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Simultaneous Biofortification of Wheat with Zinc, Iodine, Selenium, and Iron through Foliar Treatment of a Micronutrient Cocktail in Six Countries

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ABSTRACT: Field experiments were conducted on wheat to study the effects of foliar-applied iodine(I) alone, Zn (zinc) alone, and a micronutrient cocktail solution containing I, Zn, Se (selenium), and Fe (iron) on grain yield and grain concentrations of micronutrients. Plants were grown over 2 years in China, India, Mexico, Pakistan, South Africa, and Turkey. Grain-Zn was increased from 28.6 mg kg^{-1} to 46.0 mg^{-1} kg with Zn-spray and 47.1 mg^{-1} kg with micronutrient cocktail spray. Foliar-applied I and micronutrient cocktail increased grain I from 24 $\mu g\ kg^{-1}$ to 361 $\mu g\ kg^{-1}$ and 249 $\mu g\ kg^{-1}$, respectively. Micronutrient cocktail also increased grain-Se from 90 $\mu g\ kg^{-1}$ to 338 $\mu g\ kg^{-1}$ in all countries. Average increase in grain-Fe by micronutrient cocktail solution was about 12%. The results obtained demonstrated that foliar application of a cocktail micronutrient solution represents an effective strategy to biofortify wheat simultaneously with Zn, I, Se and partly with Fe without yield trade-off in wheat.

KEYWORDS: *biofortification, hidden hunger, iodine, iron, selenium, wheat grain, zinc*

INTRODUCTION

Deficiencies of zinc (Zn), iodine (I), selenium (Se), and iron (Fe) represent a serious global health problem because these micronutrient deficiencies are affecting more than one-third of the global population, especially in developing countries.[1] Micronutrient deficiencies are commonly known as hidden hunger and constitute an important form of human malnutrition. Published reports indicate that hidden hunger causes not only serious health concerns but also an important economic burden on health care system of the developing countries. Hidden hunger may cost an average loss of up to 5% in gross domestic product of the concerned countries.[2]

Micronutrient deficiencies occur usually in regions where soils are low in plant available concentrations of micronutrients. Existence of a general geographical overlap between soil Zn deficiency and human Zn deficiency has been already postulated.[3,4] As agriculture-based food products are the major source of human nutrition, the relationship among nutrient status of soils, food crops and human health is understandable.[1,5] Historically, the agricultural systems have never been purposely designed to achieve a better human nutrition and health. Instead, producing more food was always the major aim to avoid hunger problem and famines. Staple cereal grains, like wheat, are however inherently low in micronutrient concentration and bioavailability to adequately meet human nutritional needs.[3,5] Therefore, it is widely recognized that in areas of the world where staple cereal-based foods are the main dietary source, inadequate dietary intake of micronutrients is the predominant cause of the prevalence of human micro-

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Table 1. Initial Soil Properties and Wheat Cultivars Used in Six Countries, Where the Fertilizer Experiments Were Established

country	location	pH[a]	DTPA-Zn (mg kg^{-1})	DTPA-Fe (mg kg^{-1})	TMAH-I (mg kg^{-1})	available Se (μg kg^{-1})	cultivar
China	Quzhou-I	7.7	0.45	6.7	1.35	17.5	Liangxing99
	Quzhou-II	7.7	0.45	6.7	1.35	17.5	Liangxing99
	Cele	7.9	0.52	5.4	0.25	4.20	AK58
India	Ludhiana	7.7	0.51	57.7	0.86	15.10	WH1105/PBW725
	Gurdaspur	7.8	0.59	16.1	2.16	10.10	WH1105
	Bathinda	8.1	0.42	nd[b]	nd[b]	nd[b]	WH1105
Mexico	Yaqui Valley-I	8.9	0.21	2.3	4.46	9.35	Borlaug 100
	Yaqui Valley-II	8.9	0.22	2.3	4.48	9.30	Tacupeto F2001
Pakistan	Faisalabad	7.8	0.46	3.2	0.94	37.2	Faisalabad-2008
	Gujranwala	7.9	0.41	25.6	0.30	17.9	Faisalabad-2008
	Sheikhupura	8.2	0.55	8.5	0.62	12.0	Faisalabad-2008
South Africa	Langgewens	6.2	1.66	36.8	1.32	6.14	SST056/SST027
	Roodebloem	5.6	2.14	79.7	2.92	6.14	SST027/SST056
Turkey	Institute	7.8	0.94	4.2	2.83	3.76	Bezostaya1
	Topraksu	8.0	1.26	3.6	2.95	3.80	Bezostaya1
	Yusuflar	7.8	0.57	3.7	2.88	3.66	Bezostaya1

[a]Soil pH was measured in 2.5:1 water−soil ratio. [b]nd indicates no determination.

nutrient deficiencies.[1,5,6] Recently published papers show that wheat, rice, and maize are extremely low in I concentrations and contain around 10−15 μg of I per kg of grain, which are far too low to meet the daily dietary I requirement of human populations.[7,8] The required daily amount of I for human body varies between 90 and 250 μg.[6,9] Similarly, in most of the wheat-producing regions, grain Zn usually ranges between 20 and 30 mg kg^{-1}, whereas the desirable Zn concentrations to avoid risk of human Zn deficiency are around 40−50 mg kg^{-1}.[5] Also Se delivery to food systems is greatly affected from the available Se sources in soils. Low Se soils are often associated with low Se concentrations in cereal grains as shown in a number of countries.[10,11] Thus, enhancing micronutrient densities in staple cereal grains through agricultural practices is considered an effective approach to combat micronutrient malnutrition in humans.[3,12]

In most of the developing countries, wheat is a predominant source of daily calories and micronutrients, especially in resource-poor populations.[1,3] For example, in China wheat-based food products supply more than 70% of daily calorie needs and more than 20% of Zn and Fe requirements.[14] The target countries of this study, i.e., China, India, Mexico, Pakistan, South Africa, and Turkey, produce about 278 million Mg wheat per annum, which accounts for almost 37% of total wheat production in the world.[15] In these countries, deficiencies of Zn, I, Se and Fe are prevalent in soils, cereal grains, and humans.[3,6,7,16,17] As population of these six countries accounts for about 43% of the total world,[15] increasing wheat grain with Zn, I, Se, and Fe would be of immense significance for human nutrition in these areas.

