

A simple method to evaluate genetic variation in grain zinc concentration by correcting for differences in grain yield

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Abstract Increasing the grain zinc (Zn) concentration of staple food crops will help alleviate chronic Zn deficiency in many areas of the world. Significant variation in grain Zn concentration is often reported among collections of cereals, but frequently there is a concomitant variation in grain yield. In such cases grain Zn concentration and grain yield are often inversely related. Without considering the influence of the variation in grain yield on Zn concentration, the differences in grain Zn concentration may simply represent a yield dilution effect. Data from a series of field and glasshouse experiments was used to illustrate this effect and to describe an approach that will overcome the yield dilution effect. In experiments with a wide range of bread wheat, synthetic hexaploids and accessions of durum wheat, variation in grain yield among the genotypes accounted for 30–57% of the variation in grain Zn concentration.

Variation in kernel weight also occurred, but was poorly correlated with grain Zn concentration. To account for the influence of variation in grain yield on grain Zn concentration grain Zn yield was plotted against grain yield. By defining the 95% confidence belt for the regression genotypes that have inherently low or high grain Zn concentrations at a given yield level can be identified. This method is illustrated using two data sets, one consisting of bread wheat and one comprising a collection of synthetic hexaploids.

Keywords Grain · Biofortification · Genotype · Breeding · Micronutrient

Introduction

The past decade has seen much interest in developing varieties of staple grain crops with enhanced concentration of micronutrients to improve the nutritional quality of grain for human consumption (Graham et al. 1999; Grusak and DellaPenna 1999, White and Broadly 2005; Cakmak 2008). An additional benefit of enhanced grain micronutrient concentrations is an increase in seedling vigour and grain yield when available soil nutrients are low (Yilmaz et al. 1998).

Zinc is one of the nutrients being targeted in biofortification programs. Zinc deficiency limits the grain yield of crops in many parts of the world (Graham and Welch 1996; Cakmak 2008) and it is an important nutritional problem in a large number of

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developing countries (Holtz and Brown 2004). Significant differences in grain Zn concentration have been reported and often wild relatives of wheat have been suggested to be useful sources of high grain Zn (Cakmak et al. 1999; Calderini and Ortiz-Monasterio 2003a; Cakmak et al. 2004). There are also recent studies which have shown that the expression of the NAM genes can enhance grain Zn and iron (Fe) concentrations, possibly by controlling the degree of remobilization from the leaves (Uauy et al. 2006). However, in most of these studies the discussion has focused only on grain nutrient concentrations, with little acknowledgement that grain yield may be an important factor influencing the differences in nutrient concentration. Occasionally kernel weight data are presented. In studies that use a diverse array of germplasm there is generally a significant variation in grain yield, and the differences in grain Zn concentrations may simply represent a yield dilution effect.

A number of studies have shown that the yield increases achieved by plant breeding have resulted in reduced concentrations of grain nitrogen (N) and phosphorus (P) when nutrient supply is non-limiting to yield (Slafer et al. 1990; Calderini et al. 1995; Ortiz-Monasterio et al. 1997). These data highlight the importance of the yield dilution effect when discussing breeding solutions to enhanced grain nutrient loading. There is less information on the influence of yield on micronutrient concentration. Ortiz-Monasterio and Graham (2000) (cited in Calderini and Ortiz-Monasterio 2003b) described a weak association between grain Fe and Zn concentration and yield among CIMMYT germplasm, inferring the effect may be less with some micronutrients. A comparison between a single synthetic hexaploid and two high yield bread wheat cultivars showed that the synthetic hexaploid had greater micronutrient concentrations in the grain, but part of this effect could be ascribed to its 30% lower grain yield, as total grain nutrient content did not differ significantly among the three genotypes (Calderini and Ortiz-Monasterio 2003a). Using principal component analysis, Distelfeld et al. (2007) also found a negative relationship between grain yield and grain Zn and Fe concentrations. It is clear that the yield dilution effect may have a large and sometimes overriding effect on grain nutrient concentration. Measuring grain Zn concentration without considering the yield of the genotype may result in wrong conclusions about the

true level of genetic variation in grain Zn concentration being drawn.

