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Quantitative Trait Loci for Biofortification Traits in Maize Grain

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Abstract

Detecting genes that influence biofortification traits in cereal grain could help increase the concentrations of bioavailable mineral elements in crops to solve the global mineral malnutrition problem. The aims of this study were to detect the quantitative trait loci (QTLs) for phosphorus (P), iron (Fe), zinc (Zn), and magnesium (Mg) concentrations in maize grain in a mapping population, as well as QTLs for bioavailable Fe, Zn, and Mg, by precalculating their respective ratios with P. Elemental analysis of grain samples was done by coupled plasma-optical emission spectrometry in 294 F_4 lines of a biparental population taken from field trials of over 3 years. The population was mapped using sets of 121 polymorphic markers. QTL analysis revealed 32 significant QTLs detected for 7 traits, of which some were colocalized. The Additive–dominant model revealed highly significant additive effects, suggesting that biofortification traits in maize are generally controlled by numerous small-effect QTLs. Three QTLs for Fe/P, Zn/P, and Mg/P were colocalized on chromosome 3, coinciding with simple sequence repeats marker bnlg1456, which resides in close proximity to previously identified phytase genes (ZM phys1 and phys2). Thus, we recommend the ratios as bioavailability traits in biofortification research.

Key words: *bioavailability, biofortification, maize (Zea mays), minerals, quantitative trait loci*

Biofortification aims to enhance the mineral concentrations and/or bioavailability in plants through genetic improvement to solve the global mineral malnutrition problem (for a review, Bouis and Welch 2010). The detection of genes that influence biofortification traits in staple food, particularly in cereal grain, could help to increase the concentrations of bioavailable mineral elements in crop cultivars. Bioavailability can be defined as the proportion of the total amount of mineral element that is potentially absorbable in a metabolically active form (House 1999). The main substance known to inhibit the absorption of iron (Fe), zinc (Zn), and magnesium (Mg) from cereal grain is phytate (myo-inositol hexaphosphate) (for review, White and Broadley 2009). The bioavailability of Fe is reduced at dietary phytate/Fe molar quotients greater than 1, and the bioavailability of Zn is reduced when the phytate/Zn molar quotient exceeds about 6 (Lönnerdal 2002). In particular, high phytate/zinc molar ratios are observed in the diets of children from Malawi, Kenya, Mexico, and Guatemala, who consume only unfermented products of maize (*Zea mays*) (Gibson 2006). There are low-phytate strains in maize (Raboy et al. 2000), but they express some less favorable agronomic features because phytate is important for seed germination and seedling growth. Furthermore, phytate has some beneficiary effects, such as inhibition of different types of cancers (Vucenik and Shamsudin 2003; Somasundar et al. 2005). Therefore, biofortification programs usually do not include decreasing phytate as a breeding objective beyond naturally occurring variability (Ortiz-Monasterio et al. 2007).

Phosphorus (P) could be an indicator of phytate because more than 80% of the total P in maize grain is in the form of phytate (Raboy 1997). We have proposed molar ratios between P concentrations and the respective Fe and Zn concentrations as a calculated biofortification trait for predicting Fe and Zn bioavailability (Šimić, Sudar, et al. 2009). Kutman et al. (2011) applied the molar P/Fe and P/Zn ratios in a wheat biofortification program to demonstrate that the improvement of nitrogen nutritional status affected the molar ratios. A rapid and cost-effective in vitro Fe bioavailability model system exists that mimics the gastric and intestinal digestion of humans coupled with the culture of human intestinal epithelial cells (Caco-2) showing Caco-2 formation of ferritin, as a measure of Fe bioavailability (Glahn et al. 1998). However, in vitro models usually do not determine the bioavailability of several elements simultaneously. Thus, ionomic approach (Vreugdenhil et al. 2004; Salt et al. 2008; Waters and Grusak 2008) accounting for simultaneous measurement of numerous

elements seems to be a more feasible model to elucidate the relations among elements essential for biofortification and bioavailability traits in crop plants. To date, there is a lack of published studies addressing the ionomic approach for biofortification on cereals, and just few of them have dealt with its relations with P or phytate (Stangoulis et al. 2007; Shi et al. 2008; Lung'aho et al. 2011).

