HarvestPlus-Zinc Fertilizer Project

"HarvestZinc"

Use of Zinc-Containing Fertilizers for Enriching Staple Grains with Zinc and Improving Yield

-Country Reports-

(July 2011-January 2014)

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Supporting Partners

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International Zinc Association, Belgium
OMEX Agrifluids, England
International Fertilizer Industry Association, France
International Plant Nutrition Institute, USA
Bayer CropScience, Germany
ADOB, Poland
Valagro, Italy
FBSciences, USA
ATP Nutrition, Canada

Collaborating Countries

BRAZIL: Agronomy Institute, Campinas
CHINA: China Agricultural University
INDIA: Punjab Agricultural University
PAKISTAN: Pakistan Atomic Energy Commission
THAILAND: Chiang Mai University
TURKEY: Ministry of Agriculture
ZAMBİA : Golden Valley Agricultural Research Trust(GART) and University of Zambia.

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1. EXTENDED ABSTRACT

Seven countries are participating in this 2nd phase of the project. Wheat, rice, sorghum and common beans are the crops under study. Turkey, China, India, Pakistan and Zambia are conducting wheat experiments, while rice experiments are being carried out in China, Thailand and India. Brazil is working with common beans and Zambia is setting sorghum experiments in addition to wheat. Two different experiments, first of which to compare soil application of Zn-containing fertilizers and the second for comparison of Zn-containing foliar solutions, are being conducted in each country on each crop, except for Turkey where a 3rd experiment is also being carried out. In this 3rd experiment, different timings of foliar Zn treatments, alone or in combination with urea or pesticides, are being compared for effectiveness on improving grain Zn concentrations. In the second year (2012-2013), use of pH-reducing methods by inclusion of HCl or citric acid at foliar $ZnSO_4$ applications was added to the experimental treatments, with the expectation to improve translocation of applied Zn into wheat grains.

In all experiments, the treatments are compared with the local controls (LC).The LC treatments are determined by the collaborating countries themselves based on their standard recommendations of basic fertilizers (N and P in all, K in some) for a given ecological region.

Project activities have been conducted under 3 Tasks:
TASK-1: SOIL APPLICATION OF ZINC-CONTAINING FERTILIZERS AND USE OF SEEDS DIFFERING IN ZINC CONCENTRATIONS

TASK-II: APPLICATION OF FOLIAR ZINC FERTILIZERS

TASK-III: DISSEMINATION AND IMPLEMENTATION OF THE PROJECT RESULTS

Below the results are summarized for those three Tasks

TASK-I: Soil Applied Fertilizers
Seed Zinc: Effect on stand establishment

The effect of Zn treatments on stand establishment was evaluated in all 5 countries working on wheat. Number of emerged plants was counted, and the seedling heights during early growing period were measured In Turkey, China, India and Pakistan; while visually-assessed vigor scores were used instead of seedling heights in Zambia. Early growth vigor of wheat was significantly affected in China and Pakistan. It was not significantly affected in the other countries. Although not significantly, Mosaic-MESZ treatments tended to improve early plant vigor in Zambia. In Zambia, priming had an adverse effect on emergence counts, at one of the two experimental sites in both years. It was also the case at one site, in one year in China. Considering that seed priming also tended to reduce stand establishment of rice in Thailand and common beans in Brazil, the priming-solution concentration used was reduced in 2nd year in those countries.

Both number of emerged seedlings and seedling heights were affected in China and Pakistan, the effect being much more pronounced in the latter. The main difference between the results from these 2 countries was the fact that both methods of using high-Zn seeds improved stand establishment in Pakistan, while priming seeds in Zn solution had an adverse effect on stand establishment in China, except for 1 experiment in 2012-2013, where priming also improved emergence.

The effect of using high-Zn seed on emergence counts are given in **TABLE-1** for China and Pakistan.

TABLE 1. Effect of Zn-enriched seeds on improvement of wheat seedling emergence in China and Pakistan.

TREATMENT	NUMBER OF EMERGED PLANTS m^{-2}						
	CHINA				*PAKISTAN		
	SITE 1		SITE 2		2011-2012	2012-2013	DEMO
	2011	2012	2011	2012			
CONTROL (LC)	295	360	436	275	197	192	168
LC + high seed- Zn through Foliar Zn Spray	350	425	477	351	332	368	215
IMPROVEMENT %	**18.6**	**18.1**	**n.s.**	**27.6**	**68.5**	**91.7**	**28.0**

*The values given for Pakistan are means of 3 experiments in 2011-12, 2 experiments in 2012-13 and 5 demonstration/trials in 2012-13
**Improvement due to foliarly increased seed Zn as compared to LC.

As can be seen in **TABLE-1**, using high-Zn seed improved number of emerged seedlings in 3 of the 4 experiments in China, and all tests in Pakistan. The particularly striking effect was found in Pakistan. In 2013-2014 trials of Pakistan, a better emergence has also been observed in plots where Zn-enriched seeds were used.

In the experiments conducted in India, there were also differences in emergence counts due to seed Zn-enrichment but these differences were not statistically significant. However, on the average of all 6 field experiments in 2 years, seed Zn enrichment with foliar application improved emergence rates by about 9 % over control treatment. In Zambia, there were no differences in emergence counts. In case of rice, as mentioned below, there were significant increases in grain yield by using Zn-enriched seeds in China, India and Thailand (in the second year).

Soil Applied Fertilizers and Grain Yield: Wheat
Soil application of Zn-containing fertilizers did not result in significant yield improvements in Turkey, China, and Zambia. There were, however, distinct (but non-significant) increases in grain yield of wheat in some locations of those countries. Significant changes in yield due to treatments were found in India and Pakistan. In China, the fact that rice yields responded to soil application of Zn shows the relatively higher Zn efficiency of wheat as compared to rice. In contrast to the ineffectiveness of the soil applied Zn fertilizers in experiments in China, demonstration plots on farmers' fields resulted in mostly significant increases in grain yields due to seed enrichment, ranging from 0.3% to 51 %, with a yield improvement of 18.3% as average of 5 fields.

Wheat experiments were conducted at 3 sites in each of India and Pakistan in 2011-2012; at 3 sites in India and 2 sites in Pakistan in 2012-2013. In all of these 11 experiments, soil application of Zn improved wheat grain yields as compared to control treatments. However, the superiority of the different soil applied Zn treatments varied from site to site, presumably due to variations in climatic and soil conditions.

Since the variables in the experiment are too many to make clearly visible comparisons, groups of Zn-compounds are compared with LC (Local Control) and LC + $ZnSO_4$ in **TABLE- 2** and **TABLE-3.**

TABLE 2. Effect of MOSAIC-MESZ fertilizers on grain yield of wheat in India and Pakistan as compared to the LC and LC+$ZnSO_4$ treatments. Mosaic-II represents the double rate of the Mosaic-I.

TREATMENT	*GRAIN YIELD (t ha^{-1})			
	INDIA		PAKISTAN	
	2011-2012	2012-2013	2011-2012	2012-2013
LC	5.37	5.25	4.38	3.91
LC + $ZnSO_4$	5.95	5.59	5.20	4.39
% IMPROVEMENT**	**10.8**	**6.5**	**18.7**	**12.3**
LC + MOSAIC I	6.01	5.60	4.87	4.54
% IMPROVEMENT **	**11.9**	**6.6**	**11.2**	**16.1**
LC + MOSAIC II	6.05	5.58	4.96	4.21
% IMPROVEMENT**	**12.7**	**6.5**	**13.2**	**7.7**

* Values are means of 3 experiments each, except for Pakistan 2012-2013 values, which are means of 2 experiments.
**Improvement over LC.

As can be seen in **TABLE 2,** effect of both MOSAIC-MESZ fertilizers on grain yields were generally similar to that of $ZnSO_4$ in India. But, there were differences from year to year in Pakistan. In 2011-2012, $ZnSO_4$ had a greater effect, whereas in 2012-2013, MOSAIC-I was superior to $ZnSO_4$. Two other fertilizers, Kali KornKali and ADOB-HBEDZn are compared with LC and LC + $ZnSO_4$ in **TABLE 3.** When averaged over experiments in each year, yield increases by Kali KornKali and ADOB-HBEDZn were similar to those by $ZnSO_4$, except in 2012-2013 experiments in Pakistan, where both Kali KornKali and ADOB-HBEDZn resulted in higher rates of yield increase than $ZnSO_4$ **(TABLE 3).** Therefore, these 2 fertilizers seem to be promising alternatives to $ZnSO_4$, according to the first 2 year results, at least in these countries.

TABLE 3. Effect of Kali KornKali and ADOB HBEDZn fertilizers on grain yield of wheat in India and Pakistan as compared to LC and LC + $ZnSO_4$ treatments.

TREATMENT	*GRAIN YIELD (t ha^{-1})			
	INDIA		PAKISTAN	
	2011-2012	2012-2013	2011-2012	2012-2013
LC	5.37	5.25	4.38	3.91
LC + $ZnSO_4$	5.95	5.59	5.20	4.39
% IMPROVEMENT**	**10.8**	**6.5**	**18.7**	**12.3**
LC + Kali KornKali	6.07	5.60	5.26	4.57
% IMPROVEMENT **	**11.5**	**6.7**	**20.1**	**16.9**
LC + ADOB HBEDZn	5.89	5.56	5.21	4.68
% IMPROVEMENT**	**9.7**	**5.9**	**18.9**	**19.7**

* Values are means of 3 experiments each, except for Pakistan 2012-2013 values, which are means of 2 experiments.
**Improvement over LC.

Similar to the better stand establishment of wheat, seed Zn-enrichment by foliar Zn supply in China resulted in positive (and significant in 2012) increases in grain yield, especially in the 2nd year. The increases in wheat grain yield by using Zn-enriched seeds in 2012 were 13 % and 9 % at two locations.

Soil Applied Fertilizers and Grain Yield: Rice

Rice trials have been already completed in China, India and Thailand. Grain yield of rice was affected by soil Zn applications in all those 3 countries. In the first year (2011) experiments in China, Zn-enriched seed and split urea were the best treatments resulting in 8 and 9 % yield increase, respectively, at one site, while Mosaic fertilizers resulted in the highest yield increase (11 %) over the control at the other site.

In the 2012-experiments, split urea was the best at the same location as in 2011, but results differed from the first year at the other location, where ADOB HBEDZn and use Zn-enriched seed were the best treatments. ADOB HBEDZn was the superior treatment in India, resulting in 10 % yield increase at one site and 10.9 % at the other in 2011. In case of 2012, there was 9.7 % yield increase at one site and 7.5 % at the other. However, the differences among most treatments (except for the control) were not statistically significant.

The greatest yield response of rice to soil Zn applications was obtained in Thailand in 2011-2012. Kali KornKali and ADOB HBEDZn were the most effective treatments. They resulted in 41.1 and 37.3 % yield increase, respectively, at one site; and both resulted in 18.2 % yield increase at the other. In 2012-2013, however, there was no treatment effect on grain yield at 1 site, while ADOB HBEDZn resulted in the highest yield improvement (19.5 %) at the other.

Soil Applied Fertilizers and Grain Yield: Sorghum and Common Bean

The sorghum experiments are being conducted in Zambia and the common bean experiments in Brazil only. Sorghum grain yields were not improved by soil application of Zn-containing fertilizers in 2011-2012 growing season. However, in the second year of the experiment, Mosaic MESZ II significantly improved sorghum yield, resulting in 55% yield increase, at one site. At the other site, Mosaic MESZ inclined to be one of the two best treatments, after ADOB-HBED Zn, but the yield differences at this site were not statistically significant due to high CV and LSD values and generally low yield levels. It was also the case in the common bean experiments in Brazil, no significant improvement of seed yield was obtained by soil or foliar application of Zn fertilizers. An interesting observation was reported in relation to Mosaic fertilizers. In one location MESZ fertilizer was the best in terms of seed yield, while in other (second) location it resulted in the lowest yield. Results indicated that S deficiency was an important reason for the reduced performance of MESZ fertilizer in the second location, since differential S application rates were applied by the partners in Brazil (in contrast to the protocol). Despite existence of S in MESZ, the plots of MESZ treatments were treated with less amount of S fertilizer than other treatments, which is also associated with less amount of S in leaf tissue. In the currently on-going experiments this mistake has been corrected. Hence, the yield differences in the second year were not significant.

Soil Applied Fertilizers and Grain Zinc: Wheat

The grain Zn results of wheat were evaluated by using data from Turkey, China, India, Pakistan for 2011-2012 and 2012-2013, and from Zambia for 2011-2012 (in total 20 field experiments). Of these 20 experiments, there were significant improvements due to soil treatments over control in 15 experiments. In 1 of the 4 experiments in China, 2 of the 6 experiments in India and the 2 of the 5 experiments in Pakistan, the treatments did not improve grain Zn concentrations significantly.

The most outstanding result was the distinct superiority of the Treatment 5 (Mosaic-I with foliar Zn spray) to the control treatment and, in most cases, to other treatments. While the Treatment 5 (including also foliar Zn spray) improved grain Zn of wheat in 15 experiments in 5 countries, ADOB-HBED-Zn was the second most effective chemical, resulting in significant increases in 9 experiments; followed by Treatment 2 (LC + soil-applied $ZnSO_4$) being significantly effective in 6 experiments. Other treatments were rarely effective. The effects of Treatment 5 as compared to soil application of ZnSO4, MOSAIC I and local control is given in **TABLE 4.** Since the number of experiments is too big to fit in a table, country averages are given. Data given for Turkey and China are averages of 4 experiments each, while the data for India and Pakistan are averages of 6 and 5 experiments, respectively. Zambia data belong to a single experiment.

TABLE 4. Effect of foliar $ZnSO_4$ supplement on grain Zn concentration of wheat as compared to LC and LC + $ZnSO_4$ and LC + MOSAIC I treatments.

TREATMENT	**GRAIN Zn CONCENTRATION (mg kg^{-1})**				
	TURKEY	**CHINA**	**INDIA**	**PAKISTAN**	**ZAMBIA**
LC	27.2	27.6	28.7	23.4	26.8
LC + $ZnSO_4$	28.9	30.7	30.5	28.4	34.0
% IMPROVEMENT *	**n.s.**	**n.s.**	**n.s.**	**21.4**	**26.9**
LC + MOSAIC I	28.9	25.9	30.5	26.7	25.5
% IMPROVEMENT *	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**
LC +MOSAIC I +FOLIAR-Zn	32.9	34.1	37.7	28.9	38.5
% IMPROVEMENT *	**21.0**	**23.6**	**31.4**	**23.5**	**43.7**

*Improvement over LC.

Although a combined analysis was not performed for countries, the effects were denoted as n.s. if they were not significant in more than only one experiment in a given country, and the differences were too small. As can be seen in **TABLE 4**, soil application of $ZnSO_4$ significantly increased grain Zn concentration of wheat only in Pakistan, where it was effective in all 3 experiments in the first year, and Zambia. Addition of a single foliar $ZnSO_4$ application at late stages of growth made a great contribution to grain Zn concentration in 15 experiments out of total 20 in 5 countries in two years. This confirms the previous results, including the ones from the first phase of this project, indicating superiority of foliar applications to soil applications as far as grain Zn concentrations are concerned.

Another outstanding chemical, in this aspect, was ADOB-HBEDZn. It significantly improved grain Zn concentration of wheat in 9 of the 20 experiments evaluated. The related results are shown in **TABLE 5.**

Other soil-applied fertilizers were not so effective in increasing grain Zn except for a few cases. Higher efficiency of ADOB Zn-HBED might be relied to the existence of Zn in chelated form in this fertilizer.

TABLE 5. Effect of soil ADOB-HBEDZn application on grain Zn concentration of wheat as compared to LC treatment (Only the experiments where it was effective are included).

COUNTRY	LOCATION	GRAIN Zn CONCENTRATION (mg kg^{-1})		
		LC	ADOB-HBEDZn	IMPROVEMENT* (%)
**TURKEY	SITE 1	32.6	36.5	12.0
	SITE 2	23.4	32.2	37.6
**CHINA	SITE 1	32.5	37.3	14.8
	SITE 2	22.0	30.5	38.6
**PAKISTAN	SITE 1	21.5	30.6	42.3
	SITE 2	13.5	25.3	46.6
***INDIA	SITE 1	24.6	28.6	16.3
	SITE 2	26.7	31.0	16.1
ZAMBIA	SITE 1	26.8	34.3	28.0

*Improvement over LC.
**Turkey, China, and Pakistan values belong to 2011-2012. In 2012-2013, the effect was not significant in these countries.
***Values given for India belong to 2012-2013. The effect was not significant in 2011-2012.

Soil Applied Fertilizers and Grain Zinc: Rice

When Zn was not applied, grain Zn concentrations of rice were lower than that of wheat, confirming previous results. In 1 of the total 14 experiments in 2 years (an experiment conducted in India) soil applications did not significantly affect grain Zn concentration. In the other 13 experiments, Treatment 5 (MESZ+Foliar Zn), the only treatment involving foliar Zn application, resulted in the highest grain Zn concentration, except for 1 site where soil Zn application was better. This was also seen in the wheat experiments, confirming once again the superiority of foliar applications in improving grain Zn. Corresponding results are presented shown in **TABLE 6.** Among the other soil applied fertilizers, KornKali significantly improved grain Zn in 3 of the 10 experiments conducted in two years, and ADOBHBED-Zn was effective in that aspect in two of them.

TABLE 6. Effect of foliar $ZnSO_4$ supplement on grain Zn concentration of rice as compared to LC and LC + $ZnSO_4$ and LC + MOSAIC I treatments (Each value is a mean of 2 experiments).

TREATMENT	GRAIN Zn CONCENTRATION (mg kg^{-1})						
	CHINA		INDIA			THAILAND	
	2011	2012	2011	2012	2013	2011	2012
LC	19.1	19.9	20.0	20.3	18.8	17.1	16.0
LC + $ZnSO_4$	24.4	20.7	20.3	20.5	19.2	19.8	18.0
% IMPROVEMENT*	**27.7**	**4.0**	**1.5**	**1.0**	**2.1**	**15.8**	**12.5**
LC + MOSAIC I	21.4	19.9	20.3	19.7	19.6	17.7	17.0
% IMPROVEMENT*	**12.0**	**-**	**1.5**	**-**	**4.2**	**3.4**	**6.3**
LC+MOSAIC I + FOLIAR Zn	23.9	24.2	26.9	23.7	23.3	22.6	23.7
% IMPROVEMENT*	**16.8**	**21.6**	**34.5**	**16.7**	**23.9**	**32.2**	**48.1**

*Improvement over LC.

Soil Applied Fertilizers and Grain Zinc: Sorghum and Common Beans
Grain Zn analyses of sorghum revealed some significant effects of applications of Zn containing fertilizers on grain Zn concentrations at 3 of the 4 experiments conducted in two years. The greatest improvement was obtained by the Treatment 5 (the only treatment involving foliar supplement of Zn in the EXP 1), increasing grain Zn concentration from 19.7 mg kg^{-1} in control plots to 31.0 mg kg^{-1} (57.4 % improvement) as an average of 4 experiments (**Table 7**).

TABLE 7. Effect of foliar $ZnSO_4$ supplement on grain Zn concentration of sorghum in Zambia as compared to LC and LC + $ZnSO_4$ and LC + MOSAIC I treatments.

TREATMENT	GRAIN Zn CONCENTRATION (mg kg^{-1})			
	2011-2012		2012-2013	
	SITE 1	SITE 2	SITE 1	SITE 2
LC	14.7	25.9	12.3	25.8
LC + $ZnSO_4$	15.9	24.6	11.9	26.3
% IMPROVEMENT*	**n.s.**	**n.s.**	**n.s.**	**n.s.**
LC + MOSAIC I	17.7	25.5	12.5	23.5
% IMPROVEMENT*	**n.s.**	**n.s.**	**n.s.**	**n.s.**
LC+MOSAIC I + FOLIAR Zn	17.5	42.9	28.5	35.0
% IMPROVEMENT*	**n.s.**	**65.6**	**131.7**	**35.7**

*Improvement over LC.

In one of the 4 experiments with common beans in Brazil, the Treatment 5, resulted in the highest seed Zn concentration. It increased seed Zn concentration from 29 (LC) to 37 mg kg^{-1}, with a 27.6 % improvement over control. In the same experiment, ADOBHBED-Zn was the other treatment resulting in statistically significant improvement, although the increase was small. In other experiments of soil applications, none of the treatments improved seed Zn significantly, except for one where $ZnSO_4$ increased grain Zn narrowly but significantly.

Soil Applied Fertilizers: *Outcomes / Significance*

- Foliar applications of Zn proved once more to be superior to soil applications in improving grain Zn concentrations. On the other hand, they are less effective in increasing grain yields. This makes it compulsory to search for some means to improve adoptability of agronomic biofortification of cereal grains with Zn. It is very obvious that a combination of soil and foliar applications is needed for ensuring better grain yield and higher grain Zn

- Seed enrichment through foliar application on the previous crop resulted in better emergence rates and stand establishment in most of the locations, especially in China and Pakistan in both years and Thailand in 2012. This positive effect of high seed Zn was generally reflected well in grain yields.

- Priming seeds with 5 mM $ZnSO_4$ for 1 hour resulted in adverse effects in some cases. In the second year trials 1 mM ZnSO4 has been used with less or no adverse effects. It is very obvious that seed Zn-enrichment through foliar application is superior to the seed Zn priming in terms of better stand establishment and grain yield performance

- Twice application of foliar $ZnSO_4$ generally gave better results than single application. Single foliar Zn application was, however still highly effective in resulting in significant increases in grain Zn (see Task-2).

Publications

See Task 3 regarding the publications and visibility activities of the project

Lessons Learned

The reports from some of the partners indicated that 5 mM ZnSO4 used for seed priming resulted in adverse effects on stand establishment and seedling vigor, especially in Brazil for common bean, in Zambia in sorghum and in one location in China. Based on these observations, the Zn concentration used for seed priming has been reduced to 1 mM from 5 mM.

TASK-II: Foliar Applied Fertilizers

Foliar Applied Fertilizers and Grain Yield: Wheat

Of the 5 countries where wheat experiments are conducted, foliar application of Zn-containing fertilizers did not significantly affect grain yields in Turkey, China, India and Zambia. However, in case of the most foliar treatments there were non-significant increases in grain yield

Significant increases in grain yield by foliar Zn application were found in Pakistan. Although results varied among sites in Pakistan, foliar spray of $ZnSO_4$ at early milk stage resulted in the highest yield increase (32.2%) from 3.78 to 5.00 t ha^{-1}, on the average of the 3 sites in 2011-2012; whereas Kali-EPSO-Zn and Antracol-Zn were the best treatments in 2012-2013, improving grain yield from 4.26 to 6.03 (41.5% improvement) and 5.94 (39.4% improvement) t ha^{-1}, respectively.

Foliar Applied Fertilizers and Grain Yield: Rice

Foliar application of Zn-containing fertilizers significantly affected rice yields only at some experiments. There was no significant effect in experiments in India in either year. In China, there was no effect at 1 location in either year of the experiment but the yields were affected at the other location in both years. In the first year, twice application of $ZnSO_4$ resulted in the highest yield improvement, increasing rice yield from 6.73 (Local Control) to 7.59 t ha^{-1}, with a

12.8 % improvement. In the second next year, KaliEpso resulted in the highest yield with 10.5 t ha^{-1}. It meant a 7.8 % improvement over the LC plots which gave 9.74 t ha^{-1} grain yields. In Thailand, there were significant effects in all 4 experiments in 2 years. OMEX Type III was one of the best treatments in 3 of the 4 experiments. Valagro and Antracol were the best treatments at the 2nd year experiments.

Foliar Applied Fertilizers and Grain Yield: Sorghum and Common Bean

The sorghum experiments are conducted in Zambia and the common bean experiments in Brazil only. Sorghum grain yields were not improved by foliar application of Zn solutions (EXP 2). It was also the case in the common bean experiments in Brazil, no significant improvement of seed yield was obtained by foliar application of Zn fertilizers. An interesting observation was reported in relation to the Mosaic fertilizers. In one location, MESZ fertilizer was the best in terms of grain yield, while in other (second) location it resulted in the lowest yield. Results indicated that S deficiency was an important reason for poor performance of MESZ fertilizer in the second location, since differential S application rates were applied by the partners in Brazil (in contrast to the protocol). Despite existence of S in MESZ, the plots of MESZ treatments were treated with less amount of S fertilizer than other treatments, which is also associated with less amount of S in leaf tissue. Due to existence of S deficiency, the experiment is being now repeated.

Foliar Applied Fertilizers and Grain Zinc: Wheat

Since this experiment is evaluated in several aspects, they will be summarized separately.

Effect of timing of foliar $ZnSO_4$ application

Timing of single application gave different results in different experiments. One steady result in this aspect was the superiority of twice application to single application in most cases. This comparison is shown in **TABLE 8** for Turkey and China, and in **TABLE 9** for India, Pakistan and Zambia.

TABLE 8: Comparison between effects of foliar $ZnSO_4$ application twice and single application on grain Zn concentration of wheat in Turkey and China.

TREATMENT	**GRAIN Zn CONCENTRATION (mg kg^{-1})			
	TURKEY		CHINA	
	YEAR 1	YEAR 2	YEAR 1	YEAR 2
1. Local control (LC)	31.5	27.4	26.9	25.5
2. LC+ Foliar $ZnSO_4$ (booting)	35.6	32.3	35.2	33.9
3. LC+ Foliar $ZnSO_4$ (early milk)	33.0	34.3	36.1	37.2
4. LC+ Foliar $ZnSO_4$ (booting + early milk)	39.5	38.3	44.2	40.8
***IMPROVEMENT (%)**	**25.4**	**39.8**	**64.3**	**60.0**

*Improvement over LC by twice application.
**Each value is average of two sites.

TABLE 9: Comparison between effects of foliar $ZnSO_4$ application twice and single application on grain Zn concentration of wheat in India, Pakistan and Zambia.

TREATMENT	**GRAIN Zn CONCENTRATION (mg kg^{-1})				
	INDIA		PAKISTAN		ZAMBIA
	YEAR 1	YEAR 2	YEAR 1	YEAR 2	2011-2012
1. Local control (LC)	32.1	26.4	22.1	31.1	31.2
2. LC+ Foliar $ZnSO_4$ (booting)	39.3	34.3	27.4	41.7	35.1
3. LC+ Foliar $ZnSO_4$ (early milk)	38.7	36.2	28.9	40.6	34.9
4. LC+ Foliar $ZnSO_4$ (booting + early milk)	40.4	43.3	34.0	38.9	40.9
***IMPROVEMENT (%)**	**20.6**	**64.0**	**62.9**	**25.1**	**31.1**

*Improvement over LC by twice application.
**Values for Pakistan are means of 3 experiments in 2011-2012, and 2 experiments in 2012-20
13. Values for India are means of 3 experiments each year. Zambia values are means of two sites.

Effect of other Zn-compounds

Effect of the other Zn-compounds on wheat grain Zn is shown in **TABLE 10** for Turkey and China, and in **TABLE 11** for India, Pakistan and Zambia. It is important to highlight that all Zn compounds have been sprayed to foliar only once.

TABLE 10: Effect of foliar-applied commercial Zn-containing fertilizers on grain Zn concentration of wheat in Turkey and China.

TREATMENT	*GRAIN Zn CONCENTRATION (mg kg^{-1})			
	TURKEY		CHINA	
	YEAR 1	YEAR 2	YEAR 1	YEAR 2
1. Local control (LC)	31.5	27.4	26.9	25.5
5 LC+ OMEX II	34.3	38.5	38.5	38.3
6. LC+ OMEX III	30.5	34.1	38.4	34.4
7. LC+Kali-EPSO	33.3	31.9	38.2	34.3
8. LC+ ADOB ZnIDHA	33.8	35.9	39.3	33.3
9. LC+ Valagro Brexil	35.0	35.8	44.7	33.3
10. LC+ Antracol	32.6	30.4	29.9	31.3
12. LC+ FBScience	36.5	34.2	39.3	36.4

*Each value is average of two sites.

The effect of different fertilizers, as compared to the local control can be summarized as follows: Depending on country and location, most of the foliar Zn-fertilizers/compounds appeared to be very promising in improving wheat grain Zn concentration. OMEX II improved grain Zn of wheat significantly at 18 of the total 20 experiments conducted in 5 countries in two years. ADOB-ZnIDHA and Valagro Brexil followed it in efficiency, improving grain Zn of wheat significantly in 15 experiments. FBScience, OMEX III, Kali-EPSO, and Anthracol improved grain Zn in 13, 12, 11, and 8 experiments, respectively. The most important observation here is that, in 19 of the total 20 experiments, at least one of these chemicals was effective in improving grain Zn of wheat.

TABLE 11: Effect of foliar-applied commercial Zn-containing fertilizers on grain Zn concentration of wheat in India, Pakistan and Zambia.

TREATMENT	GRAIN Zn CONCENTRATION (mg kg^{-1})				
	INDIA		PAKISTAN		ZAMBIA
	YEAR 1	YEAR 2	YEAR 1	YEAR 2	2011-2012
1. Local control (LC)	32.1	26.4	22.1	31.1	31.2
5 LC+ OMEX II	43.6	37.9	27.7	44.8	57.3
6. LC+ OMEX III	36.9	35.4	24.7	32.9	48.0
7. LC+Kali-EPSO	39.2	36.1	26.4	33.9	45.3
8. LC+ ADOB ZnIDHA	39.5	35.0	27.6	32.8	41.9
9. LC+ Valagro Brexil	40.9	36.3	27.1	31.8	49.4
10. LC+ Antracol	37.9	34.4	23.5	34.7	36.6
12. LC+ FBSci-ZicRON	37.9	34.4	26.4	32.7	39.4

*Values for Pakistan are means of 3 experiments in 2011-2012, and 2 experiments in 2012-2013. Values for India are means of 3 experiments each year. Zambia values are means of two sites.

In summary, when data averaged over experiments are studied (**TABLES 10 and 11**), it is seen that in all countries and almost all years, OMEX II was the chemical resulting in the highest grain Zn concentrations. Only exceptions to this were the first year averages of Turkey and China, where its leading position was replaced by Valagro Brexil. In one of the two years in Turkey and China, in both years in India and Pakistan, and in Zambia, OMEX II resulted in the highest grain Zn. HP 2011 and HP 2012 also increased grain Zn concentration of wheat significantly at both sites in Turkey (see **ADDENDUM:** Country Report). LC + HP 2011 (Treatment 13) and LC + HP 2012 (Treatment 14) both resulted in statistically significant improvements in grain Zn concentrations at both locations in both years. In fact, HP 2011 was the best treatment among all at Eskisehir location, resulting in the highest grain Zn (43.0 mg kg^{-1}) in the first year, and the second highest in the second year (43.5 mg kg^{-1}), after twice application of $ZnSO_4$ (43.8 mg kg^{-1}). At Konya location, HP 2012 was the second most effective treatment in the first year, after twice application of $ZnSO_4$, and the best in the second year, resulting in grain Zn concentrations of 33.6 and 40.5 mg kg^{-1}, respectively.

It was also important to highlight that foliar Zn fertilizers can be sprayed to foliar together with fungicides/insecticides tested in different countries. There was no adverse effect of those pesticides on leaf Zn penetration and seed/grain Zn deposition in wheat. Similar conclusion was also made for rice and other crops tested.

In the 3rd experiment conducted only in Turkey, 0.5 % $ZnSO_4$ application at post-flowering period resulted in higher grain Zn than 0.3 % $ZnSO_4$, particularly when urea was not added. When urea added, though, the difference due to concentration was much smaller. This means that the contribution of urea to increasing the effect of applied $ZnSO_4$ on grain Zn was greater when 0.3 % $ZnSO_4$ solution was used. Post-flowering application of 0.5 % $ZnSO_4$ with addition of urea resulted in the highest grain Zn concentration (42.5 mg kg^{-1}) among all 18 treatments, followed by post-flowering application of 0.3 % $ZnSO_4$ with addition of urea (41.1 mg kg^{-1}). This shows the significant contribution of urea to the effectiveness of applied Zn, particularly when the solution concentration is lower, presumably through facilitating the penetration of foliar-applied Zn.

Among the Zn fertilizer forms of Zn (ZnO, nanoparticle ZnO, ZnSO4, $ZnCl_2$ and ZnEDTA), all with 0.3 % concentration, $ZnCl_2$ resulted in the highest grain Zn (40.7 mg kg^{-1}), followed by $ZnSO_4$, ZnEDTA and ZnO. The ZnO forms were not effective.

Very new results obtained from greenhouse and field indicate that reducing pH of solution pH and adding some special adjuvant/tenside in the solution of the foliar Zn fertilizers substantially increased grain Zn concentrations in wheat. Several methods to reduce solution pH have been added to the 3rd experiment conducted in Turkey in the second year. Reducing the solution pH to 6.0 or 4.5 was compared with the control, which was the use of tap water with a pH of 8.3. Treatments were performed twice. pH-reducing treatments were effective in increasing grain Zn as compared to high-pH tap water at both locations, while only lowering pH to 6.0 improved grain Zn, further lowering the pH to 4.5 not being effective. At Eskisehir, the highest grain Zn values were obtained by lowering the solution pH to 6.0 with citric acid, improving grain Zn from 41.0 mg kg^{-1} with tap water to 51.5 mg kg^{-1}, with a 25.6% improvement. Addition of urea to citric acid did not make a significant contribution. HCl addition down to pH 6.0 was the best treatment at Konya improving grain Zn concentration of wheat from 32.3 to 40.5 mg kg^{-1}, with 25.4% increase.

Foliar Applied Fertilizers and Grain Zinc: Rice

Effect of timing of foliar $ZnSO_4$ application

Like in wheat, twice application of $ZnSO_4$ resulted in higher grain Zn than single applications at 11 of the 12 experiments evaluated, although the differences were not always significant (**TABLE 12**). Effect of the other Zn-compounds on grain Zn concentration of rice is presented in **TABLE 13.**

TABLE 12: Comparison between effects of foliar $ZnSO_4$ application twice and single application on grain Zn concentration of rice in China, Thailand and India (each value is a mean of 2 experiments).

TREATMENT	GRAIN Zn CONCENTRATION (mg kg^{-1})						
	CHINA		THAILAND		INDIA		
	YEAR 1	YEAR 2	YEAR 1	YEAR 2	YEAR 1	YEAR 2	YEAR 3
1. Local control (LC)	19.4	21.5	17.6	19.3	19.3	18.5	18.9
2. LC+ Foliar $ZnSO_4$ (booting)	20.5	24.2	21.8	21.6	21.2	20.1	20.5
3. LC+ Foliar $ZnSO_4$ (early milk)	22.0	24.0	22.6	23.8	23.9	22.4	22.2
4. LC+ Foliar $ZnSO_4$ (booting + early milk)	23.8	26.8	26.4	24.4	24.3	22.7	22.5

As can be seen in **TABLE 13,** nearly all Zn compounds increased grain Zn of rice in most of the experiments. The superior compound changed from country to country. Since the most effective chemicals changed with experiments, a summarized result is given below:

Kali-Epso improved grain Zn concentration of rice significantly in 11 of the 14 experiments (All 6 experiments in India, 3 in China, and 2 in Thailand). OMEX II and Valagro Brexil improved grain Zn significantly in 10 of the 14 experiments (All 4 in India, 2 in each of China and Thailand). ADOB-ZnIDHA and OMEX III improved grain Zn significantly in 9 of the 14 experiments (5 out of 6 in India, 2 in each of China and Thailand). FBScience improved grain Zn significantly in 8 experiments (3 in each of India and China, 2 in Thailand). Antracol improved grain Zn of rice in 7 experiments (5 in India, 1 in each of China and Thailand).

TABLE 13: Effect of foliar-applied commercial Zn-containing fertilizers on grain Zn concentration of rice in China, Thailand and India (each value is a mean of 2 experiments).

TREATMENT	GRAIN Zn CONCENTRATION (mg kg^{-1})						
	CHINA		THAILAND		INDIA		
	YEAR 1	YEAR 2	YEAR 1	YEAR 2	YEAR 1	YEAR 2	YEAR 3
1. Local control (LC)	19.4	21.5	17.6	19.3	19.3	18.5	18.9
5 LC+ OMEX II	22.6	26.2	20.6	22.9	25.5	21.4	22.0
6. LC+ OMEX III	22.5	24.4	21.3	21.7	24.5	22.3	21.7
7. LC+Kali-EPSO	25.0	26.4	22.2	23.3	23.8	22.3	21.0
8. LC+ADOB-ZnIDHA	23.0	21.9	22.2	22.2	24.0	21.1	20.9
9. LC+ Valagro Brexil	23.8	23.9	22.1	22.7	24.7	21.9	20.8
10. LC+ Antracol	20.8	23.4	19.4	19.8	22.8	21.3	21.0
12. LC+ FBScience	24.8	25.2	23.9	21.7	22.3	19.9	21.0

The most outstanding observation about rice grain Zn analyses results was that despite percent improvements over control, grain Zn concentrations of rice are still very low as compared to wheat. This fact was also observed and stated in the first phase of the project. However, there have been cases where foliar applications resulted in improvements close to 10 mg kg^{-1}.

Foliar Applied Fertilizers and Grain zinc: *Sorghum and Common Bean*
In sorghum foliar application experiments, most treatments significantly improved grain Zn concentration. The highest rate of increases were obtained by twice application of $ZnSO_4$ and OMEX Type II at one site, while the highest grain Zn values were obtained by OMEX II and OMEX III treatments at the other, in the first year. In the second year, Valagro Brexil, Anthracol and OMEX Type III resulted in the highest grain Zn at one site, while OMEX II and OMEX III were the most effective, in that aspect, at the other site.

In common bean experiments in Brazil, in the Experiment 2, OMEX II resulted in the highest seed Zn, in all 4 experiments in 2 years, followed by twice application of $ZnSO_4$. The increases in the plots treated with OMEX might be partly due to the yield decreases in OMEX plots in the first year.

Foliar Applied Fertilizers: *Outcomes / Significance*

- Twice application of foliar $ZnSO_4$ generally gave better results than single application. Timing of single applications gave variable results, presumably due to varying environmental conditions.

- Most of the Zn-containing foliar solutions were found to be highly promising in relation to improvement of grain Zn concentration based on the 2 year results.

- Foliar Zn fertilizers can be sprayed to foliar together with fungicides/insecticides tested in different countries. There was no adverse effect of those pesticides on leaf Zn penetration and see/grain Zn deposition.

- 0.5 % concentration of $ZnSO_4$ solution gave better results than 0.3 %. However, when urea was used together with $ZnSO_4$, this difference was greatly reduced. Urea addition was effective in increasing the effectiveness of $ZnSO_4$, particularly when lower concentration of $ZnSO_4$ was used.

- Among the other Zn forms tested (ZnO, nanoparticle ZnO, $ZnCl_2$, ZnEDTA and ZnSO4) ZnCl2 gave the best result while the ZnO forms were less effective in increasing grain Zn.

- Very new and not-reported results indicate that solution pH and use of some adjuvants markedly effect the agronomic effectiveness of foliar Zn fertilizers. Reducing solution pH from 8.3 to 6.0 by HCl or citric acid significantly improved grain Zn concentration of wheat in Turkey.

Lessons Learned
Solution pH of the foliar Zn fertilizers has unexpectedly high impact on agronomic effectiveness of foliar Zn fertilizers. This is an issue to be considered in future foliar tests and research programs.

TASK-III: DISSEMINATION AND IMPLEMENTATION OF THE PROJECT RESULTS
Delivery and implementation of the project results to farmers represent an important step for the success of this project. HarvestZinc project has already started to organize “Zinc Days” event in the target countries for the agronomists/crop consultants, extension staff, farmers, nutritionists and decision makers. The "Zinc Days" event is a major collaborative effort together with the partner countries/institutions to contribute to the mission and goal of the project. This event represents an important delivery activity of the project. From the start of the second phase of the project, 15 "Zinc Days" event has been organized including 6 Zinc Days event in the second year of the project as following:

So far following 15 "zinc days" event has been organized. The number of the participants is also given below:

1) **May 10, 2011** Quzhou, Hebei (CHINA) organized by China Agricultıral University (total participants: 235)

2) **June 2, 2011:** Ludhiana (INDIA) organized by Punjab Agricultural University (total participants: 185)

3) **July 27, 2011:** Chiang Mai (THAILAND) organized by Chiang Mai University (total participants:160)

4) **August 15, 2011:** Weinan-Xian (CHINA) organized by Northwest Agriculture and Forestry University (total participants: 285)

5) **December 6, 2011:** Campinas-SP (BRAZIL) organized by the Instituto Agronomica-Campinas (total participants: 185).

6) **March 27, 2012**: Faisalabad (PAKISTAN) Nuclear Institute for Agriculture and Biology (NIAB) (total number of participants: approx. 450)

7) **June 16, 2012:** Mazatlan (MEXICO) organized by CIMMYT(total number of participants: approx 600)

8) **November 25, 2012:** Rudong-Jiangsu (CHINA) organized by Nanjing Agricultural University and China Agricultural University (total number of participants: 200)

9) **March 16, 2012:** Lusaka (ZAMBIA) organized by GART and Zambia University (total number of the farmers close to 4000). GART is a big farmer association in Zambia and organizing every year in March a big farmer event to demonstrate on-going demonstrative and research-based trials and activities.

10) **February 20, 2013:** Bathinda (INDIA) organized by the Punjab Agricultural University (total number of the farmers 60).

11) **March 12, 2013**: Lahore (PAKISTAN) organized Nuclear Institute for Agriculture and Biology (toatal nuber of participants 250)

12) **March 21, 2013** Lusaka (ZAMBIA) organized by GART and Zambia University (total number of the farmers close to 4000)

13) **May 6, 2013:** Anhui (CHINA) organized by China Agricultural University and Anhui Academy of Agricultural Sciences (total number of the participants about 200)

14) **July 31, 2013**: Hyderabad- ICRISAT (INDIA) organized by ICRISAT (total number of the participants about 300)

15) December 26, 2013: Eskisehir (TURKEY) organized by Ministry of Agriculture and Sabanci University (Total number of participants are about 300, including 110 farmers)

Outcomes / Significance

The "Zinc Days" event represents an important delivery activity of the project. This event is highly useful forum for farmers, agronomists and other end-users and provides very valuable basic information through oral presentations and educational materials such as videos, colour brochures/booklets, factsheets, various colour pictures of Zn deficiency symptoms from different food crops. Presentations made are generally focused on the following topics:

i) soil and management factors affecting availability and root uptake of zinc

ii) why plants need zinc

iii) soil and foliar zinc fertilizers and application methods

iv) zinc fertilization of selected crops

v) role of zinc-biofortified crops in yield and human nutrition

vi) role of zinc in human nutrition and health

HarvestZinc project was represented in the 3rd International Zinc Symposium, held in Hyderabad in October, 2011. *Plant and Soil*, a leading international journal on plant-soil relationships, published a **Zinc Special Issue** in December 2012, which contains 23 zinc-related original papers, and most of them were presented at the 3rd International Zinc Symposium in October 2011. Among the papers published, 6 papers were derived from the HarvestZinc project activities.

To view the **Zinc Special Issue** of *Plant and Soil*, please visit: http://link.springer.com/journal/11104/361/1/page/1

Following HarvestZinc project papers have been published in this special Zinc Issue:

1) Zou, C.Q., Y.Q. Zhang, A. Rashid, H. Ram, E. Savasli, Z. Arisoy, I. Ortiz-Monasterio, S. Simunji, Z.H. Wang, V. Sohu, M. Hassan, Y. Kaya, O. Onder, O. Lungu, M.Y. Mujahid, A.K. Joshi, Y. Zelensky, F.S. Zang, and I. Cakmak (2012) **Biofortification of wheat with Zn through Zn fertilization in seven countries.** Plant and Soil (DOI 10.1007/s11104-012-1369-2). see: http://www.springerlink.com/content/h3984n6167887448/

2) Phattarakul N, Rerkasem B, Li L J, Wu L H, Zou C Q, Ram H, Sohu V S, Kang B S, Surek H, Kalayci M, Yazici A, Zhang F S and Cakmak I. **Biofortification of rice grain with Zn through Zn fertilization in different countries.** Plant and Soil. DOI 10.1007/s11104-012-1211 http://www.springerlink.com/content/81r1603373645565/

3) Prom-u-thai C, Rerkasem B, Yazici A and Cakmak I. 2012. **Zn-priming promotes seed germination and seedling vigor of rice**. Journal of Plant Nutrition and Soil Science. 175: 482-488. See: http://onlinelibrary.wiley.com/doi/10.1002/jpln.201100332/abstract

4) Manzeke GM, Mapfumo P, Mtambanengwe F, Chikowo R, Tendayi T, and Cakmak I. 2012. **Soil fertility management effects on maize productivity and grain zinc content in smallholder farming systems of Zimbabwe.** Plant and Soil. DOI 10.1007/s11104-012-1332-2. http://www.springerlink.com/content/8674872216343t32/

5) Kutman UB, Kutman BY, Ceylan Y, Ova EA and Cakmak I. 2012. **Contributions of root uptake and remobilization to grain zinc accumulation in wheat depending on post-anthesis zinc availability and nitrogen nutrition**. Plant and Soil. DOI 10.1007/s11104-012-1300-x. http://www.springerlink.com/content/23r7418w3067t387/

In addition, several MSc and PhD students are involved in this project and conducted their Thesis Projects under the HarvestZinc project. Total number of students who conducted their projects under this project is 10 (5 in Turkey; 2 in India; 2 in China, 1 in Thailand).

2. INTRODUCTION

HarvestPlus Zinc Fertilizer Project, initiated on April 2008, studied the potential of various Zn-containing fertilizers for increasing Zn concentration of cereal grains and improving yield in different target countries.

Based on the results obtained within the 1st Phase of the project, the reaction of cereal crops to Zn fertilization showed large variation between the countries and even within a given country in terms of grain yield response. Increase in grain yield upon Zn applications ranged between 0 to 22 %. In some locations in India, Pakistan and Turkey, wheat grain yield was increased up to 22 % by soil Zn applications.

In contrast to the large variations in grain yield among countries and even within a given country, the results with grain Zn concentrations were highly consistent. Grain Zn concentrations were significantly increased by foliar Zn applications while soil Zn application was less effective. Wheat has been found to be the most promising cereal crop for increasing

Zn in grains through foliar Zn fertilization. In this aspect, maize appears to be in-responsive. In case of wheat, particular increases in grain Zn concentration after foliar application of Zn were observed in each country. For example, foliar spray of Zn increased grain Zn concentration from 25 to 61 mg kg^{-1} in one location in Punjab, from 29 to 60 mg kg^{-1} in one location in Pakistan and from 12 to 29 mg kg^{-1} in one location in Turkey. The Zn treatments containing both soil (e.g., 50 kg $ZnSO_4$) and foliar Zn application (around 2 to 4 kg $ZnSO_4$ per ha) showed the largest increases in grain Zn concentration.

The trials conducted in Turkey showed that the timing of foliar Zn application is a critical issue in maximizing grain Zn concentration (*Cakmak et al., 2010, J. Agric. Food Chem. 58: 9092-9102*). According to the results obtained from several field tests, foliar spray of Zn late in growing season resulted in much greater increases in grain Zn concentration when compared to the earlier foliar applications of Zn. Increases in concentration of whole grain Zn through soil and/or foliar Zn applications were also well reflected (proportionally) in all grain fractions analyzed (e.g., embryo, aleurone and endosperm fractions), especially in the endosperm, the part predominantly consumed in food products in target countries. Foliar Zn spray was also very effective in reducing the phytate/Zn molar ratio in the endosperm part (e.g., from 112 to 45). The phytate/Zn molar ratio is an indicator for bioavailability of Zn in diets (*Hambidge et al., 2008, 138: 2363-2366*). Reducing this ratio is an important step in improving Zn bioavailability in human body.

Another important finding in the past 3 years under the HarvestPlus Zinc Fertilizer Project was related to the role of nitrogen (N) nutrition in enriching cereal grains with Zn (and also Fe). Nitrogen-nutritional status of plants appears to be a very critical factor in i) root uptake ii) root-to-shoot translocation and iii) grain accumulation of Zn and Fe. Increasing N supply very positively affected root uptake, shoot translocation and grain deposition of Zn. This positive relationship between the grain concentrations of Zn (and Fe) and N became stronger when N and Zn supply are sufficiently high (Kutman et al., *2011, Plant Soil, 342: 149-164; Erenoglu et al. New Phytol 189: 438–448*). This result lead us to suggest that N and Zn act synergistically in improving the grain Zn concentration when both nutrients exist at sufficient amounts either in the growth medium or in the vegetative tissues.

