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REGULAR ARTICLE

Biofortification of wheat, rice and common bean by applying foliar zinc fertilizer along with pesticides in seven countries

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Abstract
Aims Rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and common bean (*Phaseolus vulgaris* L.) are major staple food crops consumed worldwide. Zinc (Zn) deficiency represents a common micronutrient deficiency in human populations, especially in regions of the world where staple food crops are the main source of daily calorie intake. Foliar application of Zn fertilizer has been shown to be effective for enriching food crop grains with Zn to desirable amounts for human nutrition. For promoting adoption of this practice by growers, it is important to know whether foliar Zn fertilizers can be

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applied along with pesticides to wheat, rice and also common bean grown across different soil and environmental conditions.

Methods The feasibility of foliar application of zinc sulphate ($ZnSO_4.7H_2O$) to wheat, rice and common bean in combination with commonly used five fungicides and nine insecticides was investigated under field conditions at the 31 sites-years of seven countries, i.e., China, India, Pakistan, Thailand, Turkey, Brazil and Zambia.

Results Significant increases in grain yields were observed with foliar Zn/foliar Zn + pesticide (5.2–7.7 % of wheat and 1.6–4.2 % of rice) over yields with no Zn treatment. In wheat, as average of all experiments, higher grain Zn concentrations were recorded with foliar Zn alone (41.2 mg kg^{-1}) and foliar Zn + pesticide (38.4 mg kg^{-1}) as compared to no Zn treatment (28.0 mg kg^{-1}). Though the magnitude of grain Zn enrichment was lesser in rice than wheat, grain Zn concentrations in brown rice were significantly higher with foliar Zn (24.1 mg kg^{-1}) and foliar Zn + pesticide (23.6 mg kg^{-1}) than with no Zn (19.1 mg kg^{-1}). In case of common bean, grain Zn concentration increased from 68 to 78 mg kg^{-1} with foliar Zn alone and to 77 mg kg^{-1} with foliar Zn applied in combination with pesticides. Thus, grain Zn enrichment with foliar Zn, without or with pesticides, was almost similar in all the tested crops.

Conclusions The results obtained at the 31 experimental site-years of seven countries revealed that foliar Zn fertilization can be realized in combination with commonly-applied pesticides to contribute Zn biofortification of grains in wheat, rice and common bean. This agronomic approach represents a useful practice for the farmers to alleviate Zn deficiency problem in human populations.

Keywords Grain yield · Grain zinc · Rice · Wheat · Common bean · Pesticides · Zinc deficiency

Introduction

Rice and wheat are the most widely cultivated food crops worldwide, and, together with maize, they provide about 60 % of the global food energy intake (Loftas et al. 1995). Similarly, common bean is an important staple legume crop in South America and, thus, a predominant source of Zn and other micronutrients in human diet (Blair 2013).

At the FAO/WHO Second International Conference on Nutrition held on 19th–21st November 2014, it was highlighted again that micronutrient deficiencies cause diverse health complications and remain highly prevalent worldwide, affecting over two billion people, with children and women at particular risk (http://www.fao.org/3/a-ml542e.pdf). Micronutrient malnutrition not only impairs people's health, well-being and work performance, but also poses a serious economic burden, especially on poorer nations, as shown for Zn deficiency (Stein 2014). Amongst micronutrients, Zn is a particular one because it plays many critical roles in both human nutrition and crop production (Cakmak 2000; Hotz and Brown 2004; Broadley et al. 2007). For example, up to 10 % of proteins in human proteome need Zn for their stability and catalytic activity (Andreini et al. 2006), and Zn is primarily involved in detoxification of reactive oxygen species and biosynthesis of proteins (Cakmak 2000; Broadley et al. 2007).

Zinc has been reported to be deficient in 30 % of the agricultural soils worldwide (Alloway 2008), and about 50 % of cereal-cultivated soils have low chemical solubility of Zn to plant roots (Marschner 1993; Graham and Welch 1996). Zinc deficiency in humans is mainly prevalent in regions of the globe where soil Zn deficiency has been well-documented and cereals are major source of daily calorie intake (Cakmak 2008). Contribution of staple cereals to daily calorie intake reaches up to 75 % in rural areas of many developing countries, such as in Central Asia and Middle-East in case of wheat and in South-East Asia in case of rice (Welch and Graham 2005; Cakmak et al. 2010a; Fiedler 2014). Rice and wheat are known to be very low in grain Zn concentrations and rich in compounds inhibiting Zn bioavailability in diet such as phytate (Broadley et al. 2007; Wessells et al. 2012). In addition, wheat and rice are generally more prone to soil Zn deficiency leading to a substantial reduction in grain yield and nutritional quality (Graham et al. 1992; Phattarakul et al. 2012; Zou et al. 2012).

