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Fractionation and bioavailability of zinc (Zn) in the rhizosphere of two wheat cultivars with different Zn deficiency tolerance

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ABSTRACT

Zinc (Zn) deficiency is a widespread problem in wheat-cultivated lands, and understanding important mechanisms determining Zn phytoavailability is of high importance. Accordingly, a two-year field experiment was conducted to investigate the effects of two Zn-deficiency tolerant wheat cultivars on Zn binding forms in a calcareous saline soil. Changes in soil Zn fractions were also found to be related to Zn uptake by wheat. Rhizosphere and bulk soil samples and the above-ground biomass were collected at grain maturity and analyzed for Zn. By using a sequential extraction procedure, soil Zn was divided into the following operationally defined fractions which are conceptually designated: 'exchangeable Zn' (EXCH-Zn), 'Zn bound to carbonates' (CAR-Zn), 'organically bound Zn' (ORG-Zn), 'Zn bound to iron and manganese oxides' (FeMnOX-Zn), and 'residual Zn' (RES-Zn). EXCH-Zn and ORG-Zn in the rhizosphere soil were found to be positively correlated with Zn uptake into the aboveground biomass of both wheat cultivars, thereby indicating that these fractions could be considered as labile pools. Higher EXCH-Zn and ORG-Zn fractions were determined in the rhizosphere, not in the bulk soil, whereas the non-labile Zn pools was decreased, suggesting strong Zn solubilization due to the input of acidity and organic compounds by root and the associated microbial activity. The differences between rhizosphere and bulk soil in EXCH-Zn and ORG-Zn were associated with the lower pH and the higher concentrations of the total (TOC) and dissolved organic carbon (DOC) in the rhizosphere. The fact, these effects (lower pH and higher DOC in the rhizosphere, rather than in the bulk soil) were stronger for Back Cross Rushan than were for Kavir; this was in line with the higher Zn uptake of Zn-deficiency tolerant Back Cross Rushan.

1. Introduction

Zinc (Zn) uptake by roots depends on the concentration of free Zn ions in the rhizosphere. However, the concentration of Zn in soil solution is usually very small, as compared to the plants' demand; thus, it is quickly depleted without proliferation from other pools.

Zinc concentration in soil solution and its availability to plant are mostly controlled by solubility relations and adsorption-desorption reactions between the solution and solid phases (Catlett et al., 2002; Lindsay, 1991). The adsorption-desorption reactions depend on the distribution of Zn in various soil fractions.

In soils, Zn exist in several different forms which are associated with a range of components (Tack and Verloo, 1995). Information about the physicochemical forms of the elements is required for understanding their bioavailability. Although there is no generally accepted definition of the term speciation can broadly be defined as the identification and quantification of the different, defined species, forms or phases in which an element occurs. Distribution in the solid phase is related to the metal release to the solution and hence, the likelihood of bioavailability (Tack and Verloo, 1995).

The following soil Zn fractions are often distinguished with regard to chemical binding characteristics: Zn bound to ion-exchanging sites, Fe and Mn oxides, organic matter, carbonates, and residual mineral phases (Wang et al., 2009). Ion exchange is the most labile binding form, and, not surprisingly, close correlations between the exchangeable soil Zn fraction and the plant uptake of Zn have been found (Chahal et al., 2005; Li et al., 2007). Various soil properties, i.e., pH, cation exchange properties and organic matter, affect the distribution of Zn in soil solid phase, thereby regulating the amount of Zn dissolved in the soil solution (Gaudalix and Pardo, 1995).

Plants can enhance the availability of mineral nutrients in various ways; for example, through the release of root exudates (Clemens et al.,

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2002; Knight et al., 1997; Udom et al., 2004). Root exudates acidify the rhizosphere and partly solubilize metals by the formation of complexes with the organic ligands (Chen et al., 2010; Wang et al., 2009). Plants are able to influence chemical conditions in the rhizosphere in various ways; for instance, by the decrease in redox potential and the increase in pH, dissolved organic carbon (DOC), and microbial activity in the rhizosphere (Dessureault-Rompre et al., 2008; Mench and Martin, 1991; Tao et al., 2003).

Zinc-deficiency tolerance wheat genotypes use certain mechanisms, such as the release of proton, phytosiderophore and other organic ligands, to improve Zn uptake by roots; thus, they can grow on soils with very low Zn availability, while Zn-sensitive genotypes suffer from Zn deficiency on such soils (Cakmak et al., 1996; Khoshgoftarmanesh et al., 2010). Differences in metal availability and fractionation between rhizosphere and the bulk soil of several plants such as wheat (Wang et al., 2002), maize (Tao et al., 2003, 2005), soybean (Yu and Zhou, 2006), ryegrass (Wei-Hong et al., 2007) and rice (Hu et al., 2011) have been demonstrated, but little is known about the factors responsible for this variation.

The higher exudation of acidity and organic ligands, i.e., phytosiderophores from the roots of Zn-deficiency tolerance genotypes (e.g., Back Cross Rushan) in comparison with Zn-deficiency sensitive genotypes (e.g., Kavir), has been widely reported (Daneshbakhsh et al., 2012; Khoshgoftarmanesh et al., 2006, 2009; Zhang et al., 1989). We hypothesized that variation in the exudation of acidity and organic ligands from wheat roots could differently affect fractionation and solubility of Zn in the soil. There is limited information regarding the possible effects of the changes induced by wheat roots on the solubilization and mobilization of Zn in the soil under field conditions. It is important to enhance our knowledge on the effect of wheat roots on the mobilization of Zn in soil under the field conditions particularly by considering that field experiments are necessary to conclude results, and are closer to real conditions than the laboratory-studies. This is also very valuable for crop fertilizer management improvement in the context of an increasing world population. In the present study, we investigated the effects of two wheat cultivars with different Zn-deficiency tolerance on the operational Zn fractionation in the rhizosphere and the bulk soil. To the best of our knowledge, this is the first study to address the effects of wheat roots on Zn fractionation in a field soil.