The first phase of the project was supported by:

- HarvestPlus Biofortification Challenge Program,
- Mosaic Company,
- International Zinc Associations,
- K+S Kali,
- Omex Agrifluids,
- International Fertilizer Industry Association and
- International Plant Nutrition Institute.

Tasks and Challenges for the Second Phase of the Project

Based on evidence that foliar Zn application is highly effective and promising in doubling grain Zn concentration at any location tested for example in wheat, it is important to motivate and encourage farmers to spray Zn to increase grain quality as well as grain yield. Following two strategies can be employed for motivation (and encouragement) of farmers to spray Zn unless there is no Zn deficiency problem in soils:

- ***to demonstrate that the plants emerging from high Zn-seeds i) have improved seedling vigor and ii) hence better yield (besides human nutritional effects)***
- ***to evaluate the applicability of Zn together with widely used insecticides***

and/or fungicides on wheat and also rice in the target countries

In view of the positive impacts of N nutrition (especially urea) on root uptake, shoot transport and grain deposition of Zn, field tests are needed to study further the interaction between Zn and N nutrition (e.g., urea supply). In the second phase of the project, a special attention will be paid to the urea and zinc relationships, especially in their foliar spray.

Most of the foliar spray experiments conducted in the first phase of the project concentrated only on applying $ZnSO_4$ and 2 different Zn-containing foliar fertilizers. It is, therefore, important to test further foliar Zn fertilizers for their effectiveness in improving grain Zn concentration. It is also important to study the role of a single spray of Zn fertilizer (before or after flowering) on grain Zn concentration. All these aspects will be major research activities of the second phase of the HarvestPlus Zinc Fertilizer Project (HarvestZinc).

Delivery of the project results to farmers (end-users) is a vital issue for the success of this project. One of the major goals of the second phase of the HarvestZinc project is, therefore, to promote and disseminate the practical and theoretical knowledge and experiences gained during the project. An important attention will be paid to the organization of ***Zinc Days*** in the target countries for the agronomists/crop consultants, extension staff, farmers, nutritionists and decision makers at the different stages of the project.

The second phase of the zinc fertilizer project will be realized under following 3 Tasks

TASK-1: SOIL APPLICATION OF ZINC-CONTAINING FERTILIZERS AND USE OF SEEDS DIFFERING IN ZINC CONCENTRATIONS
TASK-II: APPLICATION OF FOLIAR ZINC FERTILIZERS
TASK-III: DISSEMINATION AND IMPLEMENTATION OF THE PROJECT RESULTS

Following institutions are acting as the collaborators of this project in the related target countries:
BRAZIL: Instituto Agronomica, Campinas
ZAMBIA: GART-Golden Valley Agricultural Research Trust, Lusaka
INDIA: Punjab Agricultural University (PAU), Ludhiana
PAKISTAN: Pakistan Atomic Energy Commission (PAEC) Islamabad
CHINA: China Agricultural University, Beijing
THAILAND: Chiang Mai University
TURKEY: Ministry of Agriculture

The second Phase of the Project is supported by the following institutions:

Mosaic Company, USA (www.mosaicco.com)
K+S KALI GmbH, Germany (www.kali-gmbh.com)
International Zinc Association, Belgium (www.zinc.org/crops)
OMEX Agrifluids, England (www.omex.co.uk)
International Fertilizer Industry Association, France (www.fertilizer.org)
International Plant Nutrition Institute, USA (www.ipni.net)
Bayer CropScience, Germany (www.bayercropscience.com)
ADOB, Poland (www.adob.com.pl)
Valagro, Italy (www.valagro.com)
FBSciences, USA (www. fbsciences.com)
ATP Nutrition, Canada (www.atpnutrition.ca)

3. COUNTRY REPORTS

Field trials are established on 2 or 3 different locations in each target country. The locations have been selected by the collaborating partners, but for sake of standardization, the experimental details (design etc) were planned by the coordinating institution Sabanci University, and sent to the related institutes.

Since NPK requirements of the crops are different among partner countries, the rates of N, P and, if needed, K were decided by the collaborating partners depending on the crops tested. The same experimental plan was established for all crops, since the number of treatments is the same for all crops, except for the additional experiment carried out only in Turkey.

The experimental layout used is *Randomized Complete Block Design* with 4 replications where some partners may use more replications depending on the variations in their fields. The experiments are:

EXPERIMENT 1:
An experiment involving soil application of Zn-containing fertilizers plus 2 different means of seed enrichment. It includes 10 treatments.

EXPERIMENT 2:
An experiment involving foliar application of different Zn solutions. It includes 12 treatments.

EXPERIMENT 3 (Only in Turkey):
Different combinations of $ZnSO_4$ with herbicide and pesticides, urea and H_2SO_4, plus other forms of Zn were compared in the first year, while some changes have been made in the second year of the experiment. It includes 18 treatments as shown below:

EXPERIMENT 3 (1^{st} Year):

1: LC (Local Control)
2: LC + 0.5 % $ZnS0_4$ (at tillering)
3: LC + 0.5 % $ZnS0_4$+herbicide (at tillering)
4: LC + herbicide (at tillering)

5: LC + 0.5 % $ZnS0_4$ (after flowering)
6: LC + Sunn pest pesticide +0.5 % $ZnSO_4$ (after flowering)
7: LC + Sunn pest pesticide (after flowering)

8: LC + 0.3 % $ZnS0_4$ (after flowering)
9: LC + 0.3 % $ZnS0_4$ + urea (after flowering)
10: LC + 0.5 % $ZnS0_4$ (after flowering)
11: LC + 0.5 % $ZnS0_4$ + urea (after flowering)
12: LC + 0.3 % $ZnS0_4$ (after flowering) + H_2SO_4
13: LC + 0.3 % Zn $S0_4$+urea (after flowering) + H_2SO_4
14: LC + 0.5 % Zn $S0_4$ (after flowering) + H_2SO_4
15: LC + 0.5 % Zn $S0_4$+ urea (after flowering) + H_2SO_4

16: LC + 0.3 % ZnO
17: LC + 0.3 % $ZnCl_2$
18: LC + 0.3 % ZnEDTA

EXPERIMENT 3 (2nd Year):

1. Local control (LC)
2. LC + 0.5 % $ZnS0_4$ (tillering)
3. LC + 0.5 % $ZnS0_4$+herbicide (tillering)
4. LC + herbicide (tillering)
5. LC + 0.5 % $ZnS0_4$ (post flowering)
6. LC + Suni pest pesticide +0.5 % $ZnSO_4$ (post flowering)
7. LC + Suni pest pesticide (post flowering)
8. LC + 0.3 % $ZnS0_4$ (post flowering)
9. LC + 0.3 % $ZnS0_4$ + urea (post flowering)
10. LC + 0.5 % $ZnS0_4$ (post flowering)
11. LC + 0.5 % $ZnS0_4$ + urea (post flowering)
12. LC + 0.4 %$ZnSO_4$ (pH= 8.3 by Tap water (Twice))
13. LC + 0.4 %$ZnSO_4$ (pH= 6.0 by HCl (Twice))
14. LC + 0.4 %$ZnSO_4$ (pH= 4.5 by HCl (Twice))
15. LC + 0.4 %$ZnSO_4$ (pH= 6.0 by Citric acid (Twice))
16. LC + 0.4 %$ZnSO_4$ + 0.5% Urea (pH= 6.0 by Citric acid (Twice))
17. LC + 0.4 %$ZnSO_4$ (pH= 4.5 by Citric acid (Twice))
18. LC + 0.4 %$ZnSO_4$ + 0.5% Urea (pH= 4.5 by Citric acid (Twice))

Project activities of this term are given on a country report basis below:

COUNTRY REPORT - TURKEY

1. COLLABORATING INSTITUTIONS:

NATIONAL COORDINATOR:
Mufit Kalayci: Transitional Region Agricultural Research Institute, Eskisehir

COORDINATING INSTITUTION:
Transitional Region Agricultural Research Institute, Eskisehir

COLLABORATING INSTITUTIONS:
Transitional Region Agricultural Research Institute, Eskisehir
Bahri Dagdas International Research Institute, Konya

RESEARCH ASSOCIATES:
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Oguz Onder: Transitional Region Agricultural Research Institute, Eskisehir,
Zafer Arisoy: Bahri Dagdas International Research Institute, Konya,
Yasin Kaya: Bahri Dagdas International Research Institute, Konya.

2. INTRODUCTION:

Through a global study of micronutrient status in soils, carried out for the FAO, Sillanpaa (1982) revealed that Turkey is one of the countries having highest percentage of soils with Zn deficiency. A national survey, consisting of 1511 soil samples from all over the country, showed that 49.8 % of the Turkish soils are deficient in Zn (Eyupoglu et al, 1994). Central Anatolian Plateau was the most critical region of Zn deficiency; of the 76 topsoil samples from the region, 92 % had lower than 0.5 ppm DTPA-extractable Zn. (Cakmak et al., 1999).

In the meanwhile, Cavdar et al (1983) reported widespread Zn deficiency in children in Turkey. Cavdar et al (1988) also reported that Zn deficiency is critically important in pregnant women particularly in areas where cereal based diets dominate due to poor economic status. Prasad (1982) and Bouis (1996) reported that Zn deficiency is widespread in areas where cereal based foods dominate.

The critical importance of Zn deficiency for wheat production in Turkey has been shown first in 1994 in the framework of a large-scale project supported by NATO-Science for Stability Program (Cakmak et al.,1999). Numerous experiments were carried out in the Central Anatolian Plateau to find the best solution to Zn deficiency of wheat within the scope of this project. Results of these experiments showed that soil application of 5 to 7 kg Zn ha^{-1} was enough to meet wheat crops' demands in most cases and soil applications were more effective in solving Zn deficiency as far as yields were concerned (Yilmaz et al, 1997). However, soil applications were not enough to obtain acceptable grain Zn concentrations and soil + foliar applications resulted in the highest concentrations in wheat grains, foliar applications being more effective than soil applications in this aspect (Yilmaz et al, 1997).

In Turkey, grain Zn concentrations of wheat grown on Zn-sufficient soils are generally between 20 and 30 mg kg^{-1} , whereas on the Zn-deficient soils this range is between 5 and 12 mg kg^{-1} (Erdal et al., 2002; Kalayci et al., 1999). For a measurable biological impact on human health, the concentration of Zn in whole wheat grain needs to be increased at least by approximately 10 mg kg^{-1}, assuming a 400 g per day intake for adult woman in the countries where whole grain flour is used for making food like chapatti in India (Pfeiffer and McClafferty, 2007). Generally, recommended dietary allowance for Zn is around 15 mg per day (National Research Council, 1989).

However promising genetic biofortification is due to presence of genetic resources to exploit, since breeding is a long time process, agronomic biofortification seems to be the most practical approach in the short run (Cakmak, 2008), particularly in countries like Turkey having soils with high pH, $CaCO_3$ and low organic matter (Eyuboglu, 1994). Frequently observed drought is another constraint (Ekiz et al., 1998; Bagci et al.,2007), resulting in dry top soils during grain filling period and forcing plants to grow on subsoil reserves where available Zn is even lower. These ecological constraints restrict genotypes' reaching their real potential of grain Zn.

Experiments were carried out, in the first 3 year of HarvestPlus project, to investigate the effect of various soil and foliar applications of Zn-containing fertilizers on grain Zn concentrations of wheat, maize and rice for 2 years. Results have confirmed once more that, foliar applications were much more effective in increasing grain Zn concentration than soil applications. Wheat was the most responsive among these crops to Zn application. The increases in grain Zn concentrations were 83.4, 26.9 and 7.9 % for wheat, rice and maize, respectively, as an average of 4 experiments for each crop.

3. EXPERIMENTAL ACTIVITIES

Three field experiments were established on wheat at each of Eskisehir and Konya locations which are presented in Figure 1. Some soil properties of the experimental fields are given in Table 1.

Figure 1: Experimental locations shown on the map of Turkey.

Table 1. pH values and DTPA-extractable Zn and Fe concentrations of the experimental sites (top soil).

SITE	pH	DTPA-EXTRACTABLE CONCENTRATION (mg kg^{-1})	
		Zn	Fe
ESKİŞEHİR	8.2	0.45	5.8
KONYA	7.5	0.32	4.7

The lists of treatments used in the experiments are given in Tables 2-5 below.

Table 2. List of the treatments in the Experiment 1.

Treatment	Planting	Tillering	Other
1. Local control (LC)	80 kg P_2O_5/ha 75 kg N/ha	75 kg N/ha	
2. LC + Soil $ZnSO_4.7H_2O$	80 kg P_2O_5/ha 75 kg N/ha 50 kg $ZnSO_4.7H_2O$/ha	75 kg N/ha	
3. LC+Mosaic-I	200 kg MESZ/ha 51 kg N/ha	75 kg N/ha	
4. LC+Mosaic-II	400 kg MESZ/ha 27kg N/ha	75 kg N/ha	
5. LC+MosaicI+FoliarZn	200 kg MESZ/ha 51 kg N/ha	75 kg N/ha	0.5%$ZnSO_4.7H_2O$ at early milk stage
6. LC + Kali KornKali	80 kg P_2O_5/ha 75 kg N/ha 150 kg KKL/ha	75 kg N/ha	
7. 2 x Split Urea-Zn	80 kg P_2O_5/ha 75 kg N/ha with Zn	75 kg N/ha with Zn containing	
8. LC+ADOB HBEDZn	80 kg P2O5/ha 75 kg N/ha 250 kg ZnHBED/ha	75 kg N/ha	
9. LC + high seed Zn (Foliar in last crop)	80 kg P_2O_5/ha 75 kg N/ha	75 kg N/ha	
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	80 kg P_2O_5/ha 75 kg N/ha	75 kg N/ha	

In 2011-2012, In treatment 9, seeds with Zn concentration of 74 ppm, obtained by foliar Zn application in the previous season, were used at both locations. Zn concentration of enriched seeds was 47 ppm in 2012-2013. In treatment 10, priming was performed with 5 mM $ZnSO_4$ for 1 hour on seeds before sowing.

The cultivar used at both locations was Bezostaya 1 in all experiments, the predominant hard red winter wheat in the region. The seed rate was 500 seeds m^{-2}. Plot dimensions were 1.2 m width (6 rows with 20 cm row space) and 5 m length. Rate of P was 80 kg P_2O_5 ha^{-1}, all of which was applied at planting. Half of the N (75 kg N ha^{-1}) was also applied at planting. Second half of nitrogen fertilizer (75 kg N ha^{-1}) was applied at tillering stage (Zadoks 23-24) at both locations. Zn soil applications in the experiment 1 were performed by spraying 50 kg $ZnSO_4$ (7 mol water) ha^{-1} in related treatment plots and mixed with soil using a disc plow in both experiments. The experiments were planted using plot drills. Sowing dates were late October and first week of November in both years.

In the Experiment 1, the only foliar application was involved in the Treatment 5. The application was performed at a rate of about 80 ml m^{-2}, using 0.5 % $ZnSO_4.7H_2O$ solution. Application was made in mid-June at both locations, when grains were at early milk stage. All foliar applications in all 3 experiments were performed at cooler hours of late afternoon.

Table 3. List of the treatments in the Experiment 2.

TREATMENT	Soil application (kg/ha)		Foliar Zn <g(ml) in 800 ml per 10 m^2 plot size>	
	Planting	tillering	End of booting	Early milk
1. Local control (LC)	80 P_2O_5 75 N	75 N		
2. LC+ Foliar $ZnSO_4$	80 P_2O_5 75 N	75 N	4 g $ZnSO_4.7H_2O$	
3. LC+ Foliar $ZnSO_4$	80 P_2O_5 75 N	75 N		4 g $ZnSO_4.7H_2O$
4. LC+ Foliar $ZnSO_4$	80 P_2O_5 75 N	75 N	4 g $ZnSO_4.7H_2O$	4 g $ZnSO_4.7H_2O$
5. LC+OMEX II	80 P_2O_5 75 N	75 N		3.32 ml OMEX II
6. LC+OMEX III	80 P_2O_5 75 N	75 N		6.15 ml OMEXIII
7. LC+Kali-EPSO	80 P_2O_5 75 N	75 N		18.2 g EPSO
8. LC+ ADOB ZnIDHA	80 P_2O_5 75 N	75 N		9.1 g ZnIDHA
9. LC+ Valagro Brexil	80 P_2O_5 75 N	75 N		9.1 g Brexil
10. LC+Antracol	80 P_2O_5 75 N	75 N		3 g Antracol
11 LC+Fungicide or Insecticide	80 P_2O_5 75 N	75 N		**Mixing:** Fungicide or Insecticide+ 4 g $ZnSO_4.7H_2O$
12. LC+FBScience	80 P_2O_5 75 N	75 N		**Mixing:** 5 ml FBScience (ZicRon) + 4 g $ZnSO_4.7H_2O$
13. HP 2011-ATP Nutr.	80 P_2O_5 75 N	75 N		
14. HP 2012-ATP Nutr.	80 P_2O_5 75 N	75 N		

In the Experiment 2, foliar applications at booting stage (Treatments 2 and 4) were performed in last week of May at both locations. All other foliar applications were made at early milk stage, in mid-June.

In the Experiment 3, foliar applications at tillering stage (Zn solutions in Treatments 2 and 3; herbicide in Treatments 3 and 4) were performed in the last week of April at both locations. Other foliar applications were made after flowering, around mid of June.

Table 4. List of the treatments in the Experiment 3, in 2011-2012.

1	Local control (LC)
2	LC + 0.5 % $ZnS0_4$ (tillering)
3	LC + 0.5 % $ZnS0_4$+herbicide (tillering)
4	LC + herbicide (tillering)
5	LC + 0.5 % $ZnS0_4$ (post flowering)
6	LC + Suni pest pesticide +0.5 % $ZnSO_4$ (post flowering)
7	LC + Suni pest pesticide (post flowering)
8	LC + 0.3 % $ZnS0_4$ (post flowering)
9	LC + 0.3 % $ZnS0_4$ + urea (post flowering)
10	LC + 0.5 % $ZnS0_4$ (post flowering)
11	LC + 0.5 % $ZnS0_4$ + urea (post flowering)
12	LC + 0.3 % $ZnS0_4$ (post flowering)+ H_2SO_4
13	LC + 0.3 % Zn $S0_4$**+**urea (post flowering) + H_2SO_4
14	LC + 0.5 % Zn $S0_4$ (post flowering) + H_2SO_4
15	LC + 0.5 % Zn $S0_4$+ urea (post flowering) + H_2SO_4
16	LC + 0.3 % ZnO
17	LC + 0.3 % $ZnCl_2$
18	LC + 0.3 % ZnEDTA

Table 5. List of the treatments in the Experiment 3, in 2012-2013.

1	Local control (LC)
2	LC + 0.5 % $ZnS0_4$ (tillering)
3	LC + 0.5 % $ZnS0_4$+herbicide (tillering)
4	LC + herbicide (tillering)
5	LC + 0.5 % $ZnS0_4$ (post flowering)
6	LC + Suni pest pesticide +0.5 % $ZnSO_4$ (post flowering)
7	LC + Suni pest pesticide (post flowering)
8	LC + 0.3 % $ZnS0_4$ (post flowering)
9	LC + 0.3 % $ZnS0_4$ + urea (post flowering)
10	LC + 0.5 % $ZnS0_4$ (post flowering)
11	LC + 0.5 % $ZnS0_4$ + urea (post flowering)
12	LC + 0.4 %$ZnSO_4$ (pH= 8.3 by Tap water (Twice))
13	LC + 0.4 %$ZnSO_4$ (pH= 6.0 by HCl (Twice))
14	LC + 0.4 %$ZnSO_4$ (pH= 4.5 by HCl (Twice))
15	LC + 0.4 %$ZnSO_4$ (pH= 6.0 by Citric acid (Twice))
16	LC + 0.4 %$ZnSO_4$ + 0.5% Urea (pH= 6.0 by Citric acid (Twice))
17	LC + 0.4 %$ZnSO_4$ (pH= 6.0 by Citric acid (Twice))
18	LC + 0.4 %$ZnSO_4$ + 0.5% Urea (pH= 4.5 by Citric acid (Twice))

4. RESULTS AND DISCUSSIONS

GRAIN YIELDS

Since Central Anatolian Plateau of Turkey had one of the longest and hardest winters of his history in 2011-2012 cropping year, stand establishments were not complete before April at either location, although they were planted in late October in Eskisehir and early November in Konya. Drought was also responsible for poor stands at Konya location. Monthly average temperatures and precipitations for these locations are given in Tables 6 and 7, in comparison with previous year or long time averages of the sites.

Table 6. Monthly average temperatures of Eskisehir and Konya locations in the experimental period.

MONTH	MONTHLY AVERAGE TEMPERATURE (^oC)			
	ESKISEHIR		KONYA	
	2011-2012	2010-2011	2011-2012	LONG YEARS
October	8.5	10.0	10.5	12.5
November	0.8	9.3	1.3	5.8
December	0.9	4.2	0.9	1.3
January	-3.6	0.3	-2.2	-0.2
February	-5.5	0.1	-3.6	1.2
March	1.5	3.7	3.5	5.8
April	12.0	7.2	12.6	11.0
May	14.4	12.3	14.9	15.7

As can be seen in Table 6, lower temperatures as compared to normal years, particularly in November, month of stand establishment in normal years, and in March, normally the time for restart of growth, were the main reasons for the delay in establishment of complete stand cover.

Since Eskisehir location had good rains after April, when the temperatures were raised to levels suitable for growth, the yield levels were relatively better. At Konya location, however, shortage of rainfall aggravated the adverse effect of low temperatures and the experiments were irrigated in the spring at this location to secure grain growth. Precipitations obtained at these locations are given in Table 7.

Table 7. Monthly precipitations of Eskisehir and Konya locations in the experimental period, as compared to long year averages.

MONTH	MONTHLY PRECIPITATION (mm)			
	ESKISEHIR		KONYA	
	2011-2012	LONG YEARS	2011-2012	LONG YEARS
October	57.9	25.8	1.2	33.6
November	0.0	30.3	0.3	33.8
December	46.1	45.7	0.9	41.4
January	58.0	38.2	54.2	33.3
February	42.1	32.3	25.8	25.2
March	56.4	33.1	15.0	25.8
April	22.1	35.3	9.0	38.1
May	72.7	43.3	40.0	41.1

When Tables 5 and 6 are studied together, it is seen that good rains during winter were not effective due to low temperatures. Lack of any precipitation in November, and the low temperatures in March were the reasons for the delay in stand establishment. In 2012-2013, however, this problem was not encountered since a restricted irrigation was applied after planting to ensure emergence.

Number of plants counted and average plant heights measured in early spring are given for both locations in 2011-2012 and for Eskisehir location in 2012-2013 in Table 8. Although there seems to be some variation in plant numbers, differences due to treatments were not statistically significant. Particularly treatments 9 and 10 were expected to have some effects on stand establishment since these treatments involved use of Zn-enriched seeds. However, in contrast to positive effects observed in some other collaborating countries, the effects were not seen in Turkey. This lack of response in stand establishment, also reflected in lack of response in grain yield, can be interpreted as a result of the residual effect of large-scale adoption of Zn fertilization since 1990's. In contrast, great effect of foliar Zn application on grain Zn concentrations, as will be seen in related section, shows the difference between top soil and subsoil since the subsoils of the experimental sites are still very low in available Zn due to low mobility of applied Zn in soils.

The lower numbers of plants and higher plant heights at Eskisehir in the second year resulted from earlier stand count and later height measurement than the first year.

Table 8. Number of plants counted and plant heights measured in Experiment 1 in the spring.

TREATMENT	PLANT m^{-2}			PLANT HEIGHT (cm)		
	ESKISEHIR		KONYA	ESKISEHIR		KONYA
	2011-2012	2012-2013	2011-2012	2011-2012	2012-2013	2011-2012
1. Local control (LC)	464	380	291	7.0	19.0	8.4
2. LC + Soil $ZnSO_4.7H_2O$	476	371	328	6.9	18.6	8.9
3. LC+MosaicI	481	369	320	7.2	18.5	8.6
4. LC+MosaicII	463	383	324	7.2	19.1	8.9
5. LC+MosaicI+FoliarZn	490	390	332	7.0	19.5	9.1
6. LC + Kali KornKali	478	392	325	6.8	19.6	8.6
7. 2 x Split Urea-Zn	500	371	284	7.2	18.6	8.0
8. LC+ADOB HBEDZn	459	376	336	7.2	18.8	8.0
9. LC + high seed Zn (Foliar in last crop)	435	335	277	7.1	16.7	8.3
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	474	349	326	7.0	17.4	8.4
CV (%)	10.6	5.6	15.5	6.7	5.5	8.7
F TEST	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
LSD (0.05)	-		-	-		-

Grain yields were not affected significantly either by treatments in any of the 3 experiments. Tables 9-12 shows the grain yield results from the experiments.

Table 9. Wheat grain yields obtained in Experiment 1 at Eskisehir and Konya locations of Turkey, in 2011-2012 and 2012-2013 growing years.

TREATMENT	GRAIN YIELD (t ha^{-1})			
	2011-2012		2012-2013	
	ESKISEHIR	KONYA	ESKISEHIR	KONYA
1. Local control (LC)	4.05	2.63	5.44	6.01
2. LC + Soil $ZnSO_4.7H_2O$	4.14	2.71	5.39	6.01
3. LC+Mosaic-I	4.18	2.61	5.43	6.11
4. LC+Mosaic-II	4.46	2.83	5.37	6.18
5. LC+MosaicI+FoliarZn	4.11	2.66	5.25	6.22
6. LC + Kali KornKali	4.22	2.71	5.46	5.74
7. 2 x Split Urea-Zn	4.09	2.42	5.35	5.78
8. LC+ADOB HBEDZn	4.32	2.59	5.45	5.82
9. LC + high seed Zn (Foliar in last crop)	4.22	2.49	5.08	5.96
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	4.23	2.52	5.31	6.07
CV (%)	6.5	7.2	5.9	4.4
F TEST	n.s.	n.s.	n.s.	n.s.
LSD (0.05)	-	-	-	-

Table 10. Wheat grain yields obtained in Experiment 2 at Eskisehir and Konya locations of Turkey, in 2011-2012 and 2012-2013 growing years.

TREATMENT	GRAIN YIELD (t ha^{-1})			
	2011-2012		2012-2013	
	ESKISEHIR	KONYA	ESKISEHIR	KONYA
1. Local control (LC)	4.19	2.27	5.05	3.94
2. LC+ Foliar $ZnSO_4$ (booting)	4.24	2.42	5.17	4.10
3. LC+ Foliar $ZnSO_4$ (early milk)	3.97	2.40	5.42	3.70
4. LC+ Foliar $ZnSO_4$ (booting + early milk)	4.28	2.14	4.97	4.44
5 LC+ OMEX II	4.18	2.42	5.52	4.01
6. LC+ OMEX III	3.99	2.46	4.99	4.22
7. LC+Kali-EPSO	4.40	2.47	5.45	4.44
8. LC+ ADOB ZnIDHA	4.06	2.20	4.96	4.20
9. LC+ Valagro Brexil	4.32	2.38	4.98	4.91
10. LC+ Antracol	3.88	2.47	5.42	3.94
11. LC+ Pesticide (Suni pest pesticide)	3.79	2.66	5.16	3.58
12. LC+ FBScience	4.12	2.44	5.43	4.00
13. LC+HP 2011	4.67	2.73	4.98	4.27
14. LC+HP 2012	4.39	2.60	5.22	3.94
CV (%)	11.4	13.3	6.2	17.4
F TEST	n.s.	n.s.	n.s.	n.s.
LSD (0.05)	-	-	-	-

Table 11. Wheat grain yields obtained in Experiment 3 at Eskisehir and Konya locations of Turkey, in 2011-2012 growing year.

TREATMENT	**GRAIN YIELD (t ha^{-1})**	
	ESKISEHIR	**KONYA**
1. Local control (LC)	4.54	2.55
2. LC + 0.5 % $ZnS0_4$ (tillering)	4.51	2.79
3. LC + 0.5 % $ZnS0_4$+herbicide (tillering)	4.31	2.61
4. LC + herbicide (tillering)	4.61	2.49
5. LC + 0.5 % $ZnS0_4$ (post flowering)	4.67	2.59
6. LC + Suni pest pesticide +0.5 % $ZnSO_4$ (post flowering)	5.04	2.66
7. LC + Suni pest pesticide (post flowering)	4.74	2.61
8. LC + 0.3 % $ZnS0_4$ (post flowering)	4.48	2.55
9. LC + 0.3 % $ZnS0_4$ + urea (post flowering)	4.66	2.46
10. LC + 0.5 % $ZnS0_4$ (post flowering)	4.56	2.63
11. LC + 0.5 % $ZnS0_4$ + urea (post flowering)	4.64	2.57
12. LC + 0.3 % $ZnS0_4$ (post flowering)+ H_2SO_4	4.65	2.73
13. LC + 0.3 % Zn $S0_4$**+**urea (post flowering) + H_2SO_4	4.33	2.57
14.LC + 0.5 % Zn $S0_4$ (post flowering) + H_2SO_4	4.82	2,39
15.LC + 0.5 % Zn $S0_4$+ urea (post flowering) + H_2SO_4	4.57	2,63
16.LC + 0.3 % ZnO	4.91	2.84
17. LC + 0.3 % $ZnCl_2$	4.56	2.67
18. LC + 0.3 % ZnEDTA	4.81	2.59
CV (%)	7.4	10.9
F TEST	n.s.	n.s.
LSD (0.05)	-	-

Table 12. Wheat grain yields obtained in Experiment 3 at Eskisehir and Konya locations of Turkey, in 2012-2013 growing year.

TREATMENT	GRAIN YIELD (t ha^{-1})	
	ESKISEHIR	ESKISEHIR
1. Local control (LC)	5.10	4.89
2. LC + 0.5 % $ZnS0_4$ (tillering)	5.05	4.87
3. LC + 0.5 % $ZnS0_4$+herbicide (tillering)	4.90	5.37
4. LC + herbicide (tillering)	5.18	5.25
5. LC + 0.5 % $ZnS0_4$ (post flowering)	4.94	5.19
6. LC + Suni pest pesticide +0.5 % $ZnSO_4$ (post flowering)	5.04	5.00
7. LC + Suni pest pesticide (post flowering)	5.19	5.39
8. LC + 0.3 % $ZnS0_4$ (post flowering)	4.86	4.87
9. LC + 0.3 % $ZnS0_4$ + urea (post flowering)	5.22	4.66
10. LC + 0.5 % $ZnS0_4$ (post flowering)	5.01	5.03
11. LC + 0.5 % $ZnS0_4$ + urea (post flowering)	4.81	4.95
12. LC + 0.4 %$ZnSO_4$ (pH= 8.3 by Tap water (Twice))	4.89	5.07
13. LC + 0.4 %$ZnSO_4$ (pH= 6.0 by HCl (Twice))	4.71	5.19
14. LC + 0.4 %$ZnSO_4$ (pH= 4.5 by HCl (Twice))	4.99	5.05
15. LC + 0.4 %$ZnSO_4$ (pH= 6.0 by Citric acid (Twice))	4.69	4.78
16. LC + 0.4 %$ZnSO_4$ + 0.5% Urea (pH= 6.0 by Citric acid (Twice))	5.14	4.71
17. LC + 0.4 %$ZnSO_4$ (pH= 6.0 by Citric acid (Twice))	4.90	5.13
18. LC + 0.4 %$ZnSO_4$ + 0.5% Urea (pH= 4.5 by Citric acid (Twice))	5.08	5.05
CV (%)	5.8	8.8
F TEST	n.s.	n.s.
LSD (0.05)	-	-

LEAF Zn CONCENTRATIONS

Leaf Zn concentrations obtained in Experiment 1 are given in Table 13. As can be seen in the table, treatments did not result in significant changes in leaf Zn concentrations in Turkey in either year. On the contrary, grain Zn concentrations were significantly affected by treatments in all 3 experiments at both locations in both years.

GRAIN Zn CONCENTRATIONS

EXPERIMENT 1

Grain Zn concentrations obtained in the Experiment 1 is given in Table 14.

The greatest improvements in grain Zn concentrations over control plots were obtained by Treatments 5 (LC+MosaicI+FoliarZn) and 8 (LC+ADOB HBEDZn) at Eskisehir location and by Treatments 8, 7 (2 x Split Urea-Zn) and 5 at Konya location. Therefore Treatments 5 and 8 can be said to be the best treatments in that aspect according to the first year results, because they were superior at both locations. Since Treatment 5 was the only treatment involving foliar application of Zn, this result was an expected one. ADOB ZnIDHA appeared to be the most promising chemical in the first year experiments in Turkey. However, Treatment 5 was the only treatment resulting in statistically significant increase in grain Zn in the second year of the experiment at both sites. The rates of improvement caused by Treatments 5 and 8 are given in Table 15.

Table 13. Leaf Zn concentrations of wheat in the Experiment 1 in 2011-2012 and 2012-2013 growing seasons.

TREATMENT	LEAF Zn CONCENTRATION ($mg\ kg^{-1}$)			
	2011-2012		2012-2013	
	ESKISEHIR	KONYA	ESKISEHIR	KONYA
1. Local control (LC)	15.2	12.3	20.3	17.3
2. LC + Soil $ZnSO_4.7H_2O$	15.8	12.1	20.3	15.3
3. LC+Mosaic-I	16.0	12.5	20.3	16.8
4. LC+Mosaic-II	14.6	11.6	20.0	16.3
5. LC+MosaicI+FoliarZn		12.2	20.0	16.3
6. LC + Kali KornKali	16.5	12.2	20.3	17.8
7. 2 x Split Urea-Zn	15.7	13.6	20.8	17.3
8. LC+ADOB HBEDZn	16.4	12.9	20.5	17.5
9. LC + high seed Zn (Foliar in last crop)	15.7	12.3	20.0	16.3
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	15.9	13.3	19.8	17.0
CV (%)	7.6	9.3	4.9	5.0
F TEST	n.s.	n.s.	n.s.	n.s.
LSD (0.05)	-	-	-	-

Table 14. Wheat grain Zn concentrations in Experiment 1 at Eskisehir and Konya locations of Turkey, in 2011-2012 and 2012-2013 growing seasons.

TREATMENT	GRAIN Zn CONCENTRATION ($mg\ kg^{-1}$)			
	2011-2012		2012-2013	
	ESKISEHIR	KONYA	ESKISEHIR	KONYA
1. Local control (LC)	32.6	23.4	26.8	25.8
2. LC + Soil $ZnSO_4.7H_2O$	31.8	26.8	27.8	29.0
3. LC+Mosaic-I	32.6	28.1	28.0	27.0
4. LC+Mosaic-II	32.1	28.7	28.8	25.0
5. LC+MosaicI+FoliarZn	38.2	28.8	34.3	30.3
6. LC + Kali KornKali	33.1	28.2	30.5	28.3
7. 2 x Split Urea-Zn	32.7	29.6	29.0	25.5
8. LC+ADOB HBEDZn	36.5	32.2	29.0	28.3
9. LC + high seed Zn (Foliar in last crop)	32.7	25.5	28.3	24.5
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	31.9	26.2	27.0	25.8
CV (%)	7.1	13.6	12.2	10.7
F TEST	**	*	*	**
LSD (0.05)	3.4	5.4	5.1	4.2

Table 15. Grain Zn concentrations obtained by Treatments 5 and 8 and % improvements over control treatment in Experiment 1 in 2011-2012 and 2012-2013.

TREATMENT	GRAIN Zn CONCENTRATION ($mg\ kg^{-1}$)		IMPROVEMENT OVER CONTROL (%)	
	ESKISEHIR	KONYA	ESKISEHIR	KONYA
2011-2012				
1.Local control (LC)	32.6	23.4	-	-
5.LC+MosaicI+FoliarZn	38.2	28.8	17.2	23.1
8. LC+ADOB HBEDZn	36.5	32.2	12.0	37.6
2012-2013				
1.Local control (LC)	26.8	25.8	-	-
5.LC+MosaicI+FoliarZn	34.3	30.3	28.0	17.4
8. LC+ADOB HBEDZn	29.0	28.3	n.s.	n.s.

Kornkali, Mosaic-I and Mosaic-II Treatments also resulted in some improvements (nearly significant) at Konya location in the first year, but not at Eskisehir. In the second year, Kornkali at Eskisehir, and soil applied $ZnSO_4$ at Konya were the second best treatments after Treatment 5, in that aspect, although their effects were not statistically significant..

Seed enriched with Zn either by priming or by foliar application on the previous crop, did not result in significant improvements in grain Zn concentrations. As a matter of fact, these seed enrichments were expected to be effective on early growth and yield rather than grain Zn concentrations. They were not effective on grain yields either in Turkey.

EXPERIMENT 2

Grain Zn concentrations obtained in Experiment 2 is given in Table 16.

Effect by timing of foliar Zn application:

The greatest improvement due to foliar $ZnSO_4$ obtained by twice application (booting + early milk) at both locations in both years (Table 16). It resulted in 17.7 and 35.7 % improvements in grain Zn as compared to control in the first year; 46.0 and 40.3 % in the second year, at Eskisehir and Konya locations, respectively. Single application at booting was effective only at Konya in the first year, whereas both single applications were effective only at Eskisehir in the second year. This comparison is presented graphically in Figure 2.

Effect by other Zn containing chemicals:

LC + HP 2011 (Treatment 13) and LC + HP 2012 (Treatment 14) both resulted in statistically significant improvements in grain Zn concentrations at both locations in both years (Table 16). In fact, HP 2011 was the best treatment among all at Eskisehir location, resulting in the highest grain Zn (43.0 $mg\ kg^{-1}$) in the first year, and the second highest in the second year (43.5 $mg\ kg^{-1}$), after twice application of $ZnSO_4$ (43.8 $mg\ kg^{-1}$). At Konya location, HP 2012 was the second most effective treatment in the first year, after twice application of $ZnSO_4$, and the best in the second year, resulting in grain Zn concentrations of 33.6 and 40.5 $mg\ kg^{-1}$, respectively. FBScience was another chemical causing statistically significant improvements in grain Zn at both locations (11.8 and 21.5 % at Eskisehir and Konya, respectively) in the first year. OMEX II and Valagro Brexil was effective at Eskisehir but not at Konya in the first year. Other chemicals were not effective at either location. In the second year, however, OMEX II, OMEX III, KALI-EPSO, ADOB ZnIDHA, and Valagro Brexil all improved grain Zn significantly at Eskisehir, while only ADOB ZnIDHA and FBScience were effective at Konya.

Table 16. Wheat grain Zn concentrations in Experiment 2 at Eskisehir and Konya locations of Turkey, in 2011-2012 and 2012-2013 growing seasons.

TREATMENT	**GRAIN Zn CONCENTRATION ($mg\ kg^{-1}$)**			
	2011-2012		**2012-2013**	
	ESKISEHIR	**KONYA**	**ESKISEHIR**	**KONYA**
1. Local control (LC)	35.5	27.4	30.0	24.8
2. LC+ Foliar $ZnSO_4$ (booting)	37.7	33.4	38.0	26.5
3. LC+ Foliar $ZnSO_4$ (early milk)	36.4	29.5	38.3	30.3
4. LC+ Foliar $ZnSO_4$ (booting + early milk)	41.8	37.2	43.8	34.8
5 LC+ OMEX II	41.1	27.5	42.5	34.5
6. LC+ OMEX III	33.5	27.5	38.3	29.8
7. LC+Kali-EPSO	36.9	29.6	37.5	26.3
8. LC+ ADOB ZnIDHA	37.6	30.9	39.8	32.0
9. LC+ Valagro Brexil	40.7	29.2	42.0	29.5
10. LC+ Antracol	37.9	27.2	36.0	24.8
11. LC+ Pesticide (Sunn pest pesticide)	42.3	31.1	41.8	32.5
12. LC+ FBScience	39.7	33.3	35.8	32.5
13. LC+HP 2011	43.0	32.1	43.5	32.3
14. LC+HP 2012	40.9	33.6	41.8	40.5
CV (%)	7.5	10.1	11.2	13.0
F TEST	**	**	**	**
LSD (0.05)	4.1	4.4	6.3	5.7

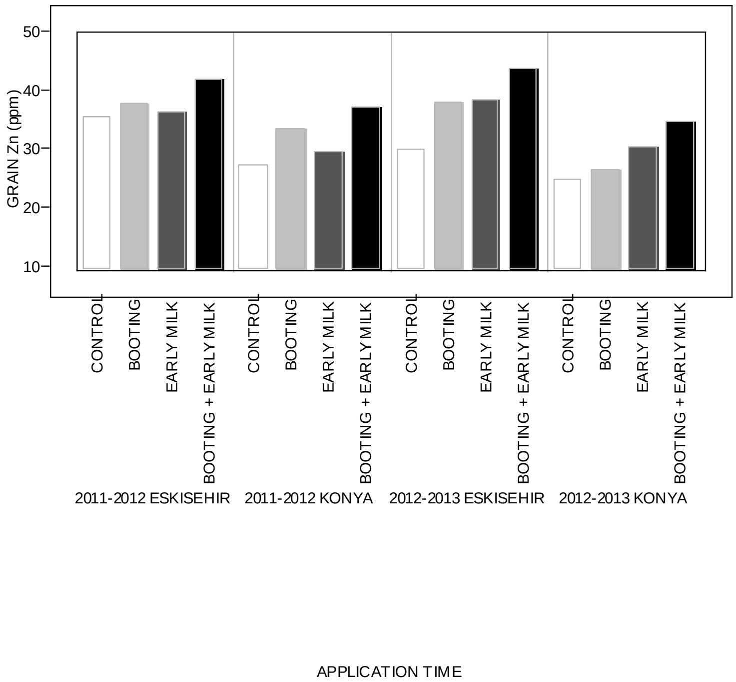

Figure 2. Effect of the application time of foliar $ZnSO_4$ on grain Zn concentrations at Eskisehir and Konya locations

EXPERIMENT 3

This experiment was carried out only in Turkey among collaborating countries. Grain Zn concentrations obtained in the experiment in the first and second year are presented in Tables 17 and 18, respectively. Changes in grain Zn due to treatments were small and not statistically significant at Konya location, except for Treatment 17 (LC + 0.3 % $ZnCl_2$) in the first year. But significant effects were observed at Eskisehir. However, in the second year of the experiment, almost all treatments, except for early foliar application at tillering stage at Konya, significantly improved grain Zn concentrations.

Effect by timing of foliar Zn application:

In the first year, both application times (tillering and post-flowering) of foliar $ZnSO_4$ resulted in significant increases in grain Zn concentrations at Eskisehir, but not at Konya. Although post-flowering application gave a slightly higher grain Zn than application at tillering, the difference due to timing was not statistically significant (Table 17). In the second year, results were similar at Eskisehir, however, postflowering application of $ZnSO_4$ improved grain Zn also at Konya, which was not the case in the first year.

Effect of solution concentration and urea and/or H_2SO_4 addition:

In the first year, 0.5 % $ZnSO_4$ application at post-flowering period resulted in higher grain Zn than 0.3 % $ZnSO_4$, at Eskisehir location, particularly when urea was not added (Table 17). When urea added, though, the difference due to concentration was much smaller. This means that the contribution of urea to increasing the effect of applied $ZnSO_4$ on grain Zn was greater when 0.3 % $ZnSO_4$ solution was used. Post-flowering application of 0.5 % $ZnSO_4$ with addition of urea resulted in the highest grain Zn concentration (42.5 mg kg^{-1}) among all 18 treatments, followed by post-flowering application of 0.3 % $ZnSO_4$ with addition of urea (41.1 mg kg^{-1}) (Table 17). This shows the significant contribution of urea to the effectiveness of applied Zn, particularly when the solution concentration is lower, presumably through facilitating the penetration of foliar-applied Zn. Similar effect of urea was also observed in the second year and this time urea was also effective at Konya location when used with lower concentration of $ZnSO_4$. This effect of urea is presented graphically in Figure 3 for the 3 of the 4 experiments where it was effective.

Effect of other Zn forms:

These forms were compared only in the first year of the experiment. Among the 3 other forms of Zn (ZnO, $ZnCl_2$ and ZnEDTA), all with 0.3 % concentration, $ZnCl_2$ resulted in the highest grain Zn (40.7 mg kg-1), followed by ZnEDTA (38.0 mg kg^{-1}), at Eskisehir. $ZnCl_2$ was the only treatment improving grain Zn significantly at Konya location. ZnO was not effective (Table 17).

Effect of solution pH:

In the first year, H_2SO_4 was added in some treatments with the expectation that lowering the phloem pH would improve the translocation of applied Zn into developing grains. However, the effect of H_2SO_4 was not significant in this particular experiment (Table 17). In the second year, effects of lowering the solution pH by use of either HCl or citric acid were searched. Use of citric acid with and without urea addition was also involved. Lowering the solution pH to 6.0 or 4.5 was compared with the control, which was the tap water with a pH of 8.3. pH-reducing treatments were effective in increasing grain Zn as compared to high-pH tap water at both locations, while only reducing pH to 6.0 improved grain Zn, further reducing the pH to 4.5 not being effective. At Eskisehir, the highest grain Zn values were obtained by reducing the solution pH to 6.0 with citric acid, whereas HCl addition down to pH 6.0 was the best treatment at Konya Addition of urea to citric acid did not make a significant difference (Table 18). These effects are shown in Figure 4.

CONCLUSIONS

- Soil applications of Zn containing fertilizers did not result in yield increases in Experiment 1 at either location, in any of the 2 years of the experiment. Yields were not expected to be affected significantly by foliar applications in Experiments 2 and 3, anyway, since our previous experiments had revealed that foliar applications are not as effective on grain yield as soil applied Zn.

- Leaf Zn concentrations were not affected by the treatments in Experiment 1, where Zn containing fertilizers were applied to soil.

- Grain Zn concentrations were significantly affected by Zn treatments in Experiment 1 at both locations. Together with Treatment 5, the only treatment also involving foliar Zn application in this experiment, ADOB HBEDZn resulted in the highest improvement in grain Zn according to the first year's results. In the second year, though, Treatment 5 was the only effective treatment.

Table 17. Wheat grain Zn concentrations in Experiment 3 at Eskisehir and Konya locations of Turkey in 2011-2012 growing year.

TREATMENT	**GRAIN Zn CONCENTRATION (mg kg^{-1})**	
	ESKISEHIR	**KONYA**
1. Local control (LC)	32.9	28.9
2. LC + 0.5 % $ZnS0_4$ (tillering)	38.2	30.6
3. LC + 0.5 % $ZnS0_4$+herbicide (tillering)	33.3	28.7
4. LC + herbicide (tillering)	32.6	26.9
5. LC + 0.5 % $ZnS0_4$ (post flowering)	40.0	28.9
6. LC + Suni pest pesticide +0.5% $ZnSO_4$ (post flowering)	39.5	27.3
7. LC + Suni pest pesticide (post flowering)	31.2	31.2
8. LC + 0.3 % $ZnS0_4$ (post flowering)	33.8	29.0
9. LC + 0.3 % $ZnS0_4$ + urea (post flowering)	41.1	31.2
10. LC + 0.5 % $ZnS0_4$ (post flowering)	38.8	28.8
11. LC + 0.5 % $ZnS0_4$ + urea (post flowering)	42.5	31.3
12. LC + 0.4 %$ZnS0_4$ (pH=8.3 by Tap water) (Twice app.)	35.9	29.2
13. LC + 0.4 % $ZnS0_4$ (pH=6.0 by HCl) (Twice app.)	37.3	31.5
14.LC + 0.5 % Zn $S0_4$ (post flowering) + H_2SO_4	38.4	29.7
15.LC + 0.5 % Zn $S0_4$+ urea (post flowering) + H_2SO_4	40.0	32.1
16.LC + 0.3 % ZnO	32.7	29.7
17. LC + 0.3 % $ZnCl_2$	40.7	37.3
18. LC + 0.3 % ZnEDTA	38.0	30.9
CV (%)	7.9	9.9
F TEST	**	**
LSD (0.05)	4.2	4.2

Table 18. Wheat grain Zn concentrations in Experiment 3 at Eskisehir and Konya locations of Turkey in 2012-2013 growing year.