Soil and foliar application of Zn fertilizers is considered an effective short-term solution to Zn deficiency-related problems in both crop production and human health (Cakmak 2008; Manzeke et al. 2014; Prasad et al. 2014). With foliar application of Zn fertilizer, increase in grain Zn is particularly high both in whole grain and in the endosperm part which can greatly contribute to dietary Zn intake (Jiang et al. 2007; Cakmak et al. 2010b; Zhang et al. 2012; Zou et al. 2012; Phattarakul et al. 2012). It is, however, important to notice that crop genotypes may respond differently to foliar Zn spray in terms of foliar absorption and loading of Zn into as shown in rice (Phattarakul et al. 2012; Mabesa et al. 2013). The timing of foliar Zn applications is also important in achieving sufficient enrichment of grains with Zn both in rice and wheat. For example, foliar Zn application at later growth stages of wheat (i.e., during anthesis and early milk stage) has been found to be highly effective in increasing grain Zn concentration while soil Zn application remained less effective (Cakmak et al. 2010b; Zou et al. 2012). Similarly in rice, application of Zn fertilizer to soil was much less effective for increasing grain Zn concentrations compared with foliar Zn application (Wissuwa et al. 2008; Phattarakul et al. 2012; Mabesa et al. 2013). Based on the meta-analysis of the published data for 10 African countries, Joy et al. (2015) reported that foliar Zn application is a cost effective approach for increasing Zn concentration in cereal grains, and the cost associated with foliar Zn spray seem to be equal to the cost of flour fortification with Zn.

Thus, it is important to motivate and encourage farmers to spray Zn fertilizer on staple food crops for improving grain Zn concentration. However, if there is no yield advantage and no premium price of Zn-enriched grains, the farmers will not be motivated to adopt foliar spray of Zn fertilizer just for enriching the grains with Zn, as this practice involves extra investment. It is known that the Zn-enriched seeds germinate better and show better crop stand and seedling vigor (Welch 1999; Harris et al. 2007; Cakmak 2008) which might be a motivating factor for the farmers to enrich grains with Zn. An additional motivation for farmers to spray Zn fertilizer to foliar would be to add Zn into their existing foliar spray program. Today, various kinds of pesticides are being sprayed on crop plants by the farmers to control foliar diseases, like leaf rust, and insect pests, like aphids (McIntosh 1996; Liu et al. 2015). Recently published evidence suggests that Zn fertilizer can be applied together with foliarly sprayed pesticides without causing adverse effect on grain Zn as shown in India (Ram et al. 2015) and China (Wang et al. 2015) in rice and wheat.

In the present study, field experiments were established to investigate the effect of foliar Zn application in form of $ZnSO_4.7H_2O$, without or with pesticides (fungicides and insecticides), in increasing grain Zn concentrations of rice and wheat grown in 26 field sites of Zambia, Thailand, China, India, Pakistan, Brazil and Turkey by using different cultivars of wheat and rice. Similar field experiments were also conducted on common bean grown in five field sites in Brazil.

Materials and methods

Experimental sites and treatments

Field experiments were carried out on rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and common bean (*Phaseolus vulgaris* L.). Rice experiments were established at eight field sites in three countries (India, China and Thailand), wheat experiments at 18 field sites in six countries (India, China, Pakistan, Brazil, Turkey and Zambia) and common bean experiment at five field sites in Brazil (Table 1). The commonly grown cultivars of these crops in the respective countries were used in the field experiments. The study included 10 different wheat, three different rice and one common bean cultivars in the experiments (Table 1). The concentration of diethylene-triaminepentaacetic acid (DTPA) extractable soil Zn, pH and organic carbon of the experimental soils are also given in Table 1. Though most soils of the wheat experimental sites contained less than 0.5 mg Zn kg^{-1}, the range of DTPA-extractable Zn was quite wide, i.e., 0.32 mg kg^{-1} soil at Konya location in Turkey and 1.40 mg kg^{-1} soil at the Capao Bonito location in Brazil. The range of DTPA-extractable Zn concentrations in the locations of the rice experiments varied from 0.33 mg kg^{-1} soil at Jiangsu location in China to 0.90 mg kg^{-1} soil at CMU location in Thailand. In case of common bean, DTPA-extractable Zn at the field sites was fairly high, ranging from 1.4 to 6.5 mg kg^{-1} soil (Table 1).

The experiments were conducted in randomized block design with four replications for rice and wheat and six replications for common bean. Field experiments comprised of three treatments as following: i) local control (basal fertilizers only, no Zn); ii) local control + two foliar sprays with 500 to 800 L per hectare of 0.5 % (*w/v*) aqueous solution of $ZnSO_4 \cdot 7H_2O$ (at boot and milk stages on rice and

Table 1 Locations, years, soil pH, soil DTPA-extractable Zn, soil organic carbon, varieties and pesticides used in the experiments with wheat, rice and common bean in 7 countries

