

PROCEEDINGS

International Workshop

Biofortification of Staple Crops

a solution to combat malnutrition

Editors
Muhammad Farooq
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Sardar Alam Cheema

Faisalabad, Pakistan
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International workshop
Biofortification of staple crops: A solution to combat malnutrition
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Preface

Humans require at least 22 mineral elements for their well-being. These can be supplied by an appropriate diet. However, it is estimated that over 60% of the world's 6 billion people are iron (Fe) deficient, over 30% are zinc (Zn) deficient, 30% are iodine (I) deficient and 15% are suffering from selenium (Se) deficiency. In addition, calcium (Ca), magnesium (Mg) and copper (Cu) deficiencies are common in many developed and developing countries.

This situation is attributed to crop production in areas with low mineral phytoavailability and/or consumption of (staple) crops with inherently low tissue mineral concentrations, compounded by a lack of fish or animal products in the diet. Currently, mineral malnutrition is among the most serious global challenges to humankind and is avoidable. Mineral malnutrition can be addressed through dietary diversification, mineral supplementation, food fortification and/or increasing mineral concentrations in edible crops (biofortification). However, strategies to increase dietary diversification, mineral supplementation and food fortification have not always been successful. Biofortification is generally considered to be the most cost effective and sustainable way to alleviate micronutrient malnutrition. Two complementary approaches can be adopted to elevate the concentrations of bioavailable mineral elements in food crops. First, agronomic biofortification is through applying mineral fertilizers and/or improving the mobilization of mineral elements from the soil to plants. Second, genetic biofortification is the development of crops with increased ability to acquire mineral elements and accumulate them in edible tissues. Plant nutritionists have been working globally to develop more nutritious and micronutrient enriched food crops. Biofortification is under the process of development in many parts of the world. However, it is relatively a new concept in Pakistan.

Biofortification of crops through the application of mineral fertilizers, combined with breeding varieties with an increased ability to acquire mineral elements, is advocated as an immediate strategy not only to increase mineral concentrations in edible crops but also to improve yields on infertile soils. The adage 'health comes from the farm, not the pharmacy' is at the heart of ongoing international biofortification research and breeding programs.

The international workshop on "*Biofortification of staple crops: a way to combat malnutrition*' was organized during March 29-31, 2015 at University of Agriculture, Faisalabad, Pakistan in collaboration with British Council. This conference provided the scientists a forum for analyzing the situation and formulating some pragmatic options to cope with the situation. Delegates from UK and Pakistan attended the workshop. Proceeding of the workshop is expected to create awareness amongst the researchers and farmers about the issue of malnutrition and global food security. This will be helpful in devising strategies and ways to harvest good and nutritious crop yields on sustainable basis to feed the global population and combat malnutrition.

Muhammad Farooq
Abdul Wakeel
Sardar Alam Cheema

Foreword

Among the major risk factors in the world responsible for diseases leading to death, the micronutrient malnutrition alone is considered the major contributing factor in approximately 30 million deaths per year. Currently, mineral malnutrition is considered to be among the most serious global challenges to humankind.

Many countries in the world are suffering from malnutrition and situation is worst in the developing and under developed countries. Taking examples of micronutrient malnutrition impact on human health in food supply caused by deficiency of essential minerals in food due to the deficiency in soil is emerging as an alarming situation for the humanity.

I believe that to address global malnutrition challenge and crisis, it is essential to improve agricultural technology to produce the nutrient rich staple crops to combat the problem of malnutrition (hidden hunger). In this context, international agricultural development projects which transfer advanced agricultural technology to each country, plays an important role with responsibility and its importance is gaining more attention.

Each country in the world, is putting tireless efforts to exchange advanced agricultural technology, and as part of these efforts, Department of Agronomy, University of Agriculture, Faisalabad has promoted various kinds of research and tries to nurture human resources. University of Agriculture, Faisalabad is emerging as a hub university in the fronts of international agricultural cooperation.

Taking this opportunity, I would like to promise that Department of Agronomy, University of Agriculture, Faisalabad will play a pivotal role in communication and co-existence between countries as a bridge through international agricultural development. In addition, I hope that this workshop will serve as a venue to contribute agricultural productivity and quality by providing new information and transferring technology of international agricultural cooperation between Pakistan and other countries.

I congratulate Dr. Sardar Alam Cheema and his team for organizing an excellent workshop at University of Agriculture, Faisalabad. This book represents primarily a collection of papers presented at the International workshop "Biofortification of staple crops 'a way to combat malnutrition' during March 29-31, 2015 at University of Agriculture, Faisalabad, Pakistan in collaboration with British Council and I hope that this volume will contribute towards escalating the vision to develop technologies, ensuring the capacity strengthening of early career researchers in field of biofortification for formulating an integrated and multi-disciplinary approach for tackling the menace of malnutrition through biofortification.

Prof Dr. Ehsanullah
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Evaluating the Potential of Zinc Application Methods to Improve Grain-Zn Concentration in Basmati rice

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Introduction

Zinc (Zn) is among the essential micronutrient required for plant growth and human health as well. It improves human immune system, cognitive growth and resistance to some contagious diseases including diarrhea. About 2.7 billion people are suffering from Zn scarcity in the world. Another report indicated that fifty percent of world's population is under the menace of Zn scarcity and its occurrence is more in developing countries of Africa and Asia (Muller and Krawinkle, 2005). In plants, its deficiency may reduce the chlorophyll biosynthesis and affects tillering, growth, and yield of crop. Rice is the second largest staple food crop in Pakistan after wheat and is a major source of export income in recent years. Rice provides 21% of the world's dietary energy and fulfills 15% of protein requirements of human population across the globe (Depar et al., 2011). In Pakistan, soils are mostly alkaline and calcareous in nature which reduces the availability of Zn and in turn causes Zn deficiency in cereals particularly in rice (Maqsood et al., 2009). About 70% area under rice cultivation in Pakistan is Zn deficient and also emerged as third utmost serious crop nutritious problem after phosphorus and nitrogen deficiency. This study was conducted to evaluate the potential of different Zn application methods in improving the grain yield and Zn biofortification of basmati rice.

Materials and methods

This experiment was conducted at agronomic research area, University of Agriculture, Faisalabad during Kharif season of 2012. The experiment was laid out in randomized complete block design with factorial arrangement having net plot size of 5.0 m × 1.84 m with three replications. The treatments consisted of hydro primed seed, soil application at the rate of 10 kg Zn ha^{-1}, foliar application at the rate of 2% Zn solution and seed primed with 0.5% Zn solution while treatment without Zn application was taken as control. Thirty days old seedlings (two seedlings per hill) were manually transplanted in puddled field with 5-7 cm standing water in 22.5 cm spaced rows. After transplanting, NPK was applied at the rate of 100-65-62 kg ha^{-1}. Whole P and K and one third of the N fertilizers were applied as basal dose while remaining nitrogen was applied in two splits. Crop was harvested and threshed to get the rice grains. Zinc in grain was analyzed on a di-acid ($HClO_4$ + HNO_3 in 3:10 ratio) digest on an Atomic Absorption Spectrophotometer (Prasad et al., 2006). Data collected were statistically analysed using Fisher's analysis of variance technique.

Results and discussion

All the Zn-application methods enhanced Zn accumulation in both the basmati rice varieties. Maximum increase in kernel Zn level (37.29 mg kg^{-1}) was observed with seed priming followed by soil addition (34.60 mg kg^{-1}) and foliage application (Table 1). In case of super basmati rice, seed priming method proved best in improving the Zn contents in rice followed by foliar application while in case of Shaheen Basmati rice, foliage spray significantly enhanced Zn concentration followed by soil application (Table 1). Minimum Zn content in rice grains was noted in non Zn-fertilized plots. However, both the varieties behaved alike in this regard (Table 1). Among all the application methods, Zn seed priming showed more potential in improving the Zn grain contents.

Table 1: Influence of zinc application methods on Zn contents in grain of basmati rice

Treatments	Super Basmati	Shaheen Basmati	Means
Control	29.41 ef	25.20 de	27.31 B
Hydropriming	24.16 f	24.42 ef	24.29 B
Foliar application	34.66 bc	39.46 ab	37.06 A
Soil application	34.47 bcd	34.73 bc	34.60 A
Zn seed priming	40.36 a	34.21 cd	37.29 A
Means	32.61	31.60	32.11

LSD (at *p* 0.05) Zinc application methods = 3.62; Interaction = 5.12; Values sharing the same case letters do not differ significantly at $p < 0.05$

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Nurturing Rice with Boron Improves Panicle Fertility, Yield and its Grain Enrichment

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Introduction

Boron (B) is one of the essential micronutrients having specific role in plant reproduction. Reproductive stage of plants is more sensitive to B deficiency than the vegetative one. Limited soil water often limits the B availability, because release of B from organic complexes is restricted; also impairs the ability of plants to extract B from soil. In alkaline soils, availability of B under aerobic conditions is substantially reduced as its availability to plants decreases with increase in soil pH, especially above pH 6.5. Under such conditions, supplemental B application is vital for adequate supply to the plants. This study was conducted to evaluate the influence of soil applied B on panicle sterility, grain yield and grain B contents in fine grain aromatic rice.

Materials and methods

The experiment was conducted in a net house under natural conditions with five replications. Seeds of two fine-grain aromatic rice cultivars Super and Shaheen Basmati were sown in soil filled earthen pots (45 × 30 cm) containing 15 kg soil. Experimental soil was sandy loam with pH 8.2, EC 0.33 (dS m^{-1}), organic matter 0.71%, N 0.044%, P_2O_5 5 ppm, K 165 ppm and B 0.45 ppm. Ten seeds were sown in each pot and plants were thinned to five plants per pot five days after emergence. Besides other recommended doses of fertilizer, B, taking boric acid (17.5% B) as source, was soil applied as basal dose at 0, 0.5, 0.75, 1.0, 1.25 and 1.5 kg ha^{-1}. Irrigation was applied every third day or according to crop requirements. However, there was never standing water in the experimental pots and the soil conditions were aerobic. Rice plants were harvested at maturity, and threshed to separated grains. Panicle sterility estimated as a ratio of un-filled spikelets and total spikelets. Boron grain contents were estimated by azomethine-H method by spectrophotometry method (Malavolta et al., 1997). Data were analysed by MSTAT-C statistical software.

Results and discussion

Boron application improved the fertility and grain B contents in both rice cultivars. A linear increase in grain B contents was observed with increase in B application rate. This suggests that B nutrition has significant effect on panicle fertility and B grain enrichment of rice. Adequate B supply improved the rice performance by reducing panicle sterility, however beyond the optimum level (i.e. 1 kg ha^{-1}), an adverse effect of B application was observed. This indicates a narrow range between the deficiency and toxicity of B.

Previously, B was applied as seed treatment, where it was immediately available to the growing seedlings. However, in this study, B was incorporated into the soil, which could have been available only after roots were developed. This shows that B biofortification is possible with either priming, coating, foliar or soil application (Farooq et al., 2011; Rehman et al., 2011). In conclusion, soil application of B is helpful not only in improving panicle fertility but also in its grain enrichment. However, application at 1 kg ha^{-1} was better.

