Available online at www.sciencedirect.com

SCIENCE DIRECT

Europ. J. Agronomy 25 (2006) 177–185

European Journal of Agronomy

www.elsevier.com/locate/eja

Genetic variability and stability of grain magnesium, zinc and iron concentrations in bread wheat

F.-X. Oury[a,*], F. Leenhardt[b], C. Rémésy[b], E. Chanliaud[c], B. Duperrier[d], F. Balfourier[a], G. Charmet[a]

[a] INRA, UMR Amélioration et Santé des Plantes, 234 avenue du Brézet, F-63 100 Clermont-Ferrand, France
[b] INRA, Unité des Maladies Métaboliques et Micronutriments, F-63 122 St-Genès-Champanelle, France
[c] ULICE, ZAC les Portes de Riom - BP173, F-63 204 Riom Cedex, France
[d] Maïs Angevin-Nickerson SA, Domaine de Mons, BP 115, F-63 203 Riom Cedex, France

Abstract

Four trials were conducted to study the grain magnesium (Mg), zinc (Zn) and iron (Fe) concentrations in bread wheat (*Triticum aestivum* L.). These trials used different sources of genotypes, including old French landraces, a worldwide germplasm collection and elite breeding lines or modern cultivars, grown in different environments. Mg concentration ranged from 600 to 1400 ppm in modern material, and reached 1890 ppm in some exotic genotypes. There was a negative correlation between grain yield and Mg concentration, but despite this dilution effect enough variability remains useful for selection purposes. Analysis of variance showed high genotype effects and Spearman rank correlations indicated moderate genotype by environment (G × E) interactions, so breeding for high Mg concentration can reasonably be envisaged. Zn concentration generally ranged from 15 to 35 ppm, but increased to 43 ppm in some genetic resources. Variation in Zn was also partly explained by a dilution effect. There was a significant effect of genotype, but also high G × E interactions, which would make direct selection more difficult than for Mg. However, as Zn and Mg concentrations appeared to be positively correlated, Zn concentration should respond favorably to selection for high Mg concentration. Fe concentration ranged from 20 to 60 ppm, and reached 88 ppm in non-adapted material. There were no significant genotype effects, very high G × E interactions, and the trait was poorly correlated to other mineral concentrations. Breeding for high Fe concentration will thus probably prove illusory.

Keywords: Dilution effect; Genetic resource; Genotype by environment interaction; Iron; Magnesium; Zinc

1. Introduction

Magnesium (Mg), iron (Fe) and zinc (Zn) are essential for good preventive nutrition. In France, the SUpplémentation en VItamines et Minéraux AntioXydants (SU-VI-MAX) study (Hercberg et al., 1998), showed that 72% of men and 77% of women had Mg intakes lower than the French recommended dietary allowances, and habitually low intakes of Mg are associated with etiologic factors in cardiovascular and nervous diseases, bone deterioration, spasmophilia and stress (Durlach, 2001). Zn is an essential trace element that has a wide range of functions in the organism due to its role as a cofactor of many enzymes. Growth retardation, immune dysfunctions and cognitive impairment are major effects of Zn deficiency that affect both industrialized and developing countries (Prasad, 1998). As diets are the only source of Zn, nutritional causes are the most common. Fe is an integral part of many proteins and enzymes that maintain good health. Fe deficiency limits oxygen delivery to cells, resulting in fatigue and decreased immunity (Bhaskaram, 2001; Haas and Brownlie, 2001). As many as 80% of the world's population may be Fe deficient, while 30% may have Fe deficiency anemia (Stoltzfus, 2001).

Mg, Fe and Zn are mainly present in the aleurone layer of bread wheat grains, and whole wheat products are an important source of the daily requirements of these mineral and trace elements in humans (Galand et al., 1997). In particular, whole grain products are the main source of Mg, which is quantitatively the predominant mineral of the grain. Diets enriched in cereal products are therefore encouraged by nutritionists in Western Europe. However, the nutritional value of cereal products needs to be improved through the general use of less refined flour and the selection of wheat varieties with high mineral density.

---

* Corresponding author. Tel.: +33 473 624 326; fax: +33 473 624 453.
E-mail address: oury@clermont.inra.fr (F.-X. Oury).

1161-0301/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.eja.2006.04.011

Awareness of the need for cultivars with higher mineral concentrations is increasing (Lopez et al., 2003), but before these micronutrients are taken into account in breeding programs, at least two questions must be answered:

(1) Does variability exist for these characters and, above all, what are the respective roles of genotype, environment, and genotype by environment (G × E) interactions, in their variability?
(2) Is there any antagonism between mineral concentrations and other important breeding characters like productivity or technological value?

Variability in mineral concentrations has already been described for different cereals like bread wheat (Zook et al., 1970; Toepfer et al., 1972; Nahapetian and Bassiri, 1976; Dikeman et al., 1982; Davis et al., 1984; McGrath, 1985; Peterson et al., 1983, 1986; Monasterio and Graham, 2000), durum wheat (Clarke et al., 2002), triticale (Feil and Fossati, 1995) and some wild relatives of wheat (Graham et al., 1999; Cakmak et al., 2000; Balint et al., 2001). However, except McGrath (1985) and Feil and Fossati (1995), all these studies dealt with mineral concentrations obtained in extensive farming conditions. So the present results obtained on a wide range of bread wheat genotypes grown with the intensive agricultural practices currently used in North-European countries are very relevant. Moreover, because only Peterson et al. (1986) and Feil and Fossati (1995) studied G × E interactions, the respective parts of genotype and environment in the control of Mg, Zn and Fe concentrations in bread wheat grown in intensive farming conditions are not well known.

