Variation in sweetcorn kernel Zn concentration a reflection of source-sink dynamics influenced by kernel number

Zhong Xiang Cheah¹*, Tim J. O’Hare², Stephen M. Harper³, Michael J. Bell¹²

¹ The University of Queensland, School of Agriculture and Food Sciences, Gatton, Queensland, 4343 Australia; ² Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Gatton, Queensland, 4343; ³ Australia Department of Agriculture and Fisheries Gatton, Queensland, 4343 Australia.

For correspondence. Email: cheah.zhong.xiang@uqconnect.edu.au

Phone: +614-32144420

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Highlight: As kernel number increase, kernel Zn concentration decreased. However total kernel Zn accumulated per cob increased, as small decreases in kernel Zn concentration were offset by increases in kernel number.
Abstract

Grain yield and mineral nutrient concentration in cereal crops are usually inversely correlated, undermining biofortification efforts. Sink size, expressed as kernel number per cob, was manipulated by controlling the time when the silks of sweetcorn (*Zea mays*) cv. Hybrix 5 and var. HiZeax 103146 were exposed to pollen. Twelve other varieties were manually pollinated to achieve maximum potential kernel number per cob, and kernel Zn concentration was correlated with kernel number and kernel mass. As kernel number increased, kernel Zn concentration decreased, with that decrease occurring to similar extents in both the embryo tissue and rest of the kernel. However, total kernel Zn accumulated per cob increased with increasing kernel number, as the small decreases in individual kernel Zn concentration were more than offset by increases in kernel number. When both kernel number and mass were considered, 90% of the variation in kernel Zn concentration was accounted for. Differential responses in assimilate and Zn distribution to sweetcorn cobs led to significant decreases in kernel Zn concentration with increasing kernel number. This suggests there will be challenges to achieving high kernel Zn concentrations in modern high-yielding sweetcorn varieties unless genotypes with higher Zn translocation rates into kernels can be identified.

**Keywords:** Biofortification, grain, kernel mass, kernel number, maize, source-sink dynamics, sweetcorn, yield, *Zea mays*, zinc
Abbreviations:

DAP  days after pollination

DM  dry mass
Introduction

Micronutrient deficiency is a severe nutritional problem affecting approximately 30% of the world’s population, mainly in developing countries (Kennedy et al., 2003). One approach to addressing this malnutrition issue has been through agronomic and genetic biofortification that aims to increase the target micronutrient concentration in the edible fraction of staple crops. Examples of species where intensive biofortification efforts have been made include rice (*Oryza sativa*), wheat (*Triticum aestivum*) and maize (*Zea mays*) (White and Broadley, 2009). Despite these efforts, achieving the combination of high yield and high micronutrient concentration has proved challenging, as crop yield is usually inversely correlated to mineral nutrient concentration (Davis et al., 2004; Murphy et al., 2008). For example, a dilution in N, protein and oil concentration of shoot and kernels has been observed with increasing maize shoot biomass and kernel yield (Scott et al., 2006; Riedell, 2010; Abdala et al., 2018). Consequently, an assessment of kernel micronutrient concentration without consideration of yield may lead to misleading genotypic selections that do not provide successful biofortification outcomes.

An increase in grain crop yield may be due to an increase in kernel number or individual kernel mass (*i.e.* larger kernels) or both, with each parameter influenced by genetic and environmental factors (Sadras, 2007). For modern maize hybrids, yield improvement has mostly been achieved through large increases in kernel number (Fischer and Palmer, 1984), which more than offsets small decreases in individual kernel mass caused by limitations in the availability of assimilates (Echarte et al., 2000). Varietal differences in kernel number and individual kernel mass between genotypes may also contribute to variation in kernel micronutrient concentration (McDonald et al., 2008). However, the evidence for these effects is not conclusive, with other studies finding the correlation between kernel Zn or Fe concentration and grain yield to be weak or non-significant (Ortiz-Monasterio and Graham, 2000; Long et al., 2004; Chakraborti et al., 2009). The opportunity for micronutrient biofortification in high yielding maize varieties is therefore not well characterised.

