

HarvestZinc Project

Annual Report: January 1-December 31, 2015

Use of Zinc- and Iodine -Containing Fertilizers for Biofortification of Cereals with Zinc and Iodine and Improving Grain Yield in Different Countries- III. PHASE

1. Introduction

At the FAO/WHO Second International Conference on Nutrition held on 19th-21st November 2014, it was highlighted again that micronutrient deficiencies cause diverse health complications and remain highly prevalent worldwide, affecting over 2 billion people, with children and women at particular risk (<http://www.fao.org/3/a-ml542e.pdf>). It was stressed that micronutrient malnutrition not only impairs people's health, well-being and work performance, but also poses a very significant economic burden, especially on poorer nations. The most common micronutrient deficiencies reported include zinc (Zn), iodine (I), iron (Fe) and selenium (Se) (Gibson, 2006, *Proc. Nutr. Soc.* 65:51-60; Zimmerman and Andersson, 2012, *Curr. Opin. Endoc. Diabet. Obesity*, 19: 382-387; Bhutta et al. 2013, *Brit. Med. Bul.* 106: 7-17). These micronutrients play critical roles in mental and physical development and immune system. Reliance on a high proportion of cereal-based diets represents major reason for micronutrient deficiency-related health problems. Today, increasing micronutrient concentration of food crops represents an important global target and humanitarian challenge. As the scale of micronutrient deficiencies becomes apparent, there is increasing recognition of the need to look beyond yield and focus on the nutritional quality of food production (Pfeiffer et al. 2007, *Crop Sci.* 47:88-105; Miller and Welch, 2013, *Food Policy*, 42: 115-128).

In most developing countries, cereals provide nearly 50 % of the daily calorie intake on average, likely increasing to more than 70 % in the rural regions as reported for Bangladesh, India and many other developing countries. It is estimated that majority of cereal-cultivated soils have low availability of Zn and also other micronutrients, such as Fe, in soils (Graham and Welch, 1996, *HarvestPlus Working Papers No: 3*; Zuo and Zhang, 2009, *Sustain. Agric.* 29: 63-71). Cereals are inherently very low in micronutrient concentrations, particularly when grown on micronutrient-deficient soils. Therefore, it is not surprising that micronutrient deficiencies in human populations are particularly widespread on the parts of the globe where soil micronutrient deficiency has been well-documented; especially in case of developing countries. Raising CO₂ concentrations in atmosphere or cultivation of high-yield cultivars also reduce concentrations of micronutrients in cereal grains, most probably due to dilution-related reasons (Cakmak, 2008, *Plant Soil*, 302: 1-17; Morgounov et al., 2013, *Can J. Plant Sci.* 93:425-433; Myers et al., *Nature*, 510: 139+).

Enhancement in grain micronutrient density by using fertilizer strategy and plant breeding approach is widely accepted cost-effective solution to the problem. As shown for Zn and Se, fertilizer strategy represents a quick and effective solution to the problem (Lyons et al., 2005, *Plant Soil*, 269: 369-380; Curtin et al., 2006, *New Zeal. J. Crop Hort.Sci.* 34:329-339; Cakmak, 2008, *Plant Soil*, 302: 1-17). Fertilizer (e.g., agronomic

biofortification) and plant breeding (e.g., genetic biofortification) approaches are synergistic and complementary in fighting against micronutrient deficiency problem in human populations. HarvestPlus program (www.harvestplus.org) started to release highly promising genotypes with high Zn (up to extra 12 ppm Zn) after long-term and successful efforts in its breeding programs. It would be of great importance to test those newly-developed biofortified genotypes for their response to foliarly applied micronutrients in terms of grain accumulation of micronutrients.

Soil and foliar zinc fertilizer application strategy aims at keeping and maintaining sufficient amount of available Zn in soil solution and in plant tissues to contribute to better root uptake and transport to the seeds of Zn during reproductive growth stage. This approach optimizes and ensures the success of plant breeding programs aiming at improving cereal grains with Zn (*Cakmak, 2008, Plant Soil, 302: 1-17; Cakmak et al., 2010a, Cereal Chem. 87:10-20*). There are several published reports developed under HarvestZinc project which show very positive effects of foliarly sprayed Zn and also Fe fertilizers (together with urea) to wheat and rice on grain Zn and Fe concentrations (*Cakmak et al., 2010b, J. Agric Food Chem. 58:9092–9102, Aciksoz et al., 2011, Plant and Soil, 349:215–225; Zou et al. 2012: Plant and Soil 361:119–130; Phattarakul et al, 2012, Plant and Soil 361:131–141*).

Encouraging results were also obtained with iodine biofortification through application of iodine-enriched fertilizers, mainly in vegetable crops (*Voogt et al, 2010, JSFA, 90: 906-913; Kiferle et al. 2013, Front. Plant Sci. 4: 1-10*), but very little information is available for cereal crops. Insufficient intake is the major reason for the widespread occurrence of iodine deficiency both in developing and well-developed countries, which is estimated to affect about 1.8 billion people in all age groups, including 240 million school children (*Andersson et al. 2012, J. Nutr. 142: 744–750; Pearce et al, 2013, Thyroid, 23: 523-528*). Iodine deficiency is known to decrease child survival, cause goiter and impair growth and development. Children may grow up stunted and with lower IQ. Today, there is an increasing demand for minimizing the extent of iodine deficiency in human populations. Due to infrastructural and/or cultural problems, the impact of salt iodization interventions on reducing iodine deficiency has failed in many places. The global efforts to reduce the daily salt intake also raise concerns for further increases in iodine deficiency in human populations. As demonstrated for Zn, agronomic biofortification approach could be an effective tool in improving iodine content of staple crops and contribute to alleviation of iodine deficiency in humans. This topic represents an important task in the 3rd phase of the project.

During the Phase II of the HarvestZinc project, activities were conducted in seven participating countries. Studies focused on the “mega-crops” wheat and rice and included sorghum and common bean. Wheat experiments were realized in Turkey, China, India, Pakistan and Zambia; rice experiments in China, Thailand and India; common beans studies in Brazil, and sorghum experiments in addition to wheat in Zambia. HarvestZinc project in its 2nd Phase has been supported by:

- **Mosaic Company**, USA
- **K+S KALI GmbH**, Germany
- **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

The project had accomplished its objectives and produced novel results and insights of practical relevance and importance. The results reveal the feasibility of a fertilizer strategy and its vast potential in alleviating Zn deficiency and positively impact on human health. Hence, it has been decided to continue this highly promising fertilizer project due to its great potential to create large short and longer term practical impacts and contribute to and complement the "biofortification concept" in the target countries. The 3rd phase of the project will also include foliar tests with iodine because very little information is available on the role of iodine fertilizer strategy in improving grain iodine concentration in cereal crops such as rice and wheat. In the past activities of the HarvestZinc project, one of the interesting results was the poor response of maize to foliar Zn application. Rice and particularly wheat respond to foliar Zn fertilization very positively and significantly; but the response of maize to Zn fertilization was much less and insignificant compared to rice and wheat. The reason of this differential response will be one of the research topics of the 3rd phase.

Delivery of the project results to farmer's (end-users) is an important component for the success of this project. One of the further major tasks of the 3rd phase of the HarvestZinc project will be promotion and dissemination of the knowledge and experiences gained during the project. An important attention will be paid to the continuation of the "Zinc Days" events and establishment of demonstration trials in the target countries.

Following institutions are the collaborators of this project in the related target countries:

BRAZIL: Universidade Federal de Lavras

SOUTH AFRICA: Stellenbosch University

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

CIMMYT-Mexico will collaborate with HarvestZinc to conduct some tests with iodine and cocktail application of micronutrients.

The 3rd phase of the project that is developed under HarvestPlus program is also supported by the following institutions:

SQM-Chile (www.sqm.com)
Bayer CropScience-Germany (www.cropscience.bayer.com)
ADOB-Poland (www.adob.com.pl)
K+S Kali-Germany (www.kali-gmbh.com)
IFA-France (www.fertilizer.org)
Valagro-Italy (www.valagro.com)
ATP Nutrition-Canada (www.atpnutrition.ca)
ICL-Israel (www.iclfertilizers.com)
Mosaic-USA (www.mosaicco.com)
Aglukon-Germany (www.aglukon.com)
IZA-Belgium (www.zinc.org)
IPNI-USA (www.ipni.net)

2. Scope of Activities: TASKS

To reach the goals of the project, the following tasks will be studied:

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

2.1. Task-I: Determine the response of newly developed high Zn lines from HarvestPlus breeding program to soil and foliar spray of Zn and other micronutrients

Participating Institutions:

INDIA: Punjab Agricultural University (PAU), Ludhiana

PAKISTAN: Pakistan Atomic Energy Commission (PAEC) Islamabad

Task description

After a long-term successful breeding effort and following the identification of high zinc lines and their release, HarvestPlus (HP) initiated the delivery of the first biofortified zinc-rice and zinc-wheat lines having up to 8 ppm (rice) to 12 ppm (wheat) added zinc in the grain. It is assumed that these biofortified genotypes have high genetic capacity to absorb more Zn from the soil and/or transport more Zn from vegetative tissues into seeds compared to currently available cultivars. It is, therefore, important to examine the individual response of these genotypes to soil- and foliar-applied micronutrient-containing fertilizers to capitalize on synergies from agronomic and genetic options. It is expected that the newly identified high-Zn candidate lines for commercialization by HarvestPlus will respond at a higher rate to soil and/or foliar applied micronutrient-containing fertilizers compared to currently cultivated varieties. This research task will build on results from the Phase I and Phase II of the project in developing crop management recommendation tailored for individual genotypes to fully exploit the genetic potential of high zinc varieties.

In this Task, up to 6 most promising newly-developed HP-rice and HP-wheat genotypes will be used both in field (in Pakistan and India) and greenhouse (at Sabanci University) experiments, and the commonly cultivated cultivars in Pakistan and India will be used as control cultivar. The studies planned will examine genotypic response to soil Zn and foliar zinc and iodine fertilizer applications in field and also in the short-term root uptake and zinc translocation experiments under greenhouse conditions. In addition, in order to assess the effect of micronutrient fertilizer treatments on Zn bioavailability of individual genotypes, phytate and the phytate-Zn-molar ratio and Zn and I concentrations will be determined both in whole grains and in milling products.

In the corresponding field experiments, plants will be treated with Zn, iodine and a cocktail of micronutrients over 2 years by using the experiences and new knowledge generated under 2nd phase of the project. Soil Zn application will be realized by applying ZnSO₄.

Annual Report of TASK-I: Determine the response of newly developed high zinc lines from HarvestPlus breeding program to soil and foliar spray of zinc and other micronutrients

Status

This Task involves field experiments in Pakistan with wheat and in India with wheat and rice. The field experiments have been started in India for rice (in June, 2015) and for wheat (in November and December, 2015). Rice nursery has been raised for 4 cultivars in Ludhiana and Gurdaspur, in India, in addition to a local cultivar used as a control cultivar.

Wheat experiments in India have been established by using 6 HP-biofortified wheat genotypes at 3 locations (Ludhiana, Gurdaspur and Bathinda). Corresponding wheat trials in Pakistan have been started by using 6 HP-biofortified wheat genotypes at 2 locations

(Faisalabad and Jhang). Major local cultivars were included in the experiments as control treatments.

Following fertilizer applications are being used to treat the experimental plants:

1. Local Treatment (LT): A common soil application of NP(K) fertilizers in the region where the trials are established.
2. LT and Soil ZnSO₄·7H₂O application (50 kg/ha)
3. LT and 2-times foliar ZnSO₄ applications
4. LT and 2-times foliar ZnSO₄+KIO₃ mixture applications:
5. LT and 2-times foliar applications of a micronutrient cocktail solution -I containing zinc (Zn), iodine (I), selenium (Se) and iron at different rates/forms
6. LT and 2-times foliar applications of a micronutrient cocktail solution-II containing zinc (Zn), iodine (I), selenium (Se) and iron at different rates/forms

Foliar applications are realized twice: i) around heading stage and ii) around early milk stage

In addition to the field trials, greenhouse tests will be conducted at Sabanci University to investigate response of the HP-biofortified genotypes to various soil- and foliar-applied Zn fertilizers in short and long-term experiments.

Outputs

Since wheat experiments have just been planted in both countries, only data on rice grain yields from India have been made available by the time of this report. Corresponding data is processed for statistical analysis. According to the available results, the fertilizer treatments mentioned have not caused statistically significant differences in rice grain yields at either location in India. Grain data for Zn and I are not available at this stage, because the corresponding seed samples will be shipped to Sabanci for analysis after official approvals. The grain yield values and grain analysis data for Zn, iodine and other nutrients will be provided in the next report (in July-2016).

Outcomes/Significance

Not applicable at this stage

2.2. Task-II: Effect of next generation foliar micronutrient and cocktail application of micronutrients (including Zn, Se, Fe and iodine) on grain concentrations of micronutrients

Participating Institutions: All partners

Task description and main milestones:

The second phase of the project generated new results and provided valuable experiences which are highly relevant for maximizing effect of foliarly-sprayed Zn fertilizers on grain Zn. The following factors were identified having a particular influence on grain Zn: i) the form of Zn in the soil applied-NP and -NPK fertilizers (e.g., chelated Zn versus inorganic Zn); ii) pH of the spray solution; iii) inclusion of urea and adjuvant in the spray solution; iv) the form and rate of Zn in foliar-applied fertilizers, and, v) timing and frequency of foliar Zn sprays. At least some of these factors will be considered and harmonized to develop the best agronomic practices of foliar spray to maximize grain Zn and other micronutrients such as iodine in targeted cereal crops.

In recent years, several fertilizer companies increasingly pay attention to the effect of their micronutrient containing fertilizers on improving the nutritional quality of the edible parts of food crops. In the past, the focus was mainly on yield and production economics. By now, next generation fertilizer products have been developed and made available for research and test marketing. Studying these new products for their agronomic effectiveness in increasing grain micronutrient concentration will be an important part of this Task.

Besides Zn deficiency, iodine deficiency (ID) is also widespread in human populations and negatively impacts on health in particular in children and pregnant women. Main effects include impaired growth and mental development, rapid growth of goiter and increases in pregnancy loss. Despite significant achievements in reducing ID incidence in human populations through the use of iodized salt, it is estimated that there are still approximately 1.8 billion people suffering from ID (Andersson et al. 2012, *J. Nutr.* 142: 744–750). Due to infrastructural and/or cultural problems, the impact of salt iodization interventions on reducing ID has failed in many places. As mentioned before, the global efforts to reduce the daily salt intake also raise concerns for further increases in ID in human populations.