The objectives of this work were to determine whether grain mineral composition is genetically controlled, and to study the relationships between Mg, Zn and Fe concentrations and two economically important traits: yield and protein concentration, which have been selected for many years.

2. Materials and methods

2.1. Plant material

Grain samples came from four trials:

Trial 1: 51 elite genotypes from an INRA breeding program were grown in 2002 at three locations (Clermont-Ferrand, Le Moulon – near Paris – and Rennes). This material was representative of modern germplasm adapted to North–West Europe.

Trial 2: 6 diverse genotypes were grown in 2002 at four locations (Biozat, near Clermont-Ferrand, Clermont-Ferrand, Châlons-en-Champagne and Chartainvilliers) to study the relationship between yield and mineral concentrations. The six genotypes were two widely grown French cultivars (Soissons and Shango), two strong improving cultivars (Qualital and ULI3), and two old landraces: Blé des Dômes (France) and BGW-76 (China). Soil analyses were made in the four locations, to study the relationship between quantities of minerals available in the soil and grain mineral concentrations.

Trial 3: 11 genotypes were grown at Clermont-Ferrand over 3 years (2001–2003 growing seasons). The 11 genotypes covered a wide range of variability including six high-yielding cultivars (Apache, Baltimore, Isengrain, Ornicar, Soissons, Sponsor), two strong improving cultivars (Renan and Tamaro) and three low-yielding landraces (Blé des Dômes from France; BGW-2 and BGW-76 from China). The aim of this trial was to estimate the importance of the year effect on variability in mineral concentrations.

Trial 4: 175 genetic resources were sampled from the INRA collection of bread wheat. They were chosen to cover a wide range of geographical origins: five groups of accessions (Asia, North and South America, the Mediterranean region, Europe and France), each represented by at least 32 genotypes. This material was grown in 2002 at Clermont-Ferrand in nursery plots (three rows per accession). The passport data of the 175 accessions is available on request. The aim was to compare the variability in mineral concentrations present in this broad-base material with variability in adapted material.

For trials 1–3, the yield values were from randomized complete block designs with two replicates per environment. The typical size of the plot was 7 m^2. Crop management corresponded to intensive farming methods with full insecticide and fungicide cover, and nitrogen fertilization fitted to high yield objectives. For trial 4 in nursery, there was no evaluation of yield, and crop management was quite different: low sowing density, reduced nitrogen fertilization to prevent lodging and only one replication per genotype.

2.2. Determination of mineral concentration

Dried grain samples (0.25–0.5 g) were dry-ashed (10 h at 500 °C) and then extracted at 130 °C in HNO_3/H_2O_2 (2:1, v/v; Merck, Suprapur, Darmstadt, Germany) until discoloration. Final dilutions were made in 1 g L^{-1} lanthanum chloride solution for Mg, and in 2% HNO_3 for Zn and Fe.

Mineral concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer 560, Norwalk, CT), in an acetylene–air flame at the following wavelengths: 285 nm (Mg), 248 nm (Fe) and 214 nm (Zn). A high sensitivity nebuliser was used for measuring trace elements. Appropriate quality controls (certified whole meal flour BCR-189) were performed for each set of measurements.

2.3. Statistical methods

Analysis of variance for the multi-environmental trials was performed using the following model:

$$Y_{ij} = \mu + e_i + g_j + \varepsilon_{ij}$$

where μ is the overall mean, e_i the effect of environment i (site or year), g_j the effect of genotype j and ε_{ij} an error term.

Mineral concentrations were measured on only one of the replicates for each location, so it was not possible to test G × E interactions using analysis of variance. Hence, stability was estimated by Spearman rank correlations calculated for each pair of environments. This approach is quite rough, as it takes into account only the part of G × E interactions leading to reversal in ranks (crossover interaction), and not the non-crossover interaction corresponding to scale effects (for more details on stability analysis, see for example, Becker and Léon, 1988 or Brancourt-Hulmel et al., 1997). Nevertheless, our purpose was to have a first estimation of the importance of G × E interactions in Mg, Zn and Fe concentrations. Therefore, stability of rankings over environments, as measured by Spearman rank coefficients, can be considered a useful information.

3. Results and discussion

3.1. *Variability in mineral concentrations*

For trial 1, with adapted genotypes, Fe and Zn concentrations were of the same magnitude (respectively, 19–58 and 14–35 ppm), while Mg concentration was higher (601–1388 ppm). In non-adapted material (trials 2 and 3 and especially genetic resources from trial 4), the lowest values were approximately the same as in trial 1, but the highest values rose to 88 ppm for Fe, 43 ppm for Zn and 1886 ppm for Mg (Table 1). The distributions of mineral concentrations were similar for the five geographical groups of genetic resources (Fig. 1): the only noticeable differences were the lower values obtained for Mg concentration in accessions from France and especially from Europe, and the higher values for Fe concentration obtained in accessions from France.