Biofortification of staple cereals with micronutrients by using agricultural approaches, such as plant breeding and agronomic biofortification represents a useful, cost-effective and sustainable strategy to combat micronutrient deficiencies in human populations.[3,12,16] Plant breeding and fertilizer strategies are, indeed, complementary and synergistic. Combining of these agricultural approaches would result in additive and synergistic impacts on grain micronutrient concentrations.[1,5] Multicountry research programs conducted under HarvestPlus-HarvestZinc projects have demonstrated that foliar Zn fertilization is an effective agronomic approach to attain desirable concentrations of Zn and I in wheat and rice for human nutrition.[8,16−18] In these studies, it has been, however, shown that soil Zn or I applications were much less effective on grain Zn or I, compared to foliar applications. Foliar application of Se also proved very effective to increase wheat grain Se concentration to attain the target concentration of 300 μg kg^{-1}.[10,19] By considering the high potential impact of the Zn fertilizer approach on improving grain Zn and contributing to public health, Joy et al. recommended governments to consider subsidy programs for fertilizers containing Zn.[20] However, in contrast to Zn, I, and Se, foliar application of Fe fertilizers was found to not be effective in enhancing the wheat grain Fe to the target concentration.[21] The positive effects of soil or foliar Fe fertilization on grain Fe was evident only if plants have a good nitrogen (N) nutrition. Increasing N nutrition has promoting effects on root uptake, shoot transport, and seed deposition of Zn and Fe.[13,22]

Almost all of published papers related to agronomic biofortification of food crops by spraying micronutrients foliarly have focused on application of a single or rarely two micronutrients in a given country. In a field experiment conducted in the central part of the Loess Plateau of China, Mao et al. studied the effects of combined soil application of Se, Zn, and I and combined foliar application of Se and Zn.[23] There was only a statistically significant increase in grain micronutrients following soil Se application and foliar Zn and Se spray. Very recently, Mangueze et al. showed that a simultaneous foliar spray of Zn and Se to rice cultivars grown in Mozambique significantly increased concentrations of Se and Zn in whole as well as polished grain.[24] Combined foliar spray of Zn and Se was also effective in increasing grain Zn and Se in field pea plants grown under greenhouse conditions.[25]

To our knowledge, there is no study that assesses the effect of combined spray of Zn, Se, Fe, and I on grain concentrations of different wheat cultivars grown on a range of soil types and under different environmental conditions and management practices in six countries. This study has investigated the impacts of a combined foliar spray of Zn, Se, Fe, and I on grain yield and grain concentrations of these micronutrients in wheat grown at 27-site years field experiments in six countries over 2 years.

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Table 2. Basic Application of NPK Fertilizers at Each Location in Six Countries Where the Experiments Were Conducted

country	fertilizer nutrient applied (kg ha^{-1})			fertilizer source			timing of N application
	N	P	K	N	P	K	
China							
Quzhou-I	225	33	62	urea	$NH_4H_2PO_4$	K_2SO_4	1/3 at planting and 2/3 at early jointing
Quzhou-II	170	39	75	urea	$NH_4H_2PO_4$	KCl	1/2 at planting and 1/2 at early jointing
Cele	274	31	7	urea	$NH_4H_2PO_4$	K_2SO_4	1/2 at planting and 1/2 at early jointing
India	150	26	30	urea	$NH_4H_2PO_4$	K_2SO_4	1/3 at planting, 1/3 at tillering and 1/3 at early jointing
Mexico	250	46	0	urea	$NH_4H_2PO_4$		all at planting
Pakistan	120	35	0	urea	$(NH_4)_2HPO_4$		1/3 at planting and 2/3 at early jointing
South Africa	100	20	0	$Ca(NO_3)_2/NH_4NO_3$	$CaH_4P_2O_8$		40% at planting, 60% at tillering
Turkey	150	36	0	urea	$NH_4H_2PO_4$		1/2 at planting and 1/2 at early jointing

Table 3. Grain Yield of Wheat Grown with Foliar Treatments of Zn Alone, I Alone, and a Micronutrient Cocktail at 27 Site-Years Field Experiments in Six Countries during 2016−2017[a]

country	location	harvest year	grain yield (Mg ha^{-1})				*F* test	$LSD_{0.05}$
			control	foliar Zn	foliar I	foliar cocktail		
China	Quzhou-I	2016	8.4	8.0	8.3	7.7	ns	
		2017	9.2	8.7	8.8	9.1	ns	
	Quzhou-II	2017	8.5	8.4	8.3	8.0	ns	
	Cele	2017	6.2	6.4	5.9	6.2	ns	
India	Ludhiana	2016	5.1	5.1	4.7	4.5	ns	
		2017	5.8	5.9	5.4	5.3	ns	
	Gurdaspur	2016	5.0	4.9	4.7	4.4	ns	
		2017	6.9	7.0	6.6	6.3	*	0.35
	Bathinda	2016	5.2	5.2	5.1	5.0	ns	
		2017	5.7	6.3	6.0	5.7	ns	
Mexico	Yaqui Valley-I	2016	6.9	6.8	6.5	6.6	ns	
	Yaqui Valley-II	2016	5.3	5.2	5.4	5.0	ns	
Pakistan	Faisalabad	2016	2.5	2.7	3.0	3.0	*	0.34
		2017	5.3	5.3	5.4	5.5	ns	
	Gujranwala	2016	4.8	5.2	4.9	4.9	ns	
		2017	5.3	5.4	5.1	4.9	*	0.47
	Sheikhupura	2017	3.7	3.7	3.8	3.8	ns	
South Africa	Langgewens	2016	2.9	3.1	2.7	2.9	ns	
		2017	1.4	1.4	1.5	1.3	ns	
	Roodebloem	2016	3.8	4.4	3.9	3.4	ns	
		2017	4.6	4.8	5.1	4.8	ns	
Turkey	Institute	2016	4.9	4.9	4.7	4.7	ns	
		2017	5.7	5.7	5.8	5.8	ns	
	Topraksu	2016	5.7	5.8	5.7	5.2	ns	
		2017	5.2	5.1	5.4	5.1	ns	
	Yusuflar	2016	3.1	3.3	3.2	3.2	ns	
		2017	2.2	2.0	1.9	1.9	ns	
	grand mean		5.2	5.2	5.1	5.0		
			the *P* value of paired *t* test					
control				0.305	0.289	0.004		
foliar Zn					0.048	0.001		
foliar I						0.008		
foliar cocktail								