Crop/country	Location	Year	Soil pH	DTPA-Zn (mg kg^{-1} soil)	Organic carbon (%)	Variety	Pesticide used[1]
Wheat							
India	Ludhiana	2011–13	7.6	0.58	0.25	PBW 621	Propiconazole*
India	Gurdaspur	2011–13	7.5	0.55	0.29	PBW 621	Propiconazole*
India	Bathinda	2011–13	7.9	0.45	0.15	PBW 621	Propiconazole*
Pakistan	Faisalabad–I	2011–12	8.3	0.56	0.29	Sehar-2006	Imidacloprid**
Pakistan	Muridke–I	2011–12	8.0	0.45	0.30	Sehar-2006	Imidacloprid**
Pakistan	Kabirwala	2011–12	8.1	0.52	0.38	Lasani-2008	Imidacloprid**
Pakistan	Faisalabad–II	2012–13	7.8	0.35	0.38	Faisalabad-2008	Imidacloprid**
Pakistan	Muridke-II	2012–13	8.0	0.88	0.70	Faisalabad-2008	Imidacloprid**
Brazil	Capão Bonito–I	2009	5.9	1.40	1.16	IAC 375	pyraclostrobin + epoxiconazol*
Brazil	Capão Bonito–II	2009	6.6	1.20	1.69	IAC 375	pyraclostrobin + epoxiconazol*
Brazil	Capão Bonito	2010	6.2	0.60	1.16	IAC 370	pyraclostrobin + epoxiconazol*
China	Hebei-Quzhou	2011–12	7.8	0.33	0.13	Liangxing 99	Omethoate**
China	Hebei–Quzhou	2012–13	8.2	0.40	0.15	Liangxing 99	Omethoate**
China	Shaanxi-Yongshu	2011–12	7.8	0.37	0.14	Jimai 47	Imidacloprid**
China	Shaanxi-Yongshu	2012–13	7.8	0.37	0.12	Jimai 47	Imidacloprid**
Turkey	Eskisehir	2011–13	8.2	0.45	0.37	Bezostaja01	Deltamethrin **
Turkey	Konya	2011–13	7.5	0.32	0.30	Bezostaja01	Deltamethrin **
Zambia	Chisamba	2012–13	5.3	1.17	2.00	Lorrie-II	Mancozeb*
Rice							
India	Ludhiana	2011–13	7.6	0.58	0.25	PR 120	Propiconazole*
India	Gurdaspur	2011–13	7.5	0.55	0.29	PR 120	Propiconazole*
China	Jiangsu	2011–12	8.2	0.33	1.38	Zhendao 11	Carbendazim*
China	Jiangsu	2012–13	8.4	0.33	0.82	Zhendao 11	Carbendazim*
China	Anhui	2011–12	6.3	0.37	0.61	Zhendao 11	Carbendazim*
China	Anhui	2012–13	6.4	0.37	0.46	Zhendao 11	Carbendazim*
Thailand	CMU	2011–12	7.7	0.90	1.50	Chainat 1	Fiproni**
Thailand	Takli	2011–12	6.2	0.50	3.70	Chainat 1	Fipronil**
Common bean							
Brazil	Votuporanga	2012	6.0	4.2	0.97	Perola	Thiamethoxam**
Brazil	Votuporanga	2013	5.3	6.5	0.63	Perola	cyantraniliprole **
Brazil	Campos Novos	2012	5.4	1.4	1.11	Perola	Clorantraniliprole + Lambda-cyhalothrin **
Brazil	Mirestrela	2013	5.1	2.7	0.63	Perola	cyantraniliprole **
Brazil	Capão Bonito	2012–13	5.6	1.5	1.11	Perola	Thiamethoxam **

[1] Pesticides applied at the rates recommended on the packages (*Fungicide; **Insecticide)

wheat and after flowering on common bean); and iii) local control + two foliar sprays of $ZnSO_4 \cdot 7H_2O$ in combination with pesticides as applied in the treatment two. The pesticides sprayed as either only fungicide or insecticides are shown in Table 1. The detail of the application of N (nitrogen), P (phosphorus) and potassium (K) fertilizers in different countries has been given in the Table 2 as per recommended management practice. The insecticides and fungicides used in the experiments were

Table 2 Rate of nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) fertilizers applied and N application schedule in 7 countries

Country	Basal fertilizers (kg ha^{-1})			N application schedule
	N	P_2O_5	K_2O	
Wheat				
India	150	62.5	30	1/3 at sowing + 1/3 at first irrigation (25 DAS - days after sowing) + 1/3 at second irrigation (50 DAS)
Pakistan	120	80	–	1/2 at sowing + 1/2 at tillering
Brazil	56	60	34	16 kg N/ha at sowing + 20 kg/ha N at 35 DAS + 20 kg/ha N at 48 DAS
China	200	35	124	1/2 at planting and 1/2at early jointing
Turkey	150	80	–	1/2 N at sowing + 1/2 N at tillering stage
Zambia	168	60	30	30 kg N/ha at planting + 138 kg N/ha at tillering (28 DAS)
Rice				
India	150	40	–	1/3 N at transplanting + 1/3 N at 21 DAT - days after transplanting + 1/3 at 42 DAT
China	200	80	150	2/5 at transplanting and 3/5 at panicle initiation
Thailand	150	80	–	1/2 N at transplanting + 1/2 N at tillering (40–45 DAT)
Common Bean				
Votuporanga	110	87	60	40 kg ha^{-1} at planting + 70 kg ha^{-1} 15–20 days after emergence
Campos Novos	88	38	38	28 kg ha^{-1} at planting + 60 kg ha^{-1} at 3 weeks after emergence
Capão Bonito	90	70	80	20 kg ha^{-1} at planting + 70 kg ha^{-1} 15 days after emergence
Mirestrela	60	150	70	20 kg ha^{-1} at planting + 40 kg ha^{-1} at 3 weeks after emergence

different in various countries (Table 1), and applied according to the manufacturers' recommended rates together with $ZnSO_4.7H_2O$.

