

## Effects of Low Iron Stress on Root Growth and Iron Uptake and Utilization in Maize Seedlings: Postprint

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### Abstract

To elucidate differences in root growth and iron uptake and utilization during the seedling stage among maize varieties with contrasting low-iron tolerance, and to provide a basis for genetic improvement of low-iron tolerance in maize, the low-iron tolerant maize variety ‘Zhenghong 2’ and the low-iron sensitive maize variety ‘Chuandan 418’ were used as materials. Maize seedlings at the three-leaf-and-one-heart stage were treated with iron nutrient solutions under severe [10 mol(Fe<sup>3+</sup>)·L<sup>-1</sup>], moderate [30 mol(Fe<sup>3+</sup>)·L<sup>-1</sup>], and mild [50 mol(Fe<sup>3+</sup>)·L<sup>-1</sup>] low-iron stress conditions, as well as a control [100 mol(Fe<sup>3+</sup>)·L<sup>-1</sup>], to analyze the effects of low-iron stress on seedling root growth and iron uptake and utilization in these varieties. The results showed that as the iron concentration in the nutrient solution decreased, root length, root volume, root activity, dry matter accumulation, iron content, iron accumulation, and relative iron absorption capacity of seedlings of both maize varieties decreased significantly. However, root mugineic acid secretion increased, the ability to transfer and distribute iron to the shoot was enhanced, and the physiological efficiency of iron improved, which represents an important physiological mechanism for maize adaptation to low-iron stress. Iron accumulation in maize seedlings showed extremely significant or significant positive correlations with root traits such as root length, root volume, root dry weight, and root activity. Under moderate and severe low-iron stress, the low-iron tolerant maize variety exhibited higher root length, root volume, root dry weight, and root activity than the low-iron sensitive maize variety, which represents an important reason for its higher iron absorption and accumulation. Root mugineic acid secretion was positively correlated with iron distribution rate to stems and leaves, and the iron distribution rate to stems and leaves was extremely significantly positively correlated with iron physiological efficiency. Increasing root mugineic acid secretion could, to a certain extent,

enhance the iron distribution rate to stems and leaves in maize, thereby improving iron physiological efficiency. Under moderate and severe low-iron stress, the increase in mugineic acid secretion in the low-iron tolerant maize variety was higher than that in the low-iron sensitive maize variety, which represents the main reason for its higher iron physiological efficiency.

## Full Text

## Preamble

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### **Effect of low iron stress on root growth and iron uptake and utilization of different maize cultivars at seedling stage\***

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**Abstract:** Iron is an essential mineral element for plant growth that plays crucial roles in chlorophyll synthesis, redox reactions, photosynthesis, respiration, and substance/energy metabolism. In calcareous soils of arid and semi-arid regions, low available iron content frequently causes iron deficiency chlorosis in maize. Understanding the mechanisms of maize tolerance to low iron stress is critical for addressing this problem. To elucidate differences in root growth and iron uptake/utilization between low-iron-tolerant and sensitive maize cultivars at the seedling stage, and to provide a basis for genetic improvement of low-iron tolerance, we conducted a hydroponic experiment using the tolerant cultivar ‘Zhenghong 2’ and sensitive cultivar ‘Chuandan 418’. Three-leaf-stage seedlings were subjected to four iron treatments: severe ( $10 \text{ mol} \cdot \text{L}^{-1} \text{ Fe}^{3+}$ ), moderate ( $30 \text{ mol} \cdot \text{L}^{-1} \text{ Fe}^{3+}$ ), mild ( $50 \text{ mol} \cdot \text{L}^{-1} \text{ Fe}^{3+}$ ) low-iron stress, and control ( $100 \text{ mol} \cdot \text{L}^{-1} \text{ Fe}^{3+}$ ). After 14 and 28 days of treatment, we analyzed the effects of low iron stress on root growth and iron uptake/utilization.