[a]ns, indicates no significant difference; * indicates significant difference at $p < 0.05$.

■ MATERIALS AND METHODS

Field Experiment. Field experiments were conducted on bread wheat (*Triticum aestivum* L.) during 2015−2016 and 2016−2017 cropping seasons at 27 field locations in the following six countries: China, India, Mexico, Pakistan, South Africa, and Turkey. The wheat cultivars tested in the field experiments were commonly cultivated in the respective countries (Table 1). Soil pH, soil available Zn and Fe (i.e., diethylene-triamine pentaacetic acid [DTPA] extractable-Zn and Fe), total I, and 0.1 M KH_2PO_4-extractable Se concentrations are given in Table 1. The DTPA-extractable micronutrients and 0.1 M KH_2PO_4-extractable Se concentrations of the experimental soils were measured according to Lindsay and Norvell[26] and Dhillon et al.,[27] respectively. Extraction and measurement of soil I concentration was carried out as described by Cakmak et al.[8]

Treatments. The experiment comprised of four treatments: (i) local control (i.e., basal N, P, K fertilizers only and no foliar application of any micronutrient); (ii) local control + foliar application of Zn (0.5% $ZnSO_4{\cdot}7H_2O$, w/v); (iii) local control +

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Table 4. Grain Zn Concentration of Wheat Grown with Foliar Treatments of Zn Alone, I Alone, and a Micronutrient Cocktail at 27 Site-Years Field Experiments in Six Countries during 2016−2017[a]

			grain Zn concentration (mg kg^{-1})					
country	location	harvest year	control	foliar Zn	foliar I	foliar cocktail	*F* test	$LSD_{0.05}$
China	Quzhou-I	2016	37.3	51.4	38.2	48.1	***	5.8
		2017	31.6	42.5	32.8	42.6	***	4.7
	Quzhou-II	2016	31.7	42.0	28.7	50.4	***	5.6
	Cele	2017	24.0	40.1	23.3	30.6	**	8.0
India	Ludhiana	2016	29.8	43.8	45.3	44.5	***	4.3
		2017	27.5	49.5	29.3	44.8	***	4.3
	Gurdaspur	2016	27.9	45.4	41.5	51.6	***	5.2
		2017	27.8	37.2	27.9	44.0	***	5.2
	Bathinda	2016	30.5	49.2	42.5	51.2	***	3.6
		2017	28.1	38.4	27.5	46.1	***	3.8
Mexico	Yaqui Valley-I	2016	33.4	54.0	33.2	53.8	***	3.0
	Yaqui Valley-II	2016	33.1	51.1	32.0	53.4	***	3.5
Pakistan	Faisalabad	2016	30.8	47.6	32.7	43.3	***	3.7
		2017	32.5	53.3	34.0	49.8	***	3.4
	Gujranwala	2016	22.4	45.6	25.1	40.3	***	3.3
		2017	26.2	44.4	28.3	44.5	***	2.8
	Sheikhupura	2017	28.0	51.8	31.4	55.6	***	2.7
South Africa	Langgewens	2016	17.4	38.3	19.2	42.1	***	6.0
		2017	32.5	52.8	31.2	59.7	***	6.6
	Roodebloem	2016	16.6	39.3	18.5	45.0	***	4.0
		2017	32.8	51.1	32.4	62.0	***	6.8
Turkey	Institute	2016	31.8	45.2	31.8	43.0	***	3.5
		2017	31.9	53.3	30.4	53.6	***	5.3
	Topraksu	2016	26.9	42.5	27.1	40.5	***	3.7
		2017	32.8	49.8	34.0	48.2	***	5.4
	Yusuflar	2016	21.2	35.4	21.1	33.7	***	2.3
		2017	26.6	48.2	28.7	49.7	***	4.5
	grand mean		28.6	46.0	30.7	47.1		
			the *P* value of paired *t* test					
control				<0.001	0.026	<0.001		
foliar Zn					<0.001	0.265		
foliar I						<0.001		
foliar cocktail								

[a]*** and ** indicate significant differences at $p < 0.001$ and <0.01, respectively.

foliar application of I (0.05% KIO_3, w/v); and (iv) local control + foliar application of a micronutrient cocktail including 0.5% $ZnSO_4\cdot 7H_2O$ + 0.05% KIO_3 + 0.2% FeEDTA and 0.001% $NaSeO_4$, w/v. The field experiments were established in a randomized complete block design with four replications, except for Mexico where replications were three. The aqueous solution of each treatment, ranging between 600 and 800 L ha^{-1}, was applied twice as following: the first spray was conducted 1 week prior to heading and the second spray at the early milk stage until most of the leaves got wet.[28] The spray was realized either under cloudy day or during the sunset. Basal NPK fertilizers were applied before crop sowing. The given dose of N fertilizer was split-applied, twice or thrice, at preplanting, tillering, or early jointing stage. The rates and forms of NPK fertilizers were based on respective country's recommendations, as shown in Table 2.

