Use of wheat genetic resources to develop biofortified wheat with enhanced grain zinc and iron concentrations and processing quality

Carlos Guzmán[1], Ana Sofía Medina-Larqué[1], Hector González-Santoyo[1], Roberto Javier Peña[1], Ravi P. Singh[1], J. Huerta-Espino[3], Ivan Ortiz-Monasterio[2], Govindan Velu[1*]

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

* Author for correspondence (Phone: +525959521900 ext. 2200; Fax:+525558047558; E-mail: velu@cgiar.org)

With 1 Table, 4 Figures and 1 supplementary table S1

Abstract

A major cereal crop worldwide, wheat contributes on average one-fifth of the calories in the human diet and is the main source of protein and nutrients for much of the world’s population. Wheat varieties with improved nutritional quality, high grain yield and desirable processing quality attributes in the adapted genetic backgrounds can help alleviate nutrient deficiencies among resource poor people. This paper reports advances in targeted crosses of landraces and ancestors of common wheat (*Triticum aestivum* L.), such as *Aegilops tauschii, T. turgidum* ssp. *diccocoides, T. turgidum* ssp. *dicoccum* and *T.aestivum* ssp. *spelta* species, which feature significant genetic variation for grain zinc and iron, with high-yielding bread wheat lines from the CIMMYT breeding program that have desirable processing and end-use quality. Resulting high-yielding lines possessed preferred processing quality traits and 10-90% higher grain micronutrient concentrations than popular commercial varieties.

Keywords: Malnutrition; Iron; Zinc; wheat quality; genetic resources.

Abbreviations: GH, Grain Hardness; GPRO%, Grain Protein percentage; FPRO%, Flour Protein percentage; Fe, iron; LV, Loaf Volume; M%TQ, Mixograph Torque; FSDS, Flour SDS Sedimnetation; TW, Test Weight; Zn, zinc;

1. Introduction

Micronutrient deficiency is one of the most important challenges facing humanity. The lack of adequate levels of essential vitamins and minerals (iron [Fe], zinc [Zn] and vitamin A) affects more than 2 billion people (UNSCN 2006). Pregnant women and young children are prone to acute micronutrient deficiency, which reduces physical and mental development in children below 5 years of age, and malnutrition is considered as the largest single contributor to disease in persons of any age (UNSCN 2006). Micronutrient deficiency is common in developing countries, where staple cereals (wheat, maize or rice) provide most calories and diets are poor in meat, poultry, fish, fruits or vegetables (Bouis et al. 2011).

Plant breeding to develop biofortified crops with enhanced micronutrient concentrations has emerged as a sustainable solution to complement strategies such as supplementation or fortification, especially for micronutrient-deficient rural inhabitants with limited access to formal markets or health care and who rely heavily on locally-grown staple food crops (Bouis et al. 2011). With the funding from the HarvestPlus CGIAR Challenge Program and the CGIAR Research Program on Agriculture for Nutrition and Health, the International Maize and Wheat Improvement Center (CIMMYT) is leading a global effort to develop and disseminate to partners in South Asia high-yielding wheat varieties that contain high levels of grain Zn and Fe. South Asia suffers from high population densities and alarming rates of malnutrition (Velu et al. 2012).

Breeding competitive high-Fe and -Zn varieties requires source materials that feature adequate genetic variation in concentrations of those micronutrients. Screening studies have shown that modern wheat cultivars are not a good source of genes for high Zn and Fe (Monasterio and Graham 2000; Zhao et al. 2009), probably because the breeding programs in which they were developed focused on maximizing yield rather than improving nutritional composition. However, wheat landraces and selected accessions of wheat ancestors such as *Aegilops tauschii, T. turgidum* ssp. *diccocoides*, *T. turgidum* ssp. *dicoccum*, and *T.aestivum* ssp. *spelta* do feature significant genetic variation for grain Fe and Zn concentrations (Cakmak et al. 2004; Gomez-Becerra et al. 2009; Suchowilska et al. 2012). Previous studies have explored the use of wide wheat genetic resources as sources of genes to enhance grain micronutrients (Fe and Zn) concentrations in the adapted high-yielding backgrounds (Cakmak et al. 2000; Ficco et al.

