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## Effects of nitrogen management on root morphology and zinc translocation from root to shoot of winter wheat in the field



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#### ABSTRACT

Nitrogen (N) nutrition is a critical factor in root uptake and deposition of zinc (Zn) into wheat grain. However, little is known about how the root system in the soil profile responds to various N supplies under field conditions. A two-year field experiment was conducted to investigate the temporal and spatial distributions of winter wheat roots and relationships between root morphological traits and root Zn uptake with different N supplies. Results showed that an increasing N supply improved root length density (RL), surface area (RSA) and root dry weight (DW), thereafter, resulting in an increased root Zn uptake. For example, root Zn uptake significantly and positively correlated with RL, RSA and root DW at jointing  $(r = 0.608^*, 0.540^* \text{ and } 0.785^{***}, \text{ respectively})$  and flowering  $(r = 0.611^*, 0.602^* \text{ and } 0.876^{***}, \text{ respectively})$ stages. Furthermore, substantial root DW (80-92%) and root Zn uptake (85-96%) were mainly recovered from the upper 30 cm of soil layer irrespective of N supply, showing a good spatial matching. The increased ratios of shoot-to-root Zn concentration and shoot-to-total Zn content with the increasing N supply indicate improving N supply promotes the root-to-shoot Zn transport and increases shoot Zn nutrition. Shoot Zn content was positively correlated with final grain yield and grain Zn concentration. Thus, a combination of optimum N supply with breeding for a better root system could be a promising approach to improve root uptake and accumulation of Zn in grain to maintain relatively higher grain Zn for human nutrition.

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#### 1. Introduction

Zinc (Zn) deficiency is a widespread nutritional disorder, affecting at least one-third of the world's population (Hotz and Brown, 2004; Stein, 2010). Inadequate dietary intake and little dietary diversity have been regarded as the major reasons behind this global problem, particularly in some developing countries where wheat provides more than 70% of daily calorie intake and is therefore an important Zn source for humans (Welch and Graham, 2004; Cakmak, 2008; White and Broadley, 2009). The reliance on cereal based foods induces Zn deficiency related health complications, including stunted growth of children and poor immune function, high susceptibility of children and adults to common infectious diseases such as diarrhoea and pneumonia, and high mortality and morbidity (Black, 2003; Hotz and Brown, 2004;

Gibson, 2006). Therefore, a combination of agronomic biofortification with breeding is considered to be an applicable and sustainable approach to solve the Zn deficiency problem in humans (Pfeiffer and McClafferty, 2007; Cakmak, 2008).

As one of the major agricultural practices in crop production, nitrogen (N) management appears to be a promising agronomic strategy for the biofortification of wheat with Zn. Recent studies showed that under both field and glasshouse conditions, adequate N supply could effectively enhance grain Zn concentration in wheat (Kutman et al., 2010; Shi et al., 2010; Gooding et al., 2012; Xue et al., 2012). Evidence from Erenoglu et al. (2011) using <sup>65</sup>Zn-labeling studies indicated that improved N supply significantly enhanced the rate of root Zn uptake and more evidently the root-to-shoot transport and retranslocation of <sup>65</sup>Zn from leaves into grains under controlled conditions. Mechanisms for the positive effects of N on the greater root Zn uptake in wheat have been discussed, including the improved exudation of organic acids or phytosiderophores, and increased abundance of root Zn uptake transporters (Cakmak et al., 2010; Erenoglu et al., 2011). Furthermore, Rose et al. (2013) suggest that the higher root Zn uptake could benefit from improved soil exploration, such as greater root length (RL) and root surface

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area (RSA). Although the importance of roots in water and nutrient acquisition has been widely acknowledged, less attention has been paid to the temporal and spatial dynamics of root growth of wheat in response to different N supplies (Herrera et al., 2007), especially the relationships between root Zn uptake and root morphological traits such as RL and RSA of wheat at different N application rates under field conditions.

A successful breeding program for biofortifying cereals and other food crops with Zn depends on the size of plant-available Zn pools in soil (Cakmak, 2008). However, there are some adverse chemical and physical problems that reduce the solubility and plant availability of Zn in many cereal-growing areas, especially in calcareous soils with high pH, low organic matter and poor soil moisture (Cakmak, 2008; Cakmak et al., 2010). Consequently, adequate uptake of Zn for better crop production and higher grain Zn concentrations are not achieved. Additionally, nutrient uptake, particularly for immobile nutrients such as Zn, was restricted by the RSA (Manske and Vlek, 2002). The formation of root hairs and the ability to produce longer and finer roots could stimulate Zn uptake in wheat and barley (Dong et al., 1995; Genc et al., 2007). Therefore, greater root length density (RLD) or RSA may represent a competitive advantage in the uptake of Zn available from the calcareous soils.

