Breeding Progress and Genotype × Environment Interaction for Zinc Concentration in CIMMYT Spring Wheat Germplasm

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SUMMARY

Genetic biofortification to improve zinc (Zn) and iron (Fe) concentrations in bread wheat (Triticum aestivum L.) could reduce micronutrient malnutrition. The International Maize and Wheat Improvement Center (CIMMYT) is working with South Asian national partners to develop and disseminate high-yielding, disease-resistant wheat varieties with significantly increased Zn and Fe concentrations. Biofortified wheats with superior agronomic performance and durable resistance to all three rusts including Ug99 group of races of stem rust fungus will be targeted for commercialization in Mega-Environments 1 and 5 of South Asia, where more than 26 per cent of the population have inadequate Zn intake. Current breeding efforts for enhanced Zn and Fe have focused on transferring genes governing increased Zn and Fe from Triticum spelta, T. dicoccon, Ae. Tauschii-based synthetics, landraces, and other reported high Zn and Fe sources to high-yielding elite wheat backgrounds. New hexaploid synthetic wheats and other known sources with significantly higher Zn and Fe concentrations are being used as donor parents for limited-backcross breeding approach onto adapted CIMMYT wheat parents. Preliminary analysis of F₄/F₅ lines evaluated in 2008-2009 revealed considerable variation for Zn (19–52 mg/kg) and Fe (23-52 mg/kg), and a 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 per cent of entries had more than 35 mg per kg Zn, suggesting good scope to identify candidate lines with enhanced Zn concentrations. Preliminary Genotype x Environment (GxE) testing of 100 best lines in target locations exhibited limited G x E interaction with significant genotypic effect and non-significant G x E interaction effect of entries across locations. Pooled data across locations showed up to 40 per cent increment over...
the local checks for Zn (~31 mg/kg) and about 25 per cent of entries with more than 35 mg per kg Zn concentrations. These preliminary results provide the first evidence of the proof-of-concept that competitive Zn biofortified varieties can be developed with higher yield potential and other non-negotiable core traits.

INTRODUCTION

Micronutrient malnutrition arising from zinc (Zn) and iron (Fe) deficiencies has emerged as a serious health concern worldwide, which afflicts over 3 billion people around the world (United Nations System Standing Committee on Nutrition, 2004). 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 deficiency in humans is widespread; more than 30 per cent of the world’s population are estimated to suffer from Zn deficiency. Low dietary intake of Zn and Fe 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). Thus, biofortification of cereal crops with Zn and Fe is a high-priority global issue. The Harvest Plus (www.harvestplus.org) Challenge Program of the Consultative Group on International Agricultural Research (CGIAR) has embarked upon to address this issue by developing biofortified crop varieties with high concentrations of micronutrients using classical and modern plant breeding tools (i.e. genetic biofortification).

Genetic biofortification to improve Zn and Fe concentrations in major staples including bread wheat (*Triticum aestivum* L.) could greatly reduce micronutrient malnutrition. Wheat is an important staple food for human in many parts of the world, contributing 28 per cent 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 worldwide (Welch and Graham, 2004). The International Maize and Wheat Improvement Center (CIMMYT) is working with its national partners to develop and disseminate high-yielding, disease-resistant wheat varieties with significantly increased Zn and Fe concentrations, with initial efforts targeted to South Asia, where more than 26 per cent of the population are Zn deficient.

