Euphytica (2012) 186:153–163
DOI 10.1007/s10681-011-0511-1

Mineral bioavailability in grains of Pakistani bread wheat declines from old to current cultivars

Shahid Hussain · Muhammad Aamer Maqsood · Zed Rengel · Muhammad Khawar Khan

Received: 17 July 2011 / Accepted: 6 August 2011 / Published online: 18 August 2011

Abstract Estimating variation in grain mineral concentration and bioavailability in relation to grain yield and the year of cultivar release is important for breeding wheat with increased content of bioavailable minerals. The grain yield and yield components, grain phytate concentration, and concentration and bioavailability of minerals (zinc Zn, iron Fe and calcium Ca) in wheat grains were estimated in 40 wheat cultivars released in Punjab (Pakistan) during the last five decades. Mean grain Zn and Ca concentrations in current-cultivars were significantly lower ($\geq$14%) than in obsolete cultivars released during the Green Revolution (1965–1976). Much of this variation was related to increased grain weight in current-cultivars. There was a positive correlation among minerals ($r = 0.39$ or higher, $n = 40$) and minerals with phytate in wheat grains ($r = 0.38$ or higher, $n = 40$). The tested cultivars varied widely in grain yield and grain phytate-to-mineral molar ratios (phytate:mineral). Compared to obsolete cultivars, the current-cultivars had a higher phytate:mineral ratio in grains, indicating poor bioavailability of minerals to humans. The study revealed a non-significant relationship between grain yield and phytate:mineral ratios in grains. Therefore, breeding for lower phytate:mineral ratios in wheat grains can ensure increased mineral bioavailability without significant reduction in the yield potential. Future breeding should be focused on developing new genotypes suitable for mineral biofortification and with increased mineral bioavailability in grains.

Keywords Bioavailability · Biofortification · Grain minerals · Pakistan · Phytate · *Triticum aestivum* L.

S. Hussain (✉) · M. A. Maqsood · M. K. Khan
Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad 38040, Pakistan
e-mail: id.hussain@yahoo.com

S. Hussain · Z. Rengel
School of Earth and Environment, The University of Western Australia, Crawley, WA 6009, Australia

Introduction

Bread wheat (*Triticum aestivum* L.) is currently grown on a large scale to feed millions of people around the world. Green Revolution in 1960s played a major role worldwide in balancing the supply demand equation for wheat grain. This was made possible by the introduction of high-yielding and fertilizer-responsive semi-dwarf wheat genotypes.

Wheat grains are a major dietary component in Pakistan and several other countries, especially in central and western Asia. On average, per capita reliance on wheat for daily food in Pakistan is 60% greater than in the rest of the world (FAO 2011a). Most of the wheat production in Pakistan comes from the Punjab province that has over 6.8 million ha under wheat cultivation. In 2009, Pakistani Punjab produced 18.4 million tons of wheat that was 77% of the total wheat production in Pakistan (MINFAL 2009) and 2.7% of the total wheat production in the world (FAO 2011b).

In Punjab (Pakistan) and most of the other regions of the world, higher grain yields, resistance to diseases and lodging, and lower farming costs have been the prime goals of wheat breeding (Bouis and Welch 2010; Rengel and Römheld 2000). In contrast, grain quality characteristics, especially mineral densities in wheat grains, have been ignored till now. As a result, grains of the cultivated wheat cultivars might have relatively low mineral densities (Fan et al. 2008).

Minerals such as zinc (Zn), iron (Fe) and calcium (Ca) are not only essential for plants, but also for humans and animals. However, recommended daily dietary intake of these minerals is generally not achieved by the human population in developing countries (Brown et al. 2001; Gibson 2006; WHO 2002). The heavy reliance in a diet on cereal grains that are inherently low in essential minerals has led to deficiencies and health concerns in the human population (White and Broadley 2009).

Deficiencies of Zn and Fe are major causes of diseases and deaths in the developing countries (WHO 2002). In Pakistan, about 10 million children (under 5 years of age) and 9.2 million women of child-bearing age suffer from Fe deficiency anemia, and about 10.5 million children (under 5 years of age) and 15 million women of child-bearing age have Zn deficiency (MINH 2009). Along with Zn and Fe, deficiency of Ca is also a human health concern in Pakistan and other developing countries (Fatima et al. 2009; White and Broadley 2009).

Biofortification of cereal grains with essential minerals that are deficient in a human diet has been suggested as a sustainable solution (Bouis and Welch 2010; Ficco et al. 2009). Biofortification relies on genetic and agronomic approaches to increase bioavailable amounts of minerals in edible portions of cereals (Hussain et al. 2010). Application of mineral fertilizers, breeding for mineral biofortification and genetic engineering for greater uptake from soil and increased remobilization of minerals from vegetative parts to developing grains are important approaches to increase mineral density in grains (Cakmak 2008; Graham et al. 1999; Uauy et al. 2006; Waters et al. 2009; Wang et al. 2008). On the other hand, the bioavailable contents of minerals in wheat grains can also be increased by decreasing phytate, an antinutrient, in staple food grains by diminishing activity of enzymes in the phytate biosynthetic pathway and over-expressing phytase (White and Broadley 2009). Phytate binds Zn, Fe, Ca and other minerals to form insoluble complexes and thus hinders absorption of these minerals into the human body (Weaver and Kannan 2002). Therefore, the phytate-to-mineral molar ratios (phytate:mineral) in wheat grains are used to categorize food for mineral bioavailability (Brown et al. 2001).

