HarvestZinc Project Phase 3
EXECUTIVE SUMMARY

Based on the results obtained in previous two phases, the work plan of the 3rd phase of the HarvestZinc project (www.harvestzinc.org) was organized in the following 4 Tasks:

Task 1: Determine the response of newly developed high Zn lines from HarvestPlus breeding program to soil and foliar spray of Zn and other micronutrients
Task-2: Effect of next generation foliar micronutrient fertilizers (especially Zn and iodine) and cocktail application of micronutrients (Zn, I, Fe and Se) on grain concentrations of micronutrients
Task 3: Understand the differential response of wheat and maize to foliar zinc fertilization
Task-4: Promote and create awareness to facilitate the adoption of the zinc fertilizer strategy at the farmer and policy maker levels

Task 1 experiments aimed to determine the combined effect of genetic and agronomic biofortification efforts on improving concentrations of Zn and other micronutrients in wheat and rice grains. Newly developed high-Zn lines were compared with the best adapted cultivars of the studied countries (India and Pakistan) under different micronutrient fertilizer treatments. Local Treatments, meaning the recommended NP(K) treatments of the countries with no additional treatment, were used as control. The treatments compared with local treatment were soil application of $ZnSO_4$, and foliar applications of $ZnSO_4$, $ZnSO_4$ + KIO_3, and 2 different cocktail solutions containing all 4 micronutrients of Zn, Fe, I, and Se at different rates/forms.

Experiments of Task1 were carried out on rice in India and on wheat in both India and Pakistan. India conducted total 4 experiments at 2 locations in 2 years. On wheat, 6 experiments were conducted in India and 4 in Pakistan.

In rice experiments, local cultivars were superior to high-Zn cultivars in grain yield. On the other hand, all high-Zn cultivars had higher Zn concentrations in their grains than the local cultivars. BR 7840 developed by HarvestPlus had the highest Zn on average of the 4 experiments. The best treatment resulting in the highest grain Zn of local cultivar was foliar Cocktail 1, followed by foliar Cocktail-II, and foliar $ZnSO_4$. In order to compare individual and combined effects of cultivars and treatments, BR 7840 was compared with local cultivar for grain Zn both in control and in the best treatment. These values are given in Table 1.

Table 1: Relative efficiencies of high-Zn cultivars and foliar applications in improving grain Zn concentration of rice.

CULTIVAR	GRAIN Zn (mg kg^{-1})		
	LOCAL TREATMENT	**BEST TREATMENT****	**IMPROVEMENT**
LOCAL CHECK*	21.4	27.6	**+6.2**
BR 7840	30.4	35.7	**+5.3**
IMPROVEMENT	**9.0**	**8.1**	
COMBINED IMROVEMENT		**35.7 – 21.4 = 14.3 mg kg^{-1}**	

*Local cultivar was PR 124 in the first year, PR 121 in the second.
**Foliar Cocktail-I

As can be seen in Table 1, individual contributions of cultivars and treatments to grain Zn concentration of rice are 9.0 and 6.2 mg kg^{-1}, respectively, whereas using high-Zn cultivars and foliar applications together resulted in 14.3 mg kg^{-1} net increase in grain rice. The only problem seems to be the relatively lower yields of high-Zn cultivars than local cultivar in India. Another important indication of Table 1 is the lower effectiveness of treatments on rice as compared to wheat. This confirms the results obtained in previous phases, where it was shown that rice was more responsive to foliar applications than maize but less than wheat (see below).

In wheat experiments, results were quite similar in India and Pakistan. In both countries, foliar cocktail solutions and foliar $ZnSO_4$ resulted in the highest improvements. Efficiencies of cultivars and treatments are shown in Table 2.

Table 2: Relative efficiencies of high-Zn cultivars and foliar applications in improving grain Zn concentration of wheat.