TARGET POPULATIONS AND BREEDING TARGET LEVEL

Development and dissemination of high Zn and Fe containing high-yielding, disease-resistant wheat varieties by CIMMYT and South Asian national partners is initially targeted to the Indo-Gangetic plains, a region with high population density and high micronutrient malnutrition (Harvest Plus, 2010). In Pakistan and Northern India, the per capita wheat consumption exceeds 400 gram per capita per day, much of it in whole meal form. More than 40 per cent of pre-school children (under 5 years of age) in Sub-Saharan Africa and South Asia are stunted, which is often used as proxy indicator for Zn deficiency. Most of the wheat produced today in South Asia is from modern varieties derived from germplasm originally developed by CIMMYT (Dixon
et al., 2009). Mega varieties such as PBW343 and Inquilab91 account for about half of the total wheat production in the Indian Punjab and in Pakistan. Harvest Plus focuses on two agro-ecological zones or mega-environments 1) Mega-Environment 1 (ME1), which comprises the North Western Plains Zone (NWPZ), an irrigated temperate zone covering the states of Punjab, Haryana, and Northwestern Uttar Pradesh; 2) Mega-Environment 5 (ME5), which encompasses the Eastern Gangetic Plains Zone (EGPZ), an irrigated high-temperature zone covering the states of Eastern Uttar Pradesh and Bihar. The HarvestPlus breeding strategy is to incorporate high Zn and Fe in new replacement wheat germplasm with superior agronomic performance in both ME 1 and ME 5 and durable resistance to recently evolved strains of yellow and stem rust, which threaten wheat production across South Asia. In the longer term, widely grown mega-varieties will eventually be converted into micronutrient-dense version.

Nutritionists have established that in South Asia the target is to increase Zn and Fe levels by 8 and 25 mg per kg, respectively, above the baseline (i.e., the average micronutrient concentration of commercial cultivars grown in the target region). This translates into total Zn and Fe levels in the grain of 33 and 50 mg per kg, respectively. Baseline values using wheat cultivars PBW343 in India and Inquilab91 in Pakistan approximately has 25 mg per kg Zn and Fe. The absolute target level for Fe is significantly higher than for Zn, due to lower bioavailability of Fe as compared to Zn.

**BIOFORTIFICATION**

Biofortification has been defined as the process of increasing bioavailable concentrations of essential elements in edible portions of crop plants through genetic selection or agronomic intervention (White and Broadley, 2005). The process holds great potential to improve the health of the poor in developing countries, particularly in rural areas. Additionally, increasing micronutrients in grains of staple food crops increases the yield potential of these crops when they are grown on micronutrient-poor soils (Cakmak et al., 2000). Much of the developing world has significant areas of such soils. Enhancing seeds with micronutrients acts as an incentive to farmers affected by micronutrient-poor soils to adopt seeds that are micronutrient-enriched for use in their cropping systems. Micronutrient-enriched staple food crops, either through traditional plant breeding methods or through molecular techniques, are powerful intervention tools that target the most vulnerable people especially resource-poor women, infants, and children (Pfeiffer and McClafferty, 2007).

**GERMPLASM SCREENING**

Exploiting the genetic variation for micronutrient concentration is one of the most powerful tools to change the nutrient balance of a given diet on a large scale. Success in crop improvement through conventional plant breeding strategies depends on the existence of genetic variation for the target traits in the available gene pool. Large variability for grain Zn and Fe has been reported in major staple crops. A diverse range of more than 3000 accessions including hexaploid, tetraploid, and diploid sources from CIMMYT genebank have been screened for Zn and Fe variation. The most promising materials, in order of importance, are wild relatives and primitive cultivated wheat,
landraces, bread wheat, durum wheat, and triticale (Ortiz-Monasterio and Graham 2000). The range for grain Zn concentration among hexaploid wheat, Triticum dicoccon, and landraces grown under field conditions was 25 to 65 mg per kg, with a mean of 35 mg per kg, while the range for Fe was 25 to 56 mg per kg, with a mean of 37 mg per kg. However, the genotypes with the highest levels were low yielding, unadapted materials (Ortiz-Monasterio et al., 2007). The most promising sources for grain Zn and Fe concentrations are thus wild relatives, primitive wheats, and landraces. Synthetic hexaploids were developed at CIMMYT by crossing Aegilops tauchii and high Zn and Fe containing accessions of T. dicoccon.

**BREEDING STRATEGY**

As our understanding of the underlying genetic control of grain Zn/Fe concentration is poor, breeding has focused on crossing high Zn donor parents with CIMMYT-derived high-yielding advanced lines with the aim of identifying transgressive segregants. Provided sufficiently large F₂ and F₃ population sizes are maintained and genetic drift minimized, the F₄ and later generations can be screened for Zn concentration once a higher level of homozygosity has been achieved.