Sweetcorn (*Zea mays* ssp. *saccharata*), which has a sugary endosperm conferred by a single gene mutation (Creech, 1965; Simonne et al., 1999), is a close relative of maize. The micronutrients of importance to human health such as Zn are found in high concentrations in the scutellum of sweetcorn and maize embryos (Cheah et al., 2019b). In dry milling of maize, this micronutrient-rich embryo is typically removed during maize kernel processing, whereas in sweetcorn the entire kernel is consumed. This potential dietary advantage in sweetcorn is negated by the fact that most of the embryo Zn is in the form of Zn-phytate, which is not bioavailable to humans (Cheah et al., 2019a), although Zn accumulated in the endosperm has been shown to be mostly bioavailable. Therefore, to achieve a beneficial outcome for human health, genotypes that accumulate Zn in the endosperm are preferable to those that accumulate Zn in the embryo.
In previous experiments, we have observed that apparent genotypic differences in kernel Zn concentration were negatively correlated with kernel number per cob; viz. the net Zn supplied to a cob was diluted over a greater number of kernels. The objective of this study was to quantify the response of sweetcorn kernel Zn concentration to kernel yield within and between sweetcorn varieties. The relative changes in accumulation and distribution of Zn between the embryo and rest of the kernel with increasing kernel number were also evaluated. A better understanding of the relationship between kernel number and Zn accumulation will underpin successful selection strategies in breeding programs that aim to develop sweetcorn varieties with high yield and elevated Zn accumulation.

Materials and methods

Experimental setup

The experiments were conducted at the Gatton Research Facility of the Department of Agriculture and Fisheries (Gatton, Australia) on a Vertisol soil (Soil Survey Staff, 2014) with a DTPA-extractable Zn concentration of 4.6 mg kg\(^{-1}\). Fourteen sweetcorn varieties were grown for this study (Table 3) at a density of 60,000 plants ha\(^{-1}\). A pre-planting basal fertiliser consisting of 55 kg N ha\(^{-1}\), 10 kg P ha\(^{-1}\), 55 kg K ha\(^{-1}\) and 80 kg S ha\(^{-1}\) was applied. Additionally, a urea-NH\(_4\)-NO\(_3\) mix (Easy-N\(^{®}\)) at a rate of 13.5 kg N ha\(^{-1}\), ammonium sulfate ((NH\(_4\))\(_2\)SO\(_4\)) at a rate of 125 kg ha\(^{-1}\) and magnesium sulfate (MgSO\(_4\)) at a rate of 10 kg ha\(^{-1}\) were each applied once using drip irrigation. Finally, zinc oxide (ZnO) was applied once at the 12-leaf stage at a rate of 0.6 kg Zn ha\(^{-1}\) as a foliar application. During the growth period, the average minimum and maximum temperatures were 15 and 30°C respectively, whereas the relative humidity range was 44-59%. A completely randomised design was used.

Experiment 1: Accumulation of Zn and carbohydrate in embryo and rest of the kernel at various maturity stages

Kernel number of the commercial variety cv. Hybrix 5 was manipulated by controlling the duration of silks exposure to pollen. Immediately prior to silk emergence, the sweetcorn ears of all plants were covered with a plastic bag. A visual assessment of peak pollen production (approximately two weeks after ear bagging) was used as a basis for treatment implementation. At this time bags were removed to expose the emerged silks to pollen for 0.5, 1, 2, 4, 8, 12 or 24 hours and subsequently covered with a paper bag to prevent further pollination. Ears were subsequently harvested at 18, 21, 24 and 28 days after pollination (DAP). The 21 and 24 DAP harvest times were equivalent to normal commercial sweetcorn harvest, with the kernels at 18 DAP being slightly immature and at 28 DAP being over-mature.
At harvest, cobs were categorised as well pollinated (> 350 kernels), moderately pollinated (150-350 kernels) or poorly pollinated (< 150 kernels). Five cobs were sampled from each category and 10 kernels were extracted from each cob. The kernels were dissected into embryo tissue and rest of the kernel (including the endosperm, aleurone and pericarp tissues), which were measured separately for tissue dry mass (DM) and Zn concentration. Kernel Zn content was then calculated as the sum of the products of each tissue Zn concentration (i.e. embryo or rest of the kernel) and DM content. The rates of kernel Zn and kernel dry matter accumulation were calculated using the formula:

\[ R = \frac{(x_{\text{end}} - x_{\text{start}})}{(n \times d)}, \]

where \(x_{\text{start}}\) and \(x_{\text{end}}\) represent the Zn or dry matter content at the start and end of a period, \(n\) represents the number of kernels, and \(d\) represents the number of days within that period.

**Experiment 2: Assessment of the relationship between kernel number and Zn concentration within varieties**

This experiment evaluated the relationship between kernel number and Zn concentration by comparing the sweetcorn varieties cv. Hybrix 5 and var. HiZeax 103146. Kernel number was manipulated using the method described in Experiment 1. Ears were then harvested at 21 DAP, kernel number was determined and 10 kernels were extracted for nutrient analysis. Exposing silks to varying durations of pollen exposure produced cobs with a wide variation in kernel number (a range of 1 to 554 kernels per cob). However, cobs with less than 10 kernels were excluded from the analysis due to an insufficient kernel mass for Zn determination. Total kernel Zn mass per cob was calculated using total kernel dry weight (g per cob) and Zn concentration (mg kg\(^{-1}\)).

