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**REVIEW**

# Breeding and agronomic approaches for the biofortification of zinc in wheat (*Triticum aestivum* L.) to combat zinc deficiency in millions of a population: a Bangladesh perspective

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The World Health Organization (WHO) has estimated that around 2 billion people across the globe are suffering from “hidden hunger”, where 815 million are under malnutrition. The major essential elements for humans are Fe, Zn, I, Se, Ca, F, and also vitamins. Among them, Zn is considered in the fifth place leading to causes of several deficiency diseases. At least one-third of the population in the world is facing Zn deficiency including around 450,000 children under the age of five. Vitamin A, Fe, and Zn deficiencies can be overcome through the biofortification of staple foodstuffs. This review emphasizes various breeding and agronomic approaches for the biofortification of Zn in wheat grains, which is an encouraging and cost-effective method to enhance the Zn contents of cereal grains. Recently, the Bangladesh Wheat and Maize Research Institute (BWMRI), with technical support from CIMMYT, Mexico, released a promising new Zn-biofortified wheat cultivar, ‘BARI Gom 33’, a result from a conventional breeding program. It has 32 and 50–55 mg kg<sup>-1</sup> Zn without and with soil application of ZnSO<sub>4</sub>, respectively. This cultivar could be a savior for a million people in South Asia, including Bangladesh.