Biofortifying wheat grains with essential minerals is an economical approach to solve mineral deficiencies in humans (Stein et al. 2007). This demands selection criteria to underpin integration of increased grain yields with increased mineral concentrations in grains (Morris and Sands 2006). For this purpose, determination of relationships between grain mineral concentrations and grain yield in a wide range of wheat cultivars is required (Hussain et al. 2010, 2011). A relationship among different yield components with mineral concentrations and bioavailability also needs precise description. However, such data are not available regarding wheat cultivars of Punjab (Pakistan). Therefore, a study was undertaken to evaluate the genetic variability of Zn, Fe, Ca and phytate concentrations in the grains of 40 wheat cultivars ranging from obsolete ones released during Green Revolution (1965–1976) to current-cultivars (released during 2001–2008) in Punjab (Pakistan). The relationships among grain yield, yield components, grain concentration and bioavailability of minerals, and year of cultivars release were estimated. Cultivars were finally characterized for high grain yields and low phytate:mineral ratios in grains.

Materials and methods

Wheat cultivars and field experiment

Forty bread wheat cultivars, officially released for cultivation in Punjab (Pakistan), were selected for the study. These included Green Revolution (released during 1965–1976, $n = 11$), post-Green Revolution (released during 1977–1988, $n = 9$), recently discontinued (released during 1989–2000, $n = 9$) and current-cultivars (released during 2001–2008, $n = 11$) (Table 1).

Before sowing, randomized soil samples (0–15 cm depth) were collected from the field (Typic Calciargid). Soil texture (35.6% sand, 37.5% silt and 26.9% clay), determined by the hydrometer method (Gee and Bauder 1986), was loam. Soil had pH 7.9, which was measured in saturated soil paste by a Calomel glass electrode using a Beckman pH meter. Electrical conductivity of saturated soil paste extract was 2.56 dS m^{-1}. Organic matter was 7.4 g kg^{-1} of soil according to Walkley–Black method (Nelson and Sommers 1982). Free lime (calcium carbonate; $CaCO_3$), estimated by acid dissolution (Allison and Moodie 1965), was 49 g kg^{-1} of soil. Plant-available Fe and Zn in the soil, extracted by 0.005 M DTPA (Lindsay and Norvell 1978), were 0.72 and 3.6 mg kg^{-1}, respectively. These micronutrients were determined using an atomic absorption spectrophotometer (*PerkinElmer, AAnalyst 100,* Waltham, USA).

Wheat cultivars were sown in November 2009 at the Wheat Research Institute, Faisalabad, Pakistan (31.384°N, 73.037°E). Each cultivar was sown in six row plots in three blocks; rows were 5.0 m long and 0.2 m apart. Sowing density was 400 seeds m^{-2}. Plants were supplied with uniform basal rates of nitrogen (60 kg ha^{-1}), phosphorus (90 kg ha^{-1}) and potassium (50 kg ha^{-1}) by applying urea, di-ammonium hydrogen phosphate and potassium sulphate. The remaining dose of nitrogen (60 kg ha^{-1}) was applied 45 days after sowing. Plots were irrigated with canal water throughout the growing season. Cultivars were harvested at maturity in April 2010.

Plant sampling and analyses

At maturity, 50 tillers of each cultivar from each block were randomly selected and measured for plant height. The spikes from these tillers were measured for length and threshed to count number of grains/spike. Grain weight (mean weight/kernel) was calculated from the mean weight of three replicates of 100 grains each and adjusted to 13% (w/w) moisture content for all cultivars. For grain and straw yields, whole plots were harvested manually and threshed to separate grains. Grain yield of all cultivars was adjusted to 13% (w/w) moisture content; both grain and straw yields were reported as t ha^{-1}. Harvest index was calculated as the ratio of grain yield to total above-ground biomass.

For chemical analyses, subsamples of threshed grain and straw were dried at 60°C for 48 h in an air-forced oven (Liu et al. 2006). Oven dried samples were ground with a Wiley mill to pass through a 0.5 mm sieve. Known weights of ground samples were digested in a di-acid (HNO_3:$HClO_4$) mixture (Jones and Case 1990). Concentrations of Zn, Fe and Ca in the digest were estimated by an atomic absorption spectrophotometer (*PerkinElmer, AAnalyst 100,* Waltham, USA). For phytate determination, 60 mg of finely ground grain samples were extracted with 10 mL of 0.2 N HCl at room temperature for 2 h under continuous shaking. Phytate in the extract was determined by a spectrophotometer (*Shimadzu, UV-1201,* Kyoto, Japan) measuring pink color that was developed by un-reacted Fe(III) with 2,2′-bi-pyridine (Haug and Lantzsch 1983) as detailed by Hussain et al. (2011). Relative mineral (Zn, Fe and Ca) bioavailabilities in grains of wheat cultivars were estimated as phytate:mineral ratios in grains (Brown et al. 2001).

Data analysis

The 40 wheat cultivars were separated into four groups: Green Revolution, post-Green Revolution, recently discontinued and current-cultivars (Table 1). Current-cultivars were compared with the rest using four suitable contrasts (Table 2). As desired contrasts were not orthogonal, Scheffe's F values were used to control the experiment-wise error rate. Pearson correlation coefficients, *r*, were used to study the interrelationship among the studied parameters (Steel et al. 1997). To characterize cultivars for increased grain yield and increased mineral bioavailability, grain yield of the cultivars was plotted against phytate:mineral ratios in grains.

Statistical analysis and data computations were done on *Microsoft Excel* 2007® and *Statistix 9*®. The level of significance (α) was set at 0.05 ($P \leq 0.05$).