TREATMENT	**GRAIN Zn CONCENTRATION ($mg\ kg^{-1}$)**	
	ESKISEHIR	**KONYA**
1. Local control (LC)	30.3	23.5
2. LC + 0.5 % $ZnS0_4$ (tillering)	42.0	25.5
3. LC + 0.5 % $ZnS0_4$+herbicide (tillering)	42.0	27.8
4. LC + herbicide (tillering)	32.3	25.3
5. LC + 0.5 % $ZnS0_4$ (post flowering)	43.0	30.5
6. LC +Suni pest pesticide +0.5 %$ZnSO_4$ (post flowering)	47.5	32.5
7. LC + Suni pest pesticide (post flowering)	31.5	23.0
8. LC + 0.3 % $ZnS0_4$ (post flowering)	37.8	26.3
9. LC + 0.3 % $ZnS0_4$ + urea (post flowering)	44.3	33.3
10. LC + 0.5 % $ZnS0_4$ (post flowering)	39.5	33.5
11. LC + 0.5 % $ZnS0_4$ + urea (post flowering)	43.5	30.5
12. LC + 0.4 %$ZnSO_4$ (pH= 8.3 by Tap water (Twice))	41.0	32.3
13. LC + 0.4 %$ZnSO_4$ (pH= 6.0 by HCl (Twice))	46.0	40.5
14. LC + 0.4 %$ZnSO_4$ (pH= 4.5 by HCl (Twice))	48.5	36.0
15. LC + 0.4 %$ZnSO_4$ (pH= 6.0 by Citric acid (Twice))	51.5	36.3
16. LC + 0.4 %$ZnSO_4$ + 0.5% Urea (pH= 6.0 by Citric acid (Twice)	54.8	37.8
17. LC + 0.4 %$ZnSO_4$ (pH= 4.5 by Citric acid (Twice))	44.5	35.8
18. LC + 0.4 %$ZnSO_4$ + 0.5% Urea (pH= 4.5 by Citric acid (Twice))	47.8	35.5
CV (%)	8.2	11.3
F TEST	**	**
LSD (0.05)	5.0	5.0

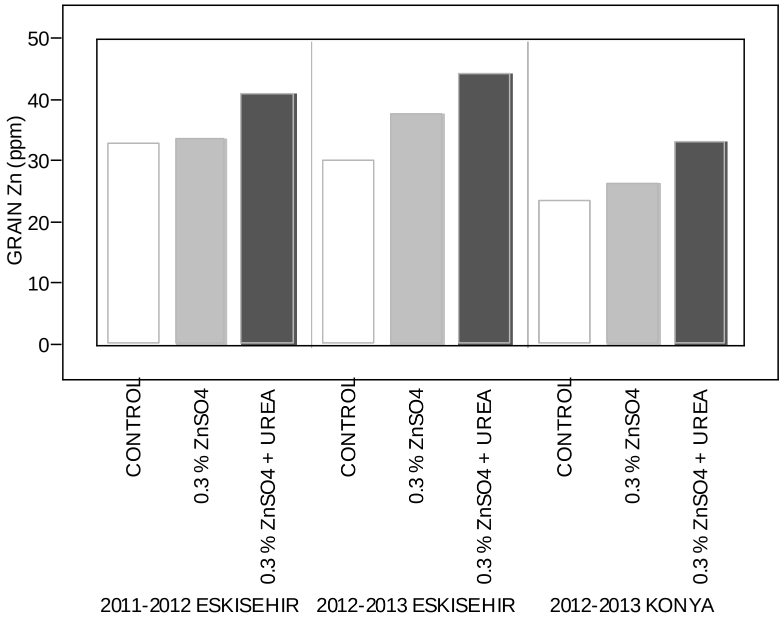

Figure 3. The effects of urea addition to $ZnSO_4$, applied at post flowering, on grain Zn concentration.

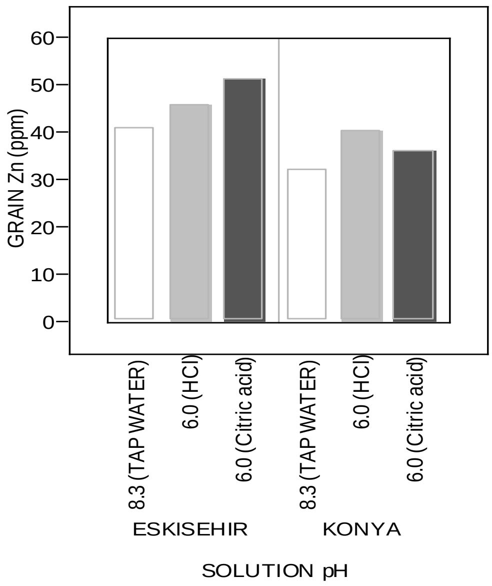

Figure 4. The effects of lowering the solution pH, by use of HCl or citric acid on grain Zn concentration.

- In Experiment 2, where foliar applications were compared, applying $ZnSO_4$ twice (at booting and early milk stages) gave higher grain Zn than applying only once, in both years.

- In Experiment 2, FBScience, HP 2011 and HP 2012 were the other foliar Zn fertilizers resulting in significant improvements in grain Zn concentrations at both locations in the first year. OMEX II and Valagro Brexil were also effective, in that aspect, at Eskisehir location but not at Konya. In the second year, HP 2011 and HP 2012 were still effective, other chemicals giving somehow different results than the first year's experiment.

- In the first year of Experiment 3, which was carried out only in Turkey, higher concentration of foliar-applied $ZnSO_4$ (0.5 %) resulted in higher grain Zn values than 0.3 % concentration when urea was not added. However, this difference diminished when urea was added. Addition of urea to foliar Zn significantly increased grain Zn when lower concentration of $ZnSO_4$ was used. This effect was interpreted as the facilitation of penetration of foliar-applied Zn.

- Among other forms of foliar applied Zn, 0.3 % $ZnCl_2$ gave the best result at both locations.

- In the second year of the experiment, reducing the solution pH from 8.3 to 6.0 significantly improved grain Zn concentrations. Citric acid was more effective, in that aspect, at Eskisehir location, whereas HCl was more effective at Konya. It is implicative of the fact that reducing the solution pH might improve translocation of the applied Zn into grains through also lowering the phloem pH.

5. TRAINING AND VISIBILITY ACTIVITIES:

Up to date 2 PhD and 3 MSc students have completed their thesis projects under the HarvestZinc project.

A zinc day, coordinated by Sabanci University and Transitional Region Agricultural Research Institute, was organized at Osmangazi University, Eskisehir, on December 26, 2013. Among approximately 300 attendants, 110 were regional farmers. Other attendants included representatives of Ministry of Agriculture, different universities, regional extension services, seed companies, regional bread and biscuit industries. The presentations given at the occasion are listed below:

- History of the Zn deficiency in Central Anatolian Plateau- M. Kalayci- Cereal Agronomist.
- Zn in soils: Availability by plants- A. Gunes- Prof. of Soils, Ankara University.
- Why do plants need Zn- I. Cakmak- Prof. of Plant Physiology, Sabanci University, Istanbul.
- Zn fertilization of crops- S. Gezgin- Prof. of Soils- Selcuk University, Konya.
- Zn in nutrition and health of animals- B. Coskun- Prof. of Veterinary Sciences- Selcuk University, Konya.
- Zn in nutrition and health of human beings- G. Pekcan- Prof. of Nutrition and Dietetics, Hacettepe University, Ankara.

Some photographs taken at the meeting are given in Figure 5.

Figure 5: Appearances from the Zinc Day organized in Eskisehir on December 26, 2013.

6. PROBLEMS ENCOUNTERED:

N:A:

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COUNTRY REPORT - CHINA

1. COLLABORATING INSTITUTIONS:

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COORDINATING INSTITUTION:
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COLLABORATING INSTITUTIONS:
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2. INTRODUCTION:

China has about 1.3 billion people, about 22% of the world population, but has only 9% of world's arable land. Agricultural production is always a big concern for the Chinese government for this enormous population with limited land. In order to feed such a big population, China has been the biggest fertilizer producer and consumer in the world. However, unbalanced fertilization, characterized by an increasingly use of nitrogen and phosphorus fertilizers, and intensified agricultural production, has created widespread Zn deficiencies throughout China.

According to the second National Soil Survey of China, conducted in 1980s, about 51% of the soils are low in Zn content. The latest soil testing results of 28258 soil samples, collected from 31 provinces during 1955-2004, indicated Zn deficiency increased to 61% nationwide (Jiyun Jin, IFA Micronutrient Fertilizer Symposium, Kunming, China, 2006). The widespread Zn deficiency in soils severely limited not only crop yield, but also crop quality and human health.

About 0.1 billion Chinese have trouble in Zn intake, and Zn malnutrition mainly impact children's growth (Ma *et al*., 2008). 30-60% of the children encounter Zn deficiency. Therefore the Zn complement is important in China.

3. EXPERIMENTAL ACTIVITIES

3.1 Experiment locations

The four wheat experiments were conducted at Wangzhuang village, Situan Town, Quzhou County, Hebei province and Yongshou town, Shaanxi province, in 2011-2012 and 2012-2013. The four rice experiments were conducted in Rudong county, Jiangsu and Anhui province, in 2011-2012. The experimental locations are shown in Figure 1.

Figure 1: The locations of experiments conducted in 2011-2012 and 2012-2013 in China. Wheat experiments were located in Hebei and Shaanxi provinces, rice experiments were located in Jiangsu and Anhui provinces.

3.2 Cultivars

Liangxing 99 and Jimai 47 wheat cultivars were used in Hebei and Shaanxi provinces, respectively. Japonica rice cultivar Zhendao 11 was used in Jiangsu and Anhui provinces.

3.3 Treatments

Two experiments were conducted for each crop, one involving soil-applied Zn-containing fertilizers and the other involving different foliar Zn treatments. The same treatments were applied for both crops at total of four locations. Experiment 1 had 10 treatments while Experiment 2 had 12. The detailed information about the treatments in these 2 experiments is listed in Table 1.

Table 1. List of the treatments of wheat and rice experiments conducted in 2011 and 2012 in China.

TREATMENTS OF EXPERİMENT 1 (SOIL TREATMENTS)	TREATMENTS OF EXPERIMENT 2 (FOLIAR TREATMENTS)
1.Local Control (LC)	1. Local control (LC)
2.LC + Soil $ZnSO_4.7H_2O$	2. LC+ Foliar $ZnSO_4$ (booting)
3.LC + Mosaic I	3. LC+ Foliar $ZnSO_4$ (early milk)
4.LC + Mosaic II	4. LC+ Foliar $ZnSO_4$ (booting + early milk)
5.LC + Mosaic I + Foliar Zn	5. LC+ OMEX II
6.LC + Kali KornKali	6. LC+ OMEX III
7.3 x Split Urea-Zn	7. LC+Kali-EPSO
8. LC+ADOB HBEDZn	8. LC+ ADOB ZnIDHA
9. LC + high seed Zn (Foliar in last crop)	9. LC+ Valagro Brexil
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	10. LC+ Antracol
	11. LC+ Pesticide
	12. LC+ FBScience

3.4 Soil properties

Before sowing, the soils were sampled to analyze the soil pH and available Zn (DTPA-Zn) concentration (Table 2).

Table 2. The soil pH and DTPA-Zn concentrations of tested soils of four locations.

Soil property	Hebei		Shaanxi		Jiangsu		Anhui	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
pH	7.8	7.8	7.8	7.8	8.2	8.4	6.3	6.4
DTPA-Zn (mg kg^{-1})	0.33	0.33	0.37	0.37	1.38	0.82	0.61	0.46

4. RESULTS AND DISCUSSIONS:

4.1. Winter wheat:

Field appearances of the wheat experiment in Hebei province are shown in Figure 2.

Figure 2. Field appearances of the wheat experiment in Hebei province, a) at regreening; b) at ripening stages.

Seedling emergence:

The seedling emergences of winter wheat were measured in Hebei and Shaanxi provinces in 2011-2012 and 2012-2013 (Table 3).

In 2011-2012, in Hebei province, soil application of different Zn fertilizer had effects on seedling emergence. Treatment 9 (LC+Seed Zn-I, where seeds were enriched with Zn by Zn spray) had the highest seedling emergence as compared to other treatments. It seems that the seeds with Zn enrichment would be benefitable for seedling emergence. However, seeds enriched with Zn by priming with Zn (Treatment 10; LC+Seed Zn-II) had the lowest seedling emergence in 2011-2012. The seedling emergences in Shaanxi province were higher than in Hebei province. The main reason for that was the higher seed rate used in Shaanxi. In Shaanxi province, there is no irrigation for winter wheat growth; therefore higher seeding rate is required for high yields.

As similar to the results in 2011-2012, the seedling emergences from Treatment 9 was the highest also in 2012-2013. Control plots and Treatment 8 had the lowest seedling emergence rate in Hebei province (Table 3).

In Shaanxi province in 2011-2012, Treatment 2 (LC+Soil $ZnSO_4{\bullet}7H_2O$) and Treatment 9 (LC+ Seed Zn-I) resulted in the highest rate of seedling emergences as Zn in seeds had the “starting fertilizer” effect on the seedling emergence. In 2012-2013, Treatments 6 and 9 gave the highest rate of seeding emergences.

Table 3. Effect of soil application of Zn fertilizers on seedling emergence (plant m^{-2}) of winter wheat in Hebei and Shaanxi in 2011-2012 and 2012-2013.

TREATMENT	HEBEI PROVINCE		SHAANXI PROVINCE	
	2011-2012	2012-2013	2011-2012	2012-2013
1. Local control (LC)	295 bc	360 cd	436 ab	275 bc
2. LC + Soil $ZnSO_4.7H_2O$	295 b	395 abc	486 a	290 bc
3. LC+Mosaic I	310 b	380 bcd	418 ab	285 bc
4. LC+Mosaic II	295 b	394 abc	464 ab	288 bc
5. LC+MosaicI+FoliarZn	310 b	383 bcd	401 b	303 bc
6. LC + Kali KornKali	305 b	392 abc	451 ab	314 ab
7. 3 x Split Urea-Zn	315 b	394 abc	462 ab	286 bc
8. LC+ADOB HBEDZn	290 bc	352 d	448 ab	296 bc
9. LC + high seed Zn (Foliar in last crop)	350 a	425 a	477 ab	351 a
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	265 c	404 ab	425 ab	273 c
LSD (0.05)	31	39	79	41
CV (%)	6.9	6.4	12.2	9.0

It was checked whether seeds enriched with zinc also had tendency to improve seedling emergences on farmers’ fields (Table 4) in 2012-2013.

Table 4. Effect of seeds enriched with Zn on seedling emergence of winter wheat in Hebei province during 2012-2013 on farmers' fields.

Treatment no	Treatment	Seedling emergence (plants m^{-2})	
		Farmer 1	Farmer 2
1	Local Control (LC)	398 a	335 a
9	LC+ Seed Zn-I	422 a	379 a
LSD (0.05)		50.9	72.1
CV (%)		7.1	11.7

Plant height:

The plant height of winter wheat was measured at regreening stage in Hebei province in 2011-2012, two months after sowing in October 2012-2013 and at elongation stage in Shaanxi province in 2011-2012 (Table 5). Zinc fertilizer improved the growth of winter wheat in both provinces. In Hebei province, Treatment 9 (LC+Seed Zn-I) and Treatment 2（LC+Soil $ZnSO_4$•$7H_2O$）increased plant height 11.8 % and 9.1%, respectively, over control treatment in 2011-2012. Similarly, the plant height was higher with Treatment 9 than other treatments at elongation stage in Shaanxi province.

But in 2012-2013, Zn application did not have a significant effect on plant height of winter wheat. Except for treatments 3 and 10, other treatments had lower plant height than that of control.

Dry weight:

The plant materials were sampled before the first foliar application of Zn fertilizers. The dry weight of shoot and flag leaves were measured (Table 6). In Hebei province, Zn fertilizers affected the dry weight of shoot and flag leaves. As compared with to control treatment, soil application of Zn fertilizers improved the dry weight of shoot and flag leaves to different extent. Treatment 4 (MESZ-Zn double amount), Treatment 6 (Kali-Zn), and Treatment 10 (seeds primed with Zn) increased shoot DW 25.4 %, 33.5 % and 32.8 %, respectively. (Table 6). Similarly, the DW of flag leaves was increased by soil application of Zn fertilizers. Treatment 9 resulted in the highest DW of flag leaves.

Similar results were obtained in 2012-2013 cropping season. Treatment 8 and Treatment 9 resulted in higher dry weight of flag leaves than the other treatments.

Table 5. Effect of soil application of different Zn fertilizers on plant height of winter wheat in Hebei and Shaanxi provinces during 2011 - 2013.

TREATMENT	PLANT HEIGHT (cm)			
	HEBEI PROVINCE		SHAANXI PROVINCE	
	2011-2012	2012-2013	2011-2012	2012-2013
1. Local control (LC)	15.6 d	15.4 c	33.8 c	28.0 a
2. LC + Soil $ZnSO_4.7H_2O$	17.1 ab	16.7 a	34.1 bc	26.2 ab
3. LC+Mosaic I	16.5 bc	16.2 ab	33.6 c	28.2 a
4. LC+Mosaic II	16.8 bc	16.4 ab	33.3 c	27.3 ab
5. LC+MosaicI+FoliarZn	16.8 bc	16.1 b	34.8 bc	25.7 b
6. LC + Kali KornKali	16.2 cd	16.1 b	34.4 bc	27.2 ab
7. 3 x Split Urea-Zn	16.5 bc	16.3 ab	33.9 c	27.1 ab
8. LC+ADOB HBEDZn	16.7 bc	16.2 ab	35.9 abc	27.9 a
9. LC + high seed Zn (Foliar in last crop)	17.5 a	16.6 ab	39.1 a	22.6 c
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	16.9 ab	16.7 a	37.5 ab	28.3 a
LSD (0.05)	0.7	0.6	3.4	2.2
CV (%)	2.5	2.3	5.4	5.4

Table 6. Effect of soil application of Zn fertilizers on shoot dry weight of winter wheat before the first foliar application (booting stage) in Hebei province during 2011-2012 and 2012-2013.

TREATMENT	DRY WEIGHT		
	SHOOTS ($t\ ha^{-1}$)	FLAG LEAVES (g / 80 leaves)	
		2011-2012	2012-2013
1. Local control (LC)	11.8 b	7.12 c	6.68 b
2. LC + Soil $ZnSO_4.7H_2O$	14.7 a	7.75 abc	7.03 b
3. LC+MosaicI	13.4 ab	8.18 a	6.92 b
4. LC+MosaicII	14.7 a	7.89 abc	7.73 ab
5. LC+MosaicI+FoliarZn	14.0 ab	7.82 abc	7.14 ab
6. LC + Kali KornKali	15.7 a	7.61 abc	7.73 ab
7. 3 x Split Urea-Zn	14.8 a	8.06 ab	6.99 b
8. LC+ADOB HBEDZn	14.0 ab	7.34 bc	8.16 a
9. LC + high seed Zn (Foliar in last crop)	14.3 ab	8.23 a	8.09 a
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	15.7 a	7.75 abc	7.23 ab
LSD (0.05)	2.7	0.79	1.06
CV (%)	13.6	6.1	9.4

SPAD reading:

The SPAD reading (chlorophyll levels) of flag leaves was performed in Hebei province at booting stage (Table 7). The SPAD values of flag leaves were slightly affected by soil application of Zn fertilizers and Treatment 1 (control) had the lowest SPAD value.

Table 7. Effect of soil application of Zn fertilizers on SPAD reading of winter wheat flag leaves at booting stage in Hebei province in 2011 – 2012 growing season.

TREATMENT	SPAD READING
1. Local control (LC)	50.9 c
2. LC + Soil $ZnSO_4.7H_2O$	52.3 a
3. LC+MosaicI	52.6 a
4. LC+MosaicII	51.9 ab
5. LC+MosaicI+FoliarZn	52.4 a
6. LC + Kali KornKali	52.1 a
7. 3 x Split Urea-Zn	52.0 a
8. LC+ADOB HBEDZn	52.1 a
9. LC + high seed Zn (Foliar in last crop)	51.1 bc
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	52.2 a
LSD (0.05)	0.9
CV (%)	1.2

Grain yield:

At harvest stage, the grain yield of winter wheat was obtained in both provinces in 2011-2012 and 2012-2013 (Table 8). The harvest index values were obtained in Hebei in both years while in Shaanxi they were calculated only in the first year.

In Hebei province, in 2011-2012, soil application of different Zn fertilizers had no significant effect on grain yield of winter wheat as compared with control, except for Treatment 10 which gave the lowest yield due to lower rate of emergence. Harvest index was not much affected by soil application of Zn-containing fertilizers, either. In Shaanxi province, soil application of Zn fertilizers had no effect on grain yield and Harvest index in 2011-2012, either (Table 8 and Table 9).

In 2012-2013, neither grain yields, nor harvest indices were significantly affected by treatments in either location.

Table 8. Effect of soil application of Zn fertilizers on wheat grain yield in Hebei and Shaanxi provinces in 2011-2012 and 2012-2013 (14 % water content).

TREATMENT	GRAIN YIELD ($t\ ha^{-1}$)			
	HEBEI		SHAANXI	
	2011-2012	2012-2013	2011-2012	2012-2013
1. Local control (LC)	8.20 ab	8.38 ab	7.06	2.65
2. LC + Soil $ZnSO_4.7H_2O$	8.71 a	8.45 ab	7.28	2.56
3. LC+Mosaic I	8.35 a	8.98 ab	7.68	2.53
4. LC+Mosaic II	8.58 a	9.47 a	7.00	2.73
5. LC+MosaicI+FoliarZn	8.28 a	7.96 b	6.98	2.16
6. LC + Kali KornKali	8.33 a	8.53 ab	7.27	2.19
7. 3 x Split Urea-Zn	8.30 a	8.81 ab	7.18	2.68
8. LC+ADOB HBEDZn	8.20 ab	9.18 a	7.51	2.68
9. LC + high seed Zn (Foliar in last crop)	8.25 ab	8.54 ab	7.03	2.18
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	7.65 b	8.62 ab	6.87	2.77
LSD (0.05)	0.60	0.78	n.s.	n.s.
CV (%)	5.4	8.5	7.7	24.7

Table 9. Effect of soil application of Zn fertilizers on harvest index (HI) in Hebei and Shaanxi provinces in 2011-2012 and 2013 (Only 2011-2012 in Shaanxi).

TREATMENT	HARVEST INDEX (%)		
	HEBEI		SHAANXI
	2011-2012	2012-2013	2011-2012
1. Local control (LC)	45.8 ab	45.0	47.8
2. LC + Soil $ZnSO_4.7H_2O$	45.7 ab	44.7	47.6
3. LC+Mosaic I	46.0 ab	44.0	47.5
4. LC+Mosaic II	45.2 b	45.1	47.6
5. LC+MosaicI+FoliarZn	45.8 ab	43.9	47.7
6. LC + Kali KornKali	45.9 ab	44.2	48.0
7. 3 x Split Urea-Zn	47.4 a	45.5	48.4
8. LC+ADOB HBEDZn	45.9 ab	44.0	48.2
9. LC + high seed Zn (Foliar in last crop)	46.6 ab	45.4	46.5
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	46.4 ab	42.9	48.6
LSD (0.05)	2.1	n.s.	n.s.
CV (%)	4.1	3.3	3.4

In Hebei province, foliar application of different Zn fertilizers (Experiment 2) had no significant effect on grain yield of winter wheat. Foliar application also had no significant effect on harvest index in Hebei province. In Shaanxi province, foliar application of Zn tended to decrease grain yield as compared to control treatment in the first year but there was no effect in the second year (Tables 10 and 11).

Table 10. Effect of foliar application of Zn fertilizers on grain yield of winter wheat in Hebei and Shaanxi provinces in 2011- 2012 and 2012-2013.

TREATMENT	GRAIN YIELD ($t\ ha^{-1}$)			
	HEBEI		SHAANXI	
	2011-2012	2012-2013	2011-2012	2012-2013
1. Local control (LC)	7.81	7.82	7.21 a	3.56
2. LC+ Foliar $ZnSO_4$ (booting)	8.00	8.12	7.03 ab	3.78
3. LC+ Foliar $ZnSO_4$ (early milk)	7.84	8.32	6.24 c	4.11
4. LC+ Foliar $ZnSO_4$ (booting + early milk)	7.63	8.66	6.39 bc	3.69
5. LC+ OMEX II	7.91	8.52	6.15 c	3.66
6. LC+ OMEX III	8.23	8.35	6.62 abc	3.76
7. LC+Kali-EPSO	7.96	8.66	6.58 abc	3.64
8. LC+ ADOB ZnIDHA	7.81	7.74	7.08 ab	3.44
9. LC+ Valagro Brexil	7.67	8.44	6.72 abc	3.83
10. LC+ Antracol	7.81	8.38	7.02 ab	3.74
11. LC+ Pesticide	7.99	8.43	6.48 bc	3.46
12. LC+ FBScience	7.92	8.15	6.48 bc	3.45
LSD (0.05)	n.s.	n.s.	0.7	n.s.
CV (%)	7.5	7.8	7.2	13.1

Table 11. Effect of foliar application of Zn fertilizers on harvest index of winter wheat in Hebei and Shaanxi provinces in 2011- 2012.

TREATMENT	HARVEST INDEX (%)	
	HEBEI	SHAANXI
1. Local control (LC)	42.6 bc	44.5 bcd
2. LC+ Foliar $ZnSO_4$ (booting)	41.3 bc	44.2 bcd
3. LC+ Foliar $ZnSO_4$ (early milk)	43.1 bc	43.8 cd
4. LC+ Foliar $ZnSO_4$ (booting + early milk)	42.3 bc	43.4 d
5. LC+ OMEX II	46.0 a	42.9 d
6. LC+ OMEX III	42.7 bc	44.7 bcd
7. LC+Kali-EPSO	43.1 bc	46.8 a
8. LC+ ADOB ZnIDHA	43.9 ab	46.1 ab
9. LC+ Valagro Brexil	40.6 c	45.5 abc
10. LC+ Antracol	43.5 ab	46.1 ab
11. LC+ Pesticide	43.6 ab	45.4 abc
12. LC+ FBScience	41.7 bc	44.7 bcd
LSD (0.05)	2.7	2.0
CV (%)	3.9	2.8

Table 12 shows that seeds enriched with Zn improved wheat yield on farmers' fields in Hebei and Shaanxi provinces. The increase rates ranged from 0.3% to 51.9% among the five experiments.

Table 12. Effect of seed enriched with Zn on yield of winter wheat in Hebei and Shaanxi provinces during 2012- 2013.

Treatment	Hebei province		Shaanxi province		
	Farmer 1	Farmer 2	Farmer 1	Farmer 2	Farmer 3
LC+ Seed Zn-I	8.28	8.57	3.86	4.46	3.99
Local Control (LC)	7.26	8.54	3.27	2.94	2.66

Figure 13 shows the effect of seed enrichment on grain Zn concentration on the farmers' fields. The effect was variable and mostly in favor of the control, presumably due to higher yields.

Table 13. Effect of seed enrichment with Zn on grain Zn concentration (mg kg^{-1}) of winter wheat in Hebei and Shaanxi provinces during 2012- 2013 on the farmer fields

Treatment	Hebei province		Shaanxi province		
	Farmer 1	Farmer 2	Farmer 1	Farmer 2	Farmer 3
LC+ Seed Zn	31.67	30.33	24.00	22.00	17.00
Local Control (LC)	34.00	35.00	20.00	21.00	22.00

Leaf Zn concentration:

Before the first foliar application (booting stage), flag leaves from soil application experiment were collected. Results are given in Table 14. Treatment 2 (LC+soil $ZnSO_4{\bullet}7H_2O$) resulted in the highest Zn concentration. It improved leaf Zn 11.6 % over control. and Treatment 8 (LC+ ZnHBED) increased leaf Zn 8.1 %. In 2012-2013, Treatments 2 and 8 were the treatments resulting in the highest leaf Zn concentrations in Hebei, like the first year. Treatment 2 was also the best in Shaanxi.

Table 14. Effect of soil application of Zn fertilizers on flag leaf Zn concentration of winter wheat before the first foliar application (booting stage) in 2011- 2012 and 2012-2013.

TREATMENT	FLAG LEAF Zn CONCENTRATION (mg kg^{-1})		
	HEBEI		SHAANXI
	2011-2012	2012-2013	2012-2013
1. Local control (LC)	28.0 bc	22.5 b	19.3 b
2. LC + Soil $ZnSO_4.7H_2O$	31.3 a	25.5 a	22.0 a
3. LC+Mosaic-I	28.5 bc	21.0 bcd	19.0 b
4. LC+Mosaic-II	27.5 c	20.0 bcd	17.8 bc
5. LC+MosaicI+FoliarZn	28.0 bc	21.8 bc	20.3 ab
6. LC + Kali KornKali	28.5 bc	21.3 bcd	18.0 bc
7. 3 x Split Urea-Zn	29.5 abc	21.5 bc	19.5 ab
8. LC+ADOB HBEDZn	30.3 b	25.8 a	19.5 ab
9. LC + high seed Zn (Foliar in last crop)	23.0 d	19.0 cd	18.3 bc
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	23.5 d	18.5 d	15.8 c
LSD (0.05)	2.6	2.8	2.7

Grain Zn concentration:

At harvest, the grain Zn concentration of winter wheat was measured in both provinces in both years (Table15).

In Hebei province, soil Zn application improved grain Zn concentration. Treatment 2 (LC+ Soil $ZnSO_4$•$7H_2O$), Treatment 5 (LC + Mosaic-III) and Treatment 8 (LC+ ZnHBED) treatments resulted in the highest grain Zn concentrations. In Shaanxi province, Treatments 5 and 8 gave the highest grain Zn (Table15). Treatment 5 also resulted in the highest grain Zn concentrations at both provinces in 2012-2013. Treatment 8 was effective at Hebei but not at Shaanxi in the second year's experiment.

Table 15. Effect of soil application of Zn fertilizers on grain Zn concentration of winter wheat in Hebei and Shaanxi provinces in 2011- 2012 and 2012-2013.

TREATMENT	**GRAIN Zn CONCENTRATION ($mg\ kg^{-1}$)**			
	HEBEI		**SHAANXI**	
	2011-2012	**2012-2013**	**2011-2012**	**2012-2013**
1. Local control (LC)	32.5 cde	36.0 abc	22.0 b	19.8 bcd
2. LC + Soil $ZnSO_4.7H_2O$	38.5 b	39.5 a	22.3 b	22.5 b
3. LC+Mosaic-I	32.3 def	31.8 c	20.8 b	18.5 cde
4. LC+Mosaic-II	28.5 fg	37.0 ab	21.5 b	17.0 de
5. LC+MosaicI+FoliarZn	42.8 a	36.0 abc	28.5 a	29.0 a
6. LC + Kali KornKali	36.3 bcd	33.5 bc	23.3 b	18.5 cde
7. 3 x Split Urea-Zn	33.3 cde	33.3 bc	23.0 b	19.0 cde
8. LC+ADOB HBEDZn	37.3 b	37.3 ab	30.5 a	21.0 bc
9. LC + high seed Zn (Foliar in last crop)	31.8 efg	32.0 c	19.5 b	18.8 cde
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	28.0 g	33.0 bc	21.3 b	16.6 e
LSD (0.05)	4.2	4.5	5.0	2.9
CV (%)	7.3	8.4	13.4	9.6

Foliar application of Zn fertilizers increased grain Zn concentration of winter wheat more than soil applications. Analysis results are given in Table 16. Rate of increase in grain Zn concentration due to treatments ranged between 5.4 % and 50.8 % in Hebei, and between 13.5 % and 96.5 % in Shaanxi in 2011-2012 (Table 16). In 2012-2013 growing season, percentage of increase in grain Zn concentration ranged from 7.7 % to 30.8 % in Hebei and from 35.1 % to 113.5 % in Shaanxi.

Table 16. Effect of foliar application of Zn fertilizers on grain Zn concentration of winter wheat in Hebei and Shaanxi provinces in 2011- 2012 and 2012-2013.

TREATMENT	GRAIN Zn CONCENTRATION ($mg\ kg^{-1}$)			
	HEBEI		SHAANXI	
	2011-2012	2012-2013	2011-2012	2012-2013
1. Local control (LC)	32.5 d	32.5 e	21.3 e	18.5 e
2. LC+ Foliar $ZnSO_4$ (booting)	42.3 bc	42.5 b	28.0 d	25.3 d
3. LC+ Foliar $ZnSO_4$ (early milk)	37.3 cd	40.5 bc	34.8 bc	33.8 b
4. LC+ Foliar $ZnSO_4$ (booting+milk)	47.5 ab	49.3 a	40.8 a	32.3 bc
5. LC+ OMEX II	41.5 bc	37.0 cde	35.5 bc	39.5 a
6. LC+ OMEX III	42.5 abc	39.0 bcd	34.3 bc	29.8 bcd
7. LC+Kali-EPSO	38.3 cd	35.5 cde	38.0 ab	33.0 bc
8. LC+ ADOB ZnIDHA	46.0 ab	38.3 bcd	32.5 c	28.3 cd
9. LC+ Valagro Brexil	49.0 a	35.0 de	40.3 a	31.5 bc
10. LC+ Antracol	34.3 d	37.5 bcde	25.5 d	25.0 d
11. LC+ Pesticide	38.5 cd	37.3 bcde	41.8 a	33.8 b
12. LC+ FBScience	37.8 cd	39.8 bcd	40.8 a	33.0 bc
LSD (0.05)	6.5	5.4	3.8	5.3
CV (%)	10.0	8.5	6.7	11.8

4.2. Rice

Rice experiments were conducted in Jiangsu and Anhui provinces in 2011 and 2012. Each treatment was replicated 4 times. There were only 9 treatments in Experiment 1 in Anhui province, in 2011.

Grain Yield

Grain yield obtained in Experiments 1 and 2 are given in Tables 17 and 18. In 2011, soil application of different Zn fertilizers had effects on rice yield. However, there are differences in responses between Jiangsu and Anhui provinces. In Jiangsu province, Treatment 7 (3xSplit Urea-Zn） and Treatment 9（LC- Seed Zn-I (seeds enriched with Zn by foliar Zn spray) resulted in the highest yields. The increase of yield by Treatment 7 may partly be due to the nitrogen management. In Anhui province, Treatments 3 and 5 gave the highest yields. Treatment 8 resulted in the lowest yields in both provinces in 2011.

In 2012, the rice grain yields were also affected by treatments. In Jiangsu province, Treatments 7 and 9 resulted in the highest yields and Treatment 8, which gave poor results in the first year, gave one of the best results in 2012 at both locations (Table 17).

Foliar applications did not affect yields at Jiangsu in either year. At Hebei, foliar applications resulted in statistically significant yield increases over control. Treatments 4, 7, and 8 were superior in 2011. In 2012 Treatments 4 and 7 were also superior together with Treatments 2 and 12 (Table 18).

Table 17. Effect of soil application of Zn fertilizers on grain yield of rice in Jiangsu and Anhui province in 2011 and 2012 (water concentration, 15%).

TREATMENT	GRAIN YIELD (t ha^{-1})			
	JIANGSU		ANHUI	
	2011	2012	2011	2012
1. Local control (LC)	7.77 bc	8.23 d	7.45 cd	7.47 b
2. LC + Soil $ZnSO_4.7H_2O$	7.80 abc	8.68 bcd	7.87 abc	7.87 ab
3. LC+Mosaic-I	7.56 c	8.62 cd	8.24 a	7.79 ab
4. LC+Mosaic-II	7.49 c	8.98 bc	8.03 ab	7.51 b
5. LC+MosaicI+FoliarZn	7.70 c	8.69 bcd	8.30 a	7.48 b
6. LC + Kali KornKali	7.86 abc	8.77 bcd	7.14 d	7.80 ab
7. 3 x Split Urea-Zn	8.45 ab	9.76 a	7.33 d	7.87 ab
8. LC+ADOB HBEDZn	7.58 c	9.11 abc	7.09 d	8.07 a
9. LC + high seed Zn (Foliar in last crop)	8.50 a	9.33 ab	7.60 bcd	8.17 a
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	7.77 bc	8.75 bcd	-	8.14 a
LSD (0.05)	0.7	0.7	0.5	0.5
CV (%)	10.6	4.8	4.6	3.9

Table 18. Effect of foliar application of Zn fertilizers on grain yield of rice in Jiangsu and Anhui provinces in 2011 and 2012 (water concentration, 15%).

TREATMENT	GRAIN YIELD (t ha^{-1})			
	JIANGSU		ANHUI	
	2011	2012	2011	2012
1. Local control (LC)	7.21	8.40	6.73 de	9.74 cd
2. LC+ Foliar $ZnSO_4$ (booting)	7.61	8.32	7.00 bcde	10.48 a
3. LC+ Foliar $ZnSO_4$ (early milk)	7.40	8.52	7.34 abcd	10.30 abc
4. LC+ Foliar $ZnSO_4$ (booting + early milk)	7.06	8.55	7.59 a	10.45 ab
5. LC+ OMEX II	7.69	8.33	7.30 abcd	9.87 bcd
6. LC+ OMEX III	7.22	8.37	6.59 ef	10.12 abc
7. LC+Kali-EPSO	7.78	8.22	7.48 ab	10.50 a
8. LC+ ADOB ZnIDHA	7.20	8.43	7.45 ab	9.82 cd
9. LC+ Valagro Brexil	7.70	8.28	7.11 abcde	10.06 abc
10. LC+ Antracol	7.55	8.21	6.04 f	9.51 d
11. LC+ Pesticide	7.76	8.55	6.56 ef	10.18 abc
12. LC+ FBScience	7.58	8.67	6.82 cde	10.46 ab
LSD (0.05)	n.s.	n.s.	0.6	0.6
CV (%)	6.6	5.1	5.5	3.9

Grain Zn concentration

Grain Zn concentration of rice grown in Jiangsu and Anhui provinces were determined (Tables 19 and 20).

Table 19. Effect of soil application of Zn fertilizer on Zn concentration of rice grain in Jiangsu and Anhui provinces in 2011 and 2012.

TREATMENT	GRAIN Zn CONCENTRATION ($mg\ kg^{-1}$)			
	JIANGSU		ANHUI	
	2011	2012	2011	2012
1. Local control (LC)	19.7 e	18.8 cd	18.5 c	21.0 d
2. LC + Soil $ZnSO_4.7H_2O$	21.5 bcd	19.3 cd	27.3 a	22.0 cd
3. LC+MosaicI	21.3 bcd	18.5 d	21.5 bc	21.3 d
4. LC+MosaicII	22.3 b	18.8 cd	22.3 b	21.3 d
5. LC+MosaicI+FoliarZn	24.2 a	23.0 a	23.5 b	25.3 a
6. LC + Kali KornKali	21.1 bcd	19.8 cd	21.5 bc	24.5 ab
7. 3 x Split Urea-Zn	20.3 ed	22.0 ab	20.3 bc	23.8 abc
8. LC+ADOB HBEDZn	21.1 bcd	20.8 bc	23.3 b	22.5 bcd
9. LC + high seed Zn (Foliar in last crop)	20.6 cde	20.3 bcd	21.0 bc	23.8 abc
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	21.6 bc	19.5 cd	18.5 c	23.5 abc
LSD (0.05)	1.2	2.1	3.5	2.2
CV (%)	3.5	5.7	8.5	5.8

Table 20. Effect of foliar application of Zn fertilizers on Zn concentration of rice grain in Jiangsu and Anhui provinces in 2011 and 2012.

TREATMENT	GRAIN Zn CONCENTRATION ($mg\ kg^{-1}$)			
	JIANGSU		ANHUI	
	2011	2012	2011	2012
1. Local control (LC)	19.0 f	20.0 e	19.8 c	23.0 c
2. LC+ Foliar $ZnSO_4$ (booting)	20.7 e	20.3 e	20.3 bc	28.0 abc
3. LC+ Foliar $ZnSO_4$ (early milk)	22.7 d	21.0 cde	21.3 bc	27.0 abc
4. LC+ Foliar $ZnSO_4$ (booting+milk)	24.8 bc	21.8 bcde	22.8 ab	31.8 a
5. LC+ OMEX II	23.3 cd	21.8 bcde	20.3 bc	32.0 a
6. LC+ OMEX III	24.1 bcd	20.8 de	20.0 bc	28.8 ab
7. LC+Kali-EPSO	27.6 a	22.3 abcd	25.0 a	27.8 abc
8. LC+ ADOB ZnIDHA	23.7 cd	22.3 abcd	20.5 bc	23.3 c
9. LC+ Valagro Brexil	24.7 bc	22.8 abc	21.5 bc	26.3 bc
10. LC+ Antracol	21.0 e	20.5 de	19.5 c	27.3 abc
11. LC+ Pesticide	23.3 cd	23.3 ab	21.0 bc	31.8 a
12. LC+ FBScience	25.5 b	24.0 a	21.5 bc	28.8 ab
LSD (0.05)	1.6	1.8	2.8	5.1
CV (%)	4.2	5.2	7.4	11.0

Soil Zn application improved grain Zn concentration at both locations. Treatment 5, the only treatment involving foliar Zn application, resulted in the highest grain Zn concentration in Jiangsu. However, the highest grain Zn was obtained by Treatment 2 (LC + Soil $ZnSO_4.7H_2O$) in Anhui (Table 19).

In 2012, Treatments 5 and 7 resulted in the highest grain Zn in Jiangsu, while Treatments 5 and 6 were the best in Anhui. Over 2 years and provinces, Treatment 5, the only treatment involving foliar application, resulted in the highest grain Zn concentrations (Table 19).

Foliar Zn application also increased grain Zn concentrations. Treatment 7 (LC+Kali-EPSO) resulted in the highest grain Zn at both locations in 2011 (Table 19). In 2012, however, Treatment 12 (LC + FBScience) was the best in Jiangsu, while Treatments 4 (LC+ Foliar $ZnSO_4$ (booting + early milk) and 5 (LC+ OMEX II) gave the best results in Anhui.

Table 21 and 22 show that seed enrichment with Zn also improves rice yield in Anhui province and Jiangsu province under farmer conditions, but has no significant effect on grain Zn concentration even decreases Zn concentration, probably due to yield increase (e.g., dilution effect)

Table 21. Effect of seed enrichment with Zn on yield (Mg ha^{-1}) of rice in Anhui and Jiangsu provinces during 2012- 2013 on the farmer fields.

TREATMENT	ANHUI			JIANGSU		
	FARMER1	FARMER 2	FARMER 3	FARMER 1	FARMER 2	FARMER 3
LC+ Seed Zn-I	10.05	8.76	7.50	8.68	8.16	8.08
Local Control	9.09	7.77	6.61	8.32	7.75	7.76

Table 22. Effect of seed enrichment with Zn on grain Zn concentration (mg kg^{-1}) of rice in Anhui and Jiangsu provinces during 2012- 2013 on the farmer fields

TREATMENT	ANHUI			JIANGSU		
	FARMER 1	FARMER 2	FARMER 3	FARMER 1	FARMER 2	FARMER 3
LC+ Seed Zn-I	24.10	20.70	23.50	19.30	15.10	17.60
Local Control	29.50	21.80	24.40	20.80	15.30	17.40

4.3 SUMMARY

According to the the results obtained in 2011–2013, Zn fertilization affected seedling emergence, grain yield and Zn concentration of wheat and rice. However, the application methods and fertilizers resulted in different responses.

Soil application of Zn fertilizers increased seedling emergence of winter wheat and had advantage to improve grain yield of wheat and rice compared with foliar application approach. However, foliar application was more advantageous to improve grain Zn concentration of winter wheat and rice as compared to soil application.

There was no significant difference in seedling emergence of winter wheat among different Zn fertilizers except ZnHBED. There was no significant difference in grain yield of winter wheat among different Zn fertilizers no matter what soil or foliar application methods. But for rice yield, Urea-Zn had advantage. For grain Zn concentration of wheat, soil application of Mosaic-III and ZnHBED seemed to have advantage as compared to the other Zn fertilizers, while foliar application of Valagro Brexil had advantage as compared to the other

Zn fertilizers. As to grain yield and Zn concentration of rice, there were different responses to Zn fertilizers.

Seeds enriched with Zn improved seedling emergences of winter wheat on farmers ‘fields. The rate of improvement ranged from 0.3% to 51.9%. Grain yields were also increased by seeds enriched with Zn in Shaanxi province which has soils with lower soil Zn availability and less irrigation.

5. TRAINING AND VISIBILITY ACTIVITIES

5.1 Project training

Some graduates took part in this project. There was one short training for graduates involved in this project before experiments started. The topic was focused on details of experiment conduction, especially on meaning of each treatment, with most important notice on application of fertilizers, sampling, and management.

5.2 Zinc farmer day

3 “Farmer Zinc Days ” were held in Hebei, Shaanxi and Jiangsu provinces in 2011 and 2012.

5.2.1 The farmer zinc day in Hebei province

The Farmer Zinc Day on zinc nutrition of crops, supported by HarvestPlus and IZA, was held at Quzhou, Hebei Province on May 10, 2011. There were more than 230 participants on the Farmer Zinc Day, which included farmers, extension workers, graduate students, agricultural researchers, government policy makers, staff at Quzhou experiment station and invited experts.

The whole activity consisted of two sections. One section mainly focused on the training program, and the other section included all participants’ visit to the field experiment and the graduate students introduced their experiments. In the opening ceremony, Prof. Fusuo Zhang reviewed the history of the collaboration between CAU and Quzhou, introduced the background of the event, talked about zinc nutrition in crops and and its importance for human health. The vice mayor Xuesong Bai gave a welcome speech. He emphasized the importance of the activity and hoped that the farmers can take advantage of the opportunity and contribute to the agricultural development in the county.

Prof. Chunqin Zou, Prof. Römheld Volker (from Hohenheim University), Prof. Liyan Ma (from college of food sciences, CAU) and Prof. Deqian Mao (from Center of disease prevention and control in China) taught farmers on Zn nutrition in crops, Zn fertilizer, Zn in improvement on stresses resistance and quality, Zn in food and zinc in disease prevention. The farmers warmly welcome these teachers and appreciate the courses.

During the field visit, participants were divided into three groups, with 80 members each. The project conductors, Yueqiang Zhang, Shanchao Yue, Yan Deng, Yanfang Xue, Lili Pang, Zhongxiang Li, Peng Yan and other graduate students made several posters and introduced the field experiments. The farmers’ interest in zinc was aroused, and they intensively talked with the graduate students and the experts.

Figure 3. A group of farmers listening to speakers on the Zn day in Hebei.

5.2.2 The Farmer Zinc Day in Shaanxi province

On August 15, 2011, “Farmer Zinc Day in China” were held by Northwest Agriculture and Forestry University (NAFU) in the city of Weinan, Shaanxi province.

In the early morning, more than 300 farmers were present. Many governors, including governors from the city of Weinan, and Zhaohui Wang, the local organizer, Prof. Ismail Cakmak, general manager Shicheng Wang and some others gave welcome speeches. Prof. Ismail Cakmak, Dr Yan Xiao, Prof. Xiaohong Tian, Dr Futong Yu, Prof. Yong Zhang, Dr Xiaojuan Wang from Xi’an Jiaotong University, Xueling Jiang from Yantai Academy of Agricultural Sciences, Prof. Qiang Gao from Jilin Agricutural University, the dean Xu from Shaanxi Academy of Agricultural Sciences and Prof. Zhaohui Wang gave a series of lectures on aspect of zinc nutrition in continuum of soil-plant-animal system.

After all lectures in the meeting room, all attendants went to the experimental field at Fengyuan Town, Lingtong District. Prof. Xueling Jiang and Prof. Ismail Cakmak talked on site about how to manage zinc nutrition in maize and apple trees (Fig.4).

Figure 4. Appearances from the meeting room and field trip on Zn day in Shaanxi.

5.2.3 The Farmer Zinc Day in Jiangsu province

The Farmer Zinc Day on zinc nutrition in crops, supported by HarvestPlus and IZA, was held at Rudong, Jiangsu Province on May 10, 2011.

There were more than 200 participants on the Farmer Zinc Day, which included farmers, extension workers, graduate students, agricultural researchers, government policy makers, staff at Rudong experiment station and invited experts.

The whole activity consisted of two sections. One section mainly focused on the training program, and the other section included Zinc fertilizer distribution and other material. In the opening ceremony, Prof. Shiwei Guo reviewed the history of the collaboration between NJAU and Rudong, introduced the background of the event. Prof. Ismail Cakmak (from Sabanci University, Turkey) encouraged and praised the excellent technical support in field experiment of HarvestPlus project, and emphasized the importance of zinc nutrition in crops and for human health. The vice mayor Changqing Xu gave a welcome speech. He emphasized the importance of the activity and hoped that the farmers can take advantage of the opportunity and contribute to the agricultural development in the county.