CULTIVAR	GRAIN Zn (mg kg^{-1})		
	LOCAL TREATMENT	BEST TREATMENT**	IMPROVEMENT
INDIA			
LOCAL CHECK*	28.7	46.1	**+17.4**
HPW01	32.5	52.3	**+19.8**
IMPROVEMENT	**3.8**	**6.2**	
COMBINED IMROVEMENT		**52.3 – 28.7 = 23.6 mg kg^{-1}**	

CULTIVAR	GRAIN Zn (mg kg^{-1})		
	LOCAL TREATMENT	BEST TREATMENT*	IMPROVEMENT
PAKISTAN			
LOCAL CHECK*	28.7	49.2	**20.5**
NR 488	33.0	54.3	**21.3**
IMPROVEMENT	**4.3**	**5.1**	
COMBINED IMROVEMENT		**54.3 – 28.7 = 25.6 mg kg^{-1}**	

*Local cultivars were HD 2967 in India, FSD-08 in Pakistan.
** Foliar Cocktail-I

The main difference in wheat trials compared with the rice results was much higher contribution of treatments to grain Zn of wheat than that of rice. Although cultivar effects were lower than in rice, highest level of improvements was obtained by combined effects in both countries (Table 2). **In India, the 2 HP-cultivars having the highest grain Zn were also the highest yielding ones among high-Zn cultivars**, with similar yield with the local check. In Pakistan, although NR 488 had lower yield than the local cultivar, the difference was not as big as in rice.

The effect of cultivars on concentration of other micronutrients in rice grains was variable. Although high-Zn cultivars also had slightly higher Fe and Se concentrations than the local cultivar, the differences were not practically significant. The most striking differences were observed in I concentrations. High-Zn cultivars, not being much different than each other, had 2.4 to 2.5 times as much I in their grains than the local cultivar. Treatments did not affect grain Fe concentrations of rice, but both foliar cocktail solutions improved grain Fe of wheat in both countries, although the improvements were not as big as in grain Zn. Cocktail-II was the best treatment in India resulting in 5.2 mg kg^{-1} increase in grain Fe, and Cocktail-I was the best in that aspect in Pakistan improving grain Fe 7.6 mg kg^{-1}. **Foliar solution applications were highly effective in increasing I and Se concentrations in both rice and wheat.** Cocktail-I was not effective on grain I (due to unexpected chemical reaction between iodine and inorganic Fe in the solution). Cocktail-II and KIO_3 greatly improved grain I, the former being superior both in wheat and rice in both countries. Both cocktails improved grain Se concentrations significantly, Cocktail-II being slightly superior. Cocktail-II application increased grain I concentration from 17 to 192 µg kg in rice, and increased it from 15 to 376, and from 24 to 365 µg kg in wheat, in India and Pakistan experiments, respectively.

Task 2 experiments aimed to study the effects of various micronutrient-containing foliar fertilizers on grain concentrations of selected micronutrients (Zn, Fe, Se and iodine) in rice (Thailand, China, India and Brazil) and wheat (China, India, Pakistan, Turkey and South Africa). In case of wheat, a seed treatment of a Zn-containing compound has been tested on seed germination and seedling development. A total of 27 experiments were conducted on wheat (6 in each of Turkey, India, and South Africa, 5 in Pakistan, and 4 in China). The total number of experiments with rice was 16 (4 in each of China, India, Thailand, and Brazil).

Except for a few cases, treatments did not affect yields of wheat and rice. The foliar Zn fertilizers tested for their impact on grain Zn were highly effective both for rice and wheat. The level of improvement was much higher in wheat than in rice, as it was also shown in Task 1 experiments. The grain Zn concentrations and treatment-based improvements in wheat and rice, as average of the treatments and all experiments conducted with these crops (27 for wheat, 16 for rice) are compared in Table 3.

Table 3: The average effect of all treatments on grain Zn concentrations of wheat and rice.

CROP	GRAIN Zn (mg kg^{-1})		IMPROVEMENT	
	TREATMENT*	LT**	(mg kg^{-1})	%
WHEAT	47.2	28.7	+18.5	64.5
RICE	27.4	22.1	+ 5.3	24.0

*Mean of 6 effective treatments
** Local treatment

There were **substantial increases in grain iodine after spray of iodine** containing solutions, indicating that fertilizer strategy could be an effective solution to the well-documented iodine deficiency problem in human populations globally.