Better root growth and synchronized N supply throughout the crop growing season are beneficial for maximizing fertilizer uptake, optimising grain yield and reducing N losses (Peng et al., 2012). Root growth can vary in response to soil conditions, such as soil moisture and N supply (Campbell et al., 1977). An important effect of N availability is an increase in root branching (Belford et al., 1987), which occurs especially in N-enriched soil patches for barley (Drew and Saker, 1975). Sattelmacher et al. (1990) reported that RSA and RL of potato were improved with the increasing N supply in the nutrient solution. Herrera et al. (2013) reported that root survivorship of spring wheat during grain filling was higher with 270 kg N ha<sup>-1</sup> compared with 20 kg N ha<sup>-1</sup>.

The objective of this work were to (i) examine temporal and spatial distribution patterns of winter wheat roots at different N application rates under field conditions, (ii) quantify the relationships between root morphological traits and root Zn uptake, and (iii) investigate the effects of N on Zn distribution in shoots and roots

#### 2. Materials and methods

#### 2.1. Field locations

The experiment was conducted at Quzhou experimental station in Hebei province (36.9° N, 115.0° E) during the 2009–2010 and 2010–2011 cropping seasons in a winter wheat–summer maize rotation system. The soil characteristics were previously described (Yue et al., 2012). Before sowing or fertilization, soil was sampled to 30 cm depth and analysed for DTPA–extractable Zn (DTPA–Zn) concentration. Before sowing, the soil DTPA–Zn concentration averaged 3.0 mg kg $^{-1}$  in 2009–2010 and 3.4 mg kg $^{-1}$  in 2010–2011.

#### 2.2. Experimental design

Winter wheat (*Triticum aestivum* L., var. Liangxing99) was planted in two cropping seasons and a randomised complete block experimental design was used with four replicate plots  $(300 \, \text{m}^2 \, \text{plot}^{-1})$ . Four N application rates were used in the field trail as follows: no N application (recorded as N<sub>0</sub>, control), lower N application (54% and 70% of optimal N application in the first and second cropping seasons, respectively; recorded as N<sub>1</sub>), optimal N application (recorded as N<sub>2</sub>) and traditional N application (recorded as

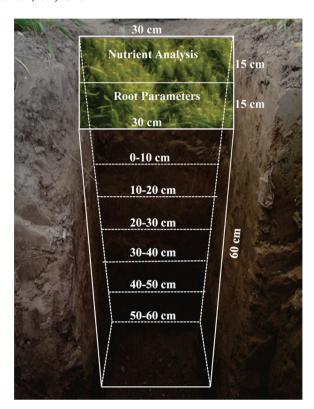


Fig. 1. Illustration of the root sampling method used in the field experiment in 2010–2011.

 $N_3$ ). The optimal N treatment  $(N_2)$  was based on the determination of soil mineral N  $(N_{min})$  of 0–90 cm at sowing, jointing (GS31, Zadocks) and flowering (GS65) stages as previously described (Cui et al., 2008; Ju et al., 2009). The four rates of N applied in form of urea were as follows: 0  $(N_0, {\rm control})$ , 75  $(N_1)$ , 138  $(N_2)$  and 300  $(N_3)$  kg N ha $^{-1}$  in 2009–2010 and 0  $(N_0, {\rm control})$ , 122  $(N_1)$ , 174  $(N_2)$  and 300  $(N_3)$  kg N ha $^{-1}$  in 2010–2011. Nitrogen fertilizer applications were split as described in Table S1. In addition, 120 kg of  $P_2O_5$  ha $^{-1}$  as superphosphate, 100 kg of  $K_2O$  ha $^{-1}$  as potassium chloride and 30 kg ha $^{-1}$  ZnSO4 $\cdot$ 7H $_2O$  were applied. All the fertilizers were broadcast after the soil was ploughed to 25 cm. The fertilizers were then incorporated into the upper 0–15 cm of the soil by rotary tillage before sowing. After the fertilizers were applied, the field plots were immediately irrigated. The irrigation rate was based on the soil water content.

#### 2.3. Sampling and nutrients analysis

In 2009–2010, shoot samples were collected at maturity (separated into grain and straw). In 2010-2011, in order to study the effects of N fertilization on root spatial distribution and root Zn distribution at different soil depths, destructive sampling was carried out to harvest roots at jointing, flowering and maturity stages. Roots were sampled from each plot by extracting them from a total soil volume of 30 cm length (two 15 cm lengths in row)  $\times$  30 cm width (adjacent two rows, 15 cm between rows) × 60 cm depth as shown in Fig. 1. Root samples were collected in four replicate plots at each N application rate. In each plot, six soil cuboids from different depths (0-10, 10-20, 20-30, 30-40, 40-50 and 50-60 cm) were sequentially extracted. The volume of each soil cuboid was  $15 \, \text{cm} \, (\text{length}) \times 30 \, \text{cm} \, (\text{width}) \times 10 \, \text{cm} \, (\text{depth})$ . Root samples were washed with tap water and kept at −20 °C before an image was captured using an optical scanner (Epson, Japan) and root measurement software (RHIZO 4b, Australia). After scanning,

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