Genetic impact of Rht dwarfing genes on grain micronutrients concentration in wheat

Govindan Velua, Ravi P. Singha, Julio Huertaa,b, Carlos Guzmánb

a Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Apdo Postal 6-641, Mexico DF, Mexico
b Campo Experimental Valle de Mexico INIFAP, Apdo. Postal 10, 56230, Chapingo, Edo de Mexico, Mexico

ARTICLE INFO
Keywords:
Rht dwarfing genes
Isogenic lines
Micronutrients
Biofortification
Wheat

ABSTRACT
Wheat is a major staple food crop providing about 20% of dietary energy and proteins, and food products made of whole grain wheat are a major source of micronutrients like Zinc (Zn), Iron (Fe), Manganese (Mn), Magnesium (Mg), Vitamin B and E. Wheat provides about 40% intake of essential micronutrients by humans in the developing countries relying on wheat based diets. Varieties with genetically enhanced levels of grain micronutrient concentrations can provide a cost-effective and sustainable option to resource poor wheat consumers. To determine the effects of commonly deployed dwarfing genes on wheat grain Zn, Fe, Mn and Mg concentrations, nine bread wheat (Triticum aestivum) and six durum wheat (T. turgidum) isoline pairs differing for Rht1 (= Rht-B1b) and one bread wheat pair for Rht2 (= Rht-D1b) dwarfing genes were evaluated for three crop seasons at N.E. Borlaug Research Station, Cd. Obregon, Sonora, Mexico. Presence of dwarfing genes have significantly reduced grain Zn concentration by 3.9 ppm (range 1.9-10.0 ppm), and Fe by 3.2 ppm (range 1.0-14.4 ppm). On the average, about 94 ppm Mg and 6 ppm Mn reductions occurred in semidwarf varieties compared to tall varieties. The thousand kernel weight (TKW) of semidwarf isolines was 2.6 g (range 0.7-5.6 g) lower than the tall counterparts whereas the plant height decreased by 25 cm (range 16–37 cm). Reductions for all traits in semidwarfs were genotype dependent and the magnitude of height reductions did not correlate with reductions in micronutrient concentrations in wheat grain. We conclude that increased grain yield potential of semidwarf wheat varieties is associated with reduced grain micronutrient concentrations; however, the magnitude of reductions in micronutrients varied depending on genetic background and their associated pleiotropic effect on yield components.

1. Introduction

Globally, over 805 million people suffer from hunger and approximately 165 million (or 1 in 4) children under the age of five are stunted due to the lack of proper nutrition received between pregnancy and a child’s second birthday (FAO, 2017). Despite the significant growth in agricultural production, a large population suffers from the dietary deficiency of essential micronutrients such as zinc (Zn) and iron (Fe). Additionally, magnesium (Mg) and manganese (Mn) deficiency is very common among resource poor consumers. In particular women and children are more vulnerable to the micronutrient deficiency. The first 1,000-days are the most important time period for a child’s cognitive, intellectual and physical development. Under nutrition contributes to 45 percent of the child deaths each year worldwide (WHO, 2017). Biofortification offers a sustainable solution to increase food and nutritional security to millions of resource poor consumers depending on major staples as the main source of their dietary energy (Bouis et al., 2011). To meet the challenge of improving nutritional food security, the HarvestPlus program of the CGIAR research program on Agriculture for Nutrition and Health (CRP-A4NH) supports the development of micronutrient-rich staple crops including common wheat (Triticum aestivum L.). The primary target nutrient for wheat is Zn, as millions of resource poor wheat consumers in the target countries in South Asia and Africa are prone to Zn deficiency. In fact, more than 400,000 children die each year due to Zn deficiency globally (Muthayya et al., 2013). Overall, an estimated 17.3% of the global population is at risk of inadequate zinc intake. The regional estimated prevalence of inadequate Zn intake ranges from 7.5% in high-income regions to 30% in South Asia (Stein, 2018). Multiple micronutrient deficiencies, including Mg and Mn, are widespread and have severe health consequences in resource poor communities. Wheat varieties with improved nutritional quality, protein content, high grain yield and desirable processing quality in adapted genetic backgrounds can help alleviate nutrient deficiencies among resource poor people (Pfeiffer and McClafferty, 2007;
Singh and Velu, 2017). For this reason, genetic resources (landraces and ancestors of common wheat) with high Zn and Fe content such as *Aegilops tauschii*, *T. turgidum ssp. dicoccoides*, *T. turgidum ssp. dicoccum* and *T. aestivum ssp. spelta* species, have been used in breeding to enhance Zn concentration (Ortiz-Monasterio et al., 2007; Guzmán et al., 2014; Velu et al., 2011, 2012, 2014).

