Field Crops Research xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

# Zinc uptake and accumulation in winter wheat relative to changes in root morphology and mycorrhizal colonization following varying phosphorus application on calcareous soil

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ARTICLE INFO

*Article history:*
Received 15 April 2016
Received in revised form 5 August 2016
Accepted 7 August 2016
Available online xxx

*Keywords:*
P supply
Root morphology
Zn uptake
Zn accumulation
Winter wheat

ABSTRACT

Although phosphorus (P) is known to reduce zinc (Zn) uptake by roots and root-to-shoot translocation, how this interaction is affected by changes in root morphology and arbuscular mycorrhizae (AM) are poorly understood. The current study determined the effects of P application rate (0, 25, 50, 100, 200, and 400 kg P $ha^{-1}$) on Zn uptake by roots and root-to-shoot translocation in a high-yielding winter wheat system on calcareous soil. Root dry weight (RDW), root length density (RLD), and root surface area (RSA) significantly increased as P application increased from 0 to 50 kg $ha^{-1}$ but were unaffected by rates >50 kg $ha^{-1}$. Zn accumulation (mg $m^{-2}$ or μg $m^{-2}$) by roots at flowering increased with application of 25 and 50 kg P $ha^{-1}$ but slightly decreased with application of 100–400 kg P $ha^{-1}$. Zn accumulation in roots and in shoots at flowering and grain yield at maturity were positively correlated with RDW, RLD, and RSA. Root Zn accumulation was increasing with increased AM colonization from low AM (<10%) to 30% AM colonization. Whereas, continuously increasing AM colonization induced root Zn accumulation decrease, which may be mainly due to the decrease of root dry weight affected by P deficiency. P application rate did not significantly affect the ratio of Zn concentrations in roots vs. shoots or the ratio of Zn accumulation in roots vs. total Zn accumulation in the plant, indicating that Zn translocation from roots to shoots is not the primary factor limiting Zn concentrations in grain. These results indicate 1) that P application affects the Zn accumulation of wheat by affecting Zn uptake by roots and 2) that the changes in Zn uptake by roots and Zn accumulation following P application reflect changes in root morphology and AM colonization of roots.



## 1. Introduction

Zinc (Zn) deficiency is a worldwide problem among humans and especially among humans who rely on cereals as a staple food (Graham et al., 1999; Welch and Graham, 2004). At least one-third of the human population suffers from deficiencies of Zn (Hotz and Brown, 2004). The low levels of Zn in soil and plant material (i.e., grain) could be a major factor contributing to the widespread occurrence of Zn deficiency in children (Cakmak et al., 1996). Therefore, soil type, crop, cultivar, and other factors should be considered with the goal of reducing micronutrient malnutrition (Rengel et al., 1999; La Frano et al., 2014; Singh and Prasad, 2014).

Application of phosphorus (P) has been reported to decrease the Zn content in plants (Haldar and Mandal, 1981; Bogdanovic et al., 1999; Izsáki, 2014), and recent studies showed that application of P fertilizer decreased the Zn concentration in wheat and maize grain (Kizilgoz and Sakin, 2010; Zhang et al., 2012). Furthermore, a pot experiment demonstrated that P application decreased Zn uptake by roots and also reduced Zn movement from roots to shoots (Yang et al., 2011). P fertilization might reduce Zn uptake by decreasing the transformation of applied Zn into a water-soluble form (Biswapati and Mandal, 1990) and by decreasing the exudation from roots of organic anions that mobilize Zn (Hoffland et al., 2006). P fertilization could also affect Zn uptake by altering root morphology. Welch and Graham (2004) indicated that the available levels of Zn at the root-soil interface could be enhanced by changing root morphology, and Rose et al. (2013) suggested that root surface area (RSA) and root length density (RLD) should be increased so as to increase the volume of soil explored by roots such that roots capture increased quantities of immobile Zn. Although effects of P fertilizer on root morphology have been reported (Chassot and Richner, 2002; Deng et al., 2014), the effect of P application rate on Zn uptake

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http://dx.doi.org/10.1016/j.fcr.2016.08.010
0378-4290/

by roots and on root morphology have not been simultaneously reported for field studies.

Root traits can be modified by P application in ways that enhance the efficiency with which P is acquired from soil (Lambers et al., 2006). Previous studies showed that P supply directly affects root biomass, RLD (De Groot et al., 2001). Ma et al. (2001) indicates that P has unique importance in the evolution of root form and function. Whether P-induced alterations in root morphology affect Zn uptake by roots, however, has not been reported.

Arbuscular mycorrhizae (AM) can improve the nutritional status of host plants by transporting slowly diffusing nutrient ions (such as P, Zn, and Cu) to roots (Kothari et al., 1991; Subramanian et al., 2008, 2013; Thompson et al., 2013). A meta-analysis by Lehmann et al. (2014) indicated that AM fungi (AMF) increased the Zn concentration in all plant tissues, and Watts-Williams et al. (2015) reported that up to 24% of the Zn in the shoots of AM colonized plants growing in soil with a low level of Zn was delivered via the AM pathway. The AM-induced increase in Zn uptake by roots and shoots could be reduced by P fertilization because P application can reduce AMF colonization of roots (Teng et al., 2013). It has been discussed whether the decreasing AM colonization decreased root and shoot Zn concentration (Ryan et al., 2008; Subramanian et al., 2008). A pot experiment by Ova et al. (2015) showed that the negative effect of increasing P supply on Zn uptake by roots and on Zn concentrations in wheat is AM-dependent. It follows that understanding the influence of P application on Zn uptake by wheat roots will require the quantification of AM variation and how P application rates decrease AM colonization then decrease root Zn concentration.