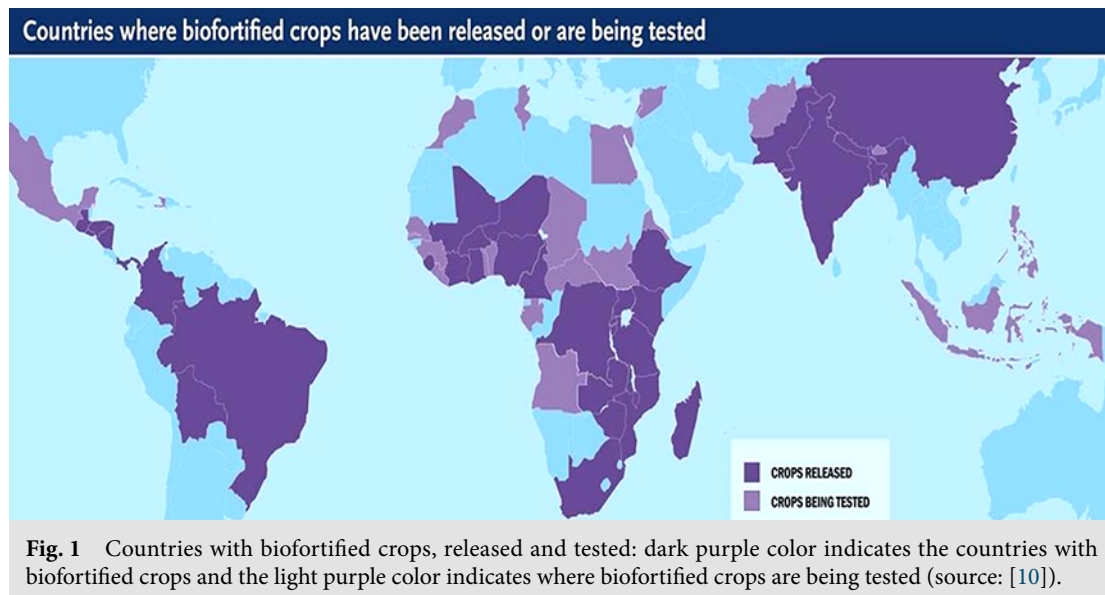
**Keywords**

Zn-biofortification; fertilizer management; cereal; human health

**Introduction**

Wheat (*Triticum aestivum* L.) is a major cereal which contributes about 28% of the world's food demand [1]. The wheat grain has a high nutritional value in protein, fats, starch, and minerals [2]. The major essential micronutrients for human health are Fe, Zn, I, Se, Cu, Ca, and F as well as vitamins [3]. The World Health Organization (WHO) has estimated that around 2 billion people across the globe are suffering from “hidden hunger”, where some 815 million are facing micronutrient deficiencies. Among them, Zn is considered as the fifth most serious leading to several deficiency diseases [4,5]. At least one-third of the population in the world is facing Zn deficiency, among them nearly half of the population live on a staple diet of rice or wheat, particularly in developing countries [6,7]. Most of the cereal-based foods usually contain a low intrinsic amount of Zn, particularly when grown in Zn-deficient soils [8,9]. Considering this burning issue, HarvestPlus, a CGIAR research program in agriculture, is working together with different national and international organizations to improve nutrition and public health by developing and promoting biofortified food crops rich in vitamins and minerals. It

provides global leadership in the development of technology for micronutrient biofortification [10]. HarvestPlus has already released more than 150 biofortified cultivars of 10 crops in 30 countries [11]. In addition, 12 further candidate biofortified cultivars are being evaluated for release in an additional 25 countries (Fig. 1).



**Fig. 1** Countries with biofortified crops, released and tested: dark purple color indicates the countries with biofortified crops and the light purple color indicates where biofortified crops are being tested (source: [10]).

In Bangladesh, wheat is the second most important cereal crop next to rice [12]. The area under wheat cultivation during 2014–2015 was about 0.44 Mha producing 1.4 Mt of wheat with an average yield of 3.08 Mt ha<sup>-1</sup> [13]. The requirement for wheat is about 24 kg person<sup>-1</sup> year<sup>-1</sup>, and >50% of this wheat needs to be imported from foreign countries. However, increasing cropping intensity in Bangladesh (by 143% in 1971–1972 and 191% in 2014–2015) coupled with the intensification in the use of modern crop cultivars have led to a deterioration in soil fertility and productivity, and ultimately the occurrence of micronutrient deficiencies in soils [13]. Zinc deficiency is presently the most visible micronutrient disorder and >70% of cultivated soils in Bangladesh have a Zn deficiency [14,15]. The major functions of Zn for normal growth and development of the crop plant have been well established and its deficiency adversely affects the final yield of crops. Zinc is essential as a cofactor for multiple enzyme systems such as carbonic anhydrase, and it is also important in auxin metabolism. Zinc sufficiency is considered to be the most important aspect in order to obtain a satisfactory yield of wheat [16]. Several studies have revealed that Zn fertilization not only improves crop yield but also enhances the Zn content in the grains [17,18].

Bangladesh is now almost self-sufficient in rice production and has not faced any severe food shortage problems since the 1970s. The number of under-nourished people who are in absolute hunger in Bangladesh has drastically been reduced due to the availability of grain. However, 41% of children under the age of five are chronically under-nourished and therefore stunted; one-third of children from 6 months to under 5 years of age are anemic and 24% of women are underweight even today, due to micronutrient deficiency. Among the micronutrient deficiencies, Zn is ranked as the fifth leading risk factor for diseases in developing countries, including Bangladesh [19]. It is therefore vital that micronutrient levels in foods are increased for improved human and animal health. Preliminary studies indicate that Zn enrichment of cereal seeds is possible through Zn fertilization, and the magnitude of this boost depends on the crop and the cultivar grown. Researchers around the world have already shown that vitamin A, Fe, and Zn deficiencies may be overcome through biofortification of staple food crops. It is a process of enhancing the essential micronutrient contents of food crops through breeding or agronomic approaches [20,21]. Increasing the Zn contents in the staple food crop is therefore an important global challenge for better production and to reduce malnutrition [22,23]. HarvestPlus, in collaboration with different international organizations, is working to improve nutrition and public health by developing and

promoting biofortified food crops with vitamins and minerals. Several biofortified crop cultivars have already released in different countries [10].

Genetic and agronomic biofortification are two important agricultural approaches that could be helpful in improving cereal grains to optimum Zn concentrations [24]. However, breeding/genetic biofortification tools strongly depend on the interaction between genotype and environment [25–27]. Similarly, agronomic biofortification also depends on the type, rate, time, and place of Zn fertilization (also referred to as “ferti-fortification”) [28,29].