In fact, there are still a small number of studies focused on the genetics of accumulation of minerals in the grains of major cereals (Tiwari et al. 2009). Objectives of this study were to detect the quantitative trait loci (QTLs) for P, Fe, Zn, and Mg concentrations in the maize grain in a mapping population as well as QTLs for bioavailable Fe, Zn, and Mg by precalculating their respective ratios with P.

Materials and Methods

Plant Material and Field Trials

Two temperate maize inbred lines B84 and Os6-2, which had significantly different ionomic profiles according to our previous study (Brkić et al. 2003), were crossed to develop a mapping population for biofortification studies. The line B84 is a well-known BSSS line, whereas OS6-2 is related to the line C103 of Lancaster origin (Liu et al. 2003). The 294 F_4 families of the population along with 6 checks, which included the parents as 2 entries each, and the subsequent F_1 generation as double entries (total of 300 entries), were grown as field trials in Osijek, Croatia, in 2005, 2006, 2007, and 2008. Details about the trials and material preparations are given elsewhere (Šimić, Sudar, et al. 2009). The experiments were conducted in 2 replications as a 30 × 10 alpha (0,1) design (Patterson and Williams 1976) planted at the end of April and harvested in October. Grain samples were taken from 5 hand-pollinated (selfed) ears to avoid xenia effect. In the statistical analysis of individual trials, the test on outliers by Anscombe and Tukey (1963) was performed to detect extreme residuals (plot errors). If significant at $P = 0.05$, they were declared as missing values and substituted by the estimated values using the iterative method of Healy and Westmacott (1956). Owing to many outliers, especially for Fe concentrations (>30%) in 2005, we discarded the 2005 trial from further statistical analysis.

Elements and Phytate Assaying

All mature grain samples were dried and ground, and the P, Fe, Zn, and Mg concentrations were determined by inductively coupled plasma-optical emission spectrometry technique after microwave digestion in the laboratory of the Research Institute for Soil Science and Agricultural Chemistry of Hungarian Academy of Science in Budapest, Hungary. Kernels were digested in 65% nitric acid (HNO_3) + 30% hydrogen peroxide (H_2O_2) by Milestone MLS 1200 microwave (Zarcinas et al. 1987). Elemental concentrations were expressed on dry matter basis. Bioavailable Fe, Zn, and Mg were estimated as the respective concentration ratios of Fe/P, Zn/P, and Mg/P equivalent to the molar ratios of P/Fe, P/Zn, and P/Mg, assuming a constant proportion of phytate in the total P. To corroborate this assumption, phytate concentrations in the grain (seed) of parent inbred lines B84 and Os6-2 grown in 2009 were measured in the chemical laboratory of the Maize Research Institute "Zemun Polje," Serbia, according to Latta and Eskin (1980) (modified by Sredojevic and Dragicevic 2009). The grain of F_4 families was not available. Phytate was determined colorimetrically in 3 samples of both the parental inbred lines with 2 subsamples of each sample based on the pink color of the Wade reagent, which was formed on the reaction of ferric ion and sulfosalicylic acid having a maximum absorbance at 500 nm. In the presence of phytate, Fe was sequestered and unavailable to react with sulfosalicylic acid, resulting in a decrease in the intensity of pink color.

