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Phytate and mineral elements concentration in a collection of Italian durum wheat cultivars

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ABSTRACT

Mineral deficiencies are prevalent in human populations and the improvement of the mineral content in cereal products represents a possible strategy to increase the human mineral intake. Nevertheless, most of the inorganic phosphorus (P_i) present in mature cereal seeds (40–80%) is stored as phytate, an anti-nutritional factor that forms complexes with minerals such as Ca, Mg, Zn and Fe reducing their bioavailability. The present study was undertaken: (i) to determine the variation in phytate and mineral concentrations in the whole grains of 84 Italian durum wheat (*Triticum durum* Desf.) cultivars representative of old and modern germplasm; (ii) to estimate the magnitude of genotype × environment interaction effects; and (iii) to examine the interrelationships among mineral concentrations in durum wheat with the final aim to identify superior durum wheat cultivars that possess low phytate content and high concentration of mineral elements in their whole-wheat flour. The cultivars were grown in field trials during 2004–2005 at Foggia, Italy and during 2005–2006 at Foggia and Fiorenzuola d'Arda—Southern and Northern Italy. The phytate content was estimated indirectly by using a microtitre plate assay evaluating the P_i absorbance at 820 nm, while the Cu, Fe, Mn, Ca, K, Mg, Na and Zn mineral contents were determined by ICP/OES. The contents of Zn and Fe across years and locations ranged from 28.5 to 46.3 mg/kg for Zn with an average of 37.4 mg/kg and from 33.6 to 65.6 mg/kg for Fe with an average of 49.6 mg/kg. P_i grain content was between 0.46 and 0.76 mg/g showing a positive correlation with all minerals except Cu and Zn. Although breeding activity for Fe and Zn would be difficult because G × E interaction is prevalent, multi-location evaluation of germplasm collection help to identify superior genotypes to achieve this objective. The results here reported open the possibility of designing a specific breeding program for improving the nutritional value of durum wheat through the identification of parental lines with low-P_i and high minerals concentration in whole grains.

1. Introduction

Durum wheat (*Triticum durum* Desf.) is an important cereal crop whose grain is predominantly used for pasta making, even if a part of the harvest is devoted to bread preparation. It is extensively cultivated in Mediterranean areas with a worldwide production of about 30 million tons. Although it contributes most significantly as a source of carbohydrate and, to a less extent, of protein, its potential contribution of micronutrients in human diets has led to a renewed interest in whole meal durum-based products. Mineral elements, in fact, are mainly present in the aleurone layer of wheat grains (Buri et al., 2004) and whole meal products have been suggested to have an important role in maintaining good health (Slavin, 2004). Unfortunately, the external grain wheat layer can also contain significant amounts of anti-nutrients, such as phytate and certain fibers (Guttieri et al., 2006). In humans, absorption of Ca (Kies, 1985; Lonnerdal et al., 1989), Fe (Hallberg et al., 1989; Larsson et al., 1996) and Zn (Sandström and Lönnerdal, 1989) can be decreased significantly from diets high in phytate. It is an established fact that most of the minerals present in wheat kernels are complexed with phytic acid (phytins) and, ultimately not nutritionally available. Therefore, breeding strategies for improving the nutritional value of cereal products should address the selection of wheat varieties with high mineral density and low phytate content in mature seeds. Because phytate plays an important role during grain germination and early seedling growth (Welch, 1986), research should verify that reducing phytate levels will not adversely impact on crop production (Graham et al., 1999). Recently, Liu et al. (2006) have studied the effect of wheat genotype on grain phytase activity, phytate,

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inorganic P, Fe and Zn content reporting a strong correlation between phytate and inorganic P. Analysis of P_i in grains is a quick, sensitive and inexpensive test for grain phytate levels, a finding essential to make plant breeding practical (Raboy et al., 2000).

In addition to the nutritional quality improvement, breeding for higher mineral density in seeds will not incur a yield penalty. In fact, bio-fortification may have important spin-off effects for increasing plant productivity in an environmentally beneficial way. As recent researches have shown, minerals are essential in helping the plants to better resist the diseases (Graham and Rovina, 1984; Sparrow and Graham, 1988; Graham and Webb, 1991; Thongbai et al., 1993) and other environmental stresses (Foy, 1992; Lynch, 1995). Micronutrient (dense) seeds are associated with greater seedling vigour, which in turn is associated with higher plant yield (Faridi et al., 1982; Flynn et al., 1987; Graham, 1991; Rengel and Graham, 1995). All this could contribute to reduce the need for fertilizers, fungicides and irrigation.

Success in crop improvement through breeding depends on the existence of genetic variation for the target traits in the available gene pool. During the last decades an important project (http://www.harvestplus.org) has been started to assess the available genetic variability for grain mineral elements that could be explored in future wheat breeding programs (reviewed by Bouis, 1996; Cakmak et al., 1996; Anglani, 1998; Graham et al., 1999, 2001; Gregorio, 2002; Welch and Graham, 2002, 2004; White and Broadley, 2005). At CIMMYT, more than 3000 GenBank accessions, including hexaploid, tetraploid, and diploid wheats have been screened for Fe and Zn contents (Monasterio and Graham, 2000). Several studies have suggested that the most promising materials, in order of importance, are wild relatives and primitive cultivated wheats, landraces, bread wheat, durum wheat, and triticale and the nutrients emphasized were Fe, Zn, and vitamin A (β-carotene) (Feil and Fossati, 1995; Bouis, 1996; Graham et al., 1999; Cakmak et al., 2000, 2004; Chhuneja et al., 2006; Ozkan et al., 2007). Notably, most of these studies which dealt with macro- and micronutrient concentrations excluded modern tetraploid species such as durum wheat from comparisons, apart from a few cases involving a very small number of genotypes (Cakmak et al., 2000; Chhuneja et al., 2006).