There is often a significant variation in kernel weight among diverse genotypes, which may also contribute to the phenotypic variation in the concentration of grain Zn. Grain nutrient concentration is affected more by grain position within the ear than by whether the grain is located on the main stem or tillers (Simmons and Moss 1978; Calderini and Ortiz-Monasterio 2003a,b). Distal grains tend to be smaller with a lower nutrient concentration. Yield increases in modern wheat cultivars are associated with higher numbers of kernels, which are largely caused by increases in grain set in the distal spikelets and florets. The variation in mean kernel weight and its relationship with grain nutrient concentration among genotypes showing large variation in grain nutrient concentration needs to be examined.

The grain Zn concentration of a genotype reflects its ability to take up Zn from the soil, to mobilize Zn from different parts of the plant and to load it in the grain (Pearson et al. 1995; Genc et al. 2006). It is desirable to combine the ability to load Zn into the grain with a high yield potential. To obtain reliable information of the level of genetic variation in grain nutrient concentration, any possible dilution and concentration effects associated with differences in yield potential need to be taken into account. In this paper we illustrate the influence of differences in yield potential on grain Zn concentration and suggest a method of data analysis that will identify genotypes with high grain Zn concentration reliably.

Materials and methods

Data on grain yield and grain Zn concentration were obtained from two field experiments that investigated the responsiveness to Zn within bread wheat (*Triticum aestivum*). These experiments were conducted at Birchip, Victoria in 1999 and 2000 and compared the response to Zn (nil Zn and + Zn treatments) among advanced breeding lines and commercial cultivars. Each plot consisted of eight rows, 15 cm apart and 5 m long. Seed was planted with a cone seeder at a rate of 180 seeds m⁻². Basal fertiliser was applied at sowing at a rate of 190 kg ha⁻¹ and consisted of 15:17:0 (N/P/K) coated with Cu, Co, Mn and Mo. No Zn was added in the basal fertiliser.

Weeds were controlled using recommended rates of herbicides and there were no significant foliar or root diseases within the trials that would influence yield. The experimental design was a split plot, randomised complete block with three or four replicates. Genotype was the main plot and Zn treatment was the sub plot. For the purpose of the analysis, only results from the +Zn treatment were used as the aim of the analysis was to examine variation in grain Zn when the supply of Zn was adequate.

Data was also obtained from three pot experiments that examined the grain Zn concentration within a collection of 192 accessions of durum wheat (*T. turgidum*) obtained from the Australian Winter Cereals Collection, a collection of primary synthetics and a range of synthetic hexaploid wheat lines (Genc and McDonald 2004). These lines were grown to evaluate their potential as sources of high grain Zn. The plants were grown in a glasshouse in a standard potting mix which provided an adequate supply of nutrients throughout the growing season.

Grain Zn concentration was measured by inductively coupled plasma atomic spectrometry (ICP-AS) after digestion in nitric acid (Zarcinas et al. 1987).

Grain Zn yield (grams per hectare for the field trials or grams per plant for the pot trials) was calculated by multiplying the grain yield by the grain Zn concentration.

Data analysis

The grain yield, grain Zn concentration and grain Zn yield were analysed by ANOVA. The relationship between grain Zn concentration and grain yield or kernel weight were examined by calculating the simple linear correlation.

To identify lines which had a high grain Zn concentration that was independent of grain yield, a linear regression method was used. The regression of grain Zn yield against grain yield, forced through the origin, was fitted. The 95% confident band around this regression was then calculated and genotypes lying outside this band were identified. The slope of the line represents the mean grain Zn concentration for the experiment. Genotypes that fall within the confidence band are not significantly different to the mean and so variation in grain Zn concentration among these genotypes largely represents a dilution

Table 1 Summary of the values for grain Zn concentration, kernel weight and grain yield of field and glasshouse experiments that examined the genetic variation in grain Zn