Chemical Analysis. At maturity, the grain yield of wheat (at 13% moisture) was recorded from the 4 to 6 m^2 central area of each plot by threshing the grains manually in China. In other countries, the grain yield was based on yield obtained at the harvesting time. A subsample from each plot was secured for analysis of Zn, I, Se, and Fe. Grain samples were washed rapidly with tap water and deionized water and then dried at about 45 °C in a forced-draft oven to constant weight. The dried grains were thereafter ground to fine flour by using an agate mill (Pulverisette 9, Fritsch GmbH, Germany) and digested with HNO_3 + H_2O_2 in a microwave accelerated reaction system (CEM Corp.) for analysis of Zn, Fe, and Se. For I analysis, grain samples were extracted in tetramethylammonium hydroxide (TMAH) at 90 °C using a closed-vessel microwave reaction system (CEM Corp.).[8] Zinc and Fe concentrations in the digested solutions were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES; Vista-Pro Axial, Varian Pty Ltd., Mulgrave, Australia), and I and Se concentrations in the digested solutions were measured by inductively coupled plasma mass spectrometry (ICPMS; 7700 series, Agilent Technologies).

Statistical Analysis. The effects of different foliar fertilization treatments on the dependent variables were determined using one-factor ANOVA at 0.05 level of least significant difference (LSD) test using SAS software (SAS 8.0). For overall effectiveness, the data sets across locations and years were compared by the paired *t* test method of SPSS 13.0 for Windows. The linear models were used to evaluate correlations among various parameters.

RESULTS

Grain Yield. Irrespective of the experimental treatments, the grain yield of wheat varied greatly in different field locations, cropping years, and countries (Table 3). In the case of the local control treatment (i.e., no foliar treatment), the overall average grain yield was 5.2 Mg ha^{-1} across all locations, years, and countries. The highest grain yields (6.2−9.2 Mg ha^{-1}) were obtained at the field locations in China, and the

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Table 5. Grain I Concentration of Wheat Grown with Foliar Treatments of Zn Alone, I Alone, and a Micronutrient Cocktail at 27 Site-Years Field Experiments in Six Countries during 2016−2017[a]

			grain I concentration (μg kg^{-1})				
country	location	harvest year	control	foliar I	foliar cocktail	*F* test	$LSD_{0.05}$
China	Quzhou-I	2016	18	633	302	***	99
		2017	31	287	213	**	110
	Quzhou-II	2017	50	252	352	**	107
	Cele	2017	17	439	344	***	112
India	Ludhiana	2016	17	1447[b]	292	***	313
		2017	7	455	242	**	197
	Gurdaspur	2016	11	220	329	***	69
		2017	4	229	141	**	77
	Bathinda	2016	24	200	325	**	107
		2017	8	304	146	**	103
Mexico	Yaqui Valley-I	2016	33	310	185	**	75
	Yaqui Valley-II	2016	16	525	213	**	141
Pakistan	Faisalabad	2016	9	nd	183	***	26
		2017	48	743	368	***	112
	Gujranwala	2016	5	nd	283	***	66
		2017	57	564	377	***	77
	Sheikhupura	2017	78	583	333	***	121
South Africa	Langgewens	2016	80	91	134	ns	
		2017	16	347	874	***	131
	Roodebloem	2016	52	156	169	**	56
		2017	5	163	422	***	82
Turkey	Institute	2016	16	155	97	***	35
		2017	16	143	77	***	32
	Topraksu	2016	19	152	65	***	39
		2017	2	135	58	***	36
	Yusuflar	2016	10	295	111	***	39
		2017	7	203	92	***	34
	grand mean		24	316[b]	249		
			the *P* value of paired *t* test				
control				<0.001	<0.001		
foliar I					0.069		
foliar cocktail							

[a]*** and ** indicate significant differences at $p < 0.001$ and <0.01, respectively; nd indicates no data recorded; and ns indicates nonsignificance. [b]The value 1447 has been considered as outlier and the grand mean given has been calculated without considering the value 1447.

lowest yields (1.4−4.6 Mg ha^{-1}) were obtained at the locations in South Africa. Across all locations, years, and experimental treatments, foliar Zn or I, applied alone, had no significant effects on grain yield over the local control treatment. Though foliar micronutrient cocktail tended to decrease overall grain yield by 3.8%, a significant decrease in yield occurred only at Gurdaspur in India and at Gujranwala in Pakistan in 2017 ($p <$ 0.05). Contrarily, foliar I and foliar micronutrient cocktail sprays significantly increased grain yield at Faisalabad in Pakistan in 2016 ($p < 0.05$; Table 3).

Grain Zinc Concentration. Without any foliar treatment, wheat grain Zn concentration across all field locations in all countries over 2 years varied from 16.6 to 37.3 mg kg^{-1}, with an overall mean of 28.6 mg kg^{-1} (Table 4). Foliar application of Zn alone or micronutrient cocktail significantly increased wheat grain Zn concentration across all site-years ($p < 0.05$). On average, for all locations over 2 years, foliar Zn spray increased grain Zn concentration from 28.6 mg kg^{-1} to 46.0 mg kg^{-1} and the foliar micronutrient cocktail spray from 28.6 mg kg^{-1} to 47.1 mg kg^{-1}. Out of the total 27 site-years field experiments, a target grain Zn concentration of 40 mg kg^{-1} was attained at the 22 field locations with foliar Zn and at 25 locations with foliar micronutrient cocktail. Apparently, there was a kind of synergistic effect of foliar I treatment on grain Zn; however, an increase in the grain Zn concentration with foliar-applied I was 7.3% only (Table 4).