Data collection

Grain yield was recorded at 13 % moisture for wheat and at 14 % moisture for rice and common bean. The grain samples were washed thoroughly with tap water, rinsed with distilled de-ionized (DDI) water, and oven dried at 45 °C. The dried grains of wheat grain, brown rice and common bean were subjected to acid-digestion (HNO_3-H_2O_2) in a closed-vessel microwave system (CEM Corp., Matthews, NC, USA), and analysed for Zn by using inductively coupled plasma optical emission spectrometry (ICP-OES) (Vista-Pro Axial; Varian Pty Ltd, Mulgrave, Australia). Measurements of Zn were checked by using a certified standard reference materials (SRM 1573a), obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA). Further details about preparation of grain samples for Zn analysis are given in Phattarakul et al. (2012) and Zou et al. (2012).

Statistical analysis

The field and laboratory data were analysed using one factor ANOVA process and means were separated by least significant difference (LSD) at $P = 0.05$. For overall effectiveness, the paired t test method was used to compare the data sets across locations and years.

Results

Grain yield in wheat and rice

Grain yield of wheat varied among the field locations of six countries (Table 3). The highest grain yield of 8.66 t ha^{-1} was recorded with foliar applied Zn at Hebei-Quzhou location in China in 2012–13 whereas the lowest grain yield of 0.75 t ha^{-1} was obtained without Zn application at Capão Bonito-II location in Brazil. At most of the field locations, wheat grain yield was increased with foliar Zn alone as well as with foliar Zn applied in combination with pesticides. However, the positive effects of foliar Zn treatments were significant

Table 3 Grain yield of wheat grown without Zn treatment and with foliar Zn treatment alone or in combination with pesticide in 24 field experiments conducted in 6 countries

Country	Location	Year	Grain yield (t ha^{-1})			L.S.D. ($P = 0.05$)
			No Zn	Foliar Zn	Foliar Zn + pesticide	
India	Ludhiana	2011–12	5.71a	5.85a	5.92a	NS
	Ludhiana	2012–13	5.50a	5.65a	5.59a	NS
	Bathinda	2011–12	4.82a	4.85a	4.82a	NS
	Bathinda	2012–13	4.52a	4.48a	4.51a	NS
	Gurdaspur	2011–12	5.60a	5.64a	5.70a	NS
	Gurdaspur	2012–13	5.53a	5.59a	5.65a	NS
Pakistan	Faisalabad-I	2011–12	3.98c	4.72b	5.49a	0.57
	Faisalabad-II	2012–13	6.04b	7.39a	7.74a	0.58
	Muredke-I	2011–12	3.55c	4.73a	4.56b	0.73
	Kabirwala	2011–12	3.82b	4.59a	5.19a	0.70
	Muredke-II	2012–13	2.48b	3.04a	3.65a	0.64
Brazil	Capão Bonito - I	2009	1.53b	1.46b	2.03a	0.20
	Capão Bonito - II	2009	0.75b	0.78b	1.21a	0.16
	Capão Bonito	2010	3.72a	3.93a	4.17a	NS
China	Hebei-Quzhou	2011–12	7.88a	7.63a	7.99a	NS
	Hebei-Quzhou	2012–13	7.82a	8.66a	8.43a	NS
	Shaanxi-Yongshou	2011–12	7.21a	6.39a	6.48a	NS
	Shaanxi-Yongshou	2012–13	3.55a	3.69a	3.46a	NS
Turkey	Eskisehir	2011–12	4.20a	4.28a	3.79a	NS
	Eskisehir	2012–13	5.05a	4.98a	5.16a	NS
	Konya	2011–12	2.27a	2.14a	2.66a	NS
	Konya	2012–13	3.94a	4.44a	3.58a	NS
Zambia	Chisamba	2012	4.37a	4.18a	4.18a	NS
	Chisamba	2013	3.66a	4.13a	3.96a	NS
Mean			4.41b	4.64a	4.75a	0.2

only at all locations in Pakistan and two locations in Brazil ($P = 0.05$; Table 3). In Pakistan, the increases in grain yield by foliar Zn applications were more pronounced. For example, at Muridke-II location of Pakistan, combined spray of Zn and insecticide enhanced grain yield by 47 % over no Zn treatment. Contrarily, at Shaanxi-Yongshou location in China, foliar Zn treatments did not increase grain yield during both years.

Based on pooled analysis across years and locations for wheat, significantly higher grain yield of 4.75 t ha^{-1} was recorded with foliar Zn applied together with pesticides. Average increases in wheat grain yield achieved across all locations and years, compared to the no Zn treatment, were 5.2 % with foliar Zn sprayed alone and 7.7 % with foliar Zn sprayed in combination with pesticides. The yield increase with foliar Zn + pesticide treatment was significant ($P = 0.05$; Table 3).