Genetic and QTL Mapping

Mixtures of the plants of each F_4 line, totally 294 F_4 lines of the population, were genotyped using sets of SNP and simple sequence repeats (SSR) molecular markers. All steps of the DNA analysis were conducted by TraitGenetics GmbH, Germany, according to the standard protocols (Šimić, Ledenčan, et al. 2009). In total, 142 SNP markers (3 multiplexes of 48/47/47 markers) were analyzed. They were derived from a proprietary SNP marker set that has been generated at TraitGenetics, identified through amplicon resequencing method and validated through the analysis of many maize lines (Ganal et al. 2009). SNPlex analysis was performed on an ABI 3730xl DNA sequencer, whereby the internal and external standards were used for size determination. A total of 65 of the 69 prescreened SSR markers were successfully mapped. Data of both the marker systems were combined and mapped using Haldane's mapping function and 121 molecular markers (56 SNP and 65 SSR) (Šimić, Ledenčan, et al. 2009). SNP markers were denoted with "ZM." Data about the SSR markers used are available via the online database, MaizeGDB (Andorf et al. 2010).

Composite interval mapping (CIM) of QTL was performed by PLABQTL computer program (Utz and Melchinger 1996) following the regression approach (Haley and Knott 1992) extended by using cofactors. Cofactors for CIM were selected automatically by the program and added to the regression model with F-to-enter = 3.5. The empirical LOD threshold for $\alpha = 0.05$ were determined by testing 1000 permutations of the data (Churchill and Doerge 1994). In a final fit, the detected QTLs were used for a simultaneous multiple regression. The proportion of phenotypic variance explained by the QTLs in the model with adjustment for the number of terms in the multiple regression model, the adjusted R^2 (R^2_{adj}), was calculated as described by Hospital et al. (1997).

Statistical Analysis

A simultaneous fit with the detected QTLs was performed for each environment. Subsequently, the results of the QTL ANOVA were presented showing the results of F tests in combined QTL-ANOVA, which included environments,

genotypes, and genotypes × environment interaction effects as the main effects. The genotypes effect was subdivided into QTL effect and residuals, whereas the genotypes × environment interaction was subdivided into QTL × environment interaction and residuals × environment interaction (latter not shown). Heritability on a genotype (entry) mean basis (Hallauer and Miranda Fo 1988) was estimated as $h^2=(s^2_G \times 100)/(s^2_{GE}/e + s^2_e/re + s^2_G)$, where s^2_G is the estimate of the genotypic variance, s^2_{GE} is the estimate of the genotype × environment interaction variance, s^2_e is the estimate of error variance, e is the number of environments, and r is the number of replications per environment.

After the combined QTL-ANOVA, percentage of the explained genotypic variance was estimated, which is generally smaller than the corresponding estimate obtained from the simultaneous fit because it is adjusted for QTL × environment interaction, avoiding an overestimation of the genetic variance explained by the QTL. This statistic refers to the proportion of the genetic variance explained by the detected putative QTL and is calculated as the ratio Q_2 = VCq/VC(genotypes) × 100, where VCq is an ad hoc estimator computed by the difference of 2 variance components and VC is the genotypic variance component. They were calculated analogously by Bliss (1967) and Knapp (1994).

For each QTL, a 1-LOD score support interval was used (Lander and Botstein 1989). QTLs for different traits were declared as colocalized QTLs when their 1-LOD support interval overlapped. Coefficient of determination or the percentage of the phenotypic variance, which is explained by a putative QTL (partial R^2), is based on the partial correlation of the putative QTL with the observed variable adjusted for cofactors. Dominance was included in the model, which affected the calculation of partial R^2, genotypic variance, and LOD scores (Utz and Melchinger 1996).

Results

All 7 grain biofortification traits in experiments over 3 years exhibited wide ranges for 294 F_4 lines of the population (Figure 1). The P concentrations were shifted upward in 2007 (Figure 1a), whereas Zn concentrations and Zn/P ratios were shifted downward in the same year (Figure 1d,e). Although no considerable shifting appeared for the Mg concentration (Figure 1f), the Mg/P ratios (Figure 1g) were shifted upward in 2008. The P, Fe/P, Zn/P, and Mg/P ratios had nearly normal frequency distributions, suggesting that these traits may be controlled by greater number of genes when compared with the mineral concentrations. The bioavailability traits are likely "normalized" by way of dividing 1 skewed trait distribution by another.

