Bulletin of Environmental Contamination and Toxicology (2021) 106:852–858
https://doi.org/10.1007/s00128-021-03183-x

Concentration and Localization of Fe and Zn in Wheat Grain as Affected by Its Application to Soil and Foliage

Aysha Kiran[1] · Abdul Wakeel[2] · Rashida Sultana[1] · Ayesha Khalid[1] · Qurrat-ul-Ain[1] · Rafia Mubaraka[2,3] · Ahmad Naeem Shahzad[4] · Shaista Jabeen Khan[5] · Mehwish Noor[1]

Received: 4 September 2020 / Accepted: 8 March 2021 / Published online: 26 March 2021

Abstract
Nutritional status of people can be improved by enhancing zinc (Zn) and iron (Fe) content in cereals used as staple mainly in poor resource countries. Zinc and Fe were applied through soil and foliage in a study to biofortify wheat grains. Foliar application of both micronutrients increased the growth and grain vigor as compared to soil application and control. Also, foliar application significantly enhanced Zn and Fe concentration in grain pre-dominantly localized in aleurone layer. Exogeneous application of Fe and Zn was found beneficial for plant growth and enhanced Fe and Zn concentrations in grain, however aleurone layer and embryonic region of the grain showed higher accumulations than that in endosperm. Therefore, understanding of physiological and molecular pathways for uptake and localization of Fe and Zn in wheat grains need to be critically examined to improve their concentration in grain to achieve the biofortification targets.

Keywords Iron · Zinc · *Triticum aestivum* · Agronomic biofortification

Micronutrient deficiencies are more prevalent among those populations of the developing and developed world which pre-dominantly rely on plant-based diets (Jeong and Guerinot 2009; Olsen and Palmgren 2014). Staple foods lacking enough nutrients are major sources of calorie intake and are therefore a cause for mineral malnutrition in over one third of the population. Modern high yielding cultivars are not only deficient in iron (Fe) and zinc (Zn) but may also have phenolics and phytic compounds that could reduce the bioavailability of Fe and Zn in both plants and humans (Welch and Graham 2004). Biofortification with Zn and Fe of the edible parts of wheat (*Triticum aestivum* L.) such as grain and endosperm is an important goal of the current research (Wakeel et al. 2018). Poor concentrations of Fe and Zn in grain-based foods occur not only due to inherent genetic capability of plants for uptake and due to deficient soils, but also when the flour is predominantly made from the endosperm of wheat grain removing bran during milling. Most of the Zn and Fe in grain are located in the embryo and aleurone layer (Ozturk et al. 2006).

Microminerals such as Fe, Zn, copper (Cu), manganese (Mn) and macro-mineral such as magnesium (Mg) act as a cofactor for many antioxidant enzymes. Their deficiency decreases the antioxidant enzyme activities which increases the sensitivity of plants for environmental stresses (Cakmak and Kutman 2018). Iron is a basic constituent of chlorophyll and hemoglobin, while Zn is essential mineral element for all forms of life due to its involvement in many functions such as gene expression regulation and cell development (Walker et al. 2005).

Biofortification by using agronomic practices such as soil or foliar application of mineral fertilizers is considered a very promising and cost-effective approach to combat malnutrition. Zayed et al. (2011) reported that foliar application of microelements is more effective than soil application in rice as crop's response is immediate in case of foliar application. Although same protein families are involved in different