Rice grain yields also exhibited a large variation among the locations of three countries (Table 4). These varied from 10.45 t ha^{-1} at Anhui-Changfeng (China) in 2013 to 4.57 t ha^{-1} at Ludhiana (India) in 2012. However, rice grain yield was not significantly influenced by any of the Zn treatments at all locations and during all years, except at Anhui-Changfeng location of China in 2013. At Anhui-Changfeng during year 2013, grain yield was 9.74 t ha^{-1} with no Zn treatment and 10.45 t ha^{-1} with foliar Zn treatment ($P = 0.05$). Although the effects were not significant, foliar Zn treatment tended to improve grain yield in all locations,

Table 4 Paddy yield of rice grown without Zn treatment and with foliar Zn treatment alone or in combination with pesticide in 12 field experiments conducted in 3 countries

Country	Location	Year	Paddy yield (t ha^{-1})			LSD ($P = 0.05$)
			No Zn	Foliar Zn	Foliar Zn + pesticide	
India	Ludhiana	2012	4.62a	4.57a	4.68a	NS
	Ludhiana	2013	5.43a	5.45a	5.46a	NS
	Gurdaspur	2012	4.91a	5.04a	4.87a	NS
	Gurdaspur	2013	6.23a	6.26a	6.23a	NS
China	Jiangsu-Rudong	2012	8.08a	8.32a	8.23a	NS
	Jiangsu-Rudong	2013	7.28a	7.41a	7.41a	NS
	Anhui-Changfeng	2012	6.23a	7.04a	6.07a	NS
	Anhui-Changfeng	2013	9.74b	10.45a	10.18a	0.36
Thailand	CMU	2011	7.19a	7.39a	7.39a	NS
	CMU	2012	6.88a	6.96a	6.83a	NS
	Takli	2011	5.58a	6.17a	5.80a	NS
	Takli	2012	4.87a	5.24a	5.13a	NS
Mean			6.42b	6.69a	6.52b	0.15

except at Ludhiana in India during 2012. Based on the overall pooled means, significantly higher rice grain yield (6.69 t ha^{-1}) was recorded with foliar Zn applied alone, which was 4.2 % higher than the no Zn treatment. However, foliar Zn applied along with pesticides enhanced rice grain yield only by 1.6 % over the no Zn treatment mean yield.

Grain zinc in wheat and rice

Wheat grain Zn concentrations without Zn application varied from 18.3 to 35.5 mg kg^{-1} at various locations of 6 countries (Table 5). Wheat grain Zn responded positively to foliar Zn applications at all locations, and in most cases the increases in grain Zn concentration with foliar Zn application were statistically significant. The highest Zn concentration in wheat grains (i.e., 53.5 mg kg^{-1}) was observed at Capao Bonito-II location of Brazil during 2009 with foliar Zn + pesticide treatment, whereas lowest grain Zn concentration (i.e., 18.3 mg kg^{-1}) was recorded in wheat grown at Shaanxi-Yongshou location of China during 2012–2013 without foliar Zn application (Table 5).

Increments in wheat grain Zn concentration with foliar Zn application were significant at all locations during all years ($P = 0.05$), with the exception of Kabirwala in Pakistan and Eskisehir in Turkey during 2011–2012 and Chisamba in Zambia during 2012. Increases in grain Zn concentrations, over the concentrations with no Zn application, were highest at the two sites of Capao Bonito in Brazil during 2009, as at least 20 mg kg^{-1} increment in grain Zn concentration was recorded with foliar Zn applied without or with pesticide at these field locations (Table 5).

In contrast to many other locations, there was a distinct decrease in grain Zn concentration when Zn was sprayed along with insecticide at Faisalabad location (during both years) and at Muridke-I location of Pakistan and at Hebei-Quzhou location of China during 2012–13, as compared to the respective grain Zn concentrations obtained with foliar Zn application alone. However, at Muridke-II location of Pakistan during 2012–13, foliar Zn sprayed alone and in combination with insecticide increased grain Zn concentration significantly over no Zn treatment ($P = 0.05$). Across all locations and years, foliar application of Zn, without as well as with pesticides increased wheat grain Zn concentration significantly ($P = 0.05$; Table 5). Mean increase in grain Zn concentration with foliar spray of Zn alone was 47.1 % and net increment was 13.2 mg Zn kg^{-1} grain over the concentration obtained with no Zn application. The net increment in grain Zn with foliar Zn + pesticide was 10.4 mg kg^{-1}.

Table 5 Grain Zn concentration of wheat grown without Zn treatment and with foliar Zn treatment alone or in combination with pesticide in 24 field experiments conducted in 6 countries

