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# Soil and foliar zinc biofortification in field pea (*Pisum sativum* L.): Grain accumulation and bioavailability in raw and cooked grains

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ABSTRACT

To evaluate the potential of cooked field peas to be used in Zn biofortification programs, all combinations of soil Zn application of 0, 4 and 8 mg $ZnSO_4 \cdot 7H_2O$ $kg^{-1}$ and foliar Zn application of 0 and two sprays of 0.25% or 0.5% (w/v) $ZnSO_4 \cdot 7H_2O$ before flowering and at early grain-filling stage were tested. Soil Zn application increased Zn-DTPA concentration 3.7- to 5.6-times depending on the Zn soil treatments. Grain Zn concentrations higher than 60 mg Zn $kg^{-1}$ were obtained with all foliar Zn applications, alone or in combination with soil Zn applications, and grain Zn bioavailability was adequate (phytate:Zn ratios lower than 15). Processing (freezing and cooking) caused a decrease of about 30% in grain Zn concentration and a 17%-increase in phytate:Zn ratios (to ⩽9.5). The combined application of 8 mg $ZnSO_4 \cdot 7H_2O$ $kg^{-1}$ soil + 0.25% (w/v) $ZnSO_4 \cdot 7H_2O$ foliarly could be a good option for biofortifying field peas.



## 1. Introduction

Zinc (Zn) is an essential nutrient in plants, animals and humans. In plants, Zn deficiency reduces plant growth, pollen viability, flowering and grain production (Cakmak, 2000; Pandey, Pathak, & Sharma, 2006). In humans, Zn deficiency is associated with severe health complications, including impairments of physical growth, immune system and learning ability, combined with increased risk of infections, DNA damage and cancer development (Gibson, 2006; Levenson & Morris, 2011). However, more than 30% of the world's population is Zn deficient, with Zn deficiency being the 11$^{th}$ most important factor causing disease or death in the world, and the 5$^{th}$ most important factor in developing countries only (WHO. Micronutrient Deficiencies, 2011). In Australia, a study with 12,153 people using a 24-h dietary recall method showed an average intake of 10.6 mg Zn per day (Auestad, Hurley, Fulgoni, & Schweitzer, 2015), which is below the recommended intake of 15 mg per day established by the National Research Council (2001). Gibson and Heath (2011) established that toddlers, adolescents (especially those of Pacific and Aboriginal ethnicities), institutionalised elderly, and possibly people with diabetes are the risks groups in Australia and New Zealand, whose daily Zn intake should be increased to the recommended values.

Food consumption provides the principal route of Zn supply in most human populations. A diet consisting of a high proportion of cereal-based food with low Zn contents is considered one of the major reasons for the widespread occurrence of Zn deficiency in humans, especially in developing countries (Gibson, 2006). Moreover, plants grown in areas with low Zn availability in soils, such as south-west, east and south-east of Australia (Alloway, 2008), suffer from Zn deficiency and show not only poor yield but also low Zn concentration in edible parts. In Zn-deficient conditions, agronomic biofortification has proved to be an effective and fast solution to increasing Zn concentration in the edible parts of several crops, particularly cereals (Cakmak et al., 2010; Ghasemi, Khoshgoftarmanesh, Afyuni, & Hadadzadeh, 2013; Gomez-Coronado, Poblaciones, Almeida, & Cakmak, 2015). Biofortification via foliar Zn application has been shown to be effective in increasing grain Zn concentration in crops grown in either Zn-sufficient or Zn-deficient soils (e.g. Hussain, Maqsoodab, Rengel, and Aziz (2012)). Secondly, soil Zn application was not effective in increasing grain Zn concentration, but increased grain yield (Cakmak et al., 2010). Therefore, soil + foliar application is the most effective method for increasing both grain Zn and grain yield (Cakmak et al., 2010).