**Experiment 3: The contribution of differences in kernel number and individual kernel mass to variation in kernel Zn concentration between sweetcorn varieties**

Fourteen sweetcorn varieties (Table 3) were manually self-pollinated to achieve maximum potential kernel number. These varieties were harvested at the sweetcorn eating stage of 21 DAP and kernel number per cob was determined. Ten kernels were extracted from each cob and DM and mineral nutrient concentration determined as per Experiment 1. The relationship between kernel number, kernel DM and kernel Zn concentration were determined.
Mineral analysis

Kernels were removed from the cob for fresh and dry mass determination, and tissue Zn concentration was determined on oven-dried (70°C) samples. Dried kernel samples were digested in 6 mL nitric acid and 2 mL perchloric acid at 150 °C before being made up to 20 ml with deionized water. The digested samples were analysed using inductively coupled plasma optical emission spectrometer (ICP-OES; Optima 7300 DV, Perkin Elmer; Wellesley, MA, USA) (Zasoski and Burau, 1977).

Results

Accumulation of dry matter and Zn during kernel development

The accumulation of Zn and dry matter in individual kernels in cobs of cv. Hybrix 5 with varying kernel numbers at different maturity stages (18-28 DAP) are shown in Fig. 1 and Table 1. The rate of accumulation of both Zn and dry matter was greatest at 21-24 DAP and was either maintained or declined to different extents at 24-28 DAP (Table 1). As kernel numbers increased, the relative changes in dry matter and Zn content were different. Specifically, the average Zn accumulation rate from 18 to 28 DAP was 0.7 µg Zn kernel⁻¹ day⁻¹ in poorly pollinated cobs (< 150 kernels), 0.5 µg kernel⁻¹ day⁻¹ in moderately pollinated cobs (150-350 kernels) and 0.3 µg kernel⁻¹ day⁻¹ in well-pollinated cobs (> 350 kernels). Kernels in well-pollinated cobs therefore accumulated Zn at ca. 40% of the rate in kernels on poorly-pollinated cobs. In contrast, the average kernel dry matter accumulation rate for the same cob classifications decreased from 17.1 mg kernel⁻¹ day⁻¹ with low kernel numbers to 14.8 and 12.2 mg kernel⁻¹ day⁻¹ in cobs with intermediate and high kernel numbers, respectively. Kernels in well-pollinated cobs therefore accumulated assimilate at ca. 70% of the rate in kernels on poorly-pollinated cobs – a much smaller reduction in accumulation than recorded for kernel Zn.

Partitioning of Zn between embryo and rest of the kernel

Given the marked difference in the relative accumulation of Zn and assimilate in kernels on cobs with different kernel numbers, the distribution of Zn between the embryo and rest of the kernel was also examined using cobs of cv. Hybrix 5 with varying kernel numbers. The Zn concentration in both embryo tissue and rest of the kernel decreased by ca. 18-33% as kernels matured over the period from 18-28 DAP (Table 2). Despite the decrease in tissue Zn concentration, the Zn content of both constituents increased over the 18-28 DAP period as tissue DM increased (Fig. 2). The rest of the kernel constituted a much larger proportion of the kernel dry matter at all stages of kernel development (viz. 95% at 18 DAP and decreasing
to 89% at 28 DAP). Hence, despite the much higher Zn concentration in embryo tissue (Table 2), the rest of
the kernel constituted the major proportion of the whole kernel Zn content at all maturity stages (Fig. 2). The
proportion of the kernel Zn content in the embryo doubled over the sampling period, from 15-21% at 18
DAP to 32-36% at 28 DAP, reflecting relatively greater increases in embryo mass over the period. It is
worth noting that kernel number had no real effect on the ratio of Zn content in the embryo and rest of the
kernel at any stage of kernel development. This similarity in response in both tissues indicated that in cobs
with poor kernel establishment, the additional Zn available to each kernel was not preferentially stored in
either tissue but distributed at similar proportions across both (Fig. 2).