Prof. Shiwei Guo, Prof. Ismail Cakmak (from Sabanci University, Turkey), Prof. Chunqin Zou (from college of resources and environmental sciences, CAU), Prof. Futong Yu (from college of resources and environmental sciences, CAU), Prof. Liyan Ma (from college of food sciences, CAU) and Prof. Deqian Mao (from Center of disease prevention and control in China) gave the presentations on zinc nutrition in crops, zinc fertilizers, importance of zinc for stress resistance and quality improvement, zinc in foods and importance of zinc in disease prevention.

Figure 5. Photographs from conference room and field trip on Zn day in Jiangsu.

5.2.4 The Farmer Zinc Day in Anhui province

The Farmer Zinc Day on zinc nutrition in crops and human health supported by HarvestPlus-Zinc project was held at Gaofu village in Panji county of Huainan city, Anhui Province on May 6, 2013. DTPA-Zn concentration of about 43.8% in the Huainan city soils are less than 0.5 mg kg^{-1}. The main crops in this area are lowland rice, wheat, maize, cotton, and oil rape.

There were more than 200 participants on the Farmer Zinc Day, which included farmers, extension workers, agricultural researchers, government policy makers, staff at Panji county and invited experts. We recruited more than 150 farmers from Panji county to attend the Farmer Zinc Day, and some farmers from around also attended it.

The local government paid great attention to the Farmer Zinc Day. During the event, the presented governors were the director of Huainan agricultural commission Xiaoming Chen, the director of Huainan agricultural technology recommendation Center Fanmei Meng, the director of Panji agricultural commission, the director of Gaofu village commission and some

other governors from different towns. Some of them gave speeches and made presentations during the meeting.

The whole activity consisted of two sections. One section mainly focused on the training program, and the other section included dissemination ofpromotional materials and fertilizers to farmers. Prof Lujiu Li chaired the whole activity. Prof. Chunqin Zou (from China Agricultural University), Prof. Levent Ozturk (from Sabanci University), Prof. Buqing Li (from Anhui Academy of Agricultural Sciences), Prof. Nianjun Yu (from Anhui University of Traditional Chinese Medicine), Dr. Ji Wu (from Anhui Academy of Agricultural Science) and Prof. Lujiu Li (from Anhui Academy of Agricultural Science) gave presentations on soil Zn status of Anhui province, zinc nutrition in crops, zinc fertilizers, zinc in food and zinc in disease prevention. The farmers were quite interested in these topics. After presentations, some promotional materials of Zn nutrition in crops, human nutrition and reasonable application of zinc fertilizers were issued to farmers. It was the first time for most of the farmers to know Zn and Zn fertilizers. Some pictures from the Farmer Zinc Day is given in Figure 6.

Figure 6. Photos taken during “Farmer Zinc Day in China” at Anhui Province, in 2013.

6. PROBLEMS ENCOUNTERED

In 2011 in Hebei province when the 0.5% $ZnSO_4 \cdot 7H_2O$ sprayed on the surface of wheat plant after sunset it also caused a little toxicity in the leaf. The reason for this may be fast evaporation of water due to high temperature in summer. In 2011, because of misunderstanding of the project, no leaf materials of wheat in Shaanxi and of rice in Jiangsu were sampled before first foliar application. In 2011, because of misunderstanding of the project, Soil 10 treatment was not applied in Anhui province.

COUNTRY REPORT – INDIA

1. COLLABORATING INSTITUTIONS:

NATIONAL COORDINATORS:
Hari Ram and Virinder Singh Sohu, Punjab Agricultural University, Ludhiana, Punjab

COORDINATING INSTITUTION:

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2. INTRODUCTION:

Zinc deficiency in crops like rice and wheat is a common micronutrient problem world over; therefore zinc malnutrition has become a major health burden among the resource poor people. One third of the world population is at the risk of zinc malnutrition due to inadequate dietary intake of zinc (Cakmak 2009). Singh (2010) reported wide spread hidden hunger of zinc in seeds and feeds which is affecting a large segment of resource poor families whose food comes mainly from cereals grown on 49 % of Indian soils which are having zinc deficiency. Vitamin A deficiency was responsible for the maximum number of deaths followed by Zn and Fe deficiencies (Black *et al.* 2008). Takkar (1996) reported that a harvest of 8 t grain/ha/yr removed 384, 744 and 320 g Zn/ha/yr in rice–wheat, maize–wheat and rice–rice cropping systems. Similarly a harvest of 6.5 t grain/ha/yr removed 416 g Zn/ha/yr in soybean– wheat cropping system. This heavy removal of Zn year after year without adequate Zn fertilization has depleted Zn from native soils and today 49 % of Indian soils are Zn deficient (Behera *et al*. 2009). Continuous intensive cropping of high yielding crop varieties has further aggravated the depletion of soil zinc leading to low zinc concentration in edible grains. Agronomic bio-fortification offers a promising strategy to address micronutrient deficiency in the diet (Cakmak, 2008). There is a scope for zinc enrichment in cereal grains which will be beneficial in reducing zinc malnutrition in India.

3. EXPERIMENTAL ACTIVITIES

The field experiments were conducted during *kharif* 2011 and 2012 at Punjab Agricultural University (PAU), Ludhiana and PAU Regional Station, Gurdaspur, Punjab (India) and during *rabi* 2011-12 and 2012-2013 at PAU, Ludhiana, PAU Regional Station Bathinda and PAU Regional Station, Gurdaspur, Punjab (India). The rice nursery was raised as per treatments. About 4 weeks old seedlings were transplanted in the field with geometry of 15 cm × 15 cm. The weeds were controlled in the experiments using butachlor 30 EC @3.0 litres/ha within two days of transplanting. The locations of the experimental sites have been given on the country map (Figure 1).

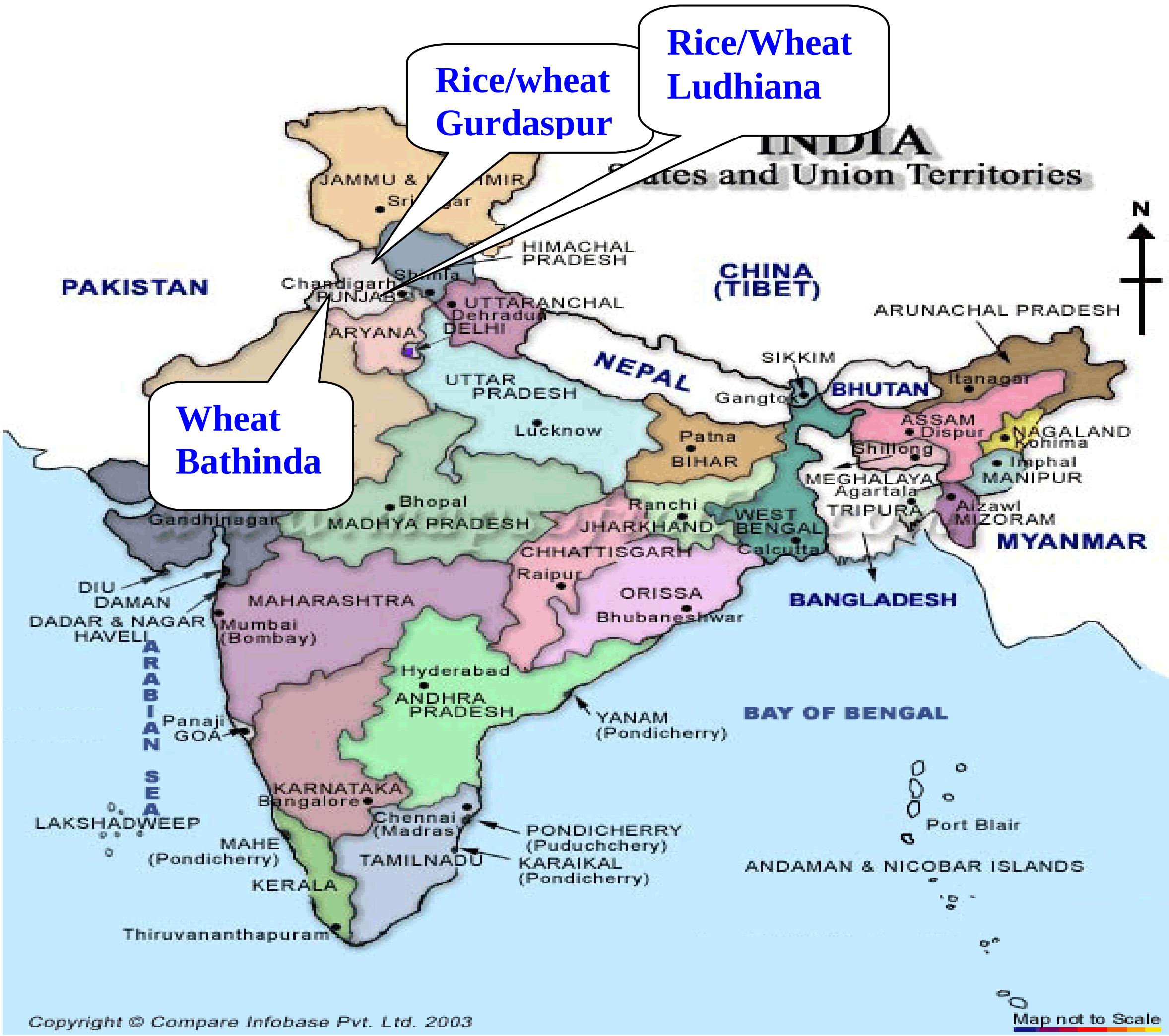

Figure 1: The experimental locations in India for Harvest Zinc rice and wheat experiments during 2011-12.

As indicated on the Indian map (Figure 1), rice experiments were conducted at Ludhiana and Gurdaspur during 2011 and 2012 rice cropping seasons. In 2011-12 and 2012-2013 seasons, wheat experiments were conducted at Ludhiana, Gurdaspur and Bathinda. The soil status in respect of micronutrients has been presented in Table 1. Soil at both the locations was low in DTPA Zn. Both of the experiments were conducted in randomized block design with four replications. The puddling was done to reduce the seepage of irrigation water. Rice was transplanted manually. Wheat crop variety PBW 621 was sown using 100 kg seed ha^{-1} at row to row spacing of 20 cm in both the years of investigations.

Table 1. DTPA-extractable Zn, Cu, Fe and Mn in soils at different locations in Punjab, India.

Location	Micronutrient (mg kg^{-1})			
	Zn	Cu	Fe	Mn
Ludhiana	0.58	1.30	10.18	9.42
Gurdaspur	0.55	2.67	22.08	10.28
Bathinda	0.45	0.40	6.35	2.21

Experiment 1: Effect of soil application of Zn-containing fertilizers and use of seeds differing in Zn concentration on productivity and grain Zn in wheat and rice

The experiment comprised of 10 treatments of 1. Recommended dose of fertilizers LC (150 kg N ha^{-1} + 40 Kg P_2O_5 ha^{-1}) 2. LC + Soil $ZnSO_4.7H_2O$ @ 50 kg ha^{-1} 3. LC + Mosaic I (P through Mosaic-MESZ) 4. LC + Mosaic-II 5. LC + Mosaic I + foliar Zn app. (0.5 % $ZnSO_4$. $7H_2O$) 6. LC + Kali Korn Kali (KCl-Zn) 7. N in three Split Zincated-Urea 8. LC + ADOB HBED Zn Chelate (250 l ha^{-1}) 9. LC + Seed Zn-I (seeds enriched with Zn) 10. LC + Seed Zn-II (seeds enriched with Zn by priming with Zn).

Experiment 2: Effect of foliar application of Zn-containing fertilizers/solutions on the productivity and grain Zn in wheat and rice

The experiment comprised of 12 treatments of 1. LC (150 Kg N ha^{-1} + 40 Kg P_2O_5 ha^{-1}) 2. LC + Foliar $ZnSO_4$ (at the booting stage) 3. LC + Foliar $ZnSO_4$ (at early milk stage) 4. LC + Foliar $ZnSO_4$ (twice foliar) (at booting stage and at milk stage). 5. LC + OMEX-Type-II Foliar Zn 6. LC + OMEX-Type-III Foliar Zn 7. LC + Kali-EPSO-Zn 8. LC + ADOB ZnIDHA 9. LC + Valagro Brexil (at milk stage) 10. LC + Bayer Antracol-Zn (3 kg ha^{-1} at milk stage) 11. LC + Propiconazole + $ZnSO_4$ foliar 12. LC + FBScience CP Foliar Zn fertilizer (at milk stage).

Table 2. Activity table of rice crop

Sr No.	Particulars	Ludhiana	Gurdaspur
2011			
1.	Variety	PR 120	PR 120
2.	Transplanting	22.06.11	29.06.11
3.	Fertilizer application (dose) N in three splits	(150 kg N ha^{-1} + 40 kg P_2O_5 ha^{-1}) 22.06.11+13.07.11+4.08.11	(150 kg N ha^{-1} + 40 kg P_2O_5 ha^{-1}) 29.06.11+10.08.11+28.08.11
4.	Treatment application dates	28.08.11 and 12.09.11	30.08.11 and 15.09.11
5.	Harvesting	10.10.2011	16.10.2011
6.	Threshing	15.10.2011	20.10.2011
2012			
1.	Variety	PR 116	PR 120
2.	Transplanting	21.06.12	21.06.12
3.	Fertilizer application (dose) N in three splits	(150 kg N ha^{-1} + 40 kg P_2O_5 ha^{-1}) 21.06.12 + 13.07.12 + 3.08.12	(150 kg N ha^{-1} + 40 kg P_2O_5 ha^{-1}) 29.06.12 + 24.07.12 + 19.08.12
4.	Treatment application dates	16.08.12 and 3.09.12	23.08.12 and 13.09.12
5.	Harvesting	3.10.2012	13.10.2012
6.	Threshing	8.10.2012	16.10.2012
2013			
1.	Variety	PR 120	PR 120
2.	Transplanting	20.06.13	22.06.13
3.	Fertilizer application (dose) N in three splits	(150 kg Nha^{-1}+ 40 kg $P_2O_5ha^{-1}$) 20.06.12 + 10.07.12 + 8.08.12	(150 kg Nha^{-1}+ 40 kg $P_2O_5ha^{-1}$) 29.06.12 + 24.07.12 + 19.08.12
4.	Treatment application dates	20.08.13 and 2.09.13	23.08.12 and 5.09.12
5.	Harvesting	6.10.2013	10.10.2013
6.	Threshing	09.10.2013	13.10.2013

Table 3. Activity table of wheat crop

Particulars	**Ludhiana**	**Gurdaspur**	**Bathinda**
Variety	PBW 621	PBW 621	PBW 621
Date of sowing	5.11.2011	15.11.2011	15.11.2011
Fertilizer application (kg ha^{-1}) N in three splits	150-62.5-30 05.11.2011+ 01.12.2011+ 14.12.2011	150-62.5-30 15.11.2011+ 09.12.2011+ 26.12.2011	150-62.5-30 15.11.2011+ 08.12.2011+ 26.12.2011
Irrigation	1) 28.11.2011 2) 25.12.2011 3) 27.01.2012 4) 14.02.2012 5) 09.03.2012 6) 27.03.2012	1) 08.12.2011 2) 10.01.2012 3) 17.02.2012 4) 29.03.2012	1) 07.11.2011 2) 10.01.2012 3) 07.02.2012 4) 22.02.2012 5) 14.03.2012 6) 28.03.2012
Treatment application date	Foliar Spray T_2 & T_4: 24.02.2012 T_3 to T_{12}: 15.03.2012	Foliar Spray T_2 & T_4: 05.03.2012 T_3 to T_{12}: 19.03.2012	Foliar Spray T_2 & T_4: 02.03.2012 T_3 to T_{12}: 16.03.2012
Harvesting	25.04.2012	09.05.2012	30.04.2012
2012-13			
1.	Variety	PBW 621	PBW 621
2.	Date of sowing	5.11.2011	15.11.2011
3.	Fertilizer application (kg/ha) N in three splits	150-62.5-30 05.11.2011+ 01.12.2011+ 14.12.2011	150-62.5-30 15.11.2011+ 09.12.2011+ 26.12.2011
4.	Irrigation	1) 25.11.2012 2) 22.12.2012 3) 26.01.2013 4) 10.02.2013 5) 12.03.2013 6) 25.03.2013	1) 06.12.2012 2) 12.01.2013 3) 14.02.2013 4) 26.03.2013
5.	Treatment application date	Foliar Spray T_2 & T_4: 22.02.2013 T_3 to T_{12}: 15.3.2013	Foliar Spray T_2 & T_4: 08.03.2013 T_3 to T_{12}: 15.03.2013
6.	Harvesting	17.04.2013	22.04.2013

4. RESULTS AND DISCUSSIONS:

Experiment 1: Effect of soil application of Zn-containing fertilizers and use of seeds differing in Zn concentration on productivity and grain Zn in rice.

A) Rice

Ludhiana

Grain yield:

The data presented in the Table 4 revealed that the highest grain yield at Ludhiana was recorded in LC + ADOB HBED Zn Chelate (250 l ha^{-1}) during 2011 (5.14 t ha^{-1}) and 2012 (5.88 t ha^{-1}). It was statistically on par with LC + Soil $ZnSO_4.7H_2O$ @ 50 kg ha^{-1} and LC+ Kali Korn Kali (KCl-Zn) but significantly higher than rest of the treatments in 2011 and 2012. The lowest grain yield was recorded in LC treatment which was significantly lower than LC + Soil $ZnSO_4.7H_2O$ @ 50 kg ha^{-1} and LC + Kali Korn Kali (KCl-Zn) but statistically on par with rest of the treatments. The zinc fertilizers might have provided better nutrition to the crop which increased the grain yield of paddy. Similar effect of zinc fertilization on grain yield of rice was also reported by Patel (2011). In 2013, the highest grain yield (6.84 t ha^{-1}) was recorded in LC + Soil $ZnSO_4.7H_2O$ @ 50 kg ha^{-1}, which was statistically on par with LC + ADOB HBED Zn Chelate (250 lha^{-1}) and LC + Kali Korn Kali (KCl-Zn) but significantly higher than rest of the treatments

Grain Zn:

The highest grain Zn was recorded in LC + Mosaic I + foliar Zn (0.5 % $ZnSO_4$. $7H_2O$) in all years of study, which was significantly higher than rest of the treatments (Table 5). It might be a result of immediate translocation of the foliar applied Zn to the developing grains due to enhanced available pools of Zn in vegetative tissues during the reproductive growth stage upon foliar spray of Zn. Soil Zn fertilizer application could not increase the grain Zn concentration significantly.

Gurdaspur

Grain yield:

The highest grain yield with 5.5 t ha^{-1} was recorded with LC + Soil $ZnSO_4.7H_2O$ @ 50 kg ha^{-1} treatment which was statistically on par with LC + Mosaic I (P through Mosaic-MESZ), LC + Mosaic-II , LC + Mosaic I + foliar Zn (0.5 % $ZnSO_4$. $7H_2O$) , LC + Kali Korn Kali (KCl-Zn) and LC + ADOB HBED Zn Chelate (250 l ha^{-1}) treatments in 2011, but in 2012 it was statistically on par with LC + Mosaic-II and RDF + Kali Korn Kali (KCl-Zn) (Table 4). LC + Soil $ZnSO_4$ also resulted in the highest grain yield in 2013.

Grain Zn

Grain Zn was not significantly influenced by any of the treatments in 2011. At this location we could not apply foliar Zn in LC + Mosaic I + foliar Zn treatment due to an overlook during the spray of foliar fertilizers. But in 2012 and 2013, LC + Mosaic I + foliar Zn resulted in significantly higher grain zinc than all other treatments.

Table 4. Effect of soil-applied Zn-containing fertilizers on grain yield of rice in 2011, 2012 and 2013.

TREATMENT	GRAIN YIELD (t ha^{-1})					
	LUDHIANA			GURDASPUR		
	2011	2012	2013	2011	2012	2013
1. Local control (LC)	4.63	5.30	6.38	5.01	6.11	6.83
2. LC + Soil $ZnSO_4.7H_2O$	5.03	5.88	6.84	5.51	6.52	7.37
3. LC+Mosaic I	4.75	5.46	6.41	5.16	6.30	6.81
4. LC+Mosaic II	4.84	5.46	6.16	5.33	6.44	7.23
5. LC+Mosaic I+FoliarZn	4.81	5.45	6.41	5.28	6.24	7.00
6. LC + Kali KornKali	5.01	5.83	6.82	5.32	6.41	7.29
7. 3 x Split Urea-Zn	4.67	5.49	6.40	5.14	6.12	7.02
8. LC+ADOB HBEDZn	5.14	5.88	6.72	5.50	6.57	7.31
9. LC + high seed Zn (Foliar in last crop)	4.70	5.45	6.40	5.19	6.12	7.00
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	4.75	5.46	6.45	5.21	6.14	6.96
CV (%)	3.7	4.6	3.87	3.6	1.9	2.37
LSD (0.05)	0.27	3.70	0.36	2.77	2.00	0.28

* Foliar Zn (0.5 % $ZnSO_4$. $7H_2O$) could not be applied at Gurdaspur location.

Table 5. Effect of soil-applied Zn-containing fertilizers on grain Zn concentration of rice in 2011, 2012 and 2013.

TREATMENT	GRAIN Zn CONCENTRATION (mg kg^{-1})					
	LUDHIANA			GURDASPUR		
	2011	2012	2013	2011	2012	2013
1. Local control (LC)	21.5	20.7	20.0	18.4	19.8	17.5
2. LC + Soil $ZnSO_4.7H_2O$	21.3	20.6	19.7	19.3	20.4	18.7
3. LC+Mosaic I	21.1	19.5	20.1	19.4	19.8	19.0
4. LC+Mosaic II	21.4	20.6	21.3	18.8	20.6	18.2
5. LC+Mosaic I+FoliarZn	26.9	24.0	24.2	18.4*	23.3	22.4
6. LC + Kali KornKali	22.7	21.3	20.0	18.9	20.6	18.0
7. 3 x Split Urea-Zn	23.2	22.3	20.9	18.5	20.8	18.5
8. LC+ADOB HBEDZn	22.6	20.9	20.7	18.6	20.3	17.8
9. LC + high seed Zn (Foliar in last crop)	22.4	21.6	19.7	20.0	19.9	18.6
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	21.8	21.7	19.8	19.1	20.4	17.3
CV (%)	9.1	5.5	7.06	5.6	3.6	6.37
LSD (0.05)	2.96	1.70	2.12	n.s.	1.28	1.71

* Foliar Zn (0.5 % $ZnSO_4$. $7H_2O$) could not be applied at Gurdaspur location in 2011.

B) Wheat

Emergence count and seedling height:

The emergence and seedling height were not significantly influenced by any of the treatment during either year of the study (Tables 6 and 7). The emergence count was 3.0 and 8.3 % higher in LC+ Seed Zn-I (seeds enriched with Zn) and LC+ Seed Zn-II (seeds Zn priming) treatments over the mean of all the other treatments across the locations and years. On an average basis of all the locations and years LC + Seed Zn-I (seeds enriched with Zn) and LC+ Seed Zn-II (seeds Zn priming) treatments recorded slightly higher seedling height than the mean of rest of the treatments.

Grain yield:

Ludhiana

The data presented in the Table 8 reveals that the highest grain yield at Ludhiana was recorded with LC + Mosaic-I which was significantly higher than LC + ADOB HBED Zn Chelate (250 l ha^{-1}), 3-Split Zincated-Urea application and LC treatments, but was statistically at par with the rest of the treatments. The lowest grain yield was recorded in LC treatment which was statistically on par with N in 3-Split Zincated-Urea.

In 2012-13, the highest grain yield (6.05 t ha^{-1}) was recorded in LC + Kali Korn Kali (KCl-Zn) which was significantly higher than LC, N in 3-Split Zincated-Urea, LC+ Seed Zn-I (seeds enriched with Zn) and LC- Seed Zn-II (seeds Zn priming) but was statistically on par with rest of the treatments (Table 8). The grain yield recorded in LC + Kali Korn Kali (KCl-Zn) was 7.7% higher over the LC treatment. Seeds enriched with Zn could increase only 2.1% grain yield over the normal seed.

Bathinda

In 2011-2012, the highest grain yield at Bathinda was recorded in LC + Mosaic-II treatment which was significantly higher than the 3-Split Zincated-Urea and LC treatments, however it was statistically at par with the rest of the treatments. The LC treatment produced lowest grain yield which was significantly lower than all the treatments. LC + Mosaic-II treatment resulted in 5.8 % and 12.6 % more grain yield than N in 3-Split Zincated-Urea and LC treatments, respectively.

In 2012-13, the highest grain yield (5.01 t ha^{-1}) was recorded in LC + Mosaic-II which was significantly higher than LC, N in 3-Split Zincated-Urea, LC + Seed Zn-I (seeds enriched with Zn) and LC- Seed Zn-II (seeds Zn priming) but was statistically on par with rest of the treatments. Seeds enriched with Zn could increase 5.3% grain yield over the normal seed.The grain yield recorded in LC+ Mosaic-II was 9.6% higher over the LC treatment.

Gurdaspur

In 2011-2012, LC + Kali Korn Kali (KCl-Zn) treatment resulted in the highest grain yield, which was significantly higher than 3-Split Zincated-Urea application and LC treatments; however it was statistically at par with the rest of the treatments. The LC treatment produced the lowest grain yield which was significantly lower than all the treatments. LC + Kali KornKali (KCl-Zn) treatment resulted in 7.1 % and 15.5 % higher grain yield than 3-Split Zincated-Urea and LC treatments, respectively.

In 2012-13, the highest grain yield (5.97 t ha^{-1}) was recorded in LC + Mosaic I + foliar Zn (0.5 % $ZnSO_4$. $7H_2O$) which was significantly higher than LC, N in 3-Split Zincated-Urea, LC + ADOB HBED Zn Chelate (250 l ha^{-1}), LC + Seed Zn-I (seeds enriched with Zn) and LC- Seed Zn-II (seeds Zn priming) but was statistically on par with rest of the treatments. This increase in grain yield in LC + Mosaic I + foliar Zn (0.5 % $ZnSO_4$. $7H_2O$) was 7.6% over the control treatment (LC). Seeds enriched with Zn could increase only 1.6% grain yield over the normal seed from LC.

Grain zinc

The highest grain zinc was recorded in LC + Mosaic I + foliar Zn (0.5%$ZnSO_4.7H_2O$) at all the locations in all years (Table 9). It was significantly higher than all other treatments except it was statistically on par with rest of the treatments in 2011-12 at Bathinda and Gurdaspur.

Table 6. Effect of soil-applied Zn-containing fertilizers on emergence count (15 days after sowing) of wheat.

TREATMENT	EMERGENCE (plants m^{-2})					
	2011-2012			2012-2013		
	Ludhiana	Bathinda	Gurdaspur	Ludhiana	Bathinda	Gurdaspur
1. Local control (LC)	211	200	201	199	193	201
2. LC + Soil $ZnSO_4.7H_2O$	210	198	202	192	200	198
3. LC+Mosaic-I	211	203	203	191	200	199
4. LC+Mosaic-II	210	201	206	196	199	195
5. LC+Mosaic-I+FoliarZn	212	202	202	201	200	197
6. LC + Kali KornKali	212	200	203	197	197	197
7. 3 x Split Urea-Zn	212	205	207	198	200	200
8. LC+ADOB HBEDZn	160	204	206	197	203	197
9. LC + high seed Zn (Foliar in last crop)	214	213	215	209	198	195
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	234	216	223	220	209	207
CV (%)	7.5	5.3	6.4	7.7	5.7	2.2
LSD (0.05)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 7. Effect of soil-applied Zn-containing fertilizers on seedling height (20 days after sowing) of wheat.

TREATMENT	SEEDLING HEIGHT (cm)					
	2011-2012			2012-2013		
	Ludhiana	**Bathinda**	**Gurdaspur**	**Ludhiana**	**Bathinda**	**Gurdaspur**
1. Local control (LC)	14.0	13.9	14.0	17.1	15.8	17.6
2. LC + Soil $ZnSO_4.7H_2O$	13.7	13.9	14.2	17.0	15.7	19.2
3. LC+Mosaic-I	13.9	14.0	14.3	18.3	16.9	19.0
4. LC+Mosaic-II	14.4	13.8	14.3	18.7	17.1	18.0
5. LC+Mosaic-I+FoliarZn	14.1	13.9	14.1	17.2	16.0	19.3
6. LC + Kali KornKali	14.3	14.3	14.3	17.6	16.4	19.5
7. 3 x Split Urea-Zn	14.5	13.2	14.2	18.5	17.0	17.2
8. LC+ADOB HBEDZn	13.2	14.5	13.6	17.6	16.4	17.5
9. LC + high seed Zn (Foliar in last crop)	14.8	14.7	14.7	18.6	17.3	18.2
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	15.9	15.8	15.1	18.3	17.0	19.6
CV (%)	7.6	7.7	8.1	9.3	9.3	9.7
LSD (0.05)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 8. Wheat grain yields obtained in Experiment 1 at 3 different locations of India, in 2011-2012 and 2012-2013 growing years.

TREATMENT	GRAIN YIELD (t ha^{-1})					
	2011-2012			2012-2013		
	Ludhiana	**Bathinda**	**Gurdaspur**	**Ludhiana**	**Bathinda**	**Gurdaspur**
1. Local control (LC)	5.68	4.78	5.66	5.62	4.57	5.55
2. LC + Soil $ZnSO_4.7H_2O$	6.30	5.33	6.23	5.99	4.91	5.86
3. LC+Mosaic-I	6.44	5.29	6.29	5.91	4.96	5.92
4. LC+Mosaic-II	6.39	5.46	6.31	5.92	5.01	5.82
5. LC+Mosaic-I+FoliarZn	6.20	5.40	6.32	5.88	5.00	5.97
6. LC + Kali KornKali	6.37	5.38	6.46	6.05	4.91	5.84
7. 3 x Split Urea-Zn	5.91	5.14	6.00	5.74	4.78	5.58
8. LC+ADOB HBEDZn	6.07	5.38	6.21	6.00	4.93	5.74
9. LC + high seed Zn (Foliar in last crop)	6.35	5.45	6.23	5.74	4.83	5.64
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	6.32	5.35	6.15	5.76	4.82	5.69
CV (%)	4.0	2.2	3.8	3.0	2.2	1.8
LSD (0.05)	0.36	0.17	0.40	2.51	1.53	1.73

Table 9. Wheat grain Zn concentrations obtained in Experiment 1 at 3 different locations of India, in 2011-2012 and 2012-2013 growing years.

TREATMENT	**GRAIN Zn CONCENTRATION (mg kg^{-1})**					
	2011-2012			**2012-2013**		
	Ludhiana	**Bathinda**	**Gurdaspur**	**Ludhiana**	**Bathinda**	**Gurdaspur**
1. Local control (LC)	34.3	28.1	31.8	26.9	24.6	26.7
2. LC + Soil $ZnSO_4.7H_2O$	38.9	28.5	32.2	29.0	25.5	28.8
3. LC+Mosaic-I	39.1	28.8	33.1	28.4	24.1	30.0
4. LC+Mosaic-II	35.1	28.8	31.7	28.6	23.9	31.4
5. LC+Mosaic-I+Fol-Zn	45.3	32.9	39.5	36.2	33.8	38.5
6. LC + Kali KornKali	38.8	28.7	31.2	27.8	27.2	27.5
7. 3 x Split Urea-Zn	35.0	29.0	35.9	27.4	26.7	30.1
8. LC+ADOB HBEDZn	34.4	29.7	32.3	29.4	28.6	31.0
9. LC + high seed Zn (Foliar in last crop)	36.7	29.2	35.5	27.6	27.8	31.1
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	36.6	28.4	33.3	29.1	24.1	29.6
CV (%)	7.5	6.3	6.3	11.3	8.5	7.1
LSD (0.05)	4.1	n.s.	n.s.	4.8	3.3	3.5

Experiment 2: Effect of foliar application of Zn-containing fertilizer on productivity and grain Zn in wheat and rice.

A.) Rice

Rice grain yield:

The grain yield of rice was not significantly influenced by any of the treatments at either of the locations during any of the years of study (Table 10). As no zinc containing fertilizers were applied at the time of planting which could correct the zinc deficiency in rice, foliar zinc applied at later stages could not influence the grain yield of paddy.

Grain Zn:

In 2011, the grain zinc in brown rice was significantly influenced by all the treatments (Table 11). The highest zinc content in rice grain was recorded in LC + OMEX-Type-II Foliar Zn which was significantly higher than LC, LC + foliar Zn (boot), LC+ Antracol-Zn (milk stage) and LC + CP Foliar Zn (at milk stage) at Ludhiana and LC, LC + Foliar Zn (boot), LC + foliar (early milk) and LC + CP Foliar Zn (at milk stage) at Gurdaspur. All the foliar zinc applications increased the grain zinc by 5.25 mg kg^{-1} Zinc at Ludhiana and 3.84 mg kg^{-1} at Gurdaspur.

In 2012, LC + Foliar (early milk) recorded the highest grain zinc in rice at Ludhiana but at Gurdaspur the highest grain zinc was recorded in LC + Kali-EPSO-Zn. All foliar zinc applications recorded significantly higher zinc than LC except RDF + Foliar Zn (boot) at Ludhiana and LC+ Foliar Zn (boot) and LC + CP Foliar Zn (at milk stage) at Gurdaspur. Phaattarkul *et al.* (2012) reported that foliar Zn application offered a practical and useful means for an effective biofortification of rice grain with Zn, irrespective of cultivars, environmental conditions and management practices in different countries.

In 2013, the highest grain Zn was recorded in LC + OMEX III at Ludhiana which was statistically on par with LC + Foliar $ZnSO_4$ (at boot + milk stage) and LC + OMEX II. At Gurdaspur, the highest grain Zn was recorded in LC + Foliar $ZnSO_4$ (at boot + milk stage) which was significantly higher than Local control but statistically on par with rest of the treatments in 2013.

Table 10. Effect of various foliar Zn solution treatments on grain yield of rice at Ludhiana and Gurdaspur

TREATMENT	GRAIN YIELD (t ha^{-1})					
	LUDHIANA			GURDASPUR		
	2011	2012	2013	2011	2012	2013
1. Local control (LC)	4.62	4.91	6.39	5.43	6.23	7.00
2. LC+ Foliar $ZnSO_4$ (booting)	4.64	5.02	6.38	5.38	6.30	6.94
3. LC+ Foliar $ZnSO_4$ (milk)	4.62	4.92	6.45	5.36	6.28	6.92
4. LC+ Foliar $ZnSO_4$ (booting + milk)	4.57	5.04	6.30	5.45	6.26	6.92
5 LC+ OMEX II	4.71	5.00	6.35	5.43	6.20	6.91
6. LC+ OMEX III	4.67	4.88	6.41	5.31	6.26	7.04
7. LC+Kali-EPSO	4.63	4.99	6.34	5.30	6.23	7.04
8. LC+ ADOB ZnIDHA	4.70	4.98	6.38	5.46	6.28	6.92
9. LC+ Valagro Brexil	4.69	4.93	6.40	5.45	6.30	6.99
10. LC+ Antracol	4.69	5.17	6.28	5.44	6.31	6.96
11. LC+ Propiconazole + Foliar $ZnSO_4$	4.68	4.87	6.36	5.46	6.23	7.05
12. LC+ FBScience	4.73	5.09	6.47	5.39	6.26	7.00
CV (%)	3.3	4.1	7.7	5.9	2.5	3.8
LSD (0.05)	n.s.	n.s.	n.s	n.s.	n.s.	n.s.

Table 11.Effect of various foliar Zn solution treatments on grain Zn concentration of rice at Ludhiana and Gurdaspuri in 2011, 2012 and 2013.

TREATMENT	GRAIN Zn CONCENTRATION (mg kg^{-1})					
	LUDHIANA			GURDASPUR		
	2011	2012	2013	2011	2012	2013
1. Local control (LC)	19.8	18.7	19.0	19.1	17.8	18.8
2. LC+ Foliar $ZnSO_4$ (booting)	23.0	19.4	19.7	20.6	19.5	21.3
3. LC+ Foliar $ZnSO_4$ (early milk)	26.1	21.6	21.6	23.6	21.2	22.8
4. LC+ Foliar $ZnSO_4$ (booting + early milk)	25.1	23.5	22.1	23.5	21.8	22.8
5 LC+ OMEX II	27.0	24.0	21.1	21.5	21.3	22.8
6. LC+ OMEX III	25.9	23.0	22.8	22.4	22.2	20.6
7. LC+Kali-EPSO	25.4	22.2	20.9	22.0	22.5	21.1
8. LC+ ADOB ZnIDHA	25.0	23.0	20.6	21.0	21.1	21.1
9. LC+ Valagro Brexil	25.5	23.8	20.1	22.1	21.6	21.4
10. LC+ Antracol	22.9	22.6	20.9	21.0	21.6	21.0
11. LC+ Propiconazole + Foliar $ZnSO_4$	26.5	23.4	20.1	23.0	22.1	21.3
12. LC+ FBScience	23.2	21.4	20.4	21.3	18.4	21.5
CV (%)	9.9	6.6	6.0	4.6	8.2	6.9
LSD (0.05)	3.5	2.1	1.8	1.7	2. 9	2.1

B) Wheat

Grain yield

The data presented in the Table 12 revealed that the grain yield was not influenced significantly by different foliar treatments of zinc at all the three locations of study in 2011-2012. The maximum grain yield was recorded in LC + Antracol-Zn (milk stage) at Ludhiana and Gurdaspur, which was 3.8 per cent and 4.6 per cent higher than LC at Ludhiana and Gurdaspur, respectively. LC treatment at Ludhiana and Gurdaspur recorded the less grain yield than all other treatments. However at Bathinda, LC + Foliar Zn (boot) treatment produced maximum grain yield, which was 3.5 per cent higher than LC + Antracol-Zn (milk stage) treatment, which recorded the lowest grain yield among all foliar Zn application treatments.

In 2012-2013, the highest grain yields were recorded in LC + OMEX-Type-II Foliar Zn at Ludhiana, in LC + OMEX-Type-II Foliar Zn and LC + Foliar (early milk) at Bathinda and in LC+ Foliar (early milk) at Gudraspur. However the differences in grain yield were non-significant.

Grain zinc

In 2011-2012, the highest grain zinc was recorded in LC + OMEX Type II foliar Zn at all the locations. It was statistically on par with LC + Foliar Zn (boot), LC + Foliar Zn (twice), LC + OMEX Type III foliar Zn and LC + Valagro Brexil (milk stage) but was significantly higher than rest of the treatments at Ludhiana.

In case of the location Bathinda, the best treatments were LC + Foliar Zn (boot), LC + Foliar Zn (twice), LC + ADOB ZnIDHA and LC+ Valagro Brexil (milk stage).

At Gurdaspur, the highest grain zinc was recorded in LC + OMEX Type II foliar Zn which was statistically on par with LC + Foliar Zn (twice), LC + Kali-EPSO-Zn, LC + ADOB ZnIDHA and LC + Valagro Brexil (milk stage) but was significantly higher than rest of the treatments in Gurdaspur. Zou *et al.* (2012) also reported higher zinc concentration in wheat grain with foliar zinc application.

In 2012-13, the highest grain zinc was recorded in LC+ foliar $ZnSO_4$ (booting + milk stage) at all the locations which was statistically on par with LC + OMEX II, LC + ADOB ZnIDHA and LC + Propiconazole + Foliar $ZnSO_4$ but was significantly higher than rest of the treatments at Ludhiana. All the treatments recorded significantly higher grain Zn than Local Control except LC+ foliar $ZnSO_4$ (booting stage) and LC + FBScience treatments at Ludhiana. At Bathinda, the highest grain Zn recorded in LC+ foliar ZnSO4 (booting + milk stage) was significantly higher than rest of the treatments. But at Gurdaspur, the highest grain Zn recorded in LC+ foliar $ZnSO_4$ (booting + milk stage) was statistically on par with LC + ADOB ZnIDHA and LC + Propiconazole + Foliar $ZnSO_4$.

Table 12. Effect of various foliar Zn treatments on grain yield of wheat at Ludhiana, Bathinda and Gurdaspur

	GRAIN YIELD (t ha^{-1})					
	2011-2012			2012-2013		
TREATMENT	**Ludhiana**	**Bathinda**	**Gurdaspur**	**Ludhiana**	**Bathinda**	**Gurdaspur**
1. Local control (LC)	5.71	4.82	5.60	5.50	4.52	5.53
2. LC+ Foliar $ZnSO_4$ (booting)	5.82	4.96	5.78	5.73	4.46	5.63
3. LC+ Foliar $ZnSO_4$ (early milk)	5.87	4.92	5.74	5.58	4.60	5.73
4. LC+ Foliar $ZnSO_4$ (booting + early milk)	5.85	4.85	5.64	5.65	4.48	5.59
5 LC+ OMEX II	5.85	4.94	5.74	5.75	4.60	5.54
6. LC+ OMEX III	5.93	4.93	5.85	5.69	4.50	5.54
7. LC+Kali-EPSO	5.83	4.85	5.78	5.68	4.57	5.66
8. LC+ ADOB ZnIDHA	5.79	4.90	5.68	5.51	4.52	5.54
9. LC+ Valagro Brexil	5.87	4.89	5.76	5.49	4.52	5.52
10. LC+ Antracol	5.94	4.78	5.87	5.51	4.52	5.54
11. LC+ Propiconazole + Foliar $ZnSO_4$	5.92	4.82	5.70	5.59	4.51	5.65
12. LC+ FBScience	5.86	4.83	5.80	5.53	4.51	5.56
CV (%)	2.3	3.2	2.8	4.2	10.1	8.0
LSD (0.05)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 13. Effect of various foliar Zn treatments on grain Zn concentration of wheat at Ludhiana, Bathinda and Gurdaspur.

TREATMENT	GRAIN Zn CONCENTRATION (mg kg^{-1})					
	2011-2012			2012-2013		
	Ludhiana	Bathinda	Gurdaspur	Ludhiana	Bathinda	Gurdaspur
1. Local control (LC)	34.6	28.4	33.2	27.2	25.4	26.5
2. LC+ Foliar $ZnSO_4$ (booting)	41.3	37.6	38.9	30.8	36.7	35.5
3. LC+ Foliar $ZnSO_4$ (early milk)	40.4	36.0	39.6	35.5	34.2	38.8
4. LC+ Foliar $ZnSO_4$ (booting + early milk)	42.7	38.2	40.3	42.3	42.2	45.5
5 LC+ OMEX II	45.5	42.0	43.3	38.2	35.8	39.7
6. LC+ OMEX III	41.6	32.5	36.6	35.9	33.5	36.8
7. LC+Kali-EPSO	41.1	32.8	43.6	36.7	34.5	37.1
8. LC+ ADOB ZnIDHA	40.4	37.8	40.4	36.8	32.3	35.9
9. LC+ Valagro Brexil	41.6	40.7	40.5	35.1	32.2	41.6
10. LC+ Antracol	40.1	32.4	41.2	34.0	30.9	38.4
11. LC+ Propiconazole + Foliar $ZnSO_4$	39.9	32.9	41.9	43.6	31.7	43.8
12. LC+ FBScience	39.2	35.5	39.1	32.6	31.7	38.8
CV (%)	7.3	8.9	5.0	10.6	9.8	8.9
LSD (0.05)	4.3	4.5	3.4	5.5	4.7	5.4

Experiment 3. Effect of zinc enrichment in seed on growth and productivity of wheat.

The experiment comprised of two treatments of 1. Recommended dose of fertilizers LC (150 Kg N ha^{-1} + 40 Kg P_2O_5 ha^{-1}) 2. LC+ Seed Zn-I (seeds enriched with Zn).

Locations of the experiment at farmers field in 2012

LOCATION	NAME OF THE FARMER	ADDRESS	DATE OF SOWING	VARIETY
Location 1	S Gurmeet Singhs s/o Joginder Singh	VPO Jodhpur Romana Bathinda	25.11.12	PBW 621
Location 2	S Gurcharan Singh s/o Lachhman Singh	VPO Jodhpur Romana Bathinda	25.11.12	PBW 621
Location 3	S. Sukhdev Singh	VPO Rattangarh	28.11.12	PBW 621

Table 14. Effect of Zinc enrichment in emergence, plant height, grain yield and grain Zn concentration of wheat (emergence and plant height observed 20 days after sowing).Control Zn concentration: 28 mg/kg)

Location	Location 1	Location 2	Location 3
		Emergence count (/m^2)	
RDF (control)	190	185	189
RDF (Zinc enriched 52 mg/kg)	199	198	198
LSD (p=0.05)	n.s.	n.s.	n.s.
CV (%)	10.5	5. 1	5.1
		Plant height (cm)	
RDF (control)	15.1	15.0	15.2
RDF (Zinc enriched 52 mg/kg)	15.6	15.7	15.5
LSD (p=0.05)	n.s.	n.s.	n.s.
CV (%)	2.2	2.3	2.4
		Grain yield (t ha^{-1})	
RDF (control)	5.53	4.66	4.51
RDF (Zinc enriched 52 mg/kg)	5.56	4.67	4.57
LSD (p=0.05)	n.s.	n.s.	n.s.
CV (%)	1.5	1.5	2.5
		Grain Zn CONCENTRATION (mg kg^{-1})	
RDF (control)	25.3	26.1	22.1
RDF (Zinc enriched 52 mg/kg)	27.2	28.0	25.3
LSD (p=0.05)	n.s.	n.s.	n.s.
CV (%)	2.1	3.5	4.3

The emergence count, plant height, grain yield and grain zinc were not-significantly influenced by any of the treatment at all the locations (Table 14). However, slightly higher emergence count and plant height were recorded in zinc enriched seed. With the use of Zn enriched seeds the increase in grain yield was only 0.65% over the normal Zn containing seed.

6 TRAINING AND VISIBILITY ACTIVITIES

1. Based on the promising results reported, two following Ph. D. students have been given the problem of micro nutrient enrichment in new recommended varieties of wheat.

S No.	Name of the student	Thesis problem
1.	PARDEEP KUMAR*	**AGRONOMIC BIOFORTIFICATION AND ENHANCEMENT OF PRODUCTIVITY AND QUALITY OF BREAD WHEAT (*Triticum aestivum* L.) VARIETIES.**
2.	DIPENDER KUMAR	**ROLE OF NITROGEN, ZINC AND IRON IN BIOFORTIFICATION OF RICE (*Oryza sativa* L.)**

*Mr Pardeep Kumar has been decorated with International Scholar award 2012 by International Plant Nutrition Institute (IPNI), USA on the basis of his academic performance, co-curricular activities and international relevance of PhD. research.

2. An Indian Zinc day was celebrated in Punjab Agricultural University, Ludhiana on June 2 2011 for the benefit of the farmers. About 450 farmers participated.

3. Farmers' Field Zinc day was celebrated in VPO Jodhpur Romana (Bathinda) on 20.02.2013 in which about 60 farmers participated in February/March.

1.7 PROBLEMS ENCOUNTERED: N.A.

1.8. FUTURE ACTIVITIES

A new experiment has been established to study impact of various foliar Zn fertilizers on grain Zn in wheat.

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COUNTRY REPORT - PAKISTAN

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SUMMARY

Zinc (Zn) deficiency in wheat is a widespread nutrient disorder in Pakistan because of the alkaline-calcareous nature of its low organic matter soils. Soil Zn deficiency not only hampers crop productivity but also the low-Zn wheat produced, the predominant staple cereal in the country, leads to malnutrition and the consequent health hazards. In Phase-I of the *HarvestPlus Zinc Fertilizer Project* (2008–11), agronomic biofortification proved to be an effective approach to optimize wheat yield and enhance grain Zn density cost-effectively.

Under Phase-II of the *HarvestPlus Zinc Fertilizer Project*, field experiments were conducted during wheat seasons 2011–12 and 2012–13 in three major cropping systems of the country (i.e. rice-wheat, mixed cropping and cotton-wheat systems) on Zn-deficient alkaline-calcareous soils (pH, 7.8–8.2; $CaCO_3$ content, 7.7–21.5%; DTPA Zn, 0.35– 0.92 mg kg^{-1}) to compare the beneficial effect of applying zincated fertilizers – by soil application as well as by foliar sprays – and using Zn-enriched wheat seed on seedling emergence, plant growth, crop productivity and grain Zn density. Salient results of these studies were as under:

Use of High Zinc Seed: Whereas as high Zn density and Zn-primed seed increased emergence of wheat seedlings ($P \leq 0.05$) and seedling height ($P \leq 0.05$), soil applied zincated fertilizers did not. Also, high Zn seed (i.e., Zn biofortified as well as Zn-primed), in the absence of using Zn fertilizer, resulted in increased grain yields in most of the field experiments ($P \leq 0.05$). High Zn seed also increased grain Zn concentration in some field experiments.