Coefficients of variation (c_v) indicating variability among the genotypes were quite high for the three minerals (Table 1): in trial 1, c_v were greater than 12% for the different site × mineral combinations, except for Zn concentration at Clermont-Ferrand ($c_v = 7.1\%$). By comparison, c_v for yield and protein concentration on the same material were nearly two times lower (6–8%). It should be noted that the increase in c_v in trials 2 or 3 compared to trial 1 was much higher for yield than for mineral concen-

Table 1
Yield, protein concentration and mineral concentrations measured on four trials carried out at Clermont-Ferrand (CF), Le Moulon (LM), Rennes (RE), Biozat (BI), Châlons-en-Champagne (CH), and Chartainvilliers (CV) in different years

Trial	Location	Year	Sample size	Yield (q ha^{-1})		Protein (%)		Mg (ppm)		Zn (ppm)		Fe (ppm)	
				Mean Min–max	c_v (%)	Mean Min–max	c_v (%)	Mean Min–max	c_v (%)	Mean min–max	c_v (%)	Mean Min–max	c_v (%)
1	CF	2002	51	75.1 59.4–87	7.9	13.6 12.2–15.7	5.5	1109 797–1388	13.8	17.3 14.3–19.7	7.1	33.1 26.1–44.7	15.1
	LM	2002	51	107.8 84.9–121.7	6.7	12.7 11.2–15.3	7.7	1006 750–1299	11.7	22.9 17.2–34.8	12.9	39.1 30.4–58.2	13.9
	RE	2002	51	115.6 82.3–134.2	8	10.5 8.7–13	8.2	894 601–1151	15.3	21.2 14.5–29	15.8	31.9 19.1–48.1	19.3
	Mean		51	99.5 78.2–110.7	6	12.3 10.8–14.2	5.8	1003 764–1243	11.4	20.5 16.1–27.2	10	34.7 27.3–41.9	10.2
2	BI	2002	6	55.7 21–78	34.8	15.4 12.8–18.1	12.3	1406 1095–1636	14.6	18.2 14–24	19.8	33.8 22–44	25.4
	CF	2002	6	70.8 33.7–90.3	33.8	15.1 12.9–17.7	13.9	1379 1163–1677	14.1	25.2 21–35	22.8	36.3 26–47	21.1
	CH	2002	6	76.8 35–103	36.1	14 12.3–17.5	13.4	1195 974–1510	15.3	22.5 15–27	21	28.8 18–40	35.3
	CV	2002	6	64 21–90	37.6	14.6 11.6–18.1	15.5	1111 918–1395	14.4	17.8 14–27	26.4	34.2 27–42	17.1
	Mean		6	66.8 27.7–89.3	34.6	14.7 12.5–17.8	13.2	1273 1038–1554	14.1	21 16–28	19.7	33.2 23-43	23.2
3	CF	2001	11	67.3 41.9–96.3	29.7			1278 997–1508	13.1	25.8 19–33	18.2	32.5 19–51	32.2
	CF	2002	11	65.7 21.4–87.1	31.2			1368 1160–1636	11.8	22.5 16–33	24.7	38.7 23–62	25.7
	CF	2003	11	54.4 39.5–64.9	15.5			1283 1080–1620	12.8	25.8 18–43	33.2	45 37–64	17.6
	Mean		11	62.5 34.3–81.5	24.6			1310 1119–1567	11.7	24.7 20–33	20.5	38.7 30.7–52	17.7
Genetic resources	CF	2002	175					1477 1098–1886	10.6	25 16.4–39.5	14.2	44.5 25.6–88.4	24.9

c_v = coefficient of variation indicating variability among genotypes.

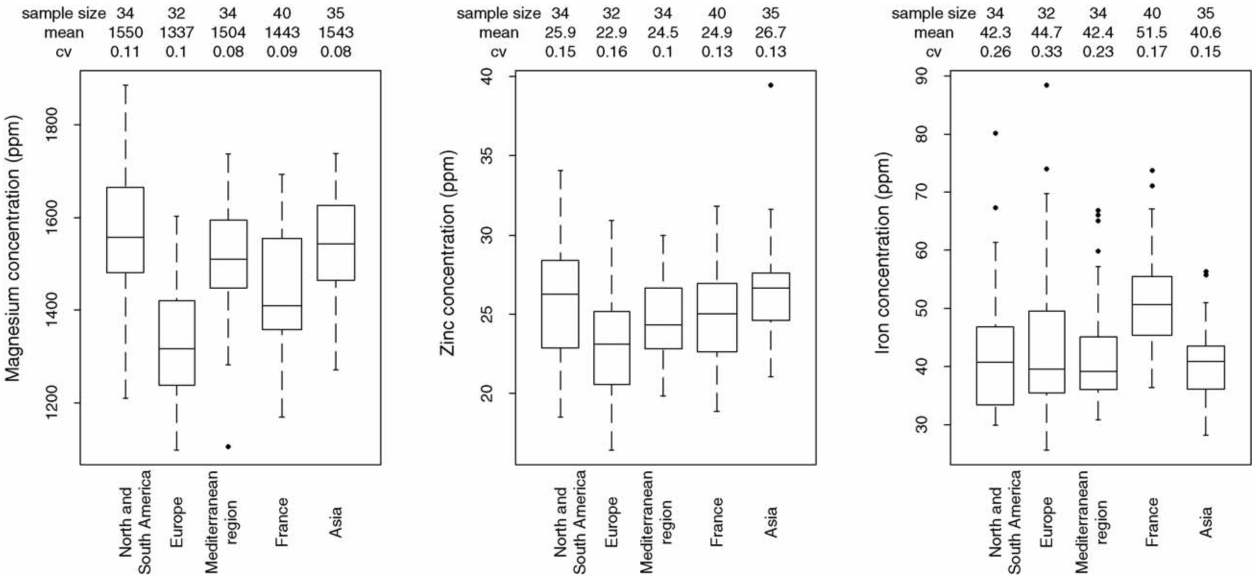

Fig. 1. Distribution of Mg, Zn and Fe concentrations for the 175 accessions of genetic resources from trial 4, as a function of their geographical origin. Coefficient of variation (c_v) indicating the variability among genotypes.

trations. This may be due to the fact that mineral have never been taken into account in breeding programs: thus, variability in Mg, Zn and Fe in modern material is expected to differ from that in older genotypes only as a consequence of random drift or indirect selection effects.

