

Research Article

Improving Grain Micronutrient Content of Durum Wheat (*Triticum turgidum* var. durum) through Agronomic Biofortification to Alleviate the Hidden Hunger

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Improvement of durum wheat grain quality through agronomic biofortification becomes a priority research area and an effective route to combat malnutrition. An experiment was conducted to evaluate the effect of micronutrient application to different varieties of durum wheat and seeding rate on final harvest grain quality under different growing locations. The treatments were arranged in split-split plot design where the varieties were assigned in the main plot, micronutrients into the subplots, and seeding rate into the sub-subplots. Each variety was sown at four levels of seeding rates and treated with ZnSO₄ and FeSO₄ applied foliarly, both at a rate of 25 Kg ha⁻¹ during flowering. Micronutrients were applied in the form of ZnSO₄ 7H₂O and FeSO₄ 7H₂O. The study confirmed that application of 25 Kg ha⁻¹ ZnSO₄-containing fertilizer has increased mineral content from 33.04 mg Kg⁻¹ to 56.73 mg kg⁻¹. The tested durum wheat varieties significantly differ in their capacity to accumulate grain Zn and Fe concentrations. Higher amount of Zn (20 mg kg⁻¹) and Fe (10 mg kg⁻¹) were accumulated by the landrace 208304 than by Asassa, an improved commercial variety. Increasing seeding rate from 100–175 Kg ha⁻¹ has reduced grain Zn and Fe concentrations. Grain mineral concentration was significantly lower at the Mekelle location than at the Melfa location. It can be concluded that foliar application of ZnSO₄ and FeSO₄ to the landrace, acc.208304, combined with 125 Kg seeds ha⁻¹ can produce better Zn and Fe denser durum wheat grain. This will help to combat the hidden hunger, especially in resource poor countries, where fortified foods are limited in access and unaffordable by small-scale farmers.

1. Introduction

The twenty-first century has faced a challenge with malnutrition, as more than 1.5 billion people are burdened with one or more forms of micronutrient deficiency [1]. Narrowing the prevalence of micronutrient deficiency down to Ethiopia, especially in rural areas of Amhara and Tigray are the most severely affected regions; about half of pregnant women and 20% of children under five are vulnerable groups [2, 3]. The poor dietary diversification, monotonous wheat-based food consumption with low concentration, loss of crop genetic

diversity, and climate change coupled with soil micronutrient depletion could be responsible for high malnutrition rate. An excessive intake of wheat-based products is frequently reported as a principal reason for micronutrient malnutrition, as wheat is innately low in zinc content and high in phytate, which further limits the bioavailability of zinc in the edible portion of wheat [4]. This food habit therefore affects the human health condition through altered grain nutritional composition. Consequently, this leads to diverse health effects such as poor growth and development and reduced immunity and tissue development, as well as poor health and increased

risk of death [5]. Thus, nutritional knowledge and food habit may play an important role through moderating the influence of household food insecurity on the diet and accordingly on nutritional outcomes.

The intervention designed to reduce the extent of micronutrient deficiency and associated health problems ranges from dietary diversification, industrial fortification, pharmaceutical supplementation, and genetic biofortification. These interventions are an immediate and effective route to ameliorate micronutrient concentration in the human body and edible plants as well. The successful implementation of these exogenous strategies however is limited due to limited access and affordability for resource-poor farmers, failure to reach all individuals, and unavailability [6, 7]. Thus, agronomic biofortification (micronutrient fertilization) can be a complimentary and effective way of agricultural strategy to improve grain nutritional composition [8].