As indicated in 3 individual experiments over the years, highly significant effects of environments and genotypes for all traits were confirmed by combined ANOVAs (Table 1). However, genotype × environment interactions were not significant for all traits due to considerable smaller genotype × environment variances than the respective genotypic variances (variances not shown). Partitioning of the genotype sum of squares revealed highly significant effects of detected QTLs for all traits, except for Zn concentrations, which was significant at $P \leq 0.5$. QTL × environment interactions were highly significant for P, Fe/P, and Zn. However, detailed analysis of QTL × environment interactions for each QTL revealed that only 2 of the 8 QTLs for P and 2 of 7 QTLs for Fe/P had significant mean squares of the QTL × environment interaction (data not shown). No particular QTL had significant QTL × environment interactions for Fe, Zn/P, Mg, and Mg/P. Heritability estimates on genotype mean basis ranged from 52.9 for Zn/P to 71.0 for Mg/P.

When pooled across the 2006, 2007, and 2008 experiments (Table 2), the means of parent lines B84 and Os6-2 differed significantly from each other only for Fe and Fe/P. The total mean of the F_4 population for P and Mg was beyond the means of both the parents, whereas that for Fe, Fe/P, Zn, Zn/P, and Mg/P was within the range of the 2 parental lines. Seed analysis from 2009 revealed no significant variation between the 2 parents for both total P and phytate concentrations. Similar proportion of phytate in the total P of about 84% was measured in both the parents.

Strict empirical LOD thresholds almost completely excluded QTLs with LOD < 4.00 (Table 3). The greatest number of QTLs was detected for P concentration and Mg/P, whereas Zn concentration and Zn/P ratio had only one significant QTL, respectively. The greatest respective percentages of phenotypic (R^2_{adj}) and genotypic (Q_2) variances were estimated for Mg/P, followed by Fe/P and P. Accordingly, the smallest R^2_{adj} and Q_2 values were estimated for Zn and Zn/P.

In the final simultaneous fit, 32 significant QTLs were detected for 7 biofortification traits, of which some were colocalized (Table 4). The detected QTLs were located on all 10 maize chromosomes, except chromosome 7. The greatest number of QTLs (6) was located on chromosome 6, where overlapping of chromosome regions occurred according to the respective support intervals for 4 respective QTLs for P, Fe, Fe/P, and Mg. Three QTLs for Fe/P, Zn/P, and Mg/P were colocalized on chromosome 3. Significant QTLs for P concentrations included 2 with LOD scores higher than 8 on chromosomes 9 and 10. The greatest LOD scores of 14.00 and 12.77 were found for 2 unique QTLs for Mg/P, with no significant dominant effect (not shown). Generally, the additive–dominant model revealed mostly highly significant additive effect with no significant dominant effect (latter not shown).

Discussion

Genetic material, soil properties, environmental conditions, and nutrient interactions affect the level of mineral concentrations in grains (House 1999). In our study, the concentrations of P, Fe, Zn, and Mg in maize grains were also influenced by environmental and genotypic effects, as observed in the results for P, Fe, and Zn obtained from the 2-year trial (Šimić, Sudar, et al. 2009). However, no significant genotype × environment interactions were detected. Our