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 (Velu et al. 2010). Limited backcross (BC₁ and BC₂) populations of 400 to 800 plants with elite materials and subsequent F₂ (1200–2400 plants) and F₃-F₄ (400–800 plants) were grown and plants with desired agronomic features selected. BC₁-F₅’s and BC₂-F₄’s were evaluated in small plots for agronomic characteristics and resistance to leaf and stem rusts in Cd Obregon, Mexico. Best performing F₄-F₅ lines exhibiting resistant to rusts, superior agronomic performance and bold and plump grain types were analyzed for Zn and Fe concentrations, initially with X-Ray Fluorescence (XRF) Spectrometer and then with Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) analysis at Waite Analytical Services Laboratory, Australia, following the nitric/perchloric acid digestion method. Grain protein per cent and grain hardness were measured using the Near Infra-Red Spectroscopy (NIRS) assay at CIMMYT Wheat Quality Laboratory.

**BREEDING PROGRESS FOR ENHANCED Zn CONCENTRATION**

Preliminary analysis of more than 1300 F₄/F₅ lines evaluated in 2007-2009 showed considerable variation for Zn (19-52 mg/kg) and Fe (23-52 mg/kg) with a mean of 27.11 ± 3.26 mg per kg for Zn and 30.52 ± 2.87 mg per kg for Fe. The second set of about 800 advanced lines screened in 2009-2010 confirmed again that large variation exists for both Zn (15-51 mg/kg) and Fe (27-43 mg/kg) (Fig. 1).

When compared to 2008-2009 season, 2009-2010 season showed several entries exceeding the target Zn concentration (33 mg/kg). This indicates making good progress and moving in the right direction in obtaining large number of high Zn lines, and suggests that there is good scope to identify high-yielding lines with enhanced Zn concentrations.
Fig. 1  Frequency distribution of grain Zn concentrations among F₄/F₅ lines screened in 2008-2009 and 2009-2010 seasons at Cd. Obregon

**Association of grain Zn with other traits:** Positive significant association between Zn and Fe concentrations (r = 0.416; P<0.01) implies that simultaneous improvement is feasible. Also, highly significant positive correlations were observed for protein content and Fe (r = 0.390; P<0.01) and Zn concentrations (r = 0.383; P<0.01) (Table 1).

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

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

*, ** Significant at 5% and 1% probability levels, respectively.

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 correlations were observed for grain Fe content and concentration (r = 0.781; P<0.01) and Zn content and concentration (r = 0.791; P<0.01). Also, the correlation coefficient 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 ($r = 0.083$). Presence of lines with higher micronutrient concentration and higher grain weight suggests that higher grain Zn and Fe concentrations are not necessarily related to smaller grain size or lesser grain weight.

**Performance of Advanced Lines Evaluated as HarvestPlus Yield Trials (HPYT):**

Set of 650 advanced $F_5-F_6$ lines with significantly enhanced Fe/Zn (mean + 1 SD) was evaluated for yield potential in 24 yield trials (Alpha-Lattice-Latinized design with 3 reps, 2 checks, and 28 entries in each trial) at Cd. Obregon in Mexico during 2009 per 2010 crop season. The overall performance of the trials were very good; many entries were agronomically similar to high-yielding checks ‘Waxwing and Roelfs F2007’. Chelated form of Zn fertilizer was applied to optimize and homogenize available soil Zn, as we experience large soil Zn heterogeneity at Cd. Obregon experimental station. Agronomic data on days-to-heading, maturity, plant height and agronomic eliteness scores were recorded for all 24 trials. Trials were harvested after physiological maturity and grain yield was measured. Yield potential ranged from 5.04 to 8.10 tons per hectare with an average yield of 6.69 tons per hectare. About 10 per cent of the entries had a yield potential similar or higher than the check’s mean (Roelfs F2007 and Waxwing) (Fig. 2), indicating that high grain yield and enhanced Zn concentration can be combined.