In the past, there were several attempts to improve iodine content of food crops by using fertilizer strategy; however, the results obtained vary much and controversial. Most of the iodine fertilization experiments conducted previously have used vegetables, and only few results are available for cereals. In this Task, field experiments will be conducted to examine role of foliarly-sprayed iodine fertilizer in increasing grain iodine. Currently, a separate project has been conducted at the Sabanci University with the support of SQM, IFA and a well-known food nutrition company to study the effect of iodine-containing soil and foliar fertilizers in increasing iodine concentration in wheat. Based on the results from this project, the form and suitable rate of iodine fertilizer have been defined and used in the HarvestZinc project activities.

There is a body of evidence in relation to the effect of selenium (Se) fertilizers on improving grain Se concentrations (*Lyons et al., 2005, Plant Soil, 269: 369-380; Curtin et al., 2006, New Zeal.J Crop Hort.Sci. 34:329-339; Broadley et al. 2010, Plant and Soil, 332:5-18*). However, information on the effect of Se fertilization on grain Se in the target countries of this project such as Pakistan and India is very limited. In the cocktail spray of the micronutrients proposed in this task, Se will be also added in solution to follow Se accumulation in rice and wheat grain. Preliminary foliar tests have been conducted in wheat at Sabanci University to develop a suitable cocktail solution of micronutrients. Based on these preliminary tests,

suitable composition and rate of micronutrients (e.g., Zn, Fe, Se and I) has been developed and being used in field experiments..

Annual Report of TASK-II: Effect of next generation foliar zinc and iodine micronutrient fertilizers and cocktail application of micronutrients (Zn, Se, Fe and I) on grain concentrations of micronutrients

Status

This Task is studying 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 is being tested on seedling development and grain yield.

As conducted before under HarvestZinc project Phase-II, the foliar treatments are being performed twice: i) around heading stage and ii) early milk stage by using following compounds:

- 1) Local Treatment (LT): A commonly-applied basal NP(K) fertilizers without Zn in the given location/region
- 2) LT and Soil ZnSO₄.7H₂O application: (50 kg ZnSO₄.7H₂O per ha)
- 3) LT and Seed Zn treatment with ATP-PreCede (only for wheat experiments)
- 4) LT and 2-times foliar ZnSO₄ applications: (0.5 % ZnSO₄.7H₂O up to 800 litres per hectare (that is equivalent to 4 kg ZnSO₄.7H₂O per hectare).
- 5) LT and 2-times foliar application of ATP Releaf Spray (only for the rice experiments).
- 6) LT and 2-times foliar application of potassium iodate (KIO₃)
- 7) LT and 2-times foliar application of potassium iodate (KIO₃) including 2 % KNO₃
- 8) LT and 2-times foliar application of ADOB-ZnIDHA
- 9) LT and 2-times foliar application of ADOB-Basfoliar
- 10) LT and 2 times foliar application of Kali-EPISO-TOP including Zn and urea
- 11) LT and 2-times foliar application of Valagro52304 solution
- 12) LT and 2 times foliar application of Bayer Antracol-Zn
- 13) LT and 2-times foliar application of a micronutrient cocktail solution-I containing zinc (Zn), iodine (I), selenium (Se) and iron (Fe) at different rates/forms
- 14) LT and 2-times foliar application of a micronutrient cocktail solution-II containing zinc (Zn), iodine (I), selenium (Se) and iron (Fe) at different rates/forms

Current Status in Partner Countries:

China:

Rice experiments:

Two lowland rice experiments were planned to be conducted at Chongqing and Jiangsu provinces. The experiment at Jiangsu has been accomplished and the grain yield results have been obtained. The cultivar used was Hendaol11. However, the other experiment at Chongqing could not be started with all treatments because all foliar products were not available. This delayed experiment will be started in 2016 in Chongqing. The results with grain yield and grain concentrations of the first experiment in Jiangsu will be presented and discussed in the next report (in July-2016). See Annex-Country Reports. For additional details.

Wheat experiments:

The two wheat experiments have been planted in Hebei and Xinjiang provinces in October, 2015. The cultivars used were Liangxing 99 in Hebei, and AK58 in Xinjiang. Further details of the experiments in China are given in the Annex-Country Reports.

India:*Rice experiments:*

The rice field experiments have been conducted during kharif 2015 at the Punjab Agricultural University (PAU), Ludhiana and at the PAU Regional Station, Gurdaspur, and Punjab. The cultivar used at both locations was PR124. The grain yield results are presented in the Annex-Country Reports. Nutrient concentration data will be provided in next term report in July 2016.

Wheat experiments:

Wheat Experiments have been planted in November 2015 at the Ludhiana, Bathinda and Gurdaspur locations, using WH 1105 cultivar at all 3 locations. Data will be collected during the growing season.

Thailand:

The field experiments in Thailand have been conducted on rice at 2 different research locations of the Chiang Mai University. The rice variety "Sanpatong 1" (SPT1) has been used at both locations. Experiments have recently been harvested. Data on grain yield and nutrient concentrations will be provided in next term report in July 2016.

South Africa:

South Africa (Stellenbosch University) conducts only wheat trials. First year's trials were conducted at two locations in the Western Cape Province of South Africa. The first trial was established on the Langgewens experimental farm of the Department of Agriculture, while the second one established on the Roodebloem experimental farm. The cultivar was SST027 at both locations. The trials were planted in May 2015 and harvested in October and November in Langgewens and Roodebloem, respectively. Yield data from these experiments are provided in the Annex-Country Reports. Chemical analysis data will be reported in next term report (in July 2016).

Brazil:

The field experiments on rice with the different soil, seed and foliar treatments will be conducted in collaboration with the Agricultural Research Company of Minas Gerais (EPAMIG) under the leadership of the Department of Soil Science at Universidade Federal de Lavras. The experiments with 13 treatments will be carried out at the Lambari experimental field and at the experimental field of Patos de Minas. Both fields are located in the state of Minas Gerais and belong to the Agricultural Research Company of Minas Gerais Establishment of both experiments are expected to be completed around mid-December, 2015.

Pakistan:

Wheat field experiments, using Faisalabad-2008 cultivar, have been started at three locations, in the districts of Faisalabad, Jhang and Gujranwala in November-2015. Data will be collected during the season.

Turkey:

Wheat experiments have been planted at 2 locations of the Eskisehir province in Central Anatolia using Bezostaya 1 cultivar in November 2015. Transitional Zone Agricultural Research Institute of the Ministry of Agriculture is coordinating the trials. Presently, early growth observations are being performed.

Outputs

Rice experiments:

Of the 4 countries conducting rice experiments, only the 3 in the northern hemisphere (China, India, and Thailand) have harvested their rice experiments yet. Brazil is just at the planting stage. Since Thailand has completed rice harvest recently, grain yield data have been provided only from the experiments in China and India. As explained in the Status section, only 1 of the 2 experiments in China has been established and the second one will be conducted in 2016. Based on the available data the experimental treatments did not result in significant yield differences statistically in any of the 3 experiments in these 2 countries in 2015, although there were some clear differences between the treatments. Since the main objective of this experiment is to improve grain concentrations of the related micronutrients, it will be possible to make assessments on the potential values of the used compounds only after chemical analysis data is available, which is expected by next term report.

China made a preliminary test to compare the effects of 2 different cocktail solutions with control treatment at the location where they could not conduct the full experiment. Some leaf injury has been reported from the use of Cocktail I solution. However, a similar problem has not been reported or observed for the other location in China and other partner countries.

Wheat experiments:

Wheat experiments are being conducted in 5 countries, but since 4 of them (the ones in the northern hemisphere) have just planted wheat in this fall, yield data has been obtained only from the experiment in South Africa. Like in rice, treatments did not significantly affect wheat grain yields at any of the 2 experiments. Early vigor observations, at the beginning of tillering stage, revealed no positive effect of seed enrichment with Zn on emergence counts in any of the 4 wheat experiments, 2 in each of South Africa and China. Observations in the other 3 countries will be provided in next term report in July 2016.

Outcomes/Significance and Publications: Not applicable at this stage

2.3. Task-III: Understand the differential response of wheat and maize to foliar zinc fertilization

Participating Institutions: Sabanci University

Task description

Previous fertilizer trials of this project conducted under field conditions in different countries revealed that wheat is very responsive to foliar Zn application and exhibits large increases in grain Zn (up to 2-fold). Significant increases were also obtained in rice grain-Zn concentrations with foliar Zn spray, but the magnitude of the increases is lower compared to wheat. In contrast to wheat and rice, the reaction of maize to Zn fertilization was variable and insignificant. It is still not clear why maize show poor response to foliar Zn fertilization in terms of increase in grain Zn. This issue will be studied under this Task.

Three aspects will be examined. Research will first study the structure of the leaf surfaces at the cuticular and stomatal level and the number of stomata on the adaxial and abaxial parts of the leaves. Second aspect will deal with the leaf penetration/absorption and transportation

of foliarly applied Zn in young wheat and maize plants. By establishing short-term experiments, leaf absorption of Zn in different pH values and with and without a selected adjuvant in wheat and maize leaves will be monitored. These experiments will clarify whether leaf penetration/absorption of Zn is an important factor in the differential response of wheat and maize to foliar Zn spray.

Since the nitrogen nutritional status of plants (e.g., grain protein) is positively associated with grain Zn accumulation (Cakmak et al., 2010a, Cereal Chem. 87:10-20; Kutman et al., 2010, Cereal. Chem. 87: 1-9; Aciksoz et al., 2011, Plant and Soil, 2011, 349: 215-225), an additional experiment will be designed to study the role of N fertilization on grain Zn accumulation in wheat and maize. It is known that maize has lower grain protein than wheat. Expected results in this task will have significant practical and agronomic implications. All experiments mentioned will be conducted at Sabanci University.

Annual Report of Task-III: Understand the differential response of wheat and maize to foliar zinc fertilization

Status

The first experiments of this Task have been started to investigate i) role of N nutritional status of plants on root Zn uptake and ii) leaf absorption and transportation of Zn (and also iodine) after immersion of leaves in a solution containing Zn or iodine. The tests were conducted by using about 3-weeks-old young seedlings. The experiment focusing on the effect of varied N nutrition has been recently harvested. The analysis of the samples related to leaf immersion experiment has been completed recently and being processed for reporting.

In the leaf immersion experiment, plants were first grown at a marginal Zn (0.5 ppm Zn) and sufficient (2 ppm Zn) Zn supply in soil under greenhouse conditions. When the plants were two weeks old, the first leaves of wheat and maize plants were dipped (immersed) into solutions containing either Zn (0.2 % ZnSO₄.7H₂O + 0.02 % Tween-20) or iodine (0.01 % KIO₃ + 0.02 % Tween-20) for 10-15 seconds and twice a day for four days. Plants were harvested next day after the final leaf treatment. At harvest, two fractions namely Fraction-I (young shoots) and Fraction-II (application leaf and remaining stem) were made. Plants were dried in oven to determine shoot dry matter production Only the Fraction I (the young part of shoot) was analyzed for Zn and iodine to measure the absorption and translocation of Zn and I after the immersion of the first leaves in the Zn- or I-containing solution.

Outputs

The available results show that there is a clear increase in Zn concentration of young parts of maize and especially in wheat plants grown under both marginal and sufficient soil Zn level compared to the control plants without leaf Zn treatment. When compared to maize, wheat plants transported more Zn from the treated leaves into young parts of plants.

However, the results with iodine were different from the results with Zn. There was no clear change in iodine accumulation in young parts of maize plants after leaf treatment of iodine. In contrast to maize, wheat plants were able to absorb iodine and translocate it into younger part of shoots. These very first results look interesting and need to be statistically evaluated. A new similar experiment will be also established to verify the results obtained.

Outcomes/Significance

Not applicable at this stage

2.4. Task-IV: Promote and create awareness to facilitate the adoption of the zinc and iodine fertilizer strategy at the farmer and policy maker levels

Participating Institutions: All partners

Task description:

Delivery and implementation of the project results will continue as an important element of the HarvestZinc project. Several activities will be organized together with the project collaborators in the target countries to achieve a successful dissemination and implementation of the project outputs and to increase public awareness of the project mission. These activities are summarized below:

Organization of the "Zinc Days Events" will continue to introduce and deliver project results and related knowledge to farmers and governmental organizations. "Zinc Days" events will be realized in selected countries and regions together with the project partners. These events will also include presentations on the importance of iodine in human nutrition and health.

Additionally, large-scale farmer-participatory field trials will be established to demonstrate to farmers the benefits of using high Zn-seeds on germination, seedling vigour and final yield. In these on-farm trials, a larger part of farmer's field (e.g. from 1/5 to 1/2 of the field depending on the size of field) will be planted with Zn-enriched seeds. At least 3 farmer fields will be targeted in the partner countries. As the farmers are engaged in monitoring crop development, they will observe and experience the benefit of high Zn seed.

Studies regarding the cost/benefit analysis of agronomic biofortification initiated in the 2nd Phase of the project will be validated in the 3rd Phase of the project. Planned activities will consider country specific circumstances and the lessons learned in the past to evaluate the costs and benefits associated with fertilizer applications and to understand the potential value of the fertilizer concept in biofortification of cereals with Zn. This program will be conducted jointly with the project partners in Pakistan and India.

Brochures/factsheets will be prepared to improve knowledge on importance of zinc and iodine nutrition for human health and crop production among the farmers, agronomists, extension people etc. These brochures will be published in local language. Based on the importance and originality of the results obtained under this project, joint publications will be made and published in international journals together with collaborators.

Annual Report of Task-IV: Promote and create awareness to facilitate the adoption of the zinc and iodine fertilizer strategy at the farmer and policy maker levels

Farmer-participatory trials

An important component of the Task-4 is the establishment of the large-scale farmer-participatory field trials for demonstration of the benefits of using high Zn-seeds on germination, seedling vigor and final yield to the growers. Partners have already designed activities to enrich rice (Brazil India and Thailand) and wheat (China, Turkey, South Africa and Pakistan) under field conditions and to use those seeds (with and without enrichment) in the following cropping season in the farmer fields. Up to 5 farmers in each partner country have been already selected for the proposed trials.