✉ Aysha Kiran
aysha.kiran@uaf.edu.pk

1 Department of Botany, University of Agriculture, Faisalabad, Pakistan

2 Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

3 Cologne Biocenter, Institute of Botany, University of Cologne, Cologne, Germany

4 Department of Agronomy, Bahauddin Zakariya University, Multan 60800, Pakistan

5 Pakistan Council for Scientific and Industrial Research, Lahore, Pakistan

steps of Fe and Zn transport but their physiological functions vary among plant organs (Borrill et al. 2014). For example in wheat, grains are storage sinks while the rest of plant body is a utilization sink for minerals i.e. Fe and Zn (Sultana et al. 2018). Thus, the availability of Zn and Fe at later stages of plant development particularly during grain filling period could increase the uptake as well as concentration of these elements in wheat grain (storage sink). Foliar application of additional Zn and Fe during grain filling period can increase activity in the source (flag leaf and stem) and thus more Zn and Fe in wheat grains (Cakmak et al. 2010). Genotypic variation for grain size and number of grains per spike is also important for grain Zn and Fe concentrations (Nowack et al. 2008; Velu et al. 2014). Combined application of both Zn and Fe could also be more effective in increasing their concentrations in grains than sole application (Habib 2012). The current study was carried out to investigate the effects of soil and foliar applications of Fe and Zn on growth, yield, grain quality and grain Fe and Zn contents of wheat and to map the localization of Fe and Zn in wheat grains. Furthermore, the localization of Fe and Zn in wheat grain in response to agronomic application of these minerals was main objective of this study.

Materials and Methods

An experiment was conducted at research area of Pakistan Council of Scientific and Industrial Research (PCSIR), Lahore, Pakistan. The standard statistical design to assure the quality of data. Randomized complete block design (RCBD) were used with factorial arrangements for field trial with plot size 3×3 m^2. For each treatment there were three replications. RCBD is commonly used in agriculture experiments for best quality data. Four wheat cultivars namely Faisalabad-2008, Saher-2006, Lasani-2011 and Punjab-2011 were evaluated. Nitrogen (N), phosphorus (P), and potassium (K) fertilizers were applied at the rate of 150, 100, 60 kg ha^{-1} in the form of urea (46% N), diammonium phosphate (18% N; 46% P_2O_5) and potassium sulfate (50% K_2O), respectively. Full doses of P and K along with 1/3 of total N were applied as basal dose. Remaining N was applied during 1st and 3rd irrigation. The 0.7% and 0.2% solution of $FeSO_4 \cdot 7H_2O$ and $ZnSO_4 \cdot 7H_2O$ were used for foliar application of Fe and Zn, respectively at tillering, booting and milking stage. About 500 mL of each solution was sprayed on each plot and equivalent amounts were applied to soil by flooding at tillering, booting and milking stage along with irrigation water. Four irrigations were applied to crop during growth period. First irrigation was applied at tillering stage, second at booting stage, third at milking and fourth at grain filling stage.

Fresh leaf samples were collected at anthesis stage. Chlorophyll content and carotenoids were estimated by using the method of (Sumanta et al. 2014). After threshing, yield parameters were measured following standard methods. Moisture contents, crude ash contents, crude protein, crude fiber and alcoholic acidity were estimated by following the standard AOAC protocols (AOAC 2016). Gluten contents were determined through a hand-washing method of AACC (AACC International 2000). For biochemical analysis, grain samples were oven-dried at 75°C until constant weight was attained. Afterwards grains were ground, and 0.25 g sample was mixed with 2.5 mL perchloric acid and nitric acid (1:2) mixture in a digestion flask. After 24 h, this mixture was heated on a hot plate until the mixture became colorless. After making the volume to 50 mL with distilled water, mixture was filtered. During analytical lab tests (determination of Fe and Zn) different suitable known concentration of standards for both elements were run to standardize the instrument along with blank sample to correct the sample from existing impurities. The instrument used for the determination of Fe and Zn concentration in the samples was Z.8200 HITACHI Atomic Absorption spectrometer (AAS). Different suitable known concentration of Fe and Zn standards Applichem® were used to standardize the instrument along with blank. The known concentration plant material was run along with samples to double check the instrument precision. The standards were also used after each 20 samples to cross check the calibration of the equipment. The coefficient of determination (R^2) value was set to 0.998, it explains how much variability can be caused of one factor relative to other. The minimum limit of detection (LOD) in sample for Fe and Zn were 0.01 and 0.05 mg L^{-1}, respectively, of this instrument. The Fe and Zn localization in grains was monitored by staining the grain sections by using the Perl's Prussian Blue Solution and diphenyl thiocarbazone (DTZ), respectively (Fig. 3). Slides of cross sections were prepared after staining and mounted with Canada Balsam and observed under digital camera supplemented stereomicroscope (Meiji MT4300H).

Data was collected from each replication and whole data set was subjected to statistical analysis by using R studio 4.0.3 (Bunny-Wunnies Freak Out) (Fox and Leanage 2016) and analysis of variance was employed under RCBD factorial design. LSD are all-pairwise test of parameters for V*T, R.