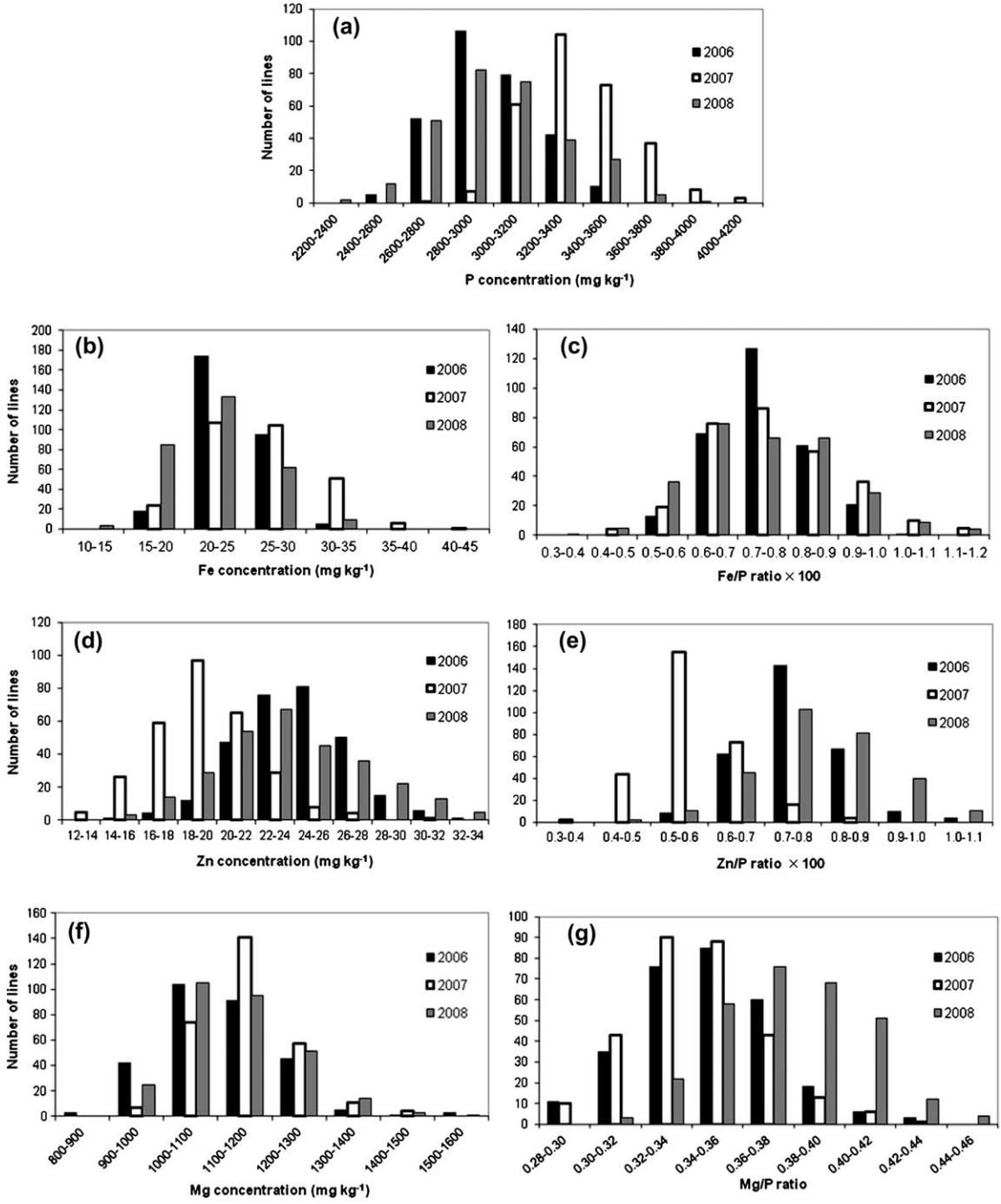

Figure 1. Histograms of 7 grain biofortification traits in 294 F_4 lines of a biparental maize population measured in field trials for 3 years. (**a**) Frequency distribution of grain P concentration. (**b**) Frequency distribution of grain Fe concentration. (**c**) Frequency distribution of Fe/P ratio. (**d**) Frequency distribution of grain Zn concentration. (**e**) Frequency distribution of Zn/P ratio. (**f**) Frequency distribution of grain Mg concentration. (**g**) Frequency distribution of Mg/P ratio.

finding agrees with the previous studies on the importance of genotype × environment interactions of P-related traits, including total P and phytate concentrations (Raboy and Dickinson 1984; Wardyn and Russell 2004; Israel et al. 2006; Lorenz et al. 2008), where the genotype × environment interactions were mostly not significant. If there were no adverse soil chemical properties (for review, Cakmak 2008) or extreme weather conditions as in our study, the genotype × environment interactions could be detected largely due to the changes in the magnitude of genotype differences rather than those in the genotype ranks. Relatively small, though partly significant, genotype × environment interactions for Fe and