Country	Location	Year	Grain Zn concentration (mg kg^{-1})			LSD ($P=0.05$)
			No Zn	Foliar Zn	Foliar Zn + pesticide	
India	Ludhiana	2011–12	34.6b	42.7a	39.9a	3.1
	Ludhiana	2012–13	27.2b	42.3a	43.6a	6.1
	Bathinda	2011–12	28.4b	38.2a	32.9b	3.5
	Bathinda	2012–13	25.4c	42.2a	31.7b	3.5
	Gurdaspur	2011–12	33.2b	40.3a	41.9a	2.9
	Gurdaspur	2012–13	26.2b	44.1a	40.2a	4.2
Pakistan	Faisalabad-I	2011–12	21.0b	40.9a	22.6b	4.9
	Faisalabad-II	2012–13	29.8b	36.8a	30.5b	2.9
	Muredke-I	2011–12	21.1b	34.9a	24.9b	6.1
	Kabirwala	2011–12	24.2a	26.2a	27.5a	NS
	Muredke-II	2012–13	30.4b	41.2a	41.5a	7.5
Brazil	Capão Bonito - I	2009	30.1b	50.0a	52.4a	4.8
	Capão Bonito - II	2009	29.5b	49.5a	53.5a	5.5
	Capão Bonito	2010	25.3a	42.7a	45.7a	7.5
China	Hebei-Quzhou	2011–12	32.4b	47.5a	38.5ab	10.0
	Hebei-Quzhou	2012–13	32.6b	49.2a	37.2b	8.4
	Shaanxi-Yongshou	2011–12	21.1b	40.7a	41.9a	2.0
	Shaanxi-Yongshou	2012–13	18.3b	32.5a	34.2a	6.3
Turkey	Eskisehir	2011–12	35.5a	41.9a	42.3a	NS
	Eskisehir	2012–13	30.0b	43.8a	41.8a	6.6
	Konya	2011–12	27.4b	37.2a	31.1a	6.2
	Konya	2012–13	24.8b	34.8a	32.5ab	8.0
Zambia	Chisamba	2012	31.8a	46.3a	48.3a	NS
	Chisamba	2013	33.8b	52.5a	51.8a	8.1
Mean			28.0c	41.2a	38.4b	2.5

Similar to wheat, brown rice (grain) Zn concentrations also varied among locations and years (Table 6). In the absence of Zn application, brown rice Zn differed greatly among the locations of Thailand during both years. Across all treatments and over all locations, maximum Zn concentration in brown rice grains, recorded at Anhui-Changfeng location of China during 2013, was 31.9 mg kg^{-1} with foliar Zn alone, whereas the minimum Zn concentration was 12.5 mg kg^{-1} without Zn application at Takli location of Thailand during 2012. Foliar Zn spray markedly improved Zn concentrations in rice grains at all locations. With the exception of year 2012 in Thailand, increases in grain Zn by foliar spray of Zn, without or with pesticide, were significant compared to the concentrations with no Zn treatment ($P=0.05$; Table 6).

Maximum increment in rice grain Zn by foliar Zn application (i.e., 9.0 mg kg^{-1}) was obtained at the CMU location of Thailand during 2011, and the minimum increment (i.e., 2.2 mg kg^{-1}) was observed at Jiangsu-Rudong location of China during 2013. When compared with the results of wheat (Table 5), the increment in grain Zn concentration with foliar Zn application to rice was clearly much less (Table 6). On the pooled analysis basis, foliar Zn application alone or with the pesticides enhanced rice grain Zn concentration by 26.2 and 23.6 % over no Zn application, respectively.

Table 6 Zinc concentrations in brown rice from plants grown without Zn treatment and with foliar Zn treatment alone or in combination with pesticide in 12 field experiments conducted in 3 countries

Country	Location	Year	Brown rice Zn (mg kg^{-1})			LSD ($P = 0.05$)
			No Zn	Foliar Zn	Foliar Zn + pesticide	
India	Ludhiana	2012	19.8b	25.1a	26.5a	3.1
	Ludhiana	2013	19.1b	23.5a	23.0a	1.5
	Gurdaspur	2012	18.7b	23.5a	23.4a	2.0
	Gurdaspur	2013	17.8b	21.8a	22.1a	2.2
China	Jiangsu-Rudong	2012	17.3b	22.7a	20.1a	2.3
	Jiangsu-Rudong	2013	19.8b	22.0a	23.2a	2.2
	Anhui-Changfeng	2012	19.8b	22.9a	21.1ab	1.9
	Anhui-Changfeng	2013	23.0b	31.9a	31.7a	3.4
Thailand	CMU	2011	21.2c	30.2a	25.4b	3.1
	CMU	2012	26.0a	28.2a	28.1a	NS
	Takli	2011	13.9b	22.5a	21.0a	2.8
	Takli	2012	12.5a	14.9a	17.3a	NS
Mean			19.1b	24.1a	23.6a	1.3

Grain yield and grain Zn in common bean

Application of foliar Zn without or with pesticide did not influence grain yield of common bean at all the locations and during all years in Brazil (Table 7). In 2012, grain yield recorded at Campos Novos location was much less than at other locations and years. Maximum grain yield was recorded at Mirestrela location during 2013. However, foliar sprays of Zn, without and with pesticide, did not increase grain yield of

Table 7 Grain yield and grain Zn concentration of common bean grown without Zn treatment and with foliar Zn treatment alone or in combination with pesticide in 5 experiments conducted in Brazil over 2012 to 2013