2009; Morgounov et al. 2007; Zhao et al. 2009). Gomez-Becerra et al. (2010) identified more than 200 *T. spelta* genotypes with Fe and Zn concentrations higher than 50 mg/kg; Monasterio and Graham (2000) showed several accessions of *T. dicoccum* and Mexican landraces with superior concentrations for both micronutrients and Chhuneja et al. (2006) reported *Ae. tauschii* and synthetic lines with concentrations higher than 60 mg/kg for Fe and Zn. The availability in the primary and secondary wheat gene pools, with genotypes containing high concentrations of micronutrients was demonstrated in various studies reported in Velu et al. (2014).

Primary driver for adoption of biofortified wheat in small-holder farmers must provide superior yields, resist important diseases and possess tolerance to heat, drought and potentially to micronutrient-poor soils (Welch and Graham 2004). This has been the strategy of CIMMYT's Global Wheat Program. Specifically, several synthetic wheat lines generated from selected *T. dicoccum, T. durum* and *A. tauschii* accessions, as well as selected spelt wheats and wheat landraces, have been crossed with high-yielding, elite wheats to develop high-yielding lines that also possess enhanced grain micronutrient concentrations, with higher Zn content as a primary target trait. To obtain varieties acceptable to farmers and commercially competitive—that is, acceptable to millers, manufacturers and consumers—rigorous selection pressure was also applied for grain yield potential, disease resistance, heat and drought tolerance and acceptable processing quality. In South Asia, biofortified wheat products should feature medium-to-hard grain texture, as well as extensible and medium-strength gluten, to produce *chappati*, the main local flat bread, of acceptable texture. Lines showing superior dough extensibility combined with medium-to-high gluten strength can also be used for products such as pan bread, thereby promoting small-to-intermediate-scale local industry. Some have raised concerns, however, about possible adverse effects on processing quality of using wheat genetic resources not previously characterized for quality traits.

The main objective of this study was thus to characterize for processing and end-use quality traits in a set of 141biofortified wheat lines with enhanced grain Zn and Fe concentrations, and to determine breeding materials derived from crosses involving wild relatives and landraces have preferred processing quality to produce different end-use products besides wheat with the higher grain Zn and Fe concentration.

2. Materials and Methods

2.1 Plant material

We used grain samples of 141 advanced lines from the HarvestPlus Yield Trial (HPYT) grown during the 2009-10 crop season in Ciudad Obregón, Sonora, México, under full irrigated condition. Trial entries were evaluated following an alpha-lattice design with three replications. The recommended dosage of N-P-K fertilizer was applied and other agronomic practices followed to raise a good crop. Plots were harvested at physiological maturity. The advanced lines were divided into five groups depending on their origin or cross: I - 22 modern bread wheat lines used as checks in this study; II – 35 lines resulting from the cross of Mexican landraces with modern cultivars; III – 26 lines derived from the cross of spelt accessions with modern cultivars; IV – 45 lines resulting from the cross of synthetic wheat lines (*T. dicoccum* accessions x *Ae. tauschii)* with modern cultivars; and V – 13 lines derived from the cross of synthetic wheat lines (*T. durum* accessions x *Ae. tauschii)* with modern cultivars. The complete pedigree of each line is shown in Supplementary Material 1.

2.2 Grain and rheological analyses

Grain Fe and Zn concentrations (mg/kg) 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), previously standardized for high-throughput screening of Zn and Fe in whole wheat grain (Paltridge et al. 2012). Grain hardness (GH), grain protein (GPRO%) and moisture content were determined using near-infrared spectroscopy (NIRS, NIR Systems 6500, Foss Denmark) according to official method AACC 39-70A (AACC 2000). Lower hardness index (%) values correspond to harder grains. Grain samples were milled using Brabender Quadrumat Jr. (C.W. Brabender OHG, Germany). Flour protein (FPRO%) and moisture content were determined by NIRS (Foss NIR systems INFRATEC 1255, FOSS-TECATOR, Denmark). Both devices were calibrated based on AACC methods (AACC 2000) for moisture (AACC Method 44-15A) and protein (AACC Method 46-11A). Grain protein and flour protein were adjusted to a 12.5% and 14% moisture basis, respectively. The SDS sedimentation test was conducted using 1 g of flour, as described in Peña et al. (1990) recording volume in ml of the sediment (FSDS). Dough development properties were determined by computerized Mixograph of Swanson (National Mfg., USA) using 35 g of flour. Two parameters were obtained: dough development time (MDDT) and % torque*min (M%TQ). The strength (ALVW) and extensibility properties (tenacity/extensibility ratio, ALVP/L) were determined in the Chopin Alveograph (Trippette and Renaud, France). The bread-making test was carried out using the AACC 10-09 method (AACC 2000) and bread loaf volume (LV) recorded. All data are given in Supplementary Material 1.