Agronomic biofortification is the application of micronutrient-containing mineral fertilizers to ameliorate concentration of essential nutrients in the edible part of food crops [9]. It can be achieved through application of micronutrient-containing fertilizers to the soil or foliarly directly to the crop leaves during specific developmental stage. However, its effectiveness is determined by the application method, growing environment, varietal response difference, and other agronomic practices [10]. In spite of its importance to reduce micronutrient deficiency, the study on how agronomic practices influence effectiveness of agronomic biofortification is scanty in northern Ethiopia. For this reason, this research was conducted the extent to which agronomic biofortification is influenced by the main and interaction effect of seeding rate, durum wheat varieties, and divergence in growing locations. Wheat varieties respond differently to the application of micronutrients during growing period probably due to their difference in micronutrient use efficiency. It has been shown that one of the effective routes to improve grain mineral composition is the application of zinc-containing fertilizer either to the soil or applying foliarly and combined use of soil and foliar application [11]. Foliar application was perceived to be more effective in increasing grain Zn and Fe concentrations in different edible crops. In wheat, the foliar-based micronutrient-containing fertilizer was reported to be effective in increasing grain zinc and iron concentrations compared to the soil-based application [12]. It is probably through the fact that foliar application of micronutrient fertilizers stimulated more nutrient uptake in the edible plant portion of cereal crops [13]. This research is, therefore, initiated (i) to evaluate the effect of foliar application of zinc- and iron-containing fertilizers on grain zinc and iron concentrations and (ii) to appraise the effectiveness of agronomic biofortification as determined by adjustment of seeding rate and difference in durum wheat varieties under two divergent agro-ecologies.

2. Materials and Methods

2.1. Description of the Study Areas. The field experiment was conducted at two locations in northern Ethiopia, at Mekelle University (MU) Research Station and Melfa Farmers

Training Center (FTC). The first location MU Research Station is suited between 13°30'N and 39°29'E. It has bimodal type of rainfall where 70 to 80% falls during July and August [14]. The other experimental location "Melfa" Farmers Training Center is also located at 13°39'N and 39°10'E. It receives an annual rainfall of 762 mm [15]. Both experimental locations are deficient in soil zinc and iron microelements [16].

2.2. Experimental Design and Treatments. The experiment was laid out in the split-split plot design. The varieties were assigned to the main plots, seeding rates to the subplots and micronutrients to the sub-subplots. The two tested durum wheat varieties were Asassa, an improved commercial variety, and farmer variety/landrace acc.208304. The varieties were planted at four levels of seeding rates (100, 125, 150, and 175 kg ha⁻¹) and treated with two levels of micronutrients including ZnSO₄ and FeSO₄. Micronutrients were applied in the form of ZnSO₄ 7H₂O and FeSO₄ 7H₂O. For each experimental plot, 20 g of Zn and Fe was applied after diluting in 1 L of water. The net plot size of subplots and sub-subplots was 4.4 m × 2.5 m and 1.2 m × 2.5 m, respectively. Each subplot and sub-subplots were separated by gang spacing of 0.5 m and 0.4 m, respectively. The spacing between replications was 0.8 m. Iron sulfate (FeSO₄) and zinc sulfate (ZnSO₄) were foliarly applied during flowering at a rate of 25 kg ha⁻¹. All experimental plots were equally treated with 46 kg ha⁻¹ of nitrogen and 20 kg ha⁻¹ of phosphorus. The nitrogen was split into two doses where the first half dose together with phosphorus was applied at planting. The remaining half dose of Nitrogen was applied at the tillering stage. Weed control was performed manually and maintained throughout the cropping period.

2.2.1. Grain Mineral Concentration Analysis. From the final grain harvested from each location, 20 gram of grain was sampled and sent to EZANA Mining Development PLC Laboratory for analysis of the grain mineral content. The grains were digested using Perten Laboratory Mill 120 to a standard sieve of 0.8 mm and grain zinc and iron concentrations were determined using Varian AA240FS Fast Sequential Atomic Absorption Photometer, a complete automated PC-controlled true double beam atomic absorption, which has fast sequential operation for fast multi-element flame AA determinations, operated with SpectrAA Base and PRO software version. All samples were digested in an automated digestion chamber. The procedure used to measure Zn and Fe concentrations (mg kg⁻¹) in the samples was as described by Kunda et al. [17].

2.3. Statistical Data Analysis. The raw data, after checking for normality and homogeneity, was analyzed for variance using GenStat statistical software ver. 14 [18], following the split-split plot design structure. This analysis allowed us to examine the main effects and the interaction effect between genotypes, seeding rates, and applied micronutrients. For significant effects, means were separated using least

significance difference (LSD) at 5% significance level. Duncan's multiple range test (DMRT) was employed for comparison of the various interaction means presented in various graphs. While some of the results presented in tabular form, most interaction results were presented as graph to make its visualization easier. The mean values were used to construct the graphs using excel graphing features. Bars representing treatments combination means were designated by error bars and separated by letters where bars separated by different letters were significantly different from each other.