Furthermore, to perform breeding for the mineral content, the availability of genetic variation is essential as well as knowledge about the genotype by environment (G × E) interaction as pointed out earlier by Peterson et al. (1986) and recently by Oury et al. (2006). To achieve new information on the genetic variability for mineral and phytate content in durum wheat, a study was undertaken: (i) to determine the variability in grain mineral cations (Ca, K, Mg, Mn, Na, Cu, Zn and Fe) and P_i concentrations in a collection of durum wheat genotypes representative of old and modern germplasm adapted to the Mediterranean conditions; (ii) to estimate the magnitude of genotype × environment interaction effects; and (iii) to examine the interrelationships among mineral concentrations in durum wheat.

2. Materials and methods

2.1. Genotypes and field experiments

A historical set of 84 Italian durum wheat cultivars (Table 1) belonging to three groups were studied: (1) "old genotypes" includes 10 entries commonly grown in Italy from 1900 until 1973; (2) "modern genotypes" contains 58 cultivars released after 1974 and carrying the *Rht* genes; and (3) "advanced breeding lines" comprises 17 breeding lines with high yield potential selected at the CRA—Centre for Cereal Research of Foggia, at the Experimental Institute for Cereal Research of Catania and at the Department of Environmental and Agro-Forestry Biology and Chemistry of the University of Bari (acronyms "L", "CTA" and "BIL", respectively). This classification was adopted to highlight the effects of the breeding progress on mineral contents. The experiments were carried out over two growing seasons (2004–2005 and 2005–2006) at Foggia, Italy (41°28′N, 15°32′E and 75 m a.s.l.) on a clay-loam soil (Typic Chromoxerert) with an average organic matter 1.97% and a water pH 7.7, where the climate is warm, sunny and dry during the spring, with a rainfall of 455 mm and 548 mm in the first and second growing season, respectively. A third experiment was carried out during 2005–2006 at Fiorenzuola d'Arda, PC, Italy (44°56′N, 12°27′E and 80 m a.s.l.) on a vertisol (Typic Calcixerert) with an average organic matter content of 2.01% and a water pH 7.9 and characterized by an average annual rainfall of 750 mm. The mineral soil contents measured in three samples at 0–30 cm collected after sowing in 2005 are reported in Table 3. The experimental design was a randomized complete block with three replications. Each experimental unit consisted of a 10.2 m^2 plot (eight rows 7.5 m long with 0.17 m between rows). Seedling density was 350 seeds m^{-2} at Foggia and 550 seeds m^{-2} at Fiorenzuola d'Arda. The sowing dates were 14 December 2004 and 22 December 2005 at Foggia and 21 December 2005 at Fiorenzuola d'Arda. The previous crop was durum wheat at Foggia and barley at Fiorenzuola d'Arda. Fertilizer applications were made at pre-sowing (36 kg ha^{-1} N and 40.5 kg ha^{-1} P as ammonium biphosphate) and top dressing (52 kg ha^{-1} N as urea) at Zadoks growth stage 2.2 and 3.1 (Zadoks et al., 1974), with a further N application (52 kg ha^{-1} N as ammonium nitrate) at Fiorenzuola d'Arda at Zadoks growth stage 3.4. Weeds within the growing season were controlled with the following herbicides: Tralcossidim (1.7 l ha^{-1}) + Clopiralid + MCPA + Fluroxypyr (2.0–2.5 l ha^{-1}). Trials at Foggia were harvested on 3 June 2005 and 4 June 2006 and at Fiorenzuola d'Arda on 3 July 2006. After harvesting the grains were cleaned and analyzed.

Table 1
List of the genotypes analysed in the study classified according to the breeding period as old, modern or advanced.

"Old" Landraces and genealogical selections (before 1910–1973)
Aziziah, Cannizzara, Capeiti8, Cappelli, Grifoni235, Matarese, Russello, Taganrog, Timilia, Trinakria
"Modern" Cultivars with Rht genes (1974–2000)
Adamello, Arcobaleno, Bradano, Brindur, Bronte, Ciccio, Cirillo, Claudio, Colorado, Colosseo, Cosmodur, Creso, Duilio, Dupri, Fortore, Gargano, Gianni, Giotto, Giusto, Grazia, Iride, Italo, Karel, Latino, Lesina, Marco, Messapia, Nefer, Neodur, Ofanto, Parsifal, Platani, Preco, Primadur, Produra, Polesine, Quadrato, Radioso, Rusticano, S. Carlo, Saadi, Sansone, Simeto, Solex, Svevo, Tiziana, Torrebianca, Tresor, Valforte, Valgerardo, Valnova, Varano, Verdi, Vesuvio, Vetrodur, Vitromax, Zenit.
"Advanced" Breeding lines (2001–)
5BIL-28, 5BIL-46, 5BIL-85, 5BIL-90, CTA432, CTA440, CTA478, CTA491, CTA503, CTA529, L102, L29, L38, L83, L89, L91, L95.

