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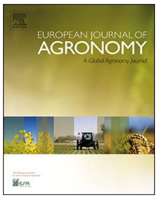

Zinc biofortification of wheat through preceding crop residue incorporation into the soil

Amir Hossein Khoshgoftarmanesh[a,*], Mojtaba Norouzi[a], Majid Afyuni[a], Rainer Schulin[b]

[a] Department of Soil Science, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran
[b] Institute of Terrestrial Ecosystems, ETH Zurich, Universitaetstrasse. 16, CH-8092 Zurich, Switzerland

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ABSTRACT

We conducted a two-year field experiment to investigate the potential benefit of preceding crop residue incorporation into the soil as a strategy to enhance the density of bioavailable grain zinc (Zn) in a subsequent wheat (*Triticum aestivum* L.) crop. Sunflower (*Heilianthus annuus* L. cv. Allstar), sorghum (*Sorghum bicolor* L. cv. Speed Feed), clover (*Trifolium pratense* L.) and safflower (*Carthamus tinctorius* L. cv. Koseh-e-Isfahan) were grown as preceding crop (precrop) on a Zn-deficient calcareous soil in central Iran, followed by a culture of two wheat cultivars i.e., Kavir and Back Cross Rushan. The harvested aboveground plant matter was air-dried, crushed into pieces of 0.5–2 cm size, mixed, and after taking a sample for analysis, incorporated manually into the upper 15 cm of the soil of one half of the same plot from which it had been harvested, while the other half received no residues. The aboveground residues of precrops were incorporated into soil or removed. A treatment with no preceding crop (fallow) and no residue incorporation, but with the same management otherwise, was implemented as control treatment. For both wheat cultivars studied, higher grain yield was obtained after clover (between 14 and 25.6%) and sunflower (between 11.3 and 19.5%) than that after safflower, sorghum and the fallow. All precrop treatments significantly increased the accumulation of grain Zn and N and decreased the phytic-acid-to-Zn (PA:Zn) molar ratio (by 5–41% in Kavir and by 11–48% in Back Cross), most effectively the clover treatment. The treatment effects on grain Zn were closely correlated with soil pH and dissolved soil organic carbon (DOC). The results show that the cultivation of appropriate precrops, especially legumes, can be an effective strategy to biofortify wheat grains with Zn without compromising yields.

1. Introduction

Zinc is an essential micro-nutrient element that is often deficient in human nutrition (Welch and Graham, 2004; White and Broadley, 2005; Graham et al., 2007). It has been estimated that over two billion people are at risk of Zn deficiency disorders (World Health Organization, 2002; Hotz and Brown, 2004; Stein, 2010). Zinc deficiency is particularly widespread in populations of developing countries depending on their nutrition on cereals for staple food and with little access to meat (Welch and Graham, 1999; Graham et al., 2001; Cakmak, 2008). Biofortification of staple food plants is considered a promising strategy to improve the Zn status in these populations (Bouis and Welch, 2010; Gibson, 2006; Mayer et al., 2008). This approach is more effective than other strategies for improving Zn status of those population groups that are most at risk of Zn-deficiency, i.e. people with low-income, populations in remote rural areas, infants, children and adolescents (Welch and Graham, 1999). Biofortification is the increase of bioavailable nutrient density in edible crop plant parts through genetic or agronomic techniques (Cakmak, 2008). One approach is agronomic biofortification. In this approach the (bioavailable) micronutrient density in plant-based food is increased by agricultural methods of crop cultivation, for example by adding the target nutrients with fertilizers or by applying amendments that increase their uptake from pools that are already in the soil but not sufficiently available (Rengel et al., 1999; Graham et al., 2001).

In this study, we were interested in the role of preceding crops and the management of their residues in Zn accumulation by subsequent wheat crops. Effects of preceding crops on the phytoavailability of micronutrients to the successive crop can for example result from residual effects of root litter and exudates on soil physical, biological, and chemical properties, generation of root channels, and other plant-soil interactions. In particular, it has been reported that legumes may enhance root uptake of immobile micronutrients by improving soil physical properties and thereby root growth conditions (Alvey et al., 2001). Wang et al. (2012) found that legume precrops improved wheat growth and enhanced the plant availability of soil nutrients. The effects

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[*] Corresponding author.
E-mail address: amirhkhosh@cc.iut.ac.ir (A.H. Khoshgoftarmanesh).

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of crop rotation on micronutrient phytoavailability can be quite different from those on total concentrations of micronutrients in soils (Wei and et al., 2006). Singh et al. (2005) found that rice (*Oriza sativa* L.) residues increased Zn and Fe phytoavailability in a sodic calcareous soil by reducing soil pH and exchangeable sodium percentage (ESP). Some studies suggest that the Zn density of wheat grains produced in organic farming systems can reach higher levels than under conventional cropping systems (Ryan et al., 2004; Mäder et al., 2011), which might be related to differences in organic matter input and Zn availability pool. Khoshgoftarmanesh and Chaney (2007) observed that wheat after sunflower accumulated more grain Zn than wheat after cotton due to higher soil depletion of Cd by sunflower in comparison with cotton.

For the purpose of biofortification it is necessary to increase the bioavailable pool of Zn in cereal grains and not just its total concentration (Cakmak, 2008). Two major factors affecting dietary Zn bioavailability are proteins and phytic acids (PA). The formation of strong complexes with PA is considered the main factor hindering the absorption of dietary Zn in the human intestine (Bosscher et al., 2001; Hotz and Brown 2004; Rimbach et al., 2008), while proteins promote Zn absorption. Several studies also found a close correlation between Zn accumulation and the concentration of N in grains, suggesting an important role of proteins in storing grain Zn (Peck et al., 2008). Nitrogen-containing compounds such as amino acids, peptides and proteins are also essential in Zn uptake, translocation and grain-loading in crop plants. Li et al. (2015) found that N fertilization significantly enhanced Zn in both whole grain and grain fractions. Furthermore, grain protein concentration is also a critical factor in bread baking (Gao et al., 2012). Therefore, targeting for high grain protein concentration in agronomic biofortification programs has the additional benefit of improving the baking quality of the flour.

