



## **Agronomic Biofortification of Zinc in Wheat**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## **ABSTRACT**

Intensive cropping system with imbalance use of fertilizers are responsible for declining soil health, underground water table, declining land and water productivity, emergence of new micronutrient deficiencies, new weed flora, and resistance to herbicides especially in emerging countries. This is further intensified when micronutrients particularly zinc (Zn), which is essential for human health, particularly in developing countries. Zn biofortification is a strategy for improving the intrinsic Zn content of the edible portion of plants via application of Zn-enriched fertilizers to soil or by foliar application at a predetermined stage and a proper dose. The most common cereal in the human diet is wheat, which make it most suitable targets for agronomic biofortification. The concentration of Zn in wheat grain is genotype-dependent and interacts with the environment, causing variation in micronutrient concentrations. Given Zn's importance in cereal-based nutrition, zinc biofortification seems to be an innovative technology for alleviating zinc deficiency in human health, particularly on the Indian subcontinent, by applying Zn as a foliar or soil application.

**Keywords:** Biofortification; Zinc; Wheat.

## **1. INTRODUCTION**

Among the cereals, wheat (*Triticum aestivum* L.) is considered as the most important field crop in

the world covering 220.10 m ha and with a production of 763.26 m t [1]. It is used as staple food by more than one third of the world's population. As one of the commonest cereal

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crops, it plays an important role in daily energy intake and in many developing nations, provides over 50% of the daily caloric intake [2]. In India, wheat is the 2<sup>nd</sup> most important food crop next to rice with a production of 99.87 mt in 29.65m ha in the year 2018-19. The share of wheat to total food grain production in India is around 35% and it occupies about 23.3% of the total area under food grains [3]. It is quite clear that sustaining wheat productivity is essential to both food as well as nutritional security in India.

Inappropriate and indiscriminate use of chemical fertilizers along with continuous cultivation of high yielding crop varieties and primary emphasis offered to macronutrients have led to the depletion of native micronutrient in most of the Indian soils [4]. In intensive cropping without balanced fertilization had led to depletion of major as well as micro nutrients from the soil. Now deficiency of zinc has become so widespread that it ranks next to N and P. Studies have reported that about 30% of the cultivated soils worldwide are deficient in zinc (Zn), and about 50% of the soils used for cereal crop production have low Zn availability for plants [5]. Nearly 50% soils in north India are deficient in Zn and likely to respond to its application.

Zinc is an essential element for all living things, including plants, people, and animals. Zn is essential for good root construction, enzyme activation, free radical detoxification, and maintaining plant stress tolerance in plants [6]. Zinc affects many essential plant functions, including (i) nitrogen intake, metabolism, and protein quality; (ii) chlorophyll synthesis, carbon anhydrase activity, and photosynthesis [7]. Zn is also required for the integrity of cellular membranes in order to maintain macromolecule structural orientation and the ion transport system [8]. Wheat yield and yield components have been observed to benefit from Zn. Application of Zn fertilizer either to the soil or as foliar application is one of the effective and productive ways to improve cereal grains [9]. Modern agricultural techniques have resulted in a severe decline in food diversity and micronutrient consumption due to the increased use of cereals and cash crops. Farmers have chosen to produce more profitable, high-yield cereal crops, resulting in a decrease in the area planted with protein- and micronutrient-rich legumes. Cereal costs have decreased proportionally, whereas prices for legumes, fruits, vegetables, and animal and fish protein have increased. Cereals are intrinsically deficient in micronutrients especially