Relationship between kernel number, kernel mass and kernel Zn concentration

The relationship between kernel Zn concentration and kernel number was similar for both varieties,
showing a consistent small decline (p = 0.003) in kernel Zn concentration as kernel number increased from
ca. 50 kernels to the respective maximums in well-pollinated cobs (ca. 450 kernels in var. HiZeax 103146
and ca. 550 kernels in cv. Hybrix 5, Fig. 3a). While the magnitude of the decline in Zn concentration with
increasing kernel number appeared to be slightly greater for var. HiZeax 103146, the difference was not
significant (p = 0.261). There appeared to be a relatively sharp increase in kernel Zn concentrations at very
low kernel numbers (< ca. 50 kernels) in cv. Hybrix 5, and although there was only one cob with kernel
numbers that low in var. HiZeax 103146, that sample also showed a similarly large increase in kernel Zn
concentration. A significant varietal effect was also observed, with var. HiZeax 103146 being 8.6 ± 1.2 mg
kg⁻¹ (p < 0.001) higher in kernel Zn concentration compared with cv. Hybrix 5 at any given kernel number
between ca. 50-550 kernels (Fig. 3a).

Due to the statistically significant but relatively small decrease in kernel Zn concentrations with
increasing kernel number for cobs with > 50 kernels, total kernel Zn mass per cob for both varieties
increased with increasing kernel number (Fig. 3b). The overall higher kernel Zn concentration resulted in a
ca. 30% higher (p = 0.002) total kernel Zn mass per cob in var. HiZeax 103146 than in cv. Hybrix 5 in cobs
with kernel number range from ca. 200 to 450 kernels.
Relationships between kernel yield and Zn concentration in a collection of sweetcorn varieties

The manually pollinated cobs from the 14 sweetcorn varieties produced different numbers of kernels, with different kernel mass and different kernel Zn concentrations (Table 3). A visual assessment of cobs showed pollination effectiveness (the proportion of total cob length hosting kernels) ranged from 70 to 100%. There was a strong correlation between kernel Zn concentration and kernel number ($R^2 = 0.57$, $p = 0.002$, Fig. 4a) across the genotypes but no correlation existed between kernel mass and Zn concentration ($R^2 = 0.05$, $p = 0.450$, Fig. 4b), suggesting that kernel number was the more important factor in determining genotypic differences in kernel Zn concentration. There appeared to be a subset of four genotypes where higher Zn concentrations were recorded than would be expected from the kernel numbers present, with a separate relationship for kernel number and kernel Zn concentration established for this subset (Fig. 4a). These genotypes recorded kernel Zn concentrations that were $11.9 \pm 2.9$ mg Zn kg$^{-1}$ higher ($p = 0.002$) for any given kernel number than the broader population, but unfortunately the combination of low kernel numbers and low kernel DM resulted in two of these genotypes (56.3-1 and 14-6) providing only low-moderate total kernel Zn mass per cob (Table 3).

The relationship between yield and kernel Zn was explored, using total kernel DM per cob as a surrogate for yield (i.e. the product of kernel number and kernel DM). As expected, there was a negative correlation between total kernel DM and kernel Zn concentration ($p = 0.017$, Fig. 5a). However, total kernel DM was positively correlated to total kernel Zn mass accumulated per cob ($p < 0.001$, Fig. 5b), with the variability in this relationship a measure of differences in the capacity of different varieties to translocate Zn into kernels. Multiple linear regression analysis showed that most of the variability in the total kernel Zn mass per cob ($R^2 = 90.3$, $p < 0.001$) across these genotypes could be accounted for by differences in kernel number per cob and individual kernel Zn concentration. Different genotypes were therefore able to be ranked in terms of their ability to partition Zn into developing kernels (Table 3).
Discussion

Effects of variability in yield influenced by source-sink dynamics on kernel Zn concentration

The dilution of mineral nutrient concentrations with high biomass production or grain yield is a well-reported phenomenon in crops (Jarrell and Beverly, 1981; Davis, 2009; Riedell, 2010). However, there are no studies that have explored the physiological mechanisms underpinning the negative correlation between micronutrient concentrations and grain yield. In this study, we examined the relationship between kernel Zn concentration and the two components of grain yield in sweetcorn, namely kernel number and kernel mass. Both these factors are important for yield improvement, as changes in kernel number may be compensated for by changes in kernel mass and vice versa (Sadras, 2007).

We demonstrated that as the sink demand for assimilates increased due to increasing numbers of established kernels, the relative change in rate of accumulation of carbohydrates in kernels was relatively insensitive, compared to that of kernel Zn (Fig. 1, Table 1). The suggestion that assimilates distribution to developing kernels was not strongly source-limited was consistent with the accumulation behaviour of assimilates in maize, where assimilate accumulation was found to be sink-limited in most growing conditions and source-limited only if assimilate availability was reduced during grain filling due to poor growing conditions (Echarte et al., 2000; Borrás et al., 2004).