The quality of previous crop residues differ in C, N, phosphorus (P), and Zn concentrations depending on plant species, and very little is known how they affect the accumulation of grain Zn in subsequent wheat crops and its bioavailability for uptake by human consumers. To test this approach also under field conditions we performed a two-year field plot experiment in which we grew different types of preceding crops and either incorporated their aboveground residues into a Zn-deficient calcareous soil or removed them to investigate their effects on yield and grain Zn, N and PA concentrations in subsequent crops of two common Iranian wheat cultivars. Preliminary results of this study have been published by Soltani et al. (2014). Here we report an analysis of the complete final data set, including also the effects of residue application vs. residue removal, the treatment effects on straw and grain yield, grain PA and PA-to-Zn molar ratio, grain N, soil N and available soil P, and mass balances for the pools of available soil Zn.

2. Materials and methods

2.1. Experimental site and soil properties

The study was conducted during 2009–2010 and 2010–2011 growing seasons on the fields of the agricultural research station at Rudasht (32°29′N, 52°10′E), approximately 40 km east of Isfahan, Iran. The mean temperature at the site was 16.3 °C in the first and 16.0 °C in the second year. The total annual rainfall was 114 mm in the first year and 126 mm in the second year, all of which was falling between November and May. The soil of the experimental plots was classified as a Typic Haplocambid (US Soil Taxonomy). Selected soil properties determined before the experimental treatments are given in Table 1. Compared to the critical deficiency level for DTPA-extractable Zn ($1.0\ mg\ kg^{-1}$) given by Mortvedt (1985), this soil was severely deficient in available Zn.

Table 1
Selected soil (0–15 cm) properties of the experimental sites.

Property	Year 1 (Field 1)	Year 2 (Field 2)
Clay ($g\ g^{-1}$)	0.414	0.425
Sand ($g\ g^{-1}$)	0.125	0.131
pH (H_2O)	7.6	7.4
Electrical Conductivity ($dS\ m^{-1}$)	5.1	6.3
$CaCO_3$ ($g\ g^{-1}$)	0.34	0.33
Organic Matter ($g\ kg^{-1}$)	3.9	4.1
Total N ($g\ kg^{-1}$)	1.2	1.1
Available P ($mg\ kg^{-1}$ soil)	11.13	12.24
DTPA-extractable Zn ($mg\ kg^{-1}$ soil)	0.15	0.18

2.2. Experimental treatments

In both experimental growing seasons, the following 4 plant species were grown as preceding crops and applied as green manure to the subsequent wheat crop: sunflower, sorghum, clover and safflower. These were chosen because they are the most popular plants cultivated before wheat in Isfahan province. In addition to these four precrop treatments, a treatment with no preceding crop (fallow) and no residue incorporation, but with the same management otherwise, was implemented as control treatment.

After chisel-plowing, mineral fertilizers were applied to all experimental plots at rates based on the recommendations of the Iranian Soil and Water Institute (Milani et al., 1998). Phosphorus was applied as triple super phosphate ($200\ kg\ P\ ha^{-1}$) and potassium in form of sulfate ($150\ kg\ K\ ha^{-1}$). Nitrogen was applied as urea at a rate of $150\ kg\ N\ ha^{-1}$ (except for the clover treatment). All four preceding crops were planted in rows with 50 cm spacing. The planting date was on 26 June 2009 in the first year and on 19 April 2010 in the second year of the experiment. Planting density was 25, 25, 50 and 700 plants m^{-2} for sunflower, sorghum, safflower and clover, respectively. The crops were irrigated to keep soil moisture at approximately 70% field capacity, using the basin irrigation method. Irrigation rates were based on evapotranspiration data collected at the local weather station of Rudasht. The irrigation water had an electrical conductivity (EC) of $1\ dS\ m^{-1}$. All four preceding crops were harvested on 18 October 2009 in the first year and on 15 August 2010 in the second year of experiment. Safflower, sunflower and sorghum were harvested at grain filling. Clover was harvested at flower bud.

The harvested aboveground plant matter was air-dried, crushed into pieces of 0.5–2 cm size, mixed, and after taking a sample for analysis, incorporated manually into the upper 30 cm of the soil of one half of the same plot from which it had been harvested, while the other half received no residues. An application rate of 7 tons per hectare was used for all four types of precrop residues. This rate is nearly equivalent to the average harvest index of preceding crops in the study area. The average aboveground biomass of precrops is shown in Table 2. Although the average harvest of precrops was different, we used the same rate of crop residue for all precrops to better compare their effects on soil and plant parameters. As Table 2 shows, the Zn, N and P concentrations and the C:N ratios were similar in each type of residue in the two experimental years, while there were large differences between the four precrop species. Clover residues contained approximately twice as much N as the other three precrops, and thus also had the lowest C:N ratio. It also had a 20% higher density of Zn than safflower and sunflower. On the other hand, clover residues had almost 20% less P than safflower and sunflower residues. It was even poorer in P than sorghum, which had the lowest concentrations in all three nutrient elements (N, P, and Zn) among the three non-legume precrops.

Three weeks after incorporation of the residues, spring wheat was planted, i.e. on 12 December 2009 in the first year and on 20 November 2010 in the second year, using the two common Iranian cultivars Kavir and Back Cross Rushan. Planting density was 350 plant m^{-2}. Fertilizers