Average Mg value in adapted material and under intensive agricultural practices (1003 ppm in trial 1) was very close to that found by McGrath (1985): 1096 ppm from 238 samples of grain collected all over the UK. These values were far below the average values obtained under extensive farming conditions, which ranged from 1330 ppm (Davis et al., 1984) to 1910 ppm (Peterson et al., 1983). This could be due to lower competition between plants, and this should be kept in mind when interpreting the very high values for Mg concentration found in some genetic resources in our study (Table 1). Indeed, in genotypes evaluated in nursery plots, as was the case for genetic resources in our study, competition between plants was reduced due to low sowing density.

The lowest mean values obtained for Fe and Zn in extensive farming conditions overlapped the mean values obtained in intensive farming conditions, and the difference between the two agricultural practices was not clear: average value for Fe concentration in trial 1 was 35 ppm (41 ppm for McGrath, 1985) compared to reported values ranging from 38 ppm (Dikeman et al., 1982) to 79 ppm (Davis et al., 1984). For Zn concentration, the average value was 21 ppm (29 ppm for McGrath, 1985) compared to reported values ranging from 22 ppm (Toepfer et al., 1972) to 47 ppm (Davis et al., 1984).

For Mg, there was a strong relationship between the quantity of Mg available in the soil and grain Mg concentration (Fig. 2).

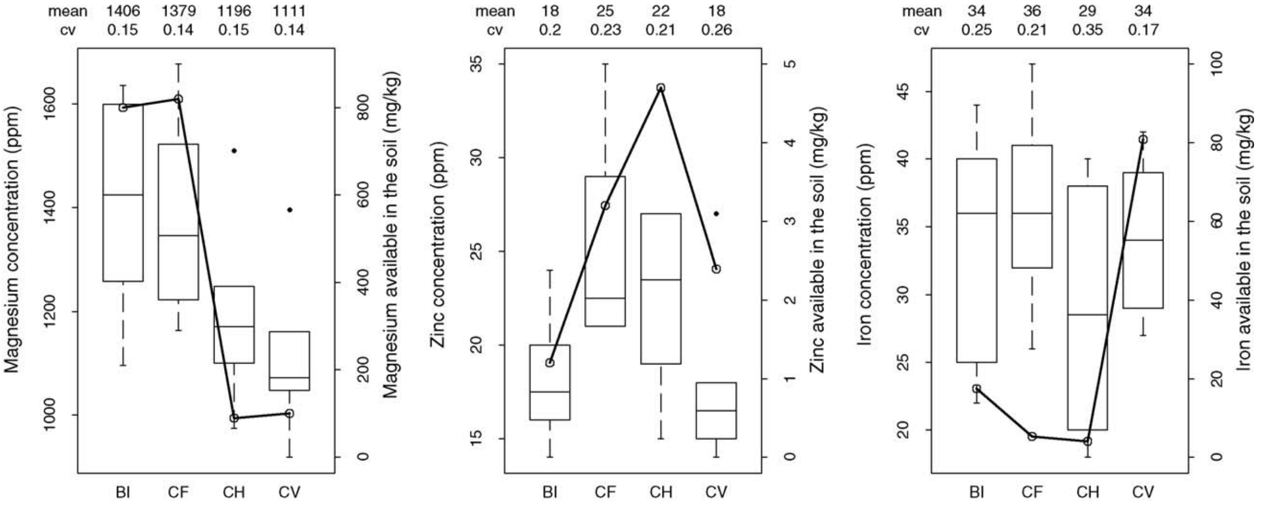

Fig. 2. Variability observed in trial 2 ($n = 6$) for the mineral concentrations of the grain (boxplots related to the left Y-axis), in relation with the available quantities of Mg, Zn and Fe in the soil (line related to the right Y-axis) at Biozat (BI), Clermont-Ferrand (CF), Châlons-en-Champagne (CH) and Chartainvilliers (CV). Coefficient of variation (c_v) indicating the variability among genotypes.

Grain Zn concentration was also higher at sites with high quantities of available Zn, even if the relationship observed was weaker than for Mg. Conversely, there was no correlation between soil available Fe and grain Fe concentration (Fig. 2).

3.2. Determinism of mineral concentrations

Analysis of variance and Spearman rank correlations were calculated in trials 1 and 3, but not in trial 2 because of the very limited size and the very particular composition of the sample of genotypes in this trial. Indeed, in trial 2, the three groups of two genotypes, each with highly contrasted yields, led to over-estimation of genotype effects and under-estimation of G × E interaction.

Significant environment effects appeared for mineral concentrations (Table 2), except for Zn in trial 3. Authors studying multi-annual and/or multi-site data (Nahapetian and Bassiri, 1976; Dikeman et al., 1982; Davis et al., 1984; Peterson et al., 1986; Feil and Fossati, 1995; Clarke et al., 2002) have already mentioned the importance of environment effects in variability in Mg, Zn and Fe concentrations. The year effect in trial 3 was observed to be weaker than site effect in trial 1, especially for Mg and Zn, which may be linked to the important role played by soil in variability in these two minerals (Fig. 2). Genotype effects were significant in trials 1 and 3 for Mg and Zn. Conversely, genotype effects were not significant for Fe in these two trials. This is in good agreement with the results of McGrath (1985), who reported significant genotype effects for Mg and Zn, but not for Fe in the same intensive farming conditions.

