belt from northeast to southwest China and represents an important factor in the occurrence of the arthritic condition called Kashin-Beck disease, which greatly reduces human well-being and working ability (Yang *et al.*, 2007).

The micronutrients Se and I can be supplied in salt form to reduce the incidence of Kashin-Beck disease. However, the form of Se that naturally occurs in food may be more effective than inorganic Se used for this purpose (Bawa *et al.*, 1992). Thus, the most efficient way to deal with this problem may be to improve the levels of these micronutrients in locally grown food crops by using an agronomic biofortification approach.

There are a range of crop biofortification approaches available. For Zn fertilization, foliar application seems to be more effective than soil application to reach the target concentration of 40 mg kg^{-1} in edible parts of crops (Cakmak *et al.*, 2010b; Wang *et al.*, 2012; Yilmaz *et al.*, 1997). For Se, both soil and foliar applications of Se fertilizers are highly effective in increasing grain Se to the target concentration of $300 \text{ } \mu\text{g kg}^{-1}$ (Lyons *et al.*, 2005b). The fertigation program in Northwestern China showed that I applied in irrigation water resulted in very positive effects on major food crop, human, and animal I status (Cao *et al.*, 1994). Increasing I level in the edible parts of vegetables through a soil fertilization strategy also seemed effective (Dai *et al.*, 2004), although the target I concentration of $500 \text{ } \mu\text{g kg}^{-1}$ in grain is hard to reach (Mackowiak and Grossl, 1999).

It has been shown that soil- and/or foliar-applied Se, Zn or I fertilizers have positive effects on the biofortification of different food crops (Cakmak *et al.*, 2010a; Cao *et al.*, 1994; Dai *et al.*, 2004; Hasani *et al.*, 2012; Lyons *et al.*, 2005a). However, the effect of a combined foliar/soil application of micronutrients on grain Zn, Se, and I in wheat grown under field conditions has not yet been reported. In the present study, we investigated the effects of soil and foliar applications of Se, Zn, and I on yield and concentrations of Se, Zn, and I in edible parts of various food crops including wheat, maize, potato, cabbage, and soybean in the Loess Plateau, a typically Zn, Se and I deficient area in China.

2. Materials and Methods

2.1. Experimental site

The field trials were conducted at Yangmazhuang village, Yongshou county (N34°49' E108°11', elevation=1127.76 m) in the central part of the Loess Plateau. Annual precipitation at the site is approximately 600 mm, and more than 65% of the rainfall occurs between July and September. Three fields were selected in the trials due to the one plant season for wheat and maize per year. The soil in the experimental fields is a calcareous loam and its basic chemical properties are presented in Table 1. Field 1 had higher fertility of OM and nutrients except N and P, but had lower total Se and higher available Se compared with fields 2 and 3. The village has around 260 inhabitants predominantly subsisting on agriculture, and is identified as a suitable place to conduct micronutrient trials because it is a typical Kashin-Beck disease area, especially with around 12% (n=31) of elderly adults suffering from health problems.

2.2. Experimental design

Two field trials investigating the role of soil application of Zn, Se, and I fertilizers (field trial 1) and foliar-applied Zn and Se fertilizers (field trial 2) on the concentrations of Zn, Se, and I in edible parts of different food crops were conducted in this study.

2.3. Field trial 1-Soil Application of Micronutrients

In the first trial, spring maize (*Zea mays* L.), soybean (*Glycine max* L.), potato (*Solanum tuberosum* L.), cabbage (*Brassica rapa* L. Chinensis Group.), winter wheat (*Triticum aestivum* L.), and canola (*Brassica napus* L.) were used to study the effect of soil application of fertilizers. The cultivars used were Zhengdan 958 for spring maize, Qianjindou for soybean, Longshu 3 for potato, Siyueman for cabbage, Jinmai 47 for winter wheat, and Ganza 1 for canola.