3. Results

3.1 Fe and Zn concentration

The average grain Fe and Zn concentration in check varieties (Group I) was 29.4 mg/kg and 21.7 mg/kg, respectively (Table 1). The maximum micronutrient concentration in this group was 36 mg/kg Fe (GID 5996190) and 24 mg/kg for Zn (GID 5994262). Group II had several entries with higher Zn and Fe grain concentrations than the highest levels found in group I (Figures 1 and 2). In groups III and IV, lines with spelt and emmer synthetics origins, respectively, showed higher mean values for Zn and Fe than those of the checks and in each of these groups at least one line was found to have higher Fe and Zn values than the maximum value found in group I. In group III and IV, highest Zn levels were 44 mg/kg (GID 6181266) and 45 mg/kg (GID 618149), respectively. In all groups there were lines with significantly higher Zn values than the maximum for the checks (Figure 1). For grain Fe content, groups III and IV showed the higher number of lines with high concentrations, while groups II and V did not show promising results compared to the checks (Figure 1). For grain Zn concentration, groups II-V had more lines with high Zn than group I. Remarkably, some genotypes in groups II, III and IV had very high Zn concentrations (up to 53 mg/kg) and there were lines with 53, 44 and 44.5 mg/kg (in groups II, III and IV, respectively), which is more than twice the grain Zn levels of the best commercial varieties.

Using group I micronutrient content averages (29.4 and 21.7 mg/kg for Fe and Zn, respectively) as a reference, groups III and IV had the highest proportion of lines with superior micronutrient concentrations (Figure 2).

3.2 Grain physical and chemical characteristics

Grain samples were tested for different processing quality traits, including the morphological index of the grain Test Weight (TW). Group I had the highest TW values (average of 81.34 kg/hl), which indicates the presence of plump grain in trials conducted under optimum conditions. All other groups except II also had an average TW over 80 kg/hl, with properly filled grains. Group II lines were mostly derivatives of Mexican landraces and showed the lowest average TW value (76.37 kg/hl), which could be due to having smaller grains or lacking adaptation to the warm, irrigated, high-production environment of Ciudad Obregón. For texture, groups I, III, IV and V had similar average grain hardness values, although there was considerable variation for hardness within each groups, with phenotypes showing a range of values from hard to soft grains. Group II comprised mainly soft grain lines, with only five lines showing hard or semi-hard texture.

No significant differences were found for mean GPRO% and FPRO% among the groups. The differences between the minimum and maximum values in all the groups were around 4%. Significant positive correlations were found between GPRO% and Fe in groups I, II and V, but only between GPRO% and Zn in group I ($r = 0.44$; $P<0.05$).

3.3 Rheological and end-use properties

The FSDS volume provides a rough estimate of sample strength. On average, all groups showed high FSDS values, although groups II and IV had some lines with low values. Performance in the mixograph was heterogeneous, with lines in each group showing acceptable values for dough development time (MDDT) and dough strength (M%TQ), while others did not reach the minimum strength required for yeast-leavened bread or even flat breads. These results were confirmed with the alveograph (ALVW), which gives a good measure of the dough strength and correlates closely with M%TQ (in this study: $r = 0.87$; $P<0.001$). As for M%TQ, the average ALVW value in each group was acceptable for bread making, with the exception of group II, in which gluten strength (ALVW) was very low. Dough extensibility (ALVP/L) was generally good in most lines of all groups, with a few exceptions (ALVP/L ≥ 1.4) that would not be useful for bread making. Group II had the most lines with moderate-to-high extensibility. In groups I, III and IV most lines had balanced gluten (ALVP/L 0.8-1.3), whereas in group V more lines showed low extensibility. The bread-making test revealed acceptable average bread loaf volumes for all groups except II, most of whose lines would not serve for making leavened bread products. Medium-to-high loaf volumes—a few exceeding 900 ml—resulted from most lines in the other groups, with a few exceptions.

