Biofortification of cereals with zinc and iron through fertilization strategy

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Abstract
Zinc (Zn) and iron (Fe) deficiencies are well-documented public health issue and an important soil constraint to crop production. Generally, there is a close geographical overlap between soil deficiency and human deficiency of Zn and Fe, indicating a high requirement for increasing concentrations of micronutrients in food crops. Breeding new plant genotypes for high grain concentrations of Fe and Zn (genetic biofortification) is the most cost-effective strategy to address the problem; but, this strategy is a long-term process. A rapid and complementary approach is therefore required for biofortification of food crops with Zn and Fe in the short term. In this regard, a fertilizer strategy (agronomic biofortification) represents an effective way for biofortification of food crops. In this paper, several examples are presented showing that application of Zn fertilizers greatly contribute to biofortification of cereal grains with Zn. By contrast, application of various inorganic and chelated Fe fertilizers remains ineffective for increasing grain Fe concentration. However, improving nitrogen (N) nutritional status of plants promoted accumulation of Fe (and also Zn) in grain. It appears that N nutritional status of plants plays a critical role in biofortification of cereal grains with Zn and Fe.

Key Words
Zinc deficiency, iron deficiency, agronomic biofortification, wheat, zinc fertilizers, iron fertilizers.

Introduction
Zinc and Fe deficiencies are a growing public health and socioeconomic issue, particularly in the developing world (Welch and Graham 2004). Recent reports indicate that nearly 500,000 children under 5 years of age die annually because of Zn and Fe deficiencies (Black *et al.* 2008). Zinc and Fe deficiencies together with vitamin A deficiency have been identified as the top priority global issue to be addressed to achieve a rapid and significant return for humanity and global stability (www.copenhagenconsensus.com). Low dietary intake of Fe and Zn appears to be the major reason for the widespread prevalence of Fe and Zn deficiencies in human populations. In countries with a high incidence of micronutrient deficiencies, cereal-based foods represent the largest proportion of the daily diet (Cakmak 2008). Cereal crops are inherently very low in grain Zn and Fe concentrations, and growing them on potentially Zn- and Fe-deficient soils further reduces Fe and Zn concentrations in grain (Cakmak *et al*. 2010). Thus, biofortification of cereal crops with Zn and Fe is a high-priority global issue. HarvestPlus (www.harvestplus.org) is the major international consortium to develop new plant genotypes with high concentrations of micronutrients by applying classical and modern breeding tools (i.e. genetic biofortification). Although plant breeding is the most sustainable solution to the problem, developing new micronutrient-rich plant genotypes is a protracted process and its effectiveness can be limited by the low amount of readily available pools of micronutrients in soil solution (Cakmak 2008). Application of Zn- and Fe-containing fertilizers (i.e. agronomic biofortification) is a short-term solution and represents a complementary approach to breeding.

Materials and Methods
The experiments described in this paper were conducted with wheat either under field or greenhouse conditions. Field trials were conducted in Central Anatolia, Turkey where soil Zn deficiency is well documented. In the case of soil application, the rate of $ZnSO_4$ application was 50 kg $ZnSO_4$ per ha, while foliar Zn was applied at the rate of 0.5 % $ZnSO_4.7H_20$ (approx. 4 kg $ZnSO_4.7H_20$ /ha) at 2 growth stages. In greenhouse studies, plants were grown with 0.05 mg Zn (low Zn plants) or 2 mg Zn (adequate Zn plants) or 10 mg Zn (high Zn plants) per kg soil, and Zn was applied in the form of $ZnSO_4 \cdot 7H_2O$. Nitrogen treatments were 50 mg N (for low N plants) or 200 mg N (for adequate N plants) in the form of $Ca(NO_3)_2 \cdot 4H_2O$. In the greenhouse experiments investigating the effectiveness of Fe fertilizers on increasing grain Fe, the following Fe compounds used : $FeSO_4$, Fe-EDTA, Fe-EDDHA and Fe-citrate, applied either into soil at the rates of 0, 5 and 10 mg Fe per kg soil or sprayed to foliar at the rate of 0.2 % Fe-EDTA at the booting and early milk stages. The rates of soil applied nitrogen ranged from 100 to 600 mg N per kg soil and applied in the form of $Ca(NO_3)_2$ (see Kutman *et al*. 2010 for further details).

Results

Field tests in Central Anatolia, where Zn deficiency is widespread, showed that soil- and foliar-applied $ZnSO_4$ significantly enhanced grain Zn concentration in wheat. The largest increases in grain Zn concentration were found in the case of combined application of soil and foliar Zn fertilizers that caused more than a 3-fold increase in grain Zn (Figure 1). Under certain conditions, Zn fertilizers were also highly effective in increasing grain yield of wheat. In contrast to Zn-deficient locations, soil application of $ZnSO_4$ in Zn-adequate soils had no or very little effect on grain Zn. However, irrespective of soil Zn status, foliar Zn applications resulted in significant increases in grain Zn, especially in the case of late-season foliar Zn application.