Status

China: Farmer Participatory trials were started with wheat, using the Liangxing99 cultivar, at a total of 6 locations (5 in Hebei and 1 in Xinjiang provinces). The first observations involving emergence and tiller counts are presented in the country report. Presently, plants are at the tillering stage.

India:

Wheat: Five farmers in Bathinda have been selected for these experiments. Plot size for Zn-enriched and untreated seed plantings are about 500 m². Zinc-enriched seeds were obtained by 3 times foliar spraying of previous year's crop at later stages of growth. The Zn concentrations of Zn-enriched and untreated seeds are about 50 mg kg⁻¹ and 24 mg kg⁻¹, respectively.

Rice: Fifteen farmers were selected from Gurdaspur and Tarantaran districts. Three foliar sprayings were applied from flowering to early milk stage. The seed samples will be analyzed to conduct the experiment on five farmers' fields in kharif 2016.

Thailand: Four farmers participated farmer's participatory trials at five different fields (about 600 m² each) within Chiang Mai Province. Seedlings were prepared on their own field with their own selected rice variety. The 2 varieties of rice (SPT1 and RD14) were used at 5 selected farmer's fields. Zinc enrichment was done by foliar application of 0.5% ZnSO₄ 3 times at flowering and early and mid milky stages. Rice samples both with and without zinc enrichment were harvested. Grain yield and zinc concentrations will be evaluated.

South Africa: Five wheat fields in the vicinity of the two sites were identified where the farmer participatory trials are to be carried out. Approximately 80 to 100 m² were separated from the rest of the fields and half of it was treated with ZnSO₄·7H₂O three times as prescribed in the protocol. Seeds were harvested to use in the Farmers Participatory Trials next year.

Brazil: Rice seeds are being planted in two plots of 500 m² each, with and without foliar Zn application at the 2 experimental stations. The purpose of these plots is to obtain Zn-enriched and untreated seeds to be used on farmer field experiments in the next cropping season.

Pakistan: Field demonstrations have been established at 6 locations of districts Faisalabad, Jhang, Gujranwala and Hafizabad to study the effect of low- and high-Zn density seed on seedling emergence and plant population of wheat. Faisalabad-2008 cultivar is being used. Early growth data will be provided in next term report in July 2016.

Turkey: A total of 7 trials, 4 on farmer fields and 3 on fields of varying soil properties at the experiment station, were established to see the relative effects of soil Zn application and Zn-enriched seed on seedling emergence and early vigor as compared to control treatment. The Zn concentrations of Zn-enriched and untreated seeds were 70 and 32 mg kg⁻¹, respectively.

Zinc Days Events

The 3rd Phase of HarvestZinc project aims to organize several "Zinc Days Events" like in the past phases. These events will also include presentations on the importance of iodine in human nutrition and health. It is planned to initiate first Zn days events in the first period of 2016 in Pakistan, Thailand and India.

Cost/Benefit Analysis

Third aspect of this Task is related to the studies regarding the cost/benefit analysis of agronomic biofortification in Pakistan and India. This activity will be realized in collaboration with the project partners in India and Pakistan in order to evaluate the costs and benefits associated with fertilizer applications, and to understand the potential value of the fertilizer concept in biofortification of wheat and rice with Zn. The first information results will be presented in the 2nd Annual Report of the project.

Visibility/Publications

The website of the project has been already updated (www.harvestzinc.org) and related brochures/factsheets will be uploaded on the website in the second year.

Outputs

The farmer participatory trials have just been started, after preparing Zn-enriched seeds in the previous season. Therefore, only data related to early growth observations on wheat from China was available so far. These results from China show some positive effect of seed enrichment with Zn on early growth on some farmer fields. Observations on farmer participatory experiments with wheat in India, Pakistan, and Turkey are going on. South Africa will start next cropping season, probably to start in May, 2016. Seed enrichments for participatory experiments with rice have been performed in China, India, and Thailand. The experiments using these seeds will start in spring 2016, while Brazil is at the stage of obtaining Zn-enriched seeds to be used in farmer participatory trials to be planted in November or December of 2016.

Outcomes/Significance

Not applicable at this stage

ANNEX: COUNTRY REPORTS

COUNTRY REPORT – CHINA

COLLABORATING INSTITUTIONS IN CHINA

NATIONAL COORDINATOR:

Chunqin Zou, China Agricultural University, Beijing

COORDINATING INSTITUTION:

China Agricultural University, Beijing

COLLABORATING INSTITUTIONS:

Wheat experiment:

-Chunqin Zou: College of Resources and Environmental Science, China Agricultural University

-Xiaopeng Gao: Cele National Station of Observation & Research for Desert Grassland Ecosystem

Rice experiment:

-Shiwei Guo: College of Resources and Environmental Sciences, Nanjing Agricultural University

-Yueqiang Zhang: Department of Plant Nutrition, College of Resources and Environment, Southwest University

INFORMATION PROVIDED BY:

Chunqin Zou and Yunfei Du: China Agricultural University

Xiaopeng Gao and Dongwei Gui: Cele National Station of Observation & Research for Desert Grassland Ecosystem

Shiwei Guo and Guangli Tian: Nanjing Agricultural University

Yueqiang Zhang and Xiaojun Shi: Southwest University

INTRODUCTION

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 Zn nutrition is an important issue in China. Regarding the iodine (I) deficiency of human beings, it was a popular topic before 1990s. As reported, about 15% suffered from the lack of iodine. However, it is becoming less critical in recent years in China because China government has recommended the salt fortified with I for all populations by policy since 1996. So, at present, about only 2 million people is suffering from I deficiency.

TASK: Effect of next generation foliar micronutrient fertilizers and cocktail application of micronutrients (Zn, I, Fe and Se) on grain concentrations of micronutrients

EXPERIMENTAL ACTIVITIES

Locations

The two lowland rice experiments were planned to conduct at Chongqing and Jiangsu provinces. However, the field experiment at Chongqing had to be stopped due to late arrival of Zn fertilizer and the test of fertilizer as cocktail-I and -II was conducted instead of all treatments. The two wheat experiments are being conducted in Hebei and Xinjiang province. The locations are marked in the map of China (Figure1)



Figure1: The locations of experiments conducted in 2015 in China. Wheat in Hebei and Xinjiang provinces, rice in Jiangsu and Chongqing provinces.

Basic information of rice experiments

The rice experiments have been conducted in Jiangsu and Chongqing provinces and the real sites are shown in Figure 2.

In Chongqing, the rice trial was established at Ciyun Town (29°3'21.6"N, 106°11'25.2"E), Jiangjin district, although this trial was not further continued due to late arrival of fertilizers. Currently we have chosen a better location for rice trial for next cropping season. The new site is located in Yuntai town, Shangshou district, Chongqing, China. The experimental field has a neutral paddy soil with low DTPA-Zn (around 0.6 mg/kg). In general, this rice trial will be started in next March. In Jiangsu, the field experiments were conducted at Agricultural Sciences Institute of Rugao (120°49' E, 32°37' N) County in Middle Jiangsu, which is shown in the picture above. The soil type is highly sandy loam and the basic physical and chemical properties of soil in 0-20 cm are as follows: 14.49 g kg⁻¹ organic matter, 1.52 g kg⁻¹ total N, 8.40 mg kg⁻¹ available phosphate, 78.40 mg kg⁻¹ available potassium and pH 7.50. The cultivar Zhendao11 was used in our experiment.



Figure 2: Experimental sites of rice trials in Chongqing (left) and Jiangsu (right) in China, 2015.

Basic information of wheat experiments

The two wheat experiments are being conducted in Hebei and Xinjiang provinces. A farmer participatory trial involving comparison of control and Zn-enriched seeds of wheat is also being conducted in these two locations.

In Hebei, the wheat trials were established in Quzhou County (36°35'43"N, 114°50'22.3"E). The cultivar Liangxing 99 was used in the trials of Quzhou. The rate of N, P, K fertilizer is 90 kg N/ha, 120 kg P₂O₅/ha and 60 kg/K₂O. The plot area is 16.2 m² in each replications.

In Xinjiang, the field experiments were conducted at Cele County (37°01'06"N, 80°43'48"E). The Cele station is located in the southern marginal zone of the Taklimakan Desert. The area is characterized by a typical continental arid climate. Long-term normal annual precipitation is only 35.1 mm, mainly distributed between May and July. Long-term average annual air temperature is 11.9°C. The soil at the experimental site is an Aeolian sandy soil. The total N, P and K are 0.58, 0.79, 12.71 g/kg, respectively. The available N determined by alkaline hydrolyze is 28.7 mg/kg. The cultivar AK58 was used in this experiment. The rate of N, P, K fertilizer is 274 kg N/ha, 166 kg P₂O₅/ha and 8.3 kg/K₂O. The plot area is 16 m².

Treatments

The detail information about treatments is listed in Table 1 below.

Table 1: Treatment information for two crops used in wheat experiments in 2015.

TREATMENT CODE	DETAIL OF TREATMENT	NOTES
1	Local treatment (LT) without Zn application	
2	LT and soil ZnSO ₄ ·7H ₂ O application	
3	LT and seed Zn treatment with ATP-PreCede	Wheat only
4	LT and 2-times foliar ZnSO ₄ ·7H ₂ O application	
5	LT and ATP Releaf Spray	Rice only
6	LT and 2-times foliar 0.05% KIO ₃	
7	LT and 2-times foliar 0.05% KIO ₃ and 2% KNO ₃	
8	LT and 2-times ADOB-ZnIDHA foliar application	
9	LT and 2-times ADOB-Basfoliar foliar applications	
10	LT and 2-times Kali-EPISO-Zn together with urea	
11	LT and 2-times Valagro 52304 solution application	
12	LT and 2-times Bayer Antracol-Zn	
13	LT and 2-times foliar cocktail micro spray-I	
14	LT and 2-times foliar cocktail micro spray-II	

RESULTS

Rice Experiments

The experiments in Rugao town, Jiangsu province have been completed. The yield results have been presented in Table 2 and the Zn analysis will be performed soon. Some pictures related to the growth and some activities in field are given in Figure 3.



Figure 3: Some pictures of rice trials in Jiangsu (upper-left, right and lower-left) and Chongqing province (lower-right).

Table 2: Effect of different treatments on grain yield of rice in TASK 2 experiment in Jiangsu province of China, 2015.

	TREATMENT	GRAIN YIELD (t ha⁻¹)
1	Local Treatment (LT)	11.46
2	LT and Soil ZnSO ₄ .7H ₂ O Application	10.82
3	LT and 2-times foliar ZnSO ₄ applications	11.11
4	LT + 2 times ATP releaf spray	11.06
5	LT and 2-times foliar potassium iodate (KIO ₃)	11.62
6	LT + 2-times foliar KIO ₃ + 2 % KNO ₃	10.98
7	LT + 2-times ADOB-ZnIDHA foliar applications	11.15
8	LT + 2-times ADOB-Basfoliar foliar applications	11.57
9	LT + Kali-EPISO-Zn together with urea	11.33
10	LT + Valagro 52304 solution	11.24
11	LT + Bayer Antracol-Zn	11.75
12	LT + Foliar Cocktail micro spray-I (Zn+Fe+I+Se):	11.16
13	LT + Foliar Cocktail micro spray-II (Zn+Fe+I+Se):	11.24
LSD (0.05)		ns

As seen in Table 2, there were no statistically significant differences in yield. Among the treatments, the Antracol treatment was the best treatment in terms of grain yield, although the effect on yield was minimal.

The progress of field rice trial with cocktail solutions at Ciyun in Chongqing

Major field experiments with 13 treatments in Chongqing on rice will be established in the coming cropping season. In this region, a few pre-tests have been conducted to check response of rice plants to 2 micronutrient-cocktail solutions. The two solutions have been sprayed for two times at later afternoon. The dates were 1st: 23th, July (previous to anthesis) and 2nd: 6th, August (early milking stage). As shown in Figure 4, after two times of foliar application, leaf injury occurred to some degree especially with cocktail-I (however, this damage was not seen in other countries).



Figure 4: Leaf injury occurred to some degree especially under cocktail-I. The treatment is CK (upper), cocktail-I (middle) and cocktail-II (lower).

Wheat experiments

Wheat experiments are being conducted in Hebei and Xinjiang provinces. There are only some basic information about the wheat experiments and progress presented.

.As shown in Table 3, there were no significant effects of soil Zn application and PreCede on seedling emergence or tillering in Hebei. Since in Xinjiang location, sowing of seeds of the Precede treatment was made with a few days of delay (due to late arrival of the product), corresponding measurements could not be made.

Table 3: Seedling emergence, plant height and tiller number of winter wheat in Hebei and Xinjiang provinces of China, 2015

Treatment code	Seedling emergence (seedling m ⁻²)		Tillers m ⁻²	
	(Hebei)	(Xinjiang)	(Hebei)	(Xinjiang)
1	464 a	620 a	1120 a	963 a
2	468 a	568 a	1156 a	888 a
3	456 a	Not performed	1200 a	Not performed

TASK-Promote and create awareness to facilitate the adoption of the zinc and iodine fertilizer strategy at the farmer and policy maker levels.

Farmer-Participatory Experiment of wheat

The farmer-participatory experiments with or without Zn-enrichment seed of wheat are being conducted in Hebei and Xinjiang provinces. The cultivar “Liangxing99” was used in both provinces. The Zn-enriched seeds came from Quzhou in 2015. The Zn concentration of the seed will be analyzed later. In these two locations, the five farmers’ fields were selected to conduct the field trials.

First results from the farmer participatory trials are given in Table 4.

Table 4: Seedling emergence and tiller number of winter wheat from the farmer participatory trials conducted in Hebei and Xinjiang provinces of China, 2015.

Farmer	Seedling emergence (seedling m ⁻²) (Hebei)		Seedling emergence (seedling m ⁻²) (Xinjiang)		Tillers m ⁻² (Hebei)	
	Zn-enriched seed	Control seed	Zn-enriched seed	Control seed	Zn-enriched seed	Control seed
1	460±48	412±10	443±41	400±42	684±62	752±98
2	448±45	416±34			844±176	740±34
3	1140±104	1340±134			1580±121	1672±62
4	456±8	432±16			644±76	620±77
5	412±48	416±25			780±48	728±93

*No tillering yet at Xinjiang due to late-planting.