in iron (Fe) and zinc (Zn) that subsequently decrease during processing. Low dietary intake of mineral nutrients due to consumption of edible portion of cereals raised on micronutrient deficient soil contributed significantly to “hidden hunger” or malnutrition. It is estimated that nearly half of the world population is affected from Zn deficiency because of low dietary intake of Zn. India alone accounts for a quarter of all under-nourished people globally. In humans, zinc deficiency causes a variety of health problems, including immune system dysfunction, learning disabilities, and physical growth, as well as an increase in mortality and infections. Zn availability in wheat cultivars in India ranges from 20 to 30 ppm [10]. Deficiency of Zn in major wheat growing areas leads to poor growth and yield attributes of wheat as well as low Zn concentration in grain and this is considered to be a major factor for low human Zn intake [11]. So, there is an urgent need to fortify the wheat grain with Zn because a large proportion of dietary calorie intake of these nutrients is derived from wheat [12]. NPK fertilization in India, which increases cereal crop yields, has often led to decline in concentration of micronutrients particularly at higher rate of application probably by a dilution effect. Balanced fertilization results in the supply of nutrients in a well-balanced ratio, leading to their efficient utilization [13]. When trace elements are added to NPK fertilisers, high fertiliser sensitive cultivars reach their full yield potential [14]. In this case, a fertilisation management approach could be a quick solution. Zinc fertilisation not only boosts yield but also increases zinc concentration in grains, according to several research [15].

## 2. EFFECT OF AGRONOMIC BIOFORTIFICATION WITH ZINC

### 2.1 Effect of Agronomic Biofortification with Zinc on Growth Parameters

In wheat crop with four levels of zinc including control (no Zn), 5 mg Zn, 10 mg Zn, and 10 mg Zn kg<sup>-1</sup> soil with urea, Nautiyal et al. [16] observed higher plant height and total dry weight with the supply of zinc added at 10 mg kg<sup>-1</sup> over the control. Gopal & Nautiyal [17] while conducting a pot experiment reported that growth of wheat plants was improved by applications of Zn (20 mg Zn kg<sup>-1</sup>) with two foliar sprays of zinc sulfate (ZnSO<sub>4</sub>) at the rate of 0.5% as compared to only Zn (20 mg Zn kg<sup>-1</sup>) and control. Nadim et al. [18] performed a field experiment to study the growth and yield responses of wheat to different

micronutrients and their application methods. They noticed a higher leaf area index and crop growth rate with the application of zinc with 10 kg ha<sup>-1</sup>. Jan et al. [19] during a field trial in the winter season of 2006–2007 observed that maximum plant height has resulted with the soil application of 15 kg Zn ha<sup>-1</sup> over control plots. At Pantnagar carried out a pot culture experiment to examine the individual and interactive effects of zinc and boron on growth, and yield in wheat (var. HD 2285). Results revealed that plant height and total dry matter content were increased with the application of zinc sulfate at 10 mg Zn kg<sup>-1</sup> soil [20]. In a soil- pot trial to examine the individual and interactive effects of zinc and boron on growth of wheat (var. HD 2285). They reported wheat crop fertilized with increasing zinc levels significantly increased the plant height and total dry-matter yield over other treatments [21]. Arafat et al. [22] found markedly highest plant height (101 cm) with soil application of Zinc (10.5 kg ha<sup>-1</sup>) and similarly higher plant height (100 cm) was noticed with side dressing method of zinc application in wheat. Bhutto et al. [23] conducted a study to examine the effect of foliar fertilization of zinc on the growth and yield response of wheat. They observed significantly higher plant height (66.1 cm) and tillers m<sup>-2</sup> (296.0) with increasing foliar zinc concentration of 2.0% over lower treatments. Chaudhary et al. [24] performed a field experiment at Kanpur to examine four levels of Zinc (0, 5, 10 and 15 kg ha<sup>-1</sup>) on wheat. They concluded that significantly higher LAI and chlorophyll intensity was with the application of 10 kg Zn ha<sup>-1</sup>.