Results

Variation in yield and concentration of minerals

Grain yield ranged from 2.6 to 4.6 t ha^{-1} with an average of 3.5 t ha^{-1} (Table 2). Contrast analysis

Table 1 Groups of bread wheat cultivars of Punjab (Pakistan) grown in the study

No.	Cultivar	Parentage	Release year
(I) Green Revolution cultivars			
1	MexiPak-65	PJ62"S"-GB-55	1965
2	Chenab-70	C271-WT(E)//SON 64	1970
3	Barani-70	PIT-GB/C271	1970
4	SA-42	C271-LR64/SON 64	1971
5	Blue Silver	II-54-388-AN(YT.54-N 10B/LR 64)	1971
6	Lyllpur-73	BB-NOR 67	1973
7	Sandal	CNO//SN64/KL.REND/3/8156	1973
8	Pari-73	CNO'S'//SON/KL.REND/M.PAK	1973
9	Yecora-70	CNO'S'//SON-KL.REND/8156	1975
10	SA-75	NAI60-CB151/S.948)M.PAK	1975
11	Punjab-76	NA160-CB151XS.948)M.PAK	1976
(II) Post-Green Revolution cultivars			
12	LU-26S	BLS-KHUSHAL 69	1977
13	WL-711	S308/CHRIS//KAL	1978
14	Punjab-81	INIA/SON 64-P.4160(E)//SON 64	1981
15	Pak-81	KVZ/BUHO//KAL/BB	1981
16	Barani-83	BB-GALLO/GTO-7C//BB-CNO	1983
17	Kohinoor-83	OREF1 158-FDL/MEXFEN'S'-TIBA63)C0C75	1984
18	Faisalabad-83	FURY/KAL-BB	1984
19	Punjab-85	KVZ/TRM//PTM/ANA	1985
20	Chakwal-86	FORLANI-ACCICO 10/ANA 75	1988
(III) Recently discontinued cultivars			
21	Pasban-90	INIA66/A.DISTT//INIA66/3/GEN81	1990
22	Rothas-90	INIA66/A.DISTT//INIA66/3/GEN81	1990
23	Inqilab-91	WL711/CROW'S'	1991
24	Parwaz-94	(V.5648)CNO'S'/LR64//SON64/3/SON/4/PRL'S'	1994
25	Shakkar-95	WL711//F3.71/TRM	1995
26	Punjan-96	SA42/3/CC/INIA//BB/INIA/4/CNO/HD832	1996
27	Aqab-2000	CROW'S'/NAC//BOW'S'	2000
28	Chenab-2000	CBRD	2000
29	Iqbal-2000	BURGUS/SORT-12-13//KAL/BB/3/PAK-81	2000
(IV) Current-cultivars			
30	SH-2002	INQLAB-91/FINK'S	2002
31	AS-2002	KHP/D-31708//CM-74-A-370/CIANO79/RL-6043/4*NAC	2002
32	Bhakkar-2002	P-20102/PIMA//SKA/3/TTR'S'/BOW'S'	2002
33	Ufaq-2002	V.84/33/V.83150	2002
34	GA-2002	PBW343	2002
35	Sehar-2006	CHILL/2*STAR/4/BOW/CROW//BUC/PVN/3/	2006
36	Shafaq-2006	LU-26/HD-2179//2*INQ-91	2006
37	Fareed-2006	PTS/3/TOB/LFN//BB/HD832-5//ON/5/4-V/ALD'S'//HPO'S'	2006
38	Faisalabad-2008	PBW62/2*PASTOR	2008
39	Lasani-2008	LUAN/KOH-97	2008
40	Miraj-08	SPARROW/INIA//V.7394/WL 711//3/BAU'S'	2008

All 40 cultivars were officially released for general cultivation in Punjab (Pakistan)

Table 2 Contrast analysis for variation in plant parameters with respect to the year of cultivar release

Parameter	Mean ± SD Min–Max	Year group means[a] I	II	III	IV	[b]Contrast i: −1, 0, 0, 1 % Increase (+) or % Decrease (−) (P)[c]	Contrast ii: 0, −1, 0, 1	Contrast iii: 0, 0, −1, 1	Contrast iv: −1, −1, −1, 3
Grain yield (t ha^{-1})	3.5 ± 0.5 2.6–4.6	3.36	3.17	3.73	3.90	+15.9 (< 0.001)	+23.0 (< 0.001)	+4.5 (0.456)	+13.9 (< 0.001)
Straw yield (t ha^{-1})	5.2 ± 0.7 3.8–6.4	5.40	5.21	4.88	5.20	−3.9 (0.644)	−0.2 (1.000)	+6.6 (0.291)	+0.7 (0.994)
Harvest index	0.41 ± 0.04 0.31–0.47	0.385	0.380	0.434	0.428	+11.2 (<0.001)	+12.8 (<0.001)	−1.4 (0.930)	+7.2 (0.001)
Grain weight (mg $kernel^{-1}$)	30 ± 6 22–42	27.7	28.2	30.1	34.1	+23.2 (<0.001)	+20.8 (<0.001)	+13.4 (0.0276)	+19.0 (<0.001)
Plant height (cm)	97 ± 8 82–116	98	105	93	93	−5.5 (0.028)	−13.2 (<0.001)	−0.4 (0.997)	−6.1 (0.001)
Grain Zn (μg g^{-1})	29 ± 3 24–36	32.2	29.8	27.3	27.3	−18.0 (<0.001)	−9.3 (0.001)	−0.1 (1.000)	−9.1 (<0.001)
Grain Fe (μg g^{-1})	38 ± 4 32–46	39.6	38.9	37.9	37.1	−6.6 (0.064)	−5.0 (0.291)	−2.2 (0.856)	−4.6 (0.162)
Grain Ca (μg g^{-1})	316 ± 39 230–405	343	324	292	401	−13.9 (<0.001)	−7.7 (0.088)	+3.0 (0.815)	−6.2 (0.076)
Grain phytate (mg g^{-1})	9 ± 1 7–11	9.40	8.72	8.45	8.75	−7.4 (0.047)	+0.3 (0.999)	+3.5 (0.613)	−1.2 (0.967)
Grain phytate: Zn ratio	27 ± 3 22–34	26.2	26.0	27.7	28.7	+9.3 (0.002)	+10.1 (0.001)	+3.5 (0.495)	+7.6 (0.002)
Grain phytate: Fe ratio	20 ± 2 14–24	20.3	19.1	19.0	20.0	−1.5 (0.949)	+4.6 (0.515)	+4.9 (0.588)	+2.6 (0.806)
Grain phytate: Ca ratio	1.7 ± 0.2 1.2–2.3	1.68	1.63	1.76	1.80	+6.9 (0.190)	+10.1 (0.038)	+1.8 (0.954)	+6.2 (0.138)