The Spearman rank correlations for each pair of sites varied considerably with the character concerned (Table 3). For yield, correlations were low for trial 1, in good agreement with the high G × E interactions usually found for this character. For trial 3 the correlations were high, which may be linked to the presence of both adapted and non-adapted genotypes in this trial: indeed, the rankings of the genotypes were more stable when low-yielding genotypes were compared to high-yielding genotypes at each location. For Mg, the correlations were quite high for the two trials, indicating a moderate level of G × E interactions. The rankings of the genotypes for Zn were not very stable from one location to another, and correlations were consequently quite low for the two trials. This indicated a relatively high level of G × E interactions. For Fe, the rankings of the genotypes were not conserved from one location to another, and Spearman rank correlations were thus very low for the two trials. This indicated a very high level of G × E interactions for this character.

Table 3
Stability of yield, protein concentration and mineral concentrations over the different environments (CF, Clermont-Ferrand; LM, Le Moulon; RE, Rennes) for trials 1 and 3

Trial	Location/year	Spearman rank correlation				
		Yield	Protein	Mg	Zn	Fe
1	CF–LM	0.284	0.605	0.687	0.337	0.276
	CF–RE	0.165	0.249	0.449	0.164	−0.042
	LM–RE	0.383	0.480	0.620	0.593	0.226
	Mean	0.277	0.445	0.585	0.365	0.153
3	2001–2002	0.755		0.764	0.039	0.023
	2001–2003	0.945		0.791	0.632	0.383
	2002–2003	0.755		0.945	0.499	0.092
	Mean	0.818		0.833	0.390	0.166
Overall		0.548		0.709	0.377	0.159

According to these results, one can expect a response to selection in breeding programs for high Mg concentration, because there are high genotype effects and moderate G × E interactions for this character. Breeding for Zn and especially Fe would be difficult because G × E interactions are more prevalent for these two minerals. For Fe, these results resembled those we obtained for phosphorus in a previous study on animal feed (Oury et al., 1998): for these two grain components, variability mainly depends on G × E interactions thus rendering selection nearly impossible.

Table 2
Analysis of variance for yield, protein concentration and mineral concentrations in trials 1 and 3

Trial	Effect	d.f.	Mean squares				
			Yield	Protein	Mg	Zn	Fe
1	Environment (sites)	2	23591 ***	132.19 ***	591165 ***	427.4 ***	765.8 ***
	Genotype	50	106 ***	1.52 ***	39446 ***	12.5 ***	37.7 NS
	Error (including G × E interaction)	100	33.3	0.37	8231	4.48	27.31
3	Environment (years)	2	540 **		28242 *	41.5 NS	432.8 **
	Genotype	10	709 ***		70826 ***	77 *	140.4 NS
	Error (including G × E interaction)	20	91.6		5177	24.58	65.25

NS, non-significant.
[*] $P < 0.05$.
[**] $P < 0.01$.
[***] $P < 0.001$.

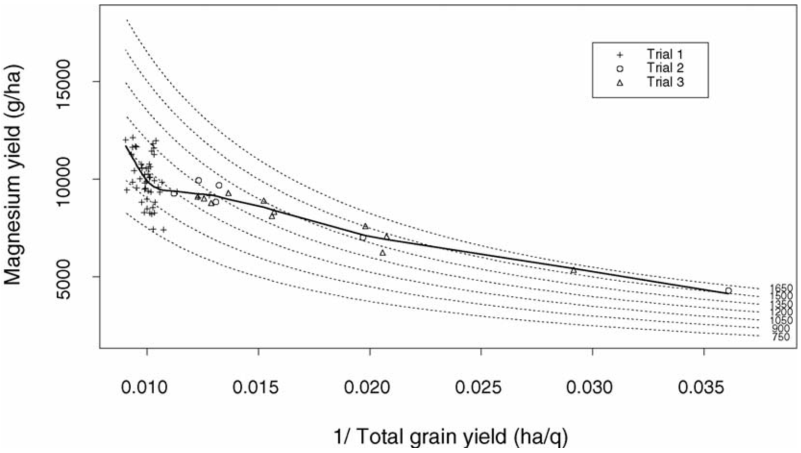

Fig. 3. Relationship between grain Mg yield and 1/(total grain yield). The symbols represent genotype means across environments for trials 1–3. The smoothing curve which best represents the scatter diagram structure (thick line) has been obtained by robust locally linear fits. Iso-concentration curves are plotted every 150 ppm, from 750 to 1650 ppm.

3.3. Relationship between yield and mineral concentration

In the presence of G × E interactions, which was the case for yield, Fe, Zn, and to a lesser extent Mg, data obtained in a single environment are not of much use. To obtain better estimates of genotypic values, we thus used the mean values calculated for each trial over the different environments. It should be kept in mind that Mg mean values were probably over-estimated in trial 3, because data averaged over the years 2001–2003 were obtained from only one site (Clermont-Ferrand) at which quantities of Mg extractible from the soil were high.

Fig. 3 shows the “1/yield” ratio against Mg yield for the three trials. Because the product of these two variables is Mg concentration, iso-concentration curves for Mg can be seen on the graph, i.e., curves representing all combinations of the two traits corresponding to a given Mg concentration. Going from low-yielding genotypes (on the right) to high-yielding ones (on the left), the smoothing curve (thick line) obtained by robust locally weighted regression (S-PLUS, 1996), crossed the iso-concentration curves from values above 1500 ppm to values of about 1050 ppm. Although Mg yield of adapted genotypes was higher than that of non-adapted material, their Mg concentration was lower, indicating a dilution of this mineral in the dry matter of the grain when breeding for productivity.

