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# High soil redox potential contributes to iron deficiency in drip-irrigated rice grown in calcareous Fluvisol

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**Abstract**: Drip-irrigated rice (*Oryza sativa* L.) is susceptible to iron (Fe) deficiency. The major possible cause of Fe deficiency is the changes in the water regime, which mainly affects the redox potential ($E_h$) of the soil dictating the solubility of Fe. However, how high soil $E_h$ affects soil available Fe and rice Fe uptake is unclear. In this paper, we investigated the effect of soil $E_h$ on rice Fe uptake under different water management strategies (drip irrigation (DI), flood irrigation (FI) and forced aeration of soil in flooding irrigation (FIO)). The results showed that the diethylenetriaminepentaacetic acid (DTPA)-extractable Fe and $Fe^{(II)}$ concentration in the soil, Fe concentration and chlorophyll contents of leaves and biomass of rice in FIO were greater than those in DI but significantly less than those in FI. The Fe uptake of the plant in DI was the lowest, but which in FI was the highest. Overall, FIO resulted in a significant reduction in Fe uptake of rice, but greater than that in DI. We concluded that both the decreased soil water content and the increased soil $E_h$ were important factors that caused Fe deficiency of drip-irrigated rice.

**Keywords**: iron deficiency chlorosis; iron uptake; water-saving cultivation; water stress; nutrition

To counteract the increasing unavailability of water for agriculture, the International Rice Research Institute has worked to develop and promote the alternate wetting and drying (AWD) water management technology (Lampayan et al. 2015). Drip irrigation (DI) of rice (*Oryza sativa* L.) is a new type of water-saving cultivation technology that combines water-saving and high-yield in Xinjiang, China (He et al. 2013); because its soil water content is generally lower than saturated field water holding capacity (Biswas et al. 2015). DI is similar to AWD, but with a higher frequency of irrigation. In practice, DI rice easily shows iron (Fe) deficiency chlorosis at the seedling stage in calcareous soils (Zhang et al. 2019a); however, this phenomenon is rare in flooding cultivation.

The precipitation and dissolution of Fe-containing-materials are driven by Fe-redox cycling (Posth et al. 2014). $Fe^{(III)}$ is reduced to $Fe^{(II)}$ at soil redox potential ($E_h$) ranging from 100 to 300 mV (Sposito 2008). Considering $E_h$ rising from –100 to +400 mV in AWD land (Johnson-Beebout et al. 2009, Tuyogon et al. 2016), so soil $E_h$ may play an important role in DI rice chlorotic. Paddy rice is suitable to grow in flooding soil; the soil water content in DI is a mild water stress condition for rice growth (Zhang et al. 2019b). Researches indicated that the water regime change results in a series of stress symptom of the plant (Lisar et al. 2012). Because Fe transports from root to shoot mainly drive by transpiration (Nikolic and Pavlovic 2018) and DI rice often has a decreased transpiration (He et al. 2013). Consequently, the soil water content may be another factor affecting Fe transport of DI rice.

Both the decreased soil water content and the increased soil $E_h$ play a role in Fe disorder in DI

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rice. Although many researches showed that these factors affect Fe availability of soil and hence disturb crop Fe nutrition (Fageria 2013), how the single soil $E_h$ affects soil Fe availability and Fe uptake of rice is rare. The objective of this simulation experiment was designed to understand the extent to which soil $E_h$ affects Fe uptake of DI rice.

## MATERIAL AND METHODS

**Soil properties**. This study was conducted at Shihezi University Agricultural Experiment Station, Xinjiang, China (44°18'N, 86°02'E) in 2017. The soil was collected from the 0–30 cm depth at Daquangou Farm in the north of Shihezi, which has been growing paddy rice for more than 30 years. The soil is a Calcareous Fluvisol according to the FAO-UNESCO classification system (FAO 1998). The experimental soil contained 7.18 g/kg $C_{Walkey–Black}$, 10.56 mg/kg $Fe_{DTPA\text{-}extraction}$, with a $pH_{H_2O}$ of 7.63 (fresh matter (FM)). The percentage of clay, silt, and sand (laser particle size analysis method) was 19.3, 32.0 and 48.7%, respectively.