Soil Applied Zinc Fertilizers: All treatments of soil-applied Zn fertilizer increased wheat yield, over the respective control yield, in all field experiments ($P \leq 0.05$). Overall maximum

yield during 2011–12 was obtained with Kali KornKali treatment, which was statistically similar to the yields obtained with most of the other soil-applied Zn treatments. During 2011–12, all soil-applied Zn treatments also resulted in increased Zn concentration in mature grains of wheat ($P \leq 0.05$). However, during 2012-13 grain Zn concentration did not increase with soil-applied Zn fertilizers.

Foliar Applied Zinc Fertilizers: Overall, all treatments of foliar-applied Zn fertilizer resulted in increased grain yields compared with the respective controls during both years ($P \leq 0.05$). Most of the foliar Zn treatments also enhanced grain Zn density during both years ($P \leq 0.05$). Foliar-fed zincated fertilizers proved more effective in increasing grain Zn concentration and soil-applied Zn fertilizers.

The use of soil-applied Zn as well as foliar-fed Zn fertilizers in wheat was highly cost effective. Additionally, the Zn-enriched wheat seed can bring additional economic benefit by way of better crop stand and yield of the succeeding crop.

Salient outcomes of these field studies were disseminated to the stakeholders by publishing crisp-clear brochures, one each in English and the national language (i.e., *Urdu*) and by organizing well attended one field day, and two national zinc days.

During the 2013–14 crop season, field experiments and demonstration trials are being conducted in three geographical districts of the Punjab province, Pakistan, i.e., Faisalabad, Lahore, and Sahiwal. No data have been recorded so far in the field experiments, as foliar Zn treatments are yet to be applied. In all the demonstration trials, use of high-Zn seed has resulted in much better seedling emergence and seedling vigor.

2. INTRODUCTION

In Pakistan, crop yield and quality losses due to soil zinc (Zn) deficiency are well recognized. Also, it is believed that low-Zn crop production, especially wheat and rice, leads to Zn malnutrition in humans and animals (Rashid, 2005). Wheat, the major staple cereal in the country, is grown on over 8.0 M ha annually and is badly affected by Zn deficiency. Though wheat is categorized as less sensitive to Zn deficiency (Rashid and Fox, 1992), significant yield increases ($P \leq 0.05$) were observed when Zn fertilizer was applied to the low-Zn soils (Rafique et al., 2006). In three-year field studies of the *Phase-I* of *HarvestZinc Fertilizer Project* as well, use of zincated fertilizers – by soil application and/or foliar feeding – resulted in significant increases in wheat yield as well as in enhancement of wheat grain Zn density ($P \leq 0.05$; Rashid et al, 2011; Zou et al., 2012).

Thus, use of Zn fertilizer is recommended for many crops, including wheat, in the country (Kausar and Rashid, 2002; Rashid, 2005). However, actual use of Zn fertilizer is limited to a few crops, like rice and citrus, and the dosage and frequency of its use is also much less than required (Rashid, 2005).

The primary objective of the field experiments under Phase-II of the HarvestZinc Fertilizer Project was to study the comparative effectiveness of using zincated fertilizers and Zn-enriched seed in optimizing wheat productivity and enhancing grain Zn density in major cropping system regions of Pakistan, i.e., rice-wheat system, mixed cropping system, and cotton-wheat system.

The objective of the 3rd year experiments during Phase-II is to study comparative effectiveness of various foliar Zn treatments in ameliorating Zn deficiency and enhancing grain Zn density in wheat. Also, the effectiveness of high-Zn seed for improving plant population, crop growth and yield is being field-demonstrated.

3. EXPERIMENTAL ACTIVITIES

3.1. Site Selection and Soil Properties

3.1.1. Years 2011–12 and 2012–13

Zn-deficient field sites were identified in the major rice-wheat system (Muridke), mixed cropping system (Faisalabad), and cotton-wheat system (Kabirwala) regions of the Punjab province, Pakistan (Fig. 1). Composite surface soils (0–30 cm) of the experimental fields were analyzed for salient physico-chemical properties (Table 1) by standard laboratory procedures (Ryan et al., 2001). Soil Zn was determined by the DTPA method of Lindsay and Norvell (1978).

3.1.2. Year 2013–14

Three field experiments have been laid out in the Punjab province of Pakistan, i.e, one each at Faisalabad, Lahore and Sabinal (Figure 1). The foliar Zn treatments of these experiments are given in Table 4. Initial soil properties of the field sites are presented in Table 1.

Figure 1. Locations of field experiments ( ) and demonstration trials (★) in the Punjab province, Pakistan.

3.2. Experimental Treatments and Crop Management

3.2.1. Years 2011-12 and 2012–13

For using low-Zn and high-Zn seed in field experiment-1 during 2011-12, Zn-enriched wheat seed (i.e., 50 mg Zn kg^{-1} seed) was produced by repeated foliar sprays of Zn to the 2010-11 crop. The low-Zn seed for 2011-12 Experiments contained 30 mg Zn kg^{-1}.

For the 2012-13 Experiment-1 and the field demonstration trials, high-Zn seed (41 mg kg^{-1}) as produced in the previous year's field experiments. The low-Zn seed for this year's experiments and trials contained 19 mg kg^{-1}. During 2011–12, Experiment-1 (on soil Zn application and high-Zn seed) and Experiemnt-2 (on foliar Zn application) were conducted in the above stated three cropping system regions. However, during 2012–13, these experiments were conducted in two cropping systems only, i.e., mixed cropping system and rice-wheat system. Treatment details are for Experiment-1 are given in Table 2, and for Experiment-2 in Table 3.

3.1.2. Year 2013–14

For the 2013-14 Experiments, low-Zn seed (21.5 mg kg^{-1}) was used. The foliar Zn treatments of these experiments are given in Table 4. For the demonstration trials, high-Zn seed (i.e., 45.5 mg Zn kg^{-1}) as well as low-Zn seed (i.e., 21.5 mg Zn kg^{-1}) was used. The experiments as well as the demonstration trials have four replications. The crop management practices were similar to the previous years.

Table 1. Surface soil (0–30 cm) properties of the experimental and demonstration fields.

Field site	pH_s	$CaCO_3$ (%)	Org. matter (%)	$NaHCO_3$-P (mg kg^{-1})	NH_4 OAc-K (mg kg^{-1})	DTPA-Zn (mg kg^{-1})
Wheat season 2011-12						
Muridke-I	8.0	12	0.52	5.4	84	0.45
Faisalabad	8.3	10	0.50	5.1	120	0.56
Kabirwala	8.1	13	0.66	9.8	110	0.52
Wheat season 2012-13						
Faisalabad-I	7.8	13.5	1.0	6.2	125	0.35
Faisalabad-II	7.8	21.5	0.2	6.8	115	0.75
Faisalabad-III	7.8	11.5	1.2	8.8	120	0.40
Muridke-II	8.0	7.7	1.2	9.4	90	0.88
Muridke-III	8.2	8.5	0.3	10	103	0.92
Wheat season 2013-14						
Faisalabad (Exp)	8.1	2.1	0.8	1.5	60	1.1
Faisalabad (Demo)	8.1	2.7	0.9	1.7	62	0.9
Lahore (Expt)	8.1	2.7	1.0	4.2	32	0.8
Lahore (Demo)	7.9	2.8	1.0	5.8	40	0.7
Sahiwal (Expt)	8.2	7.3	1.1	9.5	45	0.5
Sahiwal (Demo)	8.0	5.5	0.8	1.5	60	0.4

Table 2. Treatment details for soil zinc application (Experiment-1).

No.	Treatment
T1	**Local control (LC)** = 80 kg P_2O_5 ha^{-1} + 120 kg N ha^{-1}
T2	**LC + Soil $ZnSO_4.7H_2O$*** (@ 50 kg $ZnSO_4.7H_2O$ ha^{-1})
T3	**LC + Mosaic-MESZ#** = 80 kg P_2O_5 ha^{-1} as Mosaic-MESZ + 96 kg N ha^{-1}
T4	**LC + Mosaic-II** = 160 kg P_2O_5 ha^{-1} as MESZ + 72 kg N ha^{-1}
T5	**LC + Mosaic-III + Foliar Zn app.** = 80 kg P_2O_5 ha^{-1} as Mosaic-MESZ+ 96 kg N ha^{-1} + Zn Foliar Application @ 0.5 % $ZnSO_4.7H_2O$
T6	**LC + KornKali (KCl-Zn)§** = 80 kg P_2O_5 ha^{-1} + 120 kg N ha^{-1} +150 kg KornKali (60 kg KCl ha^{-1})
T7	**3xSplit Urea-Zn¥** = 80 kg P_2O_5 ha^{-1} + 120 kg Urea-Zn ha^{-1} (Prepared by spraying of 10 % $ZnSO_4.7H_2O$ on standard urea fertilizer)
T8	**LC + ADOB HBED Zn Chelate** = 80 kg P_2O_5 ha^{-1} + 120 kg N ha^{-1} + 250 L (kg) ha^{-1} ZnHBED
T9	**LC + *Seed Zn-I** = 80 kg P_2O_5 ha^{-1} + 120 kg N ha^{-1} + {Zn-enriched seed (2011–12, 50 mg Zn kg^{-1}, 2012-13, 41 mg Zn kg^{-1}) – by foliar sprays}
T10	**LC + Seed Zn-II** = 80 kg P_2O_5 ha^{-1} + 120 kg N ha^{-1} + (Zn-enriched seed – by priming with 5 mM $ZnSO_4$ solution for 1 hr)

[a]Low-Zn seed (2011–12, 30 **mg Zn kg^{-1}**; 2012-13, 19 mg Zn kg^{-1}) was used, unless stated otherwise
*$ZnSO_4.H_2O$ (33 % Zn) was used in place of $ZnSO_4.7H_2O$ (23 % Zn) while maintaining the above stated Zn rates.
[#]**MESZ :** 12 % N; 40 % P_2O_5, 10 % S, 1 % Zn;
[§]**Korn Kali:** 40 % K_2O; 6 % MgO; 1.5 % Zn**;** sprayed onto soil at planting and mixed with soil
[¥]For Urea-Zn (1% Zn containing Urea), 6.97 % solution of $ZnSO_4.H_2O$ was sprayed on urea granules to accomplish the desired concentration of Zn in the fertilizer
**Low-Zn seed's Zn concentration: 2011–12, 30 mg kg^{-1}; 2012–13, 19 mg kg^{-1}*

Table 3. Treatment details for foliar zinc application (Experiment-2).

T1	**Local control (LC)** = 80 kg P_2O_5 + 120 kg N ha^{-1}
T2	**LC + Foliar $ZnSO_4$** (once, before flowering**) (i.e.,** Foliar spray of 0.5 % $ZnSO_4.7H_2O$ solution @ 800 L ha^{-1})
T3	**LC + Foliar $ZnSO_4$** (once, after flowering) i.e., foliar spray of 0.5 % $ZnSO_4.7H_2O$ solution in 800 L water ha^{-1}
T4	**LC + Foliar $ZnSO_4$** (twice, i.e., once before flowering and then at early milk stage) i.e., foliar sprays of 0.5 % $ZnSO_4.7H_2O$ solution @ 800 L ha^{-1}
T5	**LC + OMEX-Type-II Foliar Zn** (i.e., OMEX-Type II @ 3.32 mL in 800 mL water per 10 m^2 (equivalent to 4 kg $ZnSO_4.7H_2O$ in 800 L water ha^{-1})
T6	**LC + OMEX-Type-III Foliar Zn** (i.e., OMEX-Type III @ 6.15 g in 800 mL water per 10 m^2 (equivalent to 4 kg $ZnSO_4.7H_2O$ in 800 L water ha^{-1})
T7	**LC + Kali-EPSO-Zn** (@ 18.2 g in 800 mL per 10 m^2 (equivalnet to 4 kg $ZnSO_4.7H_2O$ in 800 L water ha^{-1})
T8	**LC + ADOB- ZnIDHA** (i.e., ADOB ZnIDHA @ 9.1 g in 800 mL water per 10 m^2 (equivalent to 4 kg $ZnSO_4.7H_2O$ in 800 L water ha^{-1})
T9	**LC + Valagro Brexil** (@ 9.1 g in 800 mL water per 10 m^2 (eqivalent to 4 kg $ZnSO_4.7H_2O$ in 800 L water ha^{-1})
T10	**LC + Bayer Antracol-Zn** (@ 3 kg ha^{-1}), i.e., 3 g Antracol-Zn in 800 mL water for 10 m^2 area
T11	**LC + Commonly applied fungicide or insecticide** (Confidor (fungicide) mixed with same amount of $ZnSO_4.7H_2O$ (i.e., 4 kg $ZnSO_4.7H_2O$ ha^{-1} in 800 L water ha^{-1})
T12	**LC + FBScience CP Foliar Zn Fertilizer** (5 L ZicRon-F ha^{-1} mixed with 4 kg $ZnSO_4.7H_2O$ ha^{-1} in 800 L water ha^{-1}

Soil application of fertilizers was made by manual broadcasting to individual plots, followed by incorporation within the surface layer (~ 0–20 cm) with a tractor-mounted cultivator. Just prior to seed sowing, *Stomp* weedicide was applied @ 2.5 L ha^{-1}, using a manual sprayer. Wheat was sown using a single row manual drill, keeping line to line spacing of 25 cm, with seed rate of 100 kg ha^{-1}.

To control narrow- and broad-leaved weeds, during the month of January (when the crop was at tillering stage), two Zn-free weedicides (i.e., *Poma Super* @ 1.25 L ha^{-1} and *Buctril Super* @ 0.75 L ha^{-1}) were applied by using a hand sprayer. The recommended wheat cultivars were used at all field sites (Table 5). The experimental design was randomized complete block with four replications and plot size was 4 m x 9.6 m.

Table 4. The 2013–14 foliar Zn treatments for 2013–14 field experiments (to be applied at heading and early milk growth stages)[a].

Treatment
1) LC[b] (Local Control)) (NO ZINC SPRAY)
2) LC + 0.22 % $ZnS0_{4.}7H_2O$[c]
3) LC + 0.44 % $ZnS0_{4.}7H_2O$
4) LC + 0.50 % ZnIDHA-I
5) LC + 0.50 % ZnIDHA-II
6) LC + 0.22 % $ZnS0_{4.}7H_2O$ + 0.25 % Urea
7) LC + 0.44 % $ZnS0_{4.}7H_2O$ + 0.25 % Urea
8) LC + 0.22 % $ZnS0_{4.}7H_2O$ + Citric Acid[d]
9) LC + 0.44 % $ZnS0_{4.}7H_2O$ + Citric Acid
10) LC + 0.22 % $ZnS0_{4.}7H_2O$ + Citric Acid + 0.25 % Urea
11) LC + 0.44 % $ZnS0_{4.}7H_2O$ + Citric Acid + 0.25 % Urea
12) LC + 0.22 % 0.22 % $ZnS0_{4.}7H_2O$ + Adjuvant[e]
13) LC + 0.44 % 0.22 % $ZnS0_{4.}7H_2O$ + Adjuvant

[a] Low-Zn seed (i.e, 21.5 mg Zn kg-1) was used.
[b] LC = 80 kg P_2O_5 ha^{-1} + 120 kg N ha^{-1} + 70 kg K_2O ha^{-1}
[c] $ZnSO_4.H_2O$ (33 % Zn) was used in place of $ZnSO_4.7H_2O$ (23 %) while maintaining the above stated Zn concentrations
[d] An appropriate volume (i.e., about 8 mL) of 1.0 *M* Citric Acid solution was added to 3 Liter respective solution of $ZnSO_4$ (and shaken well) to bring the final solution pH between 4.5 and 5.5.
[e] 1.0 mL of ADOB Adjuvant was used in treatments 12 and 13 only; in all other Zn spray solutions, 0.05% detergent (i.e., *Surf Excel*) was added instead.

Table 5. Wheat cultivars used in the field experiments and demonstration trials.

Field site	Experiment	Wheat cultivar	Date of Sowing
	Wheat season 2011-12		
Muridke	Soil Zn and Foliar Zn	*Sehar*-2006	17 November, 2011
Faisalabad	Soil Zn and Foliar Zn	*Sehar*-2006	29 November, 2011
Kabirwala	Soil Zn and Foliar Zn	*Lasani*-2008	08 December, 2011
	Wheat season 2012-13		
Faisalabad	Soil Zn	*Sehar-2006*	11 December, 2012
	Foliar Zn	*Faisalabad-2008*	11 December, 2012
	Demonstration	*Sehar-2006*	28 December, 2012
	Demonstration	*Sehar-2006*	13 November, 2012
	Demonstration	*Sehar-2006*	13 November, 2012
Muridke	Soil Zn	*Sehar-2006*	15 November, 2012
	Foliar Zn	*Faisalabad-2008*	15 November, 2012
	Demonstration – I	*Sehar-2006*	15 November, 2012
	Demonstration – II	*Sehar-2006*	23 November, 2012

3.3. Field Demonstration Trials

To demonstrate beneficial impact of using Zn-enriched and Zn-primed seed on seedling emergence, plant growth, crop stand and crop productivity, during 2012-13 five demonstration trials were conducted in the vicinity of field experiments in rice-wheat system and mixed cropping system regions of the country. The demonstration trials had the following treatments:

No.	Treatment
T1	Local control (LC)*, #
T2	LC + Soil $ZnSO_4.7H_2O$ (@ 50 kg ha^{-1})
T3	LC + Zn-enriched seed (41 mg kg^{-1})
T4	LC + Zn-primed seed (with $ZnSO_4$)

*LC = 80 kg P_2O_5 + 120 kg N ha^{-1}

The low-Zn seed used in T1 and T2 contained 19 mg Zn kg^{-1}

The demonstration trials had three replications and plot size of 9 m x 15 m. The agronomic practices were similar to the field experiments.

During 2013–14, demonstration trials are being conducted at three field sites (Figure 1) to exhibit the impact of using high-Zn wheat seed (i.e., 45.5 mg Zn kg^{-1}) and low-Zn seed (i.e., 21.5 mg Zn kg^{-1}) on crop germination, growth and productivity. Soil properties of the field sites are given in Table 1.

4.RESULTS AND DISSCUSSION

4.1 Field Experiment-1: Soil Application of Zincated Fertilizers and Use of High Zinc Seed

4.1.1 Seedling Emergence and Vigor

Use of high-Zn density seed as well as Zn-primed seed resulted in substantial increases in seedling emergence per unit field area ($P \leq 0.05$; Table 6; Fig. 2). With high-Zn seed, increase in seedling emergence was 68% over control during 2011-12 and 92% during 2012-13. With Zn-primed seed, the magnitude of increase in seedling emergence was almost of the similar magnitude, i.e., 59% over control during first year and 99% during second year ($P \leq 0.05$). However, at any field sites, soil application of Zn fertilizer did not increase seedling emergence during either year (Table 6).

High Zn density seed also resulted in early seedling emergence (by 3–4 days), better seedling vigor and darker seedling color.

During both years, Zn-enriched seed also caused more vigorous seedling growth, resulting in higher plant height, compared with the other experimental treatments ($P \leq 0.05$; Table 6).

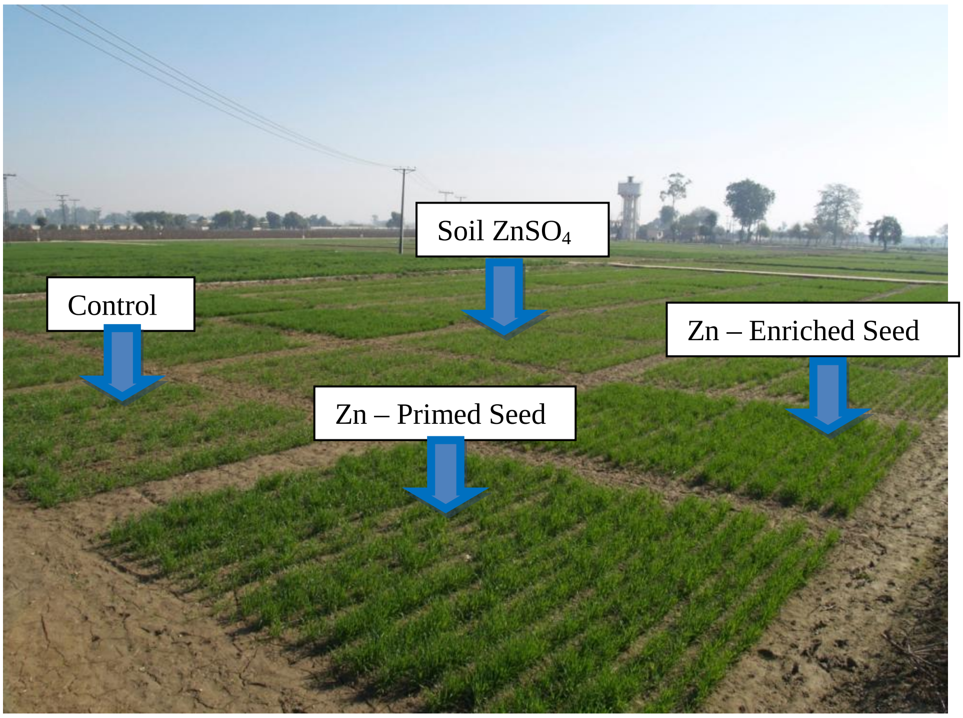

Figure. 2. Wheat seedling emergence as affected by seed Zn density.

During 2013–2014, use of high-Zn seed resulted in much better crop germination at two field sites, i.e., Faisalabad and Sahiwal (Figure 3; Table 7; $P \leq 0.05$). At the third field site as well, i.e., at Lahore, the increase in seedling emergence was appreciable.

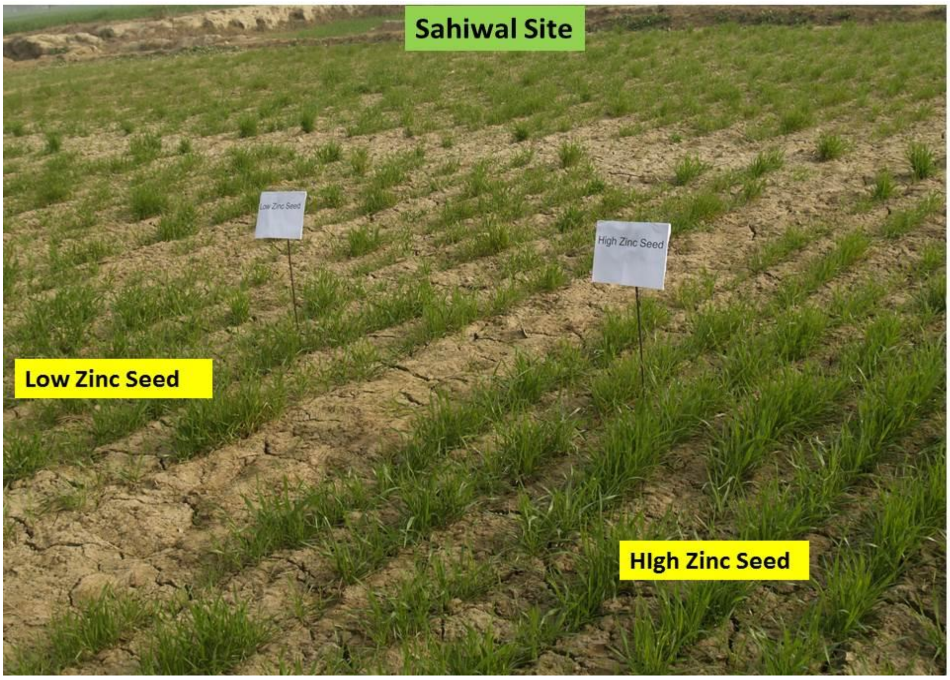

Figure 3. Wheat seedling emergence at Sahiwal, Punjab as affected by seed Zn density.

Table 6. Wheat seedling emergence and seedling height as affected by soil-applied zinc fertilizers and seed zinc density (Experiment-1).

TREATMENT	SEEDLINGS m^{-2}			SEEDLING HEIGHT (cm)		
	EXPERIMENT		DEMO	EXPERIMENT		DEMO
	2011-2012	2012-2013	2011-2012	2011-2012	2012-2013	2011-2012
1. Local control (LC)	197 b***	192 b	168 b	15.3 c	33.2 b	
2. LC + Soil $ZnSO_4.7H_2O$	205 b	199 b	154 b	15.7 bc	32.9 b	
3. LC+MosaicI	205 b	-	-	16.9 abc	34.2 b	
4. LC+MosaicII	201 b	-	-	15.8 bc	34.1 b	
5. LC+MosaicI+FoliarZn	188 b	-	-	14.8 c	33.4 b	
6. LC + Kali KornKali	211 b	-	-	15.8 bc	34.3 b	
7. 3 x Split Urea-Zn	200 b	-	-	16.4 abc	34.2 b	
8. LC+ADOB HBEDZn	209 b	-	-	15.6 c	34.3 b	
9. LC + high seed Zn (Foliar in last crop)	332 a	368 a	215 a	18.8 a	40.8 a	
10. LC + high seed Zn (Priming; 5mM $ZnSO_4$ 1 hr)	313 a	382 a	205 a	18.0 ab	39.8 a	
LSD (0.05)	53	31	17	2.4	2.9	-

*The values are means of 3 experiments in 2011-12, 2 experiments in 2012-13 and 5 demonstration/trials in 2012-13, and seedling height of one experiment in 2012-13.
**During 2012-13, seedling data were noted for the selected treatments only
***Values within a column followed by the same letter are not significantly different

Table 7. Wheat seedling emerged and seedling height as affected by seed zinc density (Demonstration Trials).

TREATMENT	SEEDLINGS m^{-2}			SEEDLING HEIGHT (cm)		
	Faisalabad	**Lahore**	**Sahiwal**	**Faisalabad**	**Lahore**	**Sahiwal**
Low Zn Seed	264 b	312	281 b	18.6	24.6	22.7
High Zn Seed	335 a	388	340 a	19.8	25.5	25.3
LSD ($P \leq 0.05$)	52	NS	115			

4.1.2. Grain Yield

Soil application of Zn fertilizers increased wheat grain yield in all field experiments during both years ($P \leq 0.05$; Table 8). During 2011-12, maximum mean grain yield (i.e., 5.26 t ha^{-1}) was obtained with KornKali (KCl-Zn), followed by 5.21 t ha^{-1} with ADOB HBED Chelated-Zn. Grain yields with all other zincated fertilizer treatments were also higher than the control yield ($P \leq 0.05$; Table 8). During 2012-13, maximum mean yield was recorded with Zn-primed seed (5.30 t ha^{-1}), which was followed by T7, i.e., Zn-enriched urea fertilizer, (5.17 t ha^{-1}) and high-Zn density seed (5.16 t ha^{-1}). Much better crop stand established with high Zn seed (Zn-fortified as well as Zn-primed; Table 6) led to better crop productivity, even in the absence of Zn fertilizer use.

4.1.3. Leaf Zn Concentration

During 2011-12, all experimental treatments resulted in increased Zn concentration in recently matured leaves of wheat prior to heading ($P \leq 0.05$; Table 9). Increases in Zn leaf concentration with Zn fertilizers and high-Zn seed were of the similar magnitude , except for Zn-primed seed at the Kabirwala field site which resulted in maximum increase in concentration ($P \leq 0.05$; Table 9). As DTPA extractable soil Zn levels at the field sites were in the deficient range (Rashid and Ryan, 2008), with control treatment Zn concentrations in wheat leaves were also in the deficient range (Rashid and Ryan, 2008). In such field situations use of Zn fertilizer is warranted.

Table 8. Wheat grain yield (t ha^{-1}) as affected by soil-applied zinc fertilizers and seed zinc density (Experiment-1).

	Wheat season 2011-12			
Treatment*	Faisalabad-I	Muridke-I	Kabirwala	Mean
Local control (LC)**	4.67 c***	4.34 c	4.14 e	4.38 c
LC + Soil $ZnSO_4.7H_2O$	4.84 abc	4.47 c	6.30 a	5.20 a
LC + Mosaic-MESZ	4.90 abc	5.03 bc	4.69 cd	4.87 ab
LC + Mosaic-II	5.18 ab	4.67 bc	5.04 cd	4.96 ab
LC + Mosaic-III + Foliar Zn	4.82 bc	5.66 a	4.92 cd	5.13 ab
LC + Kali KornKali	5.21 a	5.42 ab	5.14 bc	5.26 a
LC + 3xSplit Urea-Zn	4.90 abc	4.90abc	4.57 de	4.79 b
LC + ADOB HBED Zn	4.87 abc	5.61 a	5.15 c	5.21 a
LC + Seed Zn-I	4.87 abc	5.40 ab	4.74 cd	5.00 ab
LC + Seed Zn-II	5.08 ab	4.36 c	5.62 b	5.02 ab
LSD ($P \leq 0.05$)	0.38	0.81	0.55	0.39
	Wheat season 2012-13			
Treatment*	Faisalabad-I	Muridke-II	-	Mean
Local control (LC)	5.27 d	2.55 cd	-	3.91 d
LC + Soil $ZnSO_4.7H_2O$	6.26 bc	2.52 cd	-	4.39 bc
LC + Mosaic-MESZ	6.44 abc	2.65 bc	-	4.54 bc
LC + Mosaic-II	6.09 c	2.32 d	-	4.21 cd
LC + Mosaic-III + Foliar Zn	6.53 ab	2.90 b	-	4.71 b
LC + Kali KornKali	6.62 ab	2.53 cd	-	4.57 b
LC + 3xSplit Urea-Zn	6.74 a	3.56 a	-	5.17 a
LC + ADOB HBED Zn	6.74 a	2.63 bc	-	4.68 b
LC + Seed Zn-I	6.61 ab	3.72 a	-	5.16 a
LC + Seed Zn-II	6.84 a	3.75 a	-	5.30 a
LSD ($P \leq 0.05$)	0.42	0.28	-	0.35

*Treatment details in Table 2

**LC = 80 kg P_2O_5 + 120 kg N ha^{-1}

***Values within a column followed by the same letter are not significantly different

Table 9. Wheat leaf zinc concentration (mg kg^{-1}), one week before heading, as affected by soil-applied zincated fertilizers and seed zinc density (Experiment-1).

Wheat season 2011-12[a]				
Treatments*	**Faisalabad-I**	**Muridke-I**	**Kabirwala**	**Mean**
Local control (LC)	16.0 c**	18.6 cd	16.5 e	17.0 e
LC + Soil $ZnSO_4.7H_2O$	19.3 a	20.8 abc	27.2 bc	22.4 abc
LC + Mosaic-MESZ	20.3 a	18.6 cd	22.9 cde	20.6 cd
LC + Mosaic-II	16.9 bc	22.5 a	21.1 cde	20.2 cd
LC + Mosaic-III + foliar Zn	18.4 ab	21.6 ab	31.1 ab	23.7 ab
LC + Kali KornKali	18.4 ab	17.7 d	24.4 bcd	20.2 cd
3xSplit Urea-Zn	19.4 a	20.7 abc	17.7 de	19.3 de
LC + ADOB HBED Zn Chelate	19.9 a	19.8 bcd	27.3 bc	22.3 abc
LC + Seed Zn-I	19.4 a	17.7 d	27.4 bc	21.5 bcd
LC + Seed Zn-II	17.2 bc	19.8 bcd	36.2 a	24.4 a
LSD ($P \leq 0.05$)	1.9	2.4	7.1	2.4

[a] The 2012-13 leaf samples got contaminated
*Treatment details in Table 2.
**Values in a column followed by the same letter are not significantly different
*** ns = Non-significant

4.1.4. Wheat Grain Zn Concentration

Soil-applied zincated fertilizers as well as high Zn seed increased Zn concentration in mature wheat grains ($P<0.05$; Table 10). Maximum grain Zn concentration of 27.3 mg kg^{-1} was attained with 50 kg $ZnSO_4.7H_2O$ ha^{-1}, followed by 26.7 mg kg^{-1} Zn with Mosaic-III + Foliar Zn. Grain Zn concentrations with other zincated fertilizers were also significantly higher than the Zn concentration in wheat grain produced with control treatment. Even with Zn-enriched seeds (Zn-biofortified and Zn-primed), grain Zn concentrations were also higher than with the control treatment ($P<0.05$; Table 10).

Table 10. Wheat grain zinc concentration (mg kg^{-1}) as affected by soil-applied zinc fertilizers and seed zinc density (Experiment-1).

	Wheat season 2011-12			
Treatment*	**Faisalabad-I**	**Muridke-I**	**Kabirwala**	**Mean**
Local control (LC)**	13.5 f***	19.9 b	21.5 d	18.3 f
LC + Soil $ZnSO_4.7H_2O$	28.9 a	25.2 a	28.0 abc	27.3 a
LC + Mosaic-MESZ	21.4 cde	21.9 ab	28.1 abc	23.8 de
LC + Mosaic-II	21.4 de	23.6 ab	24.5 cd	23.2 e
LC + Mosaic-III + Foliar Zn	27.3 ab	25.3 a	27.6 abc	26.7 ab
LC + Kali KornKali	20.5 de	23.6 ab	24.7 cd	22.9 e
LC + 3xSplit Urea-Zn	23.8 bcd	22.2 ab	28.9 ab	25.0 cd
LC + ADOB HBED Zn Chelate	25.3 abc	21.3 ab	30.6 a	25.8 bc
LC + Seed Zn-I	19.4 e	23.8 ab	24.7 cd	22.6 e
LC + Seed Zn-II	22.5 cde	21.8 ab	25.3 bcd	23.2 e
LSD ($P < 0.05$)	3.9	3.9	3.9	1.51
	Wheat season 2012-13			
Treatment*	**Faisalabad-I**	**Muridke-II**	**-**	**Mean**
Local control (LC)	29.3 ab	32.9 ab	-	31.1 abc
LC + Soil $ZnSO_4.7H_2O$	28.9 ab	31.0 ab	-	24.0 bc
LC + Mosaic-MESZ	28.7 ab	33.2 ab	-	30.9 abc
LC + Mosaic-II	29.9 ab	31.5 ab	-	30.7 abc
LC + Mosaic-III + Foliar Zn	31.4 ab	32.7 ab	-	32.0 ab
LC + Kali KornKali	27.4 b	32.4 ab	-	29.9 bc
LC + 3xSplit Urea-Zn	29.4 ab	34.9 a	-	32.1 ab
LC + ADOB HBED Zn Chelate	31.2 ab	31.9 ab	-	31.5 abc
LC + Seed Zn-I	28.2 b	28.4 b	-	28.3 c
LC + Seed Zn-II	33.9 a	34.2 a	-	34.1 a
LSD ($P \leq 0.05$)	5.4	5.68		3.6

*Treatment details in Table 2

**LC = 80 kg P_2O_5 + 120 kg N ha^{-1}

***Values within a column followed by the same letter are not significantly different

4.2 Field Experiment-2: Foliar Application of Zincated Fertilizers

4.2.1 Grain Yield

All foliar Zn application treatments resulted in increased grain yield over control at all field sites during both years ($P<0.05$; Table 11). Figure 3 depict positive impact of zincated fertilizers on spike length and thickness. Overall, maximum grain yield of 5.08 t ha^{-1} was obtained with foliar application of Zn (mixed with insecticide) at early milk stage; while foliar feeding of $ZnSO_4$ at early milk stage resulted in grain yield of 5.00 t ha^{-1}, which was next to the maximum yield.

Table 11. Wheat grain yield (t ha^{-1}) as affected by foliar-applied zinc fertilizers (Experiment-2).

Wheat season 2011-12				
Treatment*	**Faisalabad-**	**Muridke**	**Kabirwala**	**Mean**
Local control (LC)**	3.98 f***	3.55 c	3.81 d	3.78 e
LC + Foliar $ZnSO_4.7H_2O$, at booting stage	4.98 abc	5.20 ab	4.75 abc	4.98 ab
LC + Foliar $ZnSO_4.7H_2O$, at early milk stage	5.13 ab	4.57 b	5.30 a	5.00 ab
LC + Foliar $ZnSO_4.7H_2O$, at early milk & booting	4.72 bcd	4.72 b	4.59 c	4.68 bc
LC + OMEX-Type-II, Foliar at early milk stage	4.60 bcd	5.60 a	4.62 bc	4.94 abc
LC + OMEX-Type-III, Foliar at early milk stage	4.38 def	4.51 b	3.70 d	4.20 d
LC + Kali-EPSO-Zn, Foliar Zn at early milk stage	4.73 bcd	4.94 ab	5.04 abc	4.91 abc
LC + ADOB-ZnIDHA, Foliar at early milk stage	4.41 def	5.07 ab	4.46 c	4.65 bc
LC + Valagro Brexil, Foliar at early milk stage	4.35 def	4.77 b	4.59 c	4.57 cd
LC + Antracol-Zn, Foliar at early milk stage	4.55 cde	4.97 ab	4.91 abc	4.81 abc
LC + Insecticide mixed with Zn at early milk stage	5.49 a	4.56 b	5.19 ab	5.08 a
LC + FBScience CP Foliar Zn at early milk stage	4.00 ef	5.15 ab	4.86 abc	4.67 bc
LSD (P ≤ 0.05)	**0.57**	**0.75**	**0.59**	**0.40**

Wheat season 2012-13				
Treatment*	**Faisalabad-**	**Muridke**	**-**	**Mean**
Local control (LC)	6.04 g	2.48 e	-	4.26 e
LC + Foliar $ZnSO_4.7H_2O$, at booting stage	7.07 ef	3.28 cd	-	5.18 d
LC + Foliar $ZnSO_4.7H_2O$, at early milk stage	7.27 def	3.62 bcd	-	5.45 bcd
LC + Foliar $ZnSO_4.7H_2O$, at early milk & booting	7.39 cde	3.04 de	-	5.22 d
LC + OMEX-Type-II, Foliar at early milk stage	6.86 f	3.66 bc	-	5.26 cd
LC + OMEX-Type-III, Foliar at early milk stage	7.71 bcd	3.78 abc	-	5.74 ab
LC + Kali-EPSO-Zn, Foliar Zn at early milk stage	8.19 a	3.87 ab	-	6.03 a
LC + ADOB-ZnIDHA, Foliar at early milk stage	7.12 ef	4.34 a	-	5.73 ab
LC + Valagro Brexil, Foliar at early milk stage	7.15 ef	3.68 bc	-	5.41 bcd
LC + Antracol-Zn, Foliar at early milk stage	8.01 ab	3.86 abc	-	5.94 a
LC + Insecticide mixed with Zn at early milk stage	7.74 abc	3.65 bc	-	5.69 ab
LC + FBScience CP Foliar Zn at early milk stage	7.81 abc	3.49 bcd	-	5.65 abc
LSD (P ≤ 0.05)	0.45	0.58	-	0.42

**LC = 80 kg P_2O_5 + 120 kg N ha^{-1}

***Values within a column followed by the same letter are not different from each other

4.2.2 Grain Zinc Concentration

Foliar-applied zincated fertilizers resulted in appreciable increases in Zn concentration in mature wheat grains in all field experiments during both years ($P \leq 0.05$; Table 12). During 2011-12, maximum mean grain Zn concentration of 34.0 mg kg^{-1} was attained with two foliar sprays of $ZnSO_4$, at milk and booting stages, followed by 28.9 mg kg^{-1} with foliar feeding of $ZnSO_4$ at early milk stage. Much higher grain Zn concentrations were achieved with foliar feeding of Zn compared with soil Zn applications in all field experiments during both years (Tables 10 and 12).

4.3. Economic Benefit of Using Zinc Fertilizer

Based on the prevalent market prices of wheat and fertilizer zinc sulfate in the country and the mean yield increases with Zn use over the past two crop seasons, soil applied as well as foliar fed Zn proved highly cost-effective (Table 13). As the uptake of soil applied Zn fertilizer by the current wheat crop is very low (i.e., less than 1% of the applied dose; Rafique et al., 2012), the soil applied Zn pays off in terms of its beneficial residual effect for the succeeding 2-3 crops in the rotation. Additionally, the Zn-enriched grains produced can serve as a superior quality seed for the next year wheat crop – for attaining better crop stand and higher yield. Thus, the actual economic benefit of using Zn fertilizer is much greater compared to the computations exhibited in Table 13.

4.4 CONCLUSIONS

- Use of all the Zn fertilizer products included in this study resulted in increased cop productivity per unit field area as well as in enhanced grain Zn density. Foliar feeding of Zn fertilizers was more effective in increasing Zn concentration in mature grains.

- Use of Zn-biofortified as well as Zn-primed seed resulted in more plant population per unit field area and, thus, led to increased crop yields even in the absence of Zn fertilizer.

- Use of Zn fertilizer in wheat is highly cost effective.

- The high-Zn seed produced by using Zn fertilizer can help attain higher yield of the next crop.

Table 12. Wheat grain zinc concentration (mg kg^{-1}) as affected by foliar-applied zinc fertilizers (Experiment-2).

Wheat season 2011-12

Treatment*	Faisalabad	Muridke	Kabirwala	Mean
Local control (LC)**	21.0 f***	21.1 d	24.2 ab	22.1 e
LC + Foliar $ZnSO_4.7H_2O$, at booting stage	26.5 bcd	27.2 bc	28.5 a	27.4 bc
LC + Foliar $ZnSO_4.7H_2O$, at early milk stage	29.6 bcd	29.7 b	27.6 ab	28.9 B
LC + Foliar $ZnSO_4.7H_2O$, at early milk & booting	40.9 a	34.9 a	26.2 ab	34.0 a
LC + OMEX-Type-II, Foliar at early milk stage	30.3 bc	28.5 bc	23.9 ab	27.7 bc
LC + OMEX-Type-III, Foliar at early milk stage	23.6 def	27.4 bc	23.0 b	24.7 cde
LC + Kali-EPSO-Zn, Foliar Zn at early milk stage	24.6 cde	29.6 b	24.8 ab	26.4 bcd
LC + ADOB-ZnIDHA, Foliar at early milk stage	31.3 b	27.1 bc	24.4 ab	27.6 bc
LC + Valagro Brexil, Foliar at early milk stage	30.1 bc	28.4 bc	22.9 b	27.1 bc
LC + Antracol-Zn, Foliar at early milk stage	22.7 ef	24.9 cd	22.9 b	23.5 de
LC + Insecticide mixed with Zn at early milk stage	22.6 ef	24.9 cd	27.5 ab	25.0 cde
LC + FBScience CP Foliar Zn at early milk stage	27.8 bcd	25.1 cd	26.3 ab	26.4 bcd
LSD ($P \leq 0.05$)	6.2	4.3	5.1	3.5

Wheat season 2012-13

Treatment*	Faisalabad	Muridke-	-	Mean
Local control (LC)	29.8 f	32.4 d	-	31.1 e
LC + Foliar $ZnSO_4.7H_2O$, at booting stage	34.2 bcde	49.1 a	-	41.7 ab
LC + Foliar $ZnSO_4.7H_2O$, at early milk stage	37.6 b	43.6 ab	-	40.6 ab
LC + Foliar $ZnSO_4.7H_2O$, at early milk & booting	36.8 bc	41.2 bc	-	38.9 bc
LC + OMEX-Type-II, Foliar at early milk stage	45.1 a	44.6 ab	-	44.8 a
LC + OMEX-Type-III, Foliar at early milk stage	31.9 def	33.9 d	-	32.9 de
LC + Kali-EPSO-Zn, Foliar Zn at early milk stage	35.2 bcd	32.6 d	-	33.9 de
LC + ADOB-ZnIDHA, Foliar at early milk stage	33.2 cdef	32.4 d	-	32.8 de
LC + Valagro Brexil, Foliar at early milk stage	30.9 ef	32.7 d	-	31.8 e
LC + Antracol-Zn, Foliar at early milk stage	36.4 bc	33.1 d	-	34.7 cde
LC + Insecticide mixed with Zn at early milk stage	30.5 ef	44.4 ab	-	37.5 bcd
LC + FBScience CP Foliar Zn at early milk stage	31.1 def	34.3 cd	-	32.7 de
LSD ($P \leq 0.05$)	4.2	7.3	-	4.9

Table 13. Economic analysis of using zinc fertilizer for wheat in Pakistan.

Treatment	Grain Yield (t ha^{-1})	Value of Additional Yield ha^{-1} (USD**)	Cost of Zinc Sulfate + Labor ha^{-1} (USD)	Value-Cost Ratio
Local Control*	4.08	-		
Soil Zn Application (25 kg Zinc Sulfate ha^{-1})***	4.70	178.25	38.50 +0 = 38.50	4.6:1
2 Foliar Spays of Zinc Sulfate	4.71	181.13	12.32 + 8.0 = 20.32	8.9:1
Soil Application + 2 Foliar Sprays	4.81	209.88	38.50 + 20.32 = 58.82	3.6:1

*Basal N and P only; Zn not applied
** 1 USD = Pakistan Ruppee 100
***As Zn fertilizer was applied along with N and P fertilizers, no labor cost involved

5. DEMONSTRATION TRIALS

In the five large plot sized demonstration trials conducted during 2012-13 as well high Zn density seed as well as Zn-primed seed resulted in enhanced seedling emergence ($P \leq 0.05$; Table 6). Soil-applied zinc sulfate increased grain yield in four out of five trials ($P \leq 0.05$; Table 14). However, in the absence of using Zn fertilizer, such yield increases were observed in three trials each with high Zn density seed and Zn-primed seed ($P \leq 0.05$).

During the wheat season 2013–14 as well, use of high-Zn seed has resulted in much better seedling emergence and seedling vigor.

Table 14. Wheat grain yield (t ha-1) and grain zinc concentration (mg kg^{-1}) as affected by soil-applied zinc and Zn-enriched seed used in five demonstration trials (2012-13).

Treatment	Mixed cropping region			Wheat-rice region		Mean
	Fsd-I	Fsd-II	Fsd-III	Muridke-II	Muridke-III	
Grain yield (t ha^{-1})						
Local control (LC)*	2.33 b**	2.17 b	2.18 b	2.32 b	2.14 b	2.23 b
LC + Soil $ZnSO_4.7H_2O$ (@ 50 kg ha^{-1})	2.94 a	2.47 ab	2.51 a	2.46 b	2.68 a	2.61 a
LC + Zn-enriched seed (41 mg kg^{-1})	3.24 a	2.80 a	2.42 ab	2.83 a	2.38 ab	2.73 a
LC + Seed primed with $ZnSO_4$	2.93 a	2.65 ab	2.56 a	2.84 a	2.47 ab	2.69 a
LSD ($P \leq 0.05$)	0.52	0.51	0.31	0.28	0.47	0.17
Grain zinc concentration (mg kg^{-1})						
Local control (LC)	24.7	28.8	38.9	28.8 ab	27.3	28.5
LC + Soil $ZnSO_4.7H_2O$ @ 50 kg ha^{-1}	24.4	26.5	33.1	30.8 a	23.9	28.9
LC + Zn-enriched seed (41 mg kg^{-1})	23.1	24.1	33.3	27.5 ab	28.5	27.9
LC + Zn-primed seed	27.7	23.9	35.6	25.5 a	27.1	27.3
LSD ($P \leq 0.05$)	ns***	ns	ns	4.9	ns	ns

*LC = 80 kg P_2O_5 + 120 kg N ha^{-1}
**Values within a column followed by the same letter are not significantly different
***ns = Non-significant

6.TECHNOLOGY TRANSFER

6.1 Technology Transfer Brochures

The salient outcomes of the *HarvestPlus Zinc Fertilizer Project's* studies in Pakistan were disseminated by to the stakeholders by publishing crisp-clear two brochures, one each in English and the national language (i.e., *Urdu*) (Hassan and Rashid, 2012; Rashid et al., 2013; Figure 4) and by organizing two National Zinc Days and one Field Day. The brochures highlighted the importance of Zn in plant and human nutrition, incidence and magnitude of Zn deficiency in soils and crops of Pakistan – because of alkaline-calcareous nature of the soils, and positive impacts of using high-Zn seed and Zn fertilizers on seedling emergence, crop stand, crop yield and grain Zn density.

Figure 4. The local language (i.e., Urdu) and English brochures, highlighting the outcomes of HarvestZinc Project's studies in Pakistan.

6.2 Farmers' Field Day

For sharing the impressive impacts of using high-Zn wheat seed (i.e., Zn-biofortified and Zn-primed seed) on much better seed germination/seedling emergence and seedling vigor, a farmers' field day was organized on 29 December, 2012 in the rice-wheat cropping system region at village Muhammad Pura (i.e., near Muridke). More than 100 stakeholders participated. The participants took keen interest in the impressive positive impacts of high-Zn wheat seed by way of much higher seedling emergence and seedling vigor (Figure 5).