This review emphasizes various breeding and agronomic approaches for biofortification of Zn in wheat grain which might be an encouraging and cost-effective method to enhance the Zn contents. It is hoped that this initiative will have a major impact upon the lives of millions of people in South Asia, including Bangladesh.

## Forms and status of zinc in soils

### Zinc content and availability

Total Zn concentrations in soils vary from 10 to 300 mg kg<sup>-1</sup>, the average being 50 mg kg<sup>-1</sup> [30]. Not all Zn is available to plants out of the total; usually <10% is plant available Zn. The low solubility of Zn in soils is the major cause for the widespread occurrence of Zn deficiency in crop plants [31]. A range of soil conditions is associated with lower Zn availability in soils which includes high pH (>7.0), prolonged submergence (expressed by the Eh value), low organic matter content, high carbonate content, and interactions with other ions (Cu<sup>2+</sup>, Fe<sup>2+</sup>, Mn<sup>2+</sup>, and H<sub>2</sub>PO<sub>4</sub><sup>-</sup>) [30,32–34]. Nearly 50% of cereal-based soils in the world, particularly in rice/wheat-based soils in South Asia, have low available Zn for plant nutrition [35,36]. The various forms of Zn in soils are free Zn<sup>2+</sup> ions, adsorbed Zn<sup>2+</sup> (on clay surfaces, organic matter, carbonates, and oxide minerals), organically-complexed Zn<sup>2+</sup>, Zn<sup>2+</sup> substituted for Mg<sup>2+</sup> in the crystal lattices of clay minerals, and Zn in primary and secondary minerals [30]. The only forms of Zn directly available for plant uptake are water-soluble Zn<sup>2+</sup> and exchangeable Zn<sup>2+</sup>.

### Micronutrient deficiency in the soils of Bangladesh

About 50% soils of the world exhibit Zn deficiency [37]. In Bangladesh, Zn deficiency is the most widespread micronutrient problem. In agro-ecological zone (AEZ) 1, the Zn status of 30% of soils are “very low” and 40% of soils they are “low”. In AEZ 3, 28% soils are “very low” and 58% soils are “low”; in AEZs 19 and 22, the 16% soils are “low” and 26% soils are of “medium” status [38]. The Zn status is “very low” to “low” in AEZs 3, 9, and 22 [39]. Zinc deficiency is particularly evident in calcareous and wetland rice soils [40–42]. Maize and rice have been found to be more responsive to Zn amendment [43–45]. The critical level of DTPA-extractable soil Zn (regarded as a good measure of available Zn) for crops is considered to be 0.6 mg kg<sup>-1</sup> [39]. A value of 0.89 mg kg<sup>-1</sup> (DTPA-extractable) is thought to be the critical limit for maize [46]. However, if the soil is not deficient in Zn, crops such as maize and beans grown on those soils may still show Zn deficiency [47] since these plants are highly sensitive to Zn deficiency.

### Global consequences of zinc deficiency including Bangladesh

Poor nutrition is responsible for 45% of deaths of the under-five aged children every year [48], and some 25% of the children in the world are suffering from stunted growth [49]. It is essential to ensure the supply of the micronutrient-enriched food to defeat the problem of “hidden hunger” across the globe.

By using data on diet and bioavailability, Welch and Graham [9] estimated that at least one-third of the population in the world is the problem of Zn deficiency, particularly children. Among them, approximately 450,000 children under the age of five die per year due to Zn deficiency [50]. The WHO reported that Zn deficiency across the

globe is about 31% [51]. Under-developed nations worldwide are particularly suffering from Zn deficiency [52], and ultimately face a high rate of mortality, due to childhood diarrhoea and pneumonia [50], mainly in the countries of South-East Asia, the eastern Mediterranean and Africa [53].