At Pantnagar, Srivastava et al. [25] while studying zinc application methods on apparent utilization efficiency of zinc and K fertilizers under rice-wheat rotation concluded that plant height was increased significantly by 5.4 percent with the application of 2 kg Zn ha<sup>-1</sup> as foliar application compared to control. However, soil application of 5 kg Zn ha<sup>-1</sup> increased the total number of tillers by 9.7 percent over control. Afzal et al. [26] while evaluating different methods of zinc application (control, zinc application in soil before planting 23 kg ha<sup>-1</sup>, zinc foliar application, 4% ZnSO<sub>4</sub> solution at two stages) reported that maximum plant height at maturity and total number of tillers were recorded in treatment where zinc was applied both in the soil and by foliar application. Barut et al. [27] carried out an experiment to assess the effect of zinc treatments on the growth and yield of three wheat cultivars. They found a significant impact of soil treatments on grain zinc concentrations as

the concentration increased from 25.1 mg kg<sup>-1</sup> (control) to 29.4 mg kg<sup>-1</sup> (10 kg Zn ha<sup>-1</sup>). However, the further increments did not show any significant increase. Ali et al. [28] while studying the effect of zinc on the productivity of wheat and soil fertility noticed that application of 10 kg Zn ha<sup>-1</sup> resulted in higher plant height (85.5 cm) over control (82 cm).

## 2.2 Effect of Agronomic Biofortification with Zinc on Yield Attributes and Yield

A pot experiment conducted by Nautiyal et al. [16] at Lucknow to study four levels of zinc including control (no Zn), 5 mg Zn, 10 mg Zn, and 10 mg Zn kg<sup>-1</sup> soil with urea in wheat. They observed increasing size of ears, number of grainsear<sup>-1</sup>, seed yield, seed Zn, and starch contents with higher zinc additions over the control. By increasing soil application of zinc from 0 kg ha<sup>-1</sup> to 50 kg ha<sup>-1</sup> increased the grain yield by 23% and 21% in the consecutive two years, respectively [29]. Hussain et al. [30] while conducting an experiment to study different zinc applications to soil (0, 4.5 or 9 mg Zn kg<sup>-1</sup>), seed (100 mL of either 0 or 6.75% Zn w/v sprayed on 1 kg seed) and foliage (distilled-water-sprayed control, 1 mL of 0.05% Zn w/v at jointing, 2 × 1 mL of 0.50% Zn w/v at heading or combined jointing and heading sprays) reported an increased grain yield (29%) with soil application of zinc as compared to other application methods. Morshedi & Farahbakhsh [31] evaluating the response of three Zn levels (0, 20, 40 kg ha<sup>-1</sup>) reported that yield components and grain yield of the wheat genotypes increased linearly with increasing dose of Zn × K. Zou et al. [32] carried out an experiment in 23 different experimental sites with four Zn treatments: nil Zn, soil Zn application, foliar Zn application and soil + foliar Zn application for biofortification of wheat with Zinc. They observed 5 % increased grain yield with soil Zn application over other. Zoz et al. [33] examined response of wheat to foliar application of zinc observed that number of spikes per m<sup>-2</sup> in wheat increased by 26% with foliar application of 216 g Zn ha<sup>-1</sup> over control. Jan et al. [19] while evaluating the effect of zinc on yield of wheat during a field trial in winter 2006–2007 concluded that markedly higher grains per spike, grain yield, straw yield, biological yield and harvest index were observed in plot receiving 15 kg Zn ha<sup>-1</sup> over control. Abdoli et al. [34] conducted a field experiment during 2013 and 2014 reported that foliar application of Zn at stemming and grain filling stages was much more effective on grain yield

than foliar Zn application at the stem elongation stage.