Grain Iodine Concentration. There was a large variation in grain I concentrations among the locations without foliar treatment (i.e., the local control). The variation found in grain I concentration of the control treatment was between 2 and 80 μg kg^{-1}, with a mean concentration of 24 μg kg^{-1} (Table 5). Foliar I and foliar micronutrient cocktail treatments significantly increased grain I concentration at all locations in each country, except for the Langgewens location in South Africa in 2016. Generally, the increases in grain I after foliar spray were lower in Turkey than other countries. The reason for such differential result could not be understood. As shown in Table 5, in the Ludhiana location in 2016, there was a particular increase in grain I (i.e., from 17 to 1447 μg kg^{-1}). When the value 1447 would be excluded as an outlier; on average, over all experimental locations, grain I concentration increased from 24 to 316 μg kg^{-1} by foliar I spray with an increase of 13.1-fold. In the case of foliar micronutrient cocktail spray, grain I concentration increased from 24 to 249 μg kg^{-1} resulting in an increase of 10.3-fold, respectively.

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Grain Selenium Concentration. Grain Se concentration in wheat grown without foliar spray of micronutrients varied drastically from 4 to 549 μg kg^{-1}, with an average of 90 μg kg^{-1} (Table 6). Foliar micronutrient cocktail resulted in significant increases in grain Se concentration across all site-years. It is obvious that the net increases in grain Se after foliar spray of the cocktail solution were higher in South Africa compared to other countries (Table 6). Depending on the field site and year, the net increases in grain Se showed variation within each country.

Table 6. Grain Se Concentration of Wheat Grown with Foliar Treatments of a Micronutrient Cocktail at 27 Site-Years Field Experiments in Six Countries during 2016–2017[a]

country	location	harvest year	grain Se concentration (μg kg^{-1}) control	foliar cocktail	F test	$LSD_{0.05}$
China	Quzhou-I	2016	43	144	**	48
		2017	45	180	*	95
	Quzhou-II	2017	42	297	**	122
	Cele	2017	26	167	**	40
India	Ludhiana	2016	406	601	*	109
		2017	549	725	***	37
	Gurdaspur	2016	46	341	***	66
		2017	26	178	**	46
	Bathinda	2016	55	299	***	18
		2017	47	293	***	41
Mexico	Yaqui Valley-I	2016	33	185	*	98
	Yaqui Valley-II	2016	16	213	*	96
Pakistan	Faisalabad	2016	257	446	***	41
		2017	190	308	*	68
	Gujranwala	2016	31	289	**	68
		2017	78	222	***	22
	Sheikhupura	2017	70	249	***	37
South Africa	Langgewens	2016	62	621	**	168
		2017	86	711	**	243
	Roodebloem	2016	55	558	***	114
		2017	20	682	***	33
Turkey	Institute	2016	66	244	**	69
		2017	63	238	**	57
	Topraksu	2016	47	215	**	65
		2017	4	155	**	44
	Yusuflar	2016	44	290	***	17
		2017	22	286	**	69
	grand mean		90	338		
			the P value of paired t test			
control				<0.001		
foliar cocktail						

[a]**, *, and *** indicate significant differences at $p < 0.01$, < 0.05, and <0.001, respectively.

Grain Iron Concentration. Grain Fe concentration of wheat grown without a foliar spray of micronutrients across all field locations varied from 22.6 to 38.7 mg kg^{-1}, with a mean concentration of 32.6 mg kg^{-1} (Table 7). Foliar micronutrient cocktail spray significantly increased grain Fe concentration from an overall average of 32.6 to 36.8 mg kg^{-1} across all locations in all countries and exhibiting a mean increase of 12.9% over the local control ($p < 0.05$). While foliar I did not affect wheat grain Fe, foliar Zn application increased grain Fe concentration from 32.6 mg kg^{-1} to 35.4 mg kg^{-1}, i.e., by 9.1% ($p < 0.05$).

Relationships between Grain and Soil Concentrations of Zn, Fe, Se, and I. Figure 1 shows the correlations between soil and grain concentrations of Zn, Se, Fe, and I. Soil DTPA-extractable Zn and Fe concentrations showed a negative relation with the grain Zn and Fe concentrations, while soil extractable Se tended to show a positive relationship with the grain Se. In the case of I, there was almost no relationship between soil I and grain I concentrations (Figure 1).

DISCUSSION

In this study, the effects of foliar applications of Zn alone, I alone, and a micronutrient cocktail containing Zn + I + Se + Fe on wheat grain yield and grain concentrations of these micronutrients were investigated over two cropping seasons by using 10 different wheat cultivars at a total of 27 site-years field experiments in six countries. In general, foliar Zn, I and micronutrient cocktail applications significantly increased grain concentrations of Zn, I, and Se but not grain yield. Grain Fe concentration was only slightly increased by the micronutrient cocktail application. According to several earlier studies, wheat grain yield can be increased by foliar application of Zn, but most commonly only in soils with very low plant available Zn, such as in soils having DTPA-Zn $\leq$ 0.2 mg kg^{-1}.[29,30] In the present study, foliar application of Zn alone had nonsignificant effect on wheat grain yield, probably because the soils in the experimental sites were not severely Zn-deficient (average DTPA-Zn in 16 field soils, 0.71 mg kg^{-1}; Table 1), and Zn was applied to foliage rather at a late stage of the growth. In addition, it is known that the expression of Zn deficiency problem in plants is affected not only from the amounts of plant available Zn in soils but also from seasonal climatic variations, especially from rainfall pattern (i.e., soil moisture) and duration of hot sunny days.[29,31,32]

Foliar application of I in the form of KIO_3 and at the rate of 0.05% KIO_3 had no grain yield trade-off in the present study across all site-years in six countries, which is consistent with the results of the recently published field study on wheat conducted in Turkey and Pakistan.[8] Iodine is not an essential micronutrient for higher plants.[33] However, some published reports indicate that I may influence the growth of plants under certain growth condition by affecting different physiological processes.[34] In good agreement with the results by Cakmak et al.,[8] this study suggested that foliar spray of I at a concentration of 0.05% KIO_3 can be considered as a useful rate for biofortification of wheat grains with I without yield trade-off.

In the case of foliar application of the micronutrient cocktail, the grain yield of plants exhibited a slightly decreasing trend at some locations (Table 3). Among 27 field locations, only in 2 locations the grain yield showed a statistically significant decline by foliar application of the micronutrient cocktail while in one location there was a significant increase in the yield. Based on these observations, it can be suggested that the micronutrient cocktail spray solution used in the present study represents a useful micronutrient cocktail without causing a harmful effect on plant growth and yield.