Results

Both foliar and soil application of Fe and Zn significantly affected the plant height. Among wheat genotypes, maximum plant height was recorded for Saher-2006 (91.7 cm) followed by Lasani-2008 (90.23 cm), Punjab-2011(88.43 cm) and Faisalabad-2008 (87.7 cm) under foliar application (Table 1). Leaf area index (LAI) was significantly affected by foliar and soil applications of Fe and Zn as compared to control treatments with mean values ranging between 39.28 to 44.71 cm^2 (Table 1), respectively. Data regarding growth rate was calculated from samples collected between milking and harvesting stages. Maximum growth rate was observed for Saher-2006 (11.10 g $days^{-1}$ m^{-2}) when Zn and Fe were applied to foliage (Table 1). Foliar application was found comparatively more effective for chlorophyll contents as compared to soil application and control treatment. No significant differences were observed for carotenoids in wheat varieties under all treatments (Table 1).

Maximum number of spikelets per spike (16.33), spike length (9.9 cm), number of grains per spike (50.6) (Table 2) and 1000 grain weight (36.16 g) (Fig. 1a) were observed for Saher-2006 under foliar application of Fe and Zn. On the other hand, minimum number of grains (32.67 grains) were observed for Punjab-2011 under control conditions. Genotypic variation was observed in case of number of tillers among these four wheat varieties. Highest number of tillers, i.e. 13, were counted for Saher-2006, followed by Lasani-2008, Faisalabad-2008 and Punjab-2011 with 8, 8 and 6 tillers, respectively, under foliage application of Fe and Zn (Table 2).

Grain per spike was only significantly increased only by foliar application of Fe and Zn for Saher-2006 and Faisalabad-2008 as compared to control but not by soil application of these nutrients. Harvest index, biological and grain yield were not affected by application of Fe and Zn (Fig. 1).

Wheat grain quality parameters such as moisture content, alcoholic acidity, ash contents, crude fiber, total protein and

Table 1 Growth parameters and pigments influenced by Zn and Fe application

Variety	Treatment	Crop growth rate (mg DW day^{-1})	Plant height (cm)	Leaf area index (LAI) (cm^2)	Total chlorophyll (mg g^{-1})	Carotenoids (mg g^{-1})
Faisalabad-2008	Control	5.9 ± 0.3f	81.1 ± 0.4 g	39.4 ± 0.8de	2.2 ± 0.02e	0.6 ± 0.03a
	Soil	7.3 ± 0.2e	82..2 ± 0.2 fg	40.9 ± 0.6bcde	2.6 ± 0.06ab	0.8 ± 0.04a
	Foliar	8.8 ± 0.2c	87.7 ± 0.7bc	42.6 ± 1.1bcd	2.6 ± 0.04a	0.7 ± 0.04a
Saher-2006	Control	8.8 ± 0.2c	83.33 ± 0.8efg	40.7 ± 0.9bcde	2.3 ± 0.05d	0.6 ± 0.03a
	Soil	9.5 ± 0.1bc	86.4 ± 1.2 cd	57.6 ± 1.9a	2.6 ± 0.06ab	0.8 ± 0.1a
	Foliar	11.1 ± 0.5a	91.7 ± 0.8a	44.7 ± 0.8b	2.6 ± 0.01a	0.8 ± 0.01a
Lasani-2008	Control	7.9 ± 0.02de	83.16 ± 0.5efg	40.2 ± 1.1cde	2.3 ± 0.03de	0.7 ± 0.03a
	Soil	9.03 ± 0.4bc	85 ± 0.8de	41.7 ± 1.5bcde	2.5 ± 0.02bc	0.7 ± 0.07a
	Foliar	9.7 ± 0.3b	90.23 ± 1.4ab	43.7 ± 0.9bc	2.6 ± 0.04a	0.8 ± 0.05a
Punjab-2011	Control	4.4 ± 0.09 g	82 ± 0.5 g	38.2 ± 1.2e	2.1 ± 0.03f	0.7 ± 0.1a
	Soil	7.0 ± 0.1f	84.7 ± 1.09def	39.0 ± 1.5de	2.3 ± 0.04de	0.7 ± 0.04a
	Foliar	8.7 ± 0.2 cd	88.43 ± 0.8bc	40..6 ± 0.4bcde	2.3 ± 0.01cd	0.8 ± 0.09a