This dilution effect for Mg must be associated with the negative correlations between yield and Mg concentration, shown for each trial in Fig. 4. The remarkable alignment of the regression lines for trials 2 and 3 and the grouping of the three trials highlights the strength of the dilution effect for Mg, because the same relationship appeared very clearly in the different trials. With a coefficient of correlation $R = -0.81$ (grouping the three trials), it appeared to be of the same order as the well known dilution effect for protein concentration, for which the coefficient of determination (R^2) is usually about 50% (Oury et al., 2003). The

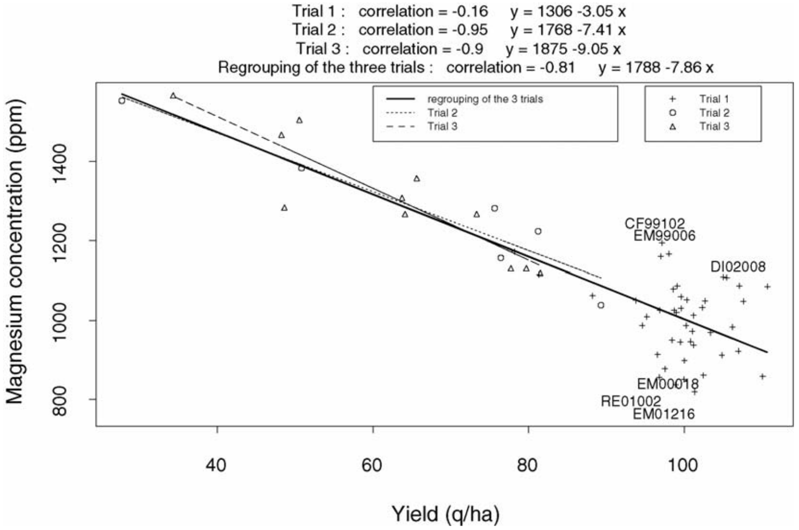

Fig. 4. Correlation between grain Mg concentration and yield. Points are genotype means across environments for trials 1–3. Genotypes that are the most apart from the regression line are identified by their name.

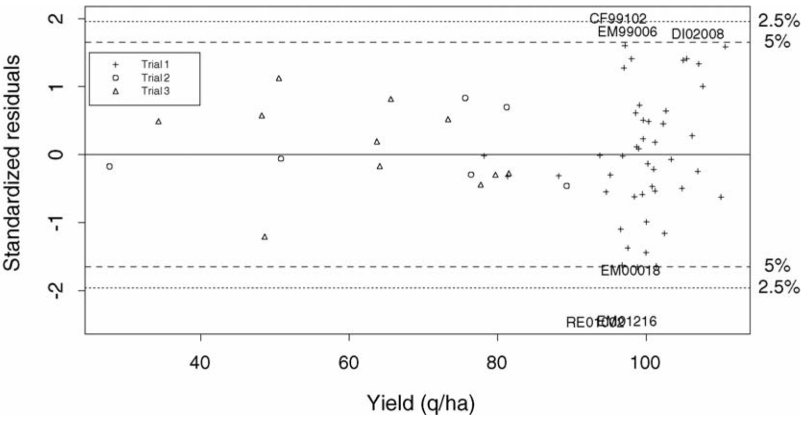

Fig. 5. Plot of the residuals to highlight the genotypes that move significantly away from the regression between grain Mg concentration and yield represented in Fig. 4.

non-significant correlation for trial 1 ($R = -0.16$) does not imply that the negative relationship between yield and Mg concentration did not exist when only modern material was analyzed. It simply reflects the fact that this relation can be masked by G × E interactions when a sample of lines exhibiting low variability for yield is considered, all the more so when means are calculated only over three environments. Nevertheless, the dispersion of the points for trial 1 in Figs. 3 and 4 indicates that, despite the dilution effect, a significant amount of variability still remained, which could be useful for breeding purposes. Advantageous genotypes for both yield and Mg concentration can be clearly distinguished in Fig. 5: a plot of standardized residuals along with quantiles of normal standard highlights genotypes (for example, CF99102) which moved significantly and positively away from the regression line between the two characters.

The increase in Mg concentration with a decrease in yield may also provide an explanation for the differences observed for Mg in the five groups of genetic resources (Fig. 1). Indeed, the hypothesis can be postulated that accessions from North and South America, the Mediterranean region and Asia were relatively less adapted to the environmental conditions of our trials than accessions from France and Europe, resulting in higher values of Mg concentration in these three exotic groups.

A dilution effect also appeared for Zn (figures not shown), but the negative correlation between yield and Zn concentration was lower: $R = -0.67$ when the three trials were grouped. This may be linked to the higher rate of G × E interactions for Zn (Table 3), which masked the negative relationship between the two characters. For Fe, as the maximal G × E interactions (Table 3) had led us to expect, the dilution effect was less clear, and the negative correlation between yield and Fe concentration was still lower ($R = -0.51$).