Plant height is an important trait in wheat, it significantly reduces lodging in higher yielding environments and increases grain yield. Extensive research has been conducted to study the effect of *Rht* genes on grain yield (Borlaug, 1968; Villareal and Rajaram, 1992) and to some extent on protein content (Gooding et al., 1999; McClung et al., 1986). The widely deployed dwarff pairs were also made for all six traits in the study. Broad-sense heritability (H²) was estimated across environments using the formula H² = σ²/σ²/σ²/σ²/σ²/σ², where σ² is the genotypic variance, σ² is the GE variance, and σ² is the residual error variance for r replicates and y years. The Principal Component Analysis (PCA) was calculated using META-R statistical package (www.data.cimmyt.org). The Pearson correlation coefficient between traits was calculated using PROC CORR procedure.

Table 1

| Source of variation | DF | Zn | Fe | Mg | Mn | TKW | PH |
|--------------------|----|----|----|----|----|-----|----|
|                    |    | Pr > F | Pr > F | Pr > F | Pr > F | Pr > F | Pr > F |
| Environment        | 2  | < 0.001 | 0.16 | < 0.001 | < 0.001 | 0.12 | 0.17 |
| Entry              | 31 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Environment × Entry| 62 | < 0.001 | 0.132 | < 0.001 | < 0.001 | < 0.001 | 1.132 |
| Isogenic           | 1  | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 0.043 | < 0.001 |
| Isogenic × Environment | 2  | 0.006 | 0.75 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Variance           |    |        |    |    |    |     |   |
| Error (a)          | 4.4 | 0.5 | 740.6 | 0.1 | 0.34 | 12.8 |
| Error (b)          | 6.4 | 2.6 | 2478.1 | 13.3 | 3.5 | 48.2 |
| Heritability       | 0.75 | 0.52 | 0.85 | 0.83 | 0.84 | 0.95 |

New research was undertaken at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico where the Green Revolution began using the *Rht* dwarfing genes. The objective of our study was to evaluate effects of dwarff genes *Rht1* and *Rht2* on four essential micronutrients (Fe, Zn, Mn and Mg) concentration and associated pleiotropic effects on grain weight and plant height by using 16 pairs of isogenic lines developed previously for 10 bread wheat (*T. aestivum*) and 6 durum wheat (*T. turgidum*) varieties.

2. Materials and methods

2.1. Plant materials

Sixteen pairs of tall and semi-dwarf isolines derived from CIMMYT historic and modern wheat varieties were used in this study. Ten pairs of isolines are derived from bread wheat (*T. aestivum*) and six were from pasta wheat (*T. durum*) varieties. The detailed procedure in developing these isolines has been described in Singh et al. (2001). All isolines carry *Rht1* dwarfing gene excepting Pavon isolines, which carries *Rht2* gene.