Table 1: Influence of soil applied boron on panicle sterility and grain B contents in fine grain aromatic rice

Application rate (kg ha^{-1} B)	Panicle sterility (%)			Grain B contents (mg kg^{-1})		
	Super Basmati	Shaheen Basmati	Mean	Super Basmati	Shaheen Basmati	Mean
Control	37.04fg	35.56g	37.04fg	1.68h	1.75g	1.72F
0.50	38.42ef	39.34de	38.42ef	1.75g	1.79f	1.77E
0.75	40.86d	39.48de	40.86d	1.89e	1.95d	1.92D
1.00	48.40a	43.20c	48.40a	1.96d	2.13c	2.05C
1.25	46.14b	41.12d	46.14b	2.21b	2.21b	2.21B
1.50	45.38b	40.62d	45.38b	2.35a	2.36a	2.35A
Mean	42.71A	39.88B	42.71A	1.97B	2.03A	

Interaction and main effect means sharing the same letter for any trait don’t differ significantly ($P < 0.05$)

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HarvestPlus Biofortification Program: High Zinc Wheat to Address Persistent Peril of Malnutrition in Pakistan

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Introduction

Chances are that the aroma of warm *chapatis* (bread) will be the first thing that greets you in Pakistan. In the country of 180 million people, some form of bread, whether as *chapatis*, *naan*, or *roti*, accompanies every meal, because no meal is complete without bread. Food is one of the fundamental requirements of life. Currently, the consumption of wheat is 128 kg/capita/annum. Despite its high consumption, is not providing sufficient daily dietary nutrient, particularly zinc (Zn) requirement (Government of Pakistan, 2013). The cereal crops including wheat is inherently low in Zn nutrient in its grains. Moreover, further its cultivation on potentially Zn deficient soils, results in reduction in Zn content in grain to a far greater proportion (Cakmak, 2014). In Pakistan, the currently grown wheat varieties contain on average 25 µg, whereas, the daily dietary requirement is around 30- 40 µg Zn per gram. The consumption of wheat containing lower quantity of Zn and/or cultivating on potentially Zn deficient agricultural soils would lead to Zn malnutrition, predominantly among the low income populations (Graham et al., 1992; Cakmak, 2008; Zou et al., 2012; Welch and Graham, 2014).

Incredibly, deficiencies of micronutrients micronutrient are afflicting over three billion people worldwide (Kennedy et al., 2003). According to FAO's food balance data, about 20% of the world population is at the risk of Zn deficiency (FAO, 2011). Pakistan is facing the crisis with some of the worst and most persistent rates of malnutrition in the world. Among the micronutrients, Zn is an essential and a component of more than 300 enzymes system, maintaining immunity, progressive growth and development (Hotz and Brown, 2004). According to NNS (2011), the prevalence of Zn deficiency is 47.6, 41.3, and 39.2 % in pregnant, non-pregnant women and children under 5 years, respectively. The rates of malnutrition in children under 5 years are wasted 15.1%, underweight 31.5% and stunted 43.7% in the impoverished communities. The stunting rate has gone up from 41.6% to 43.7%, which translates into 6.3 to 11.8 million children during 1980s and 2011, respectively. Moreover, 25% of new born babies were underweight (Bhutta, 2004). The deficiency in the daily diets results in stunting, reducing immunity, lowering IQ, risk to diarrheal disease and respiratory infection and increased risk for both mothers and infants during childbirth. The deficiency of Zn can result in young children being stunted irreversibly.

Materials and methods

In Pakistan, wheat crop is being biofortified i.e., raising the level of Zn nutrient from 25 to 37 microgram Zn per gram in its grains part. Pakistan Agricultural Research Council (PARC) under National Agriculture Research System under the Ministry of National Food Security and Research (MNFS & R) with collaboration of Consultative Group on International Agricultural Research (CGIAR), International Maize and Wheat Improvement Center (CIMMYT), International Food Policy Research Institute (IFPRI), International Center for Tropical Agriculture (CIAT) and Harvest Plus has took up this challenging task to develop and disseminate the biofortified high Zn wheat in the country. The biofortified wheat crop is produced through hybridization / genetic manipulation i.e., conventional plant breeding technique. The leadlines and the genetic stock are being tested for the agronomic performances under different ecological zones.

Results and discussion

The R & D efforts made by PARC have resulted in development of a number of biofortified high Zn wheat leadlines. The candidate variety "Zincol-2015" is high yielding, resistant to diseases and particularly to stem rust race of Ug99 and containing 37 microgram Zn per gram (+12 over the baseline). The biofortified high Zn wheat varieties are going to be released during next one or two years. After the approval of variety, about 80,000 household farming families will be cultivating and consuming biofortified high Zn wheat within following three years. The federal and provincial governments have owned the program as a potential option to improve nutrient status of the people. The program on biofortification of staple food crops has also been included in the 11th Five-Years Development Plan (2013-2018) and Pakistan Vision-2025. The Government of Punjab has also prioritized the program in its "Multi-Sectoral Nutrition Strategy Plan – 2015" to address malnutrition in the province. This program will open an era of development of biofortified crops enriched with essential minerals and vitamins to address the persistent peril of malnutrition. This product would enhance the nutritional status of the people whoever they may be and wherever they may be in the country.

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Regulating Cellular Iron Homeostasis is a Key to Iron Biofortification of Rice

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Introduction

Micronutrient biofortification of food crops has the potential to combat widespread micronutrient deficiencies in humans. Rice is poor source of micronutrients such as iron (Fe). Fe uptake from the rhizosphere and homeostasis within the plant body is extremely important for plant growth and development. As in other plants, micronutrient transport in rice is controlled at several stages, including uptake from soil, transport from root to shoot, careful control of subcellular distribution, and finally transport to seeds (Bashir et al., 2010, 2011a, 2013; Ishimaru et al., 2011; Kobayashi et al., 2014). As excess Fe in the cytoplasm may be toxic, it is either stored as ferritin in chloroplasts or is diverted to the vacuole. To enhance micronutrient accumulation in rice seeds, we need to fully understand and carefully regulate all these processes.

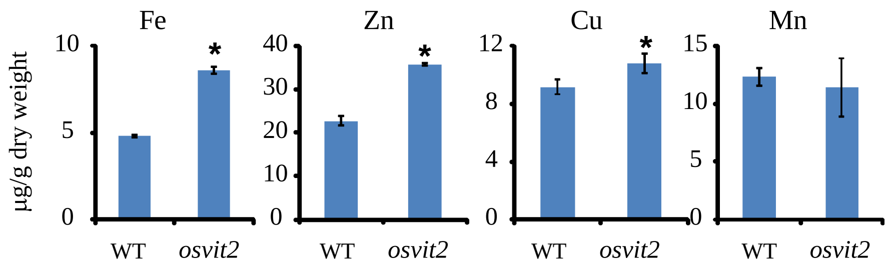
Materials and methods

We characterized knock down mutants of mitochondrial Fe transporter (MIT) and vacuolar Fe transporter 2 (VIT2). Integration of T-DNA in these mutants was confirmed through genomic PCR. Quantitative PCR analysis confirmed that the expression of *OsVIT2* and *MIT* was significantly downregulated in the *osvit2* and *mit-2* lines compared to wild-type (WT) plants. Detailed characterization of these mutants was done to understand the role of these transporters in metal homeostasis.

Results and discussion

OsVIT2 expression is upregulated by excess Fe (Bashir et al., 2011b), and the expression of *OsVIT2* could be observed through all developmental stages. In roots, its expression increased slightly from 27 days after transplantation (DAT) to 76 DAT and was not regulated diurnally. The expression pattern of *OsVIT2* through different developmental stages supported the earlier results o (Zhang et al., 2012) that OsVIT2 plays a critical role in transporting Fe and zinc (Zn) from leaves to seeds. Reduced expression of *MIT* significantly affects plant growth and development and plants completely lacking MIT are unable to survive. The reduced activity of MIT disturbs cellular Fe homeostasis and to partially mitigate this, the expression of *OsVIT2* was upregulated (Bashir et al., 2011c). Thus *mit-2* expresses *OsVIT2* at comparatively higher rates, so we assessed whether the concentration of Fe and other metals also changed in *mit-2* seeds. The concentration of Fe was higher in leaves harvested from *mit-2* plants, however *mit-2* seeds accumulated less Fe in the embryo compared to WT plants, and in *mit-2* lines complemented with *MIT*, the localization of Fe was comparable to that in WT plants. The concentrations of Fe, Zn, and copper (Cu) increased in *osvit2* seeds, while that of manganese (Mn) decreased. A knockout line of *osvit2* was previously shown to accumulate an increased amount of Fe in its seeds (Zhang et al., 2012); however, in that report, the authors did not analyze the metal concentration in polished rice. During milling, the embryo is removed during milling, leaving the endosperm as the only edible part, we measured the concentration of Fe and other metals in polished rice seeds (rice endosperm) (Figure 1). In polished *osvit2* seeds, the concentrations of Fe, Zn, and Cu were significantly elevated compared to WT seeds. Rice is poor in nutrients such as Fe, and people who depend on rice as a staple food often suffer from Fe deficiency (Bashir et al., 2010, 2013b). Thus, breeding rice plants that are capable of accumulating more Fe and Zn in the endosperm is important (Bashir et al. 2012, 2013b). These results suggest that subcellular Fe transporters affect seed Fe localization; thus, it would be interesting to regulate the expression of these transporters to biofortify rice with Fe and Zn without causing any adverse effects on plant growth and development.

(a)



(b)

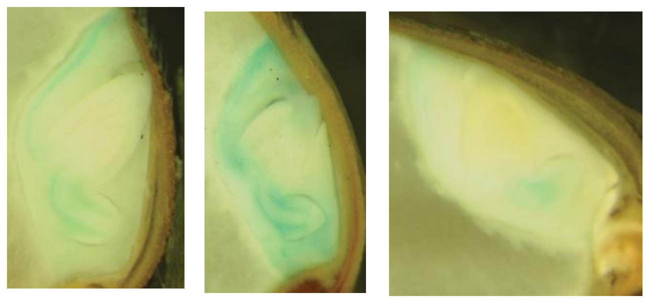


Figure 1. Metal concentration in polished WT and mutant seeds
a). Metal concentration , b). Fe localization in embryo (Perls staining)

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Perspectives of Cereals Biofortification with Folate in Pakistan

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Introduction

Folate or folic acid is form of B vitamin, soluble in water and essential in the human diet. It is involved in synthesis of nucleic acid (DNA and RNA), red blood cells and metabolism of amino acids. Folates are co-factors and co-substrates for biological methylation and nucleic acid synthesis and also function as regulatory molecules (Figure 1). Human body has no mechanism to synthesize folate (vitamin B_9) and depend on plants for these essential vitamins. The recommended dietary allowance (RDA) of folate for an adult person is 400 µg and 600 µg for a pregnant woman (Bailey and Gregory III, 1999).

Folate deficiency causes serious health problems such as megaloblastic anemia and neural tube defects (NTD). It has been expected that the neural tube is formed between 21-27 days after conception. Modern researches have shown that chances of NTD incidence can be up to 10 times more in the poorest states such as India (Figure 2). Folate deficiency also causes various neurodegenerative conditions like Alzheimer's disease, a higher risk of cardiovascular disease and development of a range of cancers. Other complications of folate deficiency are the introduction of hyper-homocysteinemia, a threat for heart attack (Terwecoren et al., 2009), missing-corporation of uracil in DNA and eventually chromosomal breakage that causes cellular degeneration. Finally, deficiency of folate causes abnormal DNA methylation sequence related with carcinogenesis.

Globally, folate deficiency induced health problems are of high concern particularly in the developing countries and developed world as well. However, fortification of industrial food and folic acid supplementation are useful approach but only in developed countries. Poor resource countries may not acquire this approach because food supplements are not cost effective for developing countries (Bekaert et al., 2007). Food diversity can be another approach, which is also limiting in developing countries because most people depend on staple foods only. Unluckily, the crops which are consumed by people as staple food contain inadequate quantity of micronutrients such as folate. Recent developments have shown that biofortification of food crops with essential food components can be a cost effective approach to combat malnutrition. As plants have ability to synthesize folate, the accumulation of it in edible parts of staple crops can be a good approach to improve folate in human nutrition in poor resource population world-wide

Folate status in Pakistani population

In Pakistan, like other developing countries, human nutrition does not meet the requirement of essential nutrients like folic acid (vitamin B). Severe anemia (hemoglobin, Hb < 70 g L^{-1}) is considered a key causative factor for mortality rate. Pregnant women and young children are mainly at highest risk of anemia. Severe anemia in pregnant women is associated with a prominent risk of maternal mortality. In Pakistan, 15% anemia cases are being reported in pregnant women, 11-12% of 6-24 month old children and iron+folate supplements showed positive effect on child birth-weight (Bhutta et al., 2009).