![Graph showing grain yield potential of 650 advanced lines](image)

**Fig. 2** Grain yield potential of 650 advanced lines expressed as percentage over the mean performance of checks (Roelfs F2007 and Waxwing) during 2009-2010 crop season, Cd. Obregon, Mexico

Based on the grain yield data, 122 entries were selected for micronutrient analysis and further promotion. All 122 entries along with checks were analyzed for Zn/Fe concentrations using ICP. Grain Zn varied from 20.1 to 44.8 mg per kg (more than
2-fold) with a mean of 28.5 mg per kg. Grain Fe varied between 24.1 and 38.8 mg per kg with a mean of 30.5 mg per kg. Among the South Asia group of entries, the highest Zn concentration (44 mg per kg) was observed, and 11 entries had above 35 mg per kg Zn (35-44 mg per kg). These results are encouraging, as these lines have higher yield potential as well as enhanced Zn levels.

**End-use Quality Test of HPYT Entries:** End-use quality tests were conducted at the CIMMYT laboratory for these 122 entries – including the South Asia group and other selected lines (excluding checks) – using 2009-2010 yield trials grain samples. Interestingly, none of the South Asian candidate lines for the international HarvestPlus Wheat Yield Trial (HPWYT) belonged to the soft grain type; most entries showed strong and balanced gluten, which is well recommended for South Asian flat breads. Grain hardness among the South Asia group of lines ranged from hard to semi-hard with protein contents of 11.8 to 15.5 per cent in the grain and 10.3 to 14.8 per cent in flour. The sedimentation index (sedimentation volume / protein per cent) in the South Asia group varied between 1.5 and 2.1, which confirms again medium strong to strong gluten combined with acceptable grain hardness among these lines. Except for a few entries, the dough development time was above 2.0 min, and the mixographic type was more than 3.0 in a scale of 1 to 5, indicating good dough mixing properties and suitability of these entries for making South Asian flat bread (chapatti) as well as yeast-leavened bread.

**GENOTYPE X ENVIRONMENT TESTING IN TARGET COUNTRIES**

The first HarvestPlus South Asia Nursery (HPAN) consists of 96 high-Zn advanced lines identified from the 2008-2009 screening nursery was evaluated in multi-environments of India, Pakistan, and Mexico during the 2009-2010 crop season. The nurseries were evaluated at three locations in India (IARI, New Delhi; BHU, Varanasi; and PAU, Ludhiana), two locations in Pakistan (PARC-Islamabad and Faisalabad), and one location in Cd Obregon, Mexico.

Combined analysis across locations revealed significant genotypic effect and non-significant G x E interaction effect, indicated by consistent rank order of entries across locations. Pooled data across locations showed up to 40 per cent increment over the local checks for Zn (31 mg/kg) and about 25 per cent of entries with more than 35 mg per kg Zn concentrations.

The Site Regression (SREG) biplot analysis is a descriptive tool where effects of genotype and G x E interaction are combined and analyzed by means of singular value decomposition. SREG is useful because it illustrates the relationships between environments, genotypes, and both combined, and it allows breeders to observe patterns of response or genotypes across environments. SREG analysis revealed that all locations except Indian Agricultural Research Institute (IARI)-India placed in the same direction further confirming low G x E interaction (Fig. 3). Lines 82 and 83 were the best performers at all the sites except IARI-India, whereas lines 20 and 64 performed well in IARI-India. The best site-specific as well as the best country and regional performers are being tested again in the 2010 2011 crop season, and the top genotypes from each group will be the choice progenitors for future crosses.
RAPID HIGH THROUGHPUT MICRONUTRIENT SCREENING TECHNIQUES

X-Ray Fluorescence (XRF) spectrometry is a technique widely used in science and industry for elemental analysis. To date, XRF is applied predominantly in manufacturing, geology, forensic science, and archaeology and to a limited extent in the analysis of plant tissues in the field of phyto-remediation. HarvestPlus partners from Flinders University, Australia, explored the potential use of two bench-top X-ray Fluorescence Spectrometer (XRF) units for non-destructive elemental analysis of whole grain wheat samples with a focus on Zn and Fe analysis. Preliminary screening of a few wheat samples scanned with XRF was very encouraging, due to the high correlation observed between ICP-Zn and XRF-Zn (r=0.9) and a coefficient of variation (CV) below 5 per cent. For accuracy testing and further validation, about 3000 samples harvested
at Obregon were analyzed for Zn and Fe with XRF and ICP at Flinders University. The relationship among the data sets for ICP-Zn and XRF-Zn ranged from $r^2=0.5$ to 0.9. Using XRF analysis, we have discarded more than 1500 samples with least Zn concentrations are retained only high Zn lines for further ICP analysis. Hence, using XRF for high-throughput, preliminary Zn screening can ultimately increase breeding effectiveness as it helps to reduce costs, save time, and facilitates getting ICP data in time before the next planting.