2.2. Trial design and management

The sixteen pairs of isolines were grown in a split-plot design with two replicates during 2014-15 (2015), 2015-16 (2016) and 2016-17 (2017) crop seasons at Norman E. Borlaug Experimental Station in Ciudad Obregon, Sonora, Mexico. Each genotype was planted in a paired row of 1 m long with a bed to bed distance of 80 cm. Trials were laid out in a paired split-plot design with the isolines (tall and semi-dwarf) as main plots and genotypes as subplot factors. All recommended agronomic practices were followed (Velu et al., 2012, 2017). The commercial form of ZnSO4·7H2O was applied in the soil as basal application along with the 50% of the recommended 200 kg/ha Nitrogen and 100% of 50 kg/ha Phosphorus fertilizers. Remaining 50% or 100 kg/ha N applied as top dressing during the second irrigation about 30 days of sowing. At maturity, whole plots were harvested.

2.3. Micronutrient analysis

About 30 g of grain samples free from dust particles, chaff, glumes and other plant materials was prepared for determining micronutrient concentration and thousand kernel weight (TKW). Grain Fe and Zn concentrations (parts per million: ppm) were measured using a bench-top, non-destructive, energy-dispersive X-ray fluorescence spectrometry (EDXRF) instrument (model X-Supreme 8000, Oxford Instruments plc, Abingdon, UK), calibrated for high-throughput screening of Zn and Fe in whole wheat grain (Paltridge et al., 2012). In addition, grain samples were analyzed for all four micronutrients with Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) at Flinders University, Australia. TKW was measured with a SeedCount digital imaging system (model SC5000, Next Instruments Pty Ltd, New South Wales, Australia). Plant height was measured from bottom of the plants to tip of the awns after physiological maturity of each plot.

2.4. Statistical analysis

Statistical analyses were conducted using Statistical Analysis System 9.2 (SAS Institute, Cary, NC, USA). Analysis of variance was done following fixed model (Gomez and Gomez, 1984), and data was analyzed as a paired split-plot design. Mean comparisons between tall and semi-dwarf pairs were also made for all six traits in the study. Broad-sense heritability (H²) (repeatability) was estimated across environments using the formula H² = σ²/σ²/σ²/σ²/σ²/σ², where σ² is the genotypic variance, σ² is the GE variance, and σ² is the residual error variance for r replicates and y years. The Principal Component Analysis (PCA) was calculated using META-R statistical package (www.data.cimmyt.org). The Pearson correlation coefficient between traits was calculated using PROC CORR procedure.

3. Results

Analysis of variance showed highly significant differences between genetic backgrounds (entries) for grain Zn, Fe, Mn, Mg, TKW and plant height (PH) (Table 1). Combined analysis across environments showed significant environment effect on grain Zn, Mn and Mg concentrations (P < 0.001). Contrast between tall and semi-dwarf isolines was significant for Fe, Zn, Mn and Mg and PH (P < 0.001) and TKW (P < 0.05). Interaction effect of *Rht* genes on environments was significant for all traits except Fe. The broad sense heritability was high for all traits (H² = 0.75 to 0.95), except intermediate heritability was observed for Fe (H² = 0.52) and coefficient of variation below 10% suggested a good management of trials across years.
3.1. Effect of Rht genes on Zn, Fe, Mn, Mg, kernel weight and plant height

Analysis of variance showed significant interaction effect between environments for grain Zn, Mn and Mg concentrations, however, significant positive correlations between environments allowed us to conduct combined analyses (averaged across 3 environments). Grain Zn averaged over three environments varied from 46 to 63 ppm with the mean of 52 ppm (Fig. 1), whereas grain Fe ranged from 29 to 52 ppm with the mean of 35 ppm (Fig. 2). On the average, dwarfing genes reduced grain Zn by 3.9 ppm and grain Fe by 3.2 ppm, respectively (Table 2). There was a significant genetic background effect on the expression of Rht genes on grain Zn and Fe concentrations, for instance highest reduction of 10 ppm occurred for Zn in bread wheat Culiacan dwarf over its tall pair, followed by durum wheat Aconchi and Bichena with about 5.9 and 5.6 ppm reductions, respectively. The lowest reduction or less effect of Rht dwarfing gene on grain Zn occurred in bread wheat Siete Cerros and durum wheat Focha with only 1.9 ppm reduction. In the case of Fe, the highest reduction, 14.4 ppm, occurred in bread wheat variety Genaro and the lowest in bread wheat varieties Seri with only 1 ppm difference between the isogenic pairs (Table 2). Grain Mg averaged over three environments varied from 965 to 1390 ppm with the trial mean of 1193 ppm, whereas grain Mn ranged from 40 to 64 ppm with a trial mean of 53 ppm. On average, about 94 ppm Mg and 6 ppm Mn reductions occurred in semi-dwarf varieties compared to tall varieties. The highest reduction of 150 ppm for Mg was in the wheat variety ‘Anza,’ whereas wheat variety ‘Galvez’ showed a maximum reduction of 11 ppm for Mn.