In South Asia, including Bangladesh, >26% of the population have inadequate dietary Zn intake. As a result, many people are suffering various diseases due to Zn deficiency, particularly in the early growth stages [53,54]. In Bangladesh, currently 36% of the under-five year-aged children are stunted, and 31% of married women aged 15–19 years are under-nourished [55]. Alarming, mostly due to iron deficiency, 92% of infants aged 6–11 months in Bangladesh suffer from anemia [56]. It has been estimated that due to massive malnutrition, Bangladesh is losing an annual labor productivity cost at US\$ 1 billion [57]. Most importantly, damage to the human body due to micronutrient deficiency at the childhood level is irreversible.

#### Most common indications of zinc deficiency

Unfortunately, many people in South-East Asia, the eastern Mediterranean, and Africa are Zn deficient [53]. Among them, women and children are the most vulnerable to micronutrient deficiencies and are completely unaware of their condition. The seven most common Zn deficiency indications are frail immunity in the human body [54], diarrhoea disease [55], limited neuro-psychologic function [56,57], allergies [58], thinning hair [59], intestinal permeability (leaky gut) [60], and skin rashes or spots [56].

#### The concept and importance of biofortification

Iron, zinc, and vitamin deficiencies may be corrected through the biofortification of staple foods. This is a new public health approach to tackle micronutrient deficiencies, particularly in developing countries. Biofortification can enrich the nutrition of staple crops through plant breeding as well as by agronomic management, particularly fertilizer application. The CGIAR has implemented the HarvestPlus program in collaboration with different national and international organizations for developing biofortified (with high micronutrient concentrations) staple crops such as wheat, rice, maize, cassava, beans, and pearl millet by plant breeding. The most promising sources for elevated grain Zn and Fe concentrations are the wild relatives, primitive wheats and landraces [61].

There are two practical agricultural approaches to increase Zn concentrations in cereal grains, genetic biofortification and agronomic biofortification (by application of a Zn-containing fertilizer) [8]. The genetic engineering approach is a lengthy and complex technique to enrich the Zn status of cereal grains but it is the most justifiable resolution. Furthermore, if Zn is available in soils, the newly developed Zn-enriched genotypes obtained from a breeding program could provide an adequate concentration of Zn in the grain more effectively than can the agronomic approach.

The dietary intake of wheat grain is generally after milling. According to a Zn-staining study of wheat seeds, Zn concentrations were found to be about 150 mg kg<sup>-1</sup> in the embryo and aleurone layer and only 15 mg kg<sup>-1</sup> in the endosperm [62]. However, the bran and embryo parts of seeds although enriched in Zn are generally removed at the time of milling [63]. Consequently, heavy consumption of a high proportion of milled wheat and other cereal products may actually result in a reduced intake of Zn. Enrichment of cereal grains with Zn is therefore a high priority area of research and will contribute to minimizing Zn deficiency-related health problems in humans. Researchers are currently attempting to improve Zn and Fe concentrations in the wheat endosperm by using molecular genetic approaches for biofortification [64].

#### Genetic biofortification of zinc

Genetic biofortification may involve both traditional breeding as well as biotechnological tools. Efforts to do this have led to the development of programs such as

HarvestPlus, Golden Rice, and the African Biofortified Sorghum Project, all focusing on the development of crop cultivars capable of producing grains richer in Zn and other micronutrients [7]. Golden Rice has been engineered to express beta-carotene by introducing a combination of genes that code for the biosynthetic pathway for the production of provitamin A in the endosperm [65]. Enhancement of Zn concentrations in modern wheat genotypes incorporates the traits from wild relatives [66,67]. In most cases, there is an inverse relationship between grain yield and grain Zn concentration [68,69] – a higher grain Zn concentration is most commonly associated with lower grain yield [70,71]. Breaking this trade-off between grain yield and Zn concentration is an important issue in programs for breeding for higher Zn concentrations in cereal grains [72–74]. Such programs require a long development time and so far no Zn-enriched cultivar of any cereal has become available. Future such cultivars may not be high yielding. Combining high grain yield and high Zn concentrations in grain requires more time.