The greater sensitivity of Zn accumulation to increasing sink size (greater kernel numbers) was manifested in declining individual kernel Zn concentration with increasing kernel numbers, and was indicative of source limitations that will constrain Zn accumulation in kernels of sweetcorn. This relationship was explored in two varieties, a commercially cultivated variety cv. Hybrix 5 and an experimental variety previously identified for its higher kernel Zn concentration, var. HiZeax 103146. We found that the same negative correlation between kernel Zn concentration and kernel number existed in both varieties (Fig. 2a), indicating that it is possible that observed differences in kernel Zn concentration between varieties could be strongly influenced by the number of kernels set. Indeed, this negative correlation between kernel Zn concentration and kernel number explained a significant proportion of the variation in kernel Zn concentration across a broader population of 14 genotypes (Fig. 3a).

Whilst the relationship between kernel number (one of the main contributors to yield increases in sweetcorn breeding) and kernel Zn concentration was observed to hold across a set of 14 genotypes, the relationship between kernel mass (the other key yield key determinant in sweetcorn) and kernel Zn concentration was weak (Fig. 4b). This suggests that the decreases in kernel Zn concentration that has been observed with increasing sweetcorn yields are likely to be mainly driven by increases in kernel number instead of kernel mass. Kernel mass in maize was reported to be strongly influenced only by reduction in potential assimilate availability leading to reduced kernel mass, but increase in potential assimilate...
availability did not result in improved kernel mass (Borrás et al., 2004). Conversely, the selection pressure for higher yields in maize, achieved mainly through increased kernel numbers (Fischer and Palmer, 1984), could have unintentionally selected for lower kernel Zn concentration in modern maize hybrids.

While individual kernel Zn concentration decreased with increasing yield, the total amount of Zn being translocated into kernels increased with yield, maximising the overall total kernel Zn mass per cob (Fig. 5b). The small decrease in individual kernel Zn concentration associated with increases in kernel number is more than offset by the increase in kernel mass per cob, and suggests that the extent of source limitations constraining kernel Zn concentration are relatively small (Fig. 3b). This implies that while both individual kernel Zn concentration and total kernel yield are both valid selection parameters, the most efficient way of increasing total Zn yield ha⁻¹ in the edible product is by increasing sweetcorn yield, regardless of whether that increase is due to higher kernel numbers, higher kernel DM or both. Future studies could explore whether the negative correlation between kernel number and Zn concentration only applies to a single cob, or to multiple cobs on a plant. If the former, there could therefore be potential to increase kernel Zn concentrations while maintaining or improving yield by selecting for genotypes with multiple, smaller cobs that each support fewer kernels.

Breeding strategies to improve dietary Zn intake from sweetcorn

From the dietary perspective, there are two different breeding strategies to achieve biofortification outcomes in high-yielding varieties, depending on the targeted market and consumption pattern. If catering to markets that consume whole cobs, selection for any combination of increased kernel number and/or kernel mass will increase the total kernel Zn mass per cob, and hence dietary intake. However, in markets where cut cobettes or processed kernels in fixed weight packaging are preferred, the dietary intake will be based on kernel concentration so higher yields will have to be achieved through larger kernel mass to avoid any negative impacts of increased kernel numbers on kernel Zn concentration. Given the dominance of increasing kernel number driving yield increases in modern maize hybrids (Fischer and Palmer, 1984), and the apparently strong environmental impacts on stability of genotypic differences in individual kernel mass, the latter strategy presents significant challenges.

On a positive note, var. HiZeax 103146 exhibited higher kernel Zn concentration than cv. Hybrix 5 at any given kernel number (Fig. 3a). This suggested that there are genotypes which are more efficient at translocating Zn into cobs for distribution across the developing kernels, a trait independent of kernel number variation, which was also observable in a subset of the 14 genotypes from Experiment 3 (Fig. 4a). Although the actual mechanisms for efficient uptake and accumulation of Zn in these varieties are unclear and warrant further investigation, these traits offer valuable resources to be exploited for genetic
biofortification of Zn in sweetcorn. Any genetic advances will need to be supported by appropriate agronomic biofortification strategies to achieve the desired enhancement of Zn partitioning and the concentration of Zn in individual kernels.

Accumulation of Zn and assimilates in embryo and rest of the kernel

Given the importance of speciation to the bioavailability of Zn for absorption after consumption, the allocation of Zn between embryo and endosperm tissues will be an important factor in securing desired biofortification outcomes. This is important because of the contrasting Zn concentrations (Table 2) and bioavailability between these kernel constituents, with Zn in embryo tissues stored predominantly as Zn-phytate which has low human bioavailability, whereas Zn stored in the endosperm is complexed with N- or S-containing ligands of higher bioavailability (Cheah et al., 2019a). In addition, the stability of that Zn allocation between kernel constituents in response to increasing source limitations caused by increasing kernel number will be particularly important for breeding programs seeking to improve both yield and bioavailable Zn content in sweetcorn.