**Experimental design**. The pot experiment consisted of three water management strategies, e.g., drip irrigation, forced aeration of soil in flooding irrigation and flooding irrigation referred to as drip irrigation (DI), forced aeration of soil in flooding irrigation (FIO), and flood irrigation (FI), respectively. Each treatment pre-sets two sampling periods (e.g., tillering (28 days after transplanting) and flowering (56 days after transplanting) stage), and which was arranged in randomized block designs with four replicates during each sampling period. In FIO, at the bottom of the pot a round-shaped aeration equipment (AEQ) was placed, the size of the AEQ (20 cm inner diameter × 3 cm height) was equal to the inner diameter of the bottom of the pots. Each AEQ was connected with an air pump (AP228, max output: 6 L/min) with plastic pipe. The plastic pot had a small drainage hole to simulate natural seepage at the rate of 5 mm/day in DI, and the plastic pot had no drainage hole in FIO and FI.

The soil described above was air-dried, passed through a 4 mm sieve, mixed with N, P and K fertilizer and packed into plastic pots (20 cm inner diameter × 30 cm height). Each plastic pot contained 7.0 kg of soil. The N (0.20 g N/kg soil) was applied in four split applications: 15% before planting, 30% at tillering, 40% at jointing-booting and 15% at the panicle stage. The P (0.34 g P/kg soil) and K (0.24 g K/kg soil) fertilizers were both applied before planting. After preparing the plastic pots, water was applied to saturate the soil to restore the natural reduction conditions.

Pre-germinate rice seeds (*Oryza sativa* L. cv. T-43) by soaking in water (20°C) for 24 h and then sown in seedling bed. Rice seedlings (40 days after emergence) were transplanted to the pots when which has four complete leaves. Each pot had four hills, and each hill contains six plants. The soil surface in all treatment was covered with 5–7 cm of water layer for one week after transplanting. Then, the water layer of DI was removed and covered with transparent plastic film (7 μm thick); while in FIO and FI a 5–7 cm of water layer was kept. The soil water content in DI was monitored by weighing the pots. When the soil moisture fell to 85% of relative field capacity, an intravenous drip apparatus was applied to supply water to the soil until the soil moisture reached 100% of relative field capacity. The FIO treatments start aerated for 1 h every 2 h with an air pump when the DI soil surface water layer was removed. The entire water management strategy continued until the entire experiment was completed. The pots were kept in a mesh room.

**Soil and plant sampling**. Four pots of rice were randomly selected for sampling during the tillering and flowering stages, respectively. A SPAD-502 chlorophyll meter (Konica Minolta SPAD-502, Osaka, Japan) was used to measure the greenness in each replication. Four hills of rice were excavated from each replication. The plant samplings were collected from two rice hills. The plant samples were divided into the shoot and root. The shoots were divided into leaf, stems, and sheaths. Leaf active Fe concentrations were determined using fresh leaves from one of these two rice hills. The remaining rice was dried in an oven at 80°C for 72 h and weighed. Plant total Fe concentrations were determined using a dry weight which was used as plant biomass described before. The soil samplings were collected from the other two rice hills, placed in coolers, and returned to the laboratory to determine soil DTPA-extractable Fe, $Fe^{(II)}$ and total Fe.