[a] Cultivar year groups included: (I) Green Revolution (released during 1965–1976, $n = 11$), (II) post-Green Revolution (released during 1977–1988, $n = 9$), (III) recently discontinued (released during 1989–2000, $n = 9$) and (IV) current-cultivars (released during 2001–2008, $n = 11$) of Pakistani bread wheat

[b] The four coefficients of each contrast are for the year group means from one to four. Cultivar group IV was compared with cultivar group I (contrast i), with cultivar group II (contrast ii), with cultivar group III (contrast iii), and with rest of the groups (II, III and III) (contrast iv)

[c] Probability (P) was calculated for the year group means on the basis of Scheffe's F-value

indicated that mean grain yield was 14% greater in current-cultivars than all the other cultivars. The mean difference in straw yield between the current-cultivars and other three cultivar groups was non-significant, although plant height of the current-cultivars was 6% less ($P = 0.001$) than in all other cultivars. Harvest index and grain weight were respectively 7.2 and 19% greater in the current-cultivars compared to all the other cultivars. Differences in yield and yield components were most obvious between current-cultivars and Green Revolution to post-Green Revolution cultivars.

In the selected 40 cultivars, grain Zn concentration ranged from 24 to 36 μg g^{-1}, with an average of 29 μg g^{-1} (Table 2). Mean grain Zn concentrations in the current-cultivars and Green Revolution cultivars were respectively 27 and 32 μg g^{-1}, with 18% lower grain Zn concentration in the current-cultivars. Grain concentrations of Ca ranged from 230 to 405 μg g^{-1} and of Fe 32 to 46 μg g^{-1}. The mean grain Ca concentration in the current-cultivars was 14% lower than in Green Revolution cultivars. However, grain Fe concentration in the current-cultivars was non-significantly different than the mean of all other cultivars.

Bioavailability of grain minerals

Mean phytate concentration in grains of 40 wheat cultivars was 9 mg g^{-1} and ranged from 7 to 11 mg g^{-1} (Table 2). Mean grain phytate concentration in the four groups of wheat cultivars ranged from 8.5 to 9.4 mg g^{-1}, maximum being in Green Revolution cultivars. Mean phytate in current-cultivars was significantly ($P = 0.047$) lower than in Green Revolution cultivars.

The mean phytate:Zn ratio in grains of 40 cultivars was 27 and ranged from 22 to 34 (Table 2). On average, the grain phytate:Zn ratio was 7.6% greater in current-cultivars than all the other cultivars. The mean phytate:Fe and phytate:Ca ratios in wheat grains were respectively 20 and 1.7. The phytate:Fe ratio in wheat grains ranged from 14 to 24; however, there were non-significant differences between the current-cultivars and groups of others cultivars. The mean grain phytate:Ca ratio was significantly ($P = 0.038$) greater in currently-cultivated than post-Green Revolution cultivars (released during 1977–1988).

Relationships among measured parameters in 40 cultivars

Grain yield (t ha^{-1}) of the selected 40 cultivars was significantly and positively related with grain weight ($r = 0.57$) and harvest index ($r = 0.66$) (Table 3). There were significant positive relationships between the year of cultivar release and grain yield ($r = 50$), harvest index ($r = 0.53$) and grain weight ($r = 0.45$). Both grain yield ($r = -0.34$) and grain weight ($r = -0.35$) had a negative relationship with the number of grains/spike. Plant height had a significant negative relationship with the year of cultivar release ($r = -0.41$), harvest index ($r = -0.61$) and grain yield ($r = -0.44$).

Concentrations of Fe and Ca in straw were positively but non-significantly, related to their respective concentrations in wheat grains (Table 3). However, straw Zn concentration had a significant positive relationship with grain Zn concentration ($r = 0.51$). Grain yield had a strong negative relationship with grain concentrations of Zn and Ca ($r = -0.49$ for both Zn and Ca). Grain weight was an important variable that negatively correlated with grain concentrations of Zn ($r = -0.44$), Fe ($r = -0.29$) and Ca ($r = -0.61$). Grain mineral concentrations were also negatively related to the year of cultivar release in Punjab; however, the relationship was significant only for Zn ($r = -0.67$) and Ca ($r = -0.52$) concentrations. Moreover, a strong positive relationship (at least $r = 0.39$) existed among the concentrations of three minerals (Zn, Fe and Ca) in wheat grains.