Each line was then classified into one of five end-use quality types established in the CIMMYT Wheat Chemistry and Quality Laboratory by Peña (2011, unpublished document): user-type 1, Pan type breads (mechanized industry); 2, leavened breads (semi-mechanized industry), flat breads, dry and fresh noodles and steam breads; 3, dense breads, flat breads (handmade); 4, steam bread, white-salted noodles and biscuits; and 5, utility wheat (poor quality). Only a few lines were included in the quality type 5. The groups III and IV were predominated by lines with quality types 1 and 2, which is linked to medium-high strength and good extensibility; in group V, most of the lines were classified in type 3, due to the prevalence of medium strong and extensible gluten; and in group II, most of the lines fall in the type 4, generally good for biscuit making, due to their soft texture and weak and extensible gluten.

3.4 Micronutrients concentration vs. end-use quality

To combine high micronutrient concentration and acceptable processing quality in adapted genetic backgrounds, we examined both traits together for each group. Group II did not show a significant increment in grain Fe concentration compared to the checks, but there was a strong increase for Zn concentration in ten lines compared to the checks. Of these ten lines only two showed slightly enhanced Fe concentration compared to the average value for the checks (29.4 mg/kg). One of these lines showed poor extensibility and was classified as quality type 5. The other one, showed excellent extensibility, weak gluten (ALVW = 94) and soft texture, and therefore had all the characteristics required to be a good biscuit making wheat cultivar. In group III, 11 lines showed significantly enhanced concentrations for both micronutrients (at least 10% more Fe and at least 20% more Zn). Three of these lines belong to quality type 1 (mechanized bread making, good extensibility and high gluten strength), six were quality type 2 (flat breads, good extensibility and medium-high gluten strength), one was quality type 3 (handmade bread) and another one was quality type 4 (biscuits, soft texture with weak and extensible gluten). In group IV, 19 lines with significantly higher Fe and Zn concentrations were divided into quality types 1 = 8 lines, type 2 = 5 lines and type 3 = 6 lines. At last, group V, with three lines showing significantly increased micronutrient concentrations, of which two of them belonged to quality type 1 and the other belongs to quality type 3.

4. Discussion

Considering the substantial genetic diversity exists for Zn and Fe, CIMMYT followed the strategy of crossing selected genetic resources and synthetic lines showing high levels of micronutrients with high-yielding modern wheat lines to develop biofortified wheat derivatives with the preferred agronomic features. In this study, the advanced breeding lines resulting from this process were analyzed for micronutrients concentration and also for processing quality traits. Ensuring acceptable processing quality would satisfy needs of target population in rural and urban areas as the biofortified wheat varieties must have the requirements of the consumer (baking quality, taste, and keeping properties must satisfy household members) and whole value chain (farmers, millers, manufacturers, and consumers).

Although grain Fe concentration is an important trait for HarvestPlus project, Zn concentration has received special attention because more than 26% of the target population in South Asia suffers from Zn deficiency. That probably explains why most lines in this study showed significantly greater grain Zn concentrations than the check varieties, as well as the practice of choosing parents with high Zn and applying greater selection pressure for Zn concentration in breeding, based on various study results suggesting that higher Zn is positively associated with higher Fe concentration (Gomez-Becerra et al. 2010; Morgounov et al. 2007; Zhao et al. 2009). In our study, the correlations between Fe and Zn in all the groups were small but statistically significant, except in group II. Apart from this general trend, several lines—particularly from groups III and IV—had micronutrient concentrations significantly higher than those of the checks, confirming the excellence of *T. spelta* and *T. dicoccum* synthetic derivatives as sources of genes for higher micronutrient concentrations as Gomez-Becerra et al. (2010) and Monasterio and Graham (2000) reported previously.

