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# Iron concentration, bioavailability, and nutritional quality of polished rice affected by different forms of foliar iron fertilizer

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ABSTRACT

The present study compared the effects of four different forms of foliar iron (Fe) fertilizers on Fe concentration, bioavailability and nutritional quality of polished rice. The results showed that foliar fertilisation at the anthesis stage was an effective way to promote Fe concentration and bioavailability of polished rice, especially in case of DTPA-Fe. Compared to the control, foliar application of DTPA-Fe increased sulphur concentration and the nutrition promoter cysteine content, whereas decreased phosphorus concentration and the antinutrient phytic acid content of polished rice, as a result increased 67.2% ferrtin formation in Caco-2 cell. Moreover, foliar DTPA-Fe application could maintain amylase, protein and minerals quality of polished rice. According to the current study, DTPA-Fe is recommended as an excellent foliar Fe form for Fe biofortification program.



## 1. Introduction

Rice is the most widely consumed staple food crop and a primary food source for 50% of the world's population (Fitzgerald, McCouch, & Hall, 2009). The nutrient-rich aleurone layer and embryo are traditionally removed before human consumption, typically leaving only the starch-rich endosperm as the edible part (Bouis & Welch, 2010; Wei, Shohag, Yang, & Zhang, 2012). Therefore, the resulting polished rice is a poor source of essential micronutrients, such as iron (Fe) and zinc (Zn) (Bouis & Welch, 2010; Khush, 1997). Jiang, Wu, Feng, Yang, and Shi (2007) found that the average Fe content in milled rice of 274 genotypes was 5.4 ± 2.9 μg/g. A survey of 11,400 rice samples revealed that brown rice contained 10.0–11.0 μg/g Fe, whereas the milled rice contained 2.0–3.0 μg/g Fe (Martínez et al., 2010). As the polished rice contains an average of only 2.0 μg/gFe (Johnson et al., 2011; Yuan, Wu, Yang, & Lv, 2012), which is much lower than the international biofortification target for polished rice Fe concentration of 14.0 μg/g. Thus, an inadequate intake of Fe from staple food leads to Fe malnutrition in humans, affecting two billion people (Sperotto, Ricachenevsky, Waldow, & Fett, 2012), most of them in developing countries (Yang, Chen, & Feng, 2007).

Many approaches have been chosen to increase the Fe content in rice grains and ameliorate the Fe malnutrition, including conventional breeding, genetic engineering and agronomic approaches (Sperotto et al., 2012; Wei et al., 2012; Yang et al., 2007). Among them, fertilisation, especially foliar Fe spray is considered as a rapid and efficient way to reach the Fe biofortification target in recent years (Aciksoz, Yazici, Ozturk, & Cakmak, 2011; Fang et al., 2008; Yuan et al., 2012). However, the Fe intake from food is not only determined by the net Fe concentration but, to a large extent, also by the Fe bioavailability (Glahn, Cheng, Welch, & Gregorio, 2002; Promüthai et al., 2009). Ideally, the Fe bioavailability in grains should be evaluated through *in vivo* human studies, but performing large-scale screenings is complex and costs limit their applicability (Glahn et al., 2002; Promüthai et al., 2009; Wei et al., 2012). In recent years, an *in vitro* digestion/Caco-2 cell model had been proposed as an alternative to *in vivo* methods for estimating mineral bioavailability in diets, as this cell line mimics the gastric and intestinal digestion of humans and has been successfully used to assess Fe bioavailability in cereal grains (Glahn et al., 2002; He, Feng, Li, & Yang, 2008; Wei et al., 2012).

$FeSO_4$ is a widely used form of foliar Fe fertilizer (Fang et al., 2008; Wei et al., 2012; Yuan et al., 2012); however, Fe(II) is highly unstable and is easily converted to plant-unavailable, solid Fe(III) forms. Therefore, increasing the stability of Fe(II) and controlling its release speed is very important for enhancing the absorption efficiency of foliar Fe fertilizer (Fernández & Ebert, 2005). Synthetic

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chelating ligands, such as EDTA and DTPA, are effective for increasing Fe bioavailability (Fernández, Río, Abadía, & Abadía, 2006; Hasegawa et al., 2012). However, to our knowledge, studies evaluating the effects of foliar application of different Fe fertilizers on the Fe content, particularly on the Fe bioavailability of polished rice are rare.

The objective of present study is to compare the effects of the foliar application of $FeSO_4$, EDTA-FeNa, DTPA-Fe, and HEDTA-Fe on the Fe concentration, bioavailability and nutritional quality of polished rice. The results can facilitate the design and development of new Fe foliar fertilizers for rice Fe biofortification program.

## 2. Materials and methods

### 2.1. Plant material and pre-culture

A *Japonica* variety HB075 was used in this experiment. The rice seeds were surface-sterilized with 1.5% (v/v) sodium hypochlorite for 10 min, rinsed thoroughly in deionized water and imbibed in a dish containing a shallow layer of deionized water at room temperature overnight. The seeds were germinated in moist quartz. The seedlings were grown in a growth chamber under a light/dark regime of 14/10 h, a temperature of 30/25 °C (day/night), 300 μmol $m^{-2}$ $s^{-1}$ photosynthetically active radiation and a relative humidity of 70–80%. Approximately 20 d old uniform and healthy seedlings were selected and transplanted.

