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**Title**

Increasing the supply of sulphur increases the grain zinc concentration in bread and durum wheat.

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## INTRODUCTION

Improving the concentration of the zinc (Zn) and iron (Fe) in the grain of cereal crops to enhance their nutritive value and improve human health, or biofortification, has been a focus of much research over the past decade ([Graham et al 1999](#), [Grusak 2002](#), [Cakmak 2008](#)). The two approaches to enhance grain nutrient density are by plant breeding and agronomic biofortification (Pfeiffer and McClafferty 2007, [Cakmak 2008](#)). However progress in implementing these strategies is hindered by our incomplete understanding of the controls and environmental influences on the uptake, transport, remobilisation and loading of Zn into the grain.

The grain Zn concentration is often significantly correlated with the concentrations of other macro- and micronutrients ([Garvin et al. 2006](#), [McDonald et al 2007](#)). Analyses of grain mineral nutrient concentrations often show the concentrations of Zn (and Fe) are significantly correlated with grain sulphur (S) concentrations. S-containing molecules, such as cysteine, glutathione and methionine, metallothioneins figure prominently Zn uptake, transport and homeostasis or are precursors of molecules that mediate Zn uptake and transport, such as nicotianamine ([Welch 1995](#), [Grusak et al 1999](#), [Suzuki et al. 2005](#)). As well, the cysteine residues in proteins are often strong binding sites of Zn and recently [Peck et al \(2008\)](#) found that the concentration of Zn in wheat grain affected the composition of the endosperm protein by influencing the degree of polymerisation within the storage proteins. Little attention has been paid to the role of S nutrition on the Zn uptake and transport to the grain and this experiment was conducted to examine this interaction in bread and durum wheat. Durum wheat is more responsive to Zn nutrition than bread wheat. The experiment tested the hypothesis that increasing the supply of S will enhance the concentration of Zn in the grain of wheat.

## MATERIALS AND METHODS

The study examined the responses to Zn and S in two species of wheat that differ in their responsiveness to zinc nutrition: bread wheat (*Triticum aestivum* L. cv Yitpi) and durum wheat (*T. turgidum* var *durum* Desf. cv Yallaroi). The experiment was conducted in a growth room under 14 hours daylength and at a day/night temperature of 20/10° C and the intensity of photosynthetically active radiation was 300-500  $\mu\text{mole quanta m}^{-2}\text{s}^{-1}$ . Plants were grown in pots containing 2 kg of infertile sand (Mt Compass sand) to which basal nutrients (with the exception of Zn and S) were added.

Prior to sowing the seed,  $\text{CaCO}_3$  was added (0.1% w/w) to raise the pH to 8 and the basal nutrients were thoroughly mixed through the soil to provide non-limiting supplies of the essential macro- and micronutrients, with the exception of Zn and S. There were two zinc treatments (2.5 and 5.0 mg Zn  $\text{kg}^{-1}$  as  $\text{ZnCl}_2$ ) and three sulphur treatments (15, 35 and 55 mg S  $\text{kg}^{-1}$  as  $\text{K}_2\text{SO}_4$  with K balanced by additional KCl at the two lower S levels). The rates of zinc and sulphur were chosen to provide a range in Zn and S concentrations in the plant tissues but not to induce deficiencies at the lowest levels. At the start of stem elongation, additional N and K were added to each pot. The pots were weighted regularly to 12% w/w and watered to weight.

Four seedlings per pot were established. At 28 days after sowing two plants per pot removed, dried at 70°C and weighed. The dried plant tissue was ground and analysed for nutrient concentration using inductively coupled plasma atomic absorption spectrometry (ICP-AES). The chlorophyll content of the flag leaf was measured regularly between ear emergence and maturity using a SPAD meter. At maturity, the shoot was cut at ground level, the ears were separated from the rest of the shoot and the plant material was dried at 70° C and weighed. The

grain was threshed from the ears, weighed to determine grain yield and samples were analysed for nutrient concentration using ICP-AES. The experimental design was a 2 x 2 x 3 factorial, randomised complete block with four replicates.

## RESULTS

There was no significant response in shoot dry matter production to Zn or S nutrition at the vegetative stage, 28 days after sowing (data not presented). Adding Zn increased the shoot Zn concentration, but the response depended on the level of S that was applied (Fig 1a). At the lowest S level there was no increase in shoot Zn concentration, but significant increases occurred at the two higher levels of S and increased with the amount of S added. This response occurred in both bread and durum wheat.

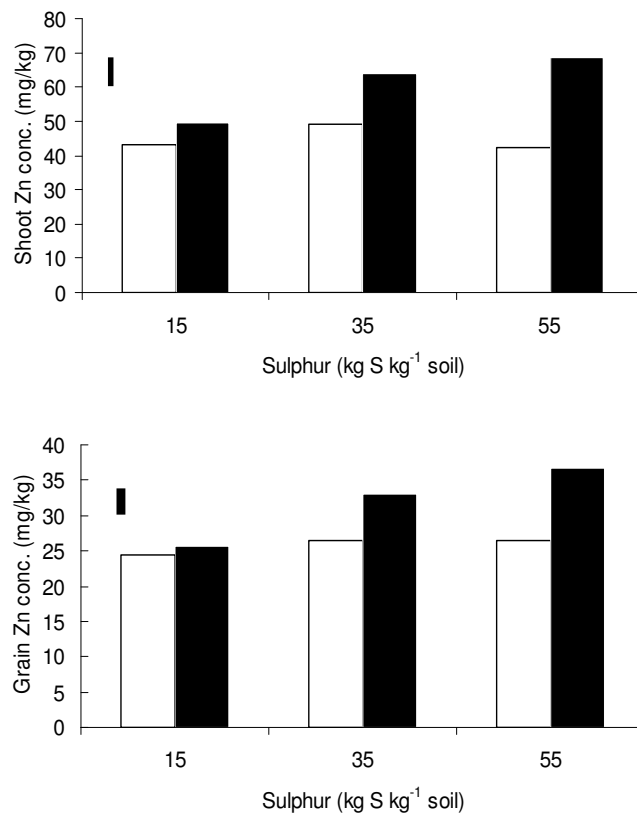


Figure 1. The effects of Zn and S nutrition on the Zn concentration of (a) the whole shoot at the commencement of stem elongation and (b) the grain at maturity. The rates of Zn are 2.5 mg Zn kg<sup>-1</sup> (□) and 5.0 mg Zn kg<sup>-1</sup> (■). Data are the averages of bread and durum wheat. The error bars are the LSD (P=0.05) for the Zn x S interaction.