Though foliar applications of Zn alone, I alone, and micronutrient cocktail did not affect grain yield of wheat, the

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Table 7. Grain Fe Concentration of Wheat Grown with Foliar Treatments of Zn Alone, I Alone, and a Micronutrient Cocktail at 27 Locations in Six Countries during 2015−2017[a]

			grain Fe concentration ($mg\ kg^{-1}$)					
country	location	harvest year	control	foliar Zn	foliar I	foliar cocktail	*F* test	$LSD_{0.05}$
China	Quzhou-I	2016	37.0	36.8	34.0	34.5	ns	
		2017	30.9	30.3	30.1	32.2	ns	
	Quzhou-II	2017	38.5	38.0	37.6	38.7	ns	
	Cele	2017	31.4	31.7	31.3	31.7	ns	
India	Ludhiana	2016	29.9	29.8	32.1	30.9	ns	
		2017	24.4	26.3	25.0	26.4	ns	
	Gurdaspur	2016	30.2	37.4	30.4	39.6	***	2.0
		2017	29.3	29.8	28.6	30.8	ns	
	Bathinda	2016	33.9	35.2	32.2	36.7	*	2.7
		2017	29.1	30.2	30.2	32.3	ns	
Mexico	Yaqui Valley-I	2016	32.7	36.3	33.0	36.1	*	3.0
	Yaqui Valley-II	2016	29.5	33.3	29.8	36.0	***	1.6
Pakistan	Faisalabad	2016	33.8	52.8	40.0	38.3	***	2.9
		2017	37.1	40.9	37.4	42.0	***	2.4
	Gujranwala	2016	22.6	31.4	24.8	41.9	***	5.9
		2017	38.7	37.6	36.9	41.6	**	2.1
	Sheikhupura	2017	36.6	37.5	33.8	43.1	**	4.6
South Africa	Langgewens	2016	27.2	33.2	26.6	32.5	***	2.3
		2017	35.3	38.9	36.3	42.3	***	2.7
	Roodebloem	2016	27.8	28.6	27.1	31.4	**	1.6
		2017	29.9	30.5	30.5	38.5	***	2.5
Turkey	Institute	2016	37.0	39.1	36.1	41.5	*	3.3
		2017	37.5	39.3	37.3	41.1	**	2.0
	Topraksu	2016	35.6	37.2	35.9	38.2	ns	
		2017	36.6	40.5	36.1	41.7	**	3.0
	Yusuflar	2016	33.9	36.5	34.3	35.9	ns	
		2017	33.1	36.0	33.5	37.2	*	2.8
	grand mean		32.6	35.4	32.6	36.8		
			the *P* value of paired *t* test					
control				0.001	0.876	<0.001		
foliar Zn					<0.001	0.089		
foliar I						<0.001		
foliar cocktail								

[a]ns indicates no significant difference; ***, *, and ** indicates significant differences at $p < 0.001$, < 0.05, and <0.01, respectively.

foliar treatment of these micronutrient significantly enhanced grain concentrations of Zn, I, and Se in all locations of six countries over 2 years (Tables 4−6). Grain Zn concentration was increased by 61% and 65% with foliar application of Zn alone and micronutrient cocktail, respectively (Table 4). It is obvious that the concentrations and forms used for Fe, I, and Se in the micronutrient cocktail solution did not exert any antagonistic effect on leaf absorption and transportation of Zn. Recently, Mangueze et al. showed that Zn and Se sprayed together significantly enhanced grain Zn and Se concentrations in rice grain.[24] Similarly, also Zhang et al. showed that spraying Fe and Zn together did not affect grain Zn accumulation in wheat.[35]

It was interesting to note that foliar Zn application tended to improve grain Fe concentrations. The average increase in grain Fe by foliar Zn spray in all locations over 2 years was about 9% (Table 7). Similarly, in our previous study, foliar Zn application had significant positive effects on grain Fe concentrations of the bran and embryo parts of the wheat grain which were not related to any Fe contamination of grain samples through soil dusts or particles.[22] Also the findings of Li et al. showed that foliar Zn application increased wheat grain Fe concentration to some extent.[36] Increases in grain Fe after foliar Zn was also found in wheat grown at the CIMMYT Research Station in Obregon (I. Ortiz-Monasterio, unpublished results). In potatoes, foliar Zn spray had no antagonistic effect on the tuber Fe concentration. There was even an increasing trend in tuber Fe concentrations after foliar Zn spray.[37] All these results in wheat and potatoes are, however, in disagreement with the results published by Saha et al., who showed a very clear decrease in grain Fe by foliar Zn application in rice.[38] Kutman et al. suggested that increases in grain Zn following foliar Zn spray probably resulted in Zn-binding compounds in grain which most likely act as a sink for Fe transport and storage.[22] Existence of highly positive correlation between grain Zn and Fe has been reported very often in several cereal germplasms.[13]

Foliar-applied Zn and micronutrient cocktail at 27 total field sites proved effective in attaining the generally accepted target grain Zn concentration of 40 $mg\ kg^{-1}$ at most of the field locations (Table 4), which is in good agreement with the earlier results reported by Cakmak et al. and Zou et al.[13,16] It has been often shown that soil Zn applications have a minimal effect on grain Zn while foliar application of Zn fertilizers are highly effective in increasing Zn both in whole grain and also in endosperm parts of the grains.[17,28] Similarly, also DTPA-