### 2.2. Field experiment

The field experiment was conducted at the Huajiachi experimental farm of Zhejiang University (Zhejiang, Hangzhou, China; 30°15′19″N, 120°10′8″E). The climate of the experimental site is subtropical humid. The soil type of experimental field was periodical water logged paddy soil. Some physico-chemical properties of the experimental soil were as follows: pH, 5.86; organic matter, 19.90 g/kg; cation exchange capacity (CEC), 14.59 cmol/kg; total nitrogen (N), 1.86 g/kg; total phosphorus (P), 0.68 g/kg; available N, 67.41 mg/kg; available P, 63.95 mg/kg; available potassium (K), 85.36 mg/kg; available Fe, 154.78 mg/kg; available Zn, 4.61 mg/kg; available Cu, 4.38 mg/kg and available manganese (Mn), 82.15 mg/kg. The experiment was arranged in randomized complete block design (RCBD), with three replicates. Thirty uniform and health seedlings were transplanted in each plot comprised a replicate. All the experimental plots received $P_2O_5$ as triple superphosphate, 120 kg/ha, and $K_2O$ as potassium chloride, 240 kg/ha, before the seedlings were transplanted. Each plot received urea-N 160 kg/ha, 2/3 as a basal fertilizer and 1/3 as top-dressing at the tillering stage.

The following foliar spray treatments were applied: (i) control, deionized water spray; (ii) 0.2% (w/v) $FeSO_4{\cdot}7H_2O$; (iii) 0.2% (w/v) EDTA-FeNa; (iv) 0.2% HEDTA-Fe and (v) 0.2% DTPA-Fe. All foliar applications contained 0.01% (v/v) Tween80 as a surfactant. Foliar spraying was performed three times using a mini-type directed air-jet sprayer once every five days, at the anthesis stage. Spray was applied after sunset; about 5:00–6:00 pm. A volume of 500 mL of the solution was sprayed per plot per treatment, with the nearby plants covered with a plastic film while spraying to prevent contamination.

### 2.3. Samples preparation

At the ripening stage, plants were harvested from the centre of each plot, manually threshed to separate grains, rinsed thoroughly with 0.01 M hydrochloric acid (HCl, AR-Grade) and then with the deionized water to eliminate residues. After air dry, the rice samples were de-husked using an electrical de-husker (JLGJ-4.5, Taizhou Cereal and Oil Instrument Co. Ltd., Zhejiang, China), polished by a polishing machine (JB-20,Taizhou Cereal and Oil Instrument Co. Ltd., Zhejiang, China) and ground using a ball mill (Retsch MM301, Germany). The ground samples of polished rice were placed in airtight plastic bags andstored in desiccators at room temperature until analysed. Part of the rice samples (100 g) were cooked for 15 min and then homogenised for 10 s using a Braun blender (type 4142) set at the maximum speed. The homogenate was frozen and lyophilized to dryness.

### 2.4. Determination of Fe bioavailability

#### 2.4.1. In vitro digestion of sample

The *in vitro* digestion of samples was according to our previously described method (He et al., 2008). Porcine pepsin (800–2500 units/mg protein), pancreatin (activity = 4 × U.S.P. specifications), and bile extract (glycine and taurine conjugates of hyodeoxycholic acid and other bile salts) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and used without further processing. The preparation of the gastric and enteric (pepsin, pancreatin and bile extract) digestion solutions and the *in vitro* digestion was performed as detailed in our previous study (He et al., 2008). The intestinal digest was heated for 4 min at 100 °C to inhibit the sample protease and then immersed in an ice bath. Aliquots of 10 g of the digested sample were transfer to polypropylene centrifuge tubes (50 mL) and centrifuged 3500*g* for 15 min at 4 °C. Supernatants were transferred and pooled. Glucose (5 mM final concentration) and HEPES (50 mM final concentration) were added into the soluble fraction, and deionized water was used to adjust the osmolarity to 310 ± 10 mOsm/kg (freezing point osmometer, Osmomat 030, Berlin, Germany). The supernatants (soluble fraction) were analysed for Fe content and used in cell uptake assays.

#### 2.4.2. Caco-2 cell culture

The available Fe from the digests was evaluated using human colon carcinoma cells (Caco-2). The Caco-2 cells were obtained from the Institute of Biochemistry and Cell Biology (SIBS, CAS, Shanghai), China and used in assays at passage between 20 and 43. The cells were grown in 25 $cm^2$ tissue culture flasks with 5 mL of Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY, USA), 4 mL/L antibiotic mixture (GIBCO, Grand Island, NY, USA), 25 mg/L amphotericin B (GIBCO, Grand Island, NY, USA), and 100 μM nonessential amino acids (GIBCO, Grand Island, NY, USA). Cells used in the Fe uptake and transport experiments were seeded on transwell (Corning, New York, USA) inserts at a density of $2.5 \times 10^5$ cells per insert (4.71 $cm^2$) and grown for 21–22 d in supplemented DMEM. Medium (1.5 mL apical, 2.5 mL basolateral) was changed every 2 d for the first 2 weeks, then daily for another 7 d prior to use for uptake and transport studies on day 21. The cells were maintained at 37 °C in an incubator (Heraeus, BB15, Germany) with 5% $CO_2$ and 95% air atmosphere at constant humidity.

#### 2.4.3. Fe bioavailability in Caco-2 cell

Fe bioavailabilility of polished rice was determined *via* subsequent exposure of the *in vitro* digested solution to the Caco-2 cell as described in our previous report (Wei et al., 2012). Shortly, immediately before the intestinal digestion period, the growth medium was removed from each culture well, and the cell layer was washed twice with Hanks' balanced salt solution (pH 7.0). The transepithelial electrical resistance (TEER) was then measured, and only those filters showing a TEER of >250 $\Omega cm^2$ at the beginning and end of the experiment were included. The bottom chamber was then filled with 2 mL of HBSS, and the upper chamber was