Additional S increased flag leaf chlorophyll content delayed senescence, but Zn had no significant effect on leaf chlorophyll content. Both grain yield and kernel weight were not significantly affected by the Zn or S treatments.

Additional soil Zn improved grain Zn when there was adequate to high levels of S, but not at the lowest concentration of S (Fig 1b). Adding S increased the grain S concentration but additional Zn did not affect the grain S concentrations. The S:Zn ratio in the grain did not vary with S treatment: the average values at 15, 35 and 55 mg S kg<sup>-1</sup> were 50, 47 and 50, respectively. High S also significantly increased the grain Fe concentration by 29%, from 21.6 mg kg<sup>-1</sup> at the lowest s concentration to 27.9 mg kg<sup>-1</sup> at the highest.

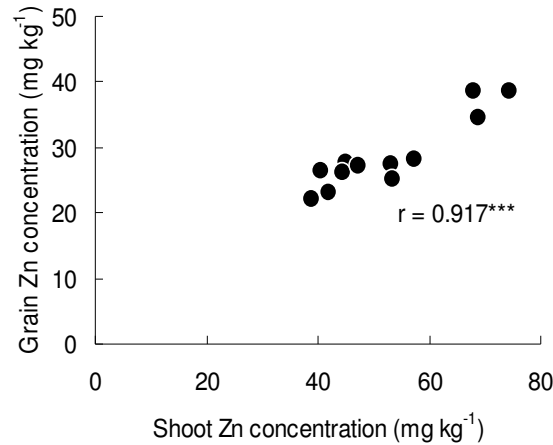


Fig 2. The relationship between the Zn concentration in the whole shoot and the Zn concentration in the grain

The concentrations of Zn in the grain were significantly correlated with the concentrations within the vegetative tissue (Fig 2), whereas this did not occur with Fe or S. Doubling the shoot Zn concentration resulted in a doubling of the grain Zn concentration. Over all the treatments, grain Zn and S concentrations and grain Fe and grain S was correlated (Table 1), but the correlation was stronger within bread wheat and between Fe and S. Grain Zn and Fe were only significantly correlated within bread wheat.

Table 1. Linear correlations between the concentrations of Zn, Fe and S in the grains of durum and bread wheat grown under different levels of soil Zn and S.

<sup>+</sup> - P<0.10, \* - P<0.05, \*\* - P<0.01, \*\*\* - P<0.001; ns = not significant

	Zn	Fe
Durum and bread wheat (n=12)		
S	0.554 <sup>+</sup>	0.819***
Zn		0.579*
Bread wheat (n=6)		
S	0.810*	0.955***
Zn		0.780 <sup>+</sup>
Durum wheat (n=6)		
S	0.433ns	0.925**
Zn		0.413ns

## **DISCUSSION**

The lack of a response to Zn or S at the vegetative stage or in grain yield and kernel weight indicates that the levels of Zn and S in the plants were adequate for growth. Adding S increased the Zn concentration in vegetative tissue suggesting the additional S enhanced the uptake of Zn by the plants. This led to a greater pool of Zn in the vegetative tissue that could subsequently be remobilised to the grain. While remobilisation of Zn from other parts of the plant and from senescing tissue is an important mechanism to improve the grain Zn concentration (eg [Uauy et al. 2006](#)), the strong relationship between the Zn concentration in the shoot and the grain emphasises the importance of accumulation of Zn during the vegetative stage as a foundation for increased grain Zn concentration. This is essentially the basis of agronomic biofortification.

Previous analysis of grain mineral concentrations in which seed S concentration has been correlated with Zn concentration has inferred a link between S and Zn transport. This experiment has shown for the first time that the level of S nutrition can affect the uptake of Zn and its deposition in the grain. The observation that the S:Zn ratio in the grain was unaffected by increased supplies of S suggests the uptake and/or transport and deposition of Zn in the grain are proportional to the supply of S. The experiment has also shown that grain Fe concentration was enhanced by about 30% with additional S. There are a number of possible reasons for this effect. Increasing the level of S in plants is likely to increase the production of methionine, which in turn may lead to increased production of phytosiderophores and nicotianamine, both of which are involved in uptake and translation of Zn. There are also a range of other S-containing molecules that are involved in Zn transport and storage and it is also likely that increasing S supply to the plants has increased their production. However, without more detailed analysis of the composition of the plants and it is not possible to state the mechanism of the observed response.

The results suggest that agronomic biofortification will be most effective when the supply of S is adequate. Since a common method of applying Zn, either as a solid fertiliser or as a foliar spray, is ZnSO<sub>4</sub>, both Zn and S are applied together, which ensures this occurs, and it may be fortuitous that the use of ZnSO<sub>4</sub> is widespread.

The two species of wheat differed in the relationships between S, Fe and Zn. Correlations with S were stronger in the bread wheat than in durum wheat which may suggest that there are genetic differences in the effects of S. Whether this is a consistent difference between bread and durum wheat requires further investigation.

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