Breeding for Enhanced Zinc and Iron Concentration in CIMMYT Spring Wheat Germplasm

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Abstract: Micronutrient malnutrition, resulting from dietary deficiency of important minerals such as zinc (Zn) and iron (Fe), is a widespread food-related health problem. Genetic enhancement of crops with elevated levels of these micronutrients is one of the most cost effective ways of solving global micronutrient malnutrition problem. Development and dissemination of high Zn and Fe containing high-yielding, disease-resistant wheat varieties by International Maize and Wheat Improvement Center (CIMMYT) is initially targeted for the Indo-Gangetic plains of South Asia, a region with high population density and high micronutrient malnutrition. The most promising sources for grain Zn and Fe concentrations are wild relatives, primitive wheats and landraces. Synthetic hexaploids were developed at CIMMYT by crossing *Aegilops taushii* and high Zn and Fe containing accessions of *T. dicoccon*. Current breeding efforts at CIMMYT have focused on transferring genes governing increased Zn and Fe from *T. spelta*, *T. dicoccon* based synthetics, land races and other reported high Zn and Fe sources to high yielding elite wheat backgrounds.

Keywords: bread wheat; correlation; genetic biofortification; grain zinc and iron

Micronutrient malnutrition arising from zinc (Zn) and iron (Fe) deficiency has emerged as a serious health concern worldwide, now it afflicts over 3 billion people around the world (United Nations System Standing Committee on Nutrition 2004). These deficiencies are caused by habitual diets that lack diversity (overly dependent on a single staple food), situations of food insecurity, where populations do not have enough to eat (WHO 2002); and low intake of vegetables, fruits, and animal and fish products, which are rich sources of minerals. Food fortification that involves adding of micronutrients to the food has played an important role in tackling the malnutrition problem to a great extent. However, fortification efforts are highly dependent on funding, and the scope is usually restricted to urban areas. Moreover, the benefits disappear if the investment not sustained. Biofortification through plant breeding, by contrast, has multiplicative advantages (Bouis *et al*. 2000). The benefits reach the total population in both urban and rural areas, and, it will not require continued investment once the bio-fortified cultivars have been developed, and do not disappear after initial successful investment and research, as long as a domestic agricultural research infrastructure is maintained. Therefore, the Consultative Group on International Agricultural Research (CGIAR) implemented HarvestPlus Challenge Program with the objective of developing biofortified (with high micronutrient concentrations) staple crops such as wheat, rice, maize, cassava etc., through plant breeding (Bouis *et al*. 2000). Wheat is an important staple food for human in many parts of the world, contributing 28% of the world edible dry matter (FAOSTAT 2010). Therefore, development of genetically enriched wheat varieties through breeding is considered as a promising and cost-effective approach for diminishing malnutrition problem (Welch & Graham 1999).

MATERIAL AND METHODS

Preliminary screening of several hundred wheat accessions showed four to five fold variability for grain Fe and Zn concentrations. The range of values for Fe concentration in grain among hexaploid wheat, *Triticum dicoccon* and landraces grown under field conditions, was from 25 to 56 mg/kg, with a mean of 37 mg/kg, while the range for Zn was 25–65 mg/kg, with a mean of 35 mg/kg. However, the genotypes with the highest levels were low yielding, unadapted genotypes (Ortiz-Monasterio *et al*. 2007). The most promising sources for grain Zn and Fe concentrations are wild relatives, primitive wheats and landraces. Synthetic hexaploids were developed at CIMMYT by crossing *Aegilops taushii* and high Zn and Fe containing accessions of *T. dicoccon*. Current breeding efforts at CIMMYT have focused on transferring genes governing increased Zn and Fe from *T. spelta*, *T. dicoccon* based synthetics, landraces and other reported high Zn and Fe sources to high yielding elite wheat backgrounds. New hexaploid synthetic wheats and other donor parents with significantly higher Zn and Fe concentrations were used as donor parents for a limited-backcross breeding approach onto adapted CIMMYT wheat parents. Limited backcross (BC1 and BC2) populations of between 400 to 800 plants with elite materials and subsequent F_2 (1200 to 2400 plants) and F_3–F_4 (400–800 plants) were grown and plants with desired agronomic features selected. A total of 3732 BC1 derived F_4 and BC2 derived F_3 lines were grown in Cd. Obregon, Mexico during 2008–2009 crop season with systematic checks in soil enriched with Zn fertilizer. $ZnSO_4$ was applied @ 100 kg/ha to optimize and homogenize available soil Zn, as we experience large soil Zn heterogeneity in the experimental station. Of the 3732 F_3–F_4 lines evaluated in 2008–2009, about 1300 F_4 and F_5 lines were harvested after selection for agronomic characteristics and resistance to leaf and stem rust. These lines were analyzed for grain Zn/Fe concentrations, and evaluated for agronomic characteristics and resistance to leaf rust and yellow rust in El Batan and Toluca Research Stations of CIMMYT, respectively during 2009 summer season. Grain samples from Cd. Obregon were analyzed for mineral concentration at Waite Analytical Services laboratory, University of Adelaide, Australia, based on the nitric/perchloric acid digestion method using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Zarcinas *et al*. 1987). Grain protein %, grain hardness was measured by NIRs assay in CIMMYT wheat quality laboratory. For thousand kernel weight (TKW), 200 grains were counted and weighed manually and converted into TKW.