**Chemical analyses**. Soil $E_h$ was determined every 7 days after transplanting using pH/ORP/ISE microcomputer multi-parameter detector (HI-98185, Hanna, Romania, Italy). To reduce the impact of soil temperature on soil $E_h$ test error, all pots were moved to a greenhouse with a stable temperature (25°C) for 2 h. Before determining the soil $E_h$, electrodes were checked and calibrated using quinhydrone redox/pH standards at pH 4 and 7. In FIO and FI, the electrode was inserted into the soil at a depth of 10 cm. In DI, the soil $E_h$ was determined as described by Fiedler et al. (2007). Briefly,

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Table 1. Physical and chemical parameters of the soil and plant

| Parameter | Method description and reference | Instrument |
|---|---|---|
| **Soil parameter** | | |
| DTPA-Fe (fresh matter) | DTPA extraction (Lindsay and Norvell 1978) | AAS (Hitachi Z-2000, Tokyo, Japan) |
| $Fe^{(II)}$ (fresh matter) | aluminium sulfate extraction (Lu 2000) | |
| Total Fe (dry matter) | HF-$HClO_4$ digestion (Tessier et al. 1979) | |
| **Plant parameter** | | |
| Leaf chlorophyll *a* and *b* (fresh matter) | acetone extraction (Zhu et al. 2018) | spectrometer (Cary 50 UV-VIS, Varian, Mulgrave, Australia) |
| Leaf active Fe (fresh matter) | HCl-soluble extraction (Takkar and Kaur 1984) | AAS (Hitachi Z-2000, Tokyo, Japan) |
| Leaf and root total Fe (dry matter) | $HNO_3$-$H_2O_2$ digestion (Zhu et al. 2018) | |

DTPA – diethylenetriaminepentaacetic acid; AAS – atomic absorption spectrophotometer

a hole (2 cm diameter) was first bored to within 10 cm of the desired depth of installation. The soil at a depth of the electrode tip was then used to make a slurry of mud with distilled water. The slurry was poured into the hole, and the electrode pushed through the slurry into natural soil. Each pot had six replicates of $E_h$ value as data. Soil pH has a significant effect on $E_h$ value, so to rectify for soil pH and enable accurate soil $E_h$ comparisons between soils at different pH, all redox values were standardized to pH 7 as equation (Matern and Mansfeldt 2016):

$$E_h \text{ (pH 7)} = (\text{measured } E_h + 205) + (\text{pH} - 7) \times 59$$

Other selected physical and chemical parameters of the soil and plant collected from these treatments are shown in Table 1.

**Statistical analysis**. Treatment effects were analyzed using a one-way ANOVA followed by Duncan's multiple range test to calculate the least significant difference (*LSD*) between means. In all cases, differences were deemed to be significant if $P < 0.05$.

## RESULTS AND DISCUSSION

**Soil $E_h$ and Fe availability**. Soil $E_h$ in DI was gradually rose, and the values ranged from 300 to 500 mV (Figure 1). This result coincides with other's study, Macías and Arbestain (2010) indicated that soil $E_h$ in cultivated soils ranged from 300 to 500 mV under aerobic soils. The soil $E_h$ in FI and FIO were declined steadily to 100 and 50 mV at the flowering stage, respectively (Figure 1). Ponnamperuma (1972) and Zhi-Guang (1985) divided soil into oxidized ($E_h$ about from 400 to 600 mV), weakly reduced ($E_h$ about from 100 to 400 mV), moderately reduced ($E_h$ about from –20 to 100 mV) and highly reduced ($E_h$ < –20 mV). Therefore, the soil oxidation-reduction state in DI was oxidized, FI was a moderately reduced soil, and FIO was a weakly reduced soil condition.