Phytate concentration in grains of 40 wheat cultivars was negatively correlated with grain yield ($r = -0.37$) and grain weight ($r = -0.26$), but the relationship was only significant for grain yield (Table 3). Moreover, grain phytate concentration was positively and significantly correlated with grain Zn ($r = 0.60$), Fe ($r = 0.38$) and Ca ($r = 0.44$) concentrations. The grain phytate:Zn ($r = 0.44$) and phytate:Ca ratios ($r = 0.31$) were significantly and positively related to the year of cultivar release. However, the relationship was non-significant for phytate:Fe ratio in wheat grains. The phytate:mineral ratio had a significant negative relationship with the number of grains/spike and a non-significant relationship with grain yield.

Table 3 Relationship (Pearson correlation coefficients, *r*) among various parameters of 40 bread wheat cultivars of Punjab (Pakistan)

Parameters				(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	
(1)	Grain yield			0.01	−0.11	0.20	−0.13	−0.15	−0.02	−0.32	−0.22	(12)
(2)	Straw yield	0.15			0.39	0.59	0.60	−0.43	0.25	−0.07	−0.67	(13)
(3)	Harvest index	0.66	−0.64			0.43	0.38	−0.01	−0.46	−0.13	−0.27	(14)
(4)	Grain weight	0.57	−0.16	0.55			0.44	−0.14	0.05	−0.64	−0.52	(15)
(5)	Grains/spike	−0.34	−0.26	−0.07	−0.35			0.46	0.64	0.39	−0.27	(16)
(6)	Grains/unit area	0.20	0.29	−0.05	−0.68	0.12			0.45	0.52	0.44	(17)
(7)	Tillers/unit area	0.37	0.41	0.00	−0.29	−0.63	0.68			0.49	−0.05	(18)
(8)	Spike length	−0.27	0.17	−0.35	−0.35	0.47	0.17	−0.21			0.31	(19)
(9)	Plant height	−0.44	0.35	−0.61	−0.22	0.10	−0.17	−0.18	0.24			
(10)	Straw Zn concentration	−0.41	0.14	−0.42	−0.45	0.05	0.20	0.14	−0.14	0.09		
(11)	Straw Fe concentration	−0.14	0.03	−0.13	−0.09	0.06	0.03	0.00	−0.04	−0.07	0.56	
(12)	Straw Ca concentration	0.18	0.09	0.08	0.02	0.00	0.15	0.17	0.04	−0.24	0.38	0.60
(13)	Grain Zn concentration	−0.49	0.31	−0.61	−0.44	0.09	0.10	0.03	0.05	0.40	0.52	0.14
(14)	Grain Fe concentration	−0.26	0.15	−0.32	−0.29	0.31	0.12	−0.13	0.11	0.13	0.20	0.08
(15)	Grain Ca concentration	−0.49	0.41	−0.68	−0.61	0.26	0.29	0.06	0.37	0.37	0.50	0.22
(16)	Grain phytate concentration	−0.37	0.40	−0.59	−0.26	−0.24	−0.01	0.13	−0.07	0.28	0.40	0.09
(17)	Grain phytate:Zn ratio	0.14	0.11	0.01	0.20	−0.38	−0.12	0.13	−0.14	−0.15	−0.12	−0.07
(18)	Grain phytate:Fe ratio	−0.13	0.25	−0.29	0.00	−0.49	−0.11	0.24	−0.16	0.15	0.22	0.03
(19)	Grain phytate:Ca ratio	0.23	−0.02	0.18	0.42	−0.47	−0.28	0.07	−0.45	−0.12	−0.16	−0.12
(20)	Cultivar release year	0.50	−0.19	0.53	0.45	−0.13	−0.08	0.00	−0.25	−0.41	−0.39	−0.06
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)

$n = 40$, $r_{(0.05)} = 0.31$, $r_{(0.01)} = 0.40$

Characterizing cultivars for increased bioavailability of minerals

Most current-cultivars had high yield and a high phytate:Zn ratio in grains (Fig. 1a). The highest grain yield was produced by Lasani-2008; however, the grain phytate:Zn ratio of this cultivar was also higher than the average of the 40 cultivars. Wheat cultivars such as Punjab-81 and MexiPak-65 had the lowest phytate:Zn ratio in grains; however, their yield potential was also low. Bhakkar-2002 was dominant wheat cultivar that had optimum grain yield and a low phytate:Zn ratio in grains.

The minimum phytate:Fe ratio in grains was in Kohinoor-83 and maximum was in SA-42 (Fig. 1b). Cultivars such as Bhakkar-2002, Rothas-90 and Yecora-70 were relatively desirable for increased grain yields and a low phytate:Fe ratio in wheat grains. Bhakkar-2002 also had a low phytate:Ca ratio in grains (Fig. 1c). The highest grain phytate:Ca ratio was in Lasani-2008, which also produced the highest grain yield. The minimum grain phytate:Ca ratio was in the oldest cultivar tested, MaxiPak-65.

Discussion

Increased grain yield and resistance to diseases have been the prime objectives of wheat breeding in Punjab (Pakistan). Therefore, current-cultivars have a higher yield potential than obsolete cultivars released during 1965–2000. Much of this appears to have been achieved through increased grain weight that also resulted in increased harvest index in the current-cultivars of bread wheat. Some authors reported very weak to no relationship between grain weight and grain yield (McDonald et al. 2008; Sadras 2007). However, when cultivars were selected on the basis of release year, grain weight was strongly related with grain yield (Morgounov et al. 2010; Zhao et al.