3. Results and Discussion

3.1. Grain Zn and Fe Concentrations as Affected by Foliar Microfertilizers Application. The foliar application of $ZnSO_4$ at the flowering stage has significantly ($p < 0.001$) enhanced grain Zn concentration from 33.04 mg kg^{-1} to 56.73 mg kg^{-1} (Figure 1). Such increase in Zn concentration in wheat grain, which ultimately improve wheat nutritional value, encourages microfertilizers application with small additional cost. The finding agreed with Cakmak et al.'s [4] finding which reported 11% grain Zn concentration gain under $ZnSO_4$ fertilization. Zhangmin et al. [19] verified that foliar-based fertilization of zinc is more phloem mobile and consequently zinc readily translocated into developing grains in wheat. In addition, improvement in grain Zn concentration under foliar zinc fertilization could be due to reduction in grain phytic acid accumulation [19]. As the application of Zn-containing fertilizers decreases, both uptake and accumulation of phosphorus consequently decrease in grain phytic acid accumulation [20] and ultimately increase the bioavailability of zinc in the grain. The concentration of zinc in wheat grain highly depends on retranslocation of zinc from vegetative tissues during the reproductive stages [21]. On the other hand, the application of $FeSO_4$ did not impact grain iron concentration. The mean value of Fe concentration was not significantly different from the unfertilized plot (Figure 1). This could be due to the characteristics of the applied fertilizer, since iron is less phloem mobile in cereals and retained in older leaves [22, 23].

The agronomic biofortification to address nutrient deficiencies is an enticing concept, but there is much to understand about the factors that determine its effectiveness. The current study showed that agronomic biofortification of zinc and iron is affected by crop variety, seeding rate, and growing locations.

3.2. Determinant Factors of Grain Zn and Fe Concentrations

3.2.1. The Varietal Difference. The concentrations of Zn and Fe were significantly ($p < 0.001$) affected by varietal differences. The maximum grain zinc concentration was accumulated more by the farmer variety "208304" (67.19 mg kg^{-1}) than Asassa, the improved variety (46.27 mg kg^{-1}) (Figure 2). This implies that the farmer variety accumulated 20.92 mg kg^{-1} of Zn more than the improved variety. This difference in varietal micronutrients accumulation under similar growing conditions is due to

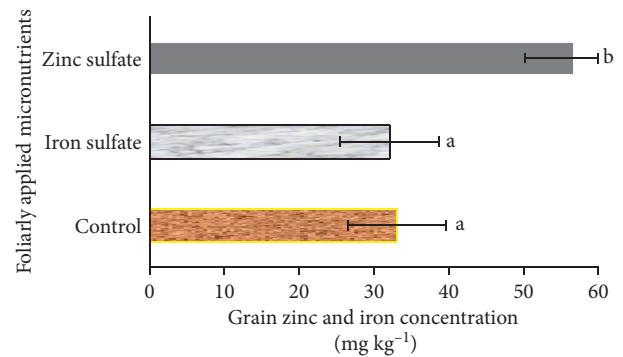


FIGURE 1: The effect of foliar application of $ZnSO_4$ and $FeSO_4$ on grain Zn and Fe concentrations (mg kg^{-1}). Bars labeled with different letters are significantly different from each other.

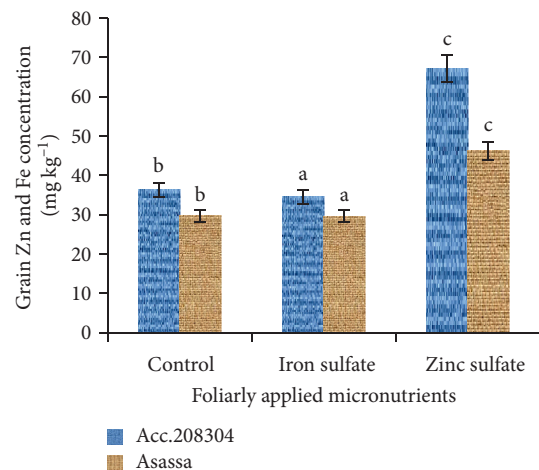


FIGURE 2: Response of the tested varieties to the applied zinc and iron fertilizers under deficit soil condition (mg kg^{-1}).

genetic difference of the varieties. This may indicate that ameliorating grain mineral concentration could also be possible through genetic biofortification through wise exploitation of existing crop genetic resources. The genetic variation in crops for micronutrient accumulation was reported by Melash et al. [24] and Mathpal et al. [25].