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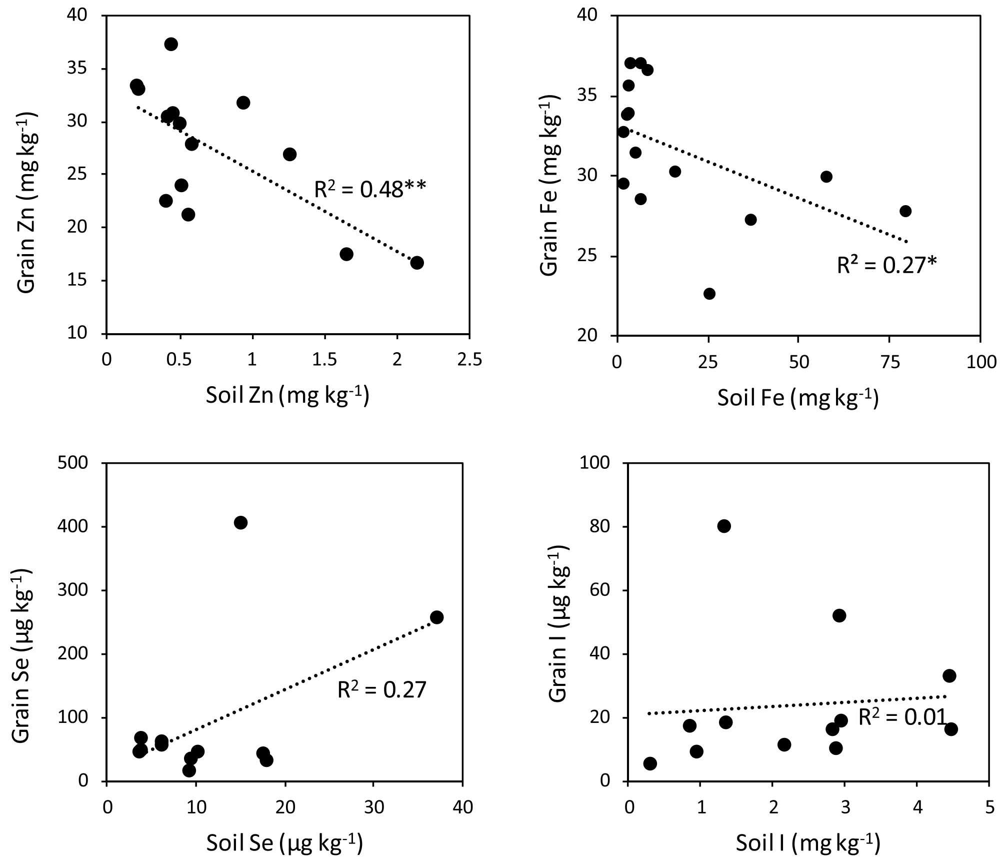

Figure 1. Correlations between soil and grain concentrations of Zn, Se, Fe, and I in the case of the plants without foliar treatments. Soil Zn and Fe show the DTPA-extractable concentrations of Zn and Fe, while soil Se and I concentrations represent the 0.1M KH_2PO_4 extractable and TMAH-extractable concentrations, respectively.

extractable Zn did not show a positive correlation with grain Zn, and there even was a negative relationship (Figure 1). Previously, existence of a poor relationship between grain Zn and extractable Zn in soils has been also shown for several plant species.[13,39,40] Zinc has relatively high phloem mobility in plants, especially in the case of a good N nutrition.[22,23] Therefore, adequate foliar supply of Zn at appropriate crop growth stages at adequate N nutrition could significantly increase the grain Zn concentration of wheat to desired levels. According to Cakmak and Kutman, keeping a high amount of readily available Zn pool in vegetative tissues during the grain filling stage (i.e., when the translocation of photoassimilates toward the grain is underway), for example, by foliar Zn spray, is of great importance for a successful biofortification of cereal grains with Zn.[5]

Foliar application of I alone or together with the micronutrient cocktail solution resulted in particular increases in the grain I concentration ($p < 0.05$; Table 5). Whereas without I application, the average grain I concentration across all field locations was 24 μg kg^{-1} only, and with foliar-applied I alone or in the micronutrient cocktail it increased to 316 μg kg^{-1} and 249 μg kg^{-1}, respectively. In the Ludhiana location in 2016, a particular increase was found in grain I (i.e., from 17 to 1447 μg kg^{-1}) that might be a consequence of an unknown contamination problem or likely a surface contamination of seeds with I during foliar treatments. The samples with extremely high I have been measured again and the results were same, indicating that very high levels of I are likely related to direct contamination (fortification) of seeds with I through foliar spray, when the florets at the spray time were open. The differential effects of foliar-applied I alone and micronutrient cocktail on enhancing grain I might be attributed to inhibitory effect of other micronutrients in the cocktail (i.e., Zn, Se or Fe) on leaf absorption and/or translocation of I from wheat leaves to grains. In a previous study, it has been shown that in hydroponically grown lettuce plants, combined application of KIO_3 and SeO_4^{-2} did not antagonize root absorption of I or Se; rather there was a synergism between these two micronutrients during their root absorption.[41] As transport of I through the xylem is more efficient than through the phloem, I loading into cereal grains has been suggested to be less efficient.[42,43] However, in a study with tomato plants it has been shown that foliar-applied I may be effective for increasing I reserves in stem and leaf tissues of tomato plants for a subsequent remobilization into fruits.[44] Recently, Cakmak et al. demonstrated that I was able to transport from older into younger leaves as well as from vegetative tissues into grain in wheat.[8] According to Hurtevent et al., I exhibits a medium phloem mobility in wheat plants.[45] In the study by Cakmak et al., it was shown that soil applied I was effective in increasing shoot I concentrations but remained less effective in improving grain I.[8] These results together with the results obtained by Hurtevent et al.[45] show that I most probably exhibits a moderate phloem-mobility. Therefore, as indicated for Zn above, a foliar spray of I during the early stage of the seed-filling (i.e., when the extensive phloem translocation of photoasasimilates into seeds takes places) would generate a

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highly available pool for I in leaf tissue for an immediate phloem loading and transport into grain.