In this study, the trends in Zn concentration and Zn content of both embryo tissue and rest of the kernel as the kernels mature on a well-pollinated cob of sweetcorn were similar to those observed in earlier studies (manuscript in review). We showed that the ratio of embryo Zn content to Zn content in rest of the kernel was constant irrespective of established kernel number, suggesting there was no preferential storage of Zn in either tissue with increasingly constrained Zn supplies. This implies that in order to further improve sweetcorn as a source of dietary Zn, there may be scope change the relative proportions of Zn in different kernel constituents by reducing the mass and/or volume ratio of the embryo relative to the endosperm or rest of the kernel (Zhang et al., 2012; Nagasawa et al., 2013; Chen et al., 2014; Golan et al., 2015; Suzuki et al., 2015).
Conclusion

As the number of established kernels on a sweetcorn cob increased, plants were able to better maintain the rate of assimilate supply to developing kernels than they were the rate of Zn supply. This resulted in a decrease in kernel Zn concentration as kernel number increased. However, increases in kernel mass per cob (the product of kernel number and dry matter content) due to increased kernel number more than offset these small decreases in individual kernel Zn concentrations, such that the total kernel Zn mass per cob was maximised when grain yield was maximised. This result suggests that in the absence of sink size limitations, increasing the supply of Zn into the kernels, either through enhanced genotypic efficiency in translocating Zn or ensuing adequate Zn availability via agronomic means, would be needed to maintain high kernel Zn concentrations. Targeted crosses of high-yielding varieties with efficient kernel Zn accumulation could potentially achieve concomitant improvements in both Zn concentration and yield in modern sweetcorn cultivars.
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Figure legends

**Fig. 1:** Accumulation of (a) Zn content and (b) dry matter content in individual kernels of sweetcorn (*Zea mays*) variety cv. Hybrix 5. Kernels were extracted from cobs that supported < 150 kernels (triangles), 150-350 kernels (squares) and > 350 kernels (circles). Samples were harvested at 18, 21, 24 and 28 days after pollination (DAP).

**Fig. 2:** Accumulation of embryo (clear) and rest of kernel (shaded) Zn content in individual kernels of sweetcorn (*Zea mays*) variety cv. Hybrix 5 at 18, 21, 24 and 28 days after pollination (DAP) for cobs supporting different numbers of established kernels (> 350 kernels, red; 150-350 kernels, green; < 150 kernels, yellow). Percentage values show the proportion of whole kernel Zn content contained in embryo tissue. Groups with different alphabets indicate significant differences in whole kernel Zn content.

**Fig. 3:** The relationships between sweetcorn (*Zea mays*) kernel number, kernel Zn concentration (on a dry mass (DM) basis) and total kernel Zn mass per cob in varieties cv. Hybrix 5 (circle) and var. HZI03146 (triangle) at 21 days after pollination (DAP).

**Fig. 4:** Relationships between kernel Zn concentration and (a) kernel number or (b) kernel dry mass for 14 sweetcorn (*Zea mays*) varieties grown in the field. Dashed lines in (a) show 95% confidence intervals.

**Fig. 5:** Relationships between (a) kernel Zn concentration or (b) total kernel Zn mass per cob and total kernel dry mass per cob for 14 sweetcorn.
Table 1: Accumulation rate of kernel Zn content and dry matter content in sweetcorn (*Zea mays*) variety cv. Hybrix 5 between 18-28 days after pollination (DAP) when cobs were well, moderately, or poorly pollinated.