The results showed that Zn-enriched seed seemed to be beneficial to improve seedlings emergence and tillering in some farmer fields in Hebei and in Xinjiang. Because of the special weather in Hebei (very early snowing), the plant height of wheat was not recorded and will be measured after green-recovering next year. Tiller number in Xinjiang was not

recorded as the farmer's experiment was planted late. Tillering will occur and be recorded in early spring, 2016.

Training and visibility activities

At present, there are no training and visibility activities related to this project.

Proposed activities in the next six months

Activities planned for the next six months include:

- (1) Zn analysis of rice leaf and grain samples from Jiangsu province;
- (2) Analysis of basic characteristics (pH, DTPA-Zn, I) of experimental field soil from Xinjiang and Hebei;
- (3) Zn analysis of Zn-enriched seed of wheat used in the farmer-participatory trials at two locations;
- (4) Analysis of emergence and height of seedlings of all field trials with winter wheat;
- (5) Collection of leaf samples before the first foliar application of Zn;
- (6) Management of field trials, including irrigation, pest control, foliar application of Zn, collections of leaf samples and harvest at maturity.
- (7) Rice trials with all Zn and I fertilizers in Chongqing will be started in next March and thus fertilizer shipping is expected soon.

COUNTRY REPORT – INDIA

COLLABORATING INSTITUTIONS IN INDIA

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INTRODUCTION

Zinc malnutrition has become a major health problem in areas where rice and wheat is consumed predominantly as food crops. Singh (2010) reported wide spread low level of zinc in seeds and feeds which is affecting a large segment of resource poor families whose food comes mainly from cereals in India. These cereals are mainly grown on 49% of Indian soils which are having zinc deficiency. Kapil and Jain (2011) reported 43.8% prevalence of zinc deficiency in Uttar Pradesh (Northern region), Karnataka (Southern region), Orissa (Eastern region), Gujarat (Western region) and Madhya Pradesh (Central region) in India. It is known that about 33% of the world population is at the risk of zinc malnutrition due to inadequate dietary intake of zinc (Cakmak 2009). Vitamin A deficiency was reported to be responsible for the maximum number of deaths followed by Zn and Fe deficiency (Black et al. 2008). Harvesting of 6.5 t grain/ha/yr removes 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 biofortification of wheat and rice offers a promising strategy to address micronutrient deficiency in the diet (Ram et al. 2015). There is a scope for zinc enrichment in cereal grains which will be beneficial in reducing zinc malnutrition in India.

In India, the considerable population is prone to iodine deficiency disorders (IDD) due to iodine deficient soil of the subcontinent and consequently the food growing on it (Pandey et al. 2013). To tackle the problem of IDD, common salt is fortified with iodine. It was further reported that about 350 million people do not consume adequate iodized salt and, therefore, they are at the risk of IDD. The people having high blood pressure problem are also consuming less of the common salt. About 263 districts out of 325 surveyed in India are IDD-endemic. Currently, household level iodized salt coverage in India is 91% out of which 71% households consuming adequate iodized salt. IDD can be prevented by providing iodized salt in Public Distribution System and strengthening the monitoring and evaluation of IDD programme. Similarly iodine enriched cereal grain is also one of the options for curing IDD in India. Keeping in view the importance and deficiency of Zn and iodine in human populations, several field experiments were planned to enrich cereal grain with Zn and iodine in India under the HarvestZinc project.

EXPERIMENTAL ACTIVITIES

The field experiments of phase III were initiated during *kharif* 2015 at PAU, Ludhiana and PAU Regional Station, Gurdaspur, Punjab (India) on rice crop. In addition to these two sites during *rabi* 2015-16, experiments on wheat were also conducted at PAU Regional Station Bathinda, Punjab, India. The locations for the PHASE III experiments have been given on the country map below (Figure 1).

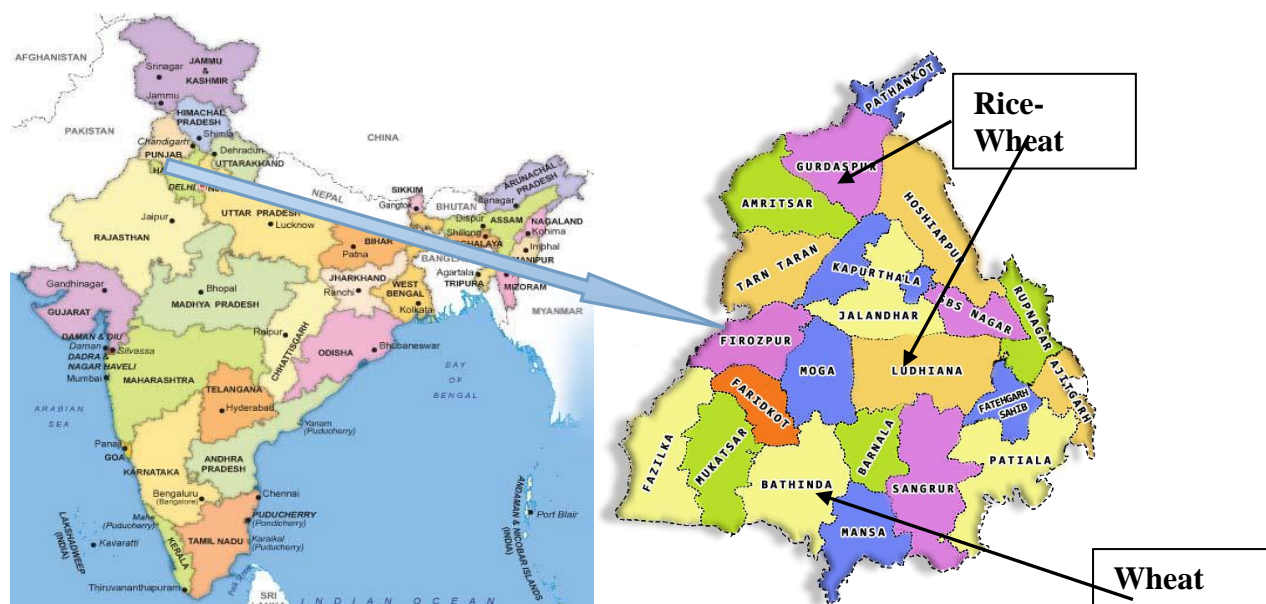


Figure 1: The experimental locations in India for HarvestZinc rice and wheat experiments during 2015-16.

The soil was slightly alkaline with 8.0 pH at Ludhiana, 7.8 at Gurdaspur and 8.3 at Bathinda. The soils at Ludhiana and Bathinda were loamy sand in texture, whereas at Gurdaspur it was sandy loam soil. The soil status in respect of micronutrients has been presented in Table 1. Soil at all the locations was low in DTPA Zn.

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

LOCATION	SOIL MICRONUTRIENT CONCENTRATION (mg kg ⁻¹)			
	Zn	Cu	Fe	Mn
Ludhiana	0.51	1.25	10.2	9.5
Gurdaspur	0.59	2.87	21.1	10.3
Bathinda	0.42	0.41	6.4	2.3

TASK: Determine the response of newly developed high Zn lines from HarvestPlus breeding program to soil and foliar spray of Zn and other micronutrients

The field experiments were conducted at the Punjab Agricultural University (PAU), Ludhiana campus and its Regional Station campus Gurdaspur in *kharif* 2015. In addition to these two locations, wheat experiment in *rabi* 2015-16 have been laid out at one more additional location of PAU Regional Station, Bathinda. The soils of the fields at both the locations were low in nitrogen, medium in P and high in K. The soil pH was slightly alkaline with 8.0 pH at Ludhiana and 7.8 at Gurdaspur. The soil texture at Ludhiana and Bathinda was loamy sand, whereas at Gurdaspur it was sandy loam soil. The soils used in the trials at all the locations were low in DTPA Zn (0.51 mg/kg at Ludhiana, 0.59 mg/kg at Gurdaspur and 0.42 mg/kg at Bathinda).

The rice experiment was laid out at Ludhiana and Gurdaspur locations including following six treatments:

1. Local Treatment (LT): A common soil application of NP(K) fertilizers in the region where the trials are established.
2. LT and Soil ZnSO₄.7H₂O application (50 kg/ha)
3. LT and 2-times foliar ZnSO₄ applications
4. LT and 2-times foliar ZnSO₄+KIO₃ mixture applications:
5. LT and 2-times foliar applications of a micronutrient cocktail solution -I containing zinc (Zn), iodine (I), selenium (Se) and iron at different rates/forms
6. LT and 2-times foliar applications of a micronutrient cocktail solution-II containing zinc (Zn), iodine (I), selenium (Se) and iron at different rates/forms

Following 5-rice varieties were used: IET 23824CGZR-1, IET 23832 IVIHP-5, IET 23829 RRHZ-7, VR 7840 and PR 124 (as Local variety). The experiments were laid out in split plot design with four replications at both locations.

The rice nursery was planted on 22 June, 2015 at Ludhiana and Gurdaspur using 20 kg seed per hectare. About 4 weeks old seedlings was transplanted in the field with geometry of 15 cm × 15 cm on 20 July, 2015 at Ludhiana and on 22.07, 2015 at Gurdaspur. The fertilizers used were 150 kg N, 40 Kg P₂O₅ per hectare at both locations. P fertilizer was applied at the time of sowing and nitrogen was applied in three split at both locations as per recommended schedule of its application (Table 3.) In zinc sulphate treatment, ZnSO₄.7H₂O @ 50 kg/ha was applied at the time of sowing. Two foliar applications of chlorpyrifos 20 EC @ 2.5 liters/ha were realized to control the stem borer and leaf folder damage on the crop at both locations. The irrigation water was allowed to stagnate for the first two weeks for the proper establishment of the crop. Later on, irrigation was applied on next day of the stagnant water leached down.

The foliar applications were made at heading and at early milk stage as per treatments at Ludhiana and Gurdaspur (Table 2 and Figure 2). The rice crop was harvested on 30.10.2015 at Ludhiana and on 05.11.2015 at Gurdaspur. The grain samples for zinc and iodine will be analyzed in January, 2016 using the ICP-OES and ICP-MS, respectively.

In the wheat experiment laid out in *rabi* 2015-16, the experiment was comprised of same six treatments mentioned above. Following six wheat varieties were used HP 1501, HP 1502, HP 1503, HP 1504, HP 1505 and HP 1506. The experiments were laid out in split plot design with four replications at all three locations (Ludhiana, Gurdaspur and Bathinda).



Figure 2: Activity in rice 2015 season Task1 experiment at Ludhiana.

Table 2: Activity of rice experiments at Ludhiana and Gurdaspur locations.

SR NO.	PARTICULARS	LUDHIANA			GURDASPUR	
1.	Variety	As per treatments			As per treatments	
2.	Nursery raising	22.06.15			22.06.15	
3.	Transplanting	20.07.15			22.07.15	
4.	Fertilizer application (dose) N in three splits	(150 kg Nha ⁻¹ + 40 kg P ₂ O ₅ ha ⁻¹) 20.07.15+10.08.15+01.09.15			(150 kg Nha ⁻¹ + 40 kg P ₂ O ₅ ha ⁻¹) 22.07.15+12.08.15+4.08.15	
5.	Treatment application*	Variety	1 st foliar	2 nd foliar	1 st foliar	2 nd foliar
		IET 23824CGZR-1	08.09.15	17.09.15	17.09.15	28.09.15
		IET 23832 IVIHP-5	24.09.15	06.10.15	30.09.15	11.10.15
		IET 23829 RRHZ-7	29.09.15	10.10.15	02.10.15	13.10.15
		VR 7840	24.09.15	06.10.15	30.09.15	10.10.15
	PR 124	18.09.15	04.10.15	22.09.15	08.10.15	
6.	Chlorpyrifos		30.08.15	10.10.15	15.09.15	15.10.15
7.	Harvesting	30.10.2015			05.11.2015	

* LT and 2-times foliar ZnSO₄ applications, LT and 2-times foliar ZnSO₄+Iodine mixture applications, LT +Foliar Cocktail micro spray-I and LT +Foliar Cocktail micro spray-II

The wheat crop using varieties as per treatments were sown on 9.11.2015, 19.11.2015 and 04.12.2015 at the locations Ludhiana, Gurdaspur and Bathinda, respectively (Table 3). Whole of the P and ½ of N fertilizer were applied at the time of sowing and ½ N will be applied at the time of first irrigation.

Table 3: Activities of the wheat experiments at three locations.

SR NO.	PARTICULARS	LUDHIANA	GURDASPUR	BATHINDA
1.	Variety	As per treatments	As per treatments	As per treatments
2.	Sowing date	09.11.2015	19.11.2015	04.12.2015

RESULTS

Rice: Ludhiana

The treatments of zinc/iodine could not influence the rice grain yield at Ludhiana (Table 4). The highest grain yield (6.23 t/ha) was recorded in soil zinc application which was followed by the control treatment. The minimum grain yield (6.07 t/ha) was recorded in LT +Foliar Cocktail micro spray-II: (2×CT-II foliar) treatment. We could not find any toxic effect of any of the treatments at this site. Among the genotypes, the highest grain yield of 7.26 t/ha was recorded in the local variety PR 124 which was statistically at par with IET23832, but was significantly superior than all other genotypes (IET23824, IET23829 and BR7840). The minimum grain yield of 5.22 t/ha was recorded in genotype BR7840 which was only statistically at par with IET23829 (5.23t /ha), but significantly inferior to all other genotypes.

None of the new genotypes could surpass the grain yield obtained in local check variety i.e. PR 124 at Ludhiana. Interaction of zinc/iodine treatments was found to be non-significant.