2. Materials and methods

2.1. Experimental site

The study was conducted in the growing seasons encompassing 2009–2010 and 2010–2011 time periods on the neighboring fields of Rudasht Agricultural Research Station (32, 29/N, 52, 10/E), east of Isfahan, Iran. The mean temperature at the site was 16.3 °C in the first year, and 16.0 °C in the second one (Isfahan Management and Planning Organization, 2010). The total annual rainfall was 114 mm in the first year, and 126 mm in the second one, with all these falling between November and May. The soil of the experimental site could be classified as a Typic Haplocambid, according to the US soil taxonomy (Table 1).

Table 1
Selected soil properties of the experimental soil.

Property	
Clay (%)	41.4
Silt (%)	45.7
pH (H_2O)	7.6
Electrical conductivity (dS m^{-1})	5.1
Organic matter (%)	0.39
Equivalent calcium carbonate (%)	34
DTPA-extractable Zn (mg kg^{-1} soil)	0.15
HNO_3-extractable-Zn (mg kg^{-1} soil)	51

The concentration of DTPA-extractable soil Zn was 0.15 mg kg^{-1}, which was below the critical threshold for Zn-deficiency of 1.0 mg kg^{-1}, as introduced by Mortvedt (1985).

2.2. Experimental procedures

Three replicate plots of 3 m length and 2 m width were installed for each of the two bread wheat cultivars. In both years, all experimental plots were chisel-plowed (0–30 depth) two weeks before sowing. We applied 200 kg triple super phosphate ha^{-1} and 150 kg K_2SO_4 ha^{-1} (Milani et al., 1998). Wheat was cultivated on December 12, 2009, in the first year, and on November 20, 2010, in the second experimental year. The seeding density was 350 seeds m^{-2}. The seeds of the two bread wheat (*Triticum aestivum* L.) cultivars including 'Back Cross Rushan' and 'Kavir' were prepared at the Soilless Culture Research Centre of the Isfahan University of Technology, Isfahan, Iran. Nitrogen (N) fertilizer was applied two times in the form of urea: the first application of 150 kg ha^{-1} was given after tillering, and the second application of 100 kg ha^{-1} was at the heading stage. During the plant growth, soil moisture was kept at about 70% field capacity by applying ponding irrigation at rates determined according to local evapotranspiration measurements at the research station. The irrigation water showed the electrical conductivity (EC) of 1 dS m^{-1}. Harvest took place on June 23, 2010, on July 1st and 9th, 2011, in the second growing season.

2.3. Soil sampling and analysis

Prior to land preparation, soil samples (0–30 cm depth) were collected from each plot in both experimental years, air-dried, crushed to pass a 2-mm sieve, and used for chemical analysis. Soil pH was measured in 1:2.5 soil:water suspensions using a digital pH meter (Model 691, Metrohm AG Herisau Switzerland), and electrical conductivity (EC) was checked with an EC meter (Model 26 Ohm-644, Metrohm AG Herisau Switzerland). Total soil organic carbon (TOC) was determined using the Walkley and Black method (Nelson and Sommers, 1982). The soil texture was determined by the hydrometer method (Gee and Bauder, 1986) known as the silty clay. $CaCO_3$ content was measured through dissolution with HCl and back-titration with NaOH (Nelson, 1982). The dissolved organic carbon (DOC) was measured in saturated-paste extracts using a Primacs SLC TOC Analyzer (Model CS22) (Bolan et al., 1996). Total nitrogen (TN) was determined using the Kjehldal method. The available phosphorus (P) was measured, as described by Olsen and Sommers (1982). The available soil Zn was extracted using diethylene triamine pentaacetic acid (DTPA) and analyzed by means of a Perkin Elmer (Wellesley, MA) atomic absorption spectrophotometer (AAS) Model 3030.

For soil Zn fractionation, rhizosphere and bulk (0–30 cm depth) samples were collected at both harvests in triplicates, from the topsoil of each plot, by pulling entire wheat plants out of the soil and gently shaking them by hand, as described by Rollwagen and Zasoski (1988). The soil that kept to be adhered to the roots was considered as the rhizosphere soil, and the rest was regarded as the bulk soil.

The sequential extraction procedure proposed by Tessier et al. (1979) separated the following five operationally defined soil Zn fractions: exchangeable Zn (EXCH-Zn), Zn bound to carbonates (CAR-Zn), organically bound Zn (ORG-Zn), Zn bound to iron and manganese oxides (FeMnOX-Zn), and residual Zn (RES-Zn) (Table 2). The extracts were analyzed for Zn using the same Perkin Elmer 3030. The total soil Zn was extracted with 6 M HNO_3 (Sposito et al., 1982) and analyzed by means of AAS. Compared with the total concentration of Zn extracted with 6 M HNO_3, Zn recovery varied from 82 to 110% for the individual samples, indicating the acceptable efficiency of the sequential extraction method. Tessier et al. (1979), Sposito et al. (1982), and Nogueira et al. (2010) reported similar percentages of Zn recovery for the Tessier sequential extraction method.