| Pollination success | Kernel number | Zn content (µg kernel\(^{-1}\) day\(^{-1}\)) | Dry matter content (mg kernel\(^{-1}\) day\(^{-1}\)) | Mean (18-28) |
|---------------------|---------------|--------------------------------|--------------------------------|--------------|
|                     | 18-21         | 21-24                        | 24-28                        |              |
| Poorly pollinated   | < 150         | 0.3                          | 0.9                          | 0.8          |
|                     | 21-24         | 0.8                          | 0.7                          | 0.8          |
|                     | 24-28         | 0.6                          | 0.5                          | 0.7 ± 0.2    |
|                     | Mean (18-28)  | 0.7 ± 0.2                    | 9.8                          | 23.1         |
|                     | 18-21         | 21-24                        | 24-28                        | 18.2         |
|                     | Mean (18-28)  | 17.1 ± 3.9                   | 14.8 ± 3.8                   |
| Moderately pollinated| 150-350      | 0.3                          | 0.7                          | 0.5          |
|                     | 21-24         | 0.5                          | 0.5                          | 0.5 ± 0.1    |
|                     | 24-28         | 0.2                          | 0.3                          | 0.3 ± 0.1    |
|                     | Mean (18-28)  | 0.5 ± 0.1                    | 9.1                          | 22.2         |
|                     | 18-21         | 21-24                        | 24-28                        | 13.6         |
|                     | Mean (18-28)  | 14.8 ± 3.8                   | 12.2 ± 2.8                   |
| Well pollinated     | > 350         | 0.3                          | 0.6                          | 0.2          |
|                     | 21-24         | 0.2                          | 0.6                          | 0.3 ± 0.1    |
|                     | 24-28         | 0.1                          | 0.2                          | 0.3 ± 0.1    |
|                     | Mean (18-28)  | 0.3 ± 0.1                    | 12.0                         | 17.7         |
|                     | 18-21         | 21-24                        | 24-28                        | 8.2          |
|                     | Mean (18-28)  | 12.2 ± 2.8                   | 12.2 ± 2.8                   |
Table 2: Zn concentration and dry mass of embryo, rest of kernel and whole kernel in sweetcorn (*Zea mays*) variety cv. Hybrix 5 at 18, 21, 24 and 28 days after pollination (DAP) on cobs with different kernel number. Means within type of tissues with different alphabets are significantly different.

| Tissue            | Zn concentration (mg kg\(^{-1}\) DM) | Kernel dry mass (mg kernel\(^{-1}\)) |
|-------------------|--------------------------------------|-------------------------------------|
|                   | 18 DAP | 21 DAP | 24 DAP | 28 DAP | 18 DAP | 21 DAP | 24 DAP | 28 DAP |
| **Embryo**        |        |        |        |        |        |        |        |        |
| > 350 kernels     | 150 ± 2.6 | 157 ± 4.9 | 114 ± 3.5 | 100 ± 7.4 | f | 3 ± 0.3 | j | 5 ± 0.1 | h | 13 ± 0.2 | e | 19 ± 0.0 | c |
| 150-350 kernels   | 136 ± 9.6 | 130 ± 4.6 | 105 ± 3.8 | 101 ± 5.3 | f | 4 ± 0.4 | i | 7 ± 0.9 | g | 17 ± 0.1 | d | 28 ± 0.8 | a |
| < 150 kernels     | 167 ± 4.2 | 135 ± 7.1 | 142 ± 6.7 | 134 ± 2.5 | cd | 2 ± 0.4 | k | 5 ± 0.2 | h | 11 ± 0.6 | f | 23 ± 0.2 | b |
| **Rest of kernel**|        |        |        |        |        |        |        |        |
| > 350 kernels     | 31 ± 1.6 | 26 ± 1.4 | 25 ± 0.9 | 23 ± 1.5 | e | 67 ± 4.0 | f | 101 ± 6.2 | d | 146 ± 5.2 | c | 173 ± 6.1 | b |
| 150-350 kernels   | 31 ± 1.6 | 28 ± 0.0 | 25 ± 0.7 | 25 ± 1.8 | de | 74 ± 2.0 | e | 99 ± 5.8 | d | 155 ± 4.1 | c | 199 ± 7.5 | a |
| < 150 kernels     | 38 ± 0.5 | 32 ± 1.1 | 32 ± 1.1 | 31 ± 0.5 | b | 60 ± 1.4 | g | 87 ± 8.9 | d | 151 ± 6.9 | c | 211 ± 9.8 | a |
| **Whole kernel**  |        |        |        |        |        |        |        |        |
| > 350 kernels     | 37 ± 1.2 | 32 ± 2.9 | 32 ± 0.7 | 30 ± 2.0 | de | 70 ± 3.1 | h | 106 ± 8.3 | e | 159 ± 6.4 | d | 192 ± 5.1 | b |
| 150-350 kernels   | 37 ± 1.6 | 35 ± 1.1 | 33 ± 1.6 | 34 ± 2.0 | cde | 78 ± 2.4 | g | 106 ± 7.2 | e | 172 ± 2.7 | c | 227 ± 6.1 | a |
| < 150 kernels     | 43 ± 1.1 | 38 ± 0.9 | 39 ± 0.4 | 41 ± 1.2 | a | 63 ± 3.8 | i | 92 ± 3.1 | f | 162 ± 6.5 | d | 234 ± 2.1 | a |
Table 3: Properties of sweetcorn (Zea mays) varieties, n = 5. Classification of Zn partitioning capacity was added based on data of total kernel Zn mass per cob obtained in this study. The first four varieties above the line expressed relatively higher kernel Zn concentration for the given kernel number (see Fig. 4).