A significant difference between tall and semidwarf isolines was observed for TKW with average reduction of 2.6 g for semidwarf compared to tall (Table 2). The highest and lowest reductions of 5.6 and 0.7 in TKW occurred in bread wheat Nesser and durum wheat Lavanco, respectively.

There was a significant positive correlation between grain Zn and Fe (r = 0.45; P < 0.01) and Mg and Mn (r = 0.80; P < 0.01) and significant positive correlation between these four micronutrients. There was no correlation between these micronutrients and TKW, which was reflected from the PCA biplot (Fig. 3) where Fe and Zn were grouped together, Mg and Mn were clustered together and TKW was distantly positioned. In case of plant height, on average the semi-dwarf isolines were 25 cm shorter than the tall pairs. The lowest and highest height differences of 16 and 37 cm observed for bread wheat Galvez and durum wheat Nehama, respectively. However, magnitude of height reductions did not influence a decreased amount of Zn and Fe in semidwarfs.

4. Discussion

Previous studies have shown that Rht dwarfing genes reduced grain Zn and Fe concentrations in wheat with a limited number of isogenic pairs (Graham et al., 1999). Our results corroborates with this finding by using 16 pairs of isolines to investigate the effect of dwarfing genes on grain Zn, Fe, Mn and Mg concentrations and kernel weight in different genetic backgrounds. On an average, the reductions of 3.9, 3.2, 6.0 and 94 ppm for grain Zn, Fe, Mn and Mg, respectively were observed in semidwarf lines over their tall counterparts. However, the magnitude of reduction varied in different genetic backgrounds. Using same 16 pairs of isolines Singh et al. (2001) found significant increase for grain yield (1 t/ha) under optimally managed environment; however, the magnitude of yield increases also varied depending on the background. Several studies have been conducted to measure effect of Rht genes on kernel weight. In our study there was a negative effect on kernel weight suggesting that Rht genes might have contributed to increase the number of kernels per spike as well as kernels per unit area and thus compensated the marginal reductions in kernel weight for increased grain yield potential. Similarly, in winter wheat background Rht alleles have reduced grain weight and it was compensated by a 10% increase in number of grains per spike and a 13% increase in tiller numbers per square meter (Kertesz et al. (1991).

Recent QTL mapping studies at CIMMYT have identified pleiotropic QTL regions that enhance kernel size and grain Zn concentration simultaneously (Hao et al., 2014; Crespo-Herrera et al., 2016). These results indicate that Zn and Fe increase in tall varieties may be due to different genetic backgrounds. Thus, the changes in micronutrients concentrations are mainly due to the associated pleiotropic effects of dwarfing genes on increased biomass partitioning and higher harvest index. Considerably this trend led to slightly lower concentration of Zn and Fe per grain in modern wheat varieties, however, the total Zn harvested from the soil or Zn harvested per unit area is higher (data not shown). Recent QTL mapping studies in wheat have shown that there are various QTL regions for grain Zn and Fe, which are not associated with height or numbers per square meter (Kertesz et al. (1991).
flowering genes (Crespo-Herrera et al., 2016; Velu et al., 2017; Krishnappa et al., 2017). These QTLs provide opportunities to select high Zn semidwarf wheat varieties for better adaptation and to achieve higher grain yield potential.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors gratefully to HarvestPlus (partly funded by Bill and Melinda Gates Foundation and others) and CGIAR research program on Agriculture for Nutrition and Health (CRP-A4NH) for financial support.