When soil $E_h$ rises to 450–500 mV, Fe is oxidized and unfavorable for plant growth, with a risk of Fe deficiency (Husson 2013). Soil $E_h$ in DI was close to 300 mV at the tillering stage (Figure 1), the corresponding $Fe^{(II)}$ and DTPA-Fe concentration of soil were 234 mg/kg and 16.9 mg/kg, respectively (Table 2). When soil $E_h$ in DI increased to 457 mV at the flowering stage (Figure 1), the $Fe^{(II)}$ and DTPA-Fe concentration of soil were decreased to 118 mg/kg and 10.2 mg/kg, respectively (Table 2). However, there were no obvious changes in $Fe^{(II)}$ concentration of soil in FI between the tillering and flowering stage, because the soil $E_h$ was always less than 100 mV (Figure 1; Table 2). The similar results were found by Frohne et al. (2011), they indicated that the $Fe^{(II)}$

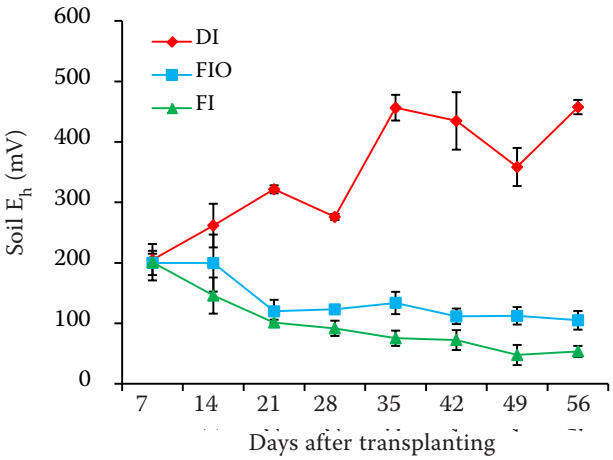


Figure 1. Changes of redox potential ($E_h$) in soil ($n$ = 4). DI – drip irrigation; FIO – forced aeration of soil in flooding irrigation; FI – flood irrigation

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Table 2. DTPA-Fe, iron ($Fe^{(II)}$) and total Fe concentration of soil at tillering and flowering stages

| Treatment | Soil DTPA-Fe | Soil $Fe^{(II)}$ | Soil total Fe |
|---|---|---|---|
| | (mg/kg) | | |
| **Tillering stage** | | | |
| DI | 16.9[c] | 234[c] | 12 876[a] |
| FIO | 24.6[b] | 270[b] | 12 717[a] |
| FI | 31.2[a] | 300[a] | 13 261[a] |
| **Flowering stage** | | | |
| DI | 10.2[c] | 118[c] | 12 971[a] |
| FIO | 47.2[b] | 300[b] | 12 338[a] |
| FI | 58.1[a] | 319[a] | 13 469[a] |

Values within a column and within a growth stage with the similar letter are not significantly different at 5% by Duncan's multiple range tests ($n$ = 4). DI – drip irrigation; FIO – forced aeration of soil in flooding irrigation; FI – flood irrigation; DTPA – diethylenetriaminepentaacetic acid

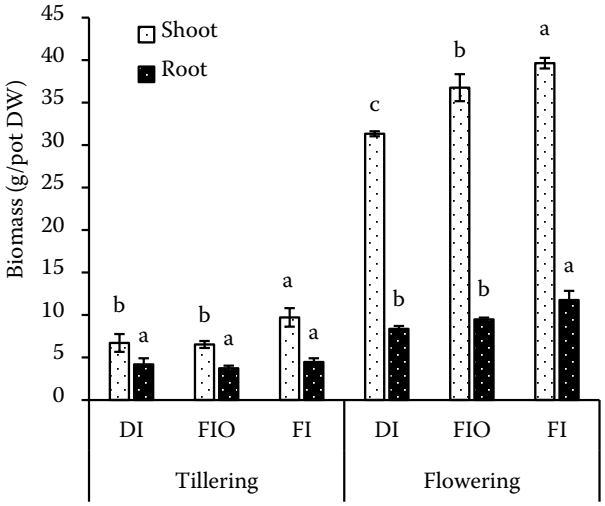


Figure 2. Rice biomass at tillering and flowering stages. Within a growth stage and plant part, bars with similar letters are not significantly different at 5% by Duncan's multiple range tests ($n$ = 4). DI – drip irrigation; FIO – forced aeration of soil in flooding irrigation; FI – flood irrigation