The participants were educated by way of display charts and technology transfer brochures about the *HarvestPlus Zinc Fertilizer Project's* salient outcomes.

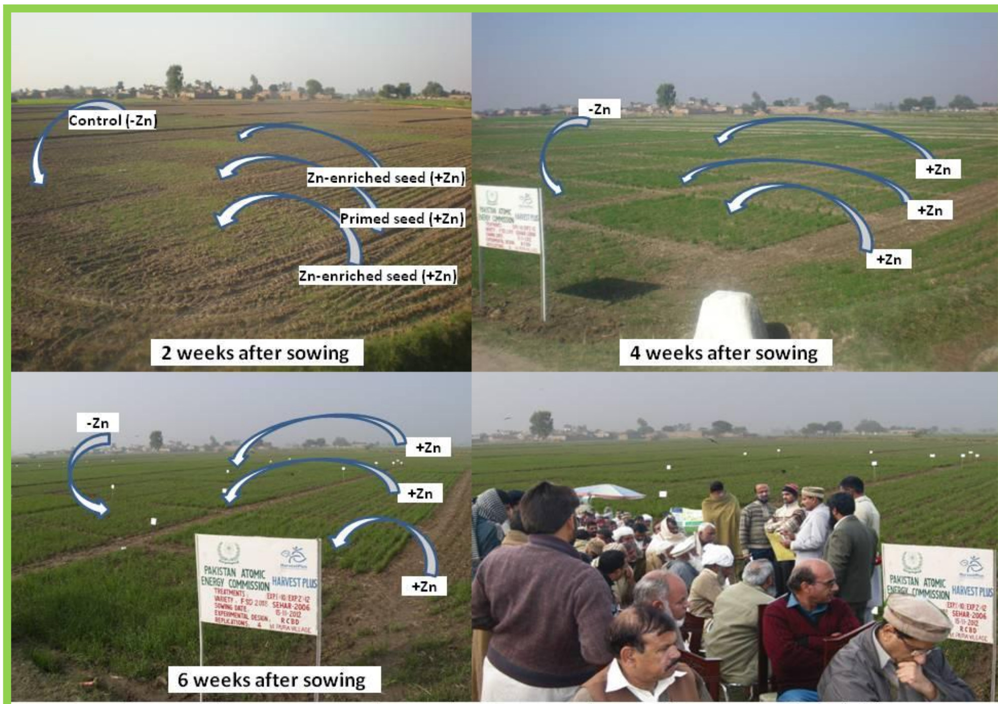

Fig. 5 Zinc effect on seedling emergence and canopy development in wheat and keen interest of stake holders in learning the Zn use technology. Location: Muhammad Pura Village near Lahore, Punjab, Pakistan

6.3 Pakistan Zinc Days

For enhancing stakeholders' awareness about the positive impacts of using Zn fertilizers and high-Zn seed on seedling emergence, crop stand and growth, wheat yield, and farmer income as well as the negative consequences of soil Zn deficiency on crop productivity and human and animal nutrition, the *National Zinc Days* were organized at NIAB, Faisalabad, Pakistan on 27th March 2012 and 12 March 2013. More than 250 participants at each of these events included farmers, agronomists, medical doctors, food technologists, agricultural extension workers, university professors, postgraduate students and the personnel of fertilizer, food and feed industry from all over the country (Figure 6).

Figure 6. Participants of the Pakistan Zinc Day with the organizers.

The learned resource persons shared latest R&D information on soil-plant-human/animal Zn deficiency problem and its cost-effective and practically feasible management strategies in agricultural soils, crops, fruits, vegetables, humans and animals. The importance and advantages of Zn biofortification of the predominant staple cereal in the country, i.e., wheat, was highlighted and a farmer-friendly technology of using zincated fertilizers for producing Zn biofortified wheat was delivered to the stakeholders.

The resource persons included the Coordinator of *HarvestPlus Zinc Fertilizer Project* in Pakistan, Dr. Abdul Rashid, a globally renowned authority of human Zn nutrition, Prof. Dr. Zulfiqar A. Bhutta of Aga Khan University, Karachi, the Vice Chancellor of University of Veterinary & Animal Sciences, Lahore, Prof. Dr. Talat N. Pasha, Chief Executive of the Punjab Agricultural Research Board, Dr, Mubarak Ali, a professor from KP Agricultural University, Peshawar, Dr. Sajida Parveen, a micronutrient researcher from Nuclear Institute for Food & Agriculture, Peshawar, Dr. M. Imtiaz, a professor from University of Agriculture, Faisalabad, Dr. M. Aamer Maqsood, and a researcher working on HarvestZinc Project, Mr. Mahmood-ul-Hassan.

Participants of the *Pakistan Zinc Days* also visited wheat field trials on agronomic biofortification of wheat. The brochures (Hassan and Rashid, 2012; Rashid et al, 2013) and souvenirs were distributed amongst the participants.

7. FUTURE R&D NEEDS

For effective utilization of the salient outcomes of this project, the following R&D activities are warranted:

- More widespread demonstration trials to disseminate beneficial impacts of using zincated fertilizers and Zn-enriched seed.
- More effective publicity of this project's outcomes by using print and electronic media.
- Improving availability of good quality Zn fertilizer products in the country.
- Facilitating use of Zn by farmers, by convincing the fertilizer industry and the Government for blending Zn with major nutrient fertilizers.
- Carrying out Zn nutrition trials on humans and animals for adequate supply of Zn to resource-poor segments of the society and livestock.

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COUNTRY REPORT - THAILAND

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2. SUMMARY AND MAIN FINDINGS

First and second year experiments were conducted at two locations in Thailand; Takli district, Nakhon Sawan (Takli site 240 km north of Bangkok: pH 7.7, DTPA Zn 0.5 ppm, P 32 ppm, K 57 ppm, Ca 3,360 ppm) and Chiang Mai University (Chiang Mai site: pH 6.2, DTPA Zn 0.9 ppm, P 54 ppm, K 58 ppm, Ca 450 ppm). Same lot of seed of Chainat 1 (CNT1 - a popular Thai rice variety, with 13 mg Zn kg^{-1}), was used in the two locations in both years. Twenty-day-old seedlings were transplanted to plots in 4 replicates in randomized complete blocks. At each site, there was one experiment with 7 soil Zn treatments, 2 seed Zn treatments and Zn- local control and one experiment with 11 foliar Zn treatments and one Zn- local control, repeating in different fields in the same vicinity in the two seasons. First year at both locations experiments were conducted in dry season of 2012, second year at Takli it was in dry season in 2013 and at Chiang Mai it was in wet season in 2012, with the following main findings.

Soil application of Zn and Zn treated seed

Grain yield and growth: Grain yield response to soil Zn varied with site, year and the Zn formulation, with the biggest increase of 18 % (0.9 t ha^{-1}) by ADOB and KKL at Takli in Year 1, with even more obvious effect on total dry weight (grain + straw), with an increase of 30% by KKL and 29% by ADOB. The grain yield was increased much less by $ZnSO_4$ applied to soil (by 9%) and planting with Zn primed seed (8%). Soil application of ADOB also significantly increased grain yield at Chiang Mai (by 19%) in the second year.

Grain Zn concentration: In the Zn- control, grain Zn was higher at Chiang Mai than at Takli in un-husked (by 43%), brown (by 42%) and white (by 24%) rice. Soil Zn treatments that increased grain Zn in rice at Takli were $ZnSO_4$ (by 24% in brown rice and 31% in white rice), KKL (by 13% in brown rice and 24% in white rice) and urea-Zn (by 21% in brown rice and

22% in white rice). These treatments also increased grain Zn at Chiang Mai, but to a much smaller extent.

Foliar Zn treatments

***Grain yield and growth**:* Grain yield response to foliar Zn varied with site, year and the Zn formulation, being largest in Chiang Mai with increases of 34% (1.3 t ha^{-1}) by Kali EPSO in year 1, and 16% by Antracol and Valagro in year 2. Other foliar Zn treatments that increased grain yield, but to a less extent, were OMEXIII and $ZnSO_4$ at booting and milky stage at Takli in both years, Antracol and Valagro at Takli in year 2, and $ZnSO_4$ at booting at Chiang Mai in year 1.

***Grain Zn concentration*:** Effects of foliar Zn on grain Zn were much larger than soil Zn treatments, and with major differences among the different Zn formulation and significant effects on the Zn in un-husked grain, brown rice and white rice. In year 1 at Takli, the Zn in un-husked rice was increased from 12.5 mg Zn kg^{-1} in Zn- control to 32.6±9.1 mg Zn kg^{-1} on the average with the foliar Zn treatments; in brown rice the increase was from 13.9 mg Zn kg^{-1} to 18.7±2.0 mg Zn kg^{-1}; and in white rice from 10.9 mg Zn $kg^{-1}$to 13.9±1.3 mg Zn kg^{-1}. In year 1 at Chiang Mai in un-husked rice the increase was from 19.6 mg Zn kg^{-1} in Zn- control to 38.2±10.3 mg Zn kg^{-1} on the average with the foliar Zn treatments; in brown rice it was from 21.2 mg Zn kg^{-1} to 26.0±2.0 mg Zn kg^{-1}; and in white rice from 15.8 mg Zn kg^{-1} to 19.7±0.9 mg Zn kg^{-1}. Among the different foliar Zn formulations, the largest and most consistent increases in grain Zn in both locations and years, in un-husked, brown and white rice, were from $ZnSO_4$ at booting and milky stage.

In both experiments grain Zn concentration, in brown and white rice, was closely and positively associated with grain yield, indicating that where rice yield is limited by Zn, the grain Zn concentration will also be low.

Capacity building and dissemination of results: Capacity in working with Zn at the collaborating lab at Chiang Mai University has been strengthened by the project with involvement of a postdoctoral fellow, 5 PhD and 2 MS students. Project results on major effects of location and genotype on grain Zn have led to more research activities on evaluation of genotypic variation in grain Zn concentration and rice production on calcareous soil. Locally funded research has also started Zn nutrition in rice and other crops of economic importance in Thailand. Research results have been disseminated in published papers and FarmNotes on Zn nutrition and management of rice and sugarcane, another of Thailand's economically important crop.

3. EXPERIMENTAL ACTIVITIES

First and second year experiments were conducted at two locations of Thailand (Figure 1), one on alkaline low-Zn soil (Takli) and one on more neutral soil with higher Zn (Chiang Mai). The Takli site was in Nakhon Sawan province (240 km north of Bangkok) and Chiang Mai site was at Chiang Mai University. The soil at Takli had pH 7.7, with 3,360 ppm extractable Ca, 31.8 ppm available P and 56.8 ppm extractable K, and the Chiang Mai soil had pH 6.2, with 450 ppm extractable Ca, 54.4 ppm available P and 58.5 ppm extractable K (Table 1). The same seed lot of a popular Thai rice variety, Chainat 1 (CNT1) with 13 mg Zn kg^{-1} in grain, was used in the two locations in both years. Twenty-day-old seedlings were transplanted to plots (4 m x 4 m each at Takli site and 3 m x 4 m at Chiang Mai site) in RCB with 4 replicates. Individual plots were separated by a low embankment, with irrigation water supplied separately. Plant spacing was 25 cm x 25 cm. Activities of two year trials are shown in Table 2.

Table 1. Soil fertility characteristics at two experimental sites.

	CHIANG MAI	TAKLI
pH	6.2	7.7
Organic matter (%)	1.5	3.7
Phosphorus (ppm)	54.4	31.8
Potassium (ppm)	58.5	56.8
Calcium (ppm)	450.3	3360.0
Zn, DTPA extractable (mg/kg)	0.9	0.5

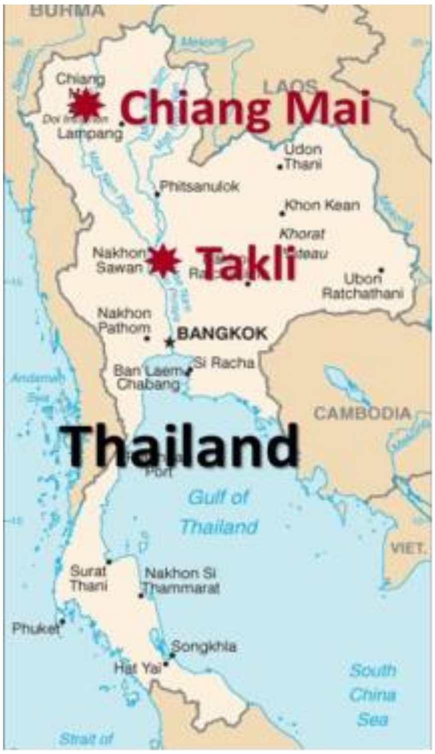


Figure 1. Location of experiments.

Table 2. Work schedule of two years' experiments at Takli and Chiang Mai locations during 2011-2013

Activities	**First year experiment (Year 1: 2011-2012)**		**Second year experiment (Year 2: 2012-2013)**	
	Takli	Chiang Mai	Takli	Chiang Mai
1. Sowing in nursery	1-12-2011	4-1-2012	15-10-2012	13-7-2012
2. Plot preparation and fertilizer application	22-12- 2011	13-2-2012	8-11-2012	5-8-2012
3. Seedling transplanting to plot	23-12- 2011	15-2-2012	9-11-2012	7-8-2012
4. Collection of leaf samples (YEB) at tillering stage and N application	21-2-2012	20-4-2012	7-1-2013	28-9-2012
5. Collection of leaf samples (flag leaf) at booting stage	3-3-2012	10-5-2012	31-1-2013	16-10-2012
6. Foliar Zn application at booting stage (Exp 2: T2, T4)	3-3-2012	11-5-2012	10-2-2013	16-10-2012
7. Foliar Zn application at early milk stage	14-3-2012	28-5-2012	10-2-2013	29-10-2012
8. Maturity	3-4-2012	19-6-2012	1-3-2013	21-11-2012

At each site, there was one experiment with 7 soil Zn treatments, 2 seed Zn treatments and Zn- local control (Table 3) and one experiment with 11 foliar Zn treatments and one Zn- local control (Table 4), repeating in different fields in the same vicinity in the two seasons (Figures 2 and 3).

Figure 2. Experimental field at Takli, year 1.

Figure 3. Experimental field at Chiang Mai, year 1.

Table 3. Soil and seed Zn treatments for experiment 1.

Treatment	Soil Application (kg ha^{-1})		Foliar Zn
	Planting	Tillering	
1. Local control (LC)	80 P_2O_5 75 N	75 N	
2. LC+ soil Zn	80 P_2O_5 75 N 50 $ZnSO_4.7H_2O$	75 N	
3. LC+MosaicI	200 MESZ 51 N	75 N	
4. LC+MosaicII	400 MESZ 27 N	75 N	
5. LC+MosaicI+FoliarZn	200 MESZ 51 N	75 N	4g $ZnSO_4.7H_2O$ g in 800 ml per 10 m^2 plot size; applied at early milk
6. LC+KKL	80 P_2O_5 75 N 150 KKL	75 kg N/ha	
7. 2xSplit Urea-Zn	80 P_2O_5 75 N with Zn containing	75 N with Zn containing	
8. LC+ADOB HBEDZn	80 P_2O_5 75 N 250 ADOB	75 N	
9. LC + high seed Zn (Foliar in last crop to 60 mg Zn kg^{-1})	80 P_2O_5 75 N	75 N	
10. LC + high seed Zn (Priming with 5 mM $ZnSO_4$ for 1 hr)	80 P_2O_5 75 N	75 N	

For leaf nutrient analysis, youngest emerged leaf blades (YEB) were collected at primordia initiation and flag leaves were collected at booting stage in experiment 1. At maturity, grain and straw yields were determined, with panicle number and weight of grain per panicle for year 1. Un-husked rice, brown rice, white rice were analyzed for nutrient content. Grains from year 2 were milled to assess the effect of Zn fertilizer on head rice yield, % un-broken grain, which is an important determinant of rice price.

Table 4. Foliar Zn treatments for experiment 2.

Treatment	Soil application ($kg\ ha^{-1}$)		Foliar Zn <g(ml) in 800 ml per 10 m^2 plot size>	
	Planting	tillering	End of booting	Early milk
1. Local control (LC)	80 P_2O_5 75 N	75 N		
2. LC+ Foliar $ZnSO_4$	80 P_2O_5 75 N	75 N	4 g $ZnSO_4.7H_2O$	
3. LC+ Foliar $ZnSO_4$	80 P_2O_5 75 N	75 N		4 g $ZnSO_4.7H_2O$
4. LC+ Foliar $ZnSO_4$	80 P_2O_5 75 N	75 N	4 g $ZnSO_4.7H_2O$	4 g $ZnSO_4.7H_2O$
5. LC+OMEX II	80 P_2O_5 75 N	75 N		3.32 ml OMEX II
6. LC+OMEX III	80 P_2O_5 75 N	75 N		6.15 ml OMEXIII
7. LC+Kali-EPSO	80 P_2O_5 75 N	75 N		18.2 g EPSO
8. LC+ ADOB ZnIDHA	80 P_2O_5 75 N	75 N		9.1 g ZnIDHA
9. LC+ Valagro Brexil	80 P_2O_5 75 N	75 N		9.1 g Brexil
10.LC+Antracol	80 P_2O_5 75 N	75 N		3 g Antracol
11.LC+ Insecticide†	80 P_2O_5 75 N	75 N		Mixing: Insecticide+ 4 g $ZnSO_4.7H_2O$
12.LC+FBScience	80 P_2O_5 75 N	75 N		Mixing: 5 ml FBScience + 4 g $ZnSO_4.7H_2O$

† Insecticide as 5% Fipronil (1 ml in 1000 ml water) was used at both locations.

4. RESULTS

4.1. Soil and seed Zn treatments

Grain yield and growth

Grain yield response to soil Zn varied with site, crop and the Zn formulation (Table 5). The greatest increase in grain yield of 18% (0.9 $t\ ha^{-1}$) was realized by ADOB and KKL in year 1 at Takli, with consistent effects of Zn treatment also on straw yield (Table 6), thus more obvious effect on total dry weight (grain + straw), with an increase of 30% by KKL and 29% by ADOB. The grain yield was also increased but to a less extent (by 9%) by $ZnSO_4$ applied to soil and planting with Zn primed seed (8%). Soil application of ADOB also significantly increased grain yield at Chiang Mai (by 19%) in year 2. The number of panicles m^{-2} and weight of grain per panicle were also significantly affected by soil/seed Zn treatments, but with increases in grain yield closely associated only with increases in grain load (Table 7). The increase in grain yield was closely associated with increase in total above ground dry weight at both sites.

Table 5. Grain yield response to soil and seed Zn treatments.

Soil/seed Zn treatment	Takli				Chiang Mai				Mean
	2011-2012		2012-2013		2011-2012		2012-2013		
	Grain yield (t ha^{-1})								
1. Control	4.84	bcd	4.22		7.74	a	6.67	bc	5.87
2. $ZnSO_4$	5.29	abc	4.51		8.10	a	6.84	bc	6.19
3. Mosaic I	5.51	ab	4.42		8.08	a	6.60	c	6.15
4. MosaicII	4.45	cd	4.28		7.74	a	7.23	b	5.92
5. MosaicI+Foliar	4.90	abcd	4.50		8.14	a	7.03	bc	6.14
6. KKL	5.72	a	4.43		7.95	a	7.22	b	6.33
7. Urea-Zn	4.57	cd	4.07		8.04	a	6.77	bc	5.86
8. ADOB	5.73	a	4.26		7.68	a	7.97	a	6.41
9. Zn+ seed (F)[1]	4.32	d	4.30		6.44	b	7.03	bc	5.52
10. Zn+ seed (P)[2]	5.25	abc	4.14		7.29	ab	7.01	bc	5.92
Mean	**5.06**		**4.31**		**7.72**		**7.04**		
F-test	$P < 0.05$		NS[3]		$P < 0.05$		$P < 0.05$		
$LSD_{0.05}$	0.86		-		1.01		0.59		

[1]Foliar Zn on seed crop [2]*Priming* with 5 mM $ZnSO_4$ for 1 hr [3]$P < 0.05$

Table 6. Straw yield response to soil and seed Zn treatments

Soil/seed Zn treatment	Takli				Chiang Mai				Mean
	2011-2012		2012-2013		2011-2012		2012-2013		
	Straw yield (t ha^{-1})								
1. Control	5.65	cd	5.38	a	7.30	bc	6.86	b	6.30
2. $ZnSO_4$	6.03	cd	5.10	ab	7.93	bc	6.75	bc	6.45
3. Mosaic I	5.24	d	5.11	ab	7.51	bc	6.51	bc	6.09
4. MosaicII	5.98	cd	4.61	bc	7.16	c	6.93	b	6.17
5. MosaicI+Foliar	7.53	ab	5.07	ab	7.27	c	6.37	c	6.56
6. KKL	7.96	a	5.66	a	8.92	a	6.33	c	7.22
7. Urea-Zn	6.75	abc	4.43	c	8.20	ab	6.55	bc	6.48
8. ADOB	7.76	a	5.12	ab	7.67	bc	7.54	a	7.02
9. Zn+ seed (F)[1]	6.82	abc	5.07	ab	7.35	bc	6.61	bc	6.46
10. Zn+ seed (P)[2]	6.32	bcd	4.58	bc	7.05	c	6.52	bc	6.12
Mean	**6.60**		**5.01**		**7.64**		**6.70**		
F-test	$P < 0.05$		$P < 0.05$		$P < 0.05$		$P < 0.05$		
$LSD_{0.05}$	1.22		0.62		0.92		0.46		

[1]Foliar Zn on seed crop [2]*Priming* with 5 mM $ZnSO_4$ for 1 hr

Table 7. Effect of soil/seed Zn treatments on panicle number and grain weight per panicle of experiment 1, and relationship between panicle number and grain weight per panicle and grain yield, year 1 at two locations in Thailand.

Soil/seed Zn treatment	Takli				Chiang Mai		
	Number/m²		Grain weight (g/panicle)		Number/m²	Grain weight (g/panicle)	
1. Control	281	ab	1.76	cd	252	3.08	a
2. $ZnSO_4$	223	c	2.41	a	241	3.40	a
3. Mosaic I	272	ab	2.08	abc	236	3.44	a
4. MosaicII	291	a	1.54	d	237	3.30	a
5. MosaicI+Foliar	257	abc	1.92	bcd	242	3.36	a
6. KKL	250	bc	2.29	ab	235	3.44	a
7. Urea-Zn	269	ab	1.71	cd	266	3.02	ab
8. ADOB	265	ab	2.16	ab	250	3.10	a
9. Zn+ seed (F)[1]	275	ab	1.56	d	254	2.54	b
10. Zn+ seed (P)[2]	254	bc	2.06	abc	241	3.02	ab
F-test	$P < 0.05$		$P < 0.05$		NS[3]	$P < 0.05$	
$LSD_{0.05}$	36		0.39		-	0.49	
CV (%)	9.4		13.9		8.0	10.7	
Relationship with grain yield							
Correlation coefficient (r)	-0.519		0.894		-0.251	0.880	
Linear regression with grain yield by f-test	NS		$P < 0.001$		NS	$P < 0.001$	

[1]Foliar Zn on seed crop [2]Priming with 5 mM $ZnSO_4$ for 1 hr [3]$P < 0.05$

Head rice yield (Year 2 only)

With head rice yield of 59.8±0.4%, the soil and seed Zn treatments did not significantly affect milling breakage at Takli, but at Chiang Mai head rice yield was slightly lower with ADOB (54.9%) and Mosaic I + Foliar (55.5%) compared with head rice yield of 57.8% in Zn- control and average of 57.3±1.3% the other soil/seed Zn treatments (Table 8)

Leaf zinc concentration

The soil and seed Zn treatments had no to small effects on Zn concentration of the youngest emerged blade at tillering and flag leaf at anthesis, which was generally higher at Chiang Mai than at Takli (Table 9).

Table 8. Effect of soil/seed Zn treatments on head rice yield (weight of full grain milled rice as percentage of un-husked rice) of experiment 1, crop 2 at two locations in Thailand.

Soil/seed Zn treatment	Takli	Chiang Mai		Mean
	Head rice yield (%)			
1. Control	59.4	57.8	abc	58.6
2. $ZnSO_4$	59.6	55.6	cde	57.6
3. Mosaic I	60.5	57.4	bcd	59.0
4. MosaicII	60.3	57.4	bcd	58.8
5. MosaicI+Foliar	59.7	54.9	e	57.3
6. KKL	59.9	59.6	a	59.7
7. Urea-Zn	59.6	55.9	bcde	57.7
8. ADOB	59.8	55.5	de	57.6
9. Zn+ seed (F)[1]	60.0	57.6	abcd	58.8
10. Zn+ seed (P)[2]	59.2	57.8	ab	58.5
Mean	**59.8**	**56.9**		
F-test	NS[1]	$P < 0.05$		
$LSD_{0.05}$	-	2.16		

[1]Foliar Zn on seed crop [2]*Priming* with 5 mM $ZnSO_4$ for 1 hr [3]$P < 0.05$

Table 9. Effect of soil/seed Zn treatment on Zn concentration in youngest emerged leaf blade (YEB) collected at tillering stage and flag leaf (FL) collected at flowering stage of experiment 1, at two locations in Thailand.

Soil/seed Zn treatment	Leaf zinc concentration (mg Zn kg^{-1})							
	Takli				Chiang Mai			
	2011-2012		2012-2013		2011-2012		2012-2013	
	YEB	FL	YEB	FL	YEB	FL	YEB	FL
1. Control	17.2	14.3	16.2	7.4	18.9	17.0	22.1	17.7
2. ZnSO4	17.8	14.1	16.8	10.0	19.9	18.1	23.0	19.4
3. Mosaic I	20.3	15.1	13.1	8.7	19.7	17.5	21.7	16.9
4. MosaicII	15.7	14.9	14.3	8.0	18.4	16.2	20.5	17.1
5. MosaicI + Foliar	17.1	14.7	14.3	65.3	20.0	16.9	21.8	17.6
6. KKL	16.2	14.3	12.8	7.8	19.4	17.4	23.0	17.4
7. Urea-Zn	16.1	14.9	13.8	9.5	20.0	18.6	22.8	18.7
8. ADOB	18.1	14.8	15.8	10.7	19.9	19.3	28.0	22.2
9. Zn+ seed (F)[1]	15.5	13.9	14.7	10.2	19.8	20.4	23.0	18.3
10. Zn+ seed (P)[2]	15.7	14.4	14.2	9.1	20.1	19.6	22.7	18.9
Mean	**17.0**	**14.5**	**14.6**	**14.7**	**19.6**	**18.1**	**22.9**	**18.4**
F-test $P <$	0.05	NS[1]	0.001	0.001	NS	0.05	0.01	0.0
LSD0.05	2.2	-	1.7	18.1	-	1.5	2.7	2.0

[1]Foliar Zn on seed crop [2]*Priming* with 5 mM $ZnSO_4$ for 1 hr [3]$P < 0.05$

Grain Zn concentration

The soil and seed Zn treatments had no to small effects on Zn concentration of the rice grain, in un-husked, brown and white rice in both crops (Tables 10 and 11) at both locations, with even milder effect in year 2. The only exception was Mosaic I + foliar Zn. For example, in year 1 at Takli, adding foliar Zn to Mosaic I increased Zn in un-husked rice from 13.5 to 38.0 mg Zn kg^{-1}, in brown rice from 14.5 to 19.2 mg Zn kg^{-1} and in white rice from 11.7 to 16.2 mg Zn kg^{-1}, and at Chiang Mai the increase in un-husked rice was from 20.0 to 40.2 mg Zn kg^{-1}, in brown rice from 20.9 to 26.1 mg Zn kg^{-1} and in white rice from 15.1 to 19.8 mg Zn kg^{-1}. In the Zn- control, grain Zn was higher at Chiang Mai than at Takli in un-husked (by 128%), brown (by 96%) and white (by 52%) rice (average over 2 years). The soil Zn treatments that increased grain Zn the most in year 1 at Takli were $ZnSO_4$ (by 24% in brown rice and 31% in white rice), KKL (by 13% in brown rice and 24% in white rice) and urea-Zn (by 21% in brown rice and 22% in white rice). These treatments also increased grain Zn at Chiang Mai, but to a much smaller extent. Grain Zn, both in brown and white rice, was closely and positively associated with grain yield (Figure 4), indicating that were rice yield is limited by Zn, the grain Zn concentration will also be low.

Table 10. Effect of soil/seed Zn treatment on Zn concentration in un-husked, brown and white rice, experiment 1, year 1 at two locations in Thailand.

Soil/seed Zn treatment	Takli Un-husked	Takli Brown rice	Takli White rice	Chiang Mai Un-husked	Chiang Mai Brown rice	Chiang Mai White rice
	(mg Zn kg^{-1})					
1. Control	13.6	14.1	11.6	19.4	20.0	14.4
2. $ZnSO_4$	16.8	18.2	15.2	20.1	21.3	15.5
3. Mosaic I	13.5	14.5	11.7	20.0	20.9	15.1
4. Mosaic II	13.3	14.8	12.2	18.9	20.0	15.0
5. Mosaic I + Foliar	38.0	19.2	16.2	40.2	26.1	19.8
6. KKL	15.3	16.6	14.4	19.4	20.5	14.7
7. Urea-Zn	16.4	18.2	14.2	19.6	20.6	14.7
8. ADOB	11.9	13.7	10.3	19.5	21.5	14.6
9. Zn+ seed (F)[1]	12.9	13.4	10.3	18.0	20.5	13.6
10. Zn+ seed (P)[2]	13.8	14.4	10.1	19.1	20.9	15.0
Mean	**16.6**	**15.7**	**12.6**	**21.4**	**21.2**	**15.2**
F-test *P* <	0.05	0.05	0.05	0.05	0.05	0.05
LSD0.05	3.8	2.3	1.7	1.6	1.7	0.9
Zn+ mean (excluding Tr 5)	14.2±1.7	15.5±1.9	12.3±2.1	19.3±0.7	20.8±0.5	14.8±0.6

[1]Foliar Zn on seed crop [2]*Priming* with 5 mM $ZnSO_4$ for 1 hr [3]$P < 0.05$

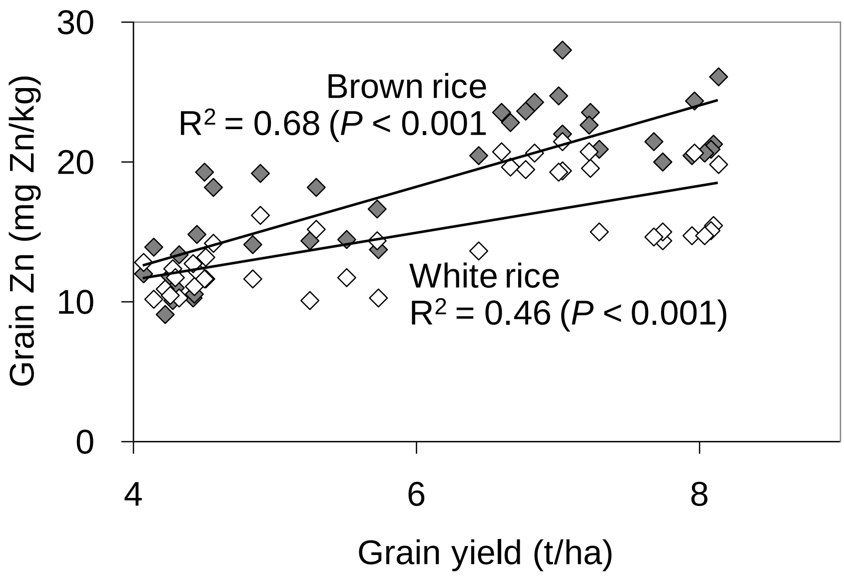


Figure 4. Relationship between grain Zn (◆, brown rice; ◇, white rice) and grain yield (un-husked rice) from different soil/seed Zn treatments (experiment 1).

Table 11. Effect of soil/seed Zn treatment on Zn concentration in un-husked, brown and white rice, experiment 1, year 2 at two locations in Thailand.

Soil/seed Zn treatment	Takli			Chiang Mai		
	Un-husked	**Brown rice**	**White rice**	**Un-husked**	**Brown rice**	**White rice**
	(mg Zn kg^{-1})					
1. Control	7.4	9.1	10.9	23.2	22.8	19.6
2. $ZnSO_4$	10.0	11.6	13.2	33.9	24.3	20.6
3. Mosaic I	8.7	10.3	12.8	37.4	23.6	20.7
4. Mosaic II	8.0	10.1	12.3	44.6	23.5	19.6
5. Mosaic I + Foliar	65.3	19.3	11.6	49.0	28.0	21.5
6. KKL	7.8	10.5	11.1	40.9	22.6	20.7
7. Urea-Zn	9.5	12.0	12.8	40.6	23.6	19.5
8. ADOB	10.7	11.8	10.4	32.5	24.4	20.6
9. Zn+ seed (F)[1]	10.2	11.0	11.7	36.5	22.0	19.4
10. Zn+ seed (P)[2]	9.1	13.9	10.2	28.6	24.7	19.3
Mean	**14.7**	**12.0**	**11.7**	**36.7**	**24.0**	**20.1**
F-test *P* <	0.0	0.0	0.1	0.0	0.0	NS
LSD0.05	18.1	4.2	2.7	2.2	2.3	
Zn+ mean±SD (excluding Tr 5)	9.3±1.0	11.4±1.2	11.8±1.1	36.9±5.2	23.6±0.9	20.0±0.7

[1]Foliar Zn on seed crop [2]Priming with with 5 mM $ZnSO_4$ for 1 hr [3]$P < 0.05$

4.2.Foliar Zn treatments

Grain yield and growth

Grain yield response to foliar Zn varied with site, crop and the Zn formulation (Table 12). The largest increases were at Chiang Mai with increases of 34% (1.3 t/ha) by Kali EPSO in year 1, and 16% by Antracol and Valagro in year 2. Other foliar Zn treatments that increased grain yield but to a less extent were OMEXIII and $ZnSO_4$ at booting and milky stage at Takli in both years, Antracol and Valagro at Takli in year 2, and $ZnSO_4$ at booting at Chiang Mai in year 1. The straw yield also responded differently to the different Zn formulations at different sites and in different years (Table 13). The foliar Zn treatments significantly affected the weight of grain per panicle at both Takli and Chiang Mai, but the effect on number of panicles m^{-2} was significant only at Chiang Mai (Table 13). The increase in grain yield was closely associated with increase in total above ground dry weight at both sites.

Table 12. Grain yield response to foliar Zn treatments.

Foliar Zn treatment	Takli				Chiang Mai				Mean
	Year 1		**Year 2**		**Year 1**		**Year 2**		
	Grain weight (t ha^{-1})								
1. Control	5.58	bcd	4.87	bc	7.19	c	6.88	b	6.13
2. $ZnSO_4$ booting	5.80	abc	4.72	c	7.95	ab	7.11	b	6.40
3. $ZnSO_4$ milky	5.81	abc	4.94	abc	7.45	bc	6.88	b	6.27
4. $ZnSO_4$ twice	6.17	ab	5.24	a	7.39	bc	6.96	b	6.44
5. OMEXII	6.13	ab	4.86	bc	7.16	c	6.98	b	6.28
6. OMEXIII	6.33	a	5.26	a	7.50	bc	7.67	a	6.69
7. Kali EPSO	6.05	ab	5.24	a	8.53	a	6.97	b	6.70
8. ADOB ZnIDHA	5.63	bcd	5.06	ab	7.61	bc	6.81	b	6.28
9. Valagro	5.03	d	5.22	a	7.58	bc	7.97	a	6.45
10. Antracol	5.26	cd	5.26	a	7.75	bc	7.96	a	6.56
11. Insecticide+$ZnSO_4$	5.80	abc	5.13	ab	7.39	bc	6.83	b	6.29
12. FBScience	5.83	abc	5.09	ab	7.85	abc	6.89	b	6.41
Mean	**5.78**		**5.07**		**7.61**		**7.16**		
F-test	*		*		*		*		
$LSD_{0.05}$	0.67		0.34		0.76		0.52		

Table 13. Straw yield response to foliar Zn treatments.

Foliar Zn treatment	Takli Year 1		Takli Year 2		Chiang Mai Year 1		Chiang Mai Year 2		Mean
	Straw yield (t ha^{-1})								
1. Control	7.57	bc	5.59	e	7.37	d	6.49	cde	6.76
2. $ZnSO_4$ booting	8.45	abc	5.66	de	8.78	abc	6.72	bcd	7.40
3. $ZnSO_4$ milky	9.38	a	5.79	de	8.40	bc	6.31	de	7.47
4. $ZnSO_4$ twice	9.13	a	6.41	ab	9.27	ab	6.49	cde	7.82
5. OMEXII	8.91	ab	5.69	de	7.81	cd	6.77	bcd	7.30
6. OMEXIII	7.48	c	6.25	abc	8.17	cd	7.05	ab	7.24
7. Kali EPSO	9.27	a	5.59	e	8.57	abc	6.30	de	7.43
8. ADOB ZnIDHA	8.29	abc	5.87	de	8.48	bc	6.43	de	7.27
9. Valagro	8.00	abc	6.59	a	8.20	cd	6.93	abc	7.43
10. Antracol	8.27	abc	6.57	a	8.64	abc	7.39	a	7.72
11. Insecticide+$ZnSO_4$	9.21	a	5.99	cd	8.38	bcd	6.57	bcd	7.54
12. FBScience	7.37	c	6.03	bcd	9.48	a	6.03	e	7.23
Mean	8.44		6.00		8.46		6.62		
F-test	*		*		*		*		
$LSD_{0.05}$	1.40		0.38		1.00		0.49		

Table 14. Effect of foliar Zn treatments on panicle number and grain weight per panicle of experiment 2, and relationship between panicle number and grain weight per panicle and grain yield, year 1 at two locations in Thailand.

Foliar Zn treatment	Takli Number/m^2	Takli Grain weight (g/panicle)		Chiang Mai Number/m^2		Chiang Mai Grain weight (g/panicle)	
1. Control	233	2.26	bcd	263	e	2.76	ab
2. $ZnSO_4$ booting	244	2.30	bcd	301	b	2.65	ab
3. $ZnSO_4$ milky	222	2.41	ab	276	cde	2.71	ab
4. $ZnSO_4$ twice	270	2.09	d	287	bcd	2.58	bc
5. OMEXII	243	2.24	bcd	282	bcde	2.54	bc
6. OMEXIII	236	2.66	a	287	bcd	2.63	ab
7. Kali EPSO	228	2.39	abc	290	bc	2.96	a
8. ADOB ZnIDHA	216	2.19	bcd	266	de	2.88	ab
9. Valagro	224	2.12	cd	280	bcde	2.71	ab
10. Antracol	220	2.14	bcd	276	cde	2.80	ab
11. Insecticide+$ZnSO_4$	242	2.14	bcd	334	a	2.21	c
12. FBScience	220	2.23	bcd	294	bc	2.67	ab
F-test	NS	$P < 0.05$		$P < 0.05$		$P < 0.05$	
$LSD_{0.05}$	-	0.28		23		0.37	
CV (%)	9.2	8.7		5.7		9.6	

Head rice yield (year 2 only)

Head rice yield of 58.2±0.5% at Takli and 60±0.8% at Chiang Mai were not affected by the foliar Zn treatments (Table 15).

Grain Zn concentration

Effects of foliar Zn on grain Zn were much larger than the soil Zn treatments, and with major differences among the different Zn formulation and significant effects on the Zn in un-husked grain, brown rice and white rice in both year 1 (Table 16) and year 2 (Table 17). In year 1 at Takli, the Zn in un-husked rice was increased from 12.5 mg Zn kg^{-1} in Zn- control to 32.6±9.1

mg Zn kg^{-1} on the average of the foliar Zn+ treatments; in brown rice the increase was from 13.9 mg Zn kg^{-1} to 18.7±2.0 mg Zn kg^{-1}; and in white rice from 10.9 mg Zn kg^{-1}to 13.9±1.3 mg Zn kg^{-1}. In year 1 at Chiang Mai, in un-husked rice the increase was from 19.6 mg Zn kg^{-1} in Zn- control to 38.2±10.3 mg Zn kg^{-1} on the average of the foliar Zn treatments; in brown rice it was from 21.2 mg Zn kg^{-1} to 26.0±2.0 mg Zn kg^{-1}; and in white rice from 15.8 mg Zn kg^{-1} to 19.7±0.9 mg Zn kg^{-1}. In year 2 the effects were much smaller, especially in white rice (Table 17).

Grain Zn, both in brown and white rice, was closely and positively associated with grain yield (Figure 5), indicating that were rice yield is limited by Zn, the grain Zn concentration will also be low.

Table 15. Effect of foliar Zn treatments on head rice yield (weight of full grain milled rice as percentage of un-husked rice) of experiment 2, year 2 at two locations in Thailand.

Foliar Zn treatment	Takli	Chiang Mai	Mean
	Head rice yield (%)		
1. Control	57.8	60.8	59.3
2. $ZnSO_4$ booting	58.8	59.1	58.9
3. $ZnSO_4$ milky	59.1	58.5	58.8
4. $ZnSO_4$ twice	58.7	59.7	59.2
5. OMEXII	58.1	59.6	58.8
6. OMEXIII	57.2	59.6	58.4
7. Kali EPSO	58.4	60.1	59.3
8. ADOB ZnIDHA	58.8	59.9	59.3
9. Valagro	57.8	61.4	59.6
10. Antracol	58.1	60.6	59.4
11. Insecticide+$ZnSO_4$	58.1	59.7	58.9
12. FBScience	57.8	60.6	59.2
Mean±SD	**58.2±0.5**	**60.0±0.8**	
F-test	NS (P < 0.05)	NS	

Table 16. Effect of foliar Zn treatment on Zn concentration in un-husked, brown and white rice, experiment 2, year 1 at two locations in Thailand.

Foliar Zn treatment	Takli Un-husked	Takli Brown rice	Takli White rice	Chiang Mai Un-husked	Chiang Mai Brown rice	Chiang Mai White rice
	(mg Zn kg^{-1})					
1. Control	12.5	13.9	10.9	19.6	21.2	15.8
2. $ZnSO_4$ booting	27.2	19.5	14.9	22.1	24.0	19.2
3. $ZnSO_4$ milky	41.4	19.3	14.0	38.3	25.9	19.6
4. $ZnSO_4$ twice	46.7	22.5	16.0	51.9	30.2	21.8
5. OMEXII	19.5	16.6	13.0	28.5	24.6	19.1
6. OMEXIII	27.3	17.1	14.4	37.0	25.4	19.8
7. Kali EPSO	33.1	17.8	14.6	46.0	26.5	20.2
8. ADOB ZnIDHA	32.8	18.9	14.1	30.2	25.5	20.6
9. Valagro	27.9	17.9	13.9	38.2	26.2	20.0
10. Antracol	20.9	15.5	10.9	33.1	23.3	18.4
11. Insect+$ZnSO_4$	44.3	21	14.2	37.5	25.4	18.9
12. FBScience	37.6	19.1	12.9	57.4	28.6	19.5
Mean	**33.1**	**18.6**	**13.8**	**39.8**	**26.1**	**19.8**
F-test P <	0.05	0.05	0.05	0.05	0.05	0.05
$LSD_{0.05}$	8.7	1.9	1.7	10.2	2.1	1.2
Zn+ mean±SD	32.6±9.1	18.7±2.0	13.9±1.3	38.2±10.3	26.0±2.0	19.7±0.9

Table 17. Effect of foliar Zn treatment on Zn concentration in un-husked, brown and white rice, experiment 2, year 2 at two locations in Thailand.

Foliar Zn treatment	Takli Un-husked	Takli Brown rice	Takli White rice	Chiang Mai Un-husked	Chiang Mai Brown rice	Chiang Mai White rice
	(mg Zn kg^{-1})					
13. Control	11.1	12.5	10.9	23.2	26.0	18.4
14. $ZnSO_4$ booting	14.9	14.9	13.2	33.9	28.2	21.7
15. $ZnSO_4$ milky	58.6	20.6	12.8	37.4	26.9	21.1
16. $ZnSO_4$ twice	56.7	20.3	12.3	44.6	28.5	20.5
17. OMEXII	25.4	15.5	11.6	49.0	30.3	21.2
18. OMEXIII	33.1	15.5	11.1	40.9	28.0	19.3
19. Kali EPSO	35.0	18.7	12.8	40.6	27.9	20.6
20. ADOB ZnIDHA	18.1	16.1	10.4	32.5	28.2	20.3
21. Valagro	39.2	17.3	11.7	36.5	28.1	20.4
22. Antracol	27.0	13.3	10.2	28.6	26.3	20.8
23. Insect+$ZnSO_4$	47.4	16.5	9.4	39.4	28.5	20.9
24. FBScience	20.7	14.0	8.6	56.8	29.3	20.3
Mean	**6.37**	**3.16**	**1.33**	**9.77**	**1.17**	**1.03**
F-test *P* <	0.001	0.01	NS	0.001	NS	NS
$LSD_{0.05}$	18.1	4.2	3.6	8.3	3.2	2.5
Zn+ mean±SD	34.2±15.0	16.6±2.4	11.3±1.5	40.0±7.9	28.2±1.1	20.6±0.6

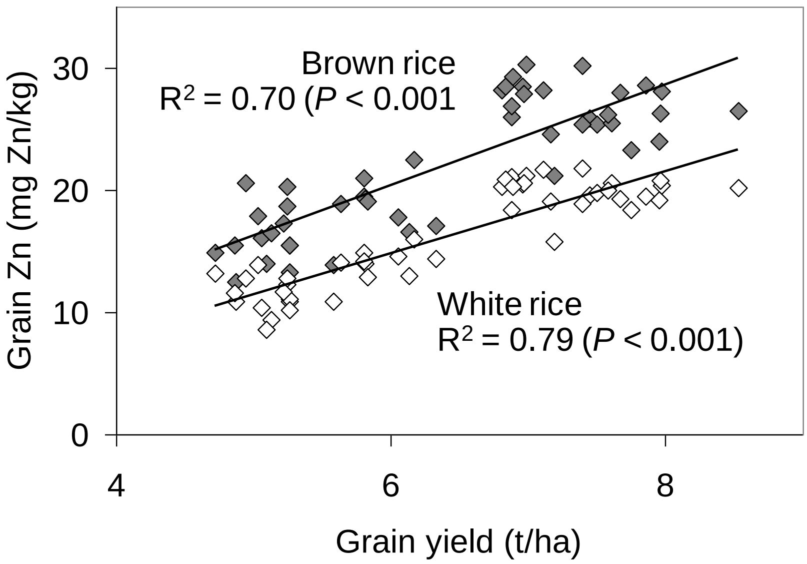


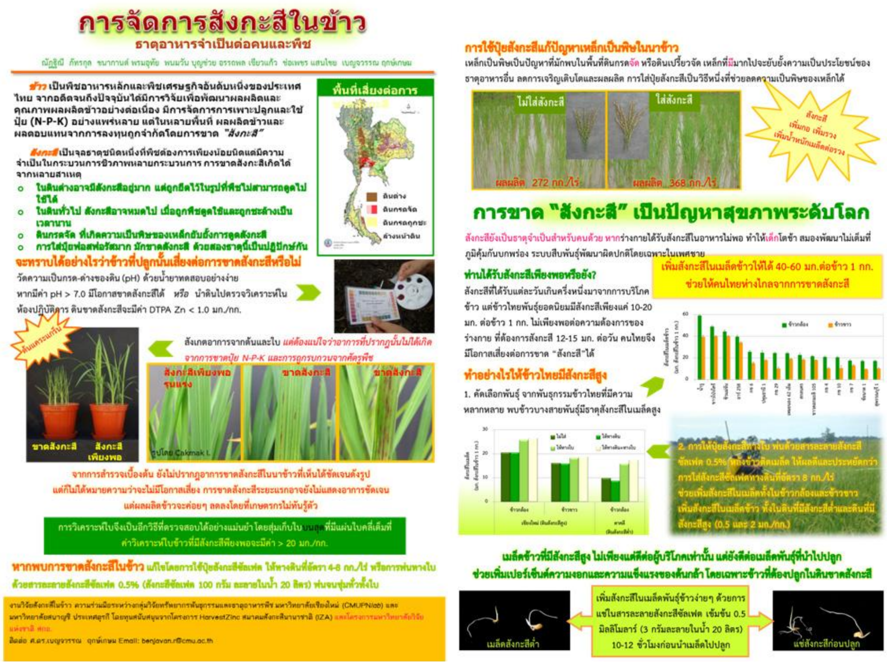
Figure 5. Relationship between grain Zn (◆, brown rice; ◇, white rice) and grain yield (un-husked rice) from different foliar Zn treatments (experiment 2).