The differential accumulation of Zn by varieties could be related to their grain phytate content [26]. Therefore, choice of micronutrient efficient varieties may have a special advantage to reduce micronutrient deficiency in humans. The target level of zinc concentration required to combat deficiency of zinc in wheat grain was estimated to be 45 mg kg^{-1} [27]. Taking the ever-increasing global food demand and high prevalence malnutrition into consideration, increasing grain Zn concentration in high-yielding wheat variety is important. Hence, the target level to reduce human micronutrient deficiency could be achieved by using the genetic resource such as the farmer variety acc.208304 (Figure 2). Exploiting crop genetic resource to enhance mineral density under deficit soils fertility can be an alternative way to overcome the problem associated with micronutrient deficiency.

3.2.2. Seeding Rate. The other important factor determining the effectiveness of agronomic biofortification was the seeding rate. The current study identified a seeding rate of 125 kg ha^{-1} as optimal for better accumulation of Zn and Fe in durum wheat grain (Table 1). As the seeding rate increases from 100 to 175 kg ha^{-1} , the concentrations of Zn and Fe in the grain declined (Table 1). From the result, it seems that grain mineral composition has an inverse relationship with seeding rate beyond 125 kg ha^{-1} . This could be due to the interplant competition for available resources such as soil moisture which reduce the uptake and translocation of minerals when water is deficient in the root zone. We have previously reported an inverse relationship between seeding rate and grain quality traits such as grain protein content and gluten content, and Zeleny index was observed [24].

3.2.3. Growing Environments (Locations). Test location was found to be the other important factor determining the accumulation of Zn and Fe in durum wheat grain. Grains harvested from MU and Melfa locations have accumulated significantly ($p < 0.001$) different amounts of Zn and Fe (Figure 3).

Both Zn and Fe grain concentrations were higher at Melfa than at MU under similar soil and crop management practices. MU growing conditions might be constrained the uptake, translocation, and accumulation of micronutrients in grains. MU location is usually subjected to terminal drought and has poor soil fertility level compared to Melfa [24]. It might be inferred that grain mineral concentration might be affected by soil and other climatic characteristics of the specific location as reported by Abrar et al. [28]. The soil chemical compositions such as high pH, high calcium carbonate content, and poor organic matter content reduce zinc bioavailability and zinc root uptake [29].

3.2.4. GEI Effect on Zn Concentration. The interaction effects between varieties, seeding rate, and microfertilizers application (GEI) were only presented for Zn as the effect did not significantly affect Fe concentration. Zn concentration in durum wheat grain was subjected to the interaction effect between varieties, seeding rate, and applied microfertilizers (Figure 4). This interactions effect has significantly ($p < 0.001$) affected grain mineral accumulation. The highest Zn concentration was obtained from farmer variety acc.208304 at seeding rate of 125 kg ha^{-1} under ZnSO_4 treatment (Figure 4). On the other hand, higher Zn concentration for the improved variety, Asassa, was obtained from 100 kg ha^{-1} seeding rate treated with ZnSO_4 . This implies that ameliorating grain mineral concentration requires varietal selection and determination of optimal seeding rate for enhanced uptake and translocation of applied microfertilizers.

The two varieties differentially interact with the seeding rate to accumulate Zn in their grain (Figure 5). The farmer variety, acc.208304, has accumulated 63.38 mg kg^{-1} of Zn at 125 kg ha^{-1} seeding rate. The amount of Zn accumulated at higher seeding rates (150 and 175 kg ha^{-1}) was not statistically different from the unfertilized accumulation

TABLE 1: Interaction effect of seeding rate and applied micronutrients on grain zinc and iron concentrations (mg kg^{-1}).