2.2. Grain sampling and mineral determination

Whole plots were harvested mechanically each year and grain yield (GY) determined at 13% moisture content. Thousand kernel weight (TKW) was calculated as the mean weight of three sets of 100 grains per plot. Approximately 100 g of grain was sampled from each field plot and stored at 4 °C. Grain was crushed in an experimental mill (Tecator Cyclotec 1093) to determine grain inorganic phosphorus (P_i) using the colorimetric method of Chen et al. (1956), modified for use on a microtiter plate as an indirect measure of phytic acid (Raboy, 1990). Dried samples were weighed (an average of 10-kernels) and extracted overnight in 10 μl 0.4N HCl mg^{-1} (approximate seed weight) at 4 °C. Following centrifugation (10,000 × g, 20 min, 4 °C), 10 μl of each supernatant

(extract) was mixed with 90 μl of distilled water, 100 μl of colorimetric reagent [1 volume 6N H_2SO_4, 1 volume 2.5% (w/v) ammonium molybdate, 1 volume 10% (v/v) ascorbic acid (daily prepared and used), and 2 volumes distilled water] made fresh daily in a microtiter plate. Assays were incubated at room temperature for 1 h, and the seed extracts were visually scored for the presence or absence of high inorganic phosphorus. Each microtiter plate included five P standards made by appropriate dilutions of 1 mM K_2HPO_4 to achieve (I) 0.0 μg P; (II) 0.15 μg P; (III) 0.46 μg P; (IV) 0.93 μg P; (V) 1.39 μg P.

Mineral element concentration (Ca, K, Mg, Mn, Na Cu, Fe, and Zn) of whole meal was determined on a Varian Vista-MPX simultaneous ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) with CCD detection (Ryan, 2005). The whole meal wheat was prepared by accurately weighing 0.5 g of sample into Teflon® PFA lined microwave digestion vessels and adding 10 ml 10 M HNO_3 (Merck Tracepur). Following digestion, the solutions were allowed to cool, transferred to 50 ml volumetric flasks, and diluted to volume with >18 MΩ/cm^3 de-ionised water. Digestions were carried out in duplicate. To avoid contamination from airborne particulates, cross contamination from sample to sample through spattering and the potential loss of volatile analytes from open acid digestions, a closed-vessel microwave sample digestion was used instead of the conventional open-vessel preparation. A closed-vessel digestion increases the pressure and effective temperature of the sample and hence increases the digestion efficiency, as well as ensuring that volatile elements are not lost in the atmosphere. The microwave digestion program settings were defined as follow: stage 1, max power 600 W, ramp 3 min, temperature 120 °C, pressure 350 PSI, time 5 min; stage 2, max power 600 W, ramp 15 min, temperature 200 °C, pressure 350 PSI, time 10 min. The reference technique (internal standardization) and ionization buffering were used throughout the analysis. All mineral determinations were compared to certified reference material (NIST SRM 8436 "Durum Wheat Flour on dry weight"). The moisture content was determined by oven drying samples of each material at 60 °C for 24 h and allowing the samples to cool in a desiccator for 4 h prior to re-weighing. Samples were prepared in duplicate and the analyses performed in triplicate.

2.3. Phytic acid analysis

Phytic acid values of 9 durum wheat genotypes selected to represent low (L83, Claudio and Ciccio), intermediate (Lesina, Arcobaleno and Vitromax), and high (Brindur, San Carlo and Trinakria) levels of P_i based on the colorimetric assay, were measured.

Seed samples were milled on a Tecator Cyclotec 1093 (International PBI, Milano, Italy) laboratory mill (1 mm screen-60 mesh) and the extraction of phytic acid was based on the Dost and Tokul procedure (Dost and Tokul, 2006): 0.2 g of dried samples were weighed in a 10 ml screw top centrifuge tube and extracted with 10 ml of 0.5 M HCl for 1 h at room temperature with continuous stirring. The samples were then centrifuged for 15 min at 4000 rpm and the supernatants were stored in 10 ml screw top bottles at −20 °C before high performance liquid chromatography (HPLC) analysis.

The HPLC analysis was performed with an Agilent Technologies 1100 HPLC system equipped with an automatic sampler and a diode array detector (DAD). The determination of phytic acid was based on metal replacement reaction of phytic acid from coloured complex of Fe(III)-thiocyanate, separating and monitoring any decrease in the concentration of the coloured complex. The Fe(III)-thiocyanate solution was prepared with 25 ml of 100 μg Fe(III)/ml, 25 ml of 500 μg ammonium thiocyanate/ml, 0.2 ml of concentrated HNO_3 and water up to 100 ml. Finally, 0.1 ml of sample extract was mixed with 0.9 ml of water and 2 ml of Fe(III)-thiocyanate complex solution. The mixture was stirred in 40 °C water bath for 2.5 h, then cooled at room temperature. After centrifugation for 5 min at 4000 × g, 50 μl of the supernatant were injected onto the HPLC system. Phytic acid concentration was calculated by using the calibration "curve". Samples were extracted and analysed in triplicate.