5.CAPACITY BUILDING

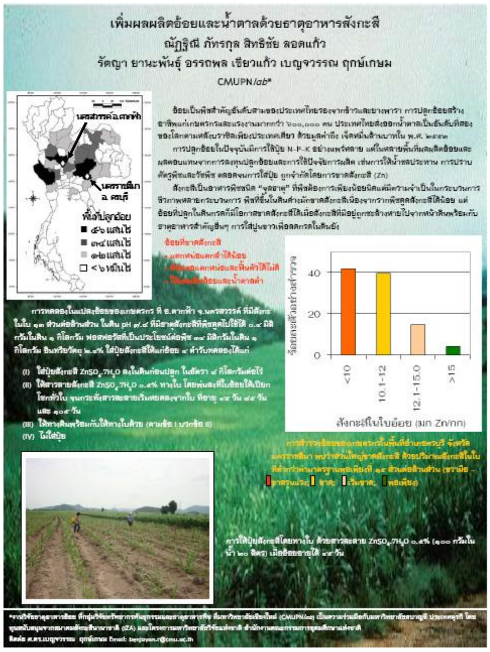
Capacity in working with Zn at the collaborating lab at Chiang Mai University has been strengthened by the project. One postdoctoral fellow visited Sabanci University to learn new methods and conducted research. Five PhD students (two having completed their studies) and two MS students conducted their thesis research in association with the project. Project results on major effects of location and genotype on grain Zn have led to more research activities on evaluation of genotypic variation in grain Zn concentration and rice production on calcareous soils. Locally funded research has also started Zn nutrition in rice and other crops of economic importance in Thailand.

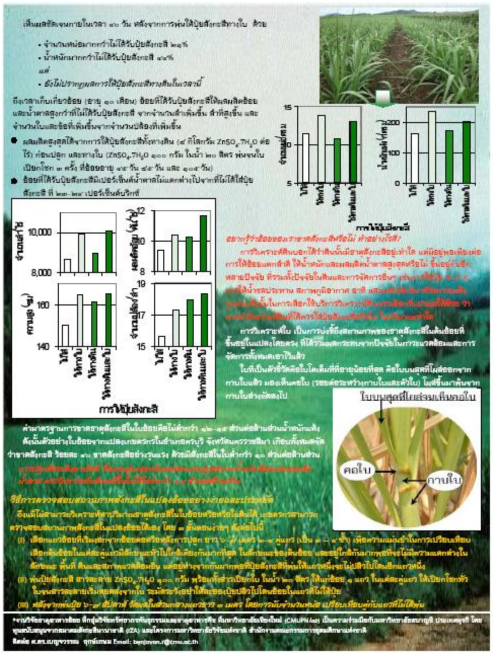
6. DISSEMINATION OF RESULTS

1) Thailand Zinc Day was organized on July 27th 2011 at Chiang Mai University, attended by 120 farmers, field agronomists from public and private sectors, students and academics, with participation and exhibition from fertilizer and chemical companies.
2) Preparation of manuscripts and publication of results from phase I and associated activities :
    i. Phattarakul N, Rerkasem B, Lijui L, Zou C, Saharan H, Sohu V, Kang BS, Surek H, Kalayci M, Yazici A, Zhang F, Cakmak I. 2012 Biofortification of rice grain with zinc through zinc fertilization in different countries. *Plant and Soil* 361: 131–141
    ii. Saenchai C, Prom-u-thai C, Jamjod S, Dell B, Rerkasem B. 2012. Genotypic variation in milling depression of iron and zinc concentration in rice grain. *Plant and Soil* 361: 271–278
3) Preparation, printing and distribution of a FarmNote in Thai on “Zinc in Rice”



4) Preparation, printing and distribution of a FarmNote in Thai on “Improving Cane and Sugar Yield from Sugarcane with Zinc Fertilizer”





5) Preparation of manuscripts for local publication on zinc in rice

   i. Management of zinc fertilizer for yield and grain quality
   ii. Zinc concentration in local rice germplasm

COUNTRY REPORT - BRAZIL

1. PARTICIPANTS

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SUMMARY

The aim of this research was to evaluate different zinc fertilizers applied to soil and bean leaves to achieve Zn-enriched grains and higher yields in Brazilian Oxisols. Ten field trials with soil and foliar applications were carried out in different locations of the São Paulo State, Brazil (Capão Bonito, Votuporanga, Campos Novos and Mirestrela), sowing in October/November 2011 (2011/12 crop season), May 2012 and September/October 2012 (2012/13 crop season), May 2013 and June 2013 . The range of available soil zinc level was medium to high. The experimental design was a randomized complete block with 6 replications and 10 and 12 treatments in the soil and foliar trials, respectively. The foliar treatments were applied after flowering, except for zinc sulfate that was also sprayed before flowering.

The effect of soil Zn treatments on yield was observed in two of five field trials. The favorable effect occurred only in Capão Bonito's 2011/12, in which the Mosaic-MESZ yielded better than Local Control treatment. But the results in Votuporanga's 2012 experiment were totally different: Mosaic-MESZ had the lowest grain yield due to sulfur deficiency as a result of low sulfur doses and very low content of this nutrient in the soil. Largest differences among seed Zn concentrations were obtained in Capão Bonito 2012/13, in accordance with soil Zn levels: Zn sulfate (10.0 kg ha^{-1} Zn) differed from all treatments, except ADOB HBED Zn (6.5 kg ha^{-1} Zn) and Mosaic-MESZ (4.0 kg ha^{-1} Zn). Also, the highest seed Zn was obtained in the soil Zn sulphate treatment at Votuporanga in 2012.

The seed yield of the foliar trials was lower at one location and the differences among treatments were observed only at Votuporanga in 2012. At this location, OMEX-II treatment had the lowest yield probably due to due to foliar damage, and the treatment FBScience + $ZnSO_4$ had higher yields compared to some other treatments. The foliar applications improved seed Zn concentrations, except in the treatments of Zinc Sulfate before flowering (all locations), Bayer Antracol-Zn, Valagro Brexil (two locations) and AdobZnIDHA (one location). The highest seed Zn concentration was observed in the treatment Omex Type II, without differing from Zinc Sulfate applied twice (all trials). The results of plant emergence indicated favorable effect of zinc-enriched seeds on plant establishment, except in the fields planted with very young seeds which had high vigor independent of the zinc enrichment. The common bean seeds treated with 1 or 5 mM $ZnSO_4$ solution for 1 h showed low plant emergence, probably because of the long time of seed treatment for this species. In conclusion, there was favorable effect of zinc-enriched seeds on plant establishment, the seed yield was rarely improved by zinc fertilizer (soil or foliar), the foliar zinc application was the method with higher potential to increase seed nutrients (19% of Zn improvement), OMEX Type II and Zinc Sulfate applied twice stood out as the best foliar treatments in that aspect

2. INTRODUCTION

Weathered Oxisoils, widely distributed in Brazil, are mostly deficient in plant nutrients, such as zinc (Zn) (Lopes & Cox, 1977). Zinc deficiency is more frequent in Savana soils (Vendrame et al., 2007).

The area planted with common beans is estimated as 3.9 million hectares (CONAB, Brazilian Crops Assessment, December 2011) and edible beans are an important staple food, eaten by almost all Brazilian people every day. As this food is sold in small bags and cooked at home, it is possible to aggregate benefits with seed biofortification to population nutrition and better income for farmers.

The aim of this research is to evaluate different zinc fertilizers applied to soil and bean leaves to achieve Zn-enriched seeds and higher yields in Brazilian Oxisols. Besides, the effects of zinc nutrition on seed germination and vigor is also being evaluated.

3. EXPERIMENTAL ACTIVITIES

The first Harvestplus Zinc Fertilizer experiments with soil applications were carried out in the South of São Paulo State, Brazil, at the Experiment Station of Capão Bonito (24º00'21" S and 48º20'58" W, 702 m altitude). The climate in the region is a Cfa (subtropical with a rainy summer and wet winter), according to the Koppen’s classification, and the soil is an Oxisol with medium zinc content (Table 1). Two field trials were performed in the same area on different dates of sowing (Table 2). The first field was sown in October 6th and the second in November 10th, 34 days after the first sowing.

Two side by side trials of Harvestplus Zinc with common beans were carried out at Votuporanga (20º25’S and 50º04’W, 500 m altitude), in the west of São Paulo State, during 2012 season: one with foliar Zn-application and the other with soil Zn-application (Tables 2 and 3). The climate of the region is a Cwa (subtropical with a rainy summer and dry winter) according to the Koppen’s classification, and the soil is an Oxisol with high zinc content (Table 1). As the growing season at Votuporanga is usually a drought season, the common bean crop was cultivated under irrigation system.

The other field was sown in Campos Novos Paulista (22º36'S and 50º00 W, 450 m altitude), in the south-west of São Paulo State, performing only the foliar treatments, under irrigation system (Tables 1 and 3). The climate of the region is transitional between Cwa and Cfa and the soil is an Oxisol.

Later field trials with Zn application to soil and foliage were performed again in Capão Bonito, during 2012/13 season, sowing in different areas (Tables 1, 2 and 3). The soil was better in the trial with Zn soil application than that in the experiment of foliar application, but both had high levels of zinc.

The last field trials were conducted in Votuporanga and Miresterela, nearby counties, both in 2013 season. The soil was similar to Votuporanga in 2012 season and had low pH in Miristrela, always with high available soil zinc in the 0-20 cm layer. While foliar treatments were applied at both locations, soil trial was performed only in Votuporanga.

The experimental design was a randomized complete block with 6 replications and 10 and 12 treatments in the soil and foliar trials, respectively (Tables 4 and 5). The zinc treatments in the soil-zinc trials were applied in accordance with the protocol, except for the first Capão Bonito soil trial, which treatment 8 (ADOB HBED Zn) was replaced by Local Control (equal to treatment 1).

The foliar treatments were applied after flowering, except for zinc sulfate that was also sprayed before flowering. The 0.5% zinc sulfate fertilizer solutions were applied with a CO_2 pressurized device, at 800 L ha^{-1} rate, except in Votuporanga. At this location the application was 600 L ha^{-1} of 1.0% zinc sulfate solution (Table 5).

Table 1. Soil chemical analysis results at Capão Bonito, Votuporanga and Campos Novos.

	Organic matter	Phosphorus	C.E.C	Bases saturation	pH	Zinc *	S
	g dm^{-3}	mg dm^{-3}	mmol$_c$ dm^{-3}	%	($CaCl_2$)	…. mg dm^{-3}	….
			Capão Bonito - Soil 2011/12 (Fields 1 and 2)				
0-20 cm	28	86	97	71	5.7	1.0	-
20-40 cm	25	14	78	56	5.4	0.3	-
			Votuporanga - Soil and Foliar, 2012				
0-20 cm	20	45	64	57	5.4	4.2	3
20-40 cm	10	23	35	57	5.0	2.9	3
			Campos Novos - Foliar, 2012				
0-20 cm	23	21	51	45	4.8	1.4	3
20-40 cm	19	8	48	48	4.8	1.4	5
			Capão Bonito - Soil, 2012				
0-20 cm	23	8	56	45	5.0	1.5	6
			Capão Bonito - Foliar, 2012				
0-20 cm	10	7	39	27	4.5	5.1	6
			Votuporanga - Soil and Foliar, 2013				
0-20 cm	13	43	41	46	4.7	6.5	2
20-40 cm	11	36	39	49	4.6	2.4	2
			Mirestrela - Foliar, 2013				
0-20 cm	13	34	44	36	4.5	2.7	6
20-40 cm	11	12	32	32	4.5	0.6	11

* Range of soil available zinc level (DTPA-extractable): Low = 0-0.5; Medium=0.6-1.2; High > 1.2

Table 2. Dates of main activities in the soil zinc trials at Capao Bonito and Votuporanga, São Paulo State, Brazil

	Capão Bonito 2011/12		Votuporanga	Capão Bonito	Votuporanga
Activity	First Field	Second Field	2012	2012/13	2013
Planting	6 October 2011	10 November 2011	3 May 2012	10 October 2012	17 May 2013
Side dress N	21 October 2011	30 November 2011	23 May 2012	31 October 2012	14 June 2013
Side dress K*	26 October 2011	30 November 2011	23 May 2012	31 October 2012	19 June 2013
Zn foliar aplication	29 November 2011 (flowering starting)	29 December 2011 (30% flowering)	29 June 2012 (small pods)	17 December 2012 (small pods)	4 July 2013 (flowering starting)
Harvesting	23 January 2012	16 February 2012	31 July 2012	24 January 2013	23 August 2013

* Except for soil treatment 6.

Table 3. Dates of main field activities in the foliar zinc trials at at Votuporanga, Campos Novos and Capão Bonito, São Paulo State, Brazil

	Votuporanga	Campos Novos	Capão Bonito	Votuporanga	Mirestrela
Activity	2012	2012	2012/13	2013	2013
Planting	4 May 2012	15 September 2012	29 October 2012	21 May 2013	20 June 2013
Side dress N	23 May 2012	13 October 2012	21 November 2012	19 June 2013	15 July 2013
Zn foliar aplication (1ª) (before flowering)	13 June 2012	30 October 2012	10 December 2012	4 July 2013	5 August 2013
Zn foliar aplication (2ª) (after flowering)	29 June 2012	19 November 2012	22 December 2012	20 July 2013	22 August 2013
Harvesting	31 July 2012	20 December 2012	24 January 2013	23 August 2013	30 September 2013

Table 4. Soil treatments and nutrients applied in common bean field trials.

	Treatments	N *	P_2O_5	K_2O **	Ca	Mg	S	Zn
		....	....	....	kg/ha	....	....	....
1	Local Control (LC)	48	80		75	19	52	
2	LC + Soil Zn	48	80		75	19	52	10.0
3	LC+Mosaic-MESZ	48	80				20	2.0
4	LC+Mosaic-MESZ (2x)	48	160				40	4.0
5	LC+Mosaic-MESZ + F0LIAR	48	80				20	2.0
6	LC+KaliKorn (KCL-Zn)	48	80	60	75	9	52	2.3
7	LC + 2 x Split Uréia - 1% Zn	48	80		75	19	52	0.7
8	LC +ADOB HBED Zn (or only LC)	48	80		75	19	52	6.5
9	LC -Seed Zn (Field)	48	80		75	19	52	
10	LC-Seed (Zn solution)	48	80		75	19	52	

* Side dress nitrogen = 70 kg ha^{-1}; ** Side dress potassium = 60 kg ha^{-1}, except for treatment 6

Table 5. Foliar treatments, zinc concentration of the products used and zinc application rates at Votuporanga, Campos Novos and Capão Bonito.

	Treatment	**Zn**	Zn Rate	
			Votuporanga 2012	Other Trials
		%	……….. kg ha^{-1}	………..
1	Local Control (LC)			
2	LC + Foliar ZnSO4 (once, before)	21	1.3	0.9
3	LC + Foliar ZnSO4 (once, after)	21	1.3	0.9
4	LC + Foliar ZnSO4 (twice foliar spray)	21	2.6	1.8
5	LC + Omex Type II Foliar Zn (D1872)	27.4	1.3	0.9
6	LC + Omex Type III Foliar Zn (SC 144)	14.8	1.3	0.9
7	LC + Kali EpsoTop - Zn	5	1.3	0.9
8	LC + Adob ZnIDHA	10	1.3	0.9
9	LC + Valagro Brexil	10	1.3	0.9
10	LC + Bayer Antracol-Zn	20	1.3	0.6
11	LC + Foliar ZnSO4 (once, after) + inseticide[(1)]	21	1.3	0.9
12	LC + FBScience (1036) + ZnSO4	21	1.3	0.9

[(1)] Engeo Pleno (125 mL ha^{-1})

To achieve Zn-enriched seeds (the soil treatment 9), one field was planted in the county of Palestina, Northwest of São Paulo State. Common beans were sown in May 2011 in the medium-zinc Oxisol and treated three times with foliar zinc sulfate 0.5% mixed with fungicides and/or insecticides: before, during and after plant flowering. The field was harvested in August 2011 and their seeds with low and high Zn (see Table 6) were used in the soil experiments. The seeds used to plant at Capão Bonito trials were stored at room temperature, while the Votuporanga's seeds were stored in a cold chamber, except for treatment 9 (enriched seeds) that was stored at room temperature. To compare zinc enriched seeds (treatment 9) with standard seeds (treatment 1), one extra treatment 1 was planted in Votuporanga with standard seeds stored at room temperature.

To stablish last soil trial in Capão Bonito, new Zn-enriched seeds were produced in Votuporanga during 2012 season, at the same time which zinc trials were performed, applying foliar zinc sulfate 1.0% twice: before and after plant flowering (Table 6).

Establishment of the field trials took place in October and November of 2011 and October of 2012 at Capão Bonito, May of 2012 and 2013 at Votuporanga, September of 2012 at Campos Novos and June of 2013 at Miristrela (Tables 2 and 3) by using conventional system to prepare the soil, except at Miristrela that was no till system. Perola was the bean cultivar used in the experiments, which is one of the most commonly used bean cultivars in Brazil.

Experimental plots had four 45 cm-rows, 1.8 m wide and 5.0 m long. The initial population was around 130 thousand plants ha^{-1}. Weed control was done with 1 L ha^{-1} of Flex herbicide (fomesafen), plus 0.5 L ha^{-1} of Fusilade herbicide (fluazifop-P-butil), or manual weeding.

In the Voruporanga's foliar-Zn trial, nitrogen and phosphorus fertilizers were applied at rates of 32-48 kg ha^{-1} and 80-94 kg ha^{-1}, respectively, at planting. The source of phosphorus was triple superphosphate and single superphosphate in the first (2012) and second (2013) trials, respectivetly. In Campos Novos, fertilization was done with 28, 38 and 38 kg ha^{-1} of N, P_2O_5 and K_2O, respectively, at planting, plus 60 kg ha^{-1} of side-dressed N, as ammonium nitrate, 3 weeks after plant emergence. In Capão Bonito foliar-Zn trial, nitrogen and phosphorus fertilizers were applied at rates of 78 kg ha^{-1} and 40 kg ha^{-1}, respectively, at planting. In Mirestrela 20, 150 and 50 kg ha^{-1} of N, P_2O_5 and K_2O were applied, respectively, at planting, plus 60 kg ha^{-1} of side-dressed N, as ammonium nitrate, 3 weeks after plant emergence. Side-dressed potassium chlorate and urea fertilizer were applied at rates of 60 kg ha^{-1} of K_2O and 70 kg ha^{-1} of N, 15-20 days after plant emergence, except in Campos Novos and Mirestrela.

The same fertilization regimes were used in the soil-Zn trials as local control treatment in all locations but application of other nutrients changed according to each treatment as shown in the related Tables (Tables 4 and 7). The soil trial fertilization was different from foliar trials only in Votuporanga 2013, where 133 kg ha^{-1} of P_2O_5 was applied as simple plus triple superphoshate to provide 40 to 49 kg ha^{-1} of S per treatment, considering that Mosaic-MESZ zinc sulphate contains sulfur and soil level of S was low.

Plant emergence was evaluated in the soil-Zn trials at 7, 8, 11 and 13 days after planting in the first trial of Capão Bonito 2011/12 and Votuporanga 2012; 8, 11, 13 and 15 days after planting in the second field of Capão Bonito 2011/12 and Capão Bonito 2012/13 and 8 days after planting at Votuporanga 2013. The number of plants with at least one leaf was counted in two central rows to calculate the average per row of 5.0 m. Also, plant population was evaluated at harvest time.

Harvest was done in the two central rows of each plot and seeds were weighed to calculate yield. Samples of seeds were sent to Sabanci University to analyze for nutrients, with exception of Votuporanga 2013 and Mirestrela 2013, where harvest was completed recently. Also, samples of leaves were taken in the Votuporanga's 2012 soil-Zn trial to analyze for nutrients.

Table 6. Seed nutrient analysis results at Palestina's and Votuoranga's production fields.

Samples	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	.... g kg^{-1}						.... mg/kg^{-1}				
	Palestina										
Foliar Zn	38.3	3.9	19.3	3.0	3.1	1.8	15.3	12	90	35	47
Control	36.0	3.8	18.0	1.8	2.1	1.6	13.4	12	80	25	38
	Votuporanga										
Foliar Zn		4.8	9.8	1.1	1.8	2.2	15.3	5.5	60	32	43
Control		0.5	9.7	1.1	1.8	2.0	13.4	5.4	57	30	37

Table 7. Soil-applied fertilizers used in Brazilian common bean trials.

Fertilizer	N-P-K	N	P_2O_5	K_2O	S	Zn	CaO (Ca)	MgO (Mg)
			.... %					
Local Control (LC)	S.SuperPhosp.	3	17	–	11	–	22.4(16.0)	6.7 (4.0)
	Urea	46	–	–	–	–	–	–
LC+Mosaic-MESZ	12-40-00	12	40	–	10	1.0	–	–
LC+KaliKorn (KCL-Zn)	00-00-40	–	–	40	5	1.5	–	6.0 (3.6)
			.... g/100mL					
ADOB HBED Zn Chelate	00-00-00	–	–	–	–	2.6	–	–

4. RESULTS AND DISCUSSIONS

4.1. Soil treatments

The results of soil-Zn trials at Capão Bonito 2011/12 (fields 1 and 2), Votuporanga and Capão Bonito 2012/13, Votuporanga and Mirestrela 2013 are presented in Tables 8 and 9.

The plant emergence data shown in Table 8 do not represent full emergence that happened around 3 days after the evaluation time, but explain the differences among treatments better. Thus, the emergence results of Table 8 are slightly lower than the one obtained at harvest time (Table 9).

The treatment 9 was the only treatment in Votuporanga that was not stored in cold chamber. Therefore, germination results of treatment 9 were excluded from the statistical analysis, and Figure 1 was included to compare high seed-Zn seed versus control (low seed-Zn). These seeds were kept under same room temperature conditions.

The results of plant emergence indicated favorable effect of zinc-enriched seeds on plant establishment in the field two (Capão Bonito) and Votuporanga (Tables 8 and 9, Figure 1). The field one was planted when seeds were very young (one month old); thus, all seeds had high vigor independent of the zinc enrichment. The plant emergence of zinc-enriched seeds (treatment 9) stood out in the second field (Table 8) and it was better than regular seeds in the third field (Figure 1).

The treatment 10 was the worst and did differ from control mainly by the evaluations done in the Votuporanga field. Thus, common bean seeds treated with 5 mM $ZnSO_4$ solution for 1 h showed low plant emergence. The lower Zn concentration was used in the seed Zn-treatment solution (1 mM Zn) in the last trial (Votuporanga 2013), but it did not reduce its negative effect on seed germination (Table 8).

Common bean crop growth was excellent at Capão Bonito in 2011/12 (Figure 2), but excessive rainfall during the reproductive plant stage reduced yield, mainly in the second field (Table 9). The Votuporanga fields developed well and the seed yields were higher than in the other fields.

Yield differences among Capão Bonito's soil treatments were observed only in the first field of 2011/12 season (Table 9), in which plots with Mosaic- MESZ produced higher yield than Local Control treatments (8 and 9) and Kali Korn (6). The treatments 3 and 4 of the MESZ fertilizers were the best treatments in Field-1 in Capao Bonito, but the results in Votuporanga were totally different (Table 9). The ADOBE-ZnHBED stood out in terms of yield results in the Votuporanga location. If salinity could be a relevant effect, it could have been seen rather at the beginning; but this was not the case. In addition, the rates used were very normal and not at a level to create a salt effect.

In general, soil treatments had very little effect on seed Zn in Capão Bonito during 2011/12 crop season (Tables 10 and 11). Differences in seed nutrient concentrations among the treatments were not found in the field 1 at Capão Bonito (Table 10). The treatment Mosaic-MESZ plus foliar application of zinc sulfate had the highest concentration of Zn in the Field 2

(Table 10). Largest differences among seed Zn values were obtained in 2012/13 crop season, in accordance with Zn doses: Zn sulfate (10.0 kg ha^{-1} Zn) differed from all other treatments, except ADOB HBED Zn (6.5 kg ha^{-1} Zn) and Mosaic-MESZ (4.0 kg ha^{-1} Zn) (Table 12). As the soil had 1.5 mg kg^{-1} of available Zn and the seed Zn concentration was improved, it is possible to enrich Zn in common bean seeds by soil fertilization when range of available Zn is low or medium. The foliar application of zinc plus Mosaic-MESZ did not increase the concentration of Zn in the seeds, which was not expected.

Yield differences among Votuporanga's soil treatments were observed only in 2012. When high rates of sulfur were applied, in 2013, yield did not differ among treatments.

In the experiment conducted in Votuporanga 2012, the application of zinc sulfate to the soil resulted in higher levels of Zn in the seeds in relation to fertilizer Mosaic-MESZ except with foliar application of Zn, in accordance with improvement of the plant's nutritional status (Table 11). However, the Zn concentration of the leaves in the Mosaic-MESZ did not differ from other zinc fertilizers. This shows that soil fertilization improved the nutritional status of the plants, but did not ensure better quality of seeds.

The Mosaic-MESZ fertilizer resulted in lower seed concentrations of S and Fe and higher P, Ca and Cu in comparison to the control in Votuporanga (Table 11), but not in Capão Bonito (2011/12 and 2012/13) (Table 10). The amount of sulfur applied per hectare was the same in all treatments (52 kg ha^{-1}), except for Mosaic-MESZ (20 kg ha^{-1}) and Mosaic-MESZ - 2x (40 kg ha^{-1}). Furthermore, this fertilizer contains half of the sulfur in elemental form which normally could be good for Oxisols.

The leaf analysis results revealed that sulfur concentrations were lower in Mosaic-MESZ treatments (Table 11) as a result of low sulfur doses and very low content of this nutrient in the soil (Table 1). Therefore, the lowest yield with the Mosaic MESZ was probably due to sulfur deficiency in plants.

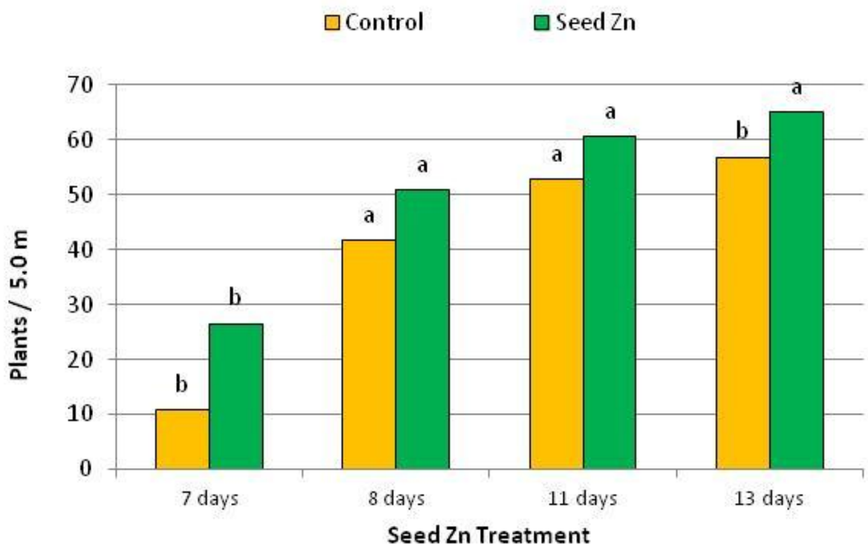

Figure 1. Seed Zn effects on plant emergence measured on various days after planting at Votuporanga (third field). Means followed by the same letters do not differ by Tukey's test (0.05).

Table 8. Averages and statistical results of the plant emergence at 8 days after planting per Zn soil treatment at Capão Bonito (fields 1 and 2), Votuporanga and Capão Bonito.

	Treatment	Capão Bonito 2011/12 Field 1 (1)	Capão Bonito 2011/12 Field 2 (2)	Votuporanga 2012 (1)	Capão Bonito 2012/13 (2)	Votuporanga 2013 (1)
1	Local Control (LC)	54 a	47 b	45 ab	53 a	33 ab
2	LC + Soil Zn	50 a	47 b	42 ab	52 a	31 ab
3	LC+Mosaic-MESZ	48 ab	46 b	49 a	50 ab	26 b
4	LC+Mosaic-MESZ (2x)	43 ab	48 b	51 a	50 ab	36 a
5	LC+Mosaic-MESZ + F0LIAR	49 ab	46 b	44 ab	54 a	35 a
6	LC+KaliKorn (KCL-Zn)	50 a	51 ab	47 ab	51 a	34 a
7	LC + Split Uréia - 1% Zn	54 a	48 b	37 ab	51 a	31 ab
8	LC +ADOB HBED (3)	48 ab	49 b	43 ab	49 ab	25 bc
9	LC -Seed Zn (Field)	52 a	63 a		52 a	35 a
10	LC-Seed (Zn solution)	37 b	40 b	28 b	42 b	17 c
	Average	49	49	43	51	30
	p<F (4)	**	**	**	**	**
	CV (%)	13.3	15.1	23.5	9.3	10.4

(1) Plant emergence was evaluated 8 days after planting
(2) Plant emergence was evaluated 13 days after planting
(3) ADOB HBED was not applied at Field 1 where treatment 9 was equal to treatment 1
(4) indicates significance at ≤ 0.01; Means followed by the same letters, in columns, do not differ by Tukey's test (0.05).

Table 9. Averages and statistical results of final plant stand (harvesting time) and yield in the soil zinc trials at Capao Bonito and Votuporanga

	Treatment	Stand	Yield	Stand	Yield	Stand	Yield
		plants / 5.0m	kg/ha	plants / 5.0m	kg/ha	plants / 5.0m	kg/ha
		C. Bonito 2011/12 - First Field		C. Bonito 2011/12- Second Field		C. Bonito 2012/13	
1	Local Control (LC)	52 ab	1174 a-c	45 b	690	55 a	3957
2	LC + Soil Zn	57 a	1186 a-c	46 b	691	53 a	3944
3	LC+Mosaic-MESZ	53 ab	1385 a	44 b	717	56 a	4201
4	LC+Mosaic-MESZ (2x)	52 ab	1402 a	43 b	749	46 a	4191
5	LC+Mosaic-MESZ + F0LIAR	55 a	1344 ab	41 b	658	52 a	4388
6	LC+KaliKorn (KCL-Zn)	55 ab	1121 bc	48 b	678	52 a	4184
7	Split Uréia - 1% Zn	52 ab	1234 a-c	46 b	720	56 a	4088
8	LC +ADOB HBED Zn [(1)]	57 a	1102 c	46 b	646	55 a	4101
9	LC -Seed Zn (Field)	59 a	1133 bc	63 a	686	53 a	4447
10	LC-Seed (Zn solution)	44 b	1242 a-c	39 b	768	46 a	4498
	Average	53	1232	46	700	52	4200
	p<F [(2)]	**	**	**	ns	*	ns
	CV (%)	10.9	10.1	13.0	13.0	11.2	10.7
		Votuporanga 2012		Votuporanga 2013			
1	Local Control (LC)	55 ab	2441 ab	44 a	3338		
2	LC + Soil Zn	53 ab	2344 ab	45 a	2985		
3	LC+Mosaic-MESZ	62 a	1861 c	44 a	3200		
4	LC+Mosaic-MESZ (2x)	60 a	2035 bc	48 a	3326		
5	LC+Mosaic-MESZ + F0LIAR	51 ab	2028 bc	47 a	3212		
6	LC+KaliKorn (KCL-Zn)	57 ab	2411 ab	48 a	3285		
7	Split Uréia - 1% Zn	53 ab	2515 a	46 a	3200		
8	LC +ADOB HBED Zn [(1)]	55 ab	2624 a	45 a	3108		
9	LC -Seed Zn (Field)	63 a	2602 a	43 a	3153		
10	LC-Seed (Zn solution)	43 b	2410 ab	32 b	3297		
	Average	55	2327	44	3210		
	p<F [(2)]	**	**	**	ns		
	CV (%)	13.9	9.6	8.8	8.2		

[(1)] HBED was not applied at Field 1 where treatment 9 was equal to treatment 1

[(2)] ** e * indicates significance at ≤ 0.01 and ≤ 0.05; Means followed by the same letters, in columns, do not differ by Tukey's test (0.05).

Table 10. Effects of various soil treatments on seed concentration of mineral nutrients in the Field 1 and Field 2 trials at Capão Bonito, 2011/12

Treatments	K	P	S	Mg	Ca	Fe	Zn	Cu	Mn	Al [3]
	............%...........					.... mg kg^{-1}................				
	Field 1									
Control	1.19	0.43	0.22	0.20	0.13	68	29	8	14	33
Soil Zn	1.19	0.43	0.22	0.20	0.13	68	30	8	13	31
Mosaic-MESZ	1.16	0.43	0.22	0.20	0.13	65	30	9	14	26
Mosaic-MESZ (2x)	1.15	0.43	0.22	0.20	0.13	67	30	9	14	29
Mosaic-MESZ + F0LIAR	1.19	0.44	0.22	0.20	0.13	68	29	9	14	31
KaliKorn (KCL-Zn)	1.15	0.43	0.23	0.21	0.15	66	30	8	13	28
Split Uréia 1% Zn	1.15	0.43	0.22	0.20	0.12	64	28	9	14	24
ADOB HBED Zn *	1.18	0.44	0.22	0.20	0.14	67	29	8	14	28
Seed Zn (Field)	1.15	0.41	0.22	0.20	0.14	65	28	8	14	25
Seed (Zn solution)	1.16	0.44	0.23	0.20	0.13	67	30	9	14	27
p<F [1]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	4.8	7.0	4.3	3.7	10.6	7.0	6.1	7.6	7.0	27.4
	Field 2									
Control	1.32	0.52	0.24	0.21	0.14	59 ab	29 c	8	12	13
Soil Zn	1.32	0.52	0.23	0.21	0.13	57 ab	31 bc	8	11	10
Mosaic-MESZ	1.32	0.51	0.23	0.21	0.14	59 ab	31 bc	8	12	13
Mosaic-MESZ (2x)	1.32	0.52	0.23	0.21	0.13	56 b	31 bc	8	12	9
Mosaic-MESZ + F0LIAR	1.32	0.52	0.23	0.21	0.14	60 ab	37 a	8	12	13
KaliKorn (KCL-Zn)	1.32	0.52	0.24	0.21	0.13	59 ab	31 bc	8	12	12
Split Uréia 1% Zn	1.30	0.50	0.23	0.22	0.14	60 ab	29 c	8	12	12
ADOB HBED Zn [1]	1.30	0.52	0.24	0.21	0.14	62 a	32 b	8	12	13
Seed Zn (Field)	1.32	0.53	0.24	0.21	0.14	60 ab	31 bc	9	12	11
Seed (Zn solution)	1.30	0.50	0.23	0.20	0.14	58 ab	29 c	8	12	12
p<F [2]	ns	ns	ns	ns	ns	*	**	ns	ns	ns
CV (%)	2.2	4.1	3.3	3.5	10.6	4.5	4.9	8.1	5.5	22.4

(1) HBED was not applied at Field 1 where treatment 9 was equal to treatment 1;
(2) ** e * indicates significance at ≤ 0.01 and ≤ 0.05; Means followed by the same letters, in columns, do not differ by Tukey's test (0.05);
(3) Data transformed in (x + 0.5) *0.5

Table 11. Effect of various soil treatments on concentrations of mineral nutrients in leaves and seeds at the Votuporanga location, 2012[1]

Treatments	N	K	P	S	Mg	Ca	Fe[2]	Zn	Cu	Mn	Al[2]
		............%...........					.... mg kg^{-1}................				
		Leaves									
Local Control (LC)	2.99	2.75 ab	0.33 ab	0.16 a-c	0.51 a	1.54	936 a	61 b	6	326 ab	766 a
LC + Soil Zn	3.00	2.92 ab	0.36 a	0.18 a	0.44 ab	1.29	820 a	82 ab	6	359 ab	643 a
LC+Mosaic-MESZ	2.72	2.74 ab	0.28 b	0.11 d	0.40 b	1.42	1198 a	75 b	5	337 ab	1007 a
LC+Mosaic-MESZ (2x)	2.81	2.83 ab	0.32 ab	0.13 cd	0.41 ab	1.33	1039 a	73 b	6	269 b	796 a
LC+Mosaic-MESZ + F0LIAR	2.85	2.64 ab	0.34 ab	0.13 b-d	0.40 ab	1.32	1147 a	104 a	6	276 b	976 a
LC+KaliKorn (KCL-Zn)	3.04	3.09 a	0.34 ab	0.18 a	0.47 ab	1.38	800 a	69 b	6	336 ab	654 a
Split Uréia 1% Zn	2.87	2.92 ab	0.33 ab	0.16 ab	0.48 ab	1.43	1014 a	67 b	6	377 ab	925 a
ADOB HBED Zn Chelate	2.94	2.84 ab	0.33 ab	0.15 a-c	0.50 ab	1.35	948 a	63 b	6	278 b	782 a
LC -Seed Zn (Field)	3.12	2.92 ab	0.35 ab	0.17 a	0.47 ab	1.42	814 a	64 b	6	308 ab	578 a
LC-Seed (Zn solution)	2.75	2.35 b	0.33 ab	0.15 a-c	0.50 ab	1.43	1202 a	72 b	6	445 a	1041 a
p<F [1]	ns	*	+	**	**	ns	*	**	ns	**	*
CV (%)	10.5	11.2	11.2	10.9	12.7	10.8	13.3	17.0	12.0	22.4	16.6
		Grains									
Local Control (LC)	-	1.05	0.43 cd	0,20 a	0.18	0.10 c	57 a	36 a-c	5 cd	25 a	1
LC + Soil Zn	-	1.06	0.44 cd	0,21 a	0.18	0.11 bc	56 ab	39 a	5 bc	31 a	1
LC+Mosaic-MESZ	-	1.09	0.51 a	0,14 b	0.18	0.13 a	51 bc	32 c	6 a	30 a	1
LC+Mosaic-MESZ (2x)	-	1.07	0.50 ab	0,15 b	0.18	0.12 ab	52 bc	33 bc	6 ab	32 a	1
LC+Mosaic-MESZ + F0LIAR	-	1.09	0.47 bc	0,14 b	0.18	0.11 a-c	50 c	37 ab	5 bc	26 a	1
LC+KaliKorn (KCL-Zn)	-	1.05	0.44 cd	0,21 a	0.18	0.10 c	54 a-c	37 ab	5 d	27 a	1
Split Uréia 1% Zn	-	1.06	0.42 d	0,20 a	0.18	0.10 c	54 a-c	36 a-c	5 cd	30 a	1
ADOB HBED Zn Chelate	-	1.06	0.43 cd	0,20 a	0.18	0.10 c	54 a-c	35 a-c	5 c	26 a	1
LC -Seed Zn (Field)	-	1.05	0.45 cd	0,21 a	0.18	0.11 bc	56 ab	37 ab	5 cd	26 a	1
LC-Seed (Zn solution)	-	1.05	0.42 d	0,20 a	0.18	0.11 bc	54 a-c	37 ab	5 cd	31 a	1
p<F [1]	-	ns	**	**	ns	**	**	**	**	**	ns
CV (%)	-	2.7	4.8	4,7	2.9	7.3	4.8	5.7	6.9	12.3	23.0

[1] ** and * indicates significance at ≤ 0.01 and ≤ 0.05, Means followed by the same letters, in columns, do not differ by Tukey's test (0.05). [2] Data transformed in (x + 0.5) *0.5

Table 12. Averages and statistical results of grain nutrient analysis in the soil zinc trials at Capão Bonito 2012/13

Treatments	K	P	S	Mg	Ca	Fe	Zn	Cu	Mn	Al [2]
	............%...........					.... mg kg^{-1}................				
Control	1.25	0.45	0.20	0.19	0.11 b	60	25 bc	9	14	0
Soil Zn	1.23	0.44	0.20	0.19	0.13 ab	61	28 a	9	14	0
Mosaic-MESZ	1.22	0.43	0.20	0.19	0.14 a	59	25 bc	9	15	0
Mosaic-MESZ (2x)	1.21	0.43	0.20	0.19	0.12 ab	60	27 ab	9	15	0
Mosaic-MESZ + F0LIAR	1.24	0.46	0.19	0.19	0.11 ab	56	25 bc	9	15	0
KaliKorn (KCL-Zn)	1.25	0.45	0.20	0.19	0.11 b	61	25 bc	8	14	0
Split Uréia 1% Zn	1.22	0.43	0.20	0.19	0.13 ab	60	25 bc	9	15	0
ADOB HBED Zn	1.23	0.43	0.20	0.19	0.14 ab	59	27 ab	9	15	1
Seed Zn (Field)	1.21	0.42	0.20	0.19	0.13 ab	58	25 c	9	15	0
Seed (Zn solution)	1.24	0.46	0.20	0.19	0.12 ab	60	25 bc	9	15	0
p<F [1]	ns	ns	ns	ns	**	ns	**	ns	ns	ns
CV (%)	4.6	6.8	4.2	3.2	15.2	6.2	4.6	7.1	9.7	33.3

[1] ** and * indicates significance at ≤ 0.01 and ≤ 0.05, Means followed by the same letters, in columns, do not differ by Tukey's test (0.05). [2] Data transformed in (x + 0.5) *0.5

4.2. Foliar treatments

In case of foliar spray of various foliar fertilizers, there were leaf toxicity symptoms with the foliar treatment 5 (OMEX-Type-II) in Votuporanga 2012 (Figure 3). As we have applied same amounts of Zn to foliage per hectare, the reason for this effect might be related to the composition of that fertilizer. In the same field, slight leaf yellowing were observed in the soil treatments 3, 4 and 5, all with Mosaic-MESZ fertilizers due to low S supply (Figure 4).

In the Votuporanga's 2012 foliar trial, the treatment FBScience + $ZnSO_4$ resulted in higher yields compared to other treatments (Table 13). However, the yield of the FBScience + $ZnSO_4$ was not statistically different from many other treatments as shown in Table 12. In case of the OmexII treatment, yield was the lowest probably due to toxicity effect on the leaves (Figure 3).

In general, all foliar treatments in Votuporanga (except FBScience treatment) tended to reduce seed yield which might be related to application of 1 % $ZnSO_4$ instead of 0.5 % $ZnSO_4$. Application of 1 % $ZnSO_4$ was made mistakenly and was corrected in the next experiments.

The seed yield of the experimental plants was much lower in Campos Novos than the Votuporanga 2013 and Mirestrela 2013 up to 3-4 times and this difference was very similar among treatments. In Campos Novos there was a strong hail following the first foliar zinc application which greatly reduced leaf area and seed yield (Table 13). Plants did not develop well in Capão Bonito, probably as a result of soil acidity.

Treatments did no differ in the combined analysis of all locations. But, in terms of numerical differences, Omex Type II Foliar Zn and Adob ZnIDHA resulted in the lowest yields and $ZnSO_4$ (once, after) and Omex Type III Foliar Zn resulted in the highest yields among fertilizers (Table 13).

In the Votuporanga 2012 trial, the foliar fertilizers increased the seed Zn concentration, except for the treatment with zinc sulfate before flowering, AdobZnIDHA and Valagro Brexil (Table 14). The highest Zn concentration was observed with OmexII treatment that was equal to the zinc sulfate treatment (twice application). Beside its possible better impact on seed Zn, other reason of higher seed Zn concentrations with OmexII could be related to lower yield in this treatment (Table 13) and thus "*concentration effect*".

The seed sulfur concentrations were higher with two applications of zinc sulfate and EPSO-TOP compared to the control, which was expected due to the greater supply of S by these fertilizers (Table 13).

The foliar applications improved seed Zn concentrations in Campos Novos and Capão Bonito trials, except in the treatments with Zinc Sulfate before flowering, Bayer Antracol-Zn and, specifically in Campos Novos, Valagro Brexil (Tables 15 and 16). The Zn dose of Bayer Antracol was the lowest among fertilizers (0.6 kg ha-1). The highest seed Zn concentration was obtained by the treatment with Omex Type II (0.9 kg ha^{-1} of Zn) in both trials, without differing from Zinc Sulfate applied twice (1.8 kg ha^{-1} of Zn) in Campos Novos.

Table 13. Averages and statistical results of yield in the foliar zinc trials at Votuporanga, Campos Novos, Capão Bonito and Mirestrela.

	Treatment	Votuporanga 2012	Campos Novos 2012	Capão Bonito 2012/13	Votuporanga 2013	Mirestrela 2013	Average
		…………………………. kg/ha ………………….……………					
3	LC + Foliar ZnSO4 (once, after)	2213 ab	722	2487	2717	4156	2459
6	LC + Omex Type III Foliar Zn (SC 144)	2241 ab	714	2292	2813	3941	2400
2	LC + Foliar ZnSO4 (once, before)	2100 b	634	2463	2856	3847	2380
7	LC + Kali EpsoTop - Zn	2272 ab	734	2037	2856	3949	2369
10	LC + Bayer Antracol-Zn	2245 ab	737	2316	2818	3702	2364
4	LC + Foliar ZnSO4 (twice foliar spray)	2036 b	640	2329	2607	4156	2354
11	LC + Foliar ZnSO4 (once, after) + inseticide	2176 ab	704	2313	2749	3800	2348
1	**Local Control (LC)**	2327 ab	595	2352	2829	3637	2348
12	LC + FBScience (1036) + ZnSO4	2498 a	732	1992	2722	3603	2310
9	LC + Valagro Brexil	2208 ab	635	1976	2704	3739	2252
5	LC + Omex Type II Foliar Zn (D1872)	1998 b	555	2411	2604	3388	2191
8	LC + Adob ZnIDHA	2122 b	637	2102	2482	3439	2156
	Average	2203	670	2256	2730	3780	2328
	p<F [1]	**	ns	ns	ns	ns	ns
	CV (%)	8.5	17.3	17.1	9.4	18.4	16.9

[1] ** e * indicates significance at ≤ 0.01 and ≤ 0.05, Means followed by the same letters, in columns, not differ by Tukey's test (0.05).

Table 14. Averages and statistical results of seed nutrient analysis in the foliar zinc trials at Votuporanga 2012

Treatments	K	P	S	Mg	Ca	Fe	Zn	Cu	Mn	Al[2]
	............%...........					.... $mg\ kg^{-1}$...............				
Local Control (LC)	0.97 ab	0.47	0.20 c	0.18 a	0.11	57	37 d	5	30	1
LC + Foliar ZnSO4 (once, before)	0.98 a	0.47	0.21 bc	0.18 a	0.10	58	40 cd	5	32	1
LC + Foliar ZnSO4 (once, after)	0.98 a	0.46	0.21 a-c	0.18 a	0.11	57	41 bc	5	31	1
LC + Foliar ZnSO4 (twice foliar spray)	0.98 a	0.48	0.22 ab	0.18 a	0.11	60	43 ab	5	32	1
LC + Omex Type II Foliar Zn (D1872)	0.98 a	0.49	0.21 bc	0.18 a	0.11	59	45 a	6	30	1
LC + Omex Type III Foliar Zn (SC 144)	0.94 b	0.46	0.21 bc	0.18 a	0.10	57	40 c	5	30	1
LC + Kali EpsoTop - Zn	0.97 a	0.47	0.23 a	0.18 a	0.10	58	42 bc	5	31	1
LC + Adob ZnIDHA	0.96 ab	0.46	0.20 c	0.18 a	0.12	57	39 cd	5	31	1
LC + Valagro Brexil	0.96 ab	0.46	0.21 bc	0.18 a	0.10	56	39 cd	5	30	1
LC + Bayer Antracol-Zn	0.97 a	0.46	0.21 bc	0.18 a	0.11	57	40 bc	5	33	1
LC + Foliar ZnSO4 (once, after) + inseticide	0.97 ab	0.46	0.21 bc	0.18 a	0.10	57	41 bc	5	30	1
LC + FBScience (1036) + ZnSO4	0.98 a	0.46	0.21 bc	0.18 a	0.11	56	40 c	5	32	1
p<F [1]	**	ns	**	*	ns	ns	**	ns	ns	ns
CV (%)	1.50	3.60	3.70	2.40	9.70	4.80	3.80	9.20	12.80	20.10

[1] ** e * indicates significance at ≤ 0.01 and ≤ 0.05, Means followed by the same letters, in columns, do not differ by Tukey's test (0.05); [2] Data transformed in (x + 0.5) *0,5

Table 15. Averages and statistical results of seed nutrient analysis in the foliar zinc trials at Campos Novos, 2012.