Recently, the Bangladesh Wheat and Maize Research Institute (BWMRI), with technical support of the International Maize and Wheat Improvement Center (CIMMYT), Mexico, has released a new Zn-enriched biofortified wheat cultivar 'BARI Gom 33', through a conventional breeding program [75]. It has 32 and 50–55 mg kg<sup>-1</sup> Zn without and with soil application of ZnSO<sub>4</sub>. This cultivar could save the lives of a million people in South Asia, including Bangladesh.

#### Agronomic biofortification of zinc

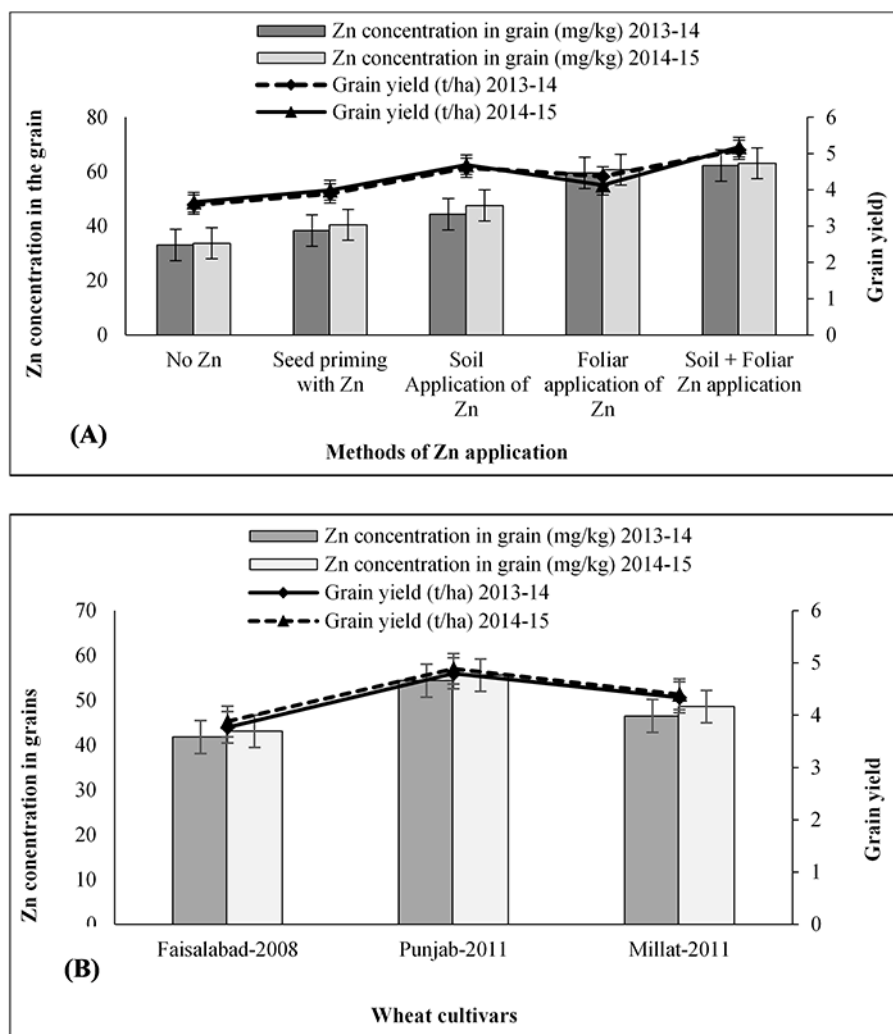
Biofortification through Zn fertilizers as a soil application or by foliar application (fertilization) can increase the crop yield as well as by increasing the Zn concentration in grains [28]. Even cultivars developed by genetic biofortification may also need Zn fertilization. Enrichment of commonly applied compound fertilizers with Zn is a further fertilizer practice useful for increasing the Zn concentration in plants. In India, the application of Zn-coated urea fertilizer significantly improved both grain yield and grain Zn concentrations of wheat [76].