In Bichpuri, Agra, Chauhan et al. [35] studied the effects of Zn (0, 2.5, 5.0 and 10 kg ha<sup>-1</sup>) on wheat in respect of yield, quality and uptake of nutrients. Results revealed application of Zn up to 5 kg ha<sup>-1</sup> increased the grain and straw yield by 9.7 and 11.5% over the control, respectively. Singh et al. [20] at Pantnagar carried out a pot culture experiment to examine the individual and interactive effects of zinc and boron on the yield of wheat (var. HD 2285). They reported that the increase in the ear length of wheat was 16.4 to 20.0% greater with Zn application of 5 or 10 mg kg<sup>-1</sup> soil over the control. They also observed an increase in the total grain weight, and 100-grain weight was with Zn application of 10 mg kg<sup>-1</sup> soil among all. At Pantnagar, Srivastava et al. [36] carried out a field trial and observed the highest pooled grain yields of wheat with soil application of 17.5 kg P ha<sup>-1</sup> and foliar applications of 2 kg Zn ha<sup>-1</sup>. Debnath et al. [37] while conducting an experiment on wheat reported the highest grain yield of 5.43 t ha<sup>-1</sup> with the application of 60 kg P<sub>2</sub>O<sub>5</sub> and 5 kg Zn ha<sup>-1</sup>. They also observed agronomic efficiency for Zn from 56.4 to 83 kg grain kg<sup>-1</sup>. Arshad et al. [38] performed an experiment to study the effect of zinc levels (0, 5, 10 and 15 kg ha<sup>-1</sup>) on the yield of wheat concluded that 10 kg Zn ha<sup>-1</sup> had resulted in significantly higher wheat spike length, 1000 grains weight, total dry matter, and grain yield. However, highest straw yield was observed with application of 5 kg Zn ha<sup>-1</sup>. Bhutto et al. [23] conducted a field experiment to examine the effect of foliar fertilization of zinc on the growth and yield response of wheat. They observed significantly higher spike length (9.2 cm), number of grains spike<sup>-1</sup> (46.4), seed index (51.0, g) and grain yield (5540.7 kg ha<sup>-1</sup>) with a higher foliar zinc concentration of 2.0% followed by lower treatments.

At Kanpur, Chaudhary et al. [39] performed a study to examine four levels of Zinc (0, 5, 10 and 15 kg ha<sup>-1</sup>) on yield and yield attributing characters of wheat. They concluded significantly highest seed yield, number of tillers m<sup>-2</sup>, test weight, with the application of 10 kg Zn ha<sup>-1</sup>. Gomez-Coronado et al. [40] conducted a field experiment with ten advanced breeding lines and three commercial varieties of wheat along with four zinc application methods and observed that zinc application, especially soil Zn application, resulted in a significant increase to about 10% in grain yield in all the studied years and

cultivars. A greenhouse experiment conducted by Keshavarz and Saadat [41] on wheat concluded that application of 10 mg kg<sup>-1</sup> Zn increased the dry weight by 25% (straw) and 32% (grain) in wheat variety 'Falat' over control. Srivastava et al. [25] at Pantnagar carried out a field experiment to study zinc application methods on apparent utilization efficiency of zinc and K fertilizers under rice-wheat rotation. Results revealed the highest mean grain yield with an application of 24.9 kg Kha<sup>-1</sup> and foliar spray of 2 kg Zn ha<sup>-1</sup>. Srivastava et al. [25] while conducting an experiment on wheat observed that soil application of Zn was more effective than foliar application of Zn in increasing test-weight. However, for yields foliar application with 2 kg Zn ha<sup>-1</sup> increased the of wheat grain yield by 9.5 percent over control. Afzal et al. [26] while evaluating different methods of zinc application (control, zinc application in soil before planting 23 kg-ha<sup>-1</sup>, zinc foliar application, 4% ZnSO<sub>4</sub> solution at two stages) reported that spike length, number of spikelets spike<sup>-1</sup>, number of grains spike<sup>-1</sup>, 1000-grain weight, biological yield, grain yield and harvest index were recorded in treatment where zinc was applied both in the soil before planting and by foliar application.