Although several landraces, spelt and even synthetic lines have shown good performances in quality analysis (Ali et al. 2013; Konvalina et al. 2013; Mondini et al. 2014), other studies have reported that those materials, as any others, can have great differences in quality traits, and some of them be completely unsatisfactory for the development of different wheat products (Lagudah et al. 1987; Mikos and Podolska 2012; Wilson et al. 2008). Strikingly, a large proportion of lines in this study possessed very good quality traits, with most showing good extensibility, a key end-use quality parameter for any wheat product. This is probably due to the use of modern lines with good-to-excellent quality as background parents in the crosses with high Zn and Fe donors, and often using 1 or 2 back-crosses or three-way crosses with the adapted good quality parents in the breeding process. Gluten strength differed among the groups. In group II, weak gluten lines predominated but most were very extensible. This is linked to their soft texture and makes them good candidates for biscuit production. In this group, the use of Mexican landraces that are known to possess soft grain texture (Ayala et al. 2013) could have led to selection of the soft grain trait in derived lines. Soft grain texture is not common in CIMMYT improved wheat germplasm. In groups III and IV, medium-strong and strong gluten lines were the most numerous, which is linked to the overall good extensibility of the lines and makes them highly acceptable for homemade flat breads, such as *chappati,* or leavened breads in mechanized or semi-mechanized local industry of South Asia. In these groups, the large number of lines with high micronutrient concentrations made it easy to find lines combining high Fe and Zn with good end-use quality. Finally, group V lines generally had slightly lower gluten quality, although they could be used as a progenitor to enhance micronutrient concentrations.

5. Conclusions

The advanced breeding lines analyzed from the HarvestPlus yield trial showed good processing quality characteristics and a significant enhancement in grain Zn and Fe concentrations, especially those originating from spelt and emmer synthetic backgrounds. Our results show that there has been progress in developing varieties that possess high Zn levels and desirable processing quality, as well as high yield potential, even though the Zn and Fe donor sources are “wild” genetic resources or landraces. This was achieved by considering processing quality and yield-related traits when designing the crosses and by applying selection pressure for various quality characteristics in the breeding lines. The final releases of biofortified wheat varieties in the target regions will help improve the livelihoods and health of numerous resource-poor, micronutrient-deficient people.

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Table 1. Average, maximum and minimum values for micronutrients and quality parameters for 5 different groups.

	Group I (Bread wheat checks)	Group II (Landrace origin)	Group III (Spelt origin)	Group IV (Emmer synthetic origin)	Group V (Durum synthetic origin)
Zn	21.7 ± 2.0 (15.8-24.3)	27.1 ± 10.3 (19.0-53.0)	30.1 ± 7.5 (14.1-44.4)	29.8 ± 7.2 (17.7-45.5)	25.8 ± 6.0 (19.6-36.7)
Fe	29.4 ± 3.3 (23.9-35.9)	27.0 ± 1.5 (24.1-31.3)	31.9 ± 3.3 (22.9-38.6)	32.8 ± 2.9 (26.9-38.1)	28.8 ± 3.0 (24.1-33.6)
TW	81.3 ± 1.0 (78.9-82.8)	76.4 ± 2.0 (75.0-82.2)	80.9 ± 2.2 (74.1-84.9)	81.4 ± 1.4 (78.3-83.9)	80.0 ± 1.1 (79.0-83.0)
GH	41.6 ± 2.6 (36.4-46.6)	61.7 ± 7.7 (40.7-68.9)	44.5 ± 4.0 (37.3-57.1)	43.2 ± 3.5 (34.4-50.9)	44.7 ± 2.5 (41.0-49.4)
GPRO%	13.0 ± 1.1 (11.1-15.0)	13.2 ± 0.4 (12.3-14.3)	13.4 ± 0.8 (11.6-15.0)	13.4 ± 0.9 (12.0-15.5)	13.6 ± 0.8 (12.3-15.0)
FPRO%	11.9 ± 1.1 (10.2-14.0)	12.0 ± 0.5 (10.0-12.7)	12.3 ± 0.9 (10.7-13.9)	12.2 ± 1.1 (10.3-15.2)	12.5 ± 0.5 (11.9-13.5)
FSDS	21.3 ± 1.0 (18.8-22.8)	21.1 ± 3.5 (10.3-23.5)	21.6 ± 1.4 (19.0-24.3)	20.9 ± 2.2 (13.3-23.5)	22.1 ± 0.6 (21.3-23.3)
MDDT	2.7 ± 0.5 (1.5-3.6)	1.5 ± 0.3 (0.9-2.5)	2.5 ± 0.6 (1.6-3.9)	2.4 ± 0.7 (1.3-3.9)	2.3 ± 0.7 (1.6-3.8)
M%TQ	108.6 ± 21.1 (67.9-152.3)	52.1 ± 9.0 (28.4-69.8)	103.6 ± 23.7 (65.2-151.4)	100.9 ± 33.8 (45.1-156.7)	91.8 ± 29.5 (60.0-150.0)
ALVW	282.6 ± 83.6 (135-431)	110.9 ± 42.2 (37-253)	298.3 ± 99.0 (126-515)	290.9 ± 126.0 (88-599)	270.4 ± 142.2 (151-549)
ALVPL	1.0 ± 0.3 (0.5-1.5)	0.6 ± 0.3 (0.3-1.8)	0.9 ± 0.2 (0.5-1.2)	0.9 ±0.2 (0.5-1.5)	0.9 ± 0.5 (0.4-2.1)
LV	775 ± 57.5 (670-900)	698.9 ± 42.9 (495-745)	802.5 ± 56.47 (660-920)	788.4 ± 66.8 (650-930)	777.3 ± 64.5 (685-910)