References

Borlaug, N.E., 1968. Wheat breeding and its impact on world food supply. In: Proc. III International Wheat Genetics Symposium. 1-36, Australian Academy of Science, Canberra.
Bouis, H.E., Hotz, C., McClafferty, B., Meenakshi, J.V., Pfeiffer, W.H., 2011. Biofortification: a new tool to reduce micronutrient malnutrition. Food Nutr. Bull. 32, 31S–40S.
Crespo-Herrera, L.A., Velu, G., Singh, R.P., 2016. Quantitative trait loci mapping reveals pleiotropic effect for grain iron and zinc concentrations in wheat. Ann. Appl. Biol. 169, 27–35.
FAO, 2017. the Food and Agriculture Organisation of the United Nations. (FAOSTAT. URL). http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Table 2
Mean differences between isolines for grain Zn, Fe, Mg, Mn, TKW and PH in Rht isogenic lines.

| Entry | Genotype Type | Zn (ppm) | Zn_diff | Fe (ppm) | Fe_diff | Mg (ppm) | Mg_diff | Mn (ppm) | Mn_diff | TKW (g) | TKW_diff | pH (CM) | pH_diff |
|-------|--------------|----------|---------|----------|---------|----------|---------|----------|---------|---------|----------|---------|---------|
| 1     | Siete cerros dwarf BW | 48.1 | 33.3 | 1155 | 49 | 42.1 | 91 |        |        |        |         |        |
| 2     | Siete cerros tall BW | 50.0 | 3.9 | 1349 | 1.6 | 1275 | 120 | 57 | 8 | 45.7 | 3.7 | 107 | 17 |
| 3     | Anza dwarf BW | 46.4 | 32.0 | 1240 | 56 | 37.3 | 88 |        |        |        |         |        |
| 4     | Anza tall BW | 49.9 | 3.5 | 1335 | 1.6 | 1390 | 150 | 63 | 7 | 40.1 | 2.8 | 113 | 25 |
| 5     | Pavon dwarf BW | 53.8 | 32.8 | 1275 | 53 | 41.5 | 100 |        |        |        |         |        |
| 6     | Pavon tall BW | 57.7 | 3.9 | 1335 | 5.2 | 1335 | 60 | 55 | 2 | 43.6 | 2.1 | 127 | 27 |
| 7     | Seri dwarf BW | 51.3 | 35.4 | 1215 | 60 | 42.9 | 92 |        |        |        |         |        |
| 8     | Seri tall BW | 54.0 | 2.7 | 1305 | 1 | 1305 | 90 | 62 | 2 | 46.9 | 4.1 | 109 | 17 |
| 9     | Kauz dwarf BW | 52.2 | 34.3 | 1075 | 49 | 40.5 | 91 |        |        |        |         |        |
| 10    | Kauz tall BW | 55.1 | 2.9 | 1095 | 1.2 | 1095 | 20 | 50 | 1 | 43.9 | 3.4 | 110 | 19 |
| 11    | Genaro dwarf BW | 58.9 | 37.6 | 1190 | 54 | 40.1 | 92 |        |        |        |         |        |
| 12    | Genaro tall BW | 61.6 | 2.7 | 1325 | 14.4 | 1325 | 135 | 64 | 10 | 43.8 | 3.7 | 113 | 21 |
| 13    | Culican dwarf BW | 53.0 | 32.0 | 1210 | 51 | 46.1 | 95 |        |        |        |         |        |