Zinc can be directly applied to soil as both organic and inorganic compounds. To obtain higher fertilizer use efficiency for Zn enrichment, ZnSO<sub>4</sub> is the most adaptable salt due to its high solubility. Other researchers have found that Zn enrichment is higher with Zn-EDTA than with inorganic Zn fertilizers [77,78]. However, due to its high cost, the use of Zn-EDTA in cereal farming is limited but it is popular due to its high solubility and the fact that it can be used as a foliar application [30]. Different Zn fertilizers, such as inorganic and organic Zn salts, play a fundamental role in the way this micronutrient is transported from leaves to the grain [79]. Unfortunately, studies evaluating the effectiveness of foliar application of different Zn forms on cereal grain Zn accumulation are limited.

The timing of foliar Zn applications is an important factor in determining their effectiveness in increasing grain Zn concentration. It is expected that large increases in the loading of Zn into the grain can be achieved when foliar Zn fertilizers are applied to plants at a late growth stage. Foliar application at the early milk plus dough stages of cereal crops increases the grain Zn concentration [80]. The Zn concentration in grains of wheat has also been increased when Zn was foliar applied at the reproductive stage [81]. It was also found that the highest concentration of Zn in the grain was when Zn was applied at the milking stage of grain development [81]. Micronutrient seed treatments, which include seed priming and seed coating, are also an attractive and easy alternative to the potential of micronutrient seed treatments for improving crop growth and grain nutrient enrichment [82]. It has also been found that seed treated with micronutrients improves the seedling stand establishment, advances phenological events, and increases yield and micronutrient concentrations in the grain [82].

The method of Zn application significantly affects economic yield and grain Zn concentrations of wheat cultivars (Fig. 2). Maximum improvements in grain yield of 24.3% and 24.1%, and for Zn concentrations a maximum increase of 50.1% and 46.6% were recorded when Zn was applied in soil + foliar in both years, respectively, whereas the minimum improvement in grain yield was recorded for the zero-Zn plot [83]. Therefore, soil + foliar application of Zn appears to be an excellent strategy to





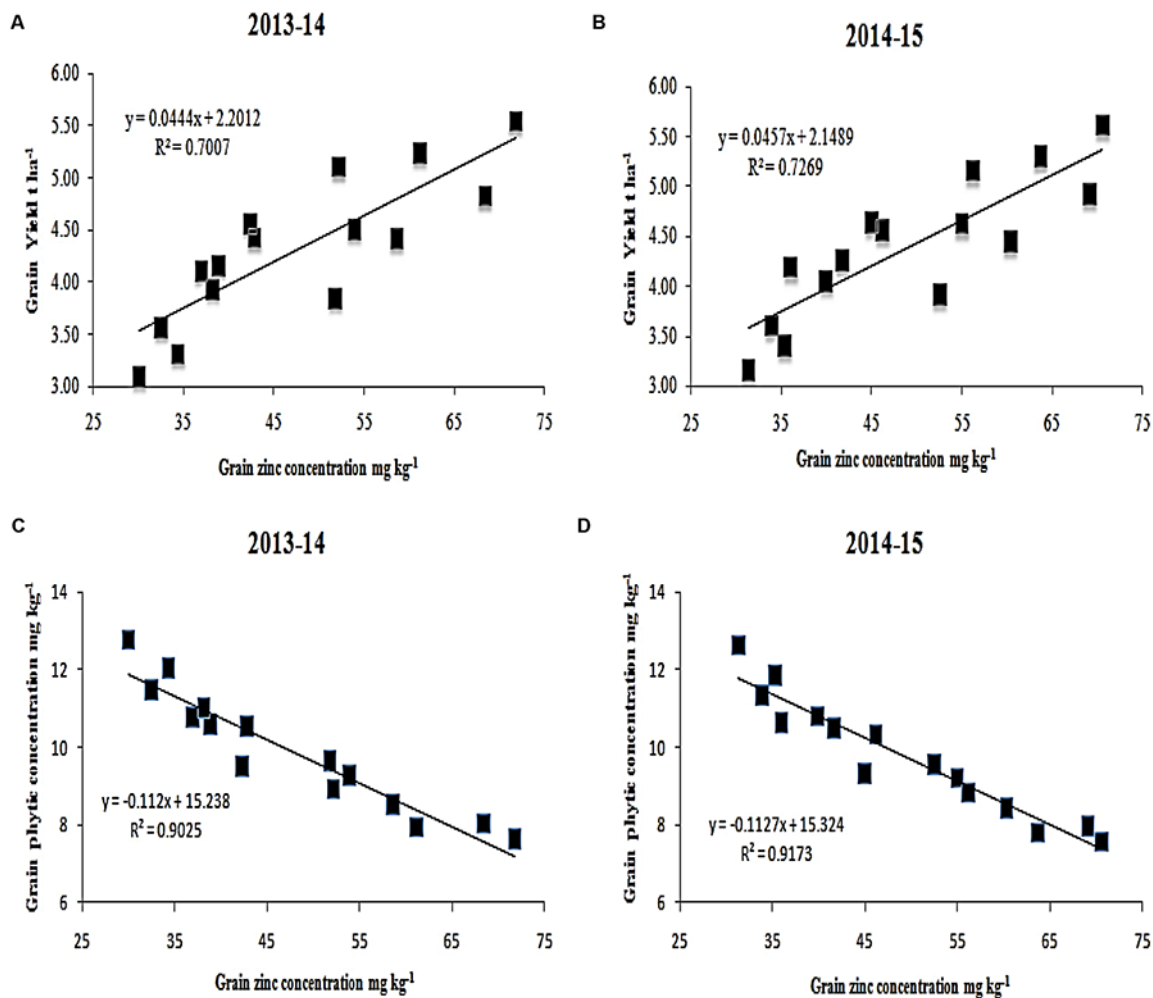
**Fig. 2** Zn concentrations in the grains of wheat as influenced by Zn application methods (A) and by wheat cultivar (B) (source: [83]).