Values are mean of three replicates. Mean values sharing the same letters are not significantly different

Table 2 Yield parameters influenced by Zn and Fe application. Values are mean of three replicates

Variety	Treatment	Spikelets per spike	Spike length	Grains per spike	Total No. of tillers	Productive tillers
Faisalabad-2008	Control	13 ± 0.5e	7.7 ± 0.5de	34.3 ± 4.6de	7 ± 0.5c	6 ± 0.5c
	Soil	15.3 ± 0.3abcd	8.5 ± 0.5bcd	39.6 ± 2.7bcde	10 ± 0.9b	6.6 ± 0.8bc
	Foliar	15.6 ± 0.8abc	9.6 ± 0.3ab	44 ± 1.9abc	11 ± 0.5ab	8 ± 0.5ab
Saher-2006	Control	14 ± 0.5cde	7.8 ± 0.1de	43 ± 1.5abcd	7.6 ± 0.8c	6.1 ± 0.7c
	Soil	15 ± 0.5abcd	9.2 ± 0.1abc	47.6 ± 1.3abc	11 ± 0.5ab	7 ± 0.2bc
	Foliar	16.3 ± 0.8a	9.9 ± 0.2a	50.6 ± 1.4a	13 ± 0.5a	9.1 ± 0.5a
Lasani-2008	Control	15.3 ± 0.3abcd	7.9 ± 0.4de	39.6 ± 2.5bcde	7.6 ± 0.8c	6 ± 0.5c
	Soil	16 ± 0.5ab	9.5 ± 0.2ab	43.3 ± 4.3abc	10.6 ± 0.3b	7.1 ± 0.4bc
	Foliar	16.3 ± 0.6a	9.8 ± 0.1a	48.3 ± 2.9ab	12 ± 1.1ab	8 ± 0.2ab
Punjab-2011	Control	13.6 ± 0.3de	7.2 ± 0.8e	32.6 ± 2.6e	7.3 ± 0.6c	6 ± 0.5c
	Soil	14.3 ± 0.6bcde	8.1 ± 0.4cde	39.3 ± 2.8cde	10 ± 0.5b	6.3 ± 0.8c
	Foliar	16 ± 0.5ab	9.5 ± 0.3ab	41.3 ± 3.6bcde	11.3 ± 0.8ab	7.3 ± 0.3bc

Mean values sharing the same letters are not significantly different

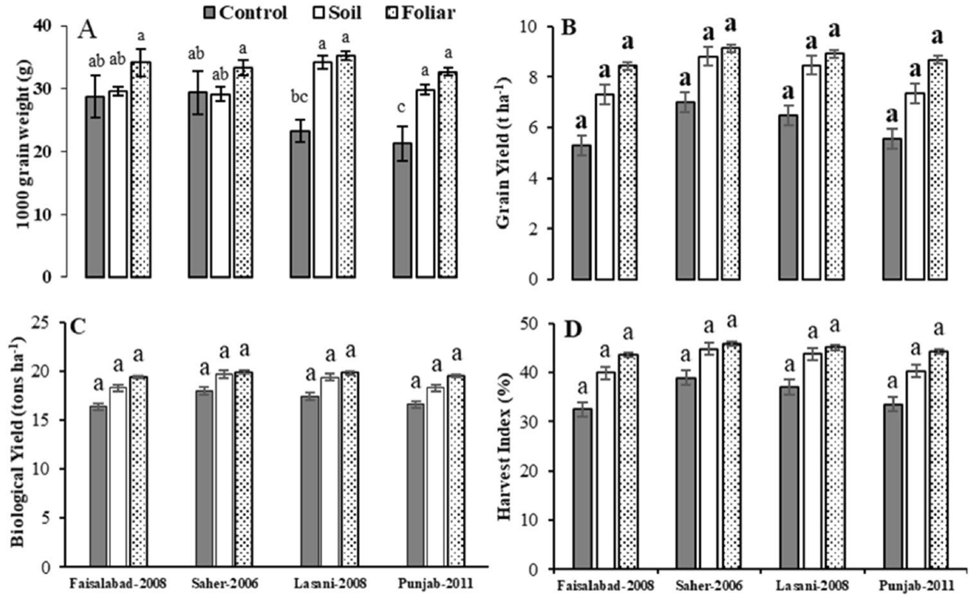

Fig. 1 Yield parameters influenced by soil and foliar application of Zn and Fe. The columns represent means of three replicates ± SE shown as bars. Different letters indicate significant differences between the treatments ($p \leq 5\%$)