Transgenic Biofortification of Cereals

Naturally staple crops are not able to synthesize folic acid in sufficient amount to fulfill the nutritional requirements of populations of developing countries. However there are certain wild plants such as sea buckthorn (*Hippophae rhamnoides* L.) have high concentrations of folate not only in shoot but also in berries. A number of wild species of sea buckthorn can be found in northern areas of Pakistan. Exploring the genes involved in folate biosynthesis in sea buckthorn, and characterizing these genes functionally after transformation into cereals crop (wheat, rice and maize) seems to be a potential approach to solve the problem of folate deficiency in Pakistan.

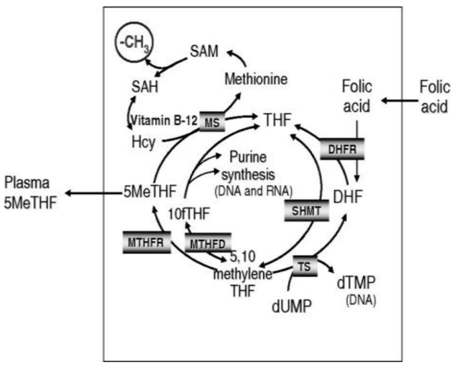

Figure 1. Folate cycle in the cytoplasm
Source: Smith et al. (2008)

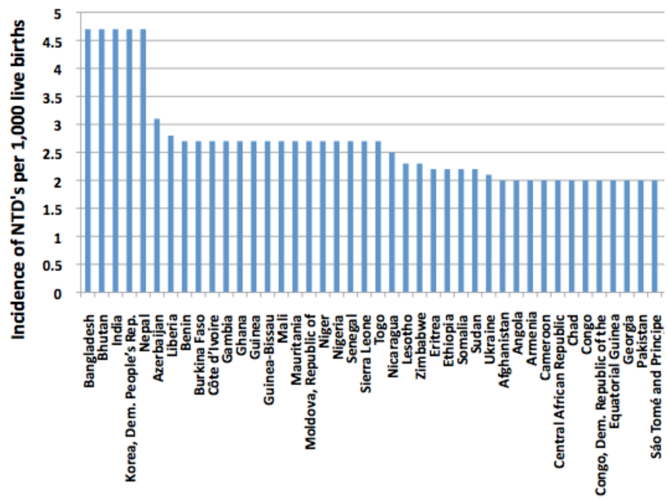

Figure 2. The top listed countries with highest annual neural tube defects (NTD) prevalence.
Source: Zaghi et al. (2013).

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Foliar Application of Zinc: An Efficient Technique for Zinc Fortified Tomato Production in Alkaline Calcareous Soils

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Introduction

Zinc (Zn) deficiency is hampering crop production and human health worldwide due to low intake of Zn in our diet. In Pakistan at present more than 50% population is Zn deficient and suffering several health problems related to Zn deficiency. Intervention of Zn enriched food in our daily life is best approach to address this problem. During recent years agronomic bio-fortification technology has been developed for wheat grains Zn-enrichment on alkaline calcareous soils of Pakistan (Hassan et al., 2012, Zou et al., 2012). Tomato (*Solanum lycopersicum* L.) is an important vegetable that is a commonly used in our daily diet. Zinc deficiency has caused yield and quality losses in tomato (Ejaz et al., 2011). Like other crops grown in Pakistan the major causes of low zinc uptake in tomato plants are; alkaline calcareous soils, rare use of Zn fertilizers and lack of tomato-specific Zn-technologies. This study was conducted study the influence of Zn application on the fruit yield and Zn fortification of tomato in alkaline calcareous soils.

Materials and methods

This pot experiment was conducted in net-house at Nuclear Institute for Agriculture and Biology (NIAB) during 2012 using Zn deficient calcareous soil (0.53 mg kg^{-1} DTPA-Zn, 8.3 pH, 10% $CaCO_3$) while growing two genotypes of tomato viz. Nagina and LB6×Nagina. Zinc treatments comprised of control (no application of Zn), soil applied Zn (7 mg Zn kg^{-1} soil), soil applied Zn +2 foliar spray of 0.5% $ZnSO_4.7H_2O$ solution (foliar application at 3 and 4 weeks after transplanting), and soil applied Zn + 4 foliar spray of 0.5% $ZnSO_4.7H_2O$ solution (foliar application at 3, 4,5, and 6 weeks after transplanting. Basal dose of NP was applied at the rate of 60 and 40 mg kg^{-1} soil. For Zn treatments, $ZnSO_4.7H_2O$ (33% Zn) was used, and for N and P treatments urea (46%) and single super phosphate (18% P_2O_5%) was applied, respectively. The experiment was laid out in randomized complete block design with four treatments and five replicates on two genotypes.

Results and discussion

Foliar Zn treatments significantly affected fruit weight, number of fruits $plant^{-1}$, dry plant weight, fruit yield, and Zn concentration in fruit as compared to control and soil Zn treatments. Foliar applied Zn treatments (2 and 4 foliar sprays) coupled with soil applied Zn gave highest fruit weight $plant^{-1}$ in two genotypes (Table 1). Highest number of fruits per plant and dry plant weight in genotypes LB6×Nagina and Nagina with highest achievements in foliar Zn treatments (2 foliar and 4 foliar sprays of Zn). In LB6×Nagina, highest fruit yield (2667 g per $plant^{-1}$) and fruit-Zn concentration (63 mg kg^{-1}) was achieved by 4 foliar sprays of Zn along with soil application of Zn. However in genotype Nagina, highest fruit yield (2493 g $plant^{-1}$) and fruit-Zn concentration (73 mg kg^{-1}) was obtained by 2 foliar and 4 foliar sprays of Zn alongwith soil applied Zn (Figures 1, 2). In both genotypes of tomato, foliar feeding of Zn showed better impact on plant's agronomic traits as compared to control (no Zn) and soil applied treatments. These results indicate that foliar feeding in tomato in combination with soil application of Zn is better approach as compared to soil application of Zn alone. This technology may help for getting higher tomato yield and production of biofortified (Zn-enriched) tomato for economic benefits of farmers and improvement of human health.

Table 1. Effects of soil and foliar applied zinc on fruit yield, fruit fresh weight, and number of fruit per plant of tomato genotypes

Treatments	Fruit weight (g per fruit)		Number of fruits per plant		Plant dry weight (g)	
Control	68 c	50 c	27 ab	18c	180 bc	145 b
Soil applied Zn (7 mg Zn kg^{-1})	72 b	55 bc	23 b	26 ab	169 c	147 b
Soil applied Zn + 2 foliar sprays (0.5% $ZnSO_4.7H_2O$)	97 a	73 a	29 ab	31a	231 a	186 a
Soil applied Zn + 4 foliar sprays (0.5% $ZnSO_4.7H_2O$)	77 b	67 ab	33 a	20 bc	213 ab	189 a
LSD (P<0.05)	8.11	12.78	6.48	7.99	27.02	10.61

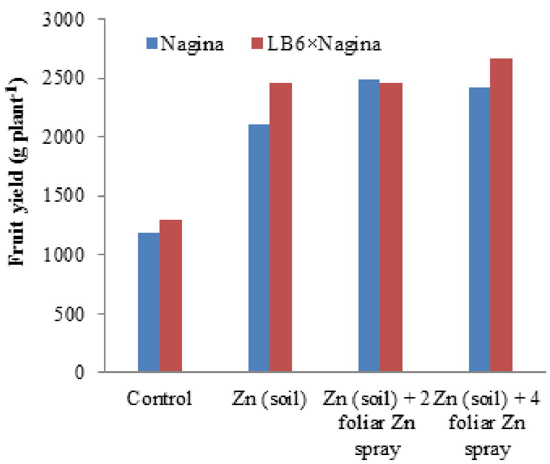

Figure 1: Fruit yield as affected by different zinc treatments

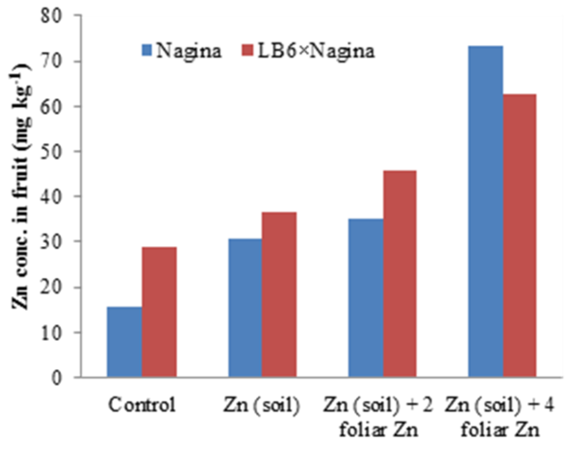

Figure 2: Zinc concentration in fruit by different zinc treatments

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Biofortification of Wheat Grain by Integrating Novel Genomic and Genetic Resources into Breeding Program

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Introduction

Biofortification of wheat could play a pivotal role in reducing hidden hunger in developing countries, where wheat is the major source of protein and nutrients. We investigated 232 D-genome synthetic hexaploid wheats (SHWs) to explore new genetic variability that may be exploited for wheat biofortification. Grain iron (Fe) ranged between 13.47 to 190.52 mg kg^{-1} with an average of 67.25 mg kg^{-} 1, and grain zinc (Zn) ranged between 13.12 to 190.79 mg kg^{-1} with an averge of 49.73 mg kg^{-1}. Some accessions (AUS34236, AUS30295, AUS30298, AUS30289 and AUS30284) showed exceptionally high Fe and Zn contents while maintaining thousand grain weight (TGW) and number of grain per spike. Positive corelation was observed between iron and zinc (r=0.14) Fe and TGW (r=0.18), while negative correlation was observed between Zn and TGW (r=-0.04). Genome-wide association analysis using DArT markers identified 12 marker-trait associations (MTAs) for Fe and Zn contents distributed over 10 loci on chromosomes 1B, 2A, 2D, 3B, 4A, 6A, 6B and 7D, of which 1B, 3B, 2D and 4A are novel. These MTAs may provide wider allelic coverage due to use of *Ae. tauschii* within SHWs, as well as a promising source of micronutrients for future breeding programs of bread wheat.

Materials and methods

In this study, the collection of 232 D genome SHWs (derived from mutual combinations of 44 durum wheat varieties and 96 *Ae. tauschii*) were used. Histrically, SHWs were produced at International Maize and Wheat Improvement Center (CIMMYT) by artificially crossing the elite durum wheat cultivars or their advanced breeding lines with different accessions of *Ae. tauschii*. The grain Zn and Fe concentrations were determined by the standard modified protocols of (Zarcinas et al., 1987). No specific permits were required for the described field trails. The field trials were performed by applying alpha lattice design with three replications with plot size of 1 x 2 m^2. Grain iron and zinc concentration were calculated from digested sample of grains using spectrophotometer in 2014 and phenotypic data for agronomic traits descriptors were averaged from cropping season in 2013-2014 at National Agriculture Research Centre, Islamabad, Pakistan.

Results and discussion

An average Fe contents were 67.25 mg kg^{-1} and 49.73 mg kg^{-1} for Zn (Table 1) were observed in present study. AUS30295 and AUS30643 carrying highest Fe and Zn content, respectively are identified as promising candidates to be used in wheat breeding. In SHW, Fe had significant positive correlation with TGW contrasting to Zn concentrations which had slightly negative correlation with TGW (Table 2). The narrow genetic background created by continuous selection over centuries by breeders especially the use of semi-dwarf verities to enhance yield has depleted the genetic variability of high micronutrient lines. SHWs characterized for grain Fe and Zn, and important agronomic traits will provide a dynamic tool for future breeding program of bread wheat i.e. new alleles identified in this study and even the already reported allele could be used in advance breeding programs. The possible exploitation of only candidate gene, *NAM-B1*, for enhancing micronutrients is very limited due to availability of functional alleles in very few genotypes and yield penalty associated with this gene (Uauy et al., 2006). Therefore, the SHWs could also be used for subsequent validation of identified MTAs which would lead to identification new genes for micronutrient enhancement. The simultaneous agronomic and biofortification characterization of SHWS will add value to genetic resource enhancement and actually not separate solution; perhaps they are complementary and synergistic.