2.4. Statistical analysis

Data from this study were reported as mean ± standard deviation (SD). Results were subjected to a combined analysis of variance (ANOVA) using STATISTICA software (StatSoft version 7.1 StatSoft, Inc., Tulsa, OK, USA) where the sources of variation were represented by environments (E) defined as a site by year combination and genotypes (G) considered as fixed factors to test their interaction. Means were identified as being significantly different on the basis of Fischer's protected least significant differences (LSD) at a probability level of 5%. Principal component analysis (PCA) was performed on the correlation matrix, calculated on the mean data across replications. Pearson correlation coefficients were used to study the relationship among the mineral elements evaluated. P_i values of genotypes selected to represent low, intermediate, and high levels of inorganic P concentration were compared to phytic acid values measured by HPLC through simple linear regression. The correlation among genotype rankings for mineral elements for each pair of environments was estimated using Spearman's rank correlation analysis (Steel and Torrie, 1980) to obtain an estimate of a the part of the G × E interaction (the crossover interaction) as a measure of stability. The year of genotypes release was taken as a continuous quantitative variable and was used for a linear regression analysis to calculate the genetic gain for potential grain Fe and Zn concentrations.

3. Results and discussion

3.1. Variability in GY and mineral concentrations

Table 2 presents the descriptive statistics (mean, range and the coefficient of variations) for the traits evaluated (GY, TKW and mineral concentrations) from 84 durum wheat cultivars ranked in three groups, as reported in Table 1, and cultivated under the same environmental conditions. Substantial differences in GY were found between environments characterized by different pedo-climatic conditions and among the evaluated durum wheat groups, with higher yield of modern cultivars and advanced breeding lines when compared to old cultivars ($p < 0.001$). On the contrary, when the genotypes were evaluated for their mineral contents no clear trends were observed between modern genotypes and advanced breeding lines compared to old genotypes.

For some mineral elements, a direct effect of the soil content on grain content was observed. The average grain Na content at Foggia during both growing seasons was about two-fold higher than the corresponding values recorded at Fiorenzuola d'Arda reflecting the different Na soil levels in the two locations (Table 3). A similar result was observed for K and, while for all other minerals the soil content was quite similar in the two experimental sites. The only exception was represented by Mn, in spite of a higher level into the soil, a lower concentration in the grains was recorded at Fiorenzuola d'Arda compared to Foggia (Table 3).

The range in mineral contents within the whole set of 84 genotypes tested in this study was 33.6–65.6 mg/kg for Fe, 28.5–46.3 mg/kg for Zn, 0.46–0.76 mg/g for P_i, 5.8–14.0 mg/kg for Cu, 41.3–59.8 mg/kg for Mn, 19.2–37.7 mg/kg for Na, 1056–1535 mg/kg for Mg, 4061–5274 mg/kg for K and 388–640 mg/kg for Ca. Thus, within the tested genotypes, there were about two-fold

Table 2
Grain yield (GY), thousand kernel weight (TKW) and mineral content in the three groups of durum wheat cultivars grown in Italy.

	Old (n = 10)			Modern (n = 57)			Advanced (n = 17)		
	Mean ± SD	Range	cv (%)	Mean ± SD	Range	cv (%)	Mean ± SD	Range	cv (%)
GY (t/ha)	4.09 ± 0.35	3.67–4.55	8.4	5.21 ± 0.41	3.56–5.86	7.9	5.45 ± 0.33	5.03–6.26	6.0
TKW (g)	43.7 ± 4.6	35.0–48.9	10.6	45.1 ± 3.7	35.8–53.0	8.3	46.2 ± 3.5	39.0–50.8	7.6
P_i (mg/g)	0.62 ± 0.0	0.48–0.69	9.9	0.59 ± 0.0	0.47–0.76	9.1	0.55 ± 0.05	0.46–0.66	9.4
Ca (mg/kg)	507 ± 61	415–619	12.1	488 ± 49	406–639	10.0	470 ± 61	388–640	13
Cu (mg/kg)	7.4 ± 1.5	5.9–10.4	19.7	7.5 ± 1.4	5.8–14.0	18.4	7.1 ± 0.9	6.0–9.6	12.4
Fe (mg/kg)	46.6 ± 5.7	41.6–60.6	12.3	43.3 ± 5.8	33.6–65.6	13.5	42.5 ± 4.3	36.6–51.3	10.1
K (mg/kg)	4420 ± 294	4061–5023	6.6	4772 ± 223	4087–5274	4.7	4587 ± 326	4062–5208	7.1
Mg (mg/kg)	1337 ± 87	1248–1535	6.5	1171 ± 67	1056–1417	5.7	1169 ± 103	1056–1481	8.9
Mn (mg/kg)	49.3 ± 4.3	43.0–57.8	8.8	47.8 ± 3.7	41.3–57.4	7.8	47.7 ± 4.3	42.9–59.8	9.0
Na (mg/kg)	29.4 ± 5.1	21.6–36.9	17.3	26.1 ± 3.7	19.2–37.7	14.0	26.6 ± 4.4	21.6–33.8	16.7
Zn (mg/kg)	36.4 ± 2.3	33.7–41.4	6.3	33.9 ± 2.8	28.5–46.3	8.3	32.7 ± 2.8	29.1–40.9	8.7

n = number of genotypes.