gluten contents were measured in grains along with Fe and Zn contents in four varieties under all treatments. Significant variation was observed for all these parameters. Grain moisture content is an important parameter to assess the shelf life. Reduction in grain moisture content was observed under Fe and Zn application as compared to control. Ash contents ranged between 1.3 and 1.6% while crude fiber between 1.12 and 1.42% across all treatments (Fig. 2).

No significant differences were observed among wheat varieties for protein and gluten contents across all treatments (Fig. 2c, e). Significant variation was observed for grain Fe and Zn concentrations under different Fe and Zn treatments (Fig. 2). Highest Fe (33.76 mg kg^{-1}) concentration in grains were observed for Saher-2006 under foliar application of these micronutrients. It was followed by Lasani-2008 (31.96 mg kg^{-1}) and Punjab-11 (30 mg kg^{-1}) and Faisalabad-2008 (28.64 mg kg^{-1}). Under soil application, Fe concentrations in grains were ranged from 24 to 27 mg kg^{-1}, while in control treatment it ranged between 16 and 20 mg kg^{-1}.

Highest Zn concentration in grain was observed for Punjab-11 (48.67 mg kg^{-1}), followed by Lasani-2008 (45.67 mg kg^{-1}), Saher-2006 (42.63 mg kg^{-1}), and Faisalabad-2008 (40.5 mg kg^{-1}) under foliar spray treatment. The grain Zn concentrations ranged between 28 and 38 mg kg^{-1} under soil Zn application while it ranged between 18 and 30 mg kg^{-1} Zn for control treatment (Fig. 2).

Microscopic observations for localized Fe and Zn with stains revealed higher concentrations in the aleurone layer. Differentially localized Fe and Zn could be observed in aleurone layer, crease, scutellum and endospermic regions of wheat kernel under different treatments. Greenish color appearance is specific to the appearance of Fe and red color is specific for Zn in wheat seeds when stained with Prussian Blue and DTZ, respectively (Ozturk et al. 2006). Higher concentrations of both Fe and Zn were observed in aleurone layer followed by crease, scutellum and embryonic region, while endospermic region showed the least concentrations (Fig. 3).

Discussion

Iron and Zinc are essential nutrients for growth and development of both plants and animals. At present, an increase in crop-nutritional value is of utmost importance because both plants and animals are suffering from the deficiency of micronutrients. A sustainable approach being used for the reduction of mineral malnutrition in developing countries is agronomic biofortification. Several studies have shown positive effects of Fe and Zn application either by soil or by foliar spray to increase their contents in cereal grains (Ozturk et al. 2006; Kyriacou et al. 2014; Cakmak and Kutman 2018). Present study also showed positive effects of combined application of Fe and Zn on morphological, yield and biochemical parameters in wheat grains. Foliar application was more effective for improvement of Fe and Zn contents in grains as compared to soil application. Habib (2012) suggested that foliar feeding of Fe + Zn could be an excellent low-cost method for biofortification in wheat grains. The enhanced concentrations of Zn and Fe through foliar spray have also been reported as an effective strategy to improve micronutrients concentration in grains (Velu et al. 2014; Naz et al. 2015; Ru et al. 2018; Sultana et al., 2018). Relationship of Fe and Zn was also studied by Sultana et al. (2018) in a field experiment and concluded that