increase grain Zn concentrations. Chattha et al. also observed that the wheat cultivars tested differed significantly in grain yield and grain Zn concentrations. Among them, 'Punjab-2011' had a higher grain yield and grain Zn concentration followed by 'Millat-2011'. The lowest grain yield and grain Zn concentrations were recorded in 'Faisalabad-2008' (Fig. 2) [83].

In summary, the strategy of fertilizer application can provide a rapid solution to the problem and can be considered as an important complementary approach to ongoing breeding programs. Fertilizer studies focusing specifically on increasing the Zn concentration of grain (or other edible parts) are, however, very rare although a large number of studies have been made on the role of soil and foliar applied Zn fertilizers for the correction of Zn deficiency and increasing plant growth and yield [84,85].

#### Varietal screening of wheat for zinc efficiency

Wheat grains inherently have a low Zn content, particularly when grown in Zn-deficient soils. Based on a range of reports and survey studies across the globe, the average Zn concentration in whole grains of wheat varies between 20 to 35 mg kg<sup>-1</sup> [86,87]. BARI evaluated eight wheat cultivars for grain enrichment of Zn and Fe and found 'BARI Gom 26' as the most promising cultivar based on its ability and stability for high Zn and Fe concentrations in the grain [88]. Zinc utilization efficiency is defined as dry matter production per unit of Zn present in the dry matter and has been reported to be linked with the activity of two Zn-regulated enzymes, namely carbonic anhydrase



**Fig. 3** Two years of study found a significant positive correlation between grain yield and grain Zn of wheat (A,B) and grain Zn and phytic acid concentrations (C,D) (source: [83]).

(CA) and superoxide dismutase (SOD). The activity of CA decreases as a consequence of Zn deficiency in a number of plant species and it is suggested as a good measure of physiologically-active Zn in the leaf tissue [89]. Tolerance of zinc deficiency in a Zn-efficient rice genotype is related to its ability to retranslocate Zn from older to emerging leaves [90]. Zinc is highly mobile within the plant system and foliar-applied Zn is translocated to leaves both above and below the treated leaf as well as to the root tips [91]. The enhanced capacity of genotypes for Zn translocation from root to shoot and its utilization under reduced Zn supply has been shown to contribute to the Zn efficiency in some wheat genotypes [92].