Table 1: Descriptive statistics and ANOVA of all traits observed in 132 SHWs of D-genome

Statistic	Minimum	Maximum	Mean	SD	ANOVA (P)
Fe (mg kg^{-1})	13.47	190.52	67.25	18.9	0.44
Zn (mg kg^{-1})	13.12	190.79	49.73	15.7	0.001**
No. of spikes	2	14	6.3	2.3	<0.0001**
Spike length (cm)	5	18.5	11.6	2.2	0.009**
Flag leaf area (cm^2)	11.8	43.5	28.7	6.3	0.986
Grains per spike	27	75	52.5	7.6	0.05*
Plant height (cm)	84.5	142.3	115.3	14.2	0.05*
Heading days	150	169	156.1	4.2	0.899
TKW (g)	25	62.4	34.9	13.2	0.192

* Significant at $P < 0.05$, ** Significant at $P < 0.01$; SD = Standard deviation

Table 2: Correlation matrix (Pearson) between grain iron, zinc and other yield components

Variables	Iron	Zinc	No. of spikes	Tillers	Spike Length	Flag leaf area	Grains per spike	Plant height	Heading days
Zinc	0.13								
No. of spikes	0.11	**0.2**							
Spike length	0.13	0.06	**0.33**	**0.34**					
Flag leaf area	**0.21**	0.02	**0.22**	**0.22**	**0.56**				
Grains per spike	-0.04	0.1	**0.32**	**0.28**	**0.56**	**0.43**			
Plant height	-0.17	0.06	0.07	0.06	-0.02	-0.00	0.09		
Heading days	-0.17	0.05	0.04	0.02	0.02	0.06	0.11	-0.001	
TKW	**0.18**	-0.04	0.06	0.12	0.1	0.16	0.05	0.02	-0.1

Values in bold are different from 0 with a significance level alpha=0.05; TKW = Thousand kernel weight

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Zinc Biofortification in Grain of Wheat Grown on Zinc Deficient Soils of Punjab (Pakistan): Findings from Six Interlinked Experiments

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Introduction

Inadequate dietary zinc (Zn) intake is a common health risk factor in Pakistan and many other developing countries. Due to their low Zn status, cereal grains are considered major cause of Zn malnutrition in population groups greatly reliant on cereal grains for daily calorie intake. Therefore, Zn biofortification of wheat and other cereals is considered an economic solution to the problem of human Zn deficiency (Cakmak, 2008). Research experiments were conducted to assess the potential of agronomic Zn biofortification strategies for wheat grown in Punjab along with comparison of wheat cultivars for grain Zn concentration and bioavailability. The findings summarized here are part of PhD thesis and salient publications produced from the projects (Hussain, 2013; Hussain et al. 2013a, b, 2012a, b, c).

Materials and methods

The research was comprised of six interlinked investigations. In the first study, grains of 65 wheat cultivars, representing local and foreign genetic pools, were analyzed to achieve new information on the genetic variability of Zn and phytate concentrations in wheat grains. In another experiment, forty wheat cultivars, released in Punjab (Pakistan) during last five decades, were grown under uniform field conditions to evaluate the genetic variability of Zn and phytate concentrations. Moreover, qualitative ([phytate]:[Zn] ratio) and quantitative (based on trivariate model of Zn absorption) estimations of Zn bioavailability were also calculated for each cultivar (Rosado et al., 2009).

In study 3, Zn indexing in wheat grains and calcareous soils of the southern Punjab was carried out to determine present levels of Zn in wheat grains and in associated soils. In another study, variation in Zn, phytate and Zn bioavailability was investigated over a wide range of Zn application rates to soil. Zn application to seed, soil and foliage were evaluated in study 5 to find out best Zn application method for Zn biofortification of wheat grains. In the final study, Zn bioavailability in different milling fractions of control and biofortified wheat grains was estimated to devise a milling strategy for efficient Zn delivery to people.

Results and discussion

Zinc concentration and Zn bioavailability was significantly lower in grains of new cultivars when compared with obsolete cultivars. The current wheat cultivars of Punjab produced higher grain yields but had lower concentration and bioavailability of Zn than the obsolete cultivars. Much of this variation was related to an increased grain weight in new cultivars. From the survey of southern Punjab, it was observed that about 75% of surface (0–15 cm) and all subsurface (15–30 cm) soil samples were deficient in plant available Zn resulting in a low grain Zn concentration (20 ± 4 μg g^{-1}, on average). Therefore, an effective breeding program with optimized agronomic approaches of Zn application for improving the Zn bioavailability in grains of cultivated bread wheat seemed imperative.

In another study, it was observed that the human requisite Zn levels in wheat grains were estimated to be achieved at very high soil Zn application rates (>12 mg Zn kg^{-1} soil). A lower rate of soil Zn application (9 mg Zn kg^{-1} soil) and foliar Zn sprays at heading also increased Zn concentration (>50 μg g^{-1}) and estimated Zn bioavailability (>3 mg Zn per 300 g flour) in wheat grains to desired levels. However, Zn in wheat grain is not uniformly distributed (Cakmak et al., 2010). For control and biofortified grains, bran had greater Zn and phytate concentrations as compared to other grain milling fractions. Bran and shorts also had greater Zn bioavailability as compared to other fractions.

It is concluded that selection and improvement of wheat germplasm, Zn application to soil and foliage, and biofortified whole-grain flour are required for better Zn nutrition (>3 mg Zn for 300 g flour) of human population living in the region.

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Zinc Application Methods Influenced the Zinc Bioavailability in Rice Grains

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Introduction

Zinc (Zn) deficiency in soils has been recognized as widespread nutritional problem throughout the rice growing countries. Low Zn phyto-availability is primarily attributed to its high fixation, due to high pH, calcareous nature of soil and submerged conditions (Alloway, 2009). In Pakistan, about 70% of soil in rice belt are Zn deficient (Hamid and Ahmad, 2001). Zinc biofortification of cereal grains is suggested for controlling widespread human Zn deficiency in developing countries. In present study, rice crop was supplied with different Zn treatments (seed priming, root dipping, soil application, foliar application and soil + foliar application) for biofortification of rice grains.

Material and methods

A field trail was carried out at Research Farm of Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad (Pakistan). Rice cv. Basmati-515 was tested for following Zn treatments: control, seed priming, root dipping, soil application, foliar application and combination of soil + foliar Zn applications were applied as $ZnSO_4{\cdot}7H_2O$ to rice grown under submerged conditions. Twenty-five day old rice seedlings were transplanted to experimental plots and Zn treatments were applied to the soil and respective parts of the plants.

At maturity, harvested grain samples were analyzed for Zn and phytate concentrations. To have a quantitative estimate of Zn bioavailability, trivariate model of Zn absorption was employed to calculate human Zn bioavailability (Miller et al., 2007).

Results and discussion

Soil and combined soil + foliar application of Zn significantly ($P \leq 0.05$) increased grain and straw yield (data not shown). Increased grain Zn concentration also significantly decreased grain phytate content. Phytate content seed^{-1} was influenced only when soil Zn was included in treatments; Zn treatments significantly influenced phytate content ha^{-1}. Whole grain Zn concentration increased from 22 (at control) to 29 mg kg^{-1} (at soil + foliar application) (Figure 1a). However, soil Zn application alone and root dipping alone had only a medium effect on grain Zn concentration. Zinc applications methods, especially soil + foliar application, decreased grain [phytate]:[Zn] ratio and increased estimated human Zn bioavailability in grains based on trivariate model of Zn absorption (Figures 1b, 1c). Similarly, soil + foliar Zn application increased trivariate model of Zn absorption based estimated Zn bioavailability from 1.1 to 1.5 mg Zn for 300 g rice grain. The physiological Zn requirement of an adult human, net Zn that need to be absorbed d^{-1} is 3 mg (Institute of Medicine, 2001), while 300 g rice biofortified grains can only ensure half of the daily Zn requirement. Conclusively, soil + foliar Zn application is suitable for optimum agronomic Zn biofortification of rice grains.

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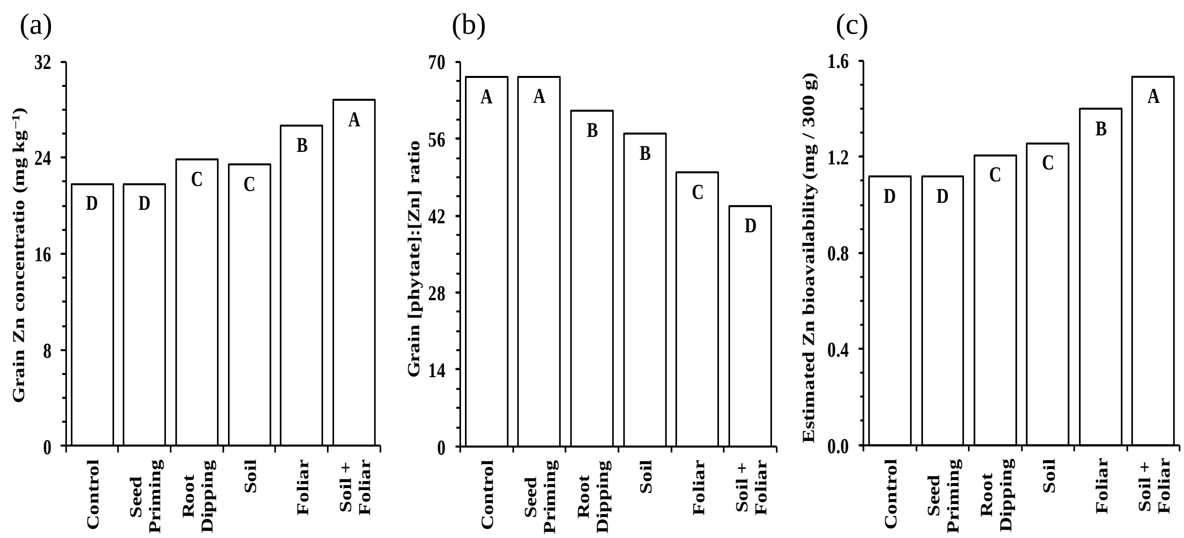

Figure 1: Grain Zinc concentration (a), Zinc bioavailability estimated as [phytate]: [Zn] ratio (b) and trivariate model of Zn absorption based human Zn bioavailability (c) in grains of rice crop grown in field and differentially treated with Zn. Different letters in the same column indicate significant differences by LSD at $P \leq 0.05$.

Seed Priming with Boron Improves Wheat Productivity and Grain Biofortification

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Introduction

Boron (B) is among the essential micronutrients, which is equally crucial for plants and human. In developing world, hidden hunger is major health controversy, which emerges due to mineral deficiency in edible grains (Buyckx, 1993). Micronutrient contents of edible grains may be improved by combined use of conventional breeding approaches as well as biotechnology which is mainly referred as biofortification (Hotz and Bonnie, 2007). Asian countries are widely becoming deficient in micronutrients. Nutrient replenishment is necessary for profitable cropping. Micronutrients can be applied by three methods i.e. foliar sprays, soil fertilization and seed treatment (seed priming and seed coating). These methods can enhance micronutrient contents of treated as well as the progeny plants (Johnson et al., 2005). Seed treatments are not only cost effective approach but are also easy to apply and have pronounced effect on plant growth (Singh et al., 2003). This study was conducted to evaluate the potential of seed priming with B solutions in improving productivity and B grain enrichment in wheat.