Little work was done on legumes regarding Zn biofortification. For example, foliar Zn application resulted in increased grain yield and grain Zn concentration in dry beans (*Phaseolus vulgaris* L.) (Ibrahim & Ramadan, 2015). However, legumes are an excellent source of protein, complex carbohydrates, vitamins and minerals in the diets of many millions of people, particularly in developing

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countries. Among legumes, field peas (*Pisum sativum* L.) are the second largest legume crop worldwide, with annual production of 17 million tonnes (FAOSTAT, 2014). In Australia, with field peas production of approximately 0.3 million tonnes annually (FAOSTAT, 2014), grain Zn concentration was between 10 and 11 mg $kg^{-1}$ (Cunningham, Milligan, & Trevisan, 2001), suggesting that field peas may be a good candidate for Zn biofortification. Indeed, much higher Zn concentrations have been found in field peas elsewhere in the world, such as 24–37 mg $kg^{-1}$ in Canada (Wang, Hatcher, & Gawalko, 2008), about 57 mg $kg^{-1}$ in India (Pandey, Gupta, & Pathak, 2013) or 34–35 mg $kg^{-1}$ in Pakistan (Rafique et al., 2015). However, in all these cases, Zn concentrations were lower than the recommended critical level, established by Huett, Maier, Sparrow, and Piggot (1997) to be 61 mg Zn $kg^{-1}$ field pea grains to achieve a sufficient Zn status in humans.

The grain Zn content is not the only important parameter. Presence of antinutritional components, mainly phytic acid, is one of the major drawbacks limiting the nutritional quality of legumes. Phytate (myo-inositol 1,2,3,4,5,6-hexakisphosphate) binds with Zn and other metal cations to form insoluble complexes that hinder Zn absorption in the human intestine. Accordingly, diets rich in phytate and low in mineral micronutrients can cause health problems, typically Fe, Zn, Mg and Ca deficiencies (Raboy, 2002). However, relatively low phytate concentration was found in field peas grown in the US (4.9–7.1 g $kg^{-1}$, Amarakoon, Thavarajah, McPhee, & Thavarajah, 2012) and Canada (6.4–8.3 g $kg^{-1}$, Wang et al., 2008).

To determine the bioavailability of minerals in food, one of the methods is measuring the molar ratio of phytate:Zn (ratios greater than 15 were associated with Zn deficiency, Morris & Ellis, 1989; Sandberg, Anderson, Carlesson, & Sandstrom, 1987). Most cereal grains and their products contain high ratios, e.g. between 25 and 34 (Morris & Ellis, 1989). Foliar Zn application was effective in decreasing the phytate:Zn ratio and thus increasing estimated Zn bioavailability (Hussain et al., 2012). Another important factor to consider is that legumes are usually cooked before intake to improve flavour and palatability. Cooking may improve nutritional value by decreasing concentration of antinutrients, such as phytate or tannins, and increasing concentration of other components such as protein and starch (Brigide, Canniatti-Brazaca, & Silva, 2014; Wang, Hatcher, Toews, & Gawalko, 2009), probably due to the loss of soluble solids during cooking, which would increase concentration or remaining grain components. However, there is inadequate knowledge on how Zn biofortification influences phytate concentration in grain, and how processing from raw to cooked grain affects content of both Zn and phytate and thus Zn bioavailability. In order to increase Zn intake by humans and clarify questions regarding the effectiveness of Zn application methods on bioavailability of Zn in field pea grains, we tested the hypothesis that combined soil and foliar application of Zn is effective in increasing both grain Zn concentration and Zn bioavailability in cooked grains of field peas. The aims of the present study were (i) to evaluate the potential of field peas to be used in the Zn biofortification programs and (ii) to promote cooked peas as a Zn source. Such evaluation consisted of analysing the effects of different soil and foliar Zn fertilization treatments on plant growth, nutrient uptake and Zn accumulation in raw grains, subsequently frozen and boiled to estimate Zn bioavailability.

## 2. Materials and methods

### 2.1. Site, experimental design and crop management

This study was conducted in a naturally-lit glasshouse located at The University of Western Australia in Perth (31°57′S, 115°47′E), Australia, between May and August 2015. During the experiment, the average temperature in the glasshouse was 23 ± 4 °C during the day and 17 ± 4 °C during the night. Light intensity varied between 250 and 1100 μmol photon $m^{-2}$ $s^{-1}$, and relative humidity varied from 40 (midday) to 85% (midnight).