Table 3
Grain yield (GY), thousand kernel weight (TKW) and mineral content for 84 genotypes recorded in 3 environments. Means and standard deviations (SD) for grains and soils.

	Grain			Soil	
	Foggia 2004–2005	Foggia 2005–2006	Fiorenzuola D'Arda 2005–2006	Foggia 2005–2006 (0–30 cm depth)	D'Arda 2005–2006 (0–30 cm depth)
GY (t/ha)	3.45 ± 0.5	3.92 ± 0.6	8.00 ± 0.9	-	-
TKW (g)	43.81 ± 4.2	38.82 ± 4.1	52.91 ± 5.1	-	-
P_i (mg/g)	0.59 ± 0.1	0.72 ± 0.1	0.45 ± 0.1	1.00 ± 0.0 g/kg[a]	0.90 ± 0.00 g/kg[a]
Ca (mg/kg)	526.97 ± 61.8	498.62 ± 52.1	434.70 ± 121.5	38.2 ± 1.4 g/kg	45.0 ± 0.9 g/kg
Cu (mg/kg)	9.17 ± 3.2	6.53 ± 0.9	6.62 ± 2.0	32.2 ± 0.3 mg/kg	33.5 ± 0.3 mg/kg
Fe (mg/kg)	47.18 ± 8.3	43.64 ± 11.9	39.78 ± 7.0	35.7 ± 0.1 mg/kg	35.5 ± 0.0 mg/kg
K (mg/kg)	4948.18 ± 283.8	5100.66 ± 322.7	4029.22 ± 619.9	12.2 ± 0.6 g/kg	7.2 ± 0.3 g/kg
Mg (mg/kg)	1232.80 ± 107.9	1182.04 ± 79.4	1156.97 ± 219.4	9.5 ± 0.3 g/kg	10.9 ± 0.2 g/kg
Mn (mg/kg)	49.73 ± 5.5	54.85 ± 6.2	39.29 ± 7.1	969.3 ± 4.8 mg/kg	1249.3 ± 4.9 mg/kg
Na (mg/kg)	33.89 ± 8.0	29.11 ± 5.8	16.92 ± 6.6	458.7 ± 36.6 mg/kg	127.3 ± 6.2 mg/kg
Zn (mg/kg)	33.97 ± 3.6	32.69 ± 4.3	35.17 ± 5.7	64.5 ± 1.1 mg/kg	78.3 ± 0.7 mg/kg

[a] P total.

differences in Fe, Zn and Mn contents, suggesting that for these elements there was some genetic potential to increase their content in durum wheat grains. The genotypes with the highest grain-Fe content (65.6 mg/kg in Sansone and 60.6 mg/kg in Grifoni 235, respectively) were by from the modern and old genotypes classes, while for Zn (46.3 mg/kg) and Cu (14.0 mg/kg) the genotypes with the highest content were within the group of modern varieties (Table 2). Overall wider ranges in mineral contents were observed among the modern genotypes (P_i, Cu, Fe, Na and Zn).

Previous studies carried out on a wide range of *Triticum* germplasm to screen for Fe and Zn content reported similar ranges (Murphy and Law, 1974; White et al., 1981; Peterson et al., 1986; Batten, 1994; Graham et al., 1999; Monasterio and Graham, 2000; Liu et al., 2006; Oury et al., 2006; Ozkan et al., 2007) suggesting that enough genetic variation exists in durum wheat germplasm to substantially increase Fe and Zn grain content. The values reported for Fe content in hexaploid wheats, wild wheats and landraces grown under field conditions, ranged from 28.8 to 56.5 mg/kg (Graham et al., 1999); 19.0–88.4 mg/kg (Oury et al., 2006); 22.9–67.6 mg/kg (Liu et al., 2006) and 10–51 mg/kg (Cakmak et al., 2000); while the ranges for Zn were 25.2–53.3 mg/kg (Graham et al., 1999), 16.4–39.5 mg/kg (Oury et al., 2006) and 16.2–32.4 mg/kg (Liu et al., 2006).

Concerning Cu and Mn grain content, Rengel et al. (1999) reported mean values ranging from 1.2 to 8.6 mg/kg and from 16 to 99 mg/kg, respectively, although some of these evaluations were conducted on fields fertilized with manure application, a treatment leading to higher Fe and Zn content values. On the contrary, Graham et al. (1999) reported mean values quite similar to our results: particularly for Mn (44.7 mg/kg), Ca (416 mg/kg), Mg (1130 mg/kg) and K (3600 mg/kg). Finally, for P_i the few available data (Liu et al., 2006) indicated a range (0.25–0.57 mg/kg) quite similar to our findings, when 186 bread wheat genotypes were evaluated.

