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# Identification of high-yield and high-Zn wheat cultivars for overcoming "yield dilution" in dryland cultivation

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ABSTRACT

The most effective measure to address human zinc (Zn) deficiency worldwide is genetic Zn biofortification of cereal grains, namely breeding crop cultivars with both high yield and high grain Zn concentration. This has been not easy due to the widely reported yield dilution effect on grain Zn, while the Zn uptake and its distribution, as sources for grain Zn filling, were much less investigated for overcoming the yield dilution. A field study was conducted in a calcareous low-Zn soil in dryland with 123 modern wheat cultivars planted for two years, and different traits were determined at maturity to characterize cultivars with high or low grain Zn at the same yield levels. For all tested cultivars, grain Zn concentration showed no significant correlation with yield in both years. Then 19 cultivars were identified with consistent yield and grain Zn performances across years, and classified into four groups: low-Zn low-yield (n = 4), high-Zn low-yield (n = 4), low-Zn high-yield (n = 7), and high-Zn high-yield (n = 4). For low-yield cultivars, grain Zn was more closely related to shoot Zn uptake, while for high-yield cultivars, both shoot Zn uptake and Zn harvest index were closely related to grain Zn. From the seven high-yield low-Zn (7142 kg ha$^{-1}$, 14.7 mg kg$^{-1}$) to the four high-yield high-Zn cultivars (7287 kg ha$^{-1}$, 20.8 mg kg$^{-1}$), there was no yield dilution effect on grain Zn because the latter possessed both high shoot Zn uptake (190 g Zn ha$^{-1}$) and high Zn harvest index (79%). In conclusion, yield dilution is not necessarily an obstacle for increasing wheat grain Zn concentration. Enhancing the Zn uptake and its distribution to grain make it possible to achieve high grain Zn and high yield simultaneously, promoting the success of genetic Zn biofortification for dryland wheat.

## 1. Introduction

Around 17% of the world population is suffering from inadequate daily zinc (Zn) intake (Wessells and Brown, 2012). For example, in rural areas of China around 20% of children, women, and the elderly can not get adequate Zn, as half of the daily Zn intake is from low-Zn wheat grains (Ma et al., 2007). After the Green Revolution, breeders have devoted most efforts to increasing grain yields, while the grain micronutrient concentration has been outside the mainstream breeding targets, so the low grain Zn concentration caused by yield dilution is very common in current commercial wheat cultivars (Fan et al., 2008; Graham et al., 2012). Therefore, breeding new cultivars with both high yield and grain Zn concentration, well known as genetic Zn biofortification (Bouis and Welch, 2010; Bouis and Saltzman, 2017), is becoming imperative in agricultural crop production.

To achieve both high yield and high grain Zn, the first step is to overcome the yield dilution effect with a good knowledge of the factors contributing to Zn accumulation in wheat grains. For one or several specific lines, grain Zn concentration is closely related to the crop Zn uptake and its distribution among shoot parts. For instance, wheat grain Zn was significantly increased by enhanced shoot Zn uptake and Zn remobilization from straw, with increased nitrogen (N) supplies in fields (Xue et al., 2012; Jin et al., 2014) or greenhouses (Erenoglu et al., 2011), and with increased Zn supply in greenhouses (Kutman et al., 2012). Besides, when the *OsNAS2* gene was overexpressed in transgenic wheat in greenhouses, the enhanced Zn remobilization alone also significantly increased grain Zn concentration (Singh et al., 2017). In addition, the grain Zn concentration was also significantly decreased by

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the inhibited Zn uptake or Zn remobilization, as observed under excess phosphorus (P) fertilization in fields (Zhang et al., 2015; Hui et al., 2017) or wheat lines with silenced *NAM* gene in greenhouses (Waters et al., 2009). However, much less information can be found on the relations of grain Zn to Zn uptake and its distribution for hundreds of wheat cultivars under field conditions, which limits the progresses to overcome yield dilution effect on grain Zn in practical production.

In the past decades, genetic Zn biofortification studies concerning many wheat cultivars have focused on grain Zn concentration and agronomic traits like yield, harvest index, ear number, grain number, and grain weight. In accordance with the yield dilution effect, grain Zn concentration is commonly reported negatively correlated with yield (Liu et al., 2014; Amiri et al., 2015). Ear number and grain number were not or negatively correlated with grain Zn for irrigated wheat cultivars (Hussain et al., 2012; Amiri et al., 2015). The relation of grain weight to grain Zn varied greatly across cultivars and areas, with positive correlation observed in American soft and hard wheat varieties (Murphy et al., 2008) and wild emmer wheat lines in Turkey and Israel (Gomez-Becerra et al., 2010), negative correlation in Australian bread and durum wheat lines (McDonald et al., 2008) and Italian durum wheat cultivars (Ficco et al., 2009), and no correlation for winter wheat cultivars in northern China (Zhang et al., 2010) and bread wheat cultivars in Mexico (Velu et al., 2016). However, most of the above studies were done in sub-humid or irrigated lands, much less are concerned with drylands which constitute around 45% of the world's arable lands (Huang et al., 2015). Given that environmental factors often have substantial influences on grain Zn concentration and its relations to agronomic traits (Guttieri et al., 2015; Velu et al., 2016), developing high-Zn cultivars with superior agronomic traits should be under targeted conditions (Gomez-Becerra et al., 2010; Velu et al., 2012). In drylands, it is highly necessary to identify promising genetic resources for wheat grain Zn biofortification.