CONCLUSION

High genetic variation exists within the wheat gene pool to substantially increase the grain Zn concentration in wheat. This genetic variation is being used by the wheat HarvestPlus breeding Program to improve modern wheat cultivars for both increased concentration and bioavailability of Zn. Preliminary G x E testing in target countries revealed low G x E interaction for Zn as indicated by the consistent ranking of best lines across locations. The data also provides the first evidence of the proof-of-concept that competitive Zn-biofortified varieties can be developed, along with high grain yield and other important agronomic traits. Under an optimistic scenario ten years after the first release of a biofortified variety in South Asia, biofortified high-Zn wheat could be consumed by 120 million resource poor people. This would eventually improve nutrition levels and could save 200,000 DALYs (disability-adjusted life years) annually.

Acknowledgments

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REFERENCES


Genotype × environment interaction for W appears to be small in peanut. Wright et al. In plant breeding, genotype ∗ environment interactions (G ∗ E) are a great challenge for GS researches, as these interactions are often evaluated within the same environment or considered as noise. However, these interactions amplify variation in phenotypes of cultivars across environments for the reason that plants respond differentially to environments and the integration of G ∗ E interactions into GS models enables the selection of stable and high-performance cultivars (Bassi et al., 2016; Rutkoski et al., 2017). Genetic gain studies for the CIMMYT/ICARDA wheat breeding program have shown continuous progress in yield and other traits (Sayre et al. 1997; Trethowan et al. 2002; Sharma et al. In addition to grain yield, significant progress has been made by the IWIN in developing resistant wheat germplasm to diseases and pests ensuring that developing and deploying genetically resistant varieties adapted to target growing environments is the best economical and environmentally friendly strategy for controlling rust diseases of wheat particularly for resource poor farmers. 2. Progress in Wheat Genetic Transformation. Bread wheat (Triticum aestivum L.), the most widespread of all wheat species, is an annual herb belonging to the family Gramineae or Poaceae. Wheat was domesticated around 8,000 years ago [29] and has since undergone hybridization and genome duplication events to generate its hexaploid genome (2n = 6x = 42, AABBDD), which is more than five times larger than the human genome.Various wheat genotypes have been used as donors. Express, Chris, Farnum, Hollis, Louise, Perigee, and WestBred 926 have all performed successfully in microspore transformation. Arabinogalactan-proteins at a concentration of 5 mg/l improved regeneration of bread (cv. Ikizce-96) and durum (cv. Genomic Prediction with Genotype by Environment Interaction Analysis for Kernel Zinc Concentration in Tropical Maize Germplasm. View ORCID ProfileEdna K. Mageto, View ORCID ProfileJose Crossa, View ORCID ProfilePaulino Pérez-Rodríguez, View ORCID ProfileThanda Dhliwayo, View ORCID ProfileNatalia Palacios-Rojas, Michael Lee, Rui Guo, FÁlix San Vicente, View ORCID ProfileXuecai Zhang and Vemuri Hindu. The ZAM panel consists of 923 inbreds from maize breeding programs of the International Maize and Wheat Improvement Center (CIMMYT). The panel represents wide genetic diversity for kernel Zn concentration (Hindu et al. 2018). @article{Velu2018BreedingFE, title={Breeding for Enhanced Zinc and Iron Concentration in CIMMYT Spring Wheat Germplasm}, author={G. Velu and R. Singh and J. Huerta-Espino and J. Peñate and I. Ortiz-Monasterio}, journal={Czech Journal of Genetics and Plant Breeding}, year={2018}, volume={47} }. G. Velu, R. Singh, +2 authors I. Ortiz-Monasterio. Develop - ment and dissemination of high Zn and Fe containing high-yielding, disease-resistant wheat varieties by International Maize and Wheat Improvement Center (CIMMYTâ€˜) CONTINUE READING. View via Publisher.