3.2. Relationship between inorganic phosphorus and phytate measurements

Phytate measurements obtained using the modified Chen colorimetric assay were validated by sampling genotypes with low, intermediate, and high levels of P_i and analysing for phytic acid determination by an HPLC method (Fig. 1). Overall, values obtained with the rapid assay well agree with the phytate concentrations determined by HPLC and the correlation between

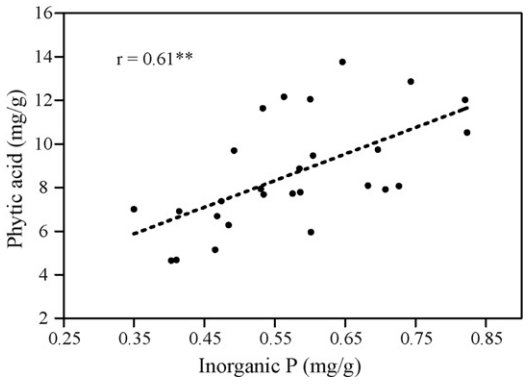

Fig. 1. Relationship between inorganic P (P_i) measurements obtained through the colorimetric assay and phytic acid content obtained by HPLC.

the two methods for phytate determination was significant (0.61**) confirming similar results reported by Liu et al. (2006) (0.43**) on wheat and by Stangoulis et al. (2006) on rice (0.53***). These results suggest that the rapid assay can be reliably used for the quantitative determination of P_i and grouping cultivars into high, medium and low phytate content and that breeders can make repeatable and thus possibly heritable measurements of P_i in an inexpensive, high-throughput manner (Aaron et al., 2007).

3.3. Genotype × environment interactions

The combined analysis of variance revealed highly significant Environment (E), Genotype (G), and Genotype × Environment (G × E) interaction effects for all evaluated traits (Table 4). GY was controlled to a large extent by E (88%), similar results were obtained for TKW (59%) and for some mineral elements (62, 52, 51 and 49% for P_i, K, Na and Mn, respectively), all characterized by a smaller G and G × E effect. For all other traits considered in this study about 50% of variance was due to G × E interaction with a very small effect of E on total variance (ranged from 4 to 23% for Zn and Cu, respectively) and a considerable influence of G on the phenotypic values observed. Considering the results on microelements, the G × E was the most important source of variation. This implies that for Fe, Zn, Cu, Mg, Ca more than Na, Mn and K, the ranking of durum wheat genotypes was most influenced by the interaction of environment with genotype. In this respect, Yang and Baker (1991) recognized two main categories of G × E interaction, crossover and noncrossover interaction, with changes in genotype rank order or differences in response scale between environments determining the nature of the interaction. To assess the nature of G × E interaction for traits evaluated in our study, Spearman's rank correlation coefficients were estimated using genotype means for all traits evaluated in the 3 environments. For most traits the correlation coefficients were not significant, suggesting that the G × E interaction for grain mineral content was not due to differences in scale among the environments. Exceptions are GY and TKW for which the Spearman's rank correlation coefficients were all highly significant (r_s = 0.30–0.59; $P \leq 0.01$). For these traits the genotypes were ranked in the same order in different environments (Table 5). On the contrary, the ranking of the genotypes for Fe, Zn, Cu, Mn and Na content were not stable from one environment to another, and the Spearman's correlations were consequently not significant. Therefore, breeding for Zn and Fe would be difficult since G × E interaction for these traits is prevalent.

3.4. Relationships between traits

PCA was used to examine the relationships among traits (Fig. 2). Given that the environment strongly affected the traits analyzed, multivariate analysis was carried out with the mean genotype values of each environment. Only the first 2 axes were considered (eigenvalues > 1), since they accounted for 65.6% of total variance: 42.8 and 22.8 for axes 1 and 2, respectively. The eigenvectors of the various components are represented in Fig. 2A. The length of each vector's projection on an axis is proportional to its contribution to the principal components of that axis, reflecting the extent to which each variable weights the two components. The first PC axis (PC1) clearly separated GY and TKW in its positive direction, while P_i, K, Na and Mn content, were separated in the negative direction. On the second PC axis (PC2), the observed variation was caused mainly by Zn, Mg, Cu and Ca while Fe showed a similar weight on PC1 and PC2. In general the PCA showed a strong association between the two components representing productivity (GY and TKW) as well as a strong association among grain mineral concentrations. Genotypic means plotted on the same plan determined by the two PC axes (Fig. 2B) are grouped in two clusters based on the environments (Foggia and Fiorenzuola d'Arda). PC1 discriminates clearly between the cultivars grown at Fiorenzuola d'Arda (right part) and those at Foggia 2005 and 2006, which are completely superimposed (left part). On the second axis (PC2), there were no clusters with respect to environments, but genotypes were largely distributed along this axis.

In order to quantitatively analyze and confirm the relationships among microelement concentrations, the Pearson correlation analysis was applied to the obtained data (Table 6). Higher GY, but not TKW, was significantly associated with a decrease in the concentration of many mineral elements, a finding suggesting that among the tested genotypes there was not a direct influence of the seed weight on the mineral concentration. Coefficient of correlation between GY and mineral elements (P_i, Ca, Mg, Na and Zn) ranged from −0.24* (Ca) to −0.53*** (Mg) while Fe, Cu, K and Mn concentrations were not significantly affected by GY. Several authors have already reported a negative correlation between yield and grain mineral concentration suggesting a "dilution effect" of

Table 4
Combined analysis of variance (ANOVA) for grain yield (GY), thousand kernel weight (TKW) and mineral content of 84 genotypes across 3 environment (year × site combination).