The present study was conducted in a typical low-Zn dryland field on the Loess Plateau, to evaluate 123 modern wheat cultivars for grain yield, yield components, Zn concentration, Zn uptake and distribution in shoot parts, and morphological traits during two growing seasons. It is aimed at the following three questions: (1) are there any wheat cultivars with stable high or low grain Zn at the same grain yield level? (2) if it is true, what are the relations of grain Zn concentration to Zn uptake and its distribution in shoot parts? (3) how to overcome the yield dilution effect to realize genetic Zn biofortification of dryland wheat?

## 2. Materials and methods

### 2.1. Experimental site

The field experiment was conducted in Yongshou (108°12′E, 34°44′N), a typical rainfed dryland area on the southern Loess Plateau of China. Under the temperate mainland monsoon climate, the annual precipitation of this area is around 550 mm and over 60% occurs during summer fallow period (July to September). Basic soil properties of the experimental field are listed in Table 1, implying that the soil was very low in available Zn.

### 2.2. Treatment design

A randomized complete block design was used for the field experiment. 123 modern wheat cultivars (Table S1) were randomly planted with four replications under the supplies of 150 kg N ha$^{-1}$ and 100 kg $P_2O_5$ ha$^{-1}$. The N was applied as urea (46% N), and P as superphosphate (16% $P_2O_5$). All the N and P fertilizers were evenly broadcasted and incorporated into soil at sowing. No potassium (K) was applied due to abundant soil available K in the experimental field. Each cultivar was manually sown in four 2-m long rows with 2.5-cm seed spacing and 20.0-cm row spacing in a 20 × 12.5 m block. The sowing dates were 28th September 2013 and 3rd October 2014, and all cultivars were harvested during 14th to 17th June 2014 and 18th to 20th June 2015. During the wheat growth periods, no irrigation was conducted and only herbicide, fungicide, and insecticide were used when necessary.

**Table 1**
Basic soil properties of the experimental field.

| Properties | Value |
|---|---|
| Sample depth (cm) | 0-20 |
| pH | 8.4 ± 0.1 |
| Cation exchange capacity (cmol kg$^{-1}$) | 20.3 ± 0.4 |
| Organic matter (g kg$^{-1}$) | 12.9 ± 0.2 |
| Total N (g N kg$^{-1}$) | 0.9 ± 0.1 |
| Mineral N (mg N kg$^{-1}$) | 26.6 ± 1.7 |
| Available P (mg P kg$^{-1}$) | 16.9 ± 1.1 |
| Available K (mg K kg$^{-1}$) | 123.4 ± 3.9 |
| Available Fe (mg Fe kg$^{-1}$) | 7.5 ± 0.1 |
| Available Mn (mg Mn kg$^{-1}$) | 18.1 ± 0.7 |
| Available Cu (mg kg$^{-1}$) | 1.3 ± 0.1 |
| Available Zn (mg Zn kg$^{-1}$) | 0.4 ± 0.1 |

### 2.3. Sampling and chemical analyses

Before sowing, the top 0–20 cm soil was sampled, air dried, and analyzed as Bao (2000) for pH (soil water ratio = 1:2.5), cation exchange capacity (1 M sodium acetate), organic matter (0.008 M potassium dichromate), total N (Kjeldahl method), mineral N (1 M potassium chloride), available P (0.5 M sodium bicarbonate), available potassium (K) (1 M ammonium acetate), available iron (Fe) (0.005 M diethylene triamine pentacetic acid (DTPA)), available manganese (Mn) (0.005 M DTPA), available copper (Cu) (0.005 M DTPA), and available Zn (0.005 M DTPA).

At specific growth stages, all cultivars were photographed and recorded for different morphological and agronomic traits, as described in Guidelines for the conduct of tests for distinctness, uniformity, and stability - Wheat (Table S2, GB/T 19,557.2-2004). At physiological maturity, the plants of 30 ears were randomly sampled from the central two rows in each cultivar plot, with roots cut off at the stem-root joint part, and then the shoots were separated into straw (including stems and leaves) and ears which were threshed after air dried into grains and husks. The air-dried straw, husk, and grain were weighed and successively washed with tap water and deionized water, and then oven dried at 65°C for moisture determination and the preparation of ground samples by a ball miller (wolfram carbide jars, Retsch MM400, Germany). The remaining plants in the central two rows were harvested to estimate the grain yield of each cultivar by adding the grain weight of 30 ears. During all the above processes, only stainless scissors, plastic or paper containers, and wooden tools were used to avoid metal contamination.

Micronutrients in plant samples were determined as described by Zhang et al. (2014) with some modifications. A ground sample of 0.2000 g was mixed with 5 ml nitric acid (65%, analytical grade, Merk) in a 50 ml teflon tube and predigested at 120 °C for 30 min, and then another 1.0 ml hydrogen peroxide (30%) was added before digestion in the microwave digester (MW Pro, Anton Paar, Austria) equipped with a 48-position rotor. For each stack, 42 samples, three blanks, and three standards (GBW10011 (GSB-2)) were randomly arranged in the rotor and each sample was digested in duplicates. After cooling down, the digestion solution was transferred into a plastic volumetric flask and diluted to 100 ml with ultrapure water (18.25 MΩ cm$^{-1}$). Zinc in the digestion solution was determined by an inductively coupled plasma mass spectrometer (ICP-MS) (iCAP Qc, Thermo Fisher Scientific, USA). The measured isotope was $^{66}$Zn and $^{73}$Ge was added as the internal standard.