concentrations in soil solution were high at low $E_h$ and dropped sharply at $E_h$ > 350 mV at pH 5 to lower values. The DTPA-Fe and $Fe^{(II)}$ concentration of soil in FIO were greatly higher than that in DI but significantly lower than in FI at both the tillering and flowering stages (Table 2), these results suggested that although the gap of soil $E_h$ between FI and FIO was not very huge, the stronger oxidation potential in FIO than in FI made it different in soil $Fe^{(II)}$ and Fe-DTPA concentration.

**Rice growth and rice Fe nutrition**. Compared with FI, a greater decrease in soil Fe availability was found in DI than that in FIO (Table 2), which in turn, affects the growth and Fe uptake of rice (e.g. compared with FI, the shoot biomass, leaf active Fe and Fe uptake of rice in DI decreased by 31, 45 and 21%, which in FIO decreased by 14, 25 and 7% at the flowering stage, respectively) (Figure 2; Tables 3 and 4). Furthermore, the leaf SPAD (soil-plant analysis development), active Fe, pigment concentration, and total Fe, especially plant Fe uptake were all lower in FIO than in FI (Tables 3 and 4; Figures 2 and 3). Therefore, these results confirmed that the increased soil $E_h$ does limit the

Table 3. Soil-plant analysis development (SPAD) value, pigment concentration and active iron (Fe) of the leaf at tillering and flowering stages

| Growth stage | Treatment | Leaf SPAD value | Leaf pigment concentration (mg/g FW) | | Leaf active Fe concentration (μg/g FW) |
|---|---|---|---|---|---|
| | | | chlorophyll *a* | chlorophyll *b* | |
| Tillering | DI | 26.6[b] | – | – | 20.3[c] |
| | FIO | 29.6[a] | – | – | 23.3[b] |
| | FI | 30.1[a] | – | – | 25.7[a] |
| Flowering | DI | 31.6[c] | 2.02[c] | 0.60[c] | 20.1[c] |
| | FIO | 35.2[b] | 2.59[b] | 0.79[b] | 26.8[b] |
| | FI | 37.3[a] | 3.12[a] | 1.06[a] | 31.3[a] |

Values within a column and a growth stage with a similar letter are not significantly different at 5% by Duncan's multiple range tests ($n$ = 4). FW – fresh weight; DI – drip irrigation; FIO – forced aeration of soil in flooding irrigation; FI – flood irrigation

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Table 4. Iron (Fe) uptake of whole rice at tillering and flowering stages

| Growth stage | Treatment | Plant Fe uptake (mg/pot) |
|---|---|---|
| Tillering | DI | 8.33[b] |
| | FIO | 9.71[b] |
| | FI | 13.05[a] |
| Flowering | DI | 15.88[c] |
| | FIO | 21.65[b] |
| | FI | 28.90[a] |

Values within a column and a growth stage with a similar letter are not significantly different at 5% by Duncan's multiple range tests ($n$ = 4). DI – drip irrigation; FIO – forced aeration of soil in flooding irrigation; FI – flood irrigation