In the control (no spray) treatment, wheat grain Se concentration varied considerably from site-year to site-year, ranging from as low as 4 $\mu g\ kg^{-1}$ (at the Topraksu location in Turkey in 2017) to as high as 549 $\mu g\ kg^{-1}$ at the Ludhiana location in India in 2017), with average Se concentration of 90 $\mu g\ kg^{-1}$ (Table 6). Wheat grown in the Ludhiana and Faisalabad locations exhibited very high Se concentrations although Se was not sprayed (Table 6), probably due to higher amount of chemically available soil Se concentrations (Table 1). The plants grown in the Ludhiana and Faisalabad locations with very high grain Se concentrations without Se spray showed similar net increases in grain Se following foliar Se spray when compared to the plants grown in other locations. It is, therefore, very likely that very high grain Se concentrations in the Ludhiana and Faisalabad locations are not related to contamination or an error problem in the Se assay. The repeated measurements showed the same Se results in those samples. As shown in Figure 1, soil Se tended to correlate grain Se although the relationship was not significant. The positive correlation between soil Se and grain Se has been already shown in wheat.[46] Plants also respond very significantly to increasing soil Se applications with corresponding increases in grain Se.[6,10,11] Foliar-applied micronutrient cocktail was quite effective in increasing grain Se concentration, on average by about 3.8-fold, from 90 $\mu g\ kg^{-1}$ to 338 $\mu g\ kg^{-1}$ (Table 6). The achieved grain Se concentrations following foliar spray of the micronutrient cocktail were close or above the estimated target grain Se level of 300 $\mu g\ kg^{-1}$ in wheat grain for better human nutrition at most of the field locations.[10] In Finland, before the start of the well-known nationwide application of Se-enriched NPK fertilizers, Se concentrations of wheat were around 25 $\mu g\ kg^{-1}$ in the early 1980s and this value increased over 150 $\mu g\ kg^{-1}$ in the past 30 years with significant impacts on human health.[11] According to a new meta-analysis, grain Se concentrations are significantly increased if an optimal Se rate, Se formulation, and Se application methodology are selected and implemented.[47] Selenium fertilizers, applied to soil or foliar either in form of Na-selenite or Na-selenate are known to be highly effective in enrichment of food crops, but it appears that the selenate form, that is used in the present study, is more effective than selenite in increasing Se accumulation in edible parts of the plants.[10] It is known that compared to selenite, selenate is better absorbed and transported into the shoot of wheat plants.[48] As agronomic biofortification of wheat grains with Se has proven tobe quite effective, being a major staple food all over the world, Se-biofortified wheat can greatly help in improving human health by reducing Se malnutrition. Most of the Se in wheat grain is known to exist in the form of selenomethionine (SeMet) which is a highly bioavailable form of Se in wheat grain.[10,49] It seems that a high proportion of Se accumulated in wheat grain after foliar spray is converted to SeMet in the grain. According to Galinha et al., up to 70−100% of the agronomically increased Se in wheat is assimilated into SeMet.[49]

In this study, grain Fe concentration was only slightly increased (by 14.0%) with foliar application of micronutrient cocktail containing Fe in form of FeEDTA (Table 7). In an earlier study conducted on wheat, foliar application of Fe was more effective in improving grain Fe and resulted in an increase in grain Fe up to 28% ($p < 0.05$).[35] Less increase in grain Fe concentration by agronomic biofortification compared to Zn may be attributed to poor phloem mobility of Fe from leaves to grains.[33] Also Aciksoz et al. showed that both soil and foliar application of Fe fertilizers has very minor effect on grain Fe concentrations.[21] As discussed by Aciksoz et al., application of Fe fertilizers to durum wheat plants in the form of $FeSO_4$ or a chelated form including Fe-EDTA, Fe-EDDHA, or Fe-citrate had no or minimal effect on shoot and grain concentrations of Fe.[21] Probably, when Fe is needed, wheat plants release Fe-chelating compounds (so-called phytosiderophores, PS) high enough to improve Fe mobilization and root absorption and to meet Fe demand. In good agreement with these results, it has been found that there is no or a very low relationship between soil Fe and grain Fe, and there is even a negative trend (Figure 1). The results showing a poor relationship between grain and soil Fe are in good agreement with the previous results[39,40]

It was encouraging to observe that increases in grain Zn, I, and Se concentrations by the foliar-applied micronutrient cocktail did not reduce the grain Fe concentration; rather, foliar-applied Zn promoted grain Fe concentration over the control treatment by 9.1%, as discussed above. Considering also the results from the literature, it can be suggested that foliar application of Fe is not an effective approach to enhance wheat grain Fe to the desired level for human nutrition. Therefore, further studies are warranted to find out more effective strategies of adequately enriching wheat grains with Fe. It is known that optimized nitrogen (N) nutrition of wheat plants could significantly contribute to grain Fe concentration.[21,50,51] Foliar application of more effective sources and formulations of Fe in combination with N fertilizer warrant investigation to increase grain Fe biofortification, rather than spraying Fe alone.[21]

In conclusion, the results of this extensive field study have clearly demonstrated that simultaneous foliar applications of Zn, I, and Se in the same cocktail solution has effectively enhanced concentrations of Zn, I, and Se in grains of different wheat cultivars grown under variable management and environmental conditions in six countries without grain yield trade-off. Since wheat is a predominant staple cereal around the globe, this agronomic biofortification strategy has huge practical relevance and importance for improving human health in resource-poor communities. Wheat grains were, however, not enriched sufficiently with Fe by foliar application of the same micronutrient cocktail, suggesting consideration of alternative agricultural approaches with high potential to contribute to grain Fe. Recent results of the long-term and successful HarvestPlus breeding programs show that new wheat, rice, and bean genotypes are now available having extra Fe (as well as Zn) up to 10 mg kg^{-1} grain thanks to breeding efforts (Andersson et al., 2017; www.harvestplus.org).[52] Combining genetic and agronomic (i.e., fertilizer) approaches may further raise grain micronutrient concentrations. Future studies should pay attention to additive and synergistic effects of genetic and agronomy on accumulation of micronutrients in food crops.

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Notes

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