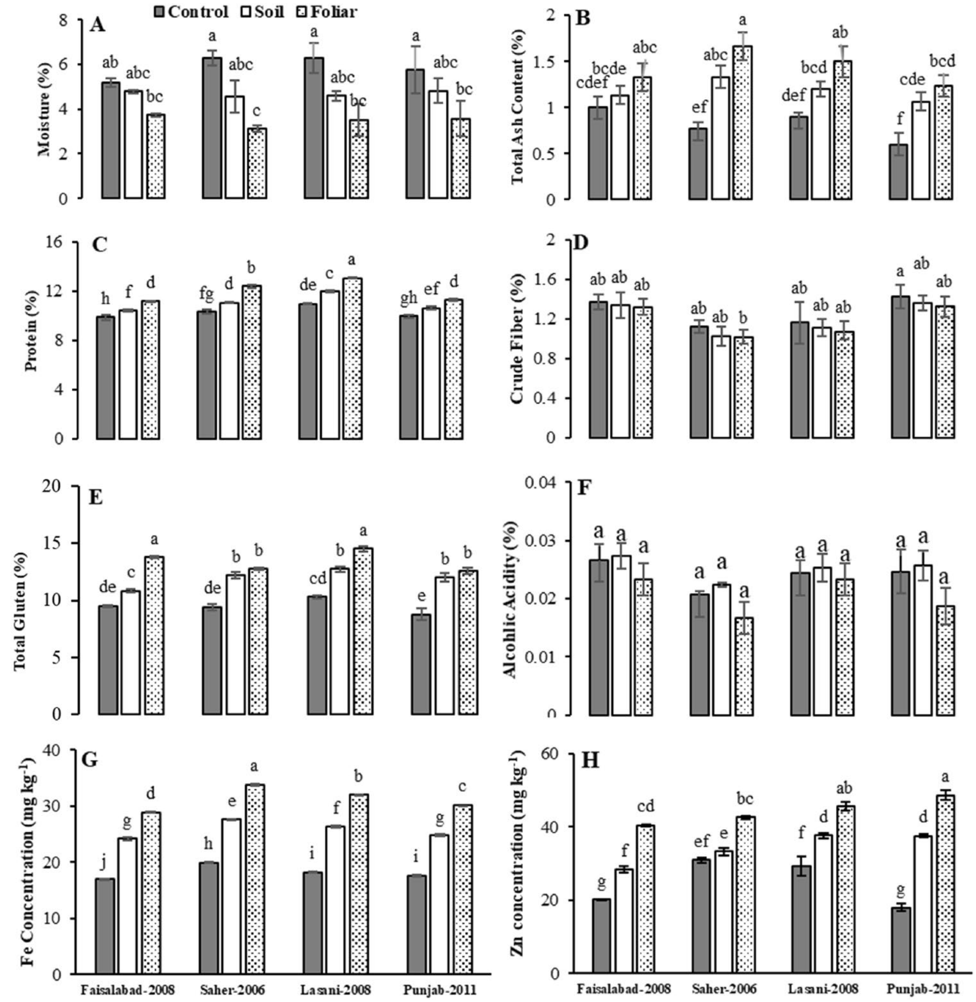

Fig. 2 Wheat grain qualitative parameters as influenced by soil and foliar application of Zn and Fe. The columns represent means of three replicates ± SE shown as bars. Different letters indicate significant differences between the treatments ($p \leq 5\%$)