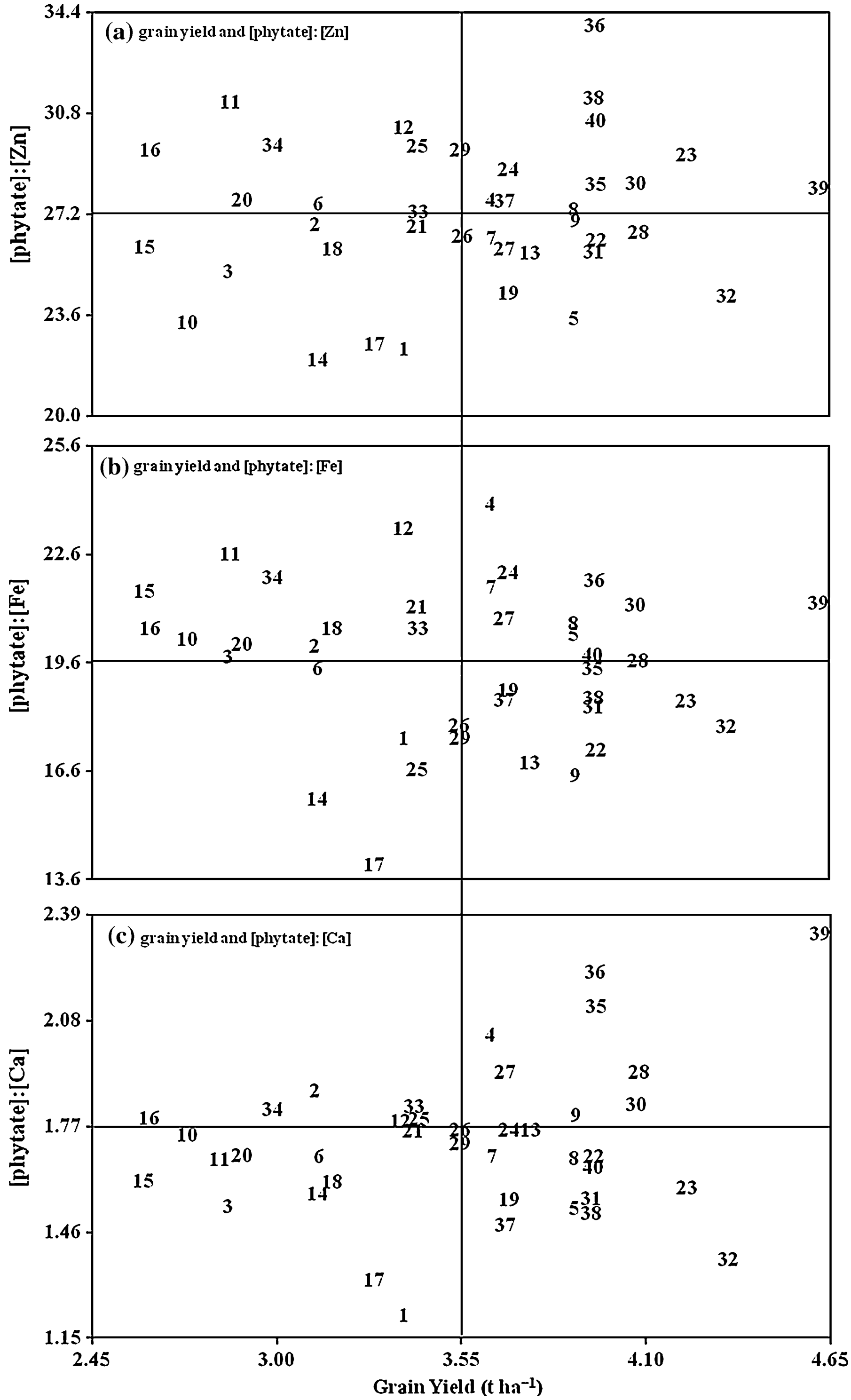

Fig. 1 Categorizing wheat cultivars for grain yield and grain phytate:mineral ratios: **a** grain yield against grain phytate:Zn ratio; **b** grain yield against grain phytate:Fe ratio; **c** grain yield against grain phytate:Ca ratio. Numbers indicate wheat cultivars released in Punjab during 1965–2008, with the oldest cultivar numbered first and the newest cultivar numbered last (as listed in Table 1)

2009), a result similar to the present study (Table 3). Morgounov et al. (2010) also reported that the number of grains/unit area is an important parameter that significantly contributed to high grain yields of new cultivars. In the present study on cultivars released in Punjab (Pakistan), however, the number of grains/unit area was only weakly related with grain yield ($r = 0.20$) (Table 3). On the other hand, number of grains/spike had a negative relationship ($r = -0.34$) with grain yield of tested 40 cultivars. This unexpected negative correlation might have been due to a deliberate selection strategy by Punjab breeders for higher grain weights over past 50 years.

The reported range of mineral concentrations in grains was similar to previous investigations on bread wheat genotypes (Hussain et al. 2011; Joshi et al. 2010; Liu et al. 2006; Murphy et al. 2008); however, Ca in wheat grains was low as compared to the old literature (Graham et al. 1999). A wide variation in grain concentrations of Zn, Fe and Ca in wheat cultivars (Table 2) can be exploited in breeding programs and in recommending cultivars for biofortification. The concentrations of Zn and Ca were negatively correlated with grain yield and the year of cultivar release (Table 3). A decrease in concentration of Zn (Hussain et al. 2011; Zhao et al. 2009) and other minerals (Fan et al. 2008) in new wheat cultivars is well established. Partially, this decreasing mineral density is due to a dilution effect caused by increased yields. McDonald et al. (2008) reported that about 30–57% of variation in grain Zn concentration was related to the difference in grain yield of wheat cultivars. A dilution effect arises if starchy endosperm of grains increases in size more than mineral-enriched embryo and bran. Zhao et al. (2009) reported that an increase in grain yield in new cultivars resulted in a significant decrease in bran yield. Our results also indicated that an increase in grain weight negatively correlated with mineral concentrations in grains of wheat cultivars (Table 3), suggesting a dilution effect.