The size of each plot was 16 m² (4m×4m), and plots with different treatments were arranged in a randomized block design with four replications for all crops in two fields. The usual local density of planting were used with 60,000, 300,000, 60,000, 112,500 seedling ha⁻¹ for maize, soybean, potato and cabbage, respectively, while 2.25 and 150 kg ha⁻¹ for canola and winter wheat, respectively. Six treatments were constructed as follows: (1) with no Se, Zn, and I fertilizer application (Control); (2) with soil Se (in the form of selenate, Se) application; (3) soil Se (in the form of selenite, Se') application used on four crops except wheat and canola; (4) soil Zn (in the form of ZnSO₄·7H₂O) application; (5) soil I (in the form of KIO₃) application, and (6) combined soil Se, Zn, and I application (Se+Zn+I).

The application rates for Se, Zn, and I were 0.21, 22.7, and 0.59 kg ha⁻¹ (as 0.50 kg Na₂SeO₄ ha⁻¹, 0.46 kg Na₂SeO₃ ha⁻¹, 100 kg ZnSO₄·7H₂O and 1 kg KIO₃, respectively), respectively. Nitrogen and phosphorus fertilizers (in the form of urea and diammonium phosphate) were applied on all of the plots. Nitrogen was applied at the rates of 160 kg ha⁻¹ for maize, wheat, canola, and cabbage, and 60 kg ha⁻¹ for soybean and potato. Phosphorus (in the form of P₂O₅) was applied on all of the plots at the rate of 100 kg ha⁻¹. Se, Zn and I fertilizers were sprayed on the soil surface in the solution form so that they could be homogeneously distributed in soil, and then were incorporated into the upper 20 cm of soil with the N and P fertilizers just before seeding using a harrow. These micronutrients are not currently applied to crops by farmers in this area.

The field experiments were conducted on the following dates: 9th of April (for potato); 23rd of April (for spring maize and soybean); 6th of May (for cabbage); 1st of September (for canola); and 18th of September (for winter wheat) in 2008. Harvesting (or sampling) was conducted on the following dates: 3rd of August (for cabbage); 24th of August (for potato); and 2nd of October (for spring maize and soybean) in 2008; 23rd of May (for canola) and 12th of June (for winter wheat) in 2009.

Table 1. Basic chemical properties of air-dried loess soil in this study.

Properties	Field 1- Maize/Potato/ Cabbage/Soybean	Field 2- Wheat/Canola (soil)	Field 3- Wheat (foliar)
pH (H ₂ O)	8.16	8.21	8.12
Organic matter (g kg ⁻¹)	13.6	12.2	12.1
Total nitrogen (g kg ⁻¹)	0.86	0.81	0.88
Nitrate nitrogen (KCl-extractable) (mg kg ⁻¹)	2.94	5.24	9.31
Ammonium nitrogen (KCl-extractable) (mg kg ⁻¹)	5.46	2.39	5.72
Available phosphorus (NaHCO ₃ -extractable) (mg kg ⁻¹)	6.63	15.9	17.9
Available potassium (NH ₄ OAc-extractable) (mg kg ⁻¹)	102	86.3	98.5
Total zinc (Jackson method) (mg kg ⁻¹)	69.3	62.8	64.1
Available zinc (DTPA-extractable)* (mg kg ⁻¹)	0.73	0.78	0.73
Total selenium (μg kg ⁻¹)	102	114	149
Available selenium (KH ₂ PO ₄ -extractable) (μg kg ⁻¹)	1.22	0.75	0.81
Total iodine (mg kg ⁻¹)	1.710	1.501	**
Available iodine (water-soluble) (mg kg ⁻¹)	0.103	0.997	**

*DTPA, diethylene triamine penta-acetic acid. In Field 1, Maize, potato, cabbage, and soybean experiments were conducted. In Field 2, wheat and canola soil application experiments were conducted. In Field 3, foliar Se and Zn application experiments on wheat were conducted. ** No data obtained.

Crop management practices were the same as those used by local farmers. None of the crops were irrigated. Weeds were manually removed. No obvious water- or pest-related stresses were observed during the growing season.

2.4. Field trial 2-Foliar Application of Se and Zn to Wheat

To assess the effect of foliar application of Se and Zn to winter wheat (*Triticum aestivum* L.), a second field experiment was conducted using a local cultivar (Jinmai 47) at the same location as the field trial 1. Similar to trial 1, a randomized block design with four replications was used. The wheat was planted on the 20th of September 2008 at 150 kg ha⁻¹ on rows with 20-cm spacing and harvested on the 10th of June 2009. Foliar Zn and Se were applied at late anthesis stage

(Zadoks scale 48 to 49, Zadoks *et al.* 1974). Zn rates were 0.00, 0.23, 0.45, 0.68, 0.91, and 1.14 kg Zn ha⁻¹ using ZnSO₄·7H₂O. Se rates were 0, 4, 20, 30, 46, and 60 g Se ha⁻¹ using selenite.