Table 4: Effect of treatments on grain yield of HarvestPlus-cultivars and local cultivar PR124 of rice at Ludhiana in 2015. CT: cocktail

GENOTYPE	GRAIN YIELD (t ha ⁻¹)						MEAN
	Local control	Soil Zinc	2×Zn Foliar)	2×Zn/I Foliar	2×CT-I Foliar	2×CT-II Foliar	
IET23824	6.25	5.98	6.13	6.19	6.07	6.01	6.11
IET23832	7.10	7.22	6.90	6.68	6.85	7.02	6.96
IET23829	5.21	5.39	5.13	5.21	5.37	5.09	5.23
BR7840	5.21	5.36	5.46	4.85	5.21	5.22	5.22
PR 124	7.23	7.23	7.15	7.78	7.15	7.03	7.26
MEAN	6.20	6.23	6.16	6.14	6.13	6.07	

LSD (0.05) Zinc/Iodine treatments : ns; Cultivars: 3.18; Interaction: ns

Rice: Gurdaspur

The treatments of zinc/iodine could not influence the grain yield of rice at Gurdaspur site, either. (Table 5). The highest grain yield of 6.76 t/ha was recorded in LT +Foliar Cocktail micro spray-II: (2×CT-II foliar) treatment which was followed by the control treatment. The minimum grain yield (6.62t/ha) was recorded in LT +Foliar Cocktail micro spray-I: (2×CT-I foliar) treatment. We could not find any phyto-toxic effect of any of the treatment at Gurdaspur. In the genotypes, the highest grain yield of 7.79 t/ha was recorded again in the local variety PR 124 which was significantly superior than all other zinc biofortified genotypes (IET23824, IET23832, IET23829 and BR7840). The minimum grain yield of 5.49 t/ha was recorded in genotype IET23829 which was significantly inferior to all other genotypes. The new genotypes IET23824 and IET23832 were statistically at par with each other but are significantly superior to the IET23829 and BR 7840 genotypes. The new genotypes IET23824 and IET23832 were not able to surpass the grain yield of the local variety PR 124 at Gurdaspur also. Interaction effects of zinc/iodine treatments were found to be non-significant.

Table 5: Effect of treatments on grain yield of cultivars of rice at Gurdaspur in 2015. CT: Cocktail

GENOTYPE	GRAIN YIELD (t ha ⁻¹)						MEAN
	Local control	Soil Zinc	2×Zn Foliar)	2×Zn/I Foliar	2×CT-I Foliar	2×CT-II Foliar	
IET23824	7.07	7.05	6.93	7.02	6.85	6.99	6.99
IET23832	7.03	7.06	6.90	6.83	6.86	7.06	6.96
IET23829	5.55	5.36	5.62	5.42	5.30	5.69	5.49
BR7840	6.14	6.12	6.11	6.22	6.20	6.19	6.16
PR 124	7.69	7.77	7.78	7.80	7.88	7.85	7.79
MEAN	6.70	6.67	6.67	6.65	6.62	6.76	

LSD (0.05) Zinc/Iodine treatments : ns; Cultivars: 2.93; Interaction: ns

Wheat:

The wheat crop has been sown as per treatments at three locations in November-2015. The data will be collected in the season.

TASK: Effect of next generation foliar micronutrient fertilizers and cocktail application of micronutrients (Zn, I, Fe and Se) on grain concentrations of micronutrients

The field experiments were conducted at the Punjab Agricultural University (PAU), Ludhiana campus and at its Regional Station campus Gurdaspur in *kharif* 2015. In addition to these two locations, wheat experiment in *rabi* 2015-16, have been laid out at one more additional location of PAU Regional Station, Bathinda. The soils of the fields at both locations were low in nitrogen, medium in P and high in K. The soil pH was slightly alkaline with 8.0 pH at Ludhiana and 7.8 at Gurdaspur. Experimental soils at all the locations were low in DTPA Zn (below 0.6 ppm Zn).

The experiment was laid out with treatments 1. Local treatment (LT) with application of NPK fertilizers without zinc (Zn) 2. LT + soil ZnSO₄.7H₂O application: (50 kg/ha ZnSO₄.7H₂O), 3. LT (for rice 2015 no PreCede treatment) and Seed Zn Treatment with PreCede (for wheat in *rabi* 2015-16 season), 4. LT and 2-times foliar ZnSO₄ applications @ 0.5 % ZnSO₄.7H₂O in 800 litres per hectare (that is equivalent to 4 kg ZnSO₄.7H₂O per hectare) at heading and at early milk stage, 5. LT and ATP Releaf spray (this treatment is only for rice experiments). 6. LT and 2-times foliar potassium iodate (KIO₃) treatments at heading and at early milk stage 7. LT+ 2-times foliar potassium iodate (KIO₃) treatments together with 2 % KNO₃ at heading stage and at flowering, 8. LT + 2-times ADOB-ZnIDHA foliar applications at heading and at early milk stage, 9. LT + 2-times ADOB-Basfoliar foliar applications at heading and at early milk stage, 10. LT + Kali-EPSo-Zn together with urea twice at the heading and at the early milk stage. , 11. LT + Valagro solution at heading and at early milk stage, 12. LT + Bayer Antracol-Zn by using three kg Antracol per ha in 800 litres sprayed twice at the heading and at the early milk stage 13. LT + foliar cocktail micro spray-I at heading and at early milk stage and 14. LT + Foliar Cocktail micro spray-II at heading and at early milk stage having different concentration of Zn. These experiments were conducted in randomized complete block (RCBD) with four replications at all the three locations

The rice nursery using a recently released variety PR 124 was raised on 22 May 2015 at Ludhiana at Gurdaspur using 20 kg seed per hectare. About 4 weeks old seedlings was transplanted in the field with geometry of 15 cm × 15 cm on 29 June 2015 at Ludhiana and on 07.07.2015 at Gurdaspur. The fertilizers used were 150 kg N, 40 Kg P₂O₅ per hectare at both the locations. P fertilizer was applied at the time of sowing and nitrogen was applied in three split at both locations as per recommended schedule of its application (Table 6.). In Zinc sulphate treatment, ZnSO₄.7H₂O @ 50 kg/ha was applied at the time of sowing. Two foliar applications of chlorpyrifos 20 EC @ 2.5 liters/ha were made to control the stem borer and leaf folder damage on the crop at both locations. The irrigation water was allowed to stagnate for the first two weeks for the proper establishment of the crop. Later on irrigation was applied on next day of the stagnant water leached down. The foliar applications were made at heading and at early milk stage as per treatments (Table 6). The rice crop was harvested on 07.10.2015 at Ludhiana and on 15.10.2015 at Gurdaspur. The samples from the field were collected manually. The net plot was harvested manually. After drying the grain samples in the sun, the grain yield was presented as t/ha. The grain samples for zinc and iodine will be analyzed in January, 2016 using ICP-OES and ICP-MS, respectively.

The wheat crop using variety WH 1105, a new released variety of PAU with high resistance to rust, was sown on 9.11.2015, 18.11.2015 and 20.11.2015 at Ludhiana, Gurdaspur and

Bathinda, respectively. Whole of the P and $\frac{1}{2}$ of N fertilizer were applied at the time of sowing and $\frac{1}{2}$ N will be applied at the time of first irrigation.

Table 6: Activity chart of the wheat and rice experiments at three locations.

SR NO.	PARTICULARS	LUDHIANA		GURDASPUR
RICE 2015				
1.	Variety	PR 124		PR 124
2.	Nursery raising	22.05.15		22.05.15
3.	Transplanting	29.06.15		07.07.15
4.	Fertilizer application (dose) N in three splits	(150 kg N ha ⁻¹ +40 kg P ₂ O ₅ ha ⁻¹) 20.06.15 + 15.07.15 + 10.08.15		(150 kg N ha ⁻¹ + 40 kg P ₂ O ₅ ha ⁻¹) 07.07.15 + 24.07.15 + 17.08.15
5.	Treatment application	27.08.15 and 12.09.15		05.09.15 and 17.09.15
6.	Chlorpyriphos	25.07.2015 and 30.08.2015		30.07.2015 and 06.09.2015
7.	Harvesting	7.10.2015		15.10.2015
WHEAT 2015-16				
		LUDHIANA	GURDASPUR	BATHINDA
1.	Variety	WH 1105	WH 1105	WH 1105
2.	Sowing date	09.11.2015	18.11.2015	20.11.2015

RESULTS

Rice:

None of the treatments at Ludhiana and Gurdaspur locations could influence the grain yield of rice significantly (Table 7). The rice grain yield ranged from 8.04 to 9.40 t/ha at Ludhiana, where the highest grain yield of rice recorded from LT+ Kali ESPO Zn used together with Urea which was followed by LT+ 2 times ADOB- Basfoliar. However, the highest grain yield recorded in Gurdaspur was in LT + Antracol-Zn which was followed by LT+ 2 times ADOB-Zn IDHA. The range of rice grain yield recorded at Gurdaspur was 7.92 to 8.11 t/ha.

Wheat:

The wheat crop has been sown as per treatments at three locations in November, 2015. The data will be collected in the season.

Table 7: Effect of different treatments on grain yield of rice in TASK 2 experiments at Ludhiana and Gurdaspur in 2015.

TREATMENT		GRAIN YIELD (t ha ⁻¹)	
		Ludhiana	Gurdaspur
1	Local Treatment (LT)	8.08	8.03
2	LT and Soil ZnSO ₄ .7H ₂ O Application	8.48	8.00
3	Local Treatment (LT)*	8.04	8.08
4	LT and 2-times foliar ZnSO ₄ applications	8.36	8.05
5	LT + 2 times ATP releaf spray	8.51	8.08
6	LT and 2-times foliar potassium iodate (KIO ₃)	7.84	7.92
7	LT + 2-times foliar KIO ₃ + 2 % KNO ₃	8.70	8.05
8	LT + 2-times foliar ADOB-ZnIDHA	8.46	8.11
9	LT + 2-times foliar ADOB-Basfoliar	9.39	8.00
10	LT + Kali-EPZO-Zn together with urea	9.40	7.98
11	LT + Valagro 52304 solution	8.80	7.96
12	LT + Bayer Antracol-Zn	9.26	8.22
13	LT + Foliar Cocktail micro spray-I	8.85	7.98
14	LT + Foliar Cocktail micro spray-II	8.31	7.98
LSD (0.05)		ns	ns
CV (%)		6.6	6.8

*ATP-Precede treatment was not applied in rice trials in 2015.

TASK: Promote and create awareness to facilitate the adoption of the zinc and iodine fertilizer strategy at the farmer and policy maker levels.

Rice: Fifteen farmers were selected from Gurdaspur and Tarantaran districts. Three foliar sprayed were applied from flowering to early milk stage. The seed samples will be analysed to conduct the experiment on five farmers' fields in *kharif* 2016.

Wheat: In *rabi* season 2015-16, five farmers in Bathinda were selected to conduct this experiment. Their wheat crop was sprayed 3-times in the last wheat crop season (*rabi* 2014-15) and seeds were obtained having 48-51 mg/kg Zn. The same seed was used to conduct this experiment in *rabi* 2015-16. The seeds without Zn enrichment had Zn around 24 mg/kg. The sizes of field selected for the trials were about 500 m².

RESULTS

No significant effect was found on the emergence count of the wheat crop in the current season 2015-16 (Table 8). The grain yield and zinc content analysis will be done later in the season.

Table 8: Effect of zinc enriched seed on the emergence of wheat crop at farmers' fields.

FARMER FIELDS	EMERGENCE COUNT (plants m ⁻²)		
	Normal seed (23-25 ppm)	Zinc enriched seed (48-51 ppm)	LSD (0.05)
Sukhpal Singh s/o Lachhman Singh	170	172	ns
Sujt Singh s/o Kapoor Singh	174	174	ns
Amarjit Singh s/o Kapoor Singh	180	178	ns
Lachhman Singh s/o Jhanda Singh	172	176	ns
Jagsir Singh S/o Darshan Singh	180	178	ns
Mean	175.2	175.6	
LSD (0.05)	ns		

TRAINING AND VISIBILITY ACTIVITIES

- A) One Zinc day event is planned at the farmers' field in wheat season.
B) Teaching material will be developed from the project.

PROPOSED ACTIVITIES IN THE NEXT SIX MONTHS

- i) The data on growth and yield characters of wheat experiments conducted at three locations (Ludhiana, Gurdaspur and Bathinda) will be taken.
ii) Wheat experiments will be harvested and samples will be collected for analysis.
iii) Sowing the nursery of rice and their transplanting will be planned for the next rice season 2016 at two locations (Ludhiana and Gurdaspur).
iv) We will collect the zinc enriched wheat seeds at the farmers' field for next season planning of experiment. We will also collect the feedback from the farmers for their overall experience with zinc bio-fortified seeds.

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COUNTRY REPORT – THAILAND

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INTRODUCTION

Zinc (Zn) is an essential mineral nutrient for human beings. It is estimated that 1/3 of the world population is affected by Zn deficiency that is associated with low dietary intake. Zinc deficiency is known to have serious adverse impacts on human health, especially in children, such as impairments in physical growth, immune system, and learning ability, and causing DNA damage and cancer development (Keen and Gershwin 1990; Ho et al. 2003; Black et al. 2008). In Thailand, there is no data on Zn deficiency among the whole country of population, but 34% of North East Thai school aged children are found to be marginally zinc deficient (Udomkesmalee et al., 1990). It has been reported that the quality of children's diets may be particularly poor in the North East region of Thailand where the per capita income and education level are among the lowest in the country (Department of Health, 1995). Moreover, dietary surveys have reported low intakes of zinc during childhood in this region (Bloem et al., 1989; Udomkesmalee et al., 1990; Egger et al., 1991).

In addition to Zn deficiency among the population in Thailand, recent studies have reported on iodine deficiency disorders (IDDs) are also a major public health problem in this region (WHO/UNICEF/ICCIDD, 2001), despite the introduction by the government of Thailand of several strategies to combat iodine deficiency (Wanaratana, 1992; Department of Health, 1998). Nevertheless, few of the children (i.e. 8%) had levels indicative of severe iodine deficiency (WHO/UNICEF/ICCIDD, 2001). Indeed the goiter rate was similar to that reported for children in North East Thailand by the 1995 Thai National Nutrition Survey (Department of Health, 1995). However, even mild to moderate iodine deficiency can have detrimental effects in children, causing impairments in both neuropsychological and physical development (van den Briel et al., 2000; WHO/ UNICEF/ICCIDD, 2001).