Liu et al. (2006) reported that grain mineral concentrations were negatively related to the number of grains/spike. In contrast, the number of grains/spike in the present study was positively related to grain concentrations of all three minerals of interest (Zn, Ca and Fe) (Table 3). However, this relationship was significant only for Fe concentration.

The range of phytate concentration, 7.0–11.3 mg g^{-1}, in the present study (Table 2) is similar to the ranges reported in previous studies on bread wheat cultivars (Erdal et al. 2002; Hussain et al. 2011), albeit little higher than those reported by Liu et al. (2006). There was a positive correlation among mineral concentrations in wheat grains ($r = 0.39$ or higher) (Table 3), indicating that breeding for one mineral can also result in higher concentrations of other minerals (see also White and Broadley 2009). However, grain phytate concentration had a strong positive relationship with mineral concentrations in wheat grains ($r = 0.38$ or higher) (Table 3). Similar to the dilution of grain minerals, this might be due to dilution of grain phytate by high grain yield ($r = -0.37$) and grain weight ($r = -0.27$) in new cultivars (Table 3). Moreover, it is partially due to minerals being bound to phytate. Hence, breeding just for increased mineral density may not greatly improve human nutrition; rather, the focus should be on increasing mineral bioavailability by breeding for a lower phytate:mineral ratio in wheat grains.

The bioavailability is considered to be greatly reduced when food has phytate:Ca ratio > 0.24 (Morris and Ellis 1985), phytate:Fe ratio > 1 (Hallberg et al. 1989) and phytate:Zn ratio > 15 (Turnlund et al. 1984). In the present study, the phytate:mineral ratios for all three minerals in grains of all 40 cultivars were greater than the critical values mentioned above (Table 2), indicating a poor bioavailability of these important minerals from wheat grains. The phytate:mineral ratios in wheat grains were non-significantly correlated with grain yield (Table 3). As cultivars with high yields and low phytate:mineral ratios are desirable in fighting hunger and mineral malnutrition, new cultivars suitable for biofortification should be bred for high yield and high mineral bioavailability.

Plotting grain yield against phytate:mineral ratios in wheat grains clearly indicated that the tested cultivars varied widely in mineral bioavailability (Fig. 1). The cultivar Sehar-2006 that currently dominates in acreage in Punjab produced above-average grain yield with mineral bioavailability being almost the same as the average of 40 cultivars. The newest cultivar Lasani-2008 had the highest grain yield and the highest phytate:Ca ratio in grains. Cultivars with relatively high grain yield and low phytate:mineral ratios in grains, such as Bhakkar-2002, should be preferred for general cultivation and for development of new genotypes.

Conclusion

The current-cultivars produced higher grain yields but had lower concentrations and bioavailabilities of

Zn and Ca than the obsolete cultivars. Much of this variation was related to an increased grain weight in new cultivars. The grain phytate concentration had a positive relationship with grain yield and mineral concentrations in the 40 cultivars. Compared to obsolete cultivars, the current-cultivars had higher phytate:mineral ratios in grains, indicating a poor bioavailability of minerals to humans. However, there was a non-significant relationship between grain yield and phytate:mineral ratios in wheat grains. Therefore, future breeding of genotypes for mineral biofortification should be focused on high mineral bioavailability.

Acknowledgments The research project was financially supported by Higher Education Commission of Pakistan through Indigenous PhD Fellowship Program. We also acknowledge the kind cooperation of Dr. Mukhdoom Hussain, director, and Mr. Mujahid Hussain Qureshi, botanist, at Wheat Research Institute, Faisalabad (Pakistan).

References

Allison LE, Moodie CD (1965) Carbonate. In: Black CA (ed) Methods of soil analysis, part 2: chemical and microbiological properties. Am Soc Agron, Madison, pp 1379–1396

Bouis HE, Welch RM (2010) Biofortification: a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Sci 50:S20–S32

Brown KH, Wuehler SE, Peerson JM (2001) The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. Food Nutr Bull 22:113–125

Cakmak I (2008) Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant Soil 302:1–17

Erdal I, Yilmaz A, Taban S, Eker S, Torun B, Cakmak I (2002) Phytic acid and phosphorus concentrations in seeds of wheat cultivars grown with and without zinc fertilization. J Plant Nutr 25:113–127

Fan MS, Zhao FJ, Fairweather-Tait SJ, Poulton PR, Dunham SJ, McGrath SP (2008) Evidence of decreasing mineral density in wheat grain over the last 160 years. J Trace Elem Med Biol 22:315–324

FAO (2011a) Food Supply Database 2007. Food and Agriculture Organization. http://faostat.fao.org/site/609/default.aspx#ancor. Accessed 16 Jul 2011

FAO (2011b) Crop Production Database 2009. Food and Agriculture Organization. http://faostat.fao.org/site/567/default.aspx#ancor. Accessed 16 Jul 2011

Fatima M, Nawaz H, Kassi M, Rehman R, Kasi PM, Kassi M, Afghan AK, Baloch SN (2009) Determining the risk factors and prevalence of osteoporosis using quantitative ultrasonography in Pakistani adult women. Singapore Med J 50:20–28