Experiment	No. lines	Mean	Minimum	Maximum	Coeff variation (%)
Grain Zn concentration (mg kg ⁻¹)					
Birchip 1999	42	25.2±2.49	19.9	30.9	9.9
Birchip 2000	28	16.3±1.83	12.4	20.1	11.2
Durum accessions	192	30.3±11.49	12.0	86.0	38.0
Primary synthetics	31	41.2±9.18	28.0	66.0	22.3
Synthetic hexaploids	45	42.5±12.89	24.0	103.3	30.3
Kernel weight (mg)					
Birchip 1999	42	30.6±3.16	24.0	39.0	10.3
Birchip 2000	28	32.9±2.35	28.4	38.2	7.1
Durum accessions	192	50.7±8.21	29.5	84.4	16.2
Primary synthetics	31	50.9±4.02	43.7	59.6	7.9
Synthetic hexaploids	45	n.a.	n.a.	n.a.	n.a.
Grain yield (t ha ⁻¹ or g plant ⁻¹) ^a					
Birchip 1999	42	3.3±0.26	2.7	3.7	7.8
Birchip 2000	28	2.4±0.31	1.7	3.1	13.1
Durum accessions	192	5.8±1.93	1.4	11.7	33.4
Primary synthetics	31	n.a.	n.a.	n.a.	n.a.
Synthetic hexaploids	45	8.6±3.33	1.1	21.0	38.7

n.a. Not available

^aUnits for grain yield: Birchip 1999 and Birchip 2000: t ha⁻¹; durum accessions and synthetics: g plant⁻¹

effect associated with the differences in grain yield. Genotypes that lie outside the confidence band have grain Zn yield either significantly higher or lower than that expected from their grain yield; that is the grain Zn concentration is inherently higher or lower than the average for the sample and is a better indicator of the level of genetic variation at a given level of yield.

Results

Within each data set there was a high level of variation for grain Zn concentration and this variation was particularly evident among the exotic germplasm collections (Table 1). Notably, one synthetic hexaploid had a grain Zn concentration of 103 mg/kg. There was also significant variation in grain yield and in kernel weight. Based on the coefficients of variation, the durum accessions and synthetics showed greater variation in grain yield and grain Zn concentration than bread wheat, but variation in kernel weight tended to be less.

Grain Zn concentration was negatively correlated with grain yield in three of the four data sets where yield was recorded (Table 2). Figure 1 illustrates the inverse relationship between grain yield and grain Zn concentration in these data sets and it is evident from this that the synthetic hexaploid with the very high grain Zn concentration had a low grain yield. The variation in grain yield accounted for 30–57% of the variation in grain Zn concentration (Fig. 1). In contrast, kernel weight had relatively little influence on grain Zn concentration and was only correlated with grain Zn concentration within the durum accessions (Table 2). In this data set grain yield and kernel weight were significantly, but weakly correlated ($r=0.243$, $P<0.001$), whereas there was no significant correlation between yield and kernel weight in the other data sets.

The relationships between grain Zn yield and grain yield for bread wheat grown in the field and the synthetic hexaploid wheat grown in the glasshouse are shown in Fig. 2. Each showed a significant linear trend, with the stronger relationship evident in the synthetic hexaploid wheat. There are a number of genotypes that lie outside the 95% confidence band and some with much greater grain Zn concentrations than would be predicted from their yield. However,

the synthetic hexaploid wheat line with the very high grain Zn concentration (103 mg/kg) lies on the confidence band, which suggests that the major reason for its high grain Zn concentration was its low yield.