2.5. Field sampling

At harvest, five maize, soybean, potato, and canola plants were randomly sampled from each plot for nutrient analysis. Wheat plants were randomly sampled from four one-meter long rows. Sampled plants of maize, soybean, canola, and wheat were air-dried and threshed, and those of potato were separated into shoots and tubers immediately after sampling. The fresh weight of shoots and tubers for potato, and the air-dried weight for the other four crops were determined. All collected plant samples were first washed by tap water and then with distilled water. Thereafter, washed

plant samples were dried to constant weight at 70 °C and stored for analysis. The remaining plants in each plot were harvested by hand and weighed to determine total biomass production and yield, which were expressed in dried weight at 70 °C for all crops.

2.6. Chemical analysis

Grain, tuber, and leaf samples collected from the trials were analyzed for Se (using inductively coupled plasma mass spectrometry (ICP-MS) following HNO_3 - H_2O_2 digestion) and B, Cu, Fe, Mn, Zn, P, K, S, Ca, and Mg (using ICP-optical emission spectrometry (Radial CIROS)) (Zarcinas *et al.*, 1987) at the University of Adelaide's Waite Analytical Services Laboratory, Adelaide, South Australia. Iodine concentrations of the samples were measured at the RJ Hill Laboratories Ltd., Hamilton, New Zealand, using ICP-MS. The nitrogen concentration of the samples was determined using the Kjeldahl method after digestion of the samples with H_2SO_4 - H_2O_2 (Ercoli *et al.*, 2011). Sample analyses were reported as the mean of four replications. The macro- and micronutrient concentrations in edible parts were expressed on a dry weight basis for all crops.

2.7. Statistical analysis

Analyses of variance were conducted using PASW statistics 17.0 Edition. The level of significance was 0.05.

3. Results

3.1. Field trial 1

Despite large variation in mean yield per treatment, there was also a high level of variability in yield (CV 5.3-27.0%) and the field trials with soil applications showed that all of the micronutrient applications had no effect on the yield of tested crop plants (Table 2). The average yields obtained (per ha) were 4101 and 9530 kg grain for wheat and maize, 1413 and 2079 kg seed for soybean and canola, 3298 kg tuber for potato, and 2399 kg biomass for cabbage (Table 2).

Both forms of Se were effective at increasing Se concentration in edible parts, but Se treatment was more effective than Se^- (Table 3). In the selenate form, the Se increases compared to the control treatment were 78 to 228 fold, and the Se application at 1 g ha^{-1} increased the Se concentration by 17.4 $\mu\text{g kg}^{-1}$ in wheat grain, 8.6 $\mu\text{g kg}^{-1}$ in maize grain, 17.3 $\mu\text{g kg}^{-1}$ in soybean seed, 10.2 $\mu\text{g kg}^{-1}$ in potato tuber, 4.1 $\mu\text{g kg}^{-1}$ in canola seed, and 76.8 $\mu\text{g kg}^{-1}$ in cabbage leaf, with the Se biofortification target of 300 $\mu\text{g kg}^{-1}$ DW reached at 16, 34, 15, 28, 71 and 3 g ha^{-1} , respectively for these crops.

In selenite form, the corresponding increases were lower and only 5 to 14 fold. The Se application of 1 g ha^{-1} increased the Se concentration by 0.2 $\mu\text{g kg}^{-1}$ for maize, 1.2 $\mu\text{g kg}^{-1}$ for soybean, 0.9 $\mu\text{g kg}^{-1}$ for potato, and 6.5 $\mu\text{g kg}^{-1}$ for cabbage, with the Se biofortification target reached at 1445, 212, 317 and 31 g ha^{-1} , respectively. No significant difference was detected between the increasing effect of Se on the edible parts of all tested crops in Se-only treatment and $\text{Se}+\text{Zn}+\text{I}$ treatment.