	DF	GY		TKW		P_i		Ca		K		Mg		Cu		Fe		Mn		Na		Zn	
		SS	(%)[a]	SS	(%)	SS	(%)	SS	(%)	SS	(%)	SS	(%)	SS	(%)	SS	(%)	SS	(%)	SS	(%)	SS	(%)
Environment (E)	2	3157.2[b]	88	25,753[b]	59	9.2[b]	62	1,125,961[b]	17	1.7E + 08[b]	52	7.5E + 05[b]	4	1134.7[b]	23	6903[b]	9	31,703[b]	49	38,627.6[b]	51	773.9[b]	4
Genotype (G)	83	227.8[b]	6	10,870[b]	25	2.4[b]	16	2,126,101[b]	31	5.9E + 07[b]	18	6.6E + 06[b]	36	1284.6[b]	26	23,373[b]	31	11,384[b]	17	12,512.1[b]	16	6331.1[b]	34
E × G	166	141.3[b]	4	4372[b]	10	2.6[b]	18	3,177,254[b]	47	8.3E + 07[b]	26	9.8E + 06[b]	53	2406.5[b]	49	41,100[b]	54	18,494[b]	28	22,812.2[b]	30	9602.3[b]	51
Error	504	63.6	2	2897	7	0.6	4	328,452	5	1.3E + 07	4	1.3E + 06	7	91.5	2	4051	5	3662	6	1915.9	3	1989.9	11
Total	755	3590.0		43,892		14.8		6,757,767		3.2E + 08		1.8E + 07		4917.3		75,427		65,243		75,867.9		18,697.1	

[a] % explained and calculated from ANOVA as factor SS (sum of squares)/total SS.
[b] Significant at $P \leq 0.001$.

Table 5
Spearman's rank correlation analysis for grain yield (GY), thousand kernel weight (TKW) and mineral content in 3 environments in Italy.

	GY	TKW	P_i	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
FG05–FG06	0.36	0.52	0.22	0.35	*0.10ns*	*−0.06ns*	0.32	*0.39*	0.29	*−0.07ns*	*0.20ns*
FG05–FIOR06	0.30	0.55	*0.13ns*	0.31	*0.03ns*	*0.03ns*	*0.14ns*	*0.08ns*	*−0.04ns*	*0.15ns*	*0.11ns*
FG06–FIOR06	0.48	0.59	0.22	*0.11ns*	*0.05ns*	0.28	*0.14ns*	0.35	*0.12ns*	*−0.06ns*	*−0.02ns*

FG05 = Foggia 2004–2005; FG06 = Foggia 2005–2006; FIOR06 = Fiorenzuola d'Arda 2005–2006.

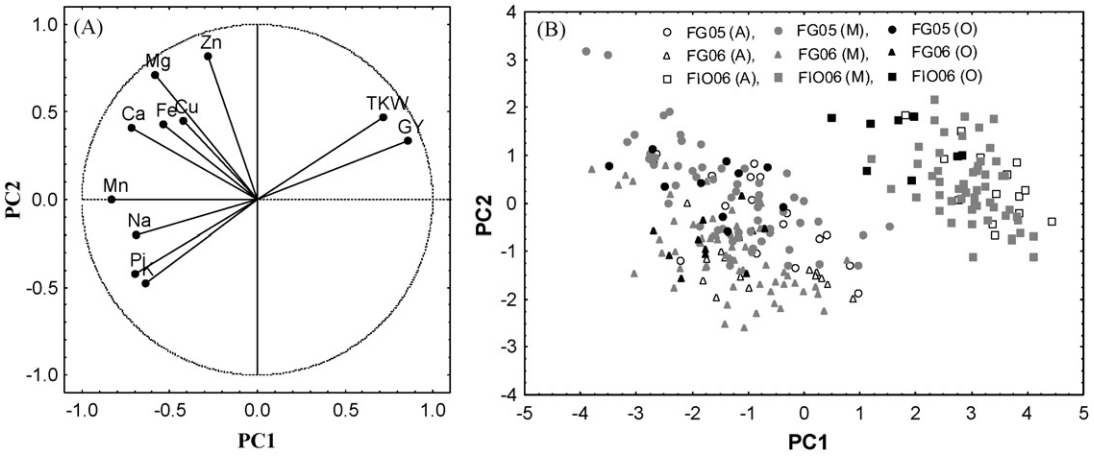

Fig. 2. Principal component analysis (PCA) projections on axes 1 and 2 accounting for 65.6% of total variance. (A) Eigenvalues of the correlation matrix are symbolized as vectors representing traits that most influence each axis. GY = grain yield, TKW = thousand kernel weight, K = potassium, P_i = inorganic phosphorus, Na = sodium, Mn = manganese, Ca = calcium, Fe = iron, Cu = cupper, Mg = magnesium, Zn = zinc. (B) Projection on planes 1–2 of 252 points representing 84 genotype means (old = O, modern = M and advanced = A) across 3 different Italian environments: (Foggia 2005 = FG05, Foggia 2006 = FG06 and Fiorenzuola d'Arda 2006 = FIOR06). Each point was obtained averaging 3 replications.

Table 6
Pearson correlation coefficients among GY, TKW, P_i, Ca, Cu, Fe, K, Mg, Mn, Na and Zn of 84 durum wheat genotypes across 3 environments in Italy.