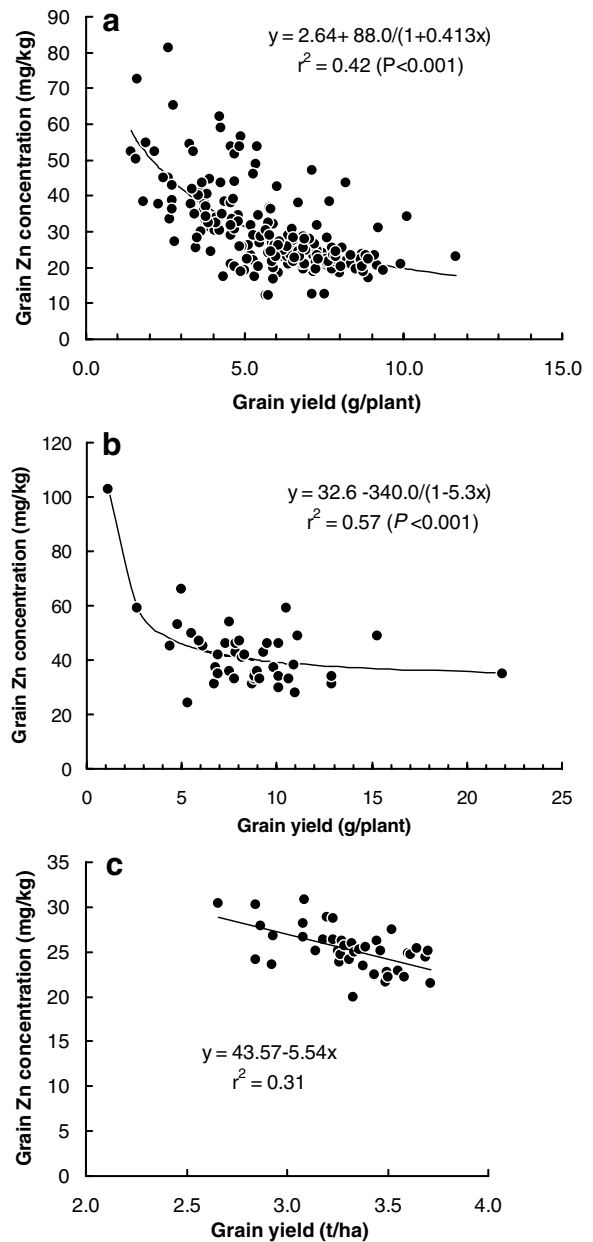


Fig. 1 The relationship between grain yield and grain Zn concentration for (a) accessions of durum wheat, (b) synthetic hexaploid wheat lines grown in the glasshouse and (c) bread wheat grown in the field at Birchip 1999

Table 2 Simple correlation coefficients between grain Zn concentration and grain yield and kernel weight within field and glasshouse experiments that examined the genetic variation in Zn nutrition

Experiment 1	Grain yield	Kernel weight
Birchip 1999	-0.570**	-0.094
Birchip 2000	0.166	-0.050
Durum accessions	-0.613**	-0.298**
Primary synthetics	n.a.	0.162
Synthetic hexaploids	-0.430*	n.a.

n.a. Not available

* $P < 0.01$

** $P < 0.001$

Discussion

Irrespective of whether plants were grown in the field or in the glasshouse, variation in grain yield influenced grain Zn concentration. The exception was at Birchip in 2000 where grain Zn concentrations, even following the addition of Zn, were low, suggesting the plants were still Zn deficient. However, in the absence of Zn stress, high grain Zn concentration was negatively correlated with grain yield, which clearly illustrates that selection for high grain Zn without consideration of the influence of grain yield will not necessarily identify genotypes that have an enhanced ability to load Zn into the grain. Up to 57% of the variation in grain Zn was found to be related to the differences in grain yield among the genotypes. In contrast there was no relationship between kernel weight and grain Zn concentration except within the durum accessions. These lines showed the greatest variation in kernel weight and kernel weight and grain yield were significantly correlated, which may help explain this result. Therefore experimental data on genetic variation in grain Zn concentrations, without supporting data on grain yield should be interpreted with some caution.

The importance of grain yield to the interpretation of grain Zn data is likely to be related to the observation that increases in grain yield in wheat are more strongly associated with increases in kernel number than in kernel weight (Sadras 2007). Higher grain numbers are due to greater grain set in distal florets and spikelets (Slafer et al. 1994), which tend to have lower micronutrient concentrations (Calderini and Ortiz-Monasterio 2003b). Consequently, the inverse relationship between yield and grain Zn

concentration within these experiments may reflect the large number of grains in distal positions with low nutrient concentrations in the high-yielding genotypes. The inverse relationship between yield and grain Zn may also reflect partitioning of a relatively fixed amount of Zn in the vegetative tissue among a variable number of grains. Increasing total Zn uptake so that there is a greater pool of Zn that can be remobilized to the grain may therefore be an avenue for improving grain Zn concentration.