Soil Zn application in the form of zinc sulphate at 22.7 kg ha^{-1} at planting was effective for biofortification of Zn concentration in cabbage leaves and canola seed (Table 3). In the Zn-treated soil, the Zn concentration in the cabbage leaf was increased by 2 fold and 0.3 fold in canola seed, and Zn application of 1 kg ha^{-1} increased the Zn concentrations respectively by 0.3 and 1.8 mg kg^{-1} , with the Zn biofortification target of 40 mg kg^{-1} reached correspondingly at 40 and 11 kg ha^{-1} . In contrast to cabbage and canola, Zn biofortification did not cause a significant increase in Zn concentration in wheat grain, maize grain, soybean seed, and potato tuber. The change in Zn concentration in the edible parts of all the tested crops caused by Zn treatment was similar to that by the $\text{Se}+\text{Zn}+\text{I}$ treatment.

Results of I soil application in the form of potassium iodate at 590 g ha^{-1} at planting were similar to that of Zn (Table 3), showing significantly increased I concentration only in cabbage.

Table 2. Effects of soil applied Se, Zn, I, and their combinations on grain/seed/tuber yield of crops in Trial 1.

Treatments	Yield (kg ha ⁻¹)					
	Wheat	Maize	Soybean	Potato (DW)	Canola	Cabbage (DW)
Control	4285(10.1)	9792(4.6)	1103(11.1)	3477(16.3)	2133(14.7)	2675(20.4)
Se (as selenate)	3991(5.3)	9705(4.5)	1452(19.7)	3071(22.1)	1558(22.5)	3205(18.9)
Se' (as selenite)	*	9536(6.8)	1192(26.9)	3461(9.5)	*	3644(13.9)
Zn	4167(14.1)	9502(10.5)	1749(27.0)	3424(18.0)	2604(12.8)	1841(15.4)
I	3957(9.7)	9432(8.4)	1636(21.6)	3191(23.4)	2283(16.1)	1441(25.2)
Se+Zn+I	4104(16.8)	9213(8.4)	1344(24.6)	3161(13.8)	1815(20.3)	1587(14.7)

*No Se' (as selenite) treatment for wheat and canola. There are no significant difference between treatments ($p>0.05$). Data are showed as means and figures in parentheses are CV% of four replications for each treatment.

Soil application of I exhibited a 2-fold increase in cabbage leaf I concentration from 580 $\mu\text{g kg}^{-1}$ to 1150 $\mu\text{g kg}^{-1}$, and I application of 1 g ha⁻¹ increased the leaf I concentration by 1.1 $\mu\text{g kg}^{-1}$, with the I biofortification target of 500 $\mu\text{g kg}^{-1}$ reached without I application. No significant increases in I concentrations of other tested crops were observed (Table 3). The change in I concentration from the I fertilizer was statistically identical to those of the I and Se+Zn+I treatments.

Soil application of Se, Zn, and I was not effective in increasing the concentrations of other micronutrients (Table 4) or macronutrients (data not shown) in the edible parts of the tested crops. Data were showed with Se+Zn+I treatments only. Cabbage contains high concentrations of Fe and Ca, whereas soybean seed contains high concentration of Fe, Cu, and Zn.

3.2. Field trial 2

Foliar application of sodium selenite and zinc sulphate on winter wheat at late-booting stage at rates of up to 60 g ha⁻¹ and 1.14 kg ha⁻¹ showed no significant effects on the biomass or grain yield (Table 5). The wheat biomass ranged from 8737 kg ha⁻¹ to 10080 kg ha⁻¹,

whereas the grain yield ranged from 3658 kg ha⁻¹ to 4278 kg ha⁻¹ (Table 5). The average values of wheat biomass and grain yield were 9481 and 3999 kg ha⁻¹, respectively.

Foliar applications of Se and Zn were both highly effective in the biofortification of wheat (Figure 1). Foliar application of Se at rates of 4 g ha⁻¹ to 60 g ha⁻¹ linearly increased the grain Se concentration, and application of Se at the rate of 1 g ha⁻¹ increased the Se concentration in wheat grain by 4.6 $\mu\text{g kg}^{-1}$, and the grain Se target of 300 $\mu\text{g kg}^{-1}$ was able to be reached at the foliar application rate of 59 g ha⁻¹ (Figure 1). Whereas, grain Zn concentration was increased with the foliar Zn application rate in a quadratic line, with the maximum grain Zn of 30 mg kg⁻¹ reached at 1.1 kg ha⁻¹ (Figure 1). This indicated that the Se and Zn concentrations in wheat grain could be simultaneously enhanced by the foliar application of Se and Zn, although the Zn concentration was still lower than the recommended value of 40 mg kg⁻¹. Zn or Se application had no effect on the concentrations of macronutrients or other micronutrients in wheat grain (data not shown).