Figure 1. Distribution of wheat lines for grain Fe and Zn concentration for each of the five

groups

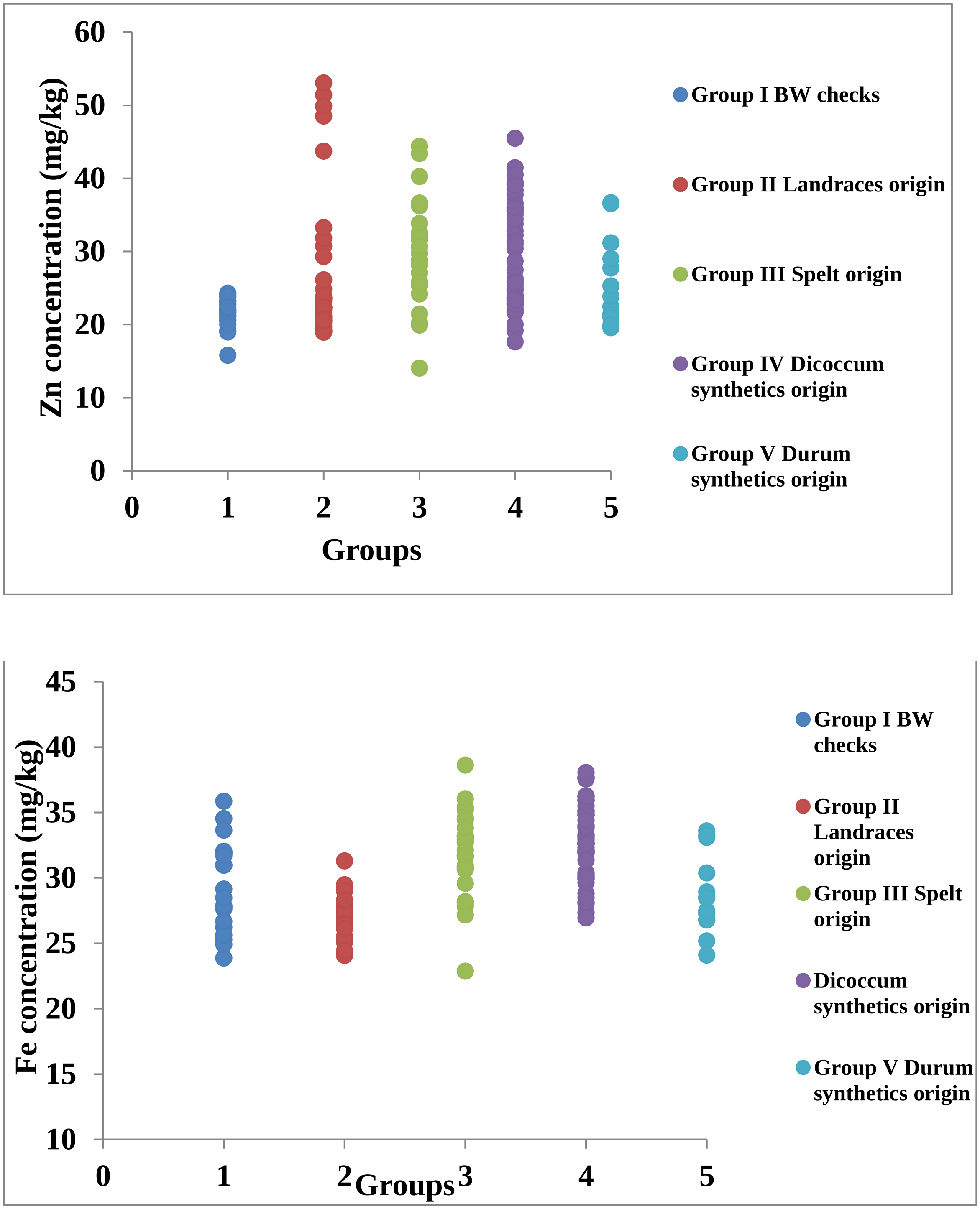

Figure 2. Frequency distribution of grain Fe and Zn concentrations in each of the give groups.