Materials and methods

This study was conducted at agronomic research farm, Department of Agronomy, University of Agriculture, Faisalabad. Seeds of wheat cultivars Mairaj-2008 and Faisalabad-2008, used in this study, were collected from Regional Agriculture Institute, Bahawalpur, Pakistan and Wheat Research Institute, AARI, Faisalabad, Pakistan, respectively. Seeds, of both wheat cultivars, were soaked in solutions of boric acid [0.1, 0.01 and 0.001% (w/v) boron]. Seeds soaked in aerated water and untreated seeds were taken as control. In both cases, soaking was done for 12 h in aerated solution (nutripriming) or water (hydropriming) keeping seed to solution ratio 1:5 (w/v). After removing with water and dried in forced air under shade till original weight. Treated and untreated seeds were sown with hand drill on November 29, 2010 using seed rate of 125 kg ha^{-1} in 22.5 cm spaced rows. Crop was harvested on April 24, 2011 at harvest maturity and was threshed to record the grain yield. Boron grain contents were estimated by azomethine-H method by spectrophotometry method (Malavolta et al., 1997). The data recorded were analyzed statistical software MSTAT-C.

Result and discussion

Seed priming with B significantly affected the grain yield; likewise, wheat cultivars differed significantly for grain yield (Table 1). However, interaction of wheat cultivars and seed priming was non-significant for grain yield (Table 1). Except seed priming in 0.1% B, seeds from all priming treatments caused increase in grain yield than control (Table 1). Seed priming with B significantly affected the grain B contents (Table 1). However, tested wheat cultivars didn't differ for grain B contents (Table 1). Moreover, interaction of wheat cultivars and seed priming was also non significant for grain B contents (Table 1). Except hydropriming, seeds from all priming treatments caused an increase in grain B contents than control (Table 1).

In conclusion, seed priming with B with lower concentration i.e. 0.001% B has a potential to improve grain yield and grain B contents in wheat.

Table 1: Influence of seed priming with boron on yield related traits and grain boron contents in wheat

Treatments	Grain yield (t ha^{-1})			Grain B contents (μg g^{-1})		
	MRJ-2008	FSD-2008	Mean	MRJ-2008	FSD-2008	Mean
Control	9.46	10.25	9.56 AB	4.53	4.57	4.49 BC
Hydropriming	8.64	10.25	9.45 B	4.36	4.53	4.45 C
Osmopriming (0.1% B)	6.10	8.64	7.82 C	4.90	4.46	4.68 BC
Osmopriming (0.01% B)	8.64	9.88	9.26 B	4.94	5.35	5.15 A
Osmopriming (0.001% B)	10.25	10.29	10.27 A	4.83	4.79	4.81 B
Mean	8.80 B	9.86 A		4.71	4.72	

Interaction and main effects not sharing the same letter for a parameter do not differ significantly at p 0.05 by the least significant difference test

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Zinc-enriched Fertilizers as a Potential Public Health Intervention in Africa

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Introduction

Dietary zinc (Zn) deficiency is widespread in sub-Saharan Africa causing a large burden of disease. Zn-enriched fertilisers are commonly used to correct crop deficiencies in areas with low phyto-available Zn (Cakmak, 2004; Ahmad et al., 2012). Recently, there has been increasing interests in deploying high application rates of Zn (~10-25 kg ha^{-1}) to cereals to increase grain Zn concentration and increase dietary Zn supplies (Cakmak, 2008; White and Broadley, 2009). In this review, we examined the potential of Zn-enriched fertilizers to alleviate dietary Zn deficiency in 10 African countries where fertiliser subsidies are routinely deployed on cereal crops, providing leverage to implement and enforce Zn enrichment of granular fertilizer.

Materials and methods

Dietary Zn supply and deficiency prevalence were quantified from food supply and composition data (Wessells and Brown, 2012). Typical effects of soil (granular) and foliar Zn applications on yields and Zn and phytic acid concentrations in maize (*Zea mays* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) grains were based on a systematic literature review. The potential efficacy of soil-applied Zn was based on current fertiliser usage while foliar strategies were modelled at 50 and 75 % coverage of cereal crops. Reductions in disease burdens attributable to Zn deficiency was estimated using a disability-adjusted life years (DALYs) approach.

Results and discussion

Baseline Zn supply in 2009 ranged from 7.1 (Zambia) to 11.9 (Mali) mg *capita*$^{-1}$ d^{-1}; prevalence of Zn deficiency ranged from 24 (Nigeria) to 66% (Zambia). In reviewed studies, soil Zn application led to an increase in median Zn concentration in maize, rice and wheat grains of 23, 7 and 19%; foliar application led to increases of 30, 25 and 63%. Enriching granular fertilisers within current subsidy schemes would be most effective in Malawi, reducing DALYs lost due to Zn deficiency by 10%. The cost *per* DALY saved ranged from US$ 624 to 5,893 via granular fertilisers and from US$ 46 to 347 via foliar fertilisers. Foliar applications are likely to be more cost effective than soil applications due to fixation of Zn in the soil but may be more difficult to deploy. Generally, the cost-effectiveness of foliar-applied Zn appears to be equivalent to fortification of staple flours at centralised milling facilities but less cost-effective than breeding in the longer term. Soil-applied Zn appears to be more expensive but has the potential advantage of reaching more households. Synergies might be realised if agronomic (fertilisation) and genetic (breeding) biofortification efforts are combined, potentially improving both impact and cost-effectiveness of these interventions.

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Table 1 Summary of the effects of zinc (Zn)-enriched fertiliser on Zn concentration in the grain of maize, rice and wheat. 'n' is the number of data points contributing to the meta-analysis in which individual studies were stratified by crop, cultivar, location and Zn application rate and pooled by application method. Q1 and Q3 = first and third quartiles, respectively

Crop	**Application *via***	**N**	**Mean**	**SD**	**Min**	**Q1**	**Median**	**Q3**	**Max**
			Zn concentration in the grain (% of control)						
Maize	Soil	12	128	18	106	114	123	139	173
Rice		30	111	15	84	102	107	119	157
Wheat		158	143	57	51	105	119	174	373
Maize	Foliar	1	130	-	130	-	130	-	130
Rice		34	127	19	107	114	125	140	172
Wheat		38	178	55	112	143	163	203	333

Table 2 Effect of different zinc (Zn) fertilisation scenarios on dietary Zn supplies and estimated risk of Zn deficiency in Malawi (for brevity, other countries are not presented here). Scenario '0' is baseline; 1 and 2 model enrichment of granular fertilisers, either subsidised or subsidised and non-subsidised; 3 and 4 model application of foliar Zn sprays to 50 and 75 % of target crops. Scenarios that are cost-effective in comparison to WHO (*) or World Bank and WHO (**) benchmarks are highlighted

Scenario	**Dietary Zn supply**	**Zn deficiency risk**	**DALYs lost due to Zn deficiency**	**Programme cost**	**Cost *per* DALY saved**
	mg *capita*$^{-1}$ d^{-1}	%	100 k population^{-1}	US$ '000s yr^{-1}	US$ yr^{-1}
0	8.9	54.8	768		
1	9.2	49.6	695	15,675	1,431
2	9.3	46.6	653	25,115	1,456
3	9.7	43.5	610	3,132	132**
4	10.1	38.7	542	4,698	138**

Zinc Supply and Deficiency Risk in Southern Asia

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Introduction

Chronic under nutrition affects >800 million people worldwide, with >2 billion people estimated to be suffering from 'hidden hunger'. Hidden hunger is the term given to mineral micronutrients deficiency (MND) which is not always visible or easy to quantify. Zinc (Zn) is one of the essential micronutrients in human nutrition making up ~2 g of human body (WHO and FAO, 2004). Zinc plays vital role in growth, sexual maturity, immunity, cognitive development. Deficiency of Zn can lead to retarded growth and cognitive development, impaired immunological functioning, and non-communicable disorders such as skeletal, cardiovascular and metabolic disorders. Zinc and iron deficiencies are estimated to reduce the Gross Domestic Products (GDP) of developing countries by 2-5 % (Stein, 2014). Human Zn nutritional status can be assessed using tissue biomarkers, dietary recall, and food balance sheets (FBS). A FBS-based method can provide a good estimate of population level Zn deficiency risks by comparing dietary Zn supplies with population requirements for Zn. Globally, Zn deficiency risk was estimated to be 17% in 2003-07 (Wessells and Brown, 2012) and 16% in 2011 (Kumssa et al., 2015). This paper reports temporal Zn supplies and deficiency risks in south Asian countries between 1992 and 2011 based on the study of Kumssa et al. (2015).

Materials and methods

FBS food composition data from the United States Department of Agriculture, Nutrient Data Laboratory (USDA-SR26) were used to determine the amount of Zn available in FBS food supplies from 1992-2011 (assuming there was no change in food Zn composition during this period). Zinc supplies were then compared with population weighted Zn requirements (WtdEAR) using the Estimated Average Requirement 'cut point' (EAR-CP) method to estimate the deficiency risks of Zn in south Asian countries: Afghanistan (AFG), Bangladesh (BGD), India (IND), Iran (IRN), Nepal (NPL), Pakistan (PAK), and Sri Lanka (LKA). The EAR-CP provides the number of healthy individuals in a given country with Zn intake below the Estimated Average requirement which is termed hereafter as "deficiency risk".

Results and discussion

Country level WtdEARs during the study period ranged from 10.2-10.6 mg capita^{-1} d^{-1}. In 2011, Zn supplies were 17, 10, 12, 20, 15, 14 and 11 mg capita^{-1} d^{-1} with a corresponding Zn deficiency risk of 6, 48, 28, 3, 12, 15 and 43% in AFG, BGD, IND, IRN, NPL, PAK and LKA respectively. In 2011, about 9, 2, 3, 2, 345, 74, and 27 million people in AFG, BGD, IND, IRN, NPL, PAK and LKA, respectively, were likely to be affected by Zn deficiency risk. Zinc deficiency risks changed by -5, -21, -1, 0, -10, 1 and -20% during the study period in AFG, BGD, IND, IRN, NPL, PAK and LKA, respectively. Cereals were the primary sources of Zn contributing 50–80% to the per capita dietary Zn supply in the region. Phytic acid (PA) to Zn (PA: Zn) ratio in south Asian countries ranged between 11-21.The prevalence of dietary Zn deficiency risk in these countries during the 20 y period was uneven. Although the overall percentage of Zn deficiency risk showed a decreasing trend, those affected by dietary Zn deficiency risk in the region increased from 377 million (30%) in 1992 to 462 million (22%) in 2011. Southern Asia accounted for 43% of the global dietary Zn deficiency risk in 2011 (Kumssa et al., 2015). The estimated dietary Zn deficiency risk in this study is likely to be conservative because >75 % of the dietary Zn in this region is derived from cereals which contain high concentration of phytic acid [PA] (myoinositol hexakisphosphate) which in turn is known to chelate Zn reducing its absorption in the gut.

The data used in this study should be interpreted with caution. For example, food supply data do not capture variations in access between households due to socio-economic factors. Furthermore, the food composition data do not capture temporal, spatial, and varietal variation in food composition data. Both these factors will have large effects on estimates of Zn deficiency risks. Further research could include assessing the health, and nutritional status of various age/gender/socioeconomic groups through biochemical, clinical and anthropometric measurements in countries with high deficiency risks of Zn. Development of localized food composition tables and updating existing ones with information on new/under-utilised food crop varieties is crucial to improve the accuracy of estimating deficiency risks of Zn.

Possible solutions to Zn deficiency include: supplementation, direct fortification of foods by fertiliser application and plant breeding. Supplementation is crucial in situations that require short term actions with high impact, for example, for pregnant women who cannot supply the developing foetus with these nutrients. However, supplementation and direct fortification of foodstuff with Zn is likely to be relatively expensive and it will not reach the majority of the population in developing countries who produce their own food. Under such circumstances, agronomic intervention by applying Zn fertiliser can help to increase the composition of Zn in food crops (Joy et al., 2015). Similarly, breeding food crop varieties with the ability to absorb and accumulate more Zn from the soil and translocate them to the edible parts, or with lower PA composition to improve bioavailability may be considered for biofortification (White and Broadley, 2009). In addition, production and provision of affordable animal products, and education to reduce the impact of PA in plant source foods on Zn bioavailability (for example, soaking, germination, and fermentation) are essential (Gibson et al., 2010). We conclude that continuing to reduce Zn deficiency risks through dietary diversification and food and agricultural interventions including fortification, crop breeding and use of micronutrient fertilizers will remain a significant challenge.