One of the most important findings of these experiments is the great effectiveness of foliar cocktail solutions on improving more than one micronutrient concentration at the same time, with the only exception of Cocktail-I not being effective on grain I. The effect of treatments on grain Fe concentrations were either small or not consistent among experiments. The only exception to this was the effect of foliar cocktail applications on grain Fe of wheat in Turkey, Brazil, and South Africa. As average of these 3 countries, foliar Cocktail-I and Cocktail-II applications increased grain Fe concentration of wheat 8.6 and 5.7 mg kg^{-1}, respectively. Cocktail-I also seemed to be better in rice experiments in Brazil, but except for 1 experiment where it improved grain Fe of rice 6.5 mg kg^{-1}, the differences were smaller and inconsistent between locations.

This superiority of Cocktail-I to Cocktail-II was also seen in grain Zn concentrations of wheat. **On the average of all 27 experiments, the improvements in grain Zn by Cocktail-I and Cocktail-II applications were 21.5 and 17.8 mg kg^{-1}, respectively**. However, in case of I and Se, Cocktail-II was superior. Cocktail-I did not affect grain I concentrations in either crop. Both cocktails improved grain Se in both crops, but Cocktail-II was superior in most cases.

In the **studies comparing maize with wheat** in terms of foliar uptake and soot translocation of Zn it was clear that wheat absorbs and transports Zn much better than maize. This finding is in good agreement with the results showing less response of maize than wheat in terms of increasing grain Zn after foliar spray.

Two joint publications have been published already, and 4 **new manuscripts** are almost ready and will be submitted by the next month (April-2018) for publication in peer-reviewed journals.

CONCLUSIONS AND FUTURE PROSPECTS:

- The results obtained in Task 1 **experiments clearly revealed the great potential benefit of combining genetic and agronomic biofortification efforts**. Although very high improvement in grain Zn seems to have been achieved by genetic biofortification (breeding) through HP activities, it is seen that it is difficult, if not impossible, to improve grain micronutrient concentrations to desired targeted levels (such as extra 20-25 ppm Zn in grain) without the contribution of agronomic biofortification. **Synergism between breeding and fertilizer strategies needs to be used more extensively in future.**

- **The HP-developed high-Zn wheat cultivars both had higher grain Zn and similar (even more yield) compared to the local cultivars**. The grain concentrations of Zn (and iodine and selenium) could be further increased significantly by using fertilizer strategy in those HP developed cereal cultivars.

- On the other hand, the greatest obstacle before adoption of agronomic biofortification lies in the difficulty of getting yield response to foliar applications. The economic analyses performed both in Pakistan and India, within the scope of this project, showed that Zn fertilizations would be economic even in case of very small yield increases particularly in case of foliar applications due to lower cost of very small amounts of fertilizer used. However, it has been achieved only in a few cases during this phase of the project. One of the presumed reasons for that is the too late applications based on previous experiences about greater effectiveness of late applications on grain Zn. However, **for the adoptability of foliar applications, it might be better to make one of the two applications at an earlier stage of growth.** Based on the previous knowledge on macronutrients, the most suitable time for this is the start of stem elongation for wheat where fast growth initiates. Besides, addition of urea (or other common N containing foliar fertilizer) might also be considered for double purpose: Both the possibility of increased penetration and/or translocation provided by urea addition and the direct effect of nitrogen to improve the probability of a yield response and increasing the chance of adoption. **When accepted, Phase 4 of the project will consider one earlier application of foliar fertilizers with and without urea addition.**

- Use of the cocktail solutions revealed a great potential of improving more than one critical micronutrient concentration at the same time. Otherwise it would be not easy to convince farmers to use a different chemical for each micronutrient. **In the next phase of the project, trials will be conducted to apply all micronutrients together with insecticides and/or fungicides.**

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