The current study investigated the Zn accumulation of winter wheat growing in the North China Plain. The aims of the study were (1) to determine whether P application affects Zn uptake by roots and Zn accumulation by shoots as a consequence of changes in root morphology, (2) to quantify the relationships between Zn concentrations in roots and shoots and AM colonization, and (3) to determine the effect of P application rate on the Zn translocation from soil to roots and then from roots to shoots and grain.

## 2. Materials and methods

### 2.1. Field location

A field experiment was conducted in the same location and under the same conditions as described in our previous report (Zhang et al., 2015). The details were as follows: the experiment was conducted at the Quzhou Experiment Station in Hebei Province (36.9°N, 115.0°E) from October to the following June in two cropping seasons (2012–2013 and 2013–2014) of a winter wheat-summer maize rotation. The experimental site is located in the center of the North China Plain (NCP) and has a calcareous alluvial soil. The soil pH (1:2.5 w/v in water) was 8.0, and the initial soil Olsen-P was 6.4 mg $kg^{-1}$ (0.5 mol $L^{-1}$ $NaHCO_3$-extractable). The soil DTPA-extractable Zn concentration before sowing averaged 0.40 mg $kg^{-1}$.

### 2.2. Experimental design

The field experiment included six P application rates (0, 25, 50, 100, 200, and 400 kg P $ha^{-1}$). Each treatment was represented by four replicate plots, and the plots were arranged in a randomized block design. Each plot was 75 $m^2$ (7.5 m × 10 m) and was treated with the same P application rate in both cropping seasons. The winter wheat (*Triticum aestivum* L.) cultivar was Liangxing 99 in both cropping seasons, and the seeding rate was 187.5 kg $ha^{-1}$. All plots received 225 kg nitrogen (N) $ha^{-1}$ as urea (46% N), 60 kg $K_2O$ $ha^{-1}$ as potassium (K) sulphate, and P fertilizer as calcium superphosphate. K and P were applied before sowing. N was applied twice per crop cycle: first as 75 kg N $ha^{-1}$ before sowing, and second as 150 kg N $ha^{-1}$ at the jointing stage. Based on soil water content, irrigation was applied at the pre-wintering stage (the stage which average daily temperature was about 0–1 °C), the jointing stage, and the flowering stage in both cropping seasons. Pests and weeds were controlled by standard practices, which pesticides and herbicide (such as omethoate, paraquat) were spraying at booting stage to control aphids and weeds, and no water, weed, or pest problems were observed during the experiment.

### 2.3. Sampling and nutrient analysis

Shoot samples were collected at the flowering stage (GS65) and the maturity stage (GS92). Entire shoots were randomly selected from two 0.5-m lengths of two adjacent rows in each plot at 209 days after sowing (DAS), and 241 DAS in 2012–2013 and at 209 DAS, and 239 DAS in 2013–2014. To reduce sampling variation, wheat shoot from uniform population density, growth height and growth stages should be gotten from entire plot. Meanwhile, wheat samples which the population density and height were too large and small, and samples have been damaged by external factor as well as edge wheat should be avoided to get. At maturity, the wheat plants were separated into grain and straw. In this report, the term "shoot" refers to all aboveground parts of wheat plants including the straw and grain. The shoot samples were rapidly washed with tap water and then with deionized water before they were dried at 60–65 °C to constant weight. All plant samples were ground with a stainless steel grinder for nutrient analysis.

In 2013–2014, roots were destructively sampled at flowering (209 DAS) in order to study the effects of P application rate on root spatial distribution and Zn distribution in roots at different soil depths. Roots were sampled from each plot by extracting them from a 0.09-$m^3$ block of soil with a 30-cm length (two 15-cm lengths in a row), a 50-cm width (adjacent two rows, 25 cm between rows), and a 60-cm depth as previously described by Xue et al. (2014). Two separate root samples were gotten from soil block: one part was used to evaluate root traits such as root length, root area and another part was used to measure root dry weight, and root nutrient concentration. Root samples were collected in four replicate plots for each P application rate. In each plot, four soil block from different depths (0–10, 10–20, 20–30, and 30–60 cm) were sequentially extracted. The dimensions of each soil block were 15 cm (length) × 50 cm (width) × 10 cm (depth). Root samples were washed with tap water and kept at −20 °C before an image was captured with an optical scanner (Epson, Japan) and the image was analyzed with software (RHIZO 4b, Australia) to obtain root measurements such as root length, root area and root volume. Soil Olsen-P concentration was evaluated at flowering stage and soil samples were collected from 0 to 10 cm, 10 to 20 cm, 20 to 30 cm, and 30 to 60 cm soil layers, respectively. The subsamples were then mixed and air dried before being ground to analyse soil Olsen-P.

The plant samples were digested with $HNO_3$-$H_2O_2$ in a microwave-accelerated reaction system (CEM, Matthews, NC, USA). The Zn and P concentrations in the digested solutions were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, OPTIMA 3300 DV, Perkin-Elmer, USA). IPE684 grain and IPE126 straw samples (Wageningen University, Netherlands) were used as reference materials to verify the digestion procedures and to calibrate the ICP-OES. The concentration of soil available P was measured by the molybdovanado phosphatase method based on extraction from air-dried soil with 0.5 M $NaHCO_3$ at pH 8.5 (180rom, 25 °C) (Olsen, 1954).

To investigate AM colonization of roots, subsamples were randomly selected from the root samples collected at 0–60 cm soil

Please cite this article in press as: Zhang, W., et al., Zinc uptake and accumulation in winter wheat relative to changes in root morphology and mycorrhizal colonization following varying phosphorus application on calcareous soil. Field Crops Res. (2016), http://dx.doi.org/10.1016/j.fcr.2016.08.010