Ficco DBM, Riefolo C, Nicastro G, Simone VD, Gesu AMD, Beleggia R, Platani C (2009) Phytate and mineral elements concentration in a collection of Italian durum wheat cultivars. Field Crop Res 111:235–242

Gee GW, Bauder JW (1986) Particle–size analysis. In: Page AL (ed) Methods of soil analysis. part 1: physical and mineralogical methods, 2nd edn, agronomy monograph, vol 9. Am Soc Agron, Madison, pp 383–409

Gibson RS (2006) Zinc: the missing link in combating micronutrient malnutrition in developing countries. Proc Nutr Soc 65:51–60

Graham R, Senadhira D, Beebe S, Iglesias C, Monasterio I (1999) Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. Field Crops Res 60:57–80

Hallberg L, Brune M, Rossander L (1989) Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. Am J Clin Nutr 49:140–144

Haug W, Lantzsch H (1983) Sensitive method for the rapid determination of phytate in cereals and cereal products. J Sci Food Agric 34:1423–1424

Hussain S, Maqsood MA, Rahmatullah (2010) Increasing grain zinc and yield of wheat for the developing world: a review. Em J Food Agric 22:326–339

Hussain S, Maqsood MA, Miller LV (2011) Bioavailable zinc in grains of bread wheat varieties of Pakistan. Cereal Res Commun. doi:10.1556/CRC.2011.003

Jones JRJ, Case VW (1990) Sampling, handling, and analysing plant tissue samples. In: Westerman RL (ed) Soil testing and plant analysis. Soil Sci Soc Am, Madison, pp 389–428

Joshi AK, Crossa J, Arun B, Chand R, Trethowan R, Vargas M, Ortiz-Monasterio I (2010) Genotype × environment interaction for zinc and iron concentration of wheat grain in eastern Gangetic plains of India. Field Crops Res 116:268–277

Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Sci Soc Am J 42:421–428

Liu ZH, Wang HY, Wang XE, Zhang GP, Chen PD, Liu DJ (2006) Genotypic and spike positional difference in grain phytase activity, phytate, inorganic phosphorus, iron and zinc contents in wheat (*Triticum aestivum* L.). J Cereal Sci 44:212–219

McDonald GK, Genc Y, Graham RD (2008) A simple method to evaluate genetic variation in grain zinc concentration by correcting for differences in grain yield. Plant Soil 306:49–55

MINFAL (2009) Agricultural statistics of Pakistan 2008–09. Ministry of Food Agriculture Livestock, Islamabad

MINH (2009) National health policy 2009: stepping towards better health. Ministry of Health, Islamabad

Morgounov A, Zykin V, Belan I, Roseeva L, Zelenskiy Y (2010) Gomez-Becerra HF, Budak H, Bekes F, Genetic gains for grain yield in high latitude spring wheat grown in Western Siberia in 1900–2008. Field Crops Res 117:101–112

Morris ER, Ellis R (1985) Bioavailability of dietary calcium-effect of phytate on adult men consuming nonvegetarian diets. In: Kies C (ed) American chemical society symposium 275: nutritional bioavailability of calcium. Am Chem Soc, Wagenington, p 63

Morris CE, Sands DC (2006) The breeder's dilemma-yield or nutrition? Nature Biotech 24:1078–1080

Murphy KM, Reeves PG, Jones SS (2008) Relationship between yield and mineral nutrient concentrations in historical and modern spring wheat cultivars. Euphytica 163:381–390

Nelson DW, Sommers LE (1982) Total carbon, organic carbon and organic matter. In: Klute A (ed) Methods of soil analysis, part 2: chemical and microbiological properties. agronomy monograph, vol 9. Soil Sci Soc Am, Madison, pp 570–571

Rengel Z, Römheld V (2000) Differential tolerance to Fe and Zn deficiencies in wheat germplasm. Euphytica 113:219–225

Sadras VO (2007) Evolutionary aspects of the trade-off between seed size and seed number in crops. Field Crops Res 100:125–138

Steel RGD, Torrie JH, Dickey DA (1997) Principles and procedures of statistics. a bio-metrical approach, 3rd edn. McGraw Hill Book Co., Inc., New York

Stein AJ, Nestel P, Meenakshi JV, Qaim M, Sachdev HPS, Bhutta ZA (2007) Plant breeding to control zinc deficiency in India: how cost-effective is biofortification? Pub Health Nutr 10:492–501

Turnlund JR, King JC, Keyes WR, Gong B, Michel MC (1984) A stable isotope study of zinc absorption in young men: effects on phytate and α-cellulose. Am J Clin Nutr 40: 1071–1077

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science 314:1298–1300

Wang Z-H, Li S-X, Malhi S (2008) Effects of fertilization and other agronomic measures on nutritional quality of crops. J Sci Food Agric 88:7–23

Waters BM, Uauy C, Dubcovsky J, Grusak MA (2009) Wheat (*Triticum aestivum*) NAM proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. J Exp Bot 60:4263–4274

Weaver CM, Kannan S (2002) Phytate and mineral bioavailability. In: Reddy NR, Sathe SK (eds) Food Phytate. CRC Press, Boca Raton, pp 211–223

White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets—iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol 182:49–84

WHO (2002) World Health Report 2002: Reducing Risks, Promoting Healthy Life. World Health Organization, Geneva

Zhao FJ, Su YH, Dunham SJ, Rakszegi M, Bedo Z, McGrath SP, Shewry PR (2009) Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. J Cereal Sci 49:290–295