High consumption of cereal-based foods with low levels and poor bioavailability of Zn is thought to be a major factor for the widespread occurrence of Zn deficiency in human, including Thailand (Welch, 2004). In most cases, it has indicated that rice cultivated soils are very low in plant-available Zn leading to further decreases in grain Zn concentration in the staple food (Alloway 2004; Cakmak 2008). Therefore, Zn deficiency in crop plants reduces both grain yield and the nutritional quality of grains which consequently affects the consumer's health. Previous studies have reported that about 30% of the cultivated soils of the world are Zn deficient and about 50% of the soils used for cereal crop production have low levels of Zn available for plants (Sillanpaa 1982; Graham et al. 1992). Zinc deficiency is one of the most common trace element deficiencies of soils in Thailand which seriously affects adequate crop production and also hinders sufficient accumulation of Zn in edible parts of food crops. Soil Zn deficiency in Thailand is usually observed in the calcareous soil series in all regions of the country such as in Chai Badan and Lop Buri soil series (Takrattanasaran et al., 2013). A wide range of soil Zn concentration (0.5-2.1 mg Zn kg⁻¹) was reported in Thailand (Phattarakul et al., 2012) which, in some areas, reach the critical Zn

deficiency limit since the critical level of DTPA extractable soil Zn for rice is 0.5–0.8 mg Zn kg⁻¹ (Randhawa et al. 1978; Sims and Johnson 1991). On the other hand, the information on soil iodine concentration in Thailand is very limited.

TASK: Effect of next generation foliar micronutrient fertilizers and cocktail application of micronutrients (Zn, I, Fe and Se) on grain concentrations of micronutrients

EXPERIMENTAL ACTIVITIES

Experiment location

The field experiments with 13 treatments were conducted at 2 different locations in different campuses of Chiang Mai University, Thailand (Figure 1) (about 10 kms. between them).

Location 1: Research demonstration field at Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University; **Location 2:** Research demonstration field at Center for Agricultural System Research, Chiang Mai University.

Plant culture

Rice variety “Sanpatong 1” (SPT1) was planted at both locations. Seedlings were prepared by soaking seeds in water overnight and incubated moist until germinated and grown for 30 days (26 June 2015–28 July 2015). The prepared seedlings were used for the experiment at both locations. Single seedlings were transplanted into hills, with 25 × 25 cm spacing, in the 3x3 m² experimental plots. 15-15-15 fertilizer was applied at 60 kg/hectare 4 times, at maximum tillering, panicle initiation, booting and flowering stages. Leaf samples were collected at both locations before foliar application. Foliar application was performed twice, at flowering and early milk stage, according to the experiment protocol. The field was kept flooded under 0.1 – 0.2 m of water until maturity. Rice seed will be harvested at maturity for grain yield and nutrient analysis, which is expected at in December 2015.

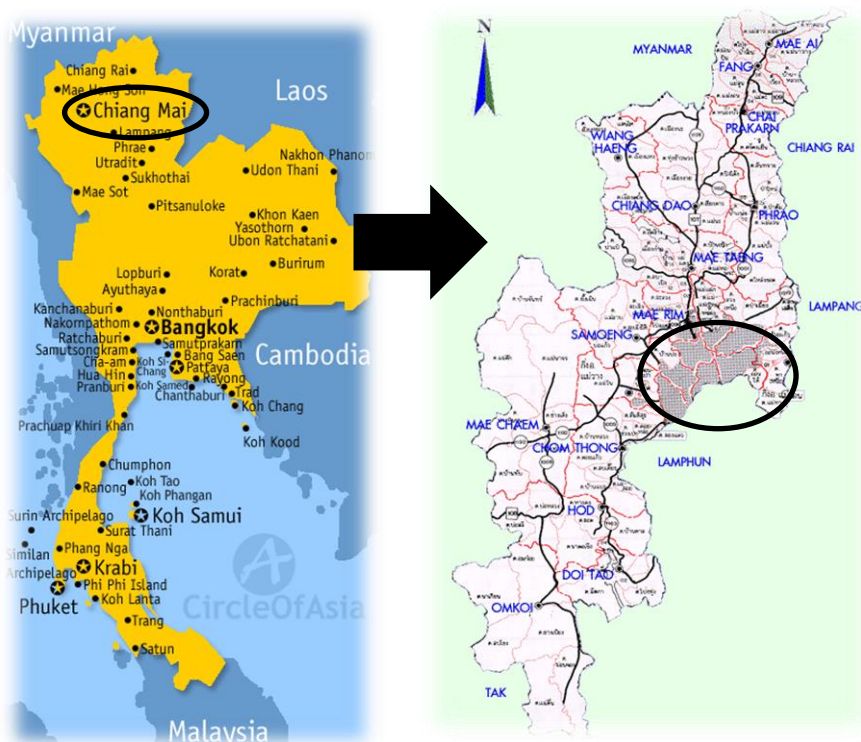


Figure 1: Map showing the locations of field experiments with 13 treatments at Chiang Mai province, THAILAND.

TASK: Promote and create awareness to facilitate the adoption of the zinc and iodine fertilizer strategy at the farmer and policy maker levels

Four farmers participated farmer's participatory trials at five different fields (600 m² each) within Chiang Mai Province. Seedlings were prepared on their own field with their own selected rice variety. The 2 varieties of rice (SPT1 and RD14) were used at 5 selected farmer's fields. Zinc enrichment was done by foliar application of 0.5% ZnSO₄ 3 times at flowering and early and mid milky stages. Rice samples both with and without zinc enrichment were harvested. Grain yield and zinc concentrations will be evaluated. Pictures from some selected farmer's rice field in farmer's participatory trials are given in Figure 2.

TRAINING AND VISIBILITY ACTIVITIES

The experiment has been involving a postdoctoral fellow and a master student at Agronomy Division, Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University.



Figure 2: Some pictures from farmer's participatory trials.

PROPOSED ACTIVITIES IN THE NEXT SIX MONTHS

Month (2016)	Activity
Jan-May	<p><u>The field experiment</u> Grain yield, yield components and zinc concentrations will be evaluated.</p> <p><u>Farmer participatory trial</u> Grain yield, yield components and zinc concentrations will be evaluated.</p>
June	<p><u>Farmer participatory trial</u> Seedling of Zn and non-Zn enriched will be prepared fields for the next crop on farmer fields.</p>

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COUNTRY REPORT – SOUTH AFRICA

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INTRODUCTION

Not much information is available about zinc deficient soils in South Africa. It is, probably, mostly evident in vertisols. Vertisols contain high calcium and magnesium and the pH is usually above 7. Phosphate fixation can be a problem and micronutrients, especially zinc and iron are often deficient. It is estimated that about 42% of South African agricultural land suffers from zinc deficiencies lowering crop production (www.farmersweekly.co.za).

In terms of human nutrient disorders at the national level, stunting and underweight remain the most common nutritional disorders affecting 1 out of 5 children and almost 1 out of 10 children respectively. Iodine and folic acid status appear to be adequate uniformly throughout the country, almost one third of women and children were anaemic, 2 out of 3 children and 1 out of 4 women had a poor vitamin A status and 45.3% of children had an inadequate zinc status. The Western Cape had the highest prevalence of inadequate zinc status, with Mpumalanga and Limpopo provinces having the lowest (www.dbsa.org).

In a study of randomly selected children between the ages of 7 and 11 years, living in a poor, peri-urban informal settlement in South Africa, it was found that 46% of the children had less than the recommended amount of serum zinc (Samuel et al. 2010). In another study amongst pre-school children aged 3-5, it was found that the prevalence of zinc deficiency was 42.6% and anemia was 28%; both were higher in girls than in boys (Motadi et al. 2015). In a study of the elderly in a rural area, Oldewage-Theron et al. (2008), found that approximately half of the subjects (51.5%) did not reach two-thirds of the recommended dietary allowance for zinc. The percentage of subjects with less than the recommended serum zinc value of 70 microg dL(-1) was 76.3%. It therefore appears that Vitamin A, zinc and iron are the main deficient nutrients in human nutrition in South Africa.

TASK: Effect of next generation foliar micronutrient fertilizers and cocktail application of micronutrients (Zn, I, Fe and Se) on grain concentrations of micronutrients

EXPERIMENTAL ACTIVITIES

Trials were established at two locations in the Western Cape Province of South Africa (See Figure 1). The first trial was established on the Langgewens experimental farm of the Department of Agriculture: West Cape at 33° 16' 33.96" S; 18° 42' 14.4" E. The second trial

was established on the Roodebloem experimental farm of the Overberg Agri agricultural co-op at 34° 13'31.55"S; 19° 26'13.76"E. The trials were planted on the 13th and 18th of May 2015 on Langgewens and Roodebloem, respectively. Both trials were planted into dry soil because the rains were extraordinary late this year. At time of planting, 40 kg N in the form of Limestone Ammonium Nitrate (28%) and 20 kg P was broadcasted on the experimental area. Trials were planted by means of a plot planter in rows 17 cm apart at a density of 100 kg seed ha⁻¹. The local cultivar SST027 was used in the trials. Weed control consisted of a pre-emergence application of prosulfocarb (3 L ha⁻¹) and triasulfuron (30 g ha⁻¹) at the time of planting. This was followed up by a post-emergence application of pinoxaden at 780 ml ha⁻¹ on 10 June 2015. Due to presence of herbicide-resistant weeds (mainly *Lolium* spp) on the Langgewens farm, poor control was achieved and in July the whole experimental area was weeded by hand to minimize competition from weeds.

On July 20 and 22, 2015, plant counts were carried out on Langgewens and Roodebloem, respectively. Two 0.5 m x 1 m metal rectangles were randomly thrown down in each plot and the number of wheat plants was counted. The mean number of plants per m² was then calculated.

On August 6 and 7, 2015, the heights of five randomly selected plants per plot were measured from the soil surface to the highest point of the plant. Only the control (Treatment 1), treatment 2 and Treatment 3 were measured since the other plots have not received any treatment yet at that stage. The mean height of the five plants was calculated for each plot. On September 1 and 2, 2015, the first foliar treatments were applied to the plots on Langgewens and Roodebloem, respectively, as prescribed in the protocol. The second application was made on September 29 and October 8, 2015, respectively, on Langgewens and Roodebloem.

The final harvesting was done by means of a plot harvester (Hege 140) on October 27, 2015 on Langgewens and on November 20, 2015 on Roodebloem. The reason for the big difference in harvesting times was the drought that occurred in the region where Langgewens is situated and the wheat was ready for harvesting much earlier than on Roodebloem, where the rain kept on falling till late in the season.

Farmer participatory experiments

Five wheat fields in the vicinity of the two sites were identified where the farmer participatory trials were to be carried out but one site was accidentally destroyed. Two localities (Locality 1 and 2) were not far from the Langgewens site (approximately 33° 15' S and 18° 42"E), one was on the farm called Altona near Durbanville (approximately 33° 41' S and 18° 37' E) and the last one near Roodebloem (approximately 34° 13' S and 19° 26' E).

Planting and management of the fields were similar to the protocols of the various farmers. Approximately 80 to 100 m² were separated from the rest of the fields and half of it was treated with ZnSO₄·7H₂O three times as prescribed in the protocol. The times of application were synchronized with the times of application in the field trials and then about two weeks after the second application a third application was added to the zinc treated areas. The harvesting was done at the same periods when the field trials were harvested. The first three locations were harvested between 26 and 29 October, 2015 and the fourth location was harvested on 20 November, 2015. The big difference in harvesting times between localities 1 to 3 and locality 4 was due to the rainfall pattern.

RESULTS

Following good rains the wheat crop was established well at both sites although the dry conditions at planting hampered the action of the pre-emergence herbicides. On Roodebloem, very little interference from weeds was observed. There was a strong presence of ryegrass (*Lolium* spp) weeds at the Langgewens site in particular. The pinoxaden was applied to try to control the ryegrass but it was ineffective due to herbicide resistance. The hand weeding, however, resulted in clean plots and the crop was not influenced too much by the weeds. Rainfall in the Roodebloem site was good but at the Langgewens site, after looking satisfactory in June, the rain decreased from July (Table 1) and the wheat had a very hard time surviving.

Table 1: Rainfall data (in mm) for the two localities from April 2015 to November 2015

LOCATIONS	MONTHLY RAINFALL (mm)							
	April	May	June	July	August	Sept	Oct	Nov
Roodebloem	20	20	140	91	54	67	18	42
Langgewens	0	18	66	58	28	10	0	22

There were no significant differences in the number of plants per m² between treatments (data not shown). The heights of the plants in Treatments 1 to 3 are given in Table 2. Also in this measurement, there were no significant differences between treatments in either experiment.

Table 2: The heights of plants (in cm) in three treatments on two localities

TREATMENT	PLANT HEIGHT (cm)	
	Roodebloem	Langgewens
1) Local treatment (LT)	26.1	24.7
2) LT and Treatment-2	27.6	24.8
3) LT and Treatment-3	24.7	24.1
LSD (0.05)	ns	ns
CV (%)	10.6	3.9

No particular signs of damage was observed after application of the zinc treatments. The yields of the wheat in the different treatments on the two locations are shown in Table 3. The grain yield on Roodebloem is more than twice higher than the yields on Langgewens due to the varying climatic conditions (See Table 1). There are, however, no significant differences between any treatments on Langgewens or Roodebloem.

Table 3: The yield of wheat (tons per ha) treated with different zinc treatments on two locations

TREATMENT		GRAIN YIELD (t ha ⁻¹)	
		Roodebloem	Langgewens
1	Local Treatment (LT)	4.29	1.69
2	LT and Soil ZnSO ₄ .7H ₂ O Application	4.12	1.87
3	LT and Seed Zn Treatment with ATP PreCede	4.36	1.91
4	LT and 2-times foliar ZnSO ₄ applications	3.87	1.67
5	LT and 2-times foliar potassium iodate (KIO ₃)	4.21	1.79
6	LT + 2-times foliar KIO ₃ + 2 % KNO ₃	4.15	1.67
7	LT + 2-times foliar ADOB-ZnIDHA	4.11	1.77
8	LT + 2-times foliar ADOB-Basfoliar	4.14	1.80
9	LT + Kali-EPZO-Zn together with urea	4.16	1.60
10	LT + Valagro 52304 solution	4.25	1.92
11	LT + Bayer Antracol-Zn	4.39	1.85
12	LT + Foliar Cocktail micro spray-I	4.15	1.97
13	LT + Foliar Cocktail micro spray-II	4.15	1.82
LSD (0.05)		ns	ns
CV (%)		8.4	14.6

TASK: Promote and create awareness to facilitate the adoption of the zinc and iodine fertilizer strategy at the farmer and policy maker levels.

The wheat in the Farmer participatory trials were harvested more or less at the same time than the field experiments in the vicinity. In the area near Langgewens, the yield was very low due to the drought but enough seed was harvested to be able to plant the Farmers Participatory Trial next year. In one of the locations, the whole area that was treated with ZnSO₄ seemed to dry out somewhat quicker than the control area so it could have been an effect of the application of the ZnSO₄.