Different authors have already mentioned a negative correlation between yield and grain mineral concentrations (Peterson et al., 1983; Feil and Fossati, 1995; Graham et al., 1999; Monasterio and Graham, 2000). However, in their studies, it was not possible to accurately measure the strength of the dilution effect because of the limited size of the sample or the experimental design with only one environment. In our study, the effect of dilution appeared to be quite strong for Mg and Zn. So breeding for high Mg or Zn concentrations and breeding for yield would be antagonistic, and to progress in both directions, yield and Mg or Zn need to be studied simultaneously. The variability present in adapted genotypes (Table 1) seems sufficient to improve Mg and Zn concentrations in wheat grain. If a need for more exotic material arises, candidate genotypes (for example, genetic resources with the highest mineral concentrations, as shown in Table 1 and Fig. 1) should be characterized for productivity, plotted on graphs like those in Figs. 4 and 5, and only the ones which move positively away from the linear regression should be used in breeding schemes. It should be noted that evaluating productivity as recommended here could be difficult, because the genetic resources concerned are often tall genotypes that are very susceptible to lodging, especially in intensive farming conditions.

3.4. Correlations between protein and mineral concentrations

Results are presented only for trial 1 (adapted material) and genetic resources from trial 4 (non-adapted material), since in trials 2 and 3 the presence of both adapted and non-adapted genotypes led to artificially high correlations. In Table 4 only positive correlations appeared, and these are in good agreement with the previous results of Peterson et al. (1983) and Feil and Fossati (1995).

Table 4
Correlations between protein and mineral concentrations, for trial 1 (above the diagonal), and for genetic resources from trial 4 (below the diagonal)

	Mg	Zn	Fe
Protein	0.44**	0.44**	0.47***
Mg	–	0.64***	0.49***
Zn	0.67***	–	0.53***
Fe	0.19*	0.30***	–

For trial 1, correlations are calculated with the genotype means across environments.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Correlations between protein and mineral concentrations were moderate ($R < 0.5$) and are probably linked to the dilution effect that affects both minerals and protein. For minerals, the correlations with Fe were quite weak, but there was a relatively high correlation ($R = 0.64$ for adapted genotypes, and $R = 0.67$ for genetic resources) between Mg and Zn. All these positive correlations suggest physiological coupling of the accumulation processes of minerals in wheat grain. QTL analysis for cationic mineral concentrations in seeds of *Arabidopsis thaliana* (Vreugdenhil et al., 2004) revealed no co-localization of QTLs for Mg, Zn and Fe. However, in their study, the total variance explained by the QTLs was low for Mg (23%) and Fe (27%) and slightly higher for Zn (42%), and the correlations between the three minerals were very low ($R < 0.18$ in all cases) compared to the correlations we observed in bread wheat. Not surprisingly, this suggests that accumulation of seed constituents is very different in cultivated species like cereals and in non-domesticated plants.

For breeding purposes, the correlation between Mg and Zn is of interest, because, as we already mentioned (see Table 3), direct selection for high Zn concentration could be difficult due to high G × E interactions. Thus, if efforts are focused on the easier selection for high Mg concentration, some indirect progress for Zn concentration can also be expected.

4. Conclusion and outlook for human nutrition

Breeding for high Mg concentration should not be too difficult, because genotype effects are high and G × E interactions moderate. For Zn concentration, higher G × E interactions would make direct selection more difficult. However, because Zn and Mg concentrations are positively correlated, Zn concentration should respond favorably to selection for high Mg concentration. For these two minerals, there are negative correlations with yield, and this dilution effect should be taken into account if progress in both productivity and mineral concentrations is the objective. Fe concentration is much more influenced by G × E interactions and poorly correlated to other minerals. Consequently improving Fe concentration by means of selection will probably prove illusory.

Mg and Zn concentrations appeared to be valuable criteria that could be met in breeding programs to improve the nutritional quality of wheat grain for human consumption. Nevertheless, any progress in Mg or Zn concentration will be of no use if white flour remains the primary use of wheat for human food. Today, most of the micronutrients are found in the bran after milling, and it has been shown that mineral intake is negatively influenced by low nutrient density foods like white bread, which are high in calories but low in vitamins and minerals. From a nutritional point of view, it is thus important to either modify the milling process to recover most of the aleurone layer in the flour, or to make better use of bran in human nutrition.

However, it should also be kept in mind that mineral bioavailability is limited by the presence of phytic acid (PA) in the aleurone layer, which forms insoluble complexes with dietary cations, thus hindering their intestinal absorption (Cheryan, 1980). PA breakdown strongly depends on the phytasic activity of the flour (Leenhardt et al., 2005). Consequently both mineral and phytase concentrations should be taken into account in breeding programs. The release of cultivars with high mineral concentrations and high intrinsic phytasic activity could greatly improve the nutritional value of bread, provided that less refined flour is utilized to preserve the source of the minerals. The adoption of bread-making processes that stimulate the phytasic activity of the flour will further enhance the mineral bioavailability of the bread.

Acknowledgements

This project has been sponsored by the French Ministry of Industry and Finance (Program ASG “Cereal genotyping and food quality” no. 01.04.90.6058).

We wish to thank Jean-Claude Tressol, Franck Vallery and Cyril Depozat for their excellent technical assistance.

References

Balint, A.F., Kovacs, G., Erdei, L., Sutka, J., 2001. Comparison of the Cu, Zn, Fe, Ca and Mg contents of the grains of wild, ancient and cultivated species. Cereal Res. Commun. 29, 375–382.

Becker, H.C., Léon, J., 1988. Stability analysis in plant breeding. Plant Breed. 101, 1–23.

Bhaskaram, P., 2001. Immunobiology of mild micronutrient deficiencies. Br. J. Nutr. 85, 75–80.

Brancourt-Hulmel, M., Biarnès-Dumoulin, V., Denis, J.-B., 1997. Points de repère dans l’analyse de la stabilité et de l’interaction génotype-milieu en amélioration des plantes. Agronomie 17, 219–246.