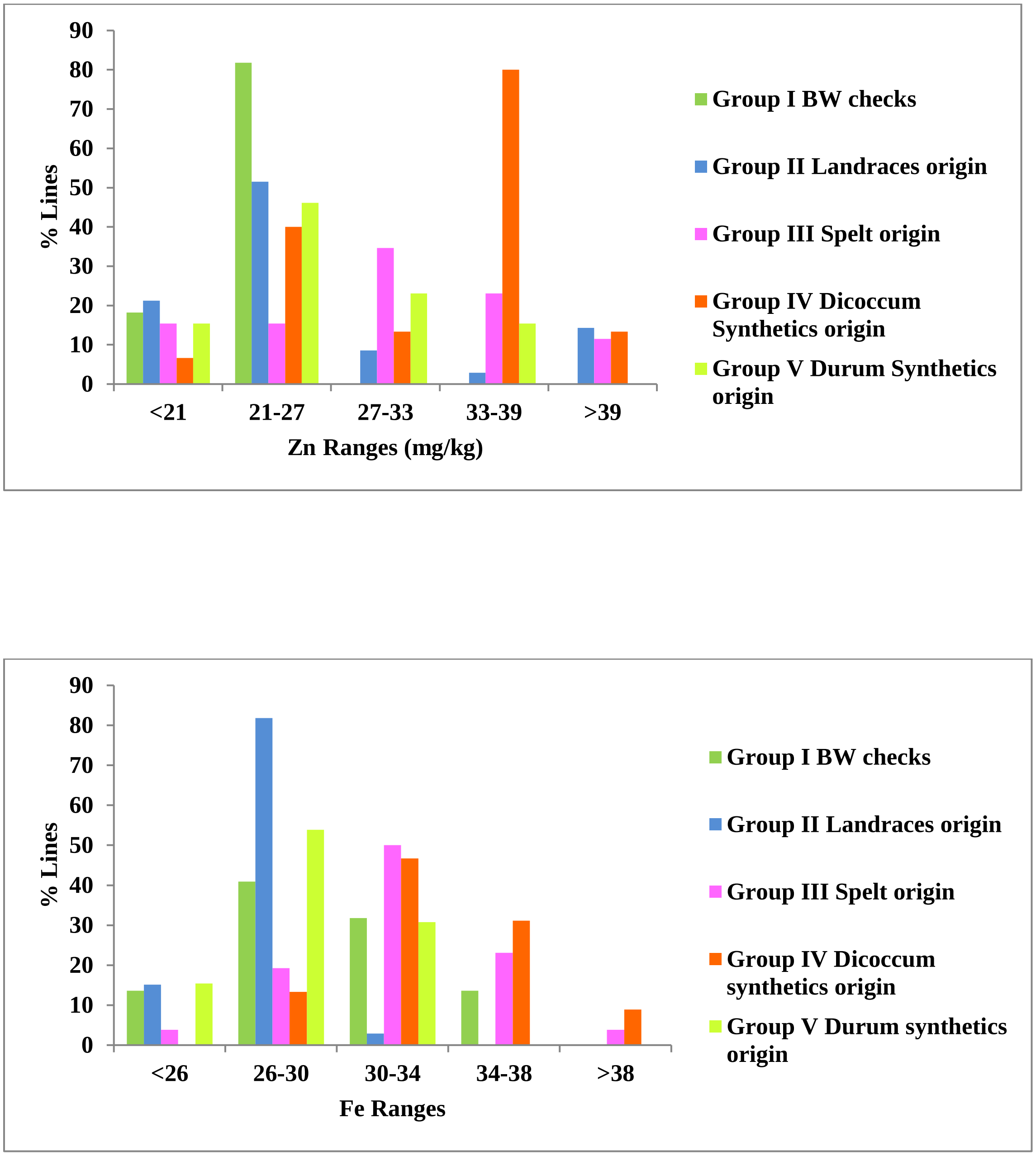

Figure 3. Percentage of wheat lines in groups II-V showing higher Fe and Zn concentrations

than the average of the checks (group I).