| 14    | Culican tall BW | 63.0 | 10 | 1315 | 3.9 | 1315 | 105 | 59 | 7 | 49.0 | 2.8 | 121 | 26 |
| 15    | Sitta dwarf BW | 49.2 | 31.4 | 1175 | 52 | 42.8 | 96 |        |        |        |         |        |
| 16    | Sitta tall BW | 51.6 | 2.4 | 34.9 | 3.5 | 1250 | 75 | 57 | 6 | 44.4 | 1.6 | 114 | 18 |
| 17    | Nesser dwarf BW | 46.9 | 29.4 | 1120 | 48 | 36.9 | 83 |        |        |        |         |        |
| 18    | Nesser tall BW | 50.6 | 3.6 | 1215 | 1.3 | 1215 | 95 | 52 | 4 | 42.5 | 5.6 | 107 | 24 |
| 19    | Galvez dwarf BW | 48.5 | 32.8 | 1135 | 46 | 44.2 | 100 |        |        |        |         |        |
| 20    | Galvez tall BW | 52.8 | 3.6 | 1255 | 3.3 | 1255 | 120 | 57 | 11 | 45.0 | 0.8 | 116 | 16 |
| 21    | Yavaros dwarf BW | 47.5 | 33.6 | 1050 | 46 | 49.5 | 89 |        |        |        |         |        |
| 22    | Yavaros tall BW | 50.2 | 2.7 | 1353 | 1.7 | 1353 | 85 | 52 | 6 | 53.2 | 3.8 | 122 | 33 |
| 23    | Aconchi dwarf BW | 51.2 | 34.1 | 965 | 52 | 48.5 | 85 |        |        |        |         |        |
| 24    | Aconchi tall BW | 57.1 | 3.9 | 1065 | 2 | 1065 | 100 | 52 | 0 | 49.5 | 1 | 121 | 35 |
| 25    | Focha dwarf BW | 49.1 | 32.0 | 1045 | 42 | 47.6 | 86 |        |        |        |         |        |
| 26    | Focha tall BW | 51.0 | 1.9 | 35.6 | 3.6 | 1145 | 100 | 53 | 10 | 50.1 | 2.5 | 118 | 32 |
| 27    | Lavanco dwarf BW | 49.2 | 33.8 | 1195 | 47 | 54.3 | 87 |        |        |        |         |        |
| 28    | Lavanco tall BW | 52.9 | 3.8 | 1275 | 1.8 | 1275 | 80 | 57 | 10 | 55.0 | 0.7 | 116 | 29 |
| 29    | Nehama dwarf BW | 49.1 | 33.2 | 1225 | 56 | 43.1 | 86 |        |        |        |         |        |
| 30    | Nehama tall BW | 54.4 | 5.3 | 1305 | 3.3 | 1305 | 80 | 59 | 4 | 45.2 | 2.2 | 123 | 37 |
| 31    | Bichena dwarf BW | 48.1 | 33.5 | 1015 | 40 | 46.0 | 98 |        |        |        |         |        |
| 32    | Bichena tall BW | 53.7 | 5.6 | 1080 | 2.1 | 1080 | 65 | 41 | 1 | 47.3 | 1.4 | 122 | 22 |
| Mean  | 52.1 | 3.9 | 1348 | 3.2 | 1193 | 94 | 53 | 6 | 45.3 | 2.6 | 104 | 25 |
| Minimum | 46.4 | 1.9 | 29.4 | 1 | 965 | 20 | 40 | 0 | 36.9 | 0.7 | 83 | 16 |
| Maximum | 63.0 | 14.4 | 1390 | 150 | 64 | 11 | 55 | 5.6 | 127 | 37 |
| Heritability | 0.75 | 0.52 | 0.85 | 0.83 | 0.84 | 0.95 |
| LSD (5%) | 4.12 | 7.63 | 116 | 7.3 | 2.12 | 7.8 |
| CV (%) | 3.88 | 10.76 | 4.8 | 6.8 | 2.29 | 3.7 |

BW: Bread Wheat, DW: Durum Wheat.
TKW = Thousand Kernel Weight (g), PH = Plant Height (cm).