A study was performed by Barut et al. [27] to study different Zinc application methods via soil application (0, 5, 10, 20, 30 and 40 kg Zn ha<sup>-1</sup>) and foliage (0.4% ZnSO<sub>4</sub>.7H<sub>2</sub>O) as foliar spray. They found the highest grain yield with soil application of 20 kg Zn ha<sup>-1</sup>, yield increment was 27.5% over control. They also reported that soil Zn application significantly (1%) increased thousand grain weight and recorded highest with 5 kg ha<sup>-1</sup> Zn, but further increments did not have significant effects. A field experiment conducted by Ghasal et al. [42] at New Delhi during 2013–14 and 2014–15 reported that the grain yields, grain Zn concentration and recovery efficiency (RE) was highest with application of 1.25 kg Zn–EDTA + 0.5% foliar spray at maximum tillering and booting stages. Singh et al. [13] while conducting an agronomical trial in Agra during *rabi* seasons of 2010 -11 and 2011-12 reported that mean yield difference of 0.41 t ha<sup>-1</sup> between 150% NPK and 150% NPK + S+ Zn was observed due to inclusion of S and Zn in treatment. Chowdhury et al. [24] working on late sown wheat reported that maximum spike length (7.28 cm), number of spikelets spike<sup>-1</sup> (11.27), number of grains per spike (23.85), 1000-grain weight (33.43 g) and number of florets spike<sup>-1</sup> (33.39) and finally highest grain yield (1.60 t ha<sup>-1</sup>) was observed with application of 6 kg ha<sup>-1</sup> Zn. At

Ranchi, Firdous et al. [43] while conducting a field trial during 2012-13 and 2013-14 revealed a significantly increase grain yield (pooled data) and straw yield with soil zinc application of 5 kg Zn ha<sup>-1</sup> + 2 foliar sprays @0.5% of ZnSO<sub>4</sub>. H<sub>2</sub>O over the control and 5 kg zinc ha<sup>-1</sup>. However, in case of straw yield it remained at par with 10 kg Zn ha<sup>-1</sup>. An experiment was conducted by Tao et al. [44] with four levels of zinc (0, 15, 30 and 45 mg Zn kg<sup>-1</sup> soil) on two wheat cultivars with different gluten levels. They found that 15 mg Zn kg<sup>-1</sup> in soil had the strongest effect on grain yield and quality as compared to other. Ali et al. [28] conducted a field experiment to study effect of zinc on the productivity of wheat and soil fertility. Results revealed that application of 10 kg Zn ha<sup>-1</sup> resulted significantly higher biological yield (6607 kg ha<sup>-1</sup>) and grain yield (2657 kg ha<sup>-1</sup>), over control (4283 kg ha<sup>-1</sup>) and (1923 kg ha<sup>-1</sup>) respectively. At New Delhi, Kumar et al. [45] carried out an experiment during 2009-10 and 2010-11. They concluded that application of 25 kg ZnSO<sub>4</sub> ha<sup>-1</sup> significantly increased effective tiller m<sup>-2</sup>, grains spike<sup>-1</sup>, 1000 grain weight, and grain diameter, also the highest straw and biological yields.

### 2.3 Effect of Agronomic Biofortification with Zinc on Nutrient Content and Uptake

An experiment conducted on wheat, Hussain et al. [30] reported that an increased whole-grain Zn concentration (95%) and whole-grain estimated Zn bioavailability (74%) was observed with soil Zn application as compared to other application methods. Cakmak et al. [29] showed that increasing foliar Zn application increased grain Zn concentration from 11 mg kg<sup>-1</sup> to 22 mg kg<sup>-1</sup> however a combined application of ZnSO<sub>4</sub> to soil and foliar spray resulted in a higher Zn content of 27 mg kg<sup>-1</sup>. Nautiyal et al. [16] at Lucknow conducted a pot experiment in wheat (*Triticum aestivum* L. CV. SP 343) with four levels of zinc including control (no Zn, 5 mg Zn, 10 mg Zn, and 10 mg Zn kg<sup>-1</sup> soil with urea). They observed increasing grain Zn contents with 10 mg Zn kg<sup>-1</sup> additions over the control. Kutman et al. [46] noticed an increased whole grain Zn concentration by up to 50% and the endosperm Zn content by over 80% with enhanced N application. Zou et al. [32] carried out an experiment in 23 different experimental sites with four Zn treatments: nil Zn, soil Zn application, foliar Zn application and soil + foliar Zn application for biofortification of wheat with Zinc. They observed a grain Zn concentration of 48

and 49 mg kg<sup>-1</sup> with foliar Zn application alone or in combination with soil application which significantly much higher than control. Bharti et al. [47] conducted field experiments at Pantnagar and observed that increasing levels of the micronutrient with 20 kg ZnSO<sub>4</sub>ha<sup>-1</sup> + foliar spray (Zn<sub>20</sub> + F), resulted in 80% increase in grain Zn content.