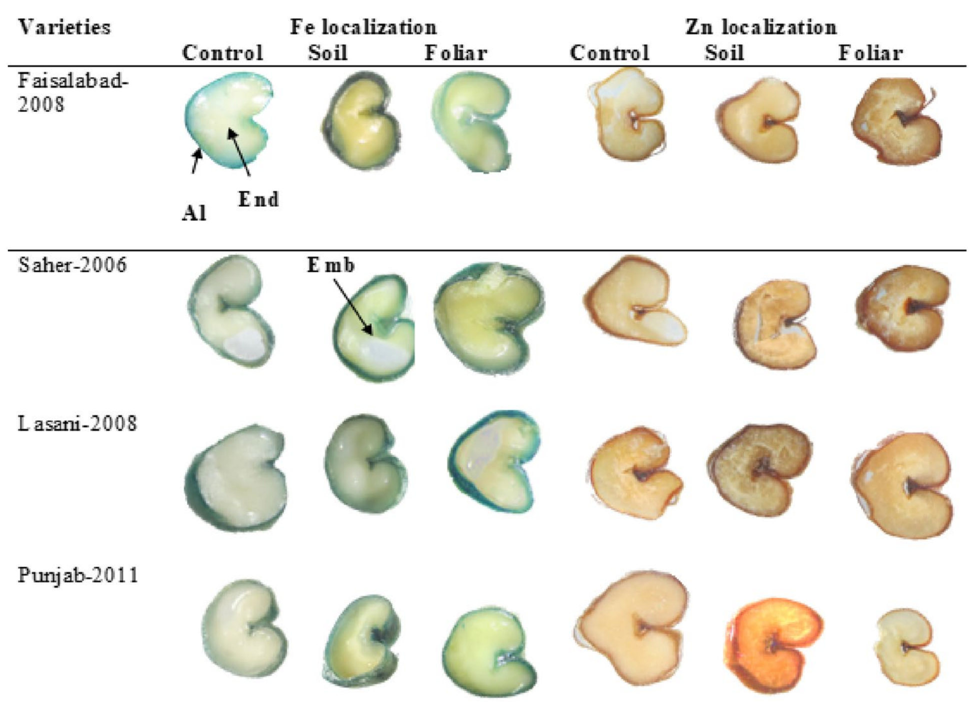

Fig. 3 Fe and Zn localization in wheat grain of four wheat cultivars under different treatments. Greenish color due to Prussian Blue Stain is specific to the appearance of Fe and red color due to diphenyl thiocarbazone (DTZ) stain is specific for Zn in wheat seeds. (*Al* aleuron layer, *End* endospermic region, *Emb* embryonic region)

Zn + Fe foliar application at grain filling stage can improve both yield and quality of wheat grains.

In present study, Fe and Zn foliar treatment improved the growth, yield and quality attributes of wheat crop as compared to soil application. The Fe and Zn are involved in many vital physiological pathways and have important role in respiration, photosynthesis and production of healthy green leaves. Higher leaf chlorophyll and carotenoids were observed under Fe and Zn application which not only upregulated the photosynthesis process but also increased the crop growth, yield and grain quality. Ru et al. (2018) found significant increase in the leaf chlorophyll-a, chlorophyll-b, total chlorophyll and total carotenoid content in wheat leaves. They also observed increase in the grain yield, protein and gluten content of wheat due to Zn and Fe application either in soil or in the form of spray on foliage. Genotypic variation for improvement in grain quality parameters is very important to consider for recommendations for field or future studies, because these quality parameters are important for usage of flour for house hold purposes or in baking industry (Ciccolini et al. 2017). Although all cultivars responded to exogenous application of Fe and Zn positively, however foliar application showed maximum concentration of Fe and Zn in grains. Higher concentration of Fe in grains of Saher-2006 and Lasani-2008 can be exploited for breeding purposes in future studies. Similarly, Punjab-2011 and Lasani-2008 should be exploited for high Zn concentration (Fig. 2).

Low inheritance and poor availability of Fe and Zn in cereal grains seems to be the root cause of mineral nutrient deficiencies, especially in regions where most important sources of calories are cereal-based diets. Endosperm is the most important part in wheat grain used in human nutrition through bread. However, Fe and Zn are concentrated in the embryo and in the aleurone layer keeping the endosperm in low concentration which can be observed by simple staining techniques (Fig. 3). Nevertheless, grinding of wheat grains between stones produced whole meal flour in traditional milling contained all parts of the grain and can improve the human nutrition. However, mostly-used roller milling has enabled the precise separation of bran (embryo and aleurone) and starchy endosperm-based flour facilitated the availability of affordable white bread, previously an expensive luxury. Ultimately, white flour has substantially lower contents of Fe and Zn than whole meal due to removal of both the embryo and the aleurone layer (Ozturk et al. 2006; Velu et al. 2014; Cakmak and Kutman 2018; Garcia-Oliveira et al. 2018; Balk et al. 2019). Genotypic variation for endospermic Fe and Zn in response to agronomic application demands precise recommendations for agronomic biofortification.

Conclusively, application of Fe and Zn, exogenously (both soil and foliar), is beneficial for plant health and to increase their concentration in grains but endospermic localization of Fe and Zn could still not be achieved as per findings of this study. Nevertheless, possible genotypic potential can be exploited to enhance endospermic concentration of these micronutrients through genetic modifications using breeding or transgenic approaches. Furthermore, combined with agronomic biofortification, understanding of the physiological and molecular pathways for uptake of Fe and Zn and their translocation in cereal grains need to be critically examined to improve bioavailable contents to combat micronutrient malnutrition.

