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Zinc and iron concentrations in grain milling fractions through combined foliar applications of Zn and macronutrients

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ABSTRACT

Agronomic biofortification of wheat with Zn and Fe can correct micronutrient deficiency in humans who consume wheat. In this study, two field experiments were conducted to investigate the effects of foliar Zn application combined with (1) macronutrients and Zn-amino acid chelates and (2) different soil N levels on Zn and Fe concentrations in whole grain and flour grown with straw incorporation. The results of the first experiment showed that flour Zn concentrations increased by 60.5%, 39.9%, and 56.2% due to the foliar application of $ZnSO_4$ combined with N (urea), PK (KH_2PO_4), and K (K_2SO_4), respectively, compared to the control, and the corresponding increases in whole grain were 65.6%, 38.1%, and 50.1%, respectively. Compared with foliar Zn application alone, foliar N and K applications caused similar increases in grain Zn and Fe concentrations, whereas the foliar application of Zn combined with PK resulted in a significant decrease (approximately 12.1% across the two cropping years) in whole-grain Zn concentrations. Flour Zn concentrations, however, were unaffected by foliar Zn combined with PK fertilization and significantly increased due to the foliar application of Zn combined with N or K (in the first cropping year) compared to foliar Zn application alone. Foliar Zn combined with macronutrient fertilization tended to increase flour and grain Fe concentrations. Additionally, reducing the soil N application rate generally had a small effect on flour and grain Zn and Fe concentrations. The results of the second experiment showed that the application of $Zn(Gly)_2$, alone or in combination with N(urea), caused similar increases in Zn and Fe concentrations in flour and whole grain. Foliar $ZnSO_4$ combined with macronutrient fertilization under 15% reduced soil N supply and straw incorporation is an economical, effective, practical, and environmentally friendly agronomic practice for enhancing Zn and Fe concentrations in flour.

1. Introduction

Zinc (Zn) and iron (Fe) deficiency caused by inadequate dietary intake is a nutritional problem in humans that affects approximately two billion people throughout the world (Kutman et al., 2011; White and Broadley, 2009). Wheat, which is inherently low in Zn, is a staple food and provides, on average, nearly 50% of humans' daily caloric intake, and this value will likely increase to more than 70% in rural regions (Cakmak, 2008; Cakmak, 2004). Additionally, most of the Zn and Fe in grain are located in the embryo and aleurone layer, which are also rich in protein (Ozturk et al., 2006), but not in the endosperm. Milling converts wheat grains into white flour, resulting in a marked reduction in flour Zn and Fe concentrations. Consequently, heavy consumption of milled wheat may lead to reduced Zn and Fe intake. In China, it has been reported that more than 85% of wheat is consumed as flour-derived products (Li, 2006). Therefore, research focusing on increasing Zn and Fe concentrations in flour is a high priority and will contribute to minimizing human Zn- and Fe-related malnutrition.

Among the interventions to alleviate Zn and Fe deficiency in humans, agricultural strategies (e.g., fertilization) are cost-effective and useful measures for improving micronutrient concentrations in grain, thereby improving human health. Directly adding Zn to extremely Zn-deficient soil (DTPA extractable Zn content: <0.2 mg kg^{-1}) improves both grain nutritional quality and

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production (Cakmak, 2008; Cakmak et al., 2010). However, in China, most wheat is grown on potentially Zn-deficient calcareous soils (DTPA-Zn: 0.5–1.0 mg kg^{-1}) and Zn-deficient soils (DTPA-Zn: 0.3–0.5 mg kg^{-1}). Although crops grown in such soils are not stressed due to Zn-deficiency, the low availability of soil Zn leads to low grain Zn concentrations. In this case, foliar Zn application with the appropriate dosage, timing, and spraying period has been shown to effectively increase grain Zn concentrations by nearly two- fold (Zhang et al., 2012b; Zhao et al., 2014; Li et al., 2014). The Zn concentrations of grain milling fractions are also improved by foliar Zn applications. Additionally, the increase in the Zn concentrations of grain and grain milling fractions due to foliar fertilization is enhanced by increased soil N levels in both fields and greenhouses (Cakmak et al., 2010; Kunman et al., 2011). This indicates that soil N application positively affects the accumulation of Zn in grains after foliar Zn application. However, with the widespread practice of wheat and maize straw return in winter wheat-summer maize rotation systems in north-central China, reducing soil N levels has become a trend to prevent fertilizer waste and protect the environment. Accordingly, it is important to study the biofortification of micronutrients (e.g., Zn and Fe) in wheat grain grown under straw incorporation, and thus reduced soil N levels. Moreover, flour-processing traits should be studied to determine possible negative effects caused by increased Zn and Fe (Gomez-Becerra et al., 2010; Peck et al., 2008).

In contrast to foliar Zn application, foliar N, P, and K fertilization has been used to increase crop production, especially in dryland areas (Gooding and Davies, 1992; Zhang et al., 2012b; Dos Santos et al., 2004; Thalooth et al., 2006). However, foliar macronutrient fertilization alone may only slightly affect or even decrease flour Zn concentrations due to the negative effects of some macronutrients on Zn (such as foliar P). Therefore, foliar Zn combined with N, P, or K fertilization may be a practical and promising measure to rectify the deficiencies of foliar Zn application alone (time and energy consuming) and compensate the reduced Zn concentrations after foliar macronutrient fertilization. Zhang et al. (2012b) demonstrated that there was no synergistic effect of combined foliar N and Zn fertilization on grain Zn concentrations. However, few studies have focused on the effects of this measure on Zn concentrations in flour. Although K is involved in activating a wide range of enzyme systems and has been shown to improve the growth and yield of Egyptian cotton when applied to soil in combination with foliar Zn fertilization, no published studies have emphasized the nutritional quality of wheat grain and its milling fractions as affected by foliar K and Zn fertilization (Nguyen et al., 2002; Sawan et al., 2008). Regarding Zn uptake by wheat receiving Zn and P amendments, most previous studies have focused on the mechanisms of soil Zn-P applications (Lu et al., 2011; Zhang et al., 2012a). The increase in wheat grain and flour Zn concentrations following foliar Zn and P fertilization has not been widely explored.