The poor association between grain Zn and mean kernel weight implies that selection for high grain Zn may not necessarily affect kernel weight. However, without more detailed analysis of the relationships between kernel number, kernel weight and grain

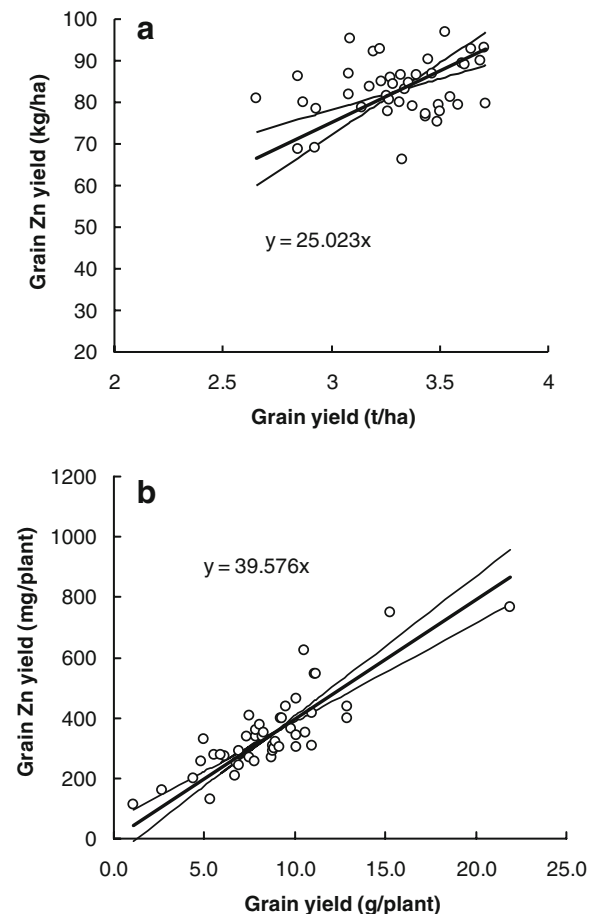


Fig. 2 The relationship between grain Zn yield and grain yield for (a) commercial bread wheat cultivars and advanced breeding lines at Birchip 2000 and (b) accessions of durum wheat grown in the glasshouse. Each point is a single genotype and the regression line \pm the 95% confidence interval is shown

nutrient concentrations among a diverse set of varieties it is difficult to draw definite conclusions. There is some support for the idea that selection for enhanced grain Zn may not alter kernel weight from the data of Calderini and Ortiz-Monasterio (2003b). They showed that grain position within the ear affected kernel weight and grain Zn concentration but there was no relationship between the two in a comparison of three genotypes. The observation that kernel weight is poorly correlated with grain Zn concentration suggests that conclusions about dilution effects in genotypes showing differences in grain Zn concentration need to be based on grain yield rather than kernel weight.

The relationship between grain yield and grain Zn yield was used as the basis of the approach because this provides a linear relationship in which the scatter of points is affected by differences in grain Zn concentration. In essence the regression describes the relationship between grain yield and grain Zn loading to the grain, and so it integrates the physiological processes of Zn uptake and partitioning to the grain. The slope represents the mean grain Zn concentration within the population and so the scatter of points represents deviations from the mean that is assessed using a confidence interval. The approach has an intuitive biological basis. Alternatively, a linear relationship could also be achieved by using the inverse of grain Zn concentration.

When regression analysis was used to explore the data, there were a number of lines in each trial with a grain Zn yield significantly greater or less than that predicted from its grain yield. This indicates there were differences in grain Zn concentration that were independent of the yield potential of the genotype and it is these lines that should be targeted as sources of improved grain Zn. The scatter of points shows that lines with the same yield but different capacities to load Zn into the grain can be identified, and these can be used for further studies to explore the physiological bases and genetic mechanisms of high grain Zn.

The method described here is based on data routinely collected when screening for enhanced grain micronutrient and so it can be easily applied without the need for further plant or grain analysis. Moreover, it can be applied retrospectively to data sets to check previous conclusions where grain yield was also measured. Grain yield shows large G×E effects and this may also affect the interpretation. Therefore,

further analysis of data in which there are common genotypes across sites and seasons is needed to examine whether lines show consistently high grain Zn concentrations. However, the method is able to overcome the problem of screening for grain Zn concentration among genotypes that differ in yield potential and it is particularly suitable for initial screening and identification of suitable parents.

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