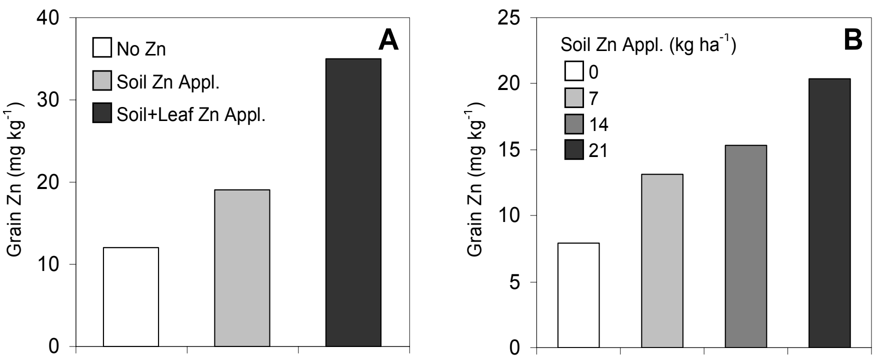

Figure 1. Grain Zn concentrations of durum wheat subjected to soil and foliar application of $ZnSO_4$ (A) and increasing rate of soil Zn fertilization (B). Plants were grown on a highly Zn-deficient calcareous soil under field conditions in Central Anatolia (Cakmak *et al.* 2010).

Enrichment of wheat grain with Zn was maximized when plants were supplied sufficiently with nitrogen (N) through soil and/or foliar (e.g., urea) application of N fertilizers. In a greenhouse study, effects of soil- and foliarly-applied N and Zn fertilizers on grain Zn concentration of durum wheat were studied when grown on a Zn-deficient soil. When Zn application was adequate, both soil and foliar N applications significantly increased grain Zn concentration. Nitrogen application remained ineffective on grain Zn when Zn supply was suboptimal (Figure 2). It seems that N and Zn act synergistically in improving the grain Zn concentration when N and Zn are present at sufficient amounts either in the growth medium or in the leaf tissues. Enrichment of commonly applied compound fertilizers with Zn is a further fertilizer practice useful for increasing Zn concentration of plants. In India, application of Zn-coated urea fertilizer significantly improved both grain yield and grain Zn concentrations (Shivay *et al*. 2008).

In contrast to Zn fertilizers (e.g. $ZnSO_4$), Fe fertilizers applied either in inorganic form (e.g. $FeSO_4$) or chelated form (e.g., Fe-EDTA, Fe-EDDHA or Fe-citrate) into soil or as a foliar were not effective in improving grain Fe concentrations of wheat grain (Aciksoz *et al.* unpublished results). Among Fe fertilizers applied, Fe-EDTA appeared to be the best Fe source for increasing grain Fe concentrations. However, increasing N applications promoted Fe accumulation in grain at each Fe application. As found with Zn, there was a close positive correlation between tissue concentrations of Fe and N.

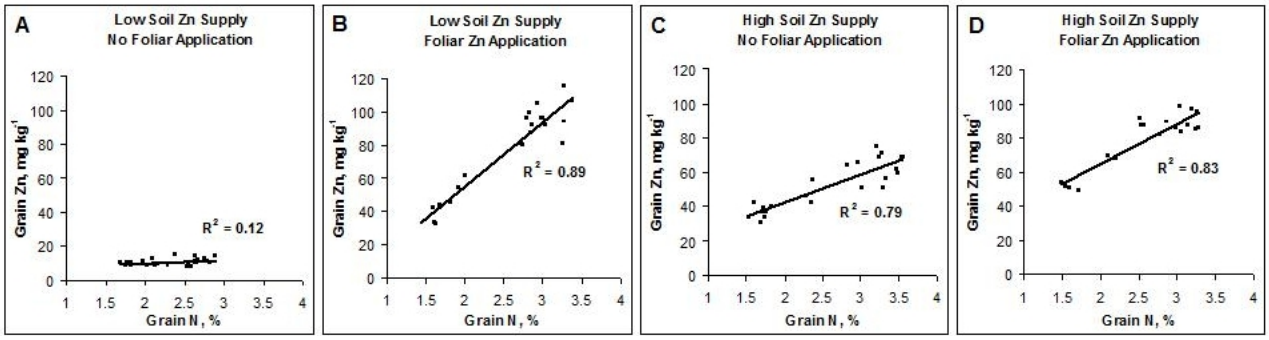

Figure 2. Correlation between grain concentrations of Zn and N in durum wheat (*Triticum durum* cv. Balcali 2000). Plants were grown at low (A and B) or high (C and D) Zn supply on a Zn-deficient calcareous soil under greenhouse conditions with (B and D) or without (A and C) foliar application of Zn (Kutman *et al.* 2010).

Conclusion

The role of N nutrition in biofortification of wheat grain with Zn and Fe is a highly relevant issue in terms of designing new fertilizer programs for increasing grain Zn and Fe and selecting the most suitable parental lines in breeding programs aimed at improving grain Zn and Fe. Improving N nutrition of plants may contribute to grain Zn and Fe concentrations by affecting the levels of Zn- or Fe-chelating nitrogenous compounds required for transport of Zn and Fe within plants and/or the abundance of Zn or Fe transporters needed for root uptake and phloem loading of Zn and Fe. Finally, the results indicate that nitrogen management represents an effective agronomic tool to contribute to grain Zn and Fe concentrations.

Acknowledgement

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