RESULTS AND DISCUSSION

Preliminary analysis of F_4/F_5 lines evaluated in 2008/2009 showed considerable variation for Zn (19–52 mg/kg) and Fe (23–52 mg/kg) with the mean of 27.11 ± 3.26 mg/kg for Zn and 30.52 ± 2.87mg/kg for Fe (Figure 1). The second set of advanced lines screened in 2009/2010 confirmed the large variation that exists for both Zn (15–51 mg/kg) and Fe (27–43 mg/kg). About 30% entries had > 35 mg/kg Zn, suggesting good scope to identify candidate lines with enhanced Zn/Fe concentrations. Among the entries, a derivative from the

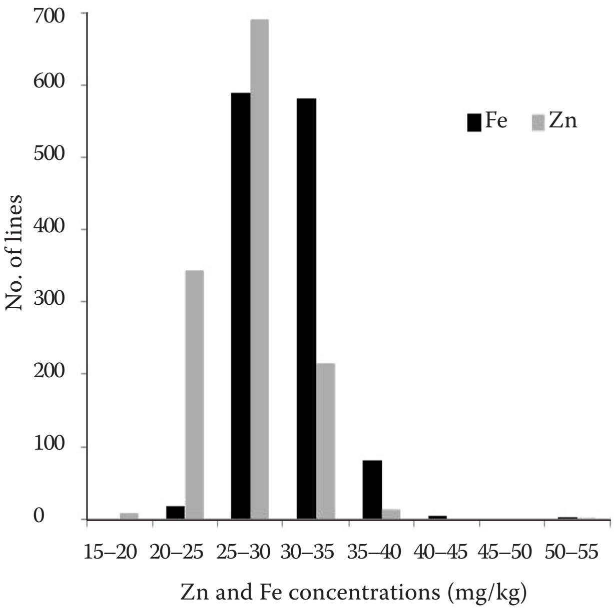

Figure 1. Frequency distribution of grain Zn and Fe concentrations in > 1300 F_4–F_5 advanced lines, Cd Obregon 2008–2009

Table 1. Correlation coefficient (r) matrix of grain Zn and Fe concentrations and other quality traits

Correlation co-efficient	Fe concentration (mg/kg)	Fe content (µg/grain)	Zn concentration (mg/kg)	Zn content (µg/grain)	Grain hardness	Protein (%)
Fe content (µg/grain)	0.781**					
Zn concentration (mg/kg)	0.416**	0.315**				
Zn content (µg/grain)	0.467**	0.725**	0.791**			
Grain hardness	0.013**	–0.152*	0.292**	0.050		
Protein (%)	0.390**	0.269**	0.383**	0.305**	0.328**	
TKW	0.332**	0.683**	0.083	0.513**	–0.232**	0.130*

TKW – thousand kernel weight

cross 'IWA 8600211//2 × PBW343 × 2/KUKUNA' showed highest Zn (51.8 mg/kg) and Fe (52.4 mg/kg) concentrations. This improved line is being used as a parent for further germplasm improvement. A total of 229 and 196 lines exhibited mean + 1 SD Fe and Zn values, respectively; whereas 43 and 41 lines contained mean + 2 SD Fe and Zn, respectively. Of the lines that had 1 SD higher Zn, 96 lines were selected for multi-location testing in South Asia considering the consumer preference of white grain type and hard to semi-hard grain textures.

Positive association between Zn and Fe concentrations (r = 0.416; P < 0.01) (Table 1) implies their simultaneous improvement in a breeding program. Also, highly significant positive correlation was observed for protein % and Fe (r = 0.390; P < 0.01) and Zn concentrations (r = 0.383; P < 0.01). The grain protein content gene *Gpc-B1*, originally found in *Triticum turgidum* ssp. *dicoccoides*, is known to enhance Zn and Fe concentrations in the grain (Uauy *et al*. 2006). Interestingly, highly significant positive correlation was observed for grain Fe content and concentrations (r = 0.781; P < 0.01) and Zn content and concentrations (r = 0.791; P < 0.01). Also, the correlation between thousand kernel weight (TKW) and Fe and Zn content (r = 0.683; P < 0.01 for Fe and r = 0.513; P < 0.01 for Zn) was much higher than the association of TKW and Fe (r = 0.332; P < 0.01) and Zn concentrations (r = 0.083) indicates there may be concentration effect due to small seed size; however, there are many lines with higher micronutrient concentrations also had higher grain weight, suggesting that higher grain Zn and Fe concentrations are not necessarily related to small grain size.

Selected advanced lines with higher Zn/Fe and desirable agronomic traits were tested for grain yield at Cd. Obregon in Mexico during 2009/2010 crop season and lines with high yield potential and enhanced Zn/Fe identified. These lines will be tested across a range of locations in target countries during 2010/2011 crop season to establish the stability of expression for the two micronutrients. Significant genotype × location interaction can be expected (Ortiz-Monasterio *et al*. 2007), and the identification of genotypes with stable expression across environments is essential. Current evidence strongly supports that competitive Zn and Fe biofortified varieties can be developed. If successful, this would dramatically contribute to improving the health and livelihood of numerous resource-poor, micronutrient-deficient people in many developing countries.

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