Table 1 *F* statistics and significance levels of the effects of mean squares as well as heritability ± standard error (SE) for 7 biofortification traits in combined QTL-ANOVA across 3 environments

Source	P	Fe	Fe/P	Zn	Zn/P	Mg	Mg/P
Environments	270.7***	93.7***	94.6***	309.4***	677.9***	21.5***	282.7***
Genotypes	2.8***	2.7***	3.0***	2.5***	2.1***	2.5***	3.4***
QTL	5.4***	4.1***	6.0***	3.4*	5.0***	7.0***	9.1***
Residuals	1.8***	2.3***	2.1***	2.5***	2.0***	1.6***	1.8***
Genotype × Environment	0.6 ns	0.7 ns	0.6 ns	0.7 ns	0.7 ns	0.7 ns	0.5 ns
QTL × Environment	2.3***	1.9**	2.3***	3.6***	0.9 ns	1.5**	1.3*
Heritability ± SE	63.9 ± 3.6	63.6 ± 3.7	66.3 ± 3.4	60.4 ± 4.0	52.9 ± 4.8	59.9 ± 4.0	71.0 ± 2.9

*significant at $P \leq 0.5$, **significant at $P \leq 0.05$,*** significant at $P \leq 0.01$, ns, not significant.

Zn grain concentrations were found in rice and wheat as reviewed by Welch and Graham (2002). However, Oikeh et al. (2004) found highly significant genotype × environment interactions for Fe and Zn concentrations in grains of 20 tropical maize genotypes, and Oikeh et al. (2003) reported about significant genotype × environment interactions for kernel–Fe in 49 tropical maize varieties grown at 6 environments in Africa where adverse soil chemical properties commonly occur.

The biparental B84 × Os6-2 population does not seem to be an ideal mapping population for biofortification studies because no significant differences between the parents were found for P, Zn, and Mg. Both the parents belong to high-phytic maize genotypes, in which 84% of the total P was likely bound by phytate, representing the fundamental fraction of total P. Raboy et al. (2001) stated when measurements were made among cultivars and breeding families, the correlation between phytate and total P was typically >0.90. This indicates that selection for reduced phytate would decrease total P without repartitioning the P bound in phytate to inorganic P, an undesirable product considering animal nutrition. Besides biofortification importance of decreasing total P because of phytate, decreasing the grain total P is also significant for the long-term goal of sustainable and environmental friendly agricultural production (Raboy 2009). Therefore, total grain P itself could be of interest in plant breeding research.

Greater number of detected QTLs for P concentration, Fe/P, and Mg/P ratios indicate that a greater number of genes than mineral concentrations might control these traits. The exception is the Zn/P ratio possibly due to a small percentage of genotypic variances explained by the detected QTL. Eight QTLs for Mg/P ratio explained 67% of the genotypic variance, indicating that these QTLs captured the majority of genes that might control bioavailable Mg in maize grain.

Three QTLs for Fe/P, Zn/P, and Mg/P were colocalized on chromosome 3, coinciding with the SSR marker bnlg1456. Although bnlg1456 was not the closest marker to the QTL for P on chromosome 3 on position 10 cM (the first QTL presented in Table 4), we may declare that the 3 QTLs for Fe/P, Zn/P, and Mg/P and the QTL for P as colocalized as well because their 1-LOD support interval overlapped. Additionally, ratio trait variation mapping to bnlg1456 and P trait variation proximal to this marker are likely controlled by the same locus because the variation is derived from the same parental contribution (smaller P derived from the parent B84; larger ratio also derived from B84) (Table 4). According to Maize Genetics and Genomics Database (Andorf et al. 2010), very near to bnlg1456 marker on chromosome 3, *phys1*, and *phys2* genes are located, which encode phytase, an enzyme that can break down the phytate and thus release digestible P, Fe, Zn, and Mg. A cDNA encoding maize phytase was cloned and characterized (Maugenest et al. 1997), as well as the structure and expression of the 2 phytase genes were presented (Maugenest et al. 1999).