TRAINING AND VISIBILITY ACTIVITIES

Up to now, no training or visibility activities has been carried out. They will be carried out in 2016 in particular when Zn-enriched seeds are planted for comparison to seeds not enriched with Zn.

PROPOSED ACTIVITIES IN THE NEXT 6 MONTHS

In February 2016, a MScAgric student will start a research project with the wheat seeds from the different treatments harvested in 2015. Attention will be given to the viability and vigour of the seeds by doing germination tests in laboratory and greenhouses. Some field trials will also be carried out. The two trials carried out on Langgewens and Roodebloem will also be repeated. Planting of zinc-treated and untreated wheat will take place in the farmers trials and the student will monitor the performance of the wheat in these areas. Students will be taken to witness the trials and some popular articles will be prepared.

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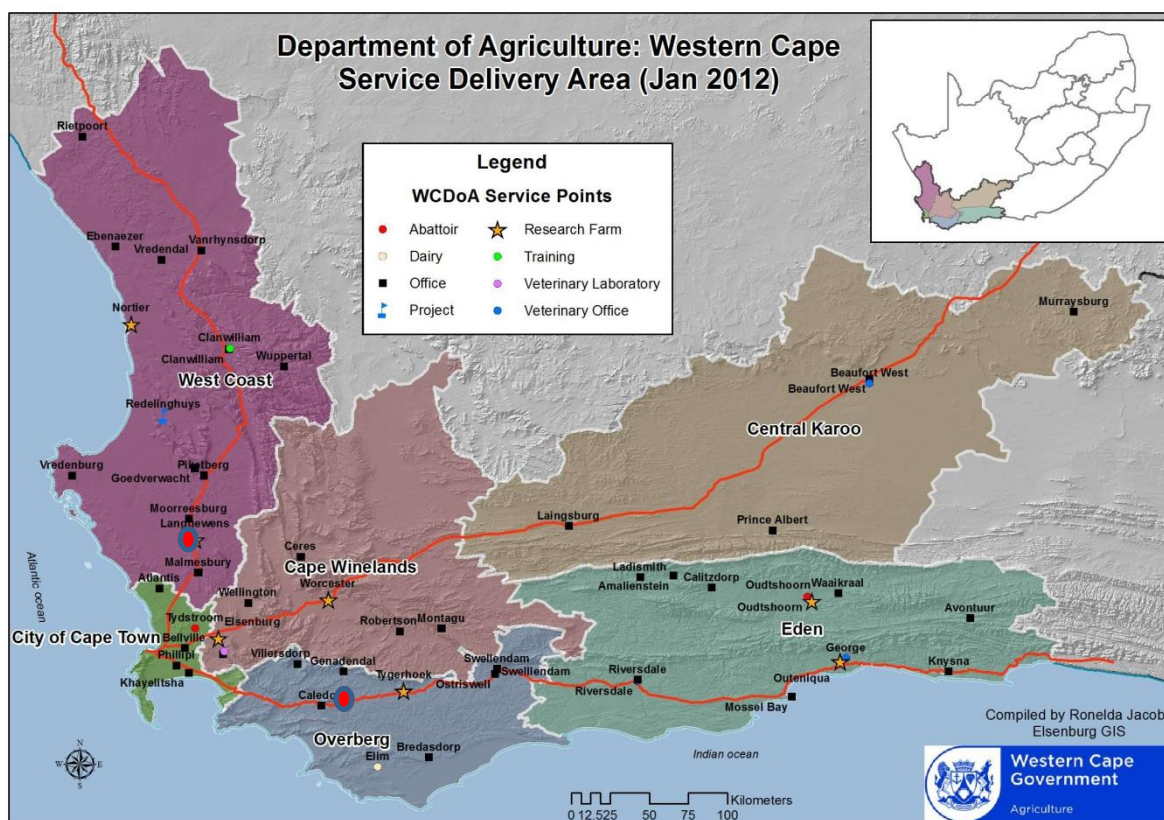


Figure 1: Location of the trials (●) for the HarvestZinc project in South Africa.

COUNTRY REPORT – BRAZIL

COLLABORATING INSTITUTIONS AND RESEARCHERS IN BRAZIL

Universidade Federal De Lavras (Coordinated By the Department of Soil Science)

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Dr. Silvio Júnio Ramos;

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Dr. Rômulo Fredson Duarte;

Dr. Ana Paula Corguinha;

Dr. Joelma Pereira.

Agricultural Research Company Of Minas Gerais (Epmig)

Fábio Aurélio Dias Martins (PhD candidate at the Plant Science Department-
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INTRODUCTION

More than 2 billion people worldwide, especially women and children in developing countries, suffer from malnutrition or “hidden hunger”, particularly deficiencies of iron, zinc, iodine and vitamin A (Hess, 2010; Thompson and Amoroso, 2014; Sala et al., 2015). Iodine (I) deficiency causes several disorders such as goiter, and it has been reported as a serious public health problem in Brazil since 1955. Because of its high relevancy, it was suggested that iodine supplementation should be performed by adding it into table salt (Medeiros-Neto, 2009). However, the National Health Surveillance Agency (ANVISA) reduced the amount of iodine in salt for humans from 40-100 mg I/kg salt to 20-60 mg/kg and a new public consultation reviewed this amount and suggested a range from 15 to 45 mg I/kg salt (RDC 23/2013, ANVISA, 2013). Besides that, recent interventions in Brazil, involving the Ministry of Health and Association of Food Industries (ABIA), have proposed the decrease of sodium content in processed foods from 12 g/day/person salt to less than 5 g/day/person until 2020. (Nilson et al., 2012), which may decrease iodine intake by the population.

Zinc is the most commonly deficient micronutrient in soils and it leads to low productivity and low Zn concentration in plant tissues (Alloway, 2008; Cakmak, 2008). The deficiency of Zn is also common in Brazilian soils, especially in Cerrado, the main region of agricultural areas in the country. Zinc deficiency has been reported as moderate and widespread for humans and soils in Brazil, respectively. This is due to high consumption of staple food with low concentration and bioavailability of Zn, especially in developing countries (Alloway, 2008; Cakmak, 2008). Recommended average daily intake of Zn ranges from 3 to 17 mg Zn per day, depending on age, gender, type of diet and other factors (Hotz and Brow, 2004; Sharma et al, 2013). Therefore, new strategies for increasing mineral content (iodine and zinc) in cereal foods (e.g., wheat and rice crops) through agricultural practices, such as genetic or agronomic biofortification, should provide adequate mineral intake for optimum nutrition.

TASK: Effect of next generation foliar micronutrient fertilizers and cocktail application of micronutrients (Zn, I, Fe and Se) on grain concentrations of micronutrients

The experiments with 13 treatments will be carried out at the Lambari experimental field (Figure 1) and at the experimental field of Patos de Minas (Figure 2). Both fields are located in the state of Minas Gerais and belong to the Agricultural Research Company of Minas Gerais. Planting of both experiments are expected to be around mid December, 2015.

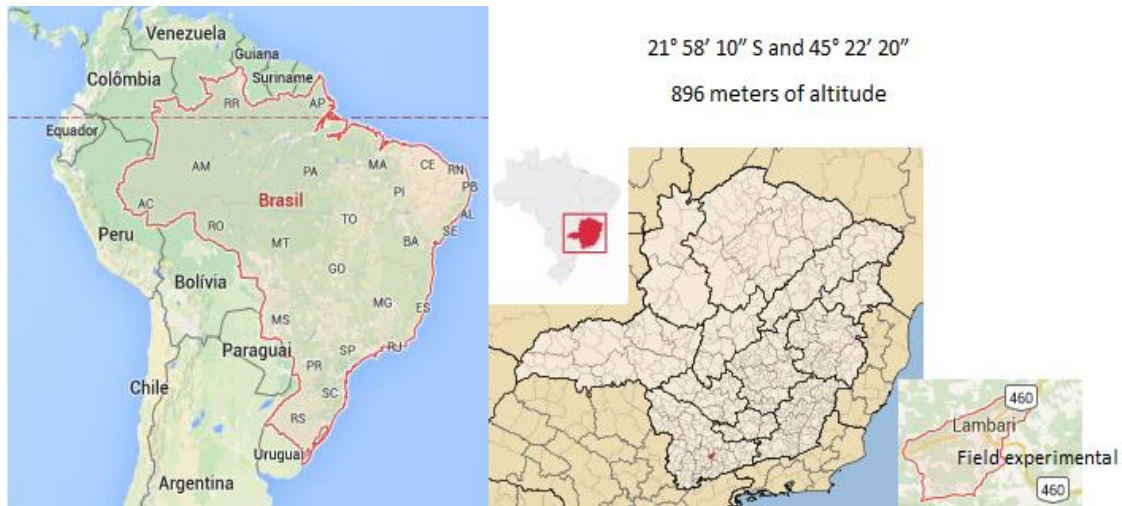


Figure 1: Field experiment station located in Lambari, MG, Brazil.

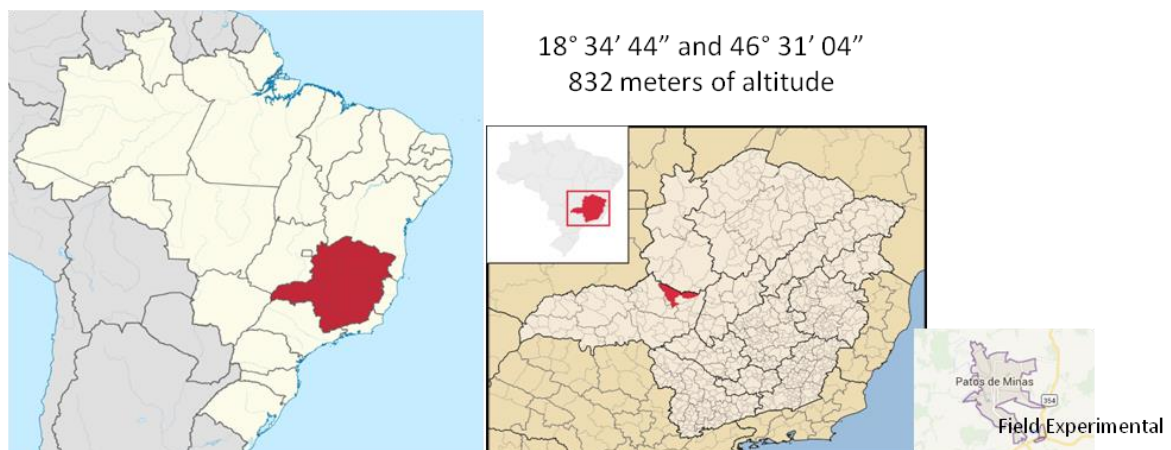


Figure 2: Field experiment station located in Patos de Minas, MG, Brazil.

Experiment at Lambari Station

The experiment will be performed in a randomized complete block design with four replications. Plot area will be 5x0.34 m with 6 rows. BRSMG Caravera cultivar will be used. The plots will be fertilized with 250 kg ha⁻¹ of commercial NPK fertilizer 08-16-16 at the time of sowing. Additional nitrogen and potassium will be top-dressed at a rate of 112 kg ha⁻¹ of urea and 34 kg ha⁻¹ potassium chloride 45 days after germination.

Experiment at Patos de Minas Station

It will also be performed in a randomized complete block design with four replications. Plot area will be 5x0.34 m with 6 rows. BRSMG Caravera cultivar will be used. The experimental plots will be fertilized by applying 250 kg ha⁻¹ of commercial NPK fertilizer 08-16-16 at the time of sowing. Additional nitrogen and potassium will be top-dressed at a rate of 112 kg ha⁻¹ of urea and 34 kg ha⁻¹ of potassium chloride 45 days after germination.

TASK: Promote and create awareness to facilitate the adoption of the zinc and iodine fertilizer strategy at the farmer and policy maker levels.

Rice seeds will be planted in two plots of 500 m² each, with and without foliar Zn application at both experimental stations. The purpose of these plots is to obtain Zn-enriched seeds and untreated seeds to be used on farmer field experiments in the next cropping season.

TRAINING AND VISIBILITY ACTIVITIES

The experiments to be conducted will enable participation of undergraduate and graduate students, as well as postdoctoral researchers. There is no possibility to involve farmers and other sectors of the productive chain of rice at this time, as most of the activities are still related to basic research.

A “Zinc Day” event will be organized next year (2016) in Brazil to provide information about Zn nutrition of crop plants and also importance of Zn and I in humans to students, agronomists, farmers, and other stakeholders. The proposed location is the city of Patos de Minas, due to its privileged location and the suggested date is the week of May 20 to 29, during a traditional Agricultural Fair called Fenamilho (<http://www.fenamilho.com.br/2016/enquete/home>).

PROPOSED ACTIVITIES IN THE NEXT SIX MONTHS

As summarized below, seeds will be multiplied at both sites in order to supply material for the upcoming farmer participatory trials to be installed in the subsequent cropping season (2016-17). Unlike the previous harvest, we intend to involve a larger number of students, as well as producers and decision makers involved in agricultural activities in the regions where the experiments will be conducted.

ACTIVITY	FIELD EXPERIMENT (TWO LOCATIONS)
Seedling preparation	November 2015
Plot preparation	November 2015
Sowing	December 2015
1 st spraying (heading)	February 2016
2 nd spraying (mid milky)	March 2016
Harvesting	April 2016

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COUNTRY REPORT – PAKISTAN

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INTRODUCTION

Zinc (Zn) deficiency in wheat is a widespread nutrient disorder in Pakistan because of the alkaline-calcareous nature and low organic matter content of the 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 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 (Kausar et al., 1976; Rashid and Fox, 1992), significant yield increases were observed when Zn fertilizer was applied to the low-Zn soils ($P \leq 0.05$; Rafique et al., 2006). In Phases I and II of the *HarvestPlus Zinc Fertilizer Project* (2008–13), agronomic biofortification proved to be an effective approach to optimize wheat yield and enhance grain Zn density cost-effectively (Zou et al. 2012; Final Report of HarvestZinc Phase-II).