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Harvest Zinc Project: Agronomic Biofortification of Zinc in Wheat in Pakistan

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Introduction

Zinc (Zn) deficiency is the most common micronutrient disorder in crop plants in Pakistan because the soils are alkaline-calcareous and low in organic matter (Rashid, 2005). Thus, wheat, grown on >8 M ha, suffers with yield loss and low-Zn density in grains (Zou et al., 2012). Widespread Zn malnutrition in Pakistan, especially in women and children (Govt. of Pakistan, 2011), is believed to be induced predominantly because of low-Zn staple cereal, wheat. Therefore, multi-location, multi-year field experiments were carried out in major cropping systems of the country to determine effectiveness of soil-applied and foliar-fed Zn in enhancing wheat yield and grain Zn density as well as to determine appropriate foliar spray strategies and impact of high-Zn wheat seed on crop establishment and productivity of the next crop.

Materials and methods

During 2008–11, field experiment on wheat (cv. Sehar-2006), with four Zn treatments and four replications, was conducted in rice-wheat tract in the Punjab (Table 1) on low Zn calcareous soils (DTPA Zn, 0.3–0.7 mg kg^{-1}; $CaCO_3$, 10–12%). Soil Zn was applied along with basal fertilizers while foliar Zn was applied one week before heading and one week after heading. During 2011–13, RCBD field experiments, with four replications, were conducted on wheat (cvs. Sehar-2006, Lasani-2008, Faisalabad-2008) in rice-wheat, mixed cropping, and cotton-wheat systems on low-Zn calcareous soils (DTPA Zn, 0.4–0.9 mg kg^{-1}; $CaCO_3$, 8–22%) to determine appropriate foliar spray strategies and the impact of high-Zn wheat seed on crop establishment and productivity of the next crop (Table 2).

Results and discussion

During 2008–11, soil applied and foliar fed Zn fertilizer increased leaf Zn concentration, grain yield and grain Zn density at all locations during all years ($P \leq 0.05$; Table 1). Grain Zn density increased significantly with soil application of $ZnSO_4$ as well as zincated urea, but was maximized with foliar feeding of Zn and soil Zn + foliar Zn use. In terms of grain yield increase, $ZnSO_4$ and zincated urea were almost equally effective; however, both of these soil-applied Zn fertilizers proved less effective in enhancing grain Zn density. Over all, use of Zn fertilizer in wheat proved highly cost-effective; value-cost ratio was 4.6:1 for soil Zn, 8.9:1 for foliar Zn, and 3.6:1 for soil Zn + foliar Zn. Thus, these studies established that Zn fertilizer use, especially in foliar sprays, can enhance wheat grain Zn density cost-effectively.

During 2011–13, soil-applied $ZnSO_4$, zincated urea, chelated Zn (Zn-HBED), high-Zn seed and Zn-primed seed enhanced grain yield ($P \leq 0.05$; Table 2). All these treatments, except for high-Zn seed, also increased grain Zn density ($P \leq 0.05$). High-Zn seed and Zn-primed seed almost doubled the seedling density per unit area. Increases in grain yield and grain Zn density were similar with one or two foliar sprays of $ZnSO_4$; however, two sprays maximized grain Zn density. Foliar Zn + pesticide increased yield

($P \leq 0.05$) but not Zn density. Chelated Zn was not superior to $ZnSO_4$ both for soil use as well as for foliar spray.

Table 1: Wheat grain yield and grain Zn density as affected by soil applied and foliar Zn fertilizer; means of 7 field trials

Zn applied	Leaf Zn (mg kg^{-1})	Grain yield		Grain Zn concentration	
		(Mg ha^{-1})	Increase (%)	(mg kg^{-1})	Increase (%)
Control[a]	20.5 a	4.08 a		39.0 a	
Soil Zn[b]	24.1 b	4.70 b	15.2	46.5 b	19.2
Foliar Zn[c]	22.9 b	4.71 b	15.4	52.2 c	33.8
Soil Zn + Foliar Zn	24.2 b	4.81 b	17.9	52.0 c	33.3
Urea Zn[d]	24.6 b	4.54 b	11.3	46.9 b	20.3
LSD ($P \leq 0.05$)	1.7	0.27		3.8	

[a] Control = 120 kg N + 34.4 kg P ha^{-1}. All other treatments were superimposed on Control. [b] 50 kg $ZnSO_4.7H_2O$ ha^{-1}.
[c] 0.5% $ZnSO_4.7H_2O$ solution. [d] Urea granules sprayed with 10% solution of $ZnSO_4.7H_2O$

Table 2. Wheat crop stand**,** grain yield and grain Zn density as affected by soil-applied Zn fertilizers and seed Zn density; means of 5 field trials

Zn applied	Seedlings (m^{-2})	Grain yield		Grain Zn concentration	
		(Mg ha^{-1})	Increase (%)	(mg kg^{-1})	Increase (%)
Control[a]	192 a	4.19 a	-	22.9 a	-
Soil Zn	199 a	4.88 b	16.5	28.4 b	24.0
3 x Split Urea-Zn[b]	-	4.93 b	17.7	27.8 b	21.4
Zn-HBED (Zn Chelate)	-	5.00 b	19.3	28.1 b	22.7
High-Zn Seed[c]	368 b	5.06 b	20.8	24.9 a	8.7
Zn-primed Seed[d]	382 b	5.13 b	22.4	27.5 b	20.1
LSD ($P \leq 0.05$)	31	0.37		2.3	

[a] Control = 120 kg N + 34.4 kg P ha^{-1}. All other treatments were superimposed on Control
[b] Urea granules sprayed with 10% solution of $ZnSO_4.7H_2O$.
[c] Seed Zn density (mg kg^{-1}): 2011-12: Low-Zn, 32.0 & 19.4; High-Zn, 50.6 & 40.9. High-Zn seed used only in this treatment.
[d] Low-Zn seed dipped in 5 mM $ZnSO_4$ solution for 1 h.

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Micronutrients Deficiencies and Management in Pakistan: An Overview

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Introduction

The nine essential plant nutrients which are classified as micronutrients are: boron (B), chloride (Cl), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn). The cultivated soils in Pakistan (~22 Mha), predominantly alluvial and loessal in origin, being alkaline, mostly calcareous and low in organic matter, are conducive to deficiency of some specific micronutrients.

Status of Micronutrient in Pakistani Soils

First-ever micronutrient deficiency in Pakistan was identified in 1969 when *Hadda* disease of rice in Kala Shah Kaku area was diagnosed to be Zn deficiency. Initially, positive cotton response to B fertilizer was observed in Sindh in1970 (Rashid, 2005). Availability of atomic absorption spectrophotometers for heavy metal determination since early 1970s, adoption of multi-nutrient soil test for Zn, Cu, Fe and Mn (i.e., AB-DTPA) and less tedious soil test for B (i.e., dilute HCl) as well as development of plant analysis diagnostic criteria for local crop genotypes since 1980s were instrumental in promoting micronutrient research and development in the country. Extensive field, greenhouse and laboratory investigations by agricultural institutions across the country have established field-scale deficiency of Zn, B, and Fe in many agronomic, vegetable and fruit crops (Table 1). Major micronutrient research and developmentmilestones in the country were: establishment of Zn deficiency in rice and Zn use recommendation in 1970s; establishment of B deficiency in cotton and B use recommendation in 1990s; establishment of B deficiency in rice and B use recommendation in 2000s; and wheat grain Zn density enhancement with Zn use in 2000s–2010s.

The most widespread micronutrient deficiency in Pakistan is of Zn, the second most extensive is of B, and the third field-scale disorder is of Fe chlorosis (Table 1). Contrary to apprehension of B toxicity in Pakistani soils by Sillanpaa (1980), widespread B deficiency has been diagnosed instead, especially in cotton and rice. Deficiencies of Cu and Mn are of localized occurrence, and Mo deficiency does not occur in Pakistan. Silicates being the source of Ni and Co, their deficiencies are not expected (Rashid, 2005).

Management options

Zinc, B and Fe fertilizers enhance crop productivity in a highly cost effective manner (Table 2) and also improve the produce quality. In general, 2–5 kg Zn ha^{-1} and 0.75–1.0 kg B ha^{-1} are adequate for 2–4 crop seasons, as crop recovery of applied micronutrients is <2% per annum. Soil-applied Fe is generally ineffective; repeated foliar sprays of $FeSO_4$ or of chelated Fe cure the chlorosis. Crop residue recycling enhances soil micronutrient availability. Despite being highly cost–effective, actual micronutrient use is negligible compared to actual requirements. Consequently, micronutrient deficiencies are hampering crop productivity and deteriorating produce quality. The low-Zn and Fe density crop produce, especially staple foods, lead to human malnutrition.

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Table 1: Observed micronutrient deficiencies in Pakistan, based on crop responses

Crop species	Zinc	Iron	Boron
Chickpea (*Cicer arietinum*)	•	•	•
Citrus (*Citrus spp.*)	•	•	•
Cotton (*Gossypium hirsutum*)	•	•	•
Deciduous fruits (Apple, Apricot, Peach, Plum)	•	•	•
Maize (*Zea mays*)	•		•
Peanut (*Arachis hypogaea*)		•	•
Potato (*Solanum tuberosum*)	•		•
Rapeseed-Mustard (*Brassica spp.*)	•		•
Rice (*Oryza sativa*)	•	•	•
Sorghum (*Sorghum bicolor*)	•		•
Sugarbeet (*Beta vulgaris*)	•		•
Wheat (*Triticum spp.*)	•	•	•

Source: Anonymous (1998); Rashid (2005)

Table 2: Some salient yield increases and economics of zinc and boron use, in field situations

Crop/Variety	Province	Zn/B applied (kg ha^{-1})	Control Yield (t ha^{-1})	Yield Increase (%)	Value: Cost Ratio[1]
		ZINC			
Rice: Basmati-types	Punjab	5	3.48	10	6-10:1
Coarse grain	Punjab, Sindh, KPK	7.5	4.78	12	6:1
Wheat	Punjab, KPK	2.5, 5/10	3.47 - 3.82	13-14	5-7:1
Cotton	Punjab, Sindh	5	2.26	8	12:1
		BORON			
Rice: Basmati-types	Punjab	1/2	3.74	20	22:1-45:1
Coarse grain	Sindh, Punjab, KPK	1	4.82	20	25:1
Cotton	Punjab, Sindh, KPK	1–2	2.38	14	15:1
.	Punjab	Foliar sprays	2.16	12	30:1
Wheat	Punjab, KPK	1–2	3.29	14	4:1

[1]Based on prices of commercial-grade micronutrient products and crop produce.
Source: Anonymous (1998); Rashid (2005).

Zinc Nutrition Improves Grain Yield and Grain Zinc in Conventional and Conservation Rice Production Systems

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Introduction

Zinc (Zn) deficiency is the most widespread micronutrient disorder in rice, which causes drastic yield reduction. In this scenario, Zn may be delivered as soil application, foliar spray or seed treatment (Farooq et al., 2012). Nevertheless, very little information is available regarding Zn application methods in different rice production systems. This study was, therefore, conducted to determine the potential of different Zn application methods in improving the grain yield and grain Zn contents under different production system of rice.

Materials and methods

This study was comprised of two independent experiments, with same set of treatments was conducted at Agronomic Research Area, University of Agriculture, Faisalabad (UAF) (31.25^{o} N, 73.06^{o}E and 183 m asl), and at farmer's field in District Sheikhupura, Pakistan during kharif season of 2013. Both experiments were laid out in randomized complete block design in split-plot arrangement. Zinc was applied at 10 kg ha^{-1} through soil application, 0.5% Zn solution was applied as foliar spray after one month of transplanting, seed were primed with 0.5 *M* $ZnSO_4$ solution for 24 h, rice seeds were coated with 2 g Zn kg^{-1} seed using arabic gum and untreated seeds were taken as control. Rice was sown in aerobic and flooded transplanted systems. Morphological and yield related traits were taken following standard procedure. Zinc in grain was analyzed on a di-acid ($HClO_4$ + HNO_3 in 3:10 ratio) digest on an Atomic Absorption Spectrophotometer (Prasad et al., 2006). Data collected were analyzed using analysis of variance technique by Computer Software Statistics. Treatment means were compared using least significant difference (LSD) test at 5% probability level.