The soil used in the experiment was topsoil (0–20 cm) collected from the area of Gingin in Western Australia (31°35′S, 115°90′E) (Cambic Arenosol). The soil was disinfected by heating to 60 °C for 1 h and was then air-dried for 2 days and sieved to <5 mm. Four subsamples of the sieved soil were analysed for various physico-chemical properties. Soil texture (determined gravimetrically) was sandy. The soil had pH 6.5 (10 g soil:25 mL deionised $H_2O$), electrical conductivity of 0.028 dS $m^{-1}$, organic carbon 2.9 g $kg^{-1}$ (Walkley, 1947), available phosphorus 14 mg $kg^{-1}$ and potassium <15 mg $kg^{-1}$, nitrate nitrogen 1 mg $kg^{-1}$ and ammonium nitrogen 2.5 mg $kg^{-1}$ (extracted with 1 M potassium chloride for 1 h at 25 °C and measured on a Lachat Flow Injection Analyzer). Plant-available Zn was 0.26 mg $kg^{-1}$ soil, determined according to Lindsay and Norvell (1978) by extraction with DTPA (diethylenetriamine pentaacetic acid) using a soil:solution ratio of 1:2 and shaking time of 2 h. Extracted Zn was determined by inductively-coupled plasma optical emission spectroscopy (Zarcinas, 1984) (ICP-OES, Vista-Pro Axial, Varian Pty Ltd, Mulgrave, Australia). Certified soil reference material and blanks were included in each batch of samples. All the results were reported on a dry weight basis.

To ensure Zn was the only nutrient limiting growth, the following basal nutrients (in mg $kg^{-1}$) were added to soil as solutions: 90.2 $KH_2PO_4$; 139.9 $K_2SO_4$; 40.1 $MgSO_4{\cdot}7H_2O$; 95.2 $NH_4NO_3$; 150.3 $CaCl_2{\cdot}2H_2O$; 10.0 $MnSO_4{\cdot}H_2O$; 2 $CuSO_4{\cdot}5H_2O$; 0.5 $CoSO_4{\cdot}7H_2O$; 0.2 $Na_2MoO_4{\cdot}2H_2O$, 0.7 $H_3BO_3$. Soil Zn treatments (see below) consisted of spraying Zn sulphate solution to the soil surface. After application of basal nutrients and different soil Zn rates, the soil in each pot was thoroughly mixed.

Zinc treatments comprised all possible combinations of soil application of 0, 4 and 8 mg $ZnSO_4{\cdot}7H_2O$ $kg^{-1}$ soil (0Soil, 4Soil and 8Soil, respectively) and foliar application of distilled-water spray (10 mL $pot^{-1}$) (NoFoliar) and two sprays (10 mL $pot^{-1}$ each) of 0.25% or 0.5% (w/v) $ZnSO_4{\cdot}7H_2O$ (0.25Foliar and 0.5Foliar, respectively), once before flowering and the second time at early grain filling stage. Foliar Zn treatments were applied in the late afternoon; spraying continued until all the leaves were wet. The pots were covered with polythene at the base of the plants so that the solution did not trickle to the soil. The treatments were arranged in completely randomized design with four replications.

The field pea cultivar used was Twilight. Seeds were surface-sterilised by soaking in 80% v/v ethanol for 60 s, washed thoroughly with sterile water and sown in 20-cm-high and 20-cm-wide pots containing 6.5 kg soil (nine seeds per pot). Seeds were inoculated with a commercial inoculant (Becker Underwood Pty Ltd, Somersby, Australia) following the manufacturer's instructions. Two weeks after emergence, the pots were thinned to leave three plants per pot. Soil moisture content was maintained around 60% of the water holding capacity by watering plants every 2 days with deionised water. Four drainage slits (2 cm wide) 2 cm above the pot base allowed drainage. There was no incidence of pests or diseases during the study.

### 2.2. Plants analysis

Plants were harvested at maturity in early August. Roots, stems with leaves, and pods including grains were separated. Fresh roots were used to determine root length, average diameter and root surface. Roots were spread out on a Perspex tray and imaged using a flat-bed scanner (EPSON Perfection V700/V750 operating resolution 400 dpi) and WinRhizo 5.0 ATM software (Regent Instruments,