Table 3. Micronutrient concentration in edible crop parts after soil application of Se, Zn, and I at planting in Trial 1.

Treatments	Micronutrient Concentration					
	Wheat	Maize	Soybean	Potato	Canola	Cabbage
Se ($\mu\text{g kg}^{-1}$)						
Control	16 b	11 c	46 c	15 c	11 c	97 c
Se (as selenate)	3667 a	1807 a	3682 a	2149 a	865 a	16234 a
Se' (as selenite)	*	63 b	297 b	196 b	*	1469 b
Se+Zn+I (Se as selenate)	4493 a	1667 a	2228 a	1701 a	700 a	19161 a
Zn (mg kg^{-1})						
Control	15 a	16 a	57 a	13 a	28 b	20 b
Zn	16 a	17 a	59 a	15 a	35 a	61 a
Se+Zn+I (Se as selenate)	16 a	18 a	59 a	13 a	36 a	48 a
I ($\mu\text{g kg}^{-1}$)						
Control	11 a	11 a	13 a	63 a	975 a	580 b
I	21 a	20 a	19 a	58 a	890 a	1150 a
Se+Zn+I (Se as selenate)	20 a	18 a	18 a	59 a	875 a	1150 a

*No Se (as selenite) treatment for wheat and canola. Means within a column followed by the same letter are not significantly different ($p < 0.05$).

Table 4. Concentrations of micronutrients B, Cu, Fe and Mn in edible crop parts after application of Se, Zn, and I to soil at planting in Trial 1.

Micronutrients (mg kg^{-1})	Crops					
	Wheat	Maize	Soybean	Potato	Canola	Cabbage
B	1.4	1.9	29.6	4.3	14.3	28.4
Cu	4.5	1.8	19.7	8.3	3.3	4.2
Fe	32.1	23.5	89.7	31.4	67.2	167.1
Mn	45.2	4.7	25.1	5.5	36.2	76.5

*Data were showed only with Se+Zn+I treatments for all crops in Trial 1.

Table 5. Effects of foliar application of Se and Zn on wheat biomass and grain yield in Trial 2.

Treatments Se (g ha ⁻¹) + Zn (kg ha ⁻¹)	Biomass (kg ha ⁻¹)	Grain yield (kg ha ⁻¹)
Nil	10080 (13.2)	4182 (12.3)
4 + 0.23	8737 (8.6)	3658 (9.8)
20 + 0.45	9314 (6.4)	3950 (6.9)
30 + 0.68	10123 (10.4)	4278 (9.2)
46 + 0.91	9538 (9.4)	4080 (8.8)
60 + 1.14	9092 (14.1)	3846 (13.6)

*There are no significant difference between treatments ($p > 0.05$). Data are showed as means and figures in parentheses are CV% of four replications for each treatment.

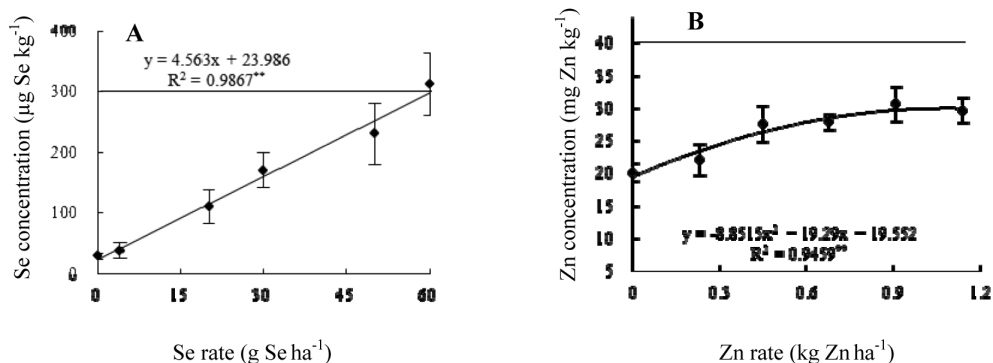


Figure 1. Effects of Se and Zn foliar applications on Se (A) and Zn (B) concentrations in wheat grain (vertical bars represent standard errors, the horizontal lines on A and B were presented with the target concentrations).