Fig. 3. Principal component analysis (PCA) for four micronutrients and TKW in Rht isogenic lines (means for 2015, 2016 and 2017).
G. Velu et al.

Field Crops Research 214 (2017) 373–377

Paltridge, N.G., Milham, P.J., Ortiz-Monasterio, J.I., Velu, G., Yasmin, Z., Palmer, L.J., Ortiz-Monasterio, I., Palacios-Rojas, N., Meng, E., Pixley, K., Trethowan, R., Pena, R.J., McClung, A.M., Cantrell, R.G., Quick, J.S., Gregory, R.S., 1986. In Muthayya, S., Rah, J.H., Sugimoto, J.D., Roos, F.F., Kraemer, K., Black, R.E., 2013. The global hidden hunger indices and maps: an advocacy tool for action. PLoS One 8, e67860. http://dx.doi.org/10.1371/journal.pone.0067860.

Ortiz-Monasterio, I., Palacios-Rojas, N., Meng, E., Pidley, K., Trehowcan, R., Pena, R.I., 2007. Enhancing the mineral and vitamin content of wheat and maize through plant breeding. J. Cereal Sci. 46, 293–307.

Paltridge, N.G., Milham, P.J., Ortiz-Monasterio, J.I., Velu, G., Yasmin, Z., Palmer, L.J., Guild, G.E., Stangoulis, J.C.R., 2012. Energy-dispersive X-ray fluorescence spectrometry as a tool for zinc, iron and selenium analysis in whole grain wheat. Plant Soil 361, 251–260.

Pfeiffer, W.H., McClafferty, B., 2007. HarvestPlus: breeding crops for better nutrition. Crop Sci. 47, 88–105.

Singh, R.P., Velu, G., 2017. Zinc-biofortified Wheat: Harnessing Genetic Diversity for Improved Nutritional Quality. Sci. Br. Biofortification Ser., pp. 1–4. (Available at:). http://www.harvestplus.org/sites/default/files/publications/Science Brief-Biofortification_1Zinc Wheat_May2017.pdf.

Singh, R., Huerta-Espino, J., Rajaram, S., Crossa, J., 2001. Grain yield and other traits of tall and dwarf isolines of modern bread and durum wheats. Euphytica 119, 241–244.

Stein, A.J., 2010. Global impacts of human mineral malnutrition. Plant Soil 335, 133–154.

Swaminathan, M.S., 2013. Genesis and growth of the yield revolution in wheat in India: lessons for shaping our agricultural destiny. Agric. Res. 2, 183–188.

Velu, G., Singh, R.P., Huerta-Espino, J., Peña, R.J., 2011. Breeding for enhanced zinc and iron concentration in CIMMYT spring wheat germplasm. Czech J. Genet Plant Breed 47, S174–S177.

Velu, G., Singh, R.P., Huerta-Espino, J., Peña-Bautista, R.J., Arun, B., Mahendra-Singh, A., Yaqub-Mujahid, M., Sohu, V.S., Mavi, G.S., Crossa, J., Alvarado, G., Joshi, A.K., Pfeiffer, W.H., 2012. Performance of biofortified spring wheat genotypes in target environments for grain zinc and iron concentrations. Field Crops Res. 137, 261–267.

Velu, G., Ortiz-Monasterio, I., Calmaka, I., Hao, Y., Singh, R.P., 2014. Biofortification strategies to increase grain zinc and iron concentrations in wheat. J. Cereal Sci. 59, 365–372.

Velu, G., Tutus, Y., Gomez-Becerra, H.F., Hao, Y., Demir, L., Kara, R., et al., 2017. QTL mapping for grain zinc and iron concentrations and zinc efficiency in a tetraploid and hexaploid wheat mapping populations. Plant Soil 411, 81–99. http://dx.doi.org/10.1007/s11104-016-3025-8.

WHO, 2017. The Double Burden of Malnutrition: Policy Brief. (Available at:). http://www.who.int/nutrition/publications/doubleburdenmalnutrition-policybrief/en/.