Treatments	K	P	S	Mg	Ca	Fe	Zn	Cu	Mn	Al(2)
	............%...........					.... mg kg-1...............				
Local Control (LC)	1.14	0.46 b	0.22	0.19	0.15	81 ab	34 c	9	15	16 a
LC + Foliar ZnSO4 (once, before)	1.15	0.49 ab	0.22	0.19	0.13	77 b	37 bc	9	15	7 a
LC + Foliar ZnSO4 (once, after)	1.16	0.50 ab	0.22	0.19	0.14	86 ab	39 b	10	15	26 a
LC + Foliar ZnSO4 (twice foliar spray)	1.16	0.47 ab	0.22	0.18	0.14	103 a	39 b	9	15	35 a
LC + Omex Type II Foliar Zn (D1872)	1.17	0.51 a	0.22	0.19	0.13	85 ab	44 a	9	15	19 a
LC + Omex Type III Foliar Zn (SC 144)	1.15	0.50 ab	0.22	0.19	0.13	74 b	38 b	9	16	6 a
LC + Kali EpsoTop - Zn	1.19	0.48 ab	0.22	0.19	0.15	78 b	38 b	9	15	16 a
LC + Adob ZnIDHA	1.18	0.50 a	0.22	0.18	0.13	95 ab	39 b	10	16	29 a
LC + Valagro Brexil	1.17	0.49 ab	0.22	0.19	0.14	90 ab	37 bc	9	15	26 a
LC + Bayer Antracol-Zn	1.15	0.47 ab	0.22	0.19	0.14	82 ab	36 bc	9	16	14 a
LC + Foliar ZnSO4 (once, after) + inseticide	1.16	0.47 ab	0.22	0.19	0.15	77 b	38 b	9	15	10 a
LC + FBScience (1036) + ZnSO4	1.15	0.49 ab	0.22	0.19	0.13	82 ab	39 b	10	15	19 a
p<F (1)	ns	**	ns	ns	ns	**	**	ns	ns	*
CV (%)	3.50	4.50	3.80	3.80	13.80	13.99	4.60	5.90	8.00	45.70

(1) ** e * indicates significance at ≤ 0.01 and ≤ 0.05, Means followed by the same letters, in columns, do not differ by Tukey's test (0.05); (2) Data transformed in (x + 0.5) *0.5

Table 16. Averages and statistical results of grain nutriet analysis in the foliar zinc trials at Capão Bonito 2012/13

Treatments	K	P	S	Mg	Ca	Fe	Zn	Cu	Mn	Al(2)
	............%...........					.... mg kg-1...............				
Local Control (LC)	1.13	0.45	0.22	0.20	0.11	53 a	27 f	7	13	3
LC + Foliar ZnSO4 (once, before)	1.13	0.43	0.22	0.21	0.10	51 a	28 f	7	13	1
LC + Foliar ZnSO4 (once, after)	1.16	0.45	0.22	0.21	0.10	56 a	35 ab	7	13	2
LC + Foliar ZnSO4 (twice foliar spray)	1.14	0.45	0.22	0.20	0.11	54 a	35 b-d	8	13	2
LC + Omex Type II Foliar Zn (D1872)	1.17	0.44	0.22	0.21	0.11	55 a	39 a	8	14	1
LC + Omex Type III Foliar Zn (SC 144)	1.13	0.45	0.22	0.21	0.10	55 a	31 de	7	13	5
LC + Kali EpsoTop - Zn	1.14	0.44	0.22	0.21	0.11	54 a	33 b-d	8	13	1
LC + Adob ZnIDHA	1.15	0.44	0.22	0.20	0.10	51 a	34 b-d	8	13	1
LC + Valagro Brexil	1.14	0.44	0.22	0.20	0.10	52 a	32 cd	8	13	2
LC + Bayer Antracol-Zn	1.14	0.44	0.22	0.20	0.10	52 a	28 ef	8	13	2
LC + Foliar ZnSO4 (once, after) + inseticide	1.15	0.45	0.22	0.20	0.11	55 a	34 b-d	8	13	1
LC + FBScience (1036) + ZnSO4	1.14	0.45	0.22	0.20	0.10	53 a	35 bc	8	13	2
p<F (1)	ns	ns	ns	ns	ns	*	**	ns	ns	ns
CV (%)	2.70	4.70	4.00	3.70	10.60	4.60	5.40	9.20	5.90	43.70

(1) ** and * indicates significance at ≤ 0.01 and ≤ 0.05, Means followed by the same letters, in columns, do not differ by Tukey's test (0.05). (2) Data transformed in (x + 0.5) *0.5

CONCLUSIONS

The results of all common bean experiments suggest that foliar zinc application represents an effective method contributing to increased seed Zn (19% of Zn improvement).

As regard to foliar Zn application, Omex type II and Zinc Sulfate applied twice were the best treatments.

Zinc biofortification of common beans is possible by soil fertilization when the range of available soil Zn is low to medium. The soil applications of Zinc Sulfate and ADOB-HBED Zn stand out as regard to seed Zn concentration.

The results related to plant emergence indicated favorable effect of zinc-enriched seeds on plant establishment, except in the fields planted with very young seeds which had high vigor independent of the zinc enrichment. The common bean seeds treated with 1 or 5 mM $ZnSO_4$ solution for 1 h showed low plant emergence, probably because of the long duration of seed treatment for this species.

5. TRAINING AND VISIBILITY ACTIVITIES

Brazilian Zinc Day was held in the Agronomic Institute, Campinas (SP), on December 6th 2011, with two hundred participants, including consultants, researchers, professors and students.

6. PROBLEMS ENCOUNTERED

Because of delayed importation of ADOB fertilizers, it could not be included as soil treatment 8 in the field 1 experiment. The treatment 8 was used as a second control treatment.

7. FUTURE ACTIVITIES

Leaf and seed samples of the last trials (Votuporanga 2013 and Mirestrela 2013) will be sent to Sabanci University Faculty of Engineering and Natural Sciences, Istanbul, Turkey.

Figure 2. Common bean trials: the first field on the left and the second field on the right.

Figure 3. Leaf toxicity symptoms in the foliar treatment OMEX-Type-II at Votuporanga (6 and 14 days after pulverization).

Figure 4. Light green leaves in the soil treatment Mosaic-MESZ (right) and green leaves in the local control (left) at Votuporanga.

COUNTRY REPORT - ZAMBIA

1. COLLABORATING INSTITUTIONS:

COORDINATING INSTITUTIONS:
Golden Valley Agricultural Research Trust (GART) and University of Zambia.

PROJECT STAFF:
Profe. Dr. Obed Lungu (Soil Management Specialist)
Simunji Simunji (Agronomist)

Summary of results and Observations:

Seedling establishment:

Among the soil applied fertilizers, seedling establishment of sorghum and wheat crops was more vigorous in plots treated with all MESZ fertilizers as well as zinc enriched seed either by seed priming or spraying with zinc sulphate.

Soil applied fertilizers:

After the second season of planting Zn trials, results revealed an increase in sorghum and wheat yields over control by applying MESZ fertilizers to the soil at planting and using Zn enriched seeds mainly by priming. The increase in sorghum grain yield was 32% in the crop treated with either MESZ fertilizer or Zn enriched seed by priming over the control which had a yield of 1.3 t ha^{-1} at Chisamba, while at Lusitu the yield increased by 44% over the control which produced 0.39 t ha^{-1}. MESZ and enriched seeds increased wheat grain yields from 3.45 t ha^{-1} under control by 11.6% to 3. 85 t ha^{-1} on the average at Chisamba location only. When considering 2012/13 wheat results, MESZ I, MESZ II and Zinc enriched seed by foliar application appear to be consistently increasing yields of wheat as compared to control.

MESZ fertilizers and $ZnSO_4$ increased Zn concentration of sorghum grain from 15 mg kg^{-1} in the control to 17 mg kg^{-1}, when applied to the soil at planting The increase in Zn concentration was 13.3% at Chisamba and 19% at Lusitu location where it was improved from 26 mg kg^{-1} to 31 mg kg^{-1}. MESZ III + foliar Zn, Kali- Korn , 2 split urea with $ZnSO_4$ and seed enriched by priming resulted in higher sorghum Zn grain concentration in 2012/13 season. Translocation of Zn from soil to sorghum plant tends to be dependent on environmental conditions of the particular location. Chisamba with heavy clay loam soils resulted in lower sorghum grain Zn concentration as compared to Lusitu with light soils.

In wheat, soil fertilizers resulted in the highest concentration of Zn in grain when MESZ III + foliar, basal $ZnSO_4$ and ADOB were applied.

Local Control Jan 2014	Mosaic MESZ III + foliar Zn Jan 2014

Figure 1: The effect of Mosaic MESZ III + foliar Zn application on plant establishment.

Foliar applied fertilizers

Foliar applied fertilizers improved sorghum grain yield over the control by 6.6% in Chisamba and 17.0% at Lusitu. The increase was caused by spraying Bayer Anthracol, $ZnSO_4$, ADOB or common Zambian fungicide on sorghum crop. The foliar fertilizers did not have significant effects on wheat grain yield as compared to untreated crop.

Spraying of $ZnSO_4$ twice and Omex Type II on sorghum crop improved the quality of sorghum grain through increasing Zn concentration by 82% from 14.0 mg kg^{-1} under control to 25.5 mg kg^{-1} under treatment at Chisamba. The improvement in sorghum grain Zn concentration was 37.3% at Lusitu from 25.6 mg kg^{-1} in the control in 2011-2012. OMEX-Type II and OMEX Type III significantly improved concentration of Zn in sorghum grain consistently at both sites during the 2012/13 season.

OMEX- Type II, OMEX Type III, Valagro Brexil and Kali- EPSO-Zn can be recommended for use as foliar fertilizers to improve wheat grain Zn concentration as given by 2011/12 season results.

2. INTRODUCTION:

Zambia through the HarvestPlus Zinc Project is participating in the global effort to address Zn deficiency that has been reported both in crops and humans. Agronomic biofortification trials are being conducted in Zambia in collaboration of the Golden Valley Agricultural ResearchTrust (GART), the University of Zambia and Sabanci University in Turkey. This report highlights the results from trials conducted during the 2011/12 ,2012/13 and 2013/14 cropping seasons (1st July, 2011 to 31st Jan 2013.

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2.1. Objective 1

To determine the concentration of zinc in sorghum grain after applying zinc fertilizers at planting and as foliar application and use of Zn enriched seeds.

2.2. Objective 2

To determine the concentration of Zn of wheat grain after agronomic biofortification with zinc sulphate at different growth stages.

3. EXPERIMENTAL ACTIVITIES

3.1 Sorghum Trials

3.1.1 Locations

Sorghum trials were planted at GART Chisamba of Chibombo district in the central part of Zambia and at Lusitu of Siavonga district in the southern part of Zambia. Chisamba is in region II of Zambian agro ecological zones receiving an average annual rainfall between 800 and 1000 mm while Lusitu receives annual rainfall of below 800 mm with high daily temperatures above 40^{o}C in some cases.

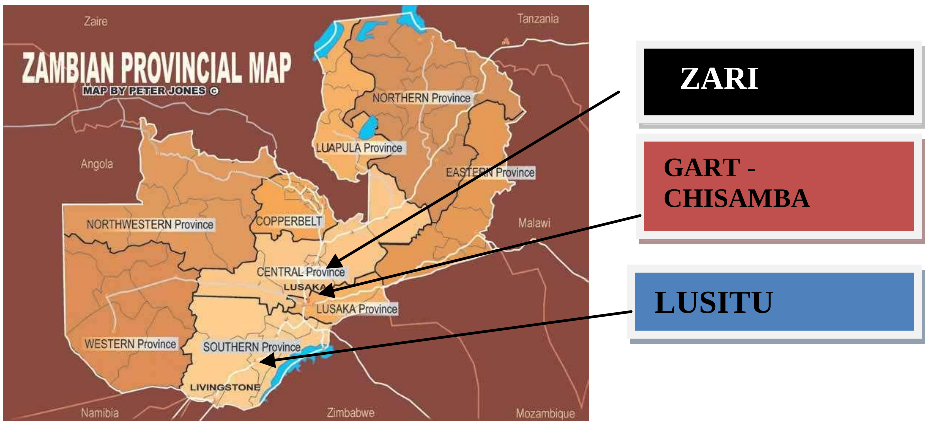

Figure 2. Map of Zambia showing the experimental sites for sorghum (GART and Lusitu) and wheat trials (GART and ZARI).

3.1.2 Zinc Fertilizers for experiments:

Fertilizers for both wheat and Sorghum trial protocols were received in time before October, 2011. These include soil fertilizers as well as foliar fertilizers of Zn. The soil fertilizers (basal) were applied at planting at different recommended nutrient rates. Top dressing in form of Urea (46% N) was added to the crop at tillering stage four weeks after planting. Foliar Zinc fertilizers were sprayed at flowering in accordance with the treatments. Basal fertilizer used in the foliar trial was applied at Zambian standard rates of 20 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1}, 20 kg K ha^{-1}.

3.1.3 Soil sampling:

Baseline soil samples were collected at 0-30 cm depth. Primary samples were taken from 7 random points in a trial plot. After mixing, a composite sample of about 1 kg was obtained and kept for analysis. Soil samples from treatment plots in the soil fertilizer trials were also collected to verify the availability of soil nutrient content in the soil after treatments. Treatment soil samples in sorghum trials were collected and analyzed.

3.1.4 Leaf Sampling:

Sampling of leaves was done at flowering stage. Leaves were collected from all treatments in the soil-treated trial and about 6 leaves were collected in the foliar treated trial. Samples, however, have not been sent anywhere for analysis having considered the grains were more important for Zn content evaluation.

3.1.5 Planting:

Planting was done in ripped furrows following a principle of minimum tillage under conservation agriculture (CA). The trials of sorghum were planted on 11th of December,2011 at Chisamba and on 13th of December, 2011 at Lusitu. During the 2012/13 season, sorghum trials were planted on the 13th of December, 2012 in Lusitu and 3rd of January, 2013 in Chisamba. While for 2013/14 season, sorghum was planted on the 13th of December, 2013 at Chisamba and on the 17th of December at Lusitu.

3.1.6 Harvesting:

The sorghum trial was harvested in the month of May, 2012 at Chisamba and in June, 2012 at Lusitu. Plot yields from the treatments were determined and converted to tons per hectare. During the 2012/13 growing season sorghum crop was harvested between May and June, 2013.

3.1.7 Plot size:

Each plot had six rows of 0.75 m row space with a row length of 5 m.

3.1.8 Treatments:

Seed used for sorghum trials during 2011/12 season was obtained from Batoka which had some plots with high Zn concentration in the grain. The high Zn concentration seed (44 mg kg^{-1}) obtained from this site was used in treatment 9 only. The rest of the plots were sown with seed of low Zn concentration of 16 mg kg^{-1} from the same site. In the subsequent seasons,, seed for treatment 9 was obtained from a plot that was enriched with Zn by repeated spraying of $ZnSO_4$ during the growing period. The seed of treatment 10 was enriched with Zn by priming in 5 mM $ZnSO_4$ solution for an hour.

Below the treatments of the soil fertilizer application (Experiment 1) are presented together with their applied rates.

1. Local control (LC): 20 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1}, 20 kg K_2O ha^{-1} at planting and 46 kg N ha^{-1} at tillering.
2. LC + Soil Zn (20 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1}, 20 kg K_2O ha^{-1}) + (50 kg $ZnSO_4.7H_2O$ ha^{-1}) at planting and 46 kg N ha^{-1} at tillering.
3. LC+ Mosaic - MESZ-I (20 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1} in form of MESZ at planting and 46 kg N ha^{-1} at tillering.
4. LC+ Mosaic – MESZ II (20 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1} in form of MESZ at planting and 46 kg N ha^{-1} at tillering.
5. LC+ Mosaic - MESZ-I (20 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1} in form of MESZ at planting and 46 kg N ha^{-1} at tillering + 0.5% $ZnSO_4.7H_2O$ foliar application.
6. LC + Kali Korn kali (20 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1} + 150kg ha^{-1} KCl -Zn at planting and 46 kg N ha^{-1} at tillering).
7. 2X Split Urea -Zn (20 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1}, 20 kg K_2O ha^{-1} at planting + 23 kg N-Zn ha^{-1} at tillering + 23 kg N-Zn ha^{-1} at flowering.
8. LC + ADOB HBED Zn Chelate (20 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1}, 20 kg K_2O ha^{-1} + 250 kg Zn HBED ha^{-1} at planting + 46 kg N ha^{-1} at tillering).
9. LC- Seed Zn -1 (seeds enriched with Zn by foliar Zn spray) + 20 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1}, 20 kg K_2O ha^{-1} at planting and 46 kg N ha^{-1} at tillering)
10. LC- Seed Zn -1 (seeds enriched with Zn by priming with Zn) + 20 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1}, 20 kg K_2O ha^{-1} at planting and 46 kg N ha^{-1} at tillering).

Foliar application treatments (Experiment 2) for sorghum were as following:

1. Local control (LC): 20 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1}, 20 kg K_2O ha^{-1} at planting and 46 kg N ha^{-1} at tillering).
2. LC + Foliar $ZnSO_4$ at the end of booting stage.
3. LC + Foliar $ZnSO_4$ once after flowering at early milk stage.
4. LC + Foliar $ZnSO_4$ (twice foliar spray) at the end of booting and at early milk stage.
5. LC+ OMEX- Type-II Foliar Zn with 27.4% Zn (3.32 ml in 800 ml water per 10 m^2) applied at early milk stage.
6. LC+ OMEX- Type-III Foliar Zn with 14.8 % Zn (6.15 ml in 800 ml water per 10 m^2) applied at early milk stage.
7. LC+ Kali- EPSO-Zn 5% Zn (18.2 gram in 800 ml water per 10 m^2) applied at early milk. stage.
8. LC +ADOB Zn IDHA applied at early milk stage.
9. LC + Valgro Brexil at early milk stage.
10. LC + Bayer Antracol- Zn at early milk stage.
11. LC + Commonly applied pesticide.
12. LC+ FBScience CP Foliar Zn fertilizer.

3.2 Wheat trials

3.2.1 Locations:

Wheat trials were planted at GART Chisamba of Chibombo district in the central part of Zambia and at Zambia Agricultural Research Institute (ZARI) of Chilanga district in Lusaka province. Chisamba and Chilanga are located region II of Zambian agro ecological zones receiving an average annual rain fall between 800 and 1000 mm.

3.2.2 Zinc Fertilizers for experiments

Fertilizers for both wheat and sorghum experiments were received in time before October, 2011. These include soil applied Zn fertilizers as well as foliar Zn fertilizers. The soil fertilizers (basal) were applied at planting at different recommended nutrient rates. Basal fertilizer used in the foliar trial was applied at Zambian standard rates of 30 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, 30 kg K ha^{-1}. Top dressing in form of Urea (46% N) is added to the crop at tillering stage about four weeks after planting at 138 kg N ha^{-1}. Foliar Zinc fertilizers are sprayed at flowering in accordance with the treatments.

3.2.3 Soil sampling:

Baseline soil samples were collected at 0-30 cm depth. Primary samples were taken from 7 random points in a trial plot. After mixing, a composite sample of about 1 kg was obtained and kept for analysis.

3.2.4 Planting:

Wheat trials were planted on 26th May,2012 at Chisamba and 29th May, 2012 at Zambia Agricultural Research Institute (ZARI) – Chilanga on disked land. Planting was done manually through drilling the seed at 120 kg ha^{-1} in shallow furrows of about 3 - 5 cm. During the 2013 wheat planting season, planting was done on 26th May at ZARI and 27th May at Chisamba.

3.2.5 Plot size:

Each plot had ten rows with row space of 0.30 m and row length of 5 m.

3.2.6 Harvesting:

The harvesting of the wheat trials was in October of each season at both sites.

3.2.7 Treatments.

The treatments used in wheat were the same as in sorghum for both soil and foliar Zn trials. The seed used in treatment 9 was enriched with Zn (46mg kg^{-1}) by spraying with $ZnSO_4$ on the previous season's wheat crop. The rest of the plots were planted with seed of low Zn concentration. Seed of treatment 10 was enriched with Zn by priming in 5 mM $ZnSO_4$ solution for one hour.

4.0. RESULTS AND DISCUSSIONS:

4.1. Sorghum trials:

4.1.1. Plant establishment on soil Zn application sorghum trial:

The results on plant establishment are mainly based on the soil application of Zn fertilizer treatments. Emergence of sorghum plants was observed to be generally lower in treatments 4 and 5 (Table 1). The Zn enriched seed either by foliar application or priming in treatments 9 and 10, respectively, did not have added advantage over the seed with lower Zn concentration in the 2011-12 growing season. In Lusitu, sorghum crop that received Kalikorn had significantly highest plant vigor score of 2.6. Other treatments with higher plant vigor included treatment 10 with seed enriched by priming in Zn sulphate and treatment 9 with seed enriched with Zn after spraying or MESZ plus foliar Zn.

Different fertilizers applied to soil at planting did not show significant variations in vigor score among the treatments at Chisamba. But a trend indicated that vigor score of sorghum plants two weeks after emergence was highest in plots that were planted with Zn enriched seed obtained by foliar application during 2011-12 growing season.

The vigor score on sorghum seedlings during the 2012-13 growing season, were observed to be highest in treatments 9, (the Zn enriched seed by spraying), 8 (soil applied ADOB) and 4 (soil applied MESZ II) in Chisamba. For Lusitu, good performance in seedling vigor was observed in treatments with MESZ fertilizers followed by Zn enriched seed by priming, the lowest score being in the control (Table2).

During the 2013/14 growing season at Chisamba, highest vigor score was observed in plots that received all three MESZ fertilizers with score of 4.3 on overage over the control that had 2.5. The MESZ fertilizers were followed by zinc enriched seed by spraying that scored 3.82 and ADOB with a score of 3.75 (Table 3).

The effects of soil applied fertilizers on the sorghum seedling vigor have generally showed that Zn enriched seeds and fertilizers containing MESZ enhance fast growth of seedlings.

Table 1. Effect of Soil Zn fertilizer treatment sorghum performance 2011/12 season

TREATMENT	CHISAMBA Plants m^{-1}	CHISAMBA Vigor score	CHISAMBA Yield ($t\ ha^{-1}$)	LUSITU Plants m^{-1}	LUSITU Vigor score	LUSITU Yield ($t\ ha^{-1}$)
LC	26	4.0	3.79	105	2.0	0.79
LC + Soil Zn	25	4.0	3.56	101	1.7	0.86
LC+ Mosaic - MESZ	23	4.0	3.64	96	2.0	0.73
LC+ Mosaic - MESZ II	18	3.5	2.59	98	1.7	0.77
LC+ Mosaic - MESZ + foliar Zn	18	3.5	3.12	108	2.1	0.66
LC+ Kalikorn	21	3.8	2.74	96	2.6	0.37
2x split+ Urea Zn	20	4.0	3.15	99	2.0	0.48
LC + ADOB	22	4.0	3.07	97	1.8	0.44
LC + Seed Zn-1 foliar	21	4.5	2.90	122	2.1	0.65
LC- Seed Zn-1 priming	23	4.0	3.62	100	2.3	0.64
LSD (0.05)	5.5	1.1	0.71	5.7	0.84	0.29
CV (%)	17.6	19.5	15.1	16.5	20.8	26.3

4.1.2 Effect of soil applied Zn fertilizers on sorghum grain yield:

The Chisamba results for 2011/12 season showed a significant difference ($P< 0.05$) among the fertilizer treatments applied to the crop at planting (Table1). Treatments 1, 2, 3 and 10 produced the best yields with an average of 3.650 t ha^{-1}. The lowest average yield of 2.73 t ha^{-1} was obtained from treatments 4, 6 and 9. Treatment 1 (control) which had nutrient application rates of 20 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1}, 20 kg K_2O ha^{-1} at planting and 46 kg N ha^{-1} at tillering produced as similar yields as MESZ applied at the same rate. Considering the processing cost of fertilizer, MESZ can replace Zambian local fertilizer (D compound) at same application rate for similar yield. Adding zinc sulphate to local basal fertilizer at planting did not have yield advantage over local fertilizer only. Sorghum seed enriched with Zn by agronomic spraying produced relatively lower grain yields (2.90 t ha^{-1}) than the control that had 3.79 t ha^{-1}.

Doubling the application rate of MESZ – treatment 4 reduced yields of sorghum probably due to nutrient toxicity. Lusitu had best yields of sorghum grain from the plots treated with MESZ fertilizers, local control and soil Zn SO_4 while a poor yield was obtained through use of Kalikorn, which was an unexpected finding under given conditions.Therefore the grain yield results in Lusitu in 2011/2012 given in Table 1 (and also in 2012/2013 in Table 2) should be very carefully interpreted.

The sorghum yields during the second season (2012/13) of experiments were the highest in plots treated with MESZ containing fertilizer and Zn enriched seed by priming, recording an average yield of 1.7 t ha^{-1} at Chisamba (Table2). At Lusitu, the sorghum yield results for 2012/13 were the best for MESZ containing fertilizers, Zn enriched seeds by priming and ADOB treatments which yielded an average of 0.5 t ha^{-1}. Between locations, yields from Chisamba outyielded Lusitu yields (Table 2). This can be attributed to differences in rainfall pattern between stations Lusitu having been affected more by drought.

Table 2. Effect of soil applied Zn fertilizers on sorghum performance in 2012/13 season.

	CHISAMBA			LUSITU		
TREATMENT	**Plants m^{-1}**	**Vigor score**	**Yield ($t\ ha^{-1}$)**	**Plants m^{-1}**	**Vigor score**	**Yield ($t\ ha^{-1}$)**
LC	24	3.5	1.27	26	2.6	0.39
LC + Soil Zn	20	3.5	1.60	25	3.0	0.40
LC+ Mosaic - MESZ	21	3.5	1.57	22	3.6	0.51
LC+ Mosaic - MESZ II	18	3.8	1.95	21	4.4	0.65
LC+ Mosaic - MESZ + foliar Zn	25	3.5	1.67	25	3.9	0.44
LC+ Kalikorn	25	3.5	1.34	20	3	0.13
2x split+ Urea Zn	19	3.5	1.13	26	2.8	0.22
LC + ADOB	22	3.8	1.30	15	3.2	0.71
LC + Seed Zn-1 foliar	22	3.8	1.23	23	2.8	0.32
LC- Seed Zn-1 priming	24	3.2	1.64	26	3.4	0.50
LSD (0.05)	7.0	5.5	0.46	7.4	1.0	0.49
CV (%)	22.1	18.5	21.4	23.0	21.0	23.9

Table 3. Effect of soil applied Zn fertilizers on sorghum performance in 2013/14 season.

TREATMENT	**Plants m^{-1}**	**Vigor score**
LC	85.7	2.50
LC + Soil Zn	81.2	3.12
LC+ Mosaic - MESZ	83.0	4.25
LC+ Mosaic - MESZ II	87.5	4.62
LC+ Mosaic - MESZ + foliar Zn	84.5	3.95
LC+ Kalikorn	70.2	3.12
2x split+ Urea Zn	78.5	3.12
LC + ADOB	56.0	3.75
LC + Seed Zn-1 foliar	118.0	3.82
LC- Seed Zn-1 priming	75.7	3.00
LSD (0.05)	23.67	0.87
CV (%)	19.9	16.9

4.1.3 Effect of foliar Zn fertilizer treatment on sorghum Grain yield:

Application of foliar zinc fertilizers on sorghum crop for 2011/12 growing season generally showed significant higher yields in plots treated with foliar $ZnSO_4$ (2.90 t ha^{-1}) applied at the end of booting stage and plots that were treated with common fungicide (2.75 t ha^{-1}) than crop treated with OMEX- type II foliar Zn or FBScience CP Foliar Zn fertilizer at Chisamba (Table 4). At Lusitu ,sorghum crop that received ADOB ZnIDHA at milk stage, Bayer Anthtracol-Zn , Valagro Brexil and common fungicide appeared to be higher yielding, producing an average yield of 2.95 t ha^{-1} in 2011/12 growing season. The results therefore show that sorghum responded similarly in yield to foliar Zn applications between the sites.

During the 2012/13 (second growing season), sorghum crop treated with Bayer Anthracol, common fungicide and foliar Zn SO_4 had yielded, on the average, 1.60 t ha^{-1} which was higher than yields from other treatments by 30% (Table 4). In Lusitu, common fungicide, foliar $ZnSO_4$ at all stages, ADOB ZnIDHA and control had better yields (1.70 t ha^{-1}).

These results have indicated that sorghum crop responds differently in grain yield to various foliar applied Zn treated fertilizers. Bayer Anthracol, common fungicide, $ZnSO_4$, and ADOB ZnIDHA consistently increased sorghum grain yields across the seasons and locations.

Table 4. Effect of foliar Zn fertilizer treatments on sorghum grain yield 2011/12 and 2012/2013 seasons.

TREATMENT	YIELD (t ha^{-1})			
	2011-2012		2012-2013	
	CHISAMBA	LUSITU	CHISAMBA	LUSITU
LC	2.58	2.64	1.24	1.71
LC+ Foliar $ZnSO_4$ at booting	2.90	2.44	1.20	1.58
LC+ Foliar $ZnSO_4$ at milk	255	2.59	0.97	1.37
LC+Foliar $ZnSO_4$ twice	2.49	1.76	1.68	1.44
LC + OMEX- TYPEII at milk	2.07	2.14	1.21	0.92
LC + OMEX- TYPEIII at milk	2.51	2.59	1.28	1.34
LC + Kali- EPSO-Zn at milk	2.45	1.54	0.83	1.04
LC+ ADOB Zn IDHA at milk	2.60	3.19	1.28	1.55
LC+ Valagro Brexil at milk	2.45	2.86	1.02	1.10
LC + Bayer Anthracol- Zn at milk	2.33	2.74	1.58	1.45
LC+ common fungicide at milk	2.75	2.99	1.49	2.09
LC+ FBScience CP	2.14	2.41	1.30	1.41
LSD (0.05)	0.69	0.76	0.50	1.02
CV %	19.2	18.0	27.9	29.0

4.1.4 Effect of soil applied Zn fertilizers on sorghum grain Zinc Concentration:

Zinc concentration of sorghum grains was relatively higher in the crop treated with MESZ fertilizers and soil zinc sulphate at Chisamba (table 5). These treatments produced 17 mg kg^{-1} of Zn concentration on the average as compared to treatments of lower Zn concentrations of 15 mg kg^{-1}. While at Lusitu, high zinc concentrations of greater than 31 mg kg^{-1} was obtained in MESZ + foliar $ZnSO_4$, Kalicorn, 2 x split + Urea $ZnSO_4$ and seed enriched with Zn by foliar application. These were followed by Zn enriched seed by priming and ADOB (27 mg kg^{-1}).

During 2012/13 season, Zn concentration of 28.5 mg kg^{-1} at Chisamba and 35.0 mg kg^{-1} at Lusitu for MESZ III + Foliar Zinc sulphate was significantly highest as compared to control with 12.3 mg kg^{-1}, 25.8 mg kg^{-1} at Chisamba and Lusitu, respectively. Korn Kali, 2 x split + Urea $ZnSO_4$ and seed enriched by priming followed MESZ III in Zn concentration at Lusitu in 2012/13 season.

The zinc concentration of sorghum grains (30 mg kg^{-1} on the average) was higher in Lusitu location than Chisamba that had 16 mg kg^{-1}, by 47% in 2011-2012 (Table 5). This difference in Zn concentration between sites can be due to differences in the inherent levels of Zn content in the soil or soil type and environmental conditions. There is higher absorption of Zn element by sorghum crop in Lusitu area and it is expected that people feeding on sorghum in this area are healthier.

Table 5. Effect of soil applied Zn fertilizers on sorghum grain Zinc concentration in 2011/12 and 2012-2013 seasons.

TREATMENT	GRAIN Zn (mg kg^{-1})			
	2011-2012		2012-2013	
	CHISAMBA	LUSITU	CHISAMBA	LUSITU
LC	14.7	25.9	12.3	25.8
LC + Soil Zn	15.9	24.6	11.9	26.3
LC+ Mosaic - MESZ	17.7	25.5	12.5	23.5
LC+ Mosaic - MESZ II	15.6	24.5	11.5	24.8
LC+ Mosaic - MESZ + foliar Zn	17.5	42.9	28.5	35.0
LC+ Kalikorn	15.2	31.4	12.3	30.8
2x split+ Urea Zn	15.0	31.0	11.8	28.3
LC + ADOB	15.2	26.9	12.0	26.5
LC + Seed Zn-1 foliar	15.1	34.6	12.0	25.5
LC- Seed Zn-1 priming	14.6	27.6	12.3	27.5
LSD (0.05)	3.1	11.4	3.5	4.6
CV %	13.7	22.5	17.4	11.7

4.1.5 Effect of foliar Zn fertilizer treatment on sorghum grain Zinc Concentration:

Level of zinc concentration in sorghum grain varied depending on the type of foliar fertilizer application used. At Chisamba, control plots had the lowest Zn concentration of 14 mg kg^{-1} in sorghum grain while the highest (26 mg kg^{-1}) was obtained in the treatments that were sprayed with $ZnSO_4$ two times and this was followed by OMEX TypeII treatment that resulted in 25 mg kg^{-1} Zn in sorghum grains in 2011-2012 season. In 2012/13, Bayer Anthracol- Zn applied at milk stage resulted in the highest grain zinc concentration with 37.5 mg kg^{-1} .This was followed by Valagro Brexil and OMEX- Type III applied at milking stage, which resulted in 36 mg kg^{-1} and 34 mg kg^{-1} Zn in sorghum grains, respectively (Table 6).

At Lusitu, the highest Zn concentrations in sorghum grains were achieved from plants sprayed with OMEX TypeII (37 mg kg^{-1}), OMEX TypeIII (37 mg kg^{-1}), Zn SO_4 applied twice (34 mg kg^{-1}) and Zn SO_4 applied at milk stage(31 mg kg^{-1}) in 2011-2012. In 2012/13 season, the highest grain Zn concentrations were obtained by use of OMEX-TypeII (33 mg kg^{-1}), OMEX TypeIII (34 mg kg^{-1}) and Kali- EPSO-Zn (32 mg kg^{-1} (Table 6).

Among the foliar Zn fertilizers, OMEX TypeII, OMEX TypeIII and twice application of $ZnSO_4$ appear to be more effective in contributing to Zn concentration in sorghum grains.

In general, foliar Zn application improved grain Zn of sorghum grain better than soil Zn treatment in heavy clay loam soils as found at Chisamba. Zn concentration in sorghum grain after soil treatment at Chisamba was 16 mg kg^{-1} on the average and this was lower than Zn concentration as a result of foliar application by 56%. At Lusitu location, Zn concentration in sorghum grain did not vary much with application methods. Both foliar and soil Zn application resulted in similar Zn concentrations. Therefore, foliar Zn application at Chisamba would be recommended in order to increase Zn concentration in sorghum grain, whereas in Lusitu either foliar or soil application could be used.

Table 6. Effect of foliar Zn fertilizer treatments on sorghum grain Zinc concentration in 2011/12 and 2012-2013 seasons.

TREATMENT	GRAIN Zn ($mg\ kg^{-1}$)			
	2011-2012		2012-2013	
	CHISAMBA	LUSITU	CHISAMBA	LUSITU
LC	14.3	25.6	14.0	23.5
LC+ Foliar $ZnSO_4$ at booting	20.9	27.4	27.3	25.8
LC+ Foliar $ZnSO_4$ at milk	21.3	30.6	29.9	23.4
LC+Foliar $ZnSO_4$ twice	25.7	33.7	22.5	26.0
LC + OMEX- TYPEII at milk	24.7	36.6	28.2	33.3
LC + OMEX- TYPEIII at milk	20.9	36.7	34.2	33.8
LC + Kali- EPSO-Zn at milk	18.6	30.1	25.5	32.0
LC+ ADOB Zn IDHA at milk	19.6	26.1	22.5	26.4
LC+ Valagro Brexil at milk	21.6	28.3	36.0	25. 8
LC + Bayer Anthracol- Zn at milk	17.8	27.6	37.5	27.3
LC+ common fungicide at milk	16.9	23.0	26.2	26.4
LC+ FBScience CP	21.5	30.3	28.5	28.8
LSD (0.05)	3.4	6.4	14.8	5.7
CV %	11.7	12.7	37.1	14.2

4.2 Wheat trials:

4.2.1 Effect of soil applied Zn on wheat seedling establishment:

On the average, MESZ fertilizers produced wheat seedlings of highest vigor at both locations during the 2011/12 growing season. Results for 2012/13 growing season at Chisamba showed highest number of seedlings in plots treated with MESZ II which had 51 seedlings and MESZ I + foliar Zn application which had 49 seedlings. These values were only significantly higher than plots treated with Zn- enriched seed by priming that had only 36 seedlings per meter. The best plant vigor was observed in plots treated with MESZ I + foliar Zn (4.0), MESZ II (3.9) and Kalicorn (3.9). Seed enriched with Zn by foliar spraying or priming had no advantage over control in vigor score (Table 7).

4.2.2 Effect of soil applied Zn fertilizers on wheat grain yield:

In 2011/12 growing season, yields of wheat varied with type of fertilizer applied at planting and good yields were obtained from the plots treated with MESZ, MESZII and high-Zn seed enriched by spraying and priming with Zinc sulphate at Chisamba. At ZARI, better yields were obtained from local control, MESZ fertilizers, Kalikorn, soil $ZnSO_4$ and seed enriched with Zn. The MESZ fertilizers and Zn-enriched seeds consistently improved the wheat yields at both sites (Table 7).

In 2012-2013, despite low vigour test at three weeks after planting, seed enriched by foliar spray and ADOB produced high yields that were statistically similar to MESZ which produced 5.0t ha^{-1} and MESZ II with 4.6 t ha^{-1} (Table 8).

Table 7. Effect of soil applied Zn fertilizers on wheat performance 2011/2012 season.

	CHISAMBA			ZARI		
TREATMENT	**Plants m^{-1}**	**Vigor score**	**Yield (t ha^{-1})**	**Plants m^{-1}**	**Vigor score**	**Yield (t ha^{-1})**
LC	169	3.0	3.45	105	2.8	4.73
LC + Soil Zn	180	2.8	3.50	101	2.3	3.94
LC+ Mosaic - MESZ	169	3.8	4.01	96	3.5	4.03
LC+ Mosaic - MESZ II	188	3.5	4.35	98	3.3	4.57
LC+ Mosaic - MESZ + foliar Zn	166	2.8	3.47	108	2.8	4.65
LC+ Kalikorn	195	2.8	3.12	96	2.3	4.45
2x split+ Urea Zn	144	2.5	3.39	99	3.0	3.70
LC + ADOB	194	2.8	3.18	97	2.3	3.83
LC + Seed Zn-1 foliar	168	2.5	4.00	122	2.5	3.88
LC- Seed Zn-1 priming	141	2.8	3.67	100	3.0	4.51
LSD (0.05)	54.1	0.78	1.01	25.3	0.84	1.04
CV (%)	21.8	18.3	19.3	17.1	20.8	17.0

Vigor score : 1= less vigor , 5 = more vigor

Table 8. Effect of soil applied Zn fertilizers on wheat performance 2012/2013 season.

	CHISAMBA			ZARI
TREATMENT	**Plants m^{-1}**	**Vigor score**	**Yield (t ha^{-1})**	**Yield (t ha^{-1})**
LC	48	3.5	4.5	4.1
LC + Soil Zn	42	3.8	4.1	4.5
LC+ Mosaic - MESZ	47	3.8	5.0	4.1
LC+ Mosaic - MESZ II	51	3.9	4.6	3.2
LC+ Mosaic - MESZ + foliar Zn	49	4.0	4.4	3.4
LC+ Kalikorn	48	3.9	4.3	4.1
2x split+ Urea Zn	41	3.6	4.5	4.7
LC + ADOB	46	3.2	4.8	3.6
LC + Seed Zn-1 foliar	46	3.2	4.8	3.2
LC- Seed Zn-1 priming	36	3.2	4.4	4.0
LSD (0.05)	12.5	0.79	1.1	1.5
CV (%)	19.2	15.2	16.6	27.2

Vigor score : 1= less vigor , 5 = more vigor

4.2.3 Effect of soil applied Zn fertilizers on wheat grain Zinc Concentration

Zinc concentration in wheat grain in 2011/12 season varied according to different treatments. Highest concentration of Zn was obtained in the crop treated with MESZ + foliar Zn, soil applied zinc sulphate and ADOB. These were significantly higher at $p<0.05$ than local control and MESZ I (Table 9). This showed that there was high efficiency in the uptake of Zn from fertilizer by the crop and translocation into grain.

Table 9 Effect of soil applied Zn fertilizers on wheat grain Zinc concentration in 2011/12 season.

	GRAIN Zn ($mg\ kg^{-1}$)
TREATMENT	**CHISAMBA**
LC	26.8
LC + Soil Zn	34.0
LC+ Mosaic - MESZ	25.5
LC+ Mosaic - MESZ II	27.5
LC+ Mosaic - MESZ + foliar Zn	38.5
LC+ Kalikorn	31.5
2x split+ Urea Zn	28.5
LC + ADOB	34.3
LC + Seed Zn-1 foliar	29.0
LC- Seed Zn-1 priming	28.0
LSD (0.05)	5.6
CV (%)	12.7

4.2.4 Effect of foliar Zn fertilizers on wheat grain yield:

There was no significant advantage ($P> 0.05$) of foliar fertilizers over control on the grain yield of wheat at either site during the 2011/12 growing season (Table 10). In 2012/13 season, OMEX Type II produced significantly higher yield (4.8t ha^{-1} of wheat grain ($p< 0.05$) at Chisamba. This yield was 23%, 25% and 35% higher than local control, Kali- EPSO-Zn and FBScience CP, respectively. There was no significant difference in wheat grain yield among foliar fertilizer treatments at ZARI. However, Bayer Antracol- Zn with 4.3 t ha^{-1} and ADOB Zn IDHA with 4.1 ha^{-1} resulted in relatively higher yields than other fertilizers (Table 10).

Table 10. Effect of foliar Zn treatments on wheat performance in 2011/2012 and 2012-2013 seasons.

TREATMENT	YIELD (t ha^{-1})			
	2011-2012		2012-2013	
	CHISAMBA	ZARI	CHISAMBA	ZARI
LC	4.37	3.56	3.7	3.4
LC+ Foliar $ZnSO_4$ at booting	4.30	3.31	4.2	3.3
LC+ Foliar $ZnSO_4$ at milk	3.70	3.33	4.2	3.7
LC+Foliar $ZnSO_4$ twice	4.18	3.27	3.9	3.9
LC + OMEX- TYPEII at milk	3.84	3.24	4.8	3.2
LC + OMEX- TYPEIII at milk	4.20	3.53	4.0	3.5
LC + Kali- EPSO-Zn at milk	4.08	3.46	3.6	4.0
LC+ ADOB Zn IDHA at milk	3.63	3.43	4.4	4.1
LC+ Valagro Brexil at milk	3.91	3.68	4.4	3.9
LC + Bayer Anthracol- Zn at milk	3.98	3.09	4.2	4.3
LC+ common fungicide at milk	4.18	3.52	4.0	4.0
LC+ FBScience CP	4.41	3.57	3.1	3.5
LSD (0.05)	1.13	0.88	1.03	1.2
CV %	19.4	17.8	17.8	21.5

4.2.5. Effect of foliar Zn fertilizers on wheat grain Zinc Concentration.

The analysis of Zn concetration in wheat grain from the crop treated with foliar fertilizers during the 2011/12 season showed that significantly higher grain Zn concentrations were obtained by treatment with OMEX-Type II (63.0 mg kg^{-1}), OMEX-Type III (58.5 mg kg^{-1}), Valagro Brexil (55.0 mg kg^{-1}) and Kali- EPSO-Zn (50.5 mg kg^{-1} as compared to control that had 31.8 mg kg^{-1} at Chisamba. While at ZARI, OMEX-Type II (51.5 mg kg^{-1}, ValagroBrexil (43.7 mg kg^{-1}) and Kali- EPSO-Zn (40.0 mg kg^{-1}) resulted in the highest Zn concentrations with the lowest from local control that had a Zn concentration of 30.5 mg kg^{-1} (Table 11).

These results have therefore indicated that OMEX- Type II, Valagro Brexil and Kali- EPSO-Zn could be recommended for use as foliar fertilizers to improve wheat grain Zn concentration. But, attention should be also given to their impact on yield.

Table11. Effect of foliar Zn fertilizer treatment on wheat grain Zn concentration in 2011/12 season.

Treatments	**GRAIN Zn ($mg\ kg^{-1}$)**	
	CHISAMBA	**ZARI**
LC	31.8	30.5
LC+ Foliar $ZnSO_4$ at booting	32.8	37.3
LC+ Foliar $ZnSO_4$ at milk	38.8	31.0
LC+ Foliar $ZnSO_4$ twice	46.2	35.5
LC + OMEX- Type II at milk	63.0	51.5
LC + OMEX- Type III at milk	58.5	37.5
LC + Kali- EPSO-Zn at milk	50.5	40.0
LC+ ADOB ZnIDHA at milk	48.0	35.8
LC+ Valagro Brexil at milk	55.0	43.7
LC +Bayer Anthracol-Zn at milk	37.3	35.8
LC+ common fungicide at milk	48.3	38.8
LC+ FBScience CP	42.0	36.8
LSD (0.05)	8.4	8.0
CV %	12.6	14.7

5.0 Training and visibility activities:

Field days: In Zambia, people were introduced to zinc trials and Zn demonstration plots through field days. In 2011/12 season, about 5000 people were invited to see the Zn trials and educated on the benefits of Zn content in human beings during the field day held on 16th March, 2012. The 2012/13 growing season Zn trials were visited by people closer to 4,000 during the field day held on the 21st March, 2013. The Minister of Agriculture officiated the opening of the 2013 field day.

Figure 3. Zinc field day at GART on 21st March, 2013

6.1 Field tours:

Groups of students on tour from Copper Belt University, University of Zambia and some agricultural colleges in Zambia such as Natural Resources Development College, Monze

College of Agriculture visited and learnt about the Zn trials. It is envisaged that this knowledge could be disseminated to many farmers upon graduation from education institutions

Professor Cakmak visited the wheat trials and nearby farmers for possibilities to implement consumption of Zn containing food in the month of September, 2012. Farmers in Kalola were educated and benefited from the Zn discussions made during his visit.

6.2 Student attachments:

During the three growing seasons, GART received 40 students on attachment at different times and for varying durations. These students attained practical knowledge on agronomic biofortification of Zn in sorghum crop. They also learnt about the importance of the trials to human nutrition as well as participating in the data collection and analysis of plant establishment (results above) and yield.

6.3 Conferences:

Zambia participated in the 3rd International Zinc Symposium in Hyderabad, India (10-14 October, 2011) where Prof. Obed Lungu presented preliminary results from Zn biofortification trials conducted in Zambia during the 2009/2010 cropping season. Results from Zn trials were prepared and were presented by Professor Cakmak in Rio, Brazil, in February, 2013. The senior agronomist, Mr. Simunji from Zambia participated in the IPNC held in Turkey between 17- 23rd August, 2013. The project under supervision of Sabanci University paid for the flight, accommodation and food.

6.4 Conservation Agriculture Association Meeting:

GART organized Conservation Agriculture Association (CAA) meeting on the 30th May, 2012 at Pamoz Hotel in Zambia. The meeting attracted a good number of stake holders in conservation agriculture. Among these, fertilizer companies such as Green Belt Fertilizers and Omnia, researchers, Non Governmental organizations, Ministry of Agriculture and farmers attended. During this meeting, the on-going research activities on Zn agronomic biofortification were presented by Prof O. Lungu.

7.0 Problems encountered:

The two sites had both serious drought spells that persisted for more than 20 days during 2012/13 season. At Lusitu, the situation was reported to be more severe than at Chisamba due to high temperatures and lack of water. A verification trip to assess the crop damage was made to evaluate the need for replanting the trial at Lusitu. The sorghum trials, affected by drought, were replanted at Lusitu. A similar situation happened also at Chisamba where replanting of sorghum was performed on 3rd January, 2013. During the 2013/14 growing season replanting of sorghum in soil fertilizer experiment was done in Lusitu on the 27th December, 2013.

8.0. Future activities:

1. Monitor, collect and analyze data on sorghum trials for 2013/14 season.
2. Send samples of wheat grains to Sabanci University for analysis of Zn content.
3. Follow up soil baseline analysis with UNZA
4. Plant wheat trials at two sites in May, 2014.
5. Write annual – final report in July, 2014.
6. Expose many people in Zambia to Zn trials and benefits of Zn in human being.