Iodine (I) deficiency is another major public health problem in Pakistan. A WHO survey on worldwide iodine status has listed Pakistan as a severely iodine-deficiency affected country with 135 million people having insufficient iodine intake (Inayat, 2009). Recently, Zia et al. (2015) have reported on iodine status in soil-plant systems in Pakistan and they believe that the origins of widespread iodine deficiency in the population are dietary. Cereal grains (e.g., wheat) are poor sources for many micronutrients including iodine; therefore, the resource-poor segments of the society relying entirely on wheat as the source of their caloric requirements are most vulnerable to iodine malnutrition. Plants grown in iodine-deficient soils do not have adequate iodine, nor does meat or other products from animals fed on iodine-deficient plant biomass. Iodized salt program was initiated in Pakistan in 1994 and has proven to be quite effective in addressing iodine deficiency disorder (IDD) in the susceptible communities. According to the National Nutrition Survey (2011), the IDD affected population in Pakistan was 76% in 2001, which has been reduced to 48% in 2011. While National Nutrition Survey in Pakistan (2011) has observed that 40% of Pakistani households consume iodized salt, UNICEF has reported that only 17% households have iodized salt and 2/3^{rds} of children have inadequate iodine uptake (Zimmermann, 2011).

The prevalence of widespread deficiencies of Zn and I are also recognized in many field crops grown in calcareous alluvial soils of Pakistan (Rashid, 2005; Zou et al., 2012; Abid et al., 2013; Zia et al. 2015). Thus, improving Zn and I status of staple cereals, i.e., wheat and rice, is believed to be an effective strategy for overcoming dietary micronutrient deficiencies in resource poor populations. The objective of the experiments during Phase-III of HarvestZinc Project is to study the comparative effectiveness of various soil and foliar Zn and I treatment strategies for ameliorating Zn deficiency in wheat crop and enhancing grain Zn and I density in wheat grains.

EXPERIMENTAL ACTIVITIES

Site Selection and Soil Properties

Soil samples were collected from 22 random fields from different farm sites surveyed in central region of the Punjab province, Pakistan. Six Zn-deficient fields (including five cultivated by farmers), having alluvial calcareous soils, were selected in the main rice-wheat cropping system (near Gujranwala) and in mixed cropping system (near Faisalabad and Jhang) areas (Figure 1). Before establishing each experiment, composite soil samples (0-30 cm) of the experimental fields were collected and analyzed for different chemical properties (Table 1).



Figure 1. Locations of field experiments (●) and demonstration trials (★) during 2015-16 in Punjab, Pakistan.

Table 1: Surface soil (0-30 cm) properties of experimental fields.

LOCATION	pH _{1:1} *	Organic matter (%)	CaCO ₃ (%)	DTPA-Zn (mg kg ⁻¹)	NO ₃ -N (mg kg ⁻¹)	NaHCO ₃ -P (mg kg ⁻¹)	NH ₄ OAc-K (mg kg ⁻¹)
Jhang	7.87±0.05*	0.64±0.02	5.7±0.5	0.26 ±0.05	15.4±4.7	20.1±2.8	339±39
Gujranwala	7.92±0.03	0.92±0.02	1.9±0.6	0.41±0.09	10.5±3.2	6.7±1.9	107±8
Faisalabad NIAB, Farm	7.76±0.13	0.66±0.04	4.3±0.7	0.46±0.05	13.8±2.9	8.1±1.4	118±13
Hafizabad	7.91±0.05	1.06±0.03	2.6±0.3	0.57±0.05	19.9±4.6	9.6±2.2	128±19
Faisalabad, Samundri	8.07±0.10	0.74±0.08	12.9±2.0	0.38±0.02	25.5±3.1	15.5±1.3	88±10
Faisalabad, Chak 30/JB	8.01±0.09	0.65±0.01	5.3±0.3	0.37±0.04	11.7±0.5	2.1±0.7	108±7

*Values are means of four replicates ± SD.

TASK: Determine the response of newly developed high Zn lines from HarvestPlus breeding program to soil and foliar spray of Zn and other micronutrients

This experiment comprises of six treatments of zinc and iodine (Table 2) to study their effect on wheat yield and grain enrichment with Zn and I of six wheat lines provided by HarvestPlus (i.e., NR-421, NR-435, NR-436, NR-457, NR-488 and NR-489). Moreover, a widely cultivated wheat cultivar in Pakistan, i.e., Faisalabad-2008, has been included as a control. The concentration of Zn in seeds of wheat lines/varieties was determined before the start of experiment (Table 3). This Experiment has been established at two locations in districts of Faisalabad and Jhang.

Table 2: Treatment plan of TASK 1 experiment.

Sr. No.	Description of Zn or I treatment
1.	Local Control (LT)
2.	LT and Soil ZnSO ₄ .7H ₂ O Application
3.	LT and 2-times foliar ZnSO ₄ Applications
4.	LT and 2-times foliar ZnSO ₄ +Iodine Mixture Applications
5.	LT +Foliar Cocktail Micro Spray-I
6.	LT +Foliar Cocktail Micro Spray-II

Table 3: Zinc concentration in wheat seed of various lines/ cultivars used in Experiment 2.

<i>Wheat Line/Cultivar</i>	Zn concentration (mg kg⁻¹)*
HarvestPlus NR-421	39±0.8
HarvestPlus NR-435	34±2.2
HarvestPlus NR-436	31±0.8
HarvestPlus NR-457	36±0.9
HarvestPlus NR-488	39±1.2
HarvestPlus NR-489	28±0.6
Faisalabad-2008, Low Zn Seed	25±1.2
Faisalabad-2008, High Zn Seed	51±1.1

*Values are means of 3 replicated analyses ± SD

As field experiments of Phase-III have been established in November, no experimental results / data are available as yet. The data to be recorded have been elaborated at the end of this Report.

TASK: Effect of next generation foliar micronutrient fertilizers and cocktail application of micronutrients (Zn, I, Fe and Se) on grain concentrations of micronutrients

The experiment here is being conducted to study the effect of 13 different treatments of zinc fertilizers and iodine salts (Table 4) on yield of wheat and their biofortification effect in terms of enhancing Zn and I in wheat grains. This Experiment, using cv. Faisalabad-2008, has been established at three locations, in districts of Faisalabad, Jhang and Gujranwala.

Table 4: Treatment plan of TASK 2 Experiment.

Sr. No.	Description of Zn or I Treatment
1.	Local Control (LT)
2.	LT + Soil ZnSO ₄ .7H ₂ O Application
3.	LT +Seed Zn Treatment with ATP-PreCede*
4.	LT + 2-times Foliar ZnSO ₄ Applications
5.	LT + 2-times Foliar Potassium Iodate (KIO ₃) Treatments
6.	LT+ 2-times Foliar Potassium Iodate (KIO ₃) +2 % KNO ₃
7.	LT + 2-times ADOB-ZnIDHA
8.	LT + 2-times ADOB-Basfoliar
9.	LT + Kali-EPSO-Zn together with Urea
10.	LT + Valagro Brexil Zn-Iodine
11.	LT +Bayer Antracol-Zn
12.	LT +Foliar Cocktail Micro Spray-I
13.	LT +Foliar Cocktail Micro Spray-II

*Faisalabad-2008, ATP Primed Seed, 267±10.9 mg Zn kg⁻¹; Faisalabad-2008. ATP Primed & washed Seed, 196±6.8 mg Zn kg⁻¹

TASK: Promote and create awareness to facilitate the adoption of the zinc and iodine fertilizer strategy at the farmer and policy maker levels.

Field demonstrations have been established at 6 locations (Figure 1) of districts Faisalabad, Jhang, Gujranwala and Hafizabad to study the effect of low- and high-Zn density seed on seedling emergence and plant population of wheat (cv. Faisalabad-2008).

TRAINING AND VISIBILITY ACITIVITES

A national Zinc Day will be organized in March 2016 at NIAB, Faisalabad to disseminate the technology on agronomic biofortification of zinc in wheat and advantage of using high-Zn wheat grains as seed for the next wheat crop. In addition to sharing the results of multi-location multi-year field trials by way of lectures, the participating stakeholders will be shown the impact of various experimental treatments in the field. Also, local language brochures will be distributed to the stakeholders, i.e., farmers, agricultural extension staff and fertilizer industry personnel. Visits of the neighboring farmers and agricultural extension straff will also be organized to the farmer participatory field trials at other field locations.

PROPOSED ACTIVITIES IN NEXT SIX MONTHS

Two replicated experiments on wheat with Zn fertilizers and I salts and a farmer participatory field demo (without replications) on comparative study of high and low Zn wheat seed are progressing at a total of six different filed locations which will be managed during the next six months. Details of the activities and the data to be recorded are as under:

- The crop will be managed in terms of weed control, irrigation, fertilizer application, etc.
- Data of seedlings density and seedling vigor will be recorded, where applicable.
- Foliar sprays of Zn and I will be carried out as per treatment plan.
- Plant leaf samples will be collected and prepared as and when necessary.
- Crop will be harvested at maturity and yield data will be recorded.
- Grain samples will be collected and prepared for Zn and I analysis.
- Leaf and grain samples will be analyzed for Zn.
- Grain samples for Iodine analysis will be sent to Sabanci University, Turkey.
- Project report will be prepared.

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COUNTRY REPORT - TURKEY

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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. Later on, a national survey, consisting of 1511 soil samples from all over the country, showed that 49.8 % of the Turkish soils were deficient in Zn (Eyupoglu et al, 1994).

Recognition of Zn deficiency in children was even earlier. 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.

After 5 years of field experiments in Central Anatolia, where Zn-deficient soils are widespread, it was shown that fertilizing soils was enough to solve the problem related to grain yield and growth under low Zn. These findings resulted in initiation of manufacturing Zn-enriched commercial fertilizers by domestic companies. However, since soil application of Zn-containing fertilizers was not enough to improve Zn concentrations of wheat grains at an expected level, a new series of experiments were started within the scope of HarvestPlus (HP) Program. Results of the first two phases of HP project, lasting six years with a total of 8 experiments, showed once more that foliar applications were more effective in improving grain Zn concentrations. Twice application of foliar ZnSO₄, at later stages of growth, improved grain Zn concentrations by 83.4 and 32.6% in the first and second phases of the project, respectively, each value being an average of 4 experiments.

The primary cause of iodine (I) deficiency in human beings is known to be low I content of soil and water. First epidemiologic studies related to I deficiency in Turkey were performed as early as 80 years ago (Atay, 1935; Onat, 1948). In an epidemiologic survey, Hatemi and Urgancioglu (1993) determined the I concentrations of 2676 water samples from 493 residential quarters throughout the country. They found that the I concentration was below 10 Jlg/l in 49 of these 493 areas, and below 20 Jlg/l in 46. Following these results, they examined 73 750 people in 115 residential districts for visible goiter. 7.5 % of the test subjects were found to have Grade 2 endemic goiter, this percentage reaching 30.5 when all goiter stages were considered.

After legislation for mandatory iodization of household salt was passed in July 1999, Turkey has been classified as a country of mild I deficiency problem by World Health Organization (WHO, 2004). However, the within-country variation seems to be quite high, occurrence of the problem increasing in resource-poor regions and/or families. As already explained for Zn,

cereal grains are also inherently low in I. This results in higher rate of occurrence of I-deficiency related health problems in people having cereal based diets. For the reasons explained above, Turkey is participating in the third phase of the project, involving Fe, I and Se fertilization of wheat, in addition to Zn, in an effort to improve grain concentrations of these elements by agronomic biofortification.

TASK: Effect of next generation foliar micronutrient fertilizers and cocktail application of micronutrients (Zn, I, Fe and Se) on grain concentrations of micronutrients

EXPERIMENTAL ACTIVITIES

The experiment with 13 treatments (excluding the treatment 5 in the protocol of 14 treatments, which is restricted to rice experiments) was planted at 3 locations within Eskisehir province in Central Anatolia (Figure 1).



Figure 1: Province of the experimental sites in Turkey.

Plantings were performed on 15th, 16th, and 21st of October, 2015, using a plot drill. The cultivar used at all locations was Bezostaya 1, 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 83 kg P₂O₅ ha⁻¹ (180 kg DAP), 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) will be applied at tillering stage (Zadoks 23-24) at all locations. Zn soil applications in the experiment were performed by spraying 50 kg ZnSO₄ (7 mol water) ha⁻¹ in related treatment plots and mixed with soil using a disc plow in all experiments. The treatment list of the experiment is given in Table 1.

Although emergences are complete at all locations, emergence counts are just being performed. In addition to manual counts of emergence, NDVI readings are also performed (Figure 2). Results will be presented in next term report.

Table 1: List of the treatments applied in TASK 2 experiments in Turkey.

1	Local Treatment (LT)
2	LT and Soil ZnSO ₄ ·7H ₂ O Application
3	LT and Seed Zn Treatment with ATP-PreCede
4	LT and 2-times foliar ZnSO ₄ applications
5	LT and 2-times foliar potassium iodate (KIO ₃) treatments
6	LT + 2-times foliar potassium iodate (KIO ₃) + 2 % KNO ₃
7	LT + 2-times ADOB-ZnIDHA foliar applications
8	LT + 2-times ADOB-Basfoliar foliar applications
9	LT + Kali-EPSO-Zn together with urea
10	LT + Valagro 52304 solution
11	LT + Bayer Antracol-Zn
12	LT + Foliar Cocktail micro spray-I (Zn+Fe+I+Se):
13	LT + Foliar Cocktail micro spray-II (Zn+Fe+I+Se):



Figure 2: NDVI recording of emerged wheat seedlings.

TASK: Promote and create awareness to facilitate the adoption of the zinc and iodine fertilizer strategy at the farmer and policy maker levels.

EXPERIMENTAL ACTIVITIES

For this task, a total of 7 trials, 4 on farmer fields and 3 on fields of varying soil properties at the experiment station, were established to see the relative effects of soil Zn application and Zn-enriched seed on seedling emergence and early vigor as compared to control treatment. The Zn concentrations of Zn-enriched and untreated seeds were 70 and 32 ppm, respectively. All other cultural practices, including the cultivar, were the same as TASK 2 experiments.

TRAINING AND VISIBILITY ACTIVITIES

No activity has been performed yet. If any visible performance differences, due to treatments, are observed, field days can be organized after the start of spring regrowth.

PROPOSED ACTIVITIES IN THE NEXT SIX MONTHS

Observations and data evaluations will continue. Since harvest of wheat crop is normally expected in mid to late July, the yield and element concentration data will be provided probably next year this time.

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