It has recently been reported that the foliar application of Zn-amino acids chelates (ZnAACs) can improve the nutritional quality of wheat and is more effective than $ZnSO_4$ in increasing grain Zn, Fe, and protein concentrations and Zn bioavailability (Ghasemi et al., 2013). Additionally, the application of AAs has also been showed to increase plant protein concentrations (Das et al., 2002; Chang et al., 2005). As nitrogen sources for plants, AAs may facilitate the uptake and translocation of Zn and Fe due to the effect of nitrogen on the number and activity of Zn- and Fe-carrier proteins on root cell membranes (Kutman et al., 2011). Moreover, AAs and certain chelating low-molecular-weight organic acids (such as citric acid) have been shown to have a promoting function on Zn concentrations, and both can increase Zn bioavailability via different mechanisms (Ekholm et al., 2003; Graham et al., 2001). However, little information is known about the impacts of foliar ZnAAC application, alone or in combination with macronutrients, on the

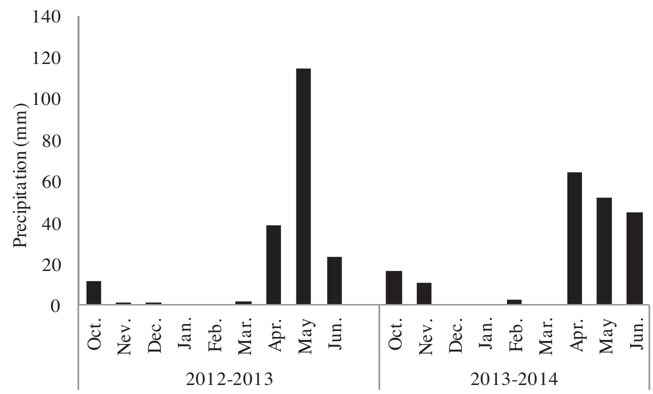

Fig. 1. Monthly precipitation during the winter wheat gro wing season.

flour nutritional quality of winter wheat grown on potentially Zn-deficient calcareous soil.

Therefore, the aims of this study were: (1) to investigate the effect of foliar $ZnSO_4$ combined with macronutrient fertilization on the nutritional quality (Zn, Fe, and protein concentration) of whole grain under reduced soil N and straw incorporation conditions; (2) to evaluate the nutritional quality and processing traits of flour influenced by foliar $ZnSO_4$ combined with macronutrient fertilization under the above soil conditions; and (3) to examine the effect of foliar Zn and macronutrient fertilization on the nutritional quality of grain and flour using ZnACCs as a Zn source.

2. Materials and methods

2.1. Field location

Two field experiments were conducted at the Sanyuan Experimental Farm (108°52'E, 34°36'N, 427 m above sea level) of Northwest A&F University, north of Xian City in Shaanxi Province during the 2012–2013 and 2013–2014 wheat cropping seasons. The climate at this site is semi-humid with an average annual temperature of 13 °C. The region's average monthly rainfall during the winter wheat growing season is shown in Fig. 1. The soil is classified as an Earth-cumuli Orthic Anthrosol (Tan et al., 2014) with a pH of 7.8, total N of 1.42 mg kg^{-1}, Olsen P of 32 mg kg^{-1}, available K of 183 mg kg^{-1}, and DTPA-Zn of 0.91 mg kg^{-1}.

2.2. Treatments and experimental design

The first experiment employed a split-plot design with four replications. The main plot (220 × 13 m) treatments included: (1) conventional soil N application rate (N_1); (2) 15% reduced conventional N rate, (N_2); and (3) 30% reduced conventional N rate (N_3). From 2008 to the start of the experiment, these plots received the same N fertilizer application rates and followed a winter wheat-summer maize rotation system. The sub-plot treatments were as follows: (1) foliar application of deionized water (CK); (2) foliar application of 0.3% (w/v) $ZnSO_4·7H_2O$ (Zn); (3) foliar application of 0.3% (w/v) $ZnSO_4·7H_2O$ plus 1.7% urea (Zn + N); (4) foliar application of 0.3% (w/v) $ZnSO_4·7H_2O$ plus 0.2% KH_2PO_4 (Zn + PK); and (5) Foliar application of 0.3% (w/v) $ZnSO_4·7H_2O$ plus 0.5% K_2SO_4 (Zn + K). Wheat was planted in the middle of October with a row spacing of 15 cm and seeding rate of 210–225 kg ha^{-1} and harvested in early June of the following year. Maize was generally planted in the middle of June after wheat harvest, with a row spacing of 50 cm and density of ~63,000 plant ha^{-1}, and harvested in early October. The cultivars of wheat and maize were Huaimai-22 and Nonghua 50, respectively. The total amount of N (as urea) used during the wheat and maize growing season was 150 and 187.5 kg ha^{-1}, respectively.