This demonstrates that all 3 calculated biofortification traits of Fe/P, Zn/P, and Mg/P ratios precisely detected a candidate gene for increasing Fe, Zn, and Mg bioavailability. However, the QTLs presumably associated with *phys1* and *phys2* genes are additive, small-effect QTLs. It seems surprising that the phytase activity is present in mature seed (grain) because its activity is usually known only during germination. However, Liu et al. (2007) found significant phytase activity in mature wheat grain. Further

Table 2 Means ± standard errors for P, Fe, Zn, Mg, and phytate concentrations (mg/kg) as well as ratios Fe/P, Zn/P, and Mg/P in the maize grain of the parental lines B84 and Os6-2 and the biparental population consisting of 294 F_4 lines

	Averaged across the 2006, 2007, and 2008 experiments							Seed 2009	
Genotype	P	Fe	Fe/P	Zn	Zn/P	Mg	Mg/P	P	Phytate
B84	3132 ± 135	24.1 ± 2.2	0.77 ± 0.08	21.77 ± 1.9	0.70 ± 0.08	1081 ± 79	0.35 ± 0.03	3553 ± 182	3002 ± 176
Os6-2	3099 ± 134	19.4 ± 1.9	0.63 ± 0.07	22.70 ± 2.0	0.73 ± 0.08	1048 ± 77	0.34 ± 0.03	3605 ± 194	3043 ± 172
F_4 population	3193 ± 109	24.1 ± 1.8	0.75 ± 0.05	22.40 ± 1.5	0.70 ± 0.05	1131 ± 47	0.35 ± 0.01	—	—

Table 3 Values of empirical LOD determined by testing 1000 permutations of data (Churchill and Doerge 1994), subsequent number of significant QTL, adjusted percentage of phenotypic variance (R^2_{adj}), and percentage of genotypic variance (Q_2) explained by the detected QTLs for 7 biofortification traits in maize grain

Trait	Empirical LOD threshold ($\alpha = 0.05$)	Number of significant QTL	R^2_{adj} (%)	Q_2 (%)
P	4.13	8	28.4	44.3
Fe	4.07	3	21.1	17.0
Fe/P	4.09	7	33.2	49.7
Zn	4.04	1	4.2	6.4
Zn/P	3.96	1	3.6	7.3
Mg	4.00	4	21.0	35.3
Mg/P	3.92	8	46.4	66.5

physiological and QTL studies on phytate concentrations in maize, including in vitro/in vivo models, should validate these findings.

On chromosome 6, overlapping of chromosome regions according to the respective support intervals occurred for 4 QTLs for P, Fe, Fe/P, and Mg. According to an abstract by Hoekenga et al. (2007) and a study of Lung'aho et al. (2011), QTLs associated with Fe bioavailability determined by an in vitro digestion/Caco-2 cell line bioassay were also identified on chromosomes 3 and 6. In our study, there were 3 unique QTLs for bioavailability traits on chromosome 1: one for Fe/P and 2 for Mg/P ratios, and there were no detected significant QTL on chromosome 1 for measured Fe and Mg concentrations. This suggests that the calculated bioavailability traits (ratios) might have partially different sets of genes controlling them. Generally, majority of all QTLs detected had no obvious candidate genes for mineral accumulation, thus providing initial information toward new gene discovery for control of biofortification traits.