Results and discussion

Zinc application, by either method, significantly improved the kernel yield and kernel Zn contents at both experimental sites (Table 1). At UAF, maximum kernel yield was harvested from Zn seed coating. However, at farmer's field, maximum kernel yield was harvested from soil applied Zn, which was followed by foliage application and Zn seed coating (Table 1). ; At UAF, maximum kernel Zn contents were recorded from foliage applied Zn, which was followed by Zn soil application (Table 1). Nonetheless, at farmer's field, maximum kernel Zn contents were recorded from soil applied Zn (Table 1). Zinc nutrition facilitates the translocation of assimilates, which contributes for yield increase (Rengel, 2001). Application of Zn also increased its grain contents as Zn is also translocation towards developing kernels. In crux, Zn application by either method improved the kernel yield and kernel Zn contents of rice; however, soil at farmer's field and was the most effective in improving the kernel yield and grain Zn contents.

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Table 1: Influence of zinc application methods on kernel yield and its kernel enrichment under different production systems of rice

Treatment	UAF						Sheikhupura					
	Kernel yield (t ha^{-1})			Kernel Zn contents (μg g^{-1})			Kernel yield (t ha^{-1})			Kernel Zn contents (μg g^{-1})		
	DSR	TR	Mean	DSR	TR	Mean	DSR	TR	Mean	DSR	TR	Mean
Control	0.83	1.85	1.34 C	20.21	26.93	23.57 D	2.4	2.7	2.6 B	24.88	25.60	25.24 D
SA	1.80	2.60	2.20 B	52.15	52.90	52.52 A	3.2	3.9	3.5 A	43.82	49.23	46.52 A
FA	2.00	1.98	1.99 B	57.83	53.04	55.44 A	3.1	3.7	3.4 A	38.83	44.04	41.44 B
SP	1.78	2.07	1.92 B	49.52	45.76	47.64 B	3.2	3.5	3.3 A	38.85	41.09	39.97 B
SC	2.71	2.88	2.79 A	40.55	35.67	38.11 C	3.2	3.6	3.4 A	36.22	30.33	33.27 C
Mean (PS)	1.82 B	2.28 A		44.05	42.86		3.0 B	3.5 A		36.52	38.06	

Interaction and main effect means not sharing same letter for any trait differ significantly ($P<0.05$); UAF= University of Agriculture, Faisalabad; DSR= Direct seeded rice; TR= Transplanted rice; T= Treatment; PS= Production systems; SA= Soil application; FA= Foliar application; SP= Seed priming; SC= Seed coating

Agronomic Biofortification to Improve Zn Bioavailability in Rice Systems

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Introduction

Zinc deficiency not only affects grain yield, farmers' income, but also human health and nutrition of billions of people worldwide using rice as staple. Flooded and irrigated lowland rice accounts for 92% of total production of 79 million ha of total harvestable area (Impa and Beebout, 2012). Due to global climate change, erratic pattern of rainfall and decreasing fresh water availability, major of part of rice growing areas are shifting toward water saving aerobic rice and alternate wetting and drying systems. With prevalence of most commonly in flooded lowland rice systems, Zn deficiency has been also found in water saving rice systems (Rehman et al., 2012). Biofortification is one of promising ways to improve Zn bioavailability in human by developing genotypes with high grain Zn contents and soil management to increase plant available soil Zn at important crop growth stages. In present study, we discuss the effects of soil and crop management strategies on paddy yield and grain Zn contents in flooded and water saving rice systems.

Materials and methods

Three experiments were conducted to evaluate the effect of soil and crop management strategies on paddy yield and grain Zn. In pot experiment, 30 days old nursery seedlings of four basmati rice genotypes (Basmati-385, Basmati-515, Basmati-2000 and Super Basmati) were transplanted under flooded greenhouse condition. Soil Zn (25 kg ha^{-1} $ZnSO_4.7H_2O$: 33% Zn) was applied at transplanting, tillering and panicle initiation. Soil was of clay loam texture with adequate DTPA extractable Zn of 1.08 mg kg^{-1}. This experiment was comprised of rice cv. Super Basmati with 22 days old nursery seedlings transplanted under flooded conditions maintained as aerobic, alternate wetting and drying, system of rice intensification (SRI) in comparison to flooding treatments. Soil Zn (15 kg ha^{-1} $ZnSO_4.7H_2O$: 33% Zn) was applied 15 days after transplanting with no Zn as control. Experimental soil was silt loam in texture and deficient in DTPA extractable Zn (0.85 mg kg^{-1}). However, after tillering, a temporary drainage was provided in each rice systems and irrigated weekly until maturity.

Results and discussion

Zn applied at transplanting in cv. Bas-385, at panicle initiation in Bas-2000 improved paddy yield and grain Zn contents. Similar increase in Zn contents was observed for basal Zn in S. Basmati and Bas-2000 cultivars as well (Table 1). Response was different when rice grown for two seasons and maximum increase in paddy yield recorded for foliar Zn at panicle initiation in CF, for Zn basal in AWD during 2008 only and for Zn applied at tillering stage in all three rice systems during both growing seasons. Likely, highest grain Zn was found for basal Zn in each rice system for first growing season and comparatively higher for Zn applied for panicle initiation stage in 2^{nd} growing season (Table 2). In addition to native soil Zn and genotype, soil redox potential seems to be major driver for Zn availability in absence of flooding, however, basal applied Zn can be beneficial to ameliorate Zn deficiency during vegetative stage (Wissuwa et al., 2008) or splitting of Zn fertilizer at different stages with nitrogen can be promising. In crux, basmati cultivars used in present study seems to tolerate Zn deficiency and when fertilized at critical crop stages respond differently, therefore, mechanisms for improved uptake at soil or plant level are warranted for their use in breeding program for development of high grain Zn rice.

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Table 1: Influence of Zn fertilization time on paddy yield and grain Zn of rice cultivars

Treatments	Paddy Yield (g pot^{-1})				Grain Zn contents (mg kg^{-1})			
	B-385	B-515	Super- B	B-2000	B-385	B-515	Super- B	B-2000
No Zn	25.65 abc	25.03 abc	24.49 abc	22.48 cd	22.13 efg	24.65 def	19.65g	21.57 fg
Zn at transplanting	29.84 ab	25.40 abc	24.41 abc	25.56 abc	32.10 a	26.98 bcd	32.23 a	30.30 ab
Zn at tillering	20.87 cd	26.29 abc	24.23 abc	22.87 bcd	26.29 cd	26.15 cd	25.87 cde	28.64 abc
Zn at panicle initiation	15.90 d	25.02 abc	21.21 cd	30.86 a	26.38 cd	25.74 cde	26.30 cd	32.49 a
HSD (P = 0.05)	7.07				3.87			

B-385= Basmati-385; B-515 = Basmati-515; Super- B = Super Basmati

Table 2: Influence of Zn application on paddy yield and grain Zn in different rice systems

Treatments	Paddy yield (kg ha^{-1})						Grain Zn contents (mg kg^{-1})					
	2008			2009			2008			2009		
	CF	AWD	DSR	CF	AWD	DSR	CF	AWD	DSR	CF	AWD	DSR
No Zn	2.7 g	2.68 g	2.51h	2.41 g	2.44 ef	2.43 f	18.80 e	22.38 cd	20.72 de	20.21 e	22.10 cde	18.41 e
Zn at basal	3.10 c	3.22 b	2.72 g	2.48 d	2.48 d	2.45 e	27.51 ab	27.85 ab	28.54 a	25.40abcde	28.29 abc	24.36abcde
Zn at tillering	3.21b	2.87 f	3.27 b	2.54 a	2.54 a	2.50 bc	25.25bc	28.64 a	27.35 ab	24.48 abcde	28.42 abc	28.75 abc
Zn at panicle initiation	2.99 de	2.97e	3.06 cd	2.44 ef	2.49 c	2.51 b	26.87ab	22.62 cd	28.02 ab	30.64 a	29.35 ab	28.59 abc
Foliar Zn at panicle initiation	3.37 a	2.84f	2.90 ef	2.40 g	2.50 bc	2.44 ef	21.19 de	28.00 ab	26.67 ab	23.51 bcde	29.35 ab	20.30 de
HSD (P = 0.05)	0.08			0.01			2.97			6.40		

Genotypic Differences of Zinc Bioavailability from Different Zinc Dense Rice Germplasms

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Introduction

Enrichment of rice with bioavailable Zn is, therefore, suggested as a way to generate major health benefits for a large number of susceptible people using rice as their staple food. The metabolizable Zn from biofortified crop grain not only depends on net Zn concentration, but also a large extent on the bioavailability of Zn. Zn bioavailability defined as the proportion of the total amount of Zn that is potentially absorbed in a metabolically active form (House 1999). Ideally, to study the bioavailability of Zn in crop grains should be evaluated through *in vivo* human studies. However, complexity to perform large-scale screening of sample and cost limit their applicability (Van Campen and Glahn 1999). *In vitro* digestion/Caco-2 cell model have been proposed as an alternative to *in vivo* methods for estimating mineral bioavailability in diets. The present study used this model to assess the bioavailability of Zn from polished rice grain with different forms of foliar Zn application. Zn bioavailability in polished rice among five selected zinc dense rice genotypes was determined using *in vitro* digestion /Caco-2 cells model.

Materials and methods

Five Zn-dense, conventional rice genotypes were selected according to our previous study (Yang et al., 1998). Selected five Genotypes were grown in the same season on paddy soil at Zhejiang university farm (Zhejiang, Hangzhou, China; 30°15´19" N, 120° 10´ 8" E). Rice sample preparation and *in vitro* digestion/Caco-2 cell model as described in our previous publication (Wei et al., 2012).

Results and discussion

Large variation in Zn concentration in polished rice of five genotypes was observed (Figure 1). The inherent Zn concentration in polished rice among five genotypes ranged from 14.42 mg/kg to 28.35 mg/kg. Among the five selected rice varieties, the genotype Biyuzaonuo contained highest Zn concentration in polished rice, followed by genotypes Bing91185, which was considered as the high-Zn density rice genotypes. The genotype Liangyoupei9 contained relatively low Zn concentration in polished rice, which was consider as the low-Zn density rice genotypes. The soluble fraction obtained from simulated gastrointestinal digestion, exposure in Caco-2 cell model. The Zn uptake efficiency in Caco-2 cell was measured as proxy of Zn bioavailability. Significant genotypic differences were found in Zn bioavailability among the selected genotypes (Figure 2), ranging from 4.91 to 9.57 %, the variation range was nearly up to 2 fold. Highest Zn bioavailability was found in genotypes Bing91185. Genotypes Lingyoupei9 contained the lowest Zn bioavailability.

These results indicated that it's possible to select rice genotype containing high Zn bioavailability by crop breeding. We conclude that increase in amount of bioavailable Zn could be done not only by increase in the absolute Zn concentration alone, but also increase in Zn bioavailability. In future study, more genotype should be used to make a better comparison with Zn concentration and Zn bioavailability.