Fe absorption of rice under waterlogging conditions, even though the increased soil $E_h$ was not very huge. In the present study, Fe availability (Table 2) in DI was over the critical level in soil (5 mg/kg) for the deficiency (Dobermann and Fairhurst 2000). But the Fe concentration of leaves (about 100 μg/mg) in DI was close to the critical values of Fe concentration (from 70 to 300 μg/kg) indicated by Fageria (2013). Also, many parameters, such as the DTPA-Fe of soil, total Fe and chlorophyll content of leaves, rice biomass and Fe uptake in DI were the lowest (Tables 2, 3 and 4; Figures 2 and 3). Therefore, it is reasonable to believe that although the soil $E_h$ has a significant effect on Fe nutrition needed for rice growth, the soil water content also plays a big role in the Fe uptake of rice in DI. The previous studies indicated that a strong root system is an important guarantee for plant nutrient uptake (Li et al. 2016). So the root biomass was higher in FI than in DI and FIO, this in return, restricts Fe uptake of rice in DI and FIO (Figure 2, Table 4). Generally, the decline in soil moisture results in a decrease in the diffusion rate of nutrients in the soil to the absorbing root surface (Hu and Schmidhalter 2005). And drought reduces both nutrient uptake by the roots and transport from root to shoot, because of restricted transpiration rates and impaired transport and membrane permeability (Abenavoli et al. 2012). DI rice has lower transpiration and root biomass, which hinders Fe uptake and easily suffer from Fe deficiency compared to flooded rice (He et al. 2013, Shrestha et al. 2015).

In conclusion, soil $E_h$ increased in FIO, which in turn, inhibited the DTPA-Fe and $Fe^{(II)}$ concentration of soil. Correspondingly, the active Fe concentration of leaves, Fe uptake, and biomass of rice declined in FIO, if compared to FI. These data proved that the increased soil $E_h$ restrict Fe nutrition needed for rice growth. On the other hand, the poorest Fe uptake and biomass of rice in DI indicated that both the decreased soil water content and the increased soil $E_h$ were important factors that caused Fe deficiency in DI rice. Therefore, from the perspective of soil Fe availability, sufficient water supply during the seedling stage of drip-irrigated rice is an important agronomic measure to ensure that drip-irrigated rice is not exposed to Fe deficiency.

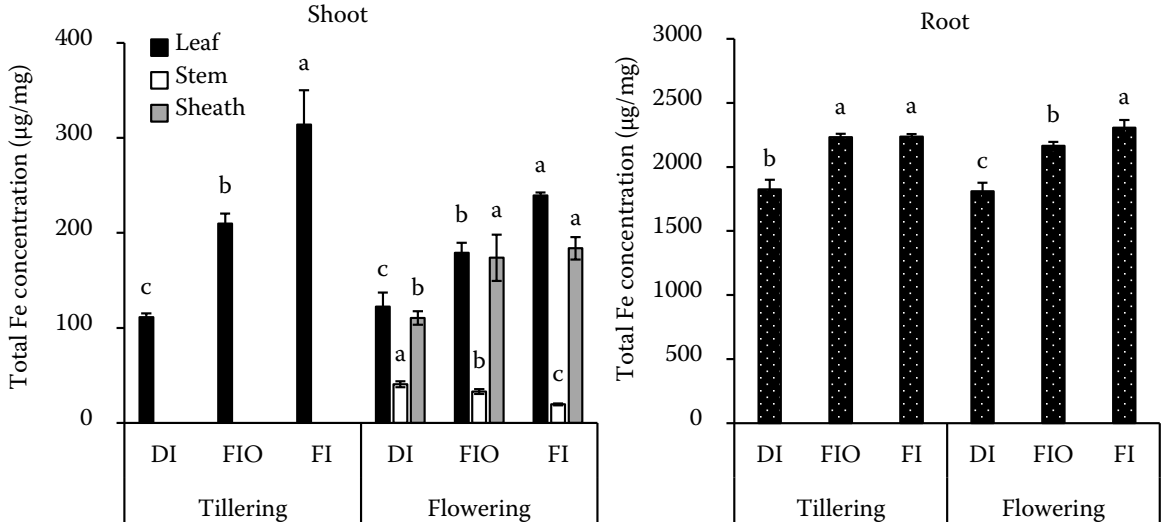


Figure 3. Total iron (Fe) concentration of rice at tillering and flowering stages. Within a growth stage and plant part, bars with similar letters are not significantly different at 5% by Duncan's multiple range tests ($n$ = 4). DI – drip irrigation; FIO – forced aeration of soil in flooding irrigation; FI – flood irrigation

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