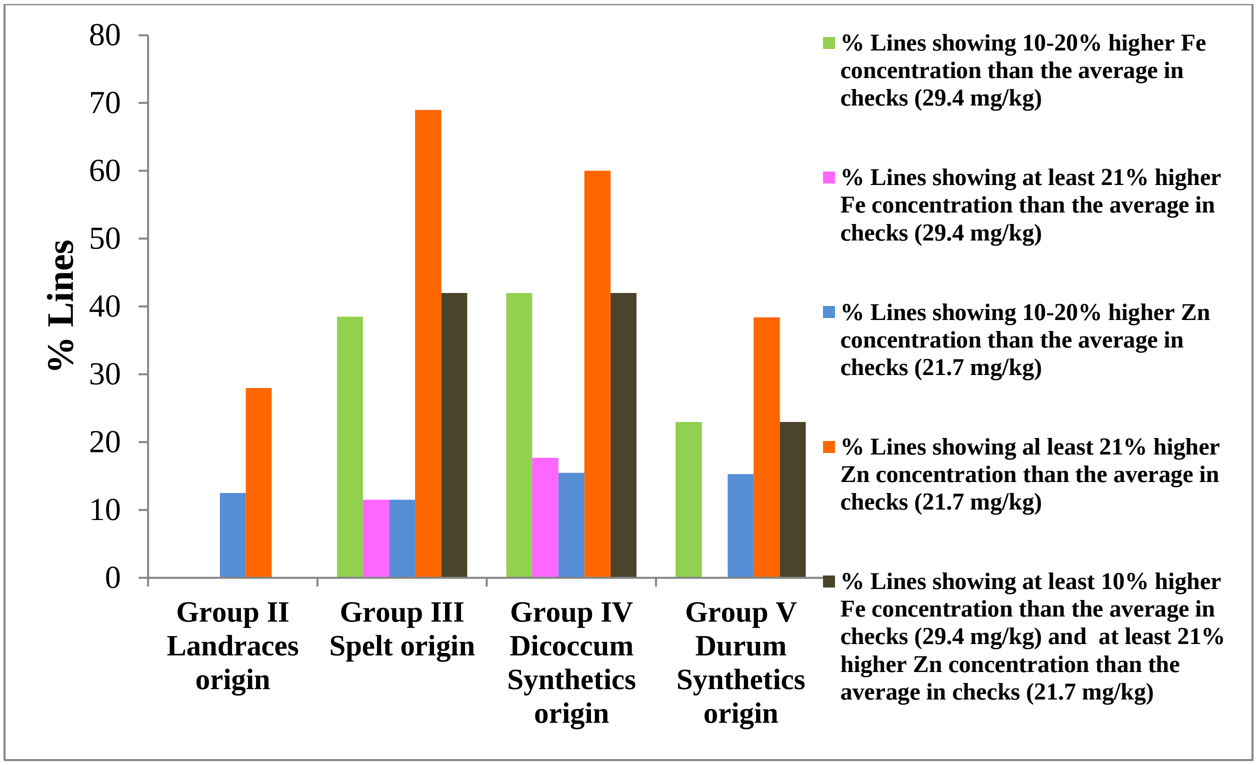

Figure 4. Percentage of wheat lines in each of the five groups with dough strength/extensibility ratio.