Significant additive effects with no significant dominant effects suggest that biofortification traits in maize are mostly controlled by numerous small-effect QTLs, and they are predicted by a simple additive model. Brkić et al. (2003)

Table 4 Significant QTL for 7 biofortification traits of grain in a maize population combined over 3 environments

Trait	Chromosome–bin	Closest marker	Position (cM)	Support interval (cM)	LOD	Partial R^2 (%)	Additive effect
P	3-05	umc59e	10	6–14	5.98	8.9	−64.34**
P	3-09	bnlg1257	48	44–52	6.48	9.7	−73.34**
P	4-08	ZM0819	48	42–52	6.69	10.2	64.18**
P	6-03	umc1887	20	14–24	4.89	7.4	−68.32**
P	6-05	ZM1367	34	30–36	5.42	8.4	64.13**
P	8-05	ZM0353	26	22–32	4.50	6.8	47.09**
P	9-02	bnlg0244	16	12–20	8.39	12.3	−71.88**
P	10-07	bnlg1839	32	28–32	8.67	12.7	65.73**
Fe	2-05	ZM1368	40	36–44	4.44	6.8	−0.86**
Fe	6-03	ZM0960	24	22–26	4.93	7.5	1.15**
Fe	8-06	ZM0825	32	26–36	4.44	6.8	−1.02*
Fe/P	1-05	ZM0845	34	30–40	4.55	6.9	8.78**
Fe/P	2-05	ZM1368	40	38–44	7.82	11.5	−11.97**
Fe/P	3-05	bnlg1456	16	12–18	6.54	9.7	11.57**
Fe/P	4-07	bnlg1784	32	30–34	4.73	7.1	−10.67**
Fe/P	6-01	bnlg0426	10	6–12	4.73	7.1	−7.64**
Fe/P	6-03	ZM0960	24	22–26	6.75	10.0	12.78**
Fe/P	10-06	ZM1315	26	20–28	5.95	8.9	−9.84**
Zn	4-08	ZM1362	44	40–46	5.19	7.8	0.88**
Zn/P	3-05	bnlg1456	16	12–18	5.21	7.8	5.87**
Mg	5-03	bnlg1046	18	16–20	4.44	6.7	23.63**
Mg	6-01	bnlg426	16	10–22	4.65	7.0	−24.29**
Mg	8-05	bnlg1782	24	22–26	5.03	7.6	26.33**
Mg	9-07	bnlg0128	44	40–48	7.96	12.1	31.82**
Mg/P	1-01	bnlg1014	0	0–4	6.00	9.3	−0.05**
Mg/P	1-03	phi109275	20	18–26	5.96	8.9	0.07**
Mg/P	3-05	bnlg1456	16	14–18	5.65	8.5	0.05**
Mg/P	4-08	ZM0819	48	42–54	5.34	8.2	−0.05**
Mg/P	5-03	ZM0215	12	8–16	14.00	19.9	0.10**
Mg/P	9-02	bnlg244	16	12–20	5.28	7.9	0.05**
Mg/P	9-07	umc1675	34	28–38	5.02	7.6	0.05**
Mg/P	10-04	ZM0363	12	6–14	12.77	19.3	−0.08**

Chromosome number-bin, relative position of the LOD peak with 1-LOD support interval, partial phenotypic variance (R^2), and significance of additive effect. Bin refers to the concept of dividing 10 maize chromosomes into 100 segments (bins) of approximately 20 cM between 2 fixed core markers (Gardiner et al. 1993). A negative value of additive effect indicates that the allele of the first parent decreases the trait value.

*Significant at $\alpha = 0.05$, **significant at $\alpha = 0.01$.

reported about simple genetic control of Fe and Zn accumulation, suggesting the possibility of more rapid developments in biofortification. As we previously stated (Šimić, Sudar, et al. 2009), a considerable increase in mineral concentrations as well as a decrease in their ratios with P seem to be achievable after a number of generations of selection. It could probably take fewer generations than improvement for protein and oil concentrations due to less number of genes involved in the control of mineral accumulation.

We conclude that the ratios of mineral elements with P as the calculated biofortification traits could be distinct traits and not merely linear functions of the respective measured concentrations having a partially different set of genes controlling them. These results support the validity of calculating the ratios of element concentrations to understand the genetic control of some complex relations in the plant ionome.

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