| Variety             | Kernel number | Kernel DM (mg kernel\(^{-1}\)) | Total kernel mass (g cob\(^{-1}\)) | Kernel Zn concentration (mg kg\(^{-1}\) DM) | Total kernel Zn mass per cob (µg cob\(^{-1}\)) | Zn partitioning capacity |
|---------------------|---------------|---------------------------------|-------------------------------------|------------------------------------------|-----------------------------------------------|-------------------------|
| O2su                | 458 ± 16      | 46 ± 6                          | 21.07 ± 0.10                        | 29.8 ± 3.2                               | 627.9 ± 0.32                                  | Very good               |
| HZ103146            | 361 ± 31      | 56 ± 1                          | 20.22 ± 0.03                        | 32.6 ± 0.8                               | 659.2 ± 0.02                                  | Very good               |
| 56.3-1              | 225 ± 31      | 40 ± 3                          | 9.00 ± 0.09                         | 41.4 ± 3.3                               | 372.6 ± 0.30                                  | Moderate                |
| 14-6                | 146 ± 47      | 34 ± 5                          | 4.96 ± 0.24                         | 43.9 ± 6.4                               | 217.7 ± 1.54                                  | Poor                    |
| Garrison (commercial)| 487 ± 14     | 57 ± 3                          | 27.76 ± 0.04                        | 19.9 ± 1.0                               | 552.4 ± 0.04                                  | Good                    |
| TF 2                | 432 ± 18      | 37 ± 3                          | 15.98 ± 0.05                        | 23.7 ± 1.2                               | 378.7 ± 0.06                                  | Moderate                |
| Hybrix 5 (commercial)| 430 ± 20     | 55 ± 2                          | 23.65 ± 0.04                        | 25.4 ± 0.7                               | 600.7 ± 0.03                                  | Very good               |
| fl2sh2              | 360 ± 36      | 26 ± 6                          | 9.36 ± 0.22                         | 24.9 ± 1.9                               | 233.1 ± 0.42                                  | Poor                    |
| EM 540              | 326 ± 47      | 42 ± 2                          | 13.69 ± 0.09                        | 24.5 ± 1.1                               | 335.4 ± 0.10                                  | Moderate                |
| 6-1 × 15-2          | 311 ± 33      | 62 ± 5                          | 19.28 ± 0.17                        | 28.3 ± 1.1                               | 545.6 ± 0.19                                  | Good                    |
| 23-6                | 269 ± 20      | 39 ± 4                          | 10.49 ± 0.08                        | 29.3 ± 1.6                               | 307.4 ± 0.13                                  | Moderate                |
| 23-1                | 230 ± 43      | 51 ± 3                          | 11.73 ± 0.13                        | 33.2 ± 2.6                               | 389.4 ± 0.34                                  | Moderate                |
| 23-7                | 190 ± 11      | 42 ± 3                          | 7.98 ± 0.03                         | 30.0 ± 0.4                               | 239.4 ± 0.01                                  | Poor                    |
| 13-2                | 181 ± 35      | 44 ± 5                          | 7.96 ± 0.18                         | 33.4 ± 2.0                               | 265.9 ± 0.36                                  | Poor                    |
Fig. 1: Accumulation of (a) Zn content and (b) dry matter content in individual kernels of sweetcorn (*Zea mays*) variety cv. Hybrix 5. Kernels were extracted from cobs that supported < 150 kernels (triangles), 150-350 kernels (squares) and > 350 kernels (circles). Samples were harvested at 18, 21, 24 and 28 days after pollination (DAP).
Fig. 2: Accumulation of embryo (clear) and rest of kernel (shaded) Zn content in individual kernels of sweetcorn (Zea mays) variety cv. Hybrix 5 at 18, 21, 24 and 28 days after pollination (DAP) for cobs supporting different numbers of established kernels (> 350 kernels, red; 150-350 kernels, green; < 150 kernels, yellow). Percentage values show the proportion of whole kernel Zn content contained in embryo tissue. Groups with different alphabets indicate significant differences in whole kernel Zn content.
Fig. 3: The relationships between sweetcorn (*Zea mays*) kernel number, kernel Zn concentration (on a dry mass (DM) basis) and total kernel Zn mass per cob in varieties cv. Hybrix 5 (circle) and var. HZ103146 (triangle) at 21 days after pollination (DAP).
Fig. 4: Relationships between kernel Zn concentration and (a) kernel number or (b) kernel dry mass for 14 sweetcorn (Zea mays) varieties grown in the field. Dashed lines in (a) show 95% confidence intervals.
Fig. 5: Relationships between (a) kernel Zn concentration or (b) total kernel Zn mass per cob and total kernel dry mass per cob for 14 sweetcorn