Location	Year	Zinc treatment			LSD ($P = 0.05$)
		No Zn	Foliar Zn	Foliar Zn + pesticide	
Grain yield (t ha^{-1})					
Votuporanga	2012	2.33	2.04	2.18	NS
Votuporanga	2013	2.83	2.61	2.75	NS
Campos Novos	2012	0.60	0.64	0.70	NS
Mirestrela	2013	3.81	4.16	3.80	NS
Capão Bonito	2012–13	2.35	2.33	2.31	NS
Mean		2.38	2.36	2.35	NS
Grain Zn concentration (mg kg^{-1})					
Votuporanga	2012	73.2c	86.9a	81.8b	1.5
Votuporanga	2013	81.2	84.8	87.0	NS
Campos Novos	2012	68.7b	77.7a	77.0a	1.4
Mirestrela	2013	62.1b	71.0a	68.9a	2.6
Capão Bonito	2012–13	53.2b	69.1a	68.2a	1.6
Mean		67.7b	77.9a	76.6a	3.9

common bean at any location during both years. Across all locations and years, foliar Zn application alone and with pesticides increased grain Zn concentration significantly ($P = 0.05$; Table 7). There was, however, no clear difference in grain Zn concentrations of common bean treated with foliar Zn with or without pesticide.

Discussion

Irrespective of foliar spray of Zn alone and foliar spray of Zn + pesticide, there was a large variation in grain yields of wheat, rice and common bean among the countries, years and even among various locations of a specific country (Tables 3, 4 and 7). This variation might be ascribed, at least partially, to variations in soil and climatic factors and productivity potential of the crop varieties used (Table 1). For example, crop responses to foliar Zn fertilization varied among the locations having different soil pH, DTPA-extractable Zn and organic carbon (Table 1). When the soil DTPA-Zn values (Table 1) are compared with the grain yield responses to foliar Zn application it can be seen that there was no clear cut relation between the DTPA-Zn and plant response to foliar Zn spray. A lack of relationship between the changes in grain yield upon Zn fertilization and soil DTPA-extractable Zn is often reported for wheat, rice and other crops (Menzies et al. 2007; Tandy et al. 2011; Phattarakul et al. 2012; Zou et al. 2012; Duffner et al. 2013). The substantial increases in wheat grain yield with foliar Zn application in Pakistan (Table 3) might be, at least, due to lower soil Zn supply to the crop as a consequence of very high soil pH values (Table 1), calcareousness (data not reported), and poor Zn acquisition capacity of the wheat genotypes used. In Pakistan, crop plants, including wheat, suffer severely with Zn deficiency because of calcareous nature of its soils (Rafique et al. 2006; Ryan et al. 2013), despite the fact that apparent soil Zn balances in these irrigated soils are positive, even without using Zn fertilizer (Rafique et al. 2012). This situation is attributed to high Zn fixation in calcareous soils rather than low total Zn content in the soils (Rafique et al. 2012). In common bean experiments, foliar Zn application with or without insecticide, did not affect grain yield (Table 7), probably due to much higher DTPA-extractable soil Zn and lower pH values of the Brazilian soils compared to the soils of other countries (Table 1).

It is known that the plant response to soil Zn deficiency or Zn fertilization is greatly affected by the seasonal changes in climatic conditions (especially high light intensity and drought conditions during reproductive growth stage) and also the crop genotypes used (Cakmak et al. 1996; Graham et al. 1999; Ekiz et al. 1998; Cakmak 2000; Karim and Rahman 2015). Plants may become more sensitive to Zn deficiency when exposed to long sunny days and water-deficient soil conditions irrespective of DTPA-extractable soil Zn status, probably due to enhanced photooxidative damage in leaves with relatively low Zn concentrations and reduced Zn diffusion to root surfaces (Marschner 1993; Cakmak 2000; Bagci et al. 2007; Sajedi et al. 2010). Karim et al. (2012) reported that foliar Zn spray increased grain yield under drought conditions, even in a soil containing sufficiently high DTPA-extractable soil Zn, indicating that foliarly sprayed Zn probably contributes to better stress tolerance of plants by improving antioxidative defense mechanisms of plants against drought-induced oxidative cell damage (Cakmak 2000) or by maintaining better pollen vitality and pollination (Sharma et al. 1990; Pandey et al. 2013).

At most of the locations, the reported wheat grain yield was generally higher with combined foliar application of Zn and insecticide, especially in case of Pakistan (Table 3). This result suggests that, besides Zn deficiency, disease or insect damage in these countries is an important yield limiting factor in wheat. For example, aphids exert an adverse effect on wheat grain yield in Faisalabad area (Mushtaq et al. 2013) which is one of the experimental locations investigated in Pakistan in this study. In 24 field locations of wheat trials across six countries, grain yield increased by 7.8 % with foliar Zn spray along with pesticides (i.e., from 4.41 to 4.75 t ha^{-1}; Table 3). In case of rice, pooled mean grain yield across 12 experiments in three countries was significantly lower without Zn application compared to the mean yield with foliar application of Zn alone ($P = 0.05$), but was similar to the pooled mean yield obtained with combined application of Zn with pesticides, suggesting that under given experimental conditions of these three countries, there was no yield-reducing problem because of fungal diseases or pest attack.