Biofortification by fertilization is of great importance in enriching seeds with Zn. Due to some degree of uncertainty as to whether a breeding strategy employed will be efficacious in enriching grains with Zn, short-term agricultural tools such as applying Zn fertilizers should be considered. In future, new research programs should be initiated focusing on the development of the most efficient methods of Zn application for promoting Zn uptake and maximizing Zn accumulation in grain. Because of the greater bioavailability of grain Zn derived from foliar application than from the soil, agronomic biofortification would be a very attractive and useful strategy in solving Zn deficiency-related health problems globally.

However, there is a significant positive correlation between grain yield and grain Zn of different wheat genotypes due to the application of different levels of Zn [83]. A 2-year study (Fig. 3A,B) found that an increase in the Zn concentration in the grain of the wheat genotypes tested also significantly improved the grain yield. However, the Zn-enriched grain did have a lower phytic acid content than in the grains with lower Zn content (Fig. 3C,D) [83].

## Summary and conclusions

It is undisputed that Zn is an essential micronutrient for human health since more than one-third of the world's population is facing various deficiency diseases. Biofortification of staple food crops is a new public health approach to overcome Fe, Zn, and vitamins deficiencies. In this review, we emphasize various breeding and agronomic approaches for biofortification of Zn in wheat grains as promising, cost-effective, and profitable tools to stimulate the Zn content of wheat grain. Recently, the Bangladesh Wheat and Maize Research Institute (BWMRI), with technical support from the HarvestPlus program of CIMMYT, released a new Zn-enriched biofortified wheat cultivar, 'BARI Gom 33' resulting from a conventional breeding program, which has 32 and 50–55 mg kg<sup>-1</sup> Zn without and with soil application of ZnSO<sub>4</sub>. This cultivar could save a million people in South Asia, including Bangladesh.

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### **Biofortyfikacja pszenicy (*Triticum aestivum* L.) cynkiem w celu ograniczenia niedoboru cynku w populacji: perspektywa Bangladeszu**

#### **Streszczenie**

WHO (Światowa Organizacja Zdrowia) oceniła, że około 2 miliardy ludzi na całym świecie cierpi z powodu „ukrytego głodu”, podczas gdy 815 milionów cierpi z powodu niedożywienia. Głównymi pierwiastkami niezbędnymi w diecie dla człowieka są Fe, Zn, J, Se, Ca, F oraz witaminy. Cynk zajmuje 5 miejsce pod względem deficytu w żywieniu, a jego niedobory prowadzą do wielu groźnych chorób. Co najmniej jedna trzecia populacji na świecie wykazuje objawy niedoboru Zn, w tym około 450000 dzieci poniżej piątego roku życia. Niedobory witaminy A, Fe i Zn można niwelować poprzez biofortyfikację podstawowych artykułów spożywczych. Niniejsza

praca przeglądowa podkreśla różne podejścia hodowlane i agronomiczne do biofortyfikacji Zn w ziarniakach pszenicy, co może stanowić zachęcające i opłacalne narzędzie służące zwiększaniu zawartości Zn. Niedawno Bangladeski Instytut Badań Pszenicy i Kukurydzy (BWMRI), przy wsparciu technicznym Międzynarodowego Centrum Ulepszania Kukurydzy i Pszenicy (CIM-MYT) w Meksyku, przekazał nową, wzbogaconą w Zn odmianę biofortyfikowanej pszenicy 'BARI Gom 33' uzyskaną w ramach konwencjonalnego programu hodowlanego. Zawartość Zn w ziarniakach wynosi odpowiednio 32,09 i 50–55  $\mu\text{g L}^{-1}$  Zn bez nawożenia i po doglebowym nawożeniu  $\text{ZnSO}_4$ . Ta odmiana może uratować przed niedożywieniem milion ludzi w Azji Południowej, w tym w Bangladeszu.