	GY	TKW	P_i	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
GY	1.00										
TKW	0.39***	1.00									
P_i	−0.31**	−0.27*	1.00								
Ca	−0.24*	−0.16ns	0.33**	1.00							
Cu	−0.06ns	−0.06ns	−0.01ns	0.33**	1.00						
Fe	−0.19ns	0.16ns	0.20ns	0.28*	0.08ns	1.00					
K	0.08ns	−0.12ns	0.03ns	0.06ns	0.07ns	−0.18ns	1.00				
Mg	−0.53***	−0.10ns	0.16ns	0.54***	0.35***	0.44***	−0.23*	1.00			
Mn	−0.15ns	−0.16ns	0.03ns	0.27*	0.19ns	0.25*	−0.13ns	0.52***	1.00		
Na	−0.37**	−0.02ns	0.19ns	0.10ns	−0.11ns	0.15ns	−0.02ns	0.16ns	0.00ns	1.00	
Zn	−0.41***	−0.08ns	0.40***	0.63***	0.28*	0.49***	−0.06ns	0.76***	0.44***	0.18ns	1.00

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$; ns = not significant.

the starch on minerals (Peterson et al., 1983; Feil and Fossati, 1995; Graham et al., 1999; Monasterio and Graham, 2000). Nevertheless, these studies did not accurately measure the strength of the dilution effect because of the limited size of the sample set or of the experimental design with only one environment. Our results, in agreement with Ortiz-Monasterio et al. (2007), suggest that the dilution effect is significant only for Mg (−0.53***) and Zn (−0.41***), while it is not significant for Fe.

In the present study, GY and TKW were not correlated with Fe concentration, and in general advanced and modern genotypes had the same Fe concentration than old genotypes. This result is in good agreement with the previous finding that no negative linkage existed between genotype yield and micronutrient density (Graham et al., 1999). In addition, the linear regression analysis to calculate the genetic gain for potential grain yield, Fe and Zn traits revealed no trend in Fe and Zn concentrations with year of release (data not shown).

Positive associations between P_i and Zn have been reported in soybean (Raboy and Dickinson, 1984), wheat (Abernethy et al., 1973), and rice (Graham et al., 1999), as well as in the present study. By contrast, no significant correlation was found between P_i and Fe concentrations. The chemical form of the two minerals might account for this difference. In wheat grain, Fe is mainly found in ferritin deposits whereas Zn is strongly bound to phytate (Grusak et al., 1999). Thus, it is implied that high GY and Fe concentration could be combined by traditional breeding strategies. Fortunately, the P_i concentration decreases with yield increase (−0.31**) confirming the previous experiences (Peterson et al., 1983; Graham et al., 1999; Monasterio and Graham, 2000; Liu et al., 2006) and the possibility for breeders to select at the same time for high GY, low P_i levels and high Fe-grain concentration. On the contrary, the selection for GY and high Mg or Zn concentration would be antagonistic, and to progress in both directions, GY and Mg or Zn need to be studied simultaneously.

Overall, a positive correlation was found among the concentrations of several important elements. Particularly interesting is the highly significant and positive correlation between Zn and P_i (0.40***), Ca (0.63***), Cu (0.28*), Fe (0.49***), Mg (0.76***) and Mn (0.44***) suggesting common genetic factors controlling the accumulation of different minerals, and that selection for one element (for example Fe) might result in an increase in other elements (such as Zn, Mg and Mn). Previous studies reported a strong correlation between Zn and Fe concentration in germplasm containing both wild wheats (Cakmak et al., 2004) and cultivated hexaploid wheats (Peterson et al., 1986), although Graham et al. (1999) reported no correlation among durum wheat genotypes (0.33ns).

These data might suggest physiological coupling of the accumulation processes of minerals in wheat grain. QTL analysis for cationic mineral concentrations revealed only a QTL on chromosome 5A with small effects on Fe, Zn, Mg and Cu

accumulation in seeds of *Triticum monococcum* (Ozkan et al., 2007), while for rice (Stangoulis et al., 2006) and *Arabidopsis thaliana* (Vreugdenhil et al., 2004) no co-localization of QTLs for phytate, Mg, Fe and Zn was revealed suggesting a more complex genetic system for these traits.

4. Conclusions

The analysis of a collection of durum wheat genotypes grown over 3 environments pointed to considerable differences in grain mineral concentrations, suggesting that there is some genetic potential to modify the levels of these components in durum wheat grains. Phytic acid has been linked to mineral deficiency because of its affinity with minerals, mainly Fe, Zn, Ca and Mg, even if recent studies have shown its role in cancer prevention (Sandström et al., 2000; Manary et al., 2000; Grases et al., 2004; Vucenik and Shamsuddin, 2006). Our findings confirmed good genotypic variability in seed phosphorus and phytic acid concentration with a small G × E effect, indicating that this variation can be exploited for breeding genotypes with low phytic acid concentrations to improve mineral bioavailability in cereal-based foods. The present data also indicate the possibility to select durum wheat cultivars with high Fe and Zn concentrations and, in contrast with a previous work (Graham et al., 1999), a positive correlation was found between Fe and Zn concentration in the grain of durum wheat, suggesting that these two traits may be combined relatively easily during breeding. Nevertheless, the complexity of the inheritance of grain mineral concentration as well as the large environment and G × E interaction effects, slow the genetic progress for these traits. Correlations among mineral concentrations suggest that the improvement of one mineral may simultaneously improve concentration of other minerals, thus multiplying the impact of the effort. The identification of genotypes high in Zn, Mn, Mg and Cu among high-yielding genotypes, demonstrated that the high mineral concentration traits can be combined with high yield.

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