At almost all field locations, there was consistently significant increase in grain Zn concentration with foliar spray of Zn in wheat and rice (Tables 5 and 6). Similar increases in grain Zn concentration upon foliar Zn spray were also reported earlier in wheat (Cakmak et al. 2010a; Zou et al. 2012; Xue et al. 2012) and in rice (Jiang et al. 2007; Phattarakul et al. 2012; Mabesa et al. 2013). In 18 of the total 24 field experiments on wheat, net increment in grain Zn with foliar Zn application was at least 10 mg kg^{-1} (Table 5). At some locations of Pakistan, Brazil, China and Zambia, net increase in wheat grain Zn was nearly 20 mg kg^{-1}, indicating a particular role of foliar Zn spray in enrichment of wheat grain with Zn. However, the extent of the increase in grain Zn concentration with foliar Zn application was much lesser in rice as compared to wheat crop (Tables 5 and 6). Differential response of rice and wheat to foliar Zn application in terms of increase in grain Zn concentration could be related to grain protein concentration. Rice grains have much lower protein than in wheat grain (Koehler and Wieser 2013). Previous studies clearly revealed that protein in cereal grains represents an important sink for Zn (Cakmak et al. 2010b; Kutman et al. 2011; Xue et al. 2012). By improving N nutritional status of plants and grain protein concentrations, grain Zn accumulation is significantly increased. Most probably, lower grain protein in rice, compared to wheat, is the possible reason for lesser increase of grain Zn in rice with foliar Zn application. In the case of common bean, there was also less increase in grain Zn with foliar Zn spray (Table 7), although common bean plants contain much more protein than wheat (Sheriff 2004). Very high Zn concentration in common bean grains even without Zn application (i.e., 67.7 mg kg^{-1}) could be an explanation for the lesser response of common bean to foliar Zn application. It would be interesting to compare common bean and wheat in terms of phloem mobility of Zn in future studies.

Of the total 24 field experiments on wheat, only in 6 experiments application of Zn together with pesticides significantly reduced effectiveness of foliar Zn application in increasing grain Zn concentration (Table 5). During both years at Faisalabad, at Muridke-II in Pakistan in 2012 and at Hebei-Quzhou location of China during 2013, application of foliar Zn in combination with pesticides reduced grain Zn concentrations over the grain Zn concentrations with foliar Zn alone (Table 5). At other locations in these countries, there was not such depression in grain Zn when Zn and pesticides were applied together. In China, at two locations different cultivars and insecticides were used which could be an explanation for the differential response in grain Zn accumulation on spraying of Zn together with insecticides. However, in Pakistan, despite the use of same insecticide on different wheat genotypes, applying Zn together with insecticide resulted in differential enrichment of wheat grain with Zn. The reason for such differential results in Pakistan could not be understood. In case of rice, at all 12 field locations of the three countries the pesticides did not hamper grain Zn accumulation when Zn fertilizer and pesticides were applied together (Table 6). When pooled rice grain Zn concentrations were considered across 12 field locations of all countries, foliar Zn application without or with pesticide resulted in 26.2 and 23.6 % increase in mean grain Zn concentration over the mean Zn concentration with no Zn application, respectively. Thus, for a vast majority of all the field locations with rice and field experiments, it can be concluded that spraying Zn along with fungicides or insecticides had no clear antagonistic effect on grain Zn accumulation. The same interpretation is true for five field experiments with common bean in Brazil (Table 7). Similar observation was also made very recently in the field experiments in China and India where the conducted trials focused more on cost effectiveness of spraying Zn fertilizer together with pesticides for increasing grain Zn in rice and wheat (Ram et al. 2015 and Wang et al. 2015). The study conducted in China on wheat showed that applying Zn together with insecticides to foliar minimized the costs associated with labor use up to 3-fold (Wang et al. 2015). Wang et al. (2015) also showed that adding Zn into insecticide spray solution had no adverse effect on the toxic impact of insecticides on aphids.

The magnitude of increase in grain Zn concentration with foliar Zn application depends largely on the growth stage of crop plants at which foliar Zn application is realized as was shown earlier in rice and wheat (Cakmak et al. 2010a; Phattarakul et al. 2012; Mabesa et al. 2013; Boonchuay et al. 2013; Stomph et al. 2014). Marked increases in grain Zn concentration occur usually when Zn is sprayed to plants before anthesis (i.e., just prior to heading) and/or right after anthesis (i.e., early milk stage). As fungicides and insecticides are also generally applied to wheat and rice around anthesis stage (Groth and Bond 2006; Wu et al. 2013; D'Angelo et al. 2014), foliar application of Zn in combination with pesticides would be advantageous for the growers.

Conclusion

Results of the present study with 31 experimental site-years in seven countries clearly show, with the exception of a few sites, that mixing of $ZnSO_4$ is compatible with the tested 14 different fungicides and insecticides and, foliar Zn can be safely applied along with these pesticides. As the governments are not expected to ensure premium price to the farmers for high-Zn grain of wheat, rice and common bean, compatibly of fertilizer Zn and pesticides may encourage the farmers to add Zn in the pesticide spray solutions, as Zn fertilization may also contribute to better crop productivity. Thus, application of Zn-containing fertilizers with pesticides appears to be a useful and cost-effective solution to address the Zn deficiency problem in human populations.

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