4. Discussion

4.1. Crop yield was not remarkably affected by micronutrients

Although some studies have reported increases in biomass from the addition of Se (Turakainen *et al.*, 2004) and I (Dai *et al.*, 2004), these studies were not

conducted in the field. The lack of yield response to Zn (whether soil- or foliar-applied) in the field indicates that the crop yields may be less constrained by the level of available Zn in the soil here and more dependent on other factors, including low water supply due to local dry climate (Kang *et al.*, 2002).

4.2. Soil-applied selenate or foliar-applied selenite were effective in increasing selenium concentration in edible parts of crops

The main aim of the trial was to biofortify food crops with key micronutrients. Selenium biofortification was very successful: sodium selenate applied to the soil at planting was efficiently transferred from root to foliage/grain/tuber and was effective for all crops tested. For winter wheat, maize, soybean, potato, canola, and cabbage, the corresponding Se concentration increase with one gram of Se applied per hectare of soil was 17.4, 8.6, 17.3, 10.2, 4.1, and 76.8 $\mu\text{g kg}^{-1}$, respectively. Therefore, in the experimental area, to reach the biofortification target Se level of 300 $\mu\text{g kg}^{-1}$, the amount of selenate applied can be reduced to only 16 g ha^{-1} for wheat, 34 g ha^{-1} for maize, 15 g ha^{-1} for soybean, 28 g ha^{-1} for potato, 71 g ha^{-1} for canola, and 3 g ha^{-1} for cabbage. Sodium selenite applied in the same way as selenate was less effective. Application of Se at 1 g ha^{-1} in the form of selenite was found to increase the Se concentration by only 0.2 $\mu\text{g kg}^{-1}$ for maize, 1.2 $\mu\text{g kg}^{-1}$ for soybean, 0.9 $\mu\text{g kg}^{-1}$ for potato, and 605 $\mu\text{g kg}^{-1}$ for cabbage, with the Se biofortification target of 300 $\mu\text{g kg}^{-1}$ reached at 1167, 213, 331 and 31 g ha^{-1} , respectively. These findings were consistent with most of the previous studies, which reported that barley grain Se concentration of 100 to 200 $\mu\text{g kg}^{-1}$ was obtained by application of Se at 10 to 20 g Se ha^{-1} as selenate, but over 100 g Se ha^{-1} as selenite was required to reach the same grain Se level (Ylärinta, 1985). On a fine sandy loam soil (pH 6.0), the barley grain Se concentration was increased from 33 (control) to 234 $\mu\text{g kg}^{-1}$ with 10 g Se ha^{-1} as selenate, while with the same dose as selenite, no increase for grain Se was found (Gupta *et al.*, 1993).

Foliar application of Se in the form of selenite at rates of 0 to 60 g ha^{-1} linearly increased grain Se concentration from 30 $\mu\text{g kg}^{-1}$ to 310 $\mu\text{g kg}^{-1}$, indicating a 4.6 $\mu\text{g kg}^{-1}$ increase of Se concentration in wheat grain for 1 g ha^{-1} foliar-applied Se. The grain Se target of 300 $\mu\text{g kg}^{-1}$ was able to be reached using a Se application rate of 59 g ha^{-1} for winter wheat. Foliar selenate was not

tested in this study, but previous research indicates that selenate is more effective, although the difference is much less than that when the two Se forms are applied to the soil. For rice, the grain Se concentration was also found to be 35.9% higher with foliar selenate application than with selenite (Chen *et al.*, 2002), and for carrot, its leaves accumulated as much as 80 $\mu\text{g Se g}^{-1}$ (DW) with foliar selenate application, while only 50 $\mu\text{g Se g}^{-1}$ (DW) with selenite application (Kápolna *et al.*, 2009).