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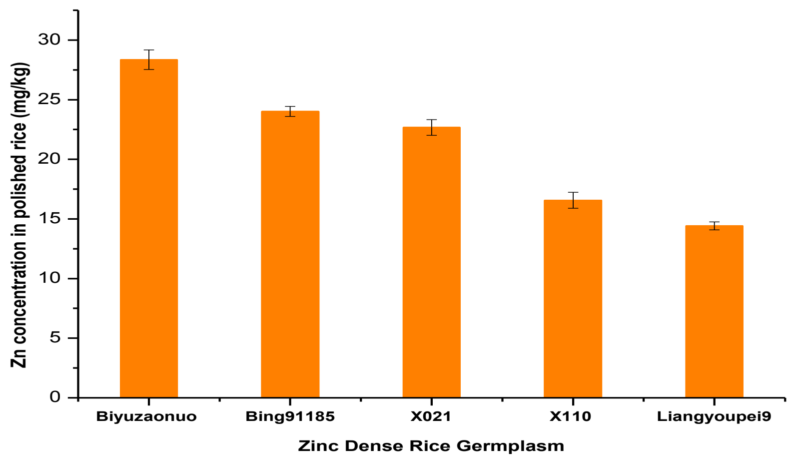

Figure 1. Zinc concentration in polished rice of five Zinc dense genotypes. Error bars indicates standard errors of the means (n = 4)

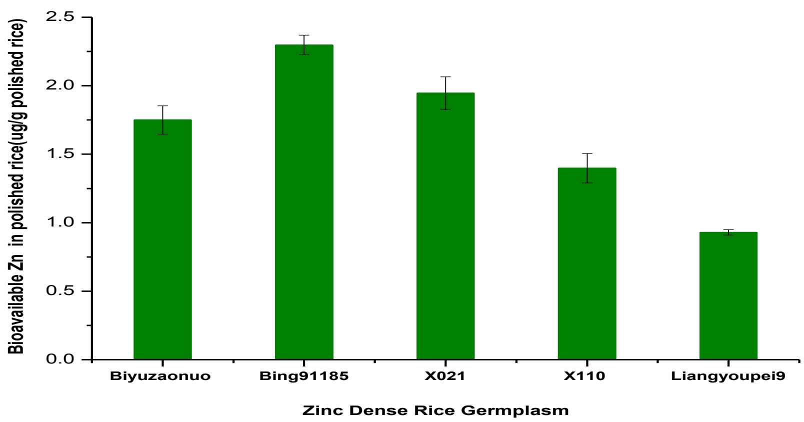

Figure 2. The amount of bioavailable Zn in polished rice of five Zinc dense genotypes. Error bars indicates standard errors of the means (n = 4).

Biofortification of Edible Crops with Essential Dietary Minerals

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Introduction

Human diets must supply adequate amounts of at least 25 essential mineral elements (White and Brown, 2010). Unfortunately, it is estimated that the diets of over two thirds of the world's population contain insufficient amounts of iron (Fe), zinc (Zn), copper (Cu), calcium (Ca), magnesium (Mg), selenium (Se) or iodine (I) (White and Broadley, 2009). This situation can be remedied through dietary diversification, mineral supplementation, food fortification, or increasing the concentrations and/or bioavailability of mineral elements in edible crops (biofortification). The latter can be achieved through two complimentary approaches: (1) improved agronomy, which seeks to increase the phytoavailability and acquisition of mineral elements by crops, and (2) the development of crop genotypes better able to acquire mineral elements and accumulate them in edible tissues (White and Broadley, 2009; White et al., 2012). This presentation will describe both agronomic and genetic strategies for increasing the concentrations of essential mineral elements commonly lacking in human diets in edible crops.

The Accumulation of Mineral Elements in Edible Tissues

Mineral elements are generally acquired by plants from the soil solution, through their roots. They traverse the root to the stele, where they are loaded into the xylem for transport to transpiring shoot tissues. This route to the shoot can be supplemented by foliar applications of mineral fertilizers. Their subsequent delivery to non-transpiring or xylem-deficient tissues occurs via the phloem. Both Mg and Se are readily mobile in the phloem, whereas Fe, Zn, Cu and I are less mobile, and Ca is effectively immobile (White, 2012). Mineral elements with low phloem mobility accumulate in tissues with high transpiration rates, such as stems and leaves, and are relatively less concentrated in tissues with low transpiration rates, such as fruits, seeds and tubers (White and Broadley, 2009). Thus, (1) the accumulation of a mineral element in the root requires efficient acquisition from the soil solution (and/or redistribution from the shoot) and sequestration within the root, (2) the accumulation of a mineral element in the shoot requires efficient acquisition from the soil solution, transport to the shoot (or foliar fertilization) and sequestration within the shoot, and (3) the accumulation of a mineral element in fruits, seeds and tubers requires efficient transport to the shoot (or foliar fertilization), mobility in the phloem, and sequestration within these tissues.

Improving the Uptake of Mineral Elements from the Soil

Low concentrations of mineral elements in plant tissues can occur because of the absence of a particular element in the soil, as might occur for Se or I in some soils, or because an element has low phytoavailability despite being abundant in the soil, as might occur for Fe, Zn and Cu in calcareous or alkaline soils (White and Broadley, 2009; White et al., 2012). In the former situation, fertilizers must be applied to biofortify edible crops. In the latter case, root exudates can be exploited to modify soil chemistry or biology to increase the phytoavailability and acquisition of the mineral element (White and Broadley, 2009). Increasing the volume of soil explored by plant roots, developing root architectures to match the spatial or temporal heterogeneities in the phytoavailability of mineral elements, and increasing the capacity and affinity of transport proteins in the plasma membrane of root cells, can also contribute to greater acquisition of phytoavailable mineral elements. These root traits can be assayed and selected for in crop improvement programmes.

Redistributing Mineral Elements within the Plant

High concentrations of mineral elements in plant tissues can be toxic (White and Brown, 2010). It is therefore important that mineral elements are accumulated and transported within plant tissues in non-toxic forms. If yield is not to be compromised, then the accumulation of mineral elements in roots and leaves of plants with ample supply is primarily limited by tissue toxicity (White and Broadley, 2011). Thus, increased tolerance of high tissue concentrations of mineral elements, for which genetic variation has been observed, could increase the potential for their biofortification. By contrast, the accumulation of mineral elements in phloem-fed tissues is generally limited by the rate of their transport in the phloem. Again, there appears to be within-species genetic variation in this trait, which might be utilized to increase the concentrations of mineral elements in fruits, seeds and tubers (White and Broadley, 2009).

Breeding for Mineral Biofortification

Sufficient genetic variation has been reported in most major crops to breed for increased concentrations of mineral elements lacking in human diets in their edible portions, and chromosomal loci (QTL) affecting these traits have been identified in several crops (White et al., 2012). In addition, targeted Genetic Modification is being pursued to increase concentrations of selected mineral elements in edible crops (White and Broadley 2009; White et al., 2011). Genetic Modification has successfully increased Fe and Zn concentrations in roots and leaves through greater exudation of phytosiderophores in graminaceous plants, increased Fe(III) reductase activity in non-graminaceous plants, increased Fe or Zn influx to root cells, increased xylem transport, or greater sequestration in vacuoles. The overexpression of nicotianamine synthase has increased concentrations of Fe and Zn in leaves and phloem-fed tissues, and the targeted overexpression of metal-binding proteins, such as ferritin and lactoferrin, has increased Fe, Zn and Cu concentrations in the tissues in which they were expressed. Successful biofortification of edible produce with Ca has been achieved by increasing the activity of vacuolar Ca^{2+}/H^{+}-antiporters in appropriate tissues.

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Selenium Biofortification: Impact of Rhizospheric Se-tolerant Microbes and Selenate Agronomic Fertilization on Se Uptake and Transport in Staple Crops

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Introduction

Selenium (Se) is present in nature and its distribution in soil is highly uneven worldwide. In most of the soils, Se concentration ranges from 0.01 to 2 mg $kg^{-1}$of soil and it is present in soil in the form of selenate, selenite, selenide, organic and elemental Se. For Se-deficient regions of the world such as Finland, New Zealand, Europe, China, UK and Australia production of Se-enriched crops is considered as nutritionally significant (Bañuelos et al., 2015). All plant species have the ability to take up Se from soil and to transport and accumulate it in various above ground plant parts such as stems, leaves, fruits, flowers and seeds. Se is an essential micronutrient for humans and animals but Se is not essential for growth of higher plants (Yasin et al., 2015). However, Se can stimulate growth of some plant species when supplied to plants in low doses and it can be toxic for most plant species when present in high concentration in soil. In Finland, selenate has been added in fertilizers in the range of 10 to 15 mg kg^{-1} of fertilizers by law since 1983 to improve Se concentrations in edible crops parts. Plants can uptake selenate through roots using sulfate transporters. Selenate is highly water soluble and during irrigation practices a major fraction of selenate leaches down and becomes unavailable for absorption by plants roots, decreasing the efficacy of selenate fertilizers. Selenium resistant plant growth promoting rhizosphere bacteria (PGPR) and endophytic bacteria can enhance plant growth and biomass production (Sura-de Jong et al., 2015), and may also improve mineral nutrients solublization through various mechanisms which result in increased uptake of nutrient. In this greenhouse study, the effect of inoculation with Se resistant bacterial strain YAP-4 was observed on wheat and corn plants growth and Se uptake under selenate supplemented conditions.

Materials and methods

This greenhouse study was conducted at the University of the Punjab, Lahore, Pakistan. Wheat and corn plants were grown in earthen pots filled with non-seleniferous soil. The treatments used were i) control, ii) Se-treated and iii) inoculated Se-treated plants. After seed germination the plants were supplied with sodium selenate (3 mg Se kg^{-1} of soil). At the time of seed sowing the seeds and potted soil were inoculated with Se-resistant bacterial strain YAP-4 (a gram + rod shaped soil bacteria isolated locally). The corn and wheat plants were grown until maturity under natural conditions and at harvest plant growth parameters were calculated. Elemental analysis was carried out with inductively coupled plasma atomic emission spectroscopy (ICP-AES) after acid digestion (Lindblom et al., 2012).

Results and discussion

Inoculation with bacterial strain YAP-4 resulted in increased plant growth and dry biomass per pot of wheat (13%) and corn (21%) plants in comparison to un-inoculated Se-treated plants. Plant growth promoting rhizosphere bacteria could enhance plant growth by nitrogen fixation, phytohormones synthesis, phosphate and potassium solublization, ammonia production, improving abiotic stress tolerance and they may also inhibit the growth of plant pathogens. Selenium concentration in stem, leaf and seed tissues of un-treated corn and wheat control plants was <1 mg kg^{-1} DW (it was below the detection limit of ICP-AES machine, which was 0.01-0.02 ppb in acid digest and 1-2 ppm in plant tissue, respectively). Selenium treatment alone or in combination with bacterial inoculation resulted in significant Se accumulation in stem, leaves and seeds compared to un-treated control (Table 1). Interestingly, bacterial inoculation in the presence of Se treatment caused significant increases in plant Se content in comparison to un-inoculated Se-treated plants (Table 1). The increase in Se concentration in inoculated plants may be liked to bacterially enhanced root growth, leading to a higher root surface area. Based on the Se concentration observed in these selenate-treated corn and wheat plants, the plant material could be considered biofortified food. However, this Se rich plant material should be diluted with low-Se plant material before consumption by humans or animals. The safe levels for daily Se intake ranges from 55 - 75 µg day^{-1} for adult humans and 100 - 200 µg day^{-1} for their livestock, respectively, which would correspond with one or a few grams DW of this plant material. In conclusion, for the purpose of Se-biofortification, the application of sodium selenate in combination with inoculation with Se-resistant bacterial strains is a good approach to improve the efficacy of Se-containing fertilizers in fields and to improve Se content in wheat and corn plants and possibly also in other crops.

Table 1. Effect of Se-treatment and inoculation with strain YAP4 on Se content in stems, leaves and seeds of corn and wheat plants. (n=3, 95% level of significance)

Treatments	**Se concentration in corn (mg kg^{-1}DW)**		**Se concentration in wheat (mg kg^{-1}DW)**	
	Stem	Leaves	Seeds	Stem
Se-treated	22 ± 3a	23 ± 3a	35 ± 2a	12 ± 2.6a
Inoculated Se-treated	18 ± 1.5a	31 ± 2b	47 ± 1.5b	66 ± 17b

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