Cakmak, I., Ozkan, H., Braun, H.J., Welch, R.M., Romheld, V., 2000. Zinc and iron concentrations in seeds of wild, primitive, and modern wheats. Food Nutr. B 21, 401–403.

Cheryan, M., 1980. Phytic acid interactions in food systems. CRC Crit. Rev. Food Sci. Nutr. 13, 297–335.

Clarke, J.M., Norvell, W.A., Clarke, F.R., Buckley, W.T., 2002. Concentration of cadmium and other elements in the grain of near-isogenic durum lines. Can. J. Anim. Sci. 82, 27–33.

Davis, K.R., Peters, L.J., Cain, R.F., Le Tourneau, D., Mac Ginnis, J., 1984. Evaluation of the nutrient composition of wheat III. Minerals. Cereal Food. World 29, 246–248.

Dikeman, E., Pomeranz, Y., Lai, F.S., 1982. Minerals and protein contents in hard red winter wheat. Cereal Chem. 59, 139–142.

Durlach, J., 2001. Importance and clinical forms of chronic primary magnesium deficiency in human beings. In: Rayssiguier, Y., Mazur, A., Durlach, J. (Eds.), Advances in Magnesium Research (Nutrition and Health). Libbey, J. and Company Ltd. Publishers, Eastleigh, England, pp. 13–20.

Feil, B., Fossati, D., 1995. Mineral composition of triticale grains as related to grain yield and grain protein. Crop Sci. 35, 1426–1431.

Galand, P., Preciozi, P., Durlach, J., Valeix, P., Ribas, L., Bouzid, D., Favier, A., Hercberg, S., 1997. Dietary magnesium intake in a French adult population. Magnesium Res. 10, 321–328.

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.

Haas, J.D., Brownlie, T., 2001. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. J. Nutr. 131, 691–696.

Hercberg, S., Preciozi, P., Briancon, S., Galand, P., Triol, I., Malvy, D., Roussel, A.M., Favier, A., 1998. A primary prevention trial using nutritional doses of antioxidant vitamins and minerals in cardiovascular diseases and cancers in a general population: the SU.VI.MAX study-design, methods, and participants characteristics. Control Clin. Trials 19, 336–351.

Leenhardt, F., Levrat-Verny, M.A., Chanliaud, E., Rémésy, C., 2005. Moderate decrease of pH by sourdough fermentation is sufficient to reduce phytate content of whole wheat flour through endogenous phytase activity. J. Agric. Food Chem. 53, 98–102.

Lopez, H.W., Krespine, V., Lemaire, A., Coudray, C., Feillet-Coudray, C., Messager, A., Demigné, C., Rémésy, C., 2003. Wheat variety has a major influence on mineral bioavailability: studies in rats. J. Cereal Sci. 37, 257–266.

McGrath, S.P., 1985. The effects of increasing yields on the macro- and microelement concentrations and off-takes in the grain of winter wheat. J. Sci. Food Agric. 36, 1073–1083.

Monasterio, I., Graham, R.D., 2000. Breeding for trace minerals in wheat. Food Nutr. B 21, 392–396.

Nahapetian, A., Bassiri, A., 1976. Variations in concentrations and interrelationships of phytate, phosphorus, magnesium, calcium, zinc and iron in wheat varieties during two years. J. Agric. Food Chem. 24, 947–950.

Oury, F.-X., Carré, B., Pluchard, P., Bérard, P., Nys, Y., Leclercq, B., 1998. Genetic variability and stability of poultry feeding related characters in wheat, in relation to environmental variation. Agronomie 18, 139–150.

Oury, F.-X., Bérard, P., Brancourt-Hulmel, M., Depatureaux, C., Doussinault, G., Galic, N., Giraud, A., Heumez, E., Lecomte, C., Pluchard, P., Rolland, B., Rousset, M., Trottet, M., 2003. Yield and grain protein concentration in bread wheat: a review and a study of multi-annual data from a French breeding program. J. Genet. Breed. 57, 59–68.

Peterson, C.J., Johnson, V.A., Mattern, P.J., 1983. Evaluation of variation in mineral element concentrations in wheat flour and bran of different cultivars. Cereal Chem. 60, 450–455.

Peterson, C.J., Johnson, V.A., Mattern, P.J., 1986. Influence of cultivar and environment on mineral and protein concentrations of wheat flour, bran, and grain. Cereal Chem. 63, 183–186.

Prasad, A.S., 1998. Zinc in human health: an update. J. Trace Elem. Exp. Med. 11, 63–87.

S-PLUS, 1996. S-Plus User's Guide Version 3.4. MathSoft, Seattle, WA.

Stoltzfus, R.J., 2001. Defining iron-deficiency anemia in public health terms: reexamining the nature and magnitude of the public health problem. J. Nutr. 131, 565–567.

Toepfer, E.W., Polansky, M.M., Eheart, J.F., Slover, H.T., Morris, E.R., 1972. Nutrient composition of selected wheats and wheat products XI. Summary. Cereal Chem. 49, 173–186.

Vreugdenhil, D., Aarts, M.G.M., Koornneef, M., Nelissen, H., Ernst, W.H.O., 2004. Natural variation and QTL analysis for cationic mineral content in seeds of *Arabidopsis thaliana*. Plant Cell Environ. 27, 828–839.

Zook, E.G., Greene, F.E., Morris, E.R., 1970. Nutrient composition of selected wheats and wheat products. VI. Distribution of manganese, copper, nickel, zinc, magnesium, lead, tin, cadmium, chromium and selenium as determined by atomic absorption spectroscopy and colorimetry. Cereal Chem. 47, 720–731.