In Bichpuri, Agra, Chauhan et al. [35] studied the effects of Zn (0, 2.5, 5.0 and 10 kg ha<sup>-1</sup>) on wheat. They observed removal of Zn by grain and straw increased from 152.8 to 202.5 and 129.5 to 182.5 g ha<sup>-1</sup> with the increase in Zn levels. The removal of N, P and K increased up to 5 kg Zn ha<sup>-1</sup>. Kumar et al. [48] at New Delhi, carried out an experiment during the winter seasons of 2013–14 and 2014–15 reported that total N, K and Zn uptake was increased with application of Zn compared to control under 1.45 mg kg<sup>-1</sup> soil available Zn. Srivastava et al. [25] at Pantnagar carried out a field experiment to study different zinc application methods on apparent utilization efficiency of zinc and K fertilizers under rice-wheat rotation. They concluded that soil application of 5 kg Zn ha<sup>-1</sup> and 2 kg Zn ha<sup>-1</sup> as foliar spray increased Zn concentration in wheat grain significantly by 8.0 and 13.5 percent, respectively, over control. Barut et al. [27] concluded that effect of soil Zn treatments on grain zinc concentrations were significant as the concentration in grain increased from 25.1 mg kg<sup>-1</sup> in control to 29.4 mg kg<sup>-1</sup> with application of 10 kg Zn ha<sup>-1</sup>. Afzal et al. [26] while evaluating different methods of zinc application (control, zinc application in soil before planting 23 kg ha<sup>-1</sup>, zinc foliar application, 4% ZnSO<sub>4</sub> solution at two stages) reported that grain zinc contents (33.11 mg kg<sup>-1</sup>), grain protein contents (10.1%) were observed in the treatment where zinc was applied both in the soil before planting and by foliar application on later growth stages, which is better than all other treatments. Ghasal et al. [42] conducted field experiments during 2013–14 and 2014–15 at New Delhi. Results revealed that grain Zn concentration was highest with application of 1.25 kg Zn-EDTA + 0.5% foliar spray at maximum tiller and booting stages. Jarallah and Amedy [49] performed an experiment to study the effect of N and Zn use efficiency on nutrients uptake by wheat, observed that the added zinc levels had a significant effect on nitrogen uptake by straw and grain. Increasing added zinc levels of 10, 20, 30 and 40 kg ha<sup>-1</sup> led to increasing nitrogen uptake by straw of 16.0, 33.5, 26.8 and 26.7%, and by grain of 17.1, 43.4, 37.3 and 31.7%, respectively,

compared with the control. Jat et al. [50] conducted a trial during *rabi* seasons of 2009-10 and 2010-11 at Bikaner. They concluded that N, K and Zn content and uptake in grain and straw by wheat significantly enhanced with application of zinc at 3 kg ha<sup>-1</sup> over control while zinc uptake in grain and straw was significantly increased up to 6 kg Zn ha<sup>-1</sup> during both the years and also in pooled analysis. Ali et al. [28] conducted a field experiment to study the effect of zinc on the productivity of wheat and soil fertility. They observed utilization of 10 kg Zn ha<sup>-1</sup> resulted in higher soil N (0.37%), soil P (2.9 mgkg<sup>-1</sup>), soil K (77.6 mgkg<sup>-1</sup>) and soil organic matter content (0.5%) over control.

### 3. CONCLUSION

Zinc biofortification of crops, either by soil or foliar application, is necessary in today's intensive agriculture. Mineral fertilisers, both macro and micro, in combination with suitable soil fertilisation procedures, are recommended for improving grain development, productivity, and nutrient concentration, particularly zinc. If the benefits of biofortified crops to human health are shown to consumers, they will be in high demand. Furthermore, in order to improve overall, we need to alter our old formulated fertiliser recommendations in light of current trends in micronutrient shortages, particularly Zn.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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