4.3. Foliar-applied Zn was more effective than soil-applied Zn for biofortification

In contrast to Se, Zn applied to the soil in the form of Zn sulphate at a rate of 232.7 kg ha^{-1} did not increase Zn concentrations in the edible parts of any crop, except for cabbage and canola, where a 2-fold increase in cabbage leaf and 0.3-fold increase in canola seed were obtained, and Zn application of 1 kg ha^{-1} increased their Zn concentration respectively by 0.3 and 1.8 mg kg^{-1} , with the Zn biofortification target of 40 mg kg^{-1} reached correspondingly at 40 and 11 kg ha^{-1} . This result indicates that Zn application on loess soil is not an effective biofortification method. Another study showed that the application of 5 mg kg^{-1} Zn to soil increased the Zn concentration of navy bean shoot from 19.93 mg kg^{-1} to 38.12 mg kg^{-1} , and of 10 mg kg^{-1} Zn, increased that to 54.48 mg kg^{-1} in a navy bean greenhouse experiment (Gonzalez *et al.*, 2008). The effect of soil Zn application on the grain Zn concentration is further related to the soil characteristics that affect Zn availability, including pH and Ca concentration (Alloway, 2009). On the loess soil, low effectiveness of soil-applied Zn application could be attributed to the adsorption by soil colloids and the limited transport of Zn absorbed by plants from phloem to grain/tubers (Erenoglu *et al.*, 2011). Compared to soil application, foliar application of Zn was shown to be a more effective and efficient way to increase Zn concentration in winter wheat grain, with the maximum grain Zn of 30 mg kg^{-1} reached at 1.14 kg ha^{-1} , although it was still lower than the recommended value of 40 mg kg^{-1} . These results are

similar to those reported by Yilmaz (Yilmaz *et al.*, 1997) in which foliar-applied Zn increased wheat grain Zn concentration by 50% from 18 to 27 mg Zn kg⁻¹, and was more effective than soil-applied Zn. Similar findings were described by Cakmak (Cakmak *et al.*, 2010b), which showed wheat grain Zn concentration was increased from 11.7 (control) to 26.9 mg kg⁻¹ with foliar-applied Zn. Thus, foliar Zn application is an effective biofortification method for wheat grain on the Loess Plateau.

4.4. Soil applied I was effective for biofortification of I in leafy vegetables

Application of I (in the form of potassium iodate) to the soil did not increase I levels in grains of wheat, maize, soybean, canola or in potato tubers, but exhibited a two fold increase in I concentration in cabbage leaf, and I application of 1 g ha⁻¹ increased the leaf I concentration by 1.1 µg kg⁻¹, with the I biofortification target of 500 µg kg⁻¹ reached without I application. Iodine levels in grain or seed are usually low, which was also the case in this trial (10–20 µg kg⁻¹), and in potato tubers it was slightly higher at around 60 µg kg⁻¹, but in the canola seed, the I level was around 1000 µg kg⁻¹. Other researchers found that I levels in rice leaves were over 100 mg kg⁻¹, but only 10 mg kg⁻¹ in grain with an application of 100 µM IO₃⁻. The reason for this may be that I is transported almost entirely in the xylem, and that only a minimal amount is loaded into the grain/seed or tubers (Mackowiak and Grossl, 1999). The control cabbage leaves had a higher I concentration (580 µg kg⁻¹) than the target I level of 500 µg kg⁻¹, estimated from the recommended I consumption of 150 µg d⁻¹ for adults by Mackowiak and Grossl (Mackowiak and Grossl, 1999).

Thus, increasing the dietary I content by agronomic biofortification of leafy vegetables such as cabbage, lettuce, and spinach may be relatively easy. For regions such as Yongshou, Shaanxi Province, where these trials were conducted, the best approach to increase human I levels is to encourage greater consumption of leafy vegetables.

5. Conclusion

Using a range of food crops in field trials in the Loess Plateau, which has a dry climate and low soil nutrient availability, soil-applied selenate and foliar-applied selenite were found to be effective for Se biofortification. Foliar zinc sulphate is effective in biofortifying winter wheat, and soil-applied Zn is effective in increasing the Zn concentration in cabbage leaf and canola seed. Soil-applied I was found to be only effective in increasing I concentration in cabbage leaf. Agronomic biofortification of the crops with Se as soil-applied sodium selenate was unaffected by co-application of Zn and/or I.

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