Micronutrients	Seeding rate (kg ha^{-1})			
	100	125	150	175
Control	33.11	37.57	30.23	31.26
Zinc sulfate	52.57	71.16	52.21	50.97
Iron sulfate	30.99	40.13	31.96	25.24
LSD _{0.05}				8.52
CV (%)				5.10

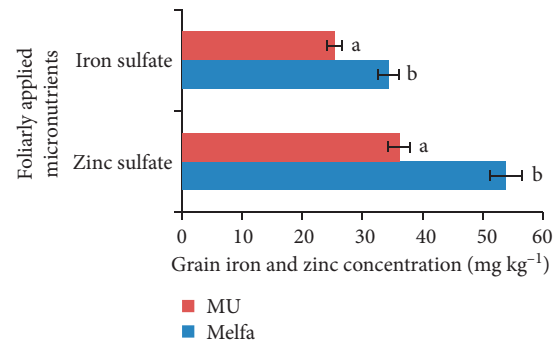


FIGURE 3: Grain Zn and Fe concentrations (mg kg^{-1}) across test locations. Bars designated by different letters are statistically different from each other.

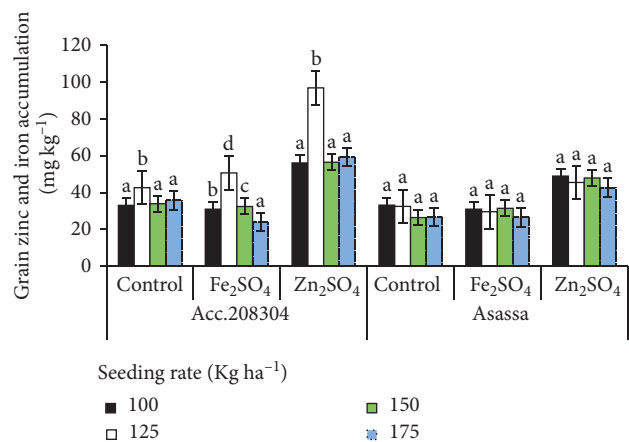


FIGURE 4: Grain Zn and Fe concentrations (mg kg^{-1}) due to the interaction between varieties, seeding rate, and microfertilizers. Bars separated with different letters are statistically different from each other.

(Figure 5). The improved variety, Asassa, has accumulated less grain Zn under all seeding rates compared to the farmer variety, acc.208304. For the improved variety, the amount of Zn concentrated decreased with increasing seeding rate. The interaction between seeding rate and variety significantly affects Zn concentration of the grain.

It seems that, there is an inverse relationship between grain micronutrients concentration and seeding rate. This universally explains that, there was a steady decrease for grain zinc concentration as the seeding rate increased from 100 to 175 kg ha^{-1} . Such inverse relationship between

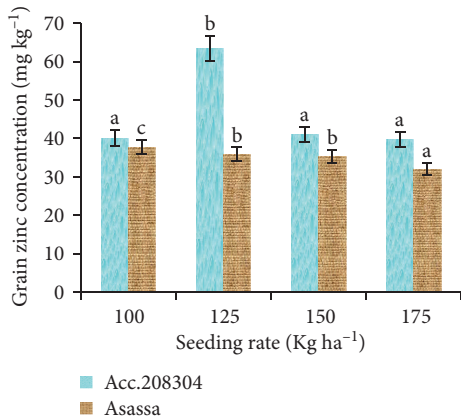


FIGURE 5: Grain Zn concentration (mg kg⁻¹) due to wheat variety by seeding rate interaction effects. Bars designated with different letters are statistically different.

seeding rate and grain quality traits was reported previously [24]. This study further indicated that for maximum concentration of grain Zn, variety-specific seeding rate recommendation is needed.

4. Conclusion

Micronutrients nutrition is one of the potentially amendable factors in human health and well-beings at any stage. These factors could be reduced directly through nutrition-specific intervention and indirectly by nutrition-sensitive interventions. The study indicated that grain zinc and iron concentrations were significantly affected by seeding rate, varietal difference, and test locations. More concentrations of Zn and Fe were found in grains harvested from Melfa than from MU. Grain Zn and Fe concentrations decreased as the seeding rate increased from 125 kg ha⁻¹ to 175 kg ha⁻¹ and higher Zn and Fe were accumulated by the landrace, acc.208304, than the improved variety, Asassa, which implies that farmer varieties could have better genetic potential for micronutrient accumulation. Exploitation of existing genetic resources for natural grain micronutrients accumulation could be an affordable and sustainable approach to mitigate Zn and Fe deficiency-related health problems. However, further research is needed as the findings cannot be conclusive from single season experimentation.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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