References

AACC International (2000) AACC International approved methods of analysis. AACC International, Saint Paul

AOAC (2016) Official methods of analysis of AOAC INTERNATIONAL, 20th edn. AOAC International, Rockville

Balk J, Connorton JM, Wan Y, Lovegrove A, Moore KL, Uauy C, Sharp PA, Shewry PR (2019) Improving wheat as a source of iron and zinc for global nutrition. Nutr Bull 44:53–59

Borrill P, Connorton JM, Balk J, Miller AJ, Sanders D, Uauy C (2014) Biofortification of wheat grain with iron and zinc: integrating novel genomic resources and knowledge from model crops. Front Plant Sci 5:53

Cakmak I, Kutman UB (2018) Agronomic biofortification of cereals with zinc: a review. Eur J Soil Sci 69:172–180

Cakmak I, Pfeiffer WH, McClafferty B (2010) REVIEW: biofortification of durum wheat with zinc and iron. Cereal Chem J 87:10–20

Ciccolini V, Pellegrino E, Coccina A, Fiaschi AI, Cerretani D, Sgherri C, Quartacci MF, Ercoli L (2017) J Agric Food Chem 65:5443–5452

Fox J, Leanage A (2016) J Stat Softw 73:1–13

Garcia-Oliveira AL, Chander S, Ortiz R, Menkir A, Gedil M (2018) Genetic basis and breeding perspectives of grain iron and zinc enrichment in cereals. Front Plant Sci 9:937

Habib M (2012) Effect of supplementary nutrition with Fe, Zn chelates and urea on wheat quality and quantity. Afr J Biotechnol 11:2661–2665

Jeong J, Lou GM (2009) Homing in on iron homeostasis in plants. Trends Plant Sci 14:280–285

Kyriacou B, Moore KL, Paterson D, De Jonge MD, Howard DL, Stangoulis J, Tester M, Lombi E, Johnson AAT (2014) Localization of iron in rice grain using synchrotron X-ray fluorescence microscopy and high resolution secondary ion mass spectrometry. J Cereal Sci 59:173–180

Naz S, Tahir MA, Qadir A (2015) Iron and zinc biofortification strategies in wheat crop by exogenous application of micronutrients. Food Sci Q Manag 35:49–55

Nowack B, Schwyzer I, Schulin R (2008) Uptake of Zn and Fe by wheat (*Triticum aestivum* var. Greina) and transfer to the grains in the presence of chelating agents (ethylenediaminedisuccinic acid and ethylenediaminetetraacetic acid). J Agric Food Chem 56:4643–4649

Olsen LI, Palmgren MG (2014) Many rivers to cross: the journey of zinc from soil to seed. Front Plant Sci 5:1–6

Ozturk L, Yazici MA, Yucel C, Torun A, Cekic C, Bagci A, Ozkan H, Braun H-J, Sayers Z, Cakmak I (2006) Concentration and localization of zinc during seed development and germination in wheat. Physiol Plant 128:144–152

Ru K, Hl S, Kunjadia BB (2018) Effect of zinc and iron application on leaf chlorophyll, carotenoid, grain yield and quality of wheat in calcareous soil of Saurashtra region. Int J Chem Stud 6:2092–2096

Sultana S, Naser HM, Quddus MA, Shil NCA, Hossain MA (2018) Effect of foliar application of iron and zinc on nutrient uptake and grain yield of wheat under different irrigation regimes. Bangladesh J Agric Res 2:395–406

Sumanta N, Imranul Haque C, Nishika J, Suprakash R (2014) Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. Res J Chem Sci 4:63–69

Velu G, Ortiz-Monasterio I, Cakmak I, Hao Y, Singh RP (2014) Biofortification strategies to increase grain zinc and iron concentrations in wheat. J Cereal Sci 59:365–372

Wakeel A, Farooq M, Bashir K, Ozturk L (2018) Micronutrient malnutrition and biofortification: recent advances and future perspectives. In: Hossain SD et al (eds) Plant micronutrition use efficiency. Academic Press, London, pp 225–243

Walker CF, Kordas K, Stoltzfus RJ, Black RE (2005) Interactive effects of iron and zinc on biochemical and functional outcomes in supplementation trials. Am J Clin Nutr 82:5–12

Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp Bot 55:353–364

Zayed BA, Salem AKM, El Sharkawy HM (2011) Effect of different micronutrient treatments on rice (*Oriza sativa* L.) growth and yield under saline soil conditions. World J Agric Sci 7:179–184