The results showed that as iron concentration decreased, both cultivars exhibited significant reductions in root length, root volume, root vitality, dry matter, iron content, iron accumulation, and relative iron absorption capacity. However, low-iron stress increased mugineic acid secretion from roots, enhanced iron translocation to shoots, and improved iron physiological efficiency—these represent important adaptive mechanisms in maize. Iron accumulation showed highly significant positive correlations with root length, root volume, root dry weight, and root vitality. Under moderate and severe low-iron stress, the tolerant cultivar ‘Zhenghong 2’ maintained greater root length, volume, dry weight,

and vitality than ‘Chuandan 418’, which contributed to its higher iron accumulation. Mugineic acid secretion was positively correlated with iron distribution to shoots, which in turn was highly significantly correlated with iron physiological efficiency. Increasing mugineic acid secretion could enhance iron allocation to shoots and thus improve physiological efficiency. Under moderate and severe stress, ‘Zhenghong 2’ showed greater increases in mugineic acid secretion than ‘Chuandan 418’, which was the main reason for its higher iron physiological efficiency.

**Keywords:** Maize; Low iron stress; Seedling stage; Root; Mugineic acid; Iron uptake and utilization

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## Introduction

Iron is an essential mineral element for plants that plays vital roles in substance and energy metabolism [1-3]. Despite being the fourth most abundant element in the earth’s crust, iron forms oxides and hydroxides under oxidizing conditions and in neutral to alkaline soils, resulting in very low concentrations of plant-available ionic iron [4]. Plants frequently exhibit iron deficiency chlorosis, particularly in calcareous soils of arid and semi-arid regions [5]. Maize (*Zea mays*) is an important food, feed, and industrial crop. Sichuan Province is a major maize production and consumption region in China [6-7], with 4.2373 million hectares of calcareous purple soil in its eastern and northern areas, accounting for 46.5% of the province’s total purple soil area. Low available iron content in these soils severely constrains maize yield improvement [8].

Practical measures such as applying iron preparations, foliar fertilization, and punch-injection methods have proven ineffective for correcting maize iron chlorosis, often causing environmental problems while being costly and labor-intensive [9-12]. Previous studies indicate that the seedling stage is one of the most sensitive periods to iron deficiency, determining whether a cultivar can subsequently grow normally in calcareous soils. Iron deficiency significantly inhibits maize seedling growth, reduces chlorophyll and active iron content [13], and triggers various physiological and metabolic changes to acquire sufficient iron for growth [14-16].

Among these responses, mugineic acid metabolism is particularly important. Under iron deficiency, maize synthesizes and actively secretes mugineic acid phytosiderophores into the rhizosphere. These siderophores chelate  $Fe^{3+}$  in the soil, and the  $Fe^{3+}$ -chelate complexes are transported to root plasma membranes and

taken up into root cells via specific transporters. However, significant genotypic differences exist among maize cultivars in mugineic acid secretion and response to low-iron stress [17-18]. Therefore, exploiting maize's inherent potential by screening and breeding low-iron-tolerant cultivars represents an economically effective approach to solving iron deficiency chlorosis.

This study used previously screened maize cultivars with contrasting low-iron tolerance to investigate the effects of low-iron stress on root growth and iron uptake/utilization, as well as differential responses between tolerant and sensitive cultivars, providing a basis for genetic improvement of low-iron tolerance in maize.

## Materials and Methods

### 1.1 Experimental Materials

Two maize cultivars with contrasting low-iron tolerance at the seedling stage were selected: the low-iron-tolerant cultivar 'Zhenghong 2' and the low-iron-sensitive cultivar 'Chuandan 418' [17]. Seeds were obtained from Sichuan Agricultural University and represented typical genotypes identified in previous screening experiments as insensitive and sensitive to low-iron stress, respectively.

### 1.2 Experimental Design

The experiment employed a two-factor completely randomized design. Factor A consisted of four levels of low-iron stress, with concentrations set according to Long et al. [13]: A1, severe low-iron stress with  $10 \text{ mol} \cdot \text{L}^{-1} \text{Fe}^{3+}\text{-EDTA}$ ; A2, moderate stress with  $30 \text{ mol} \cdot \text{L}^{-1} \text{Fe}^{3+}\text{-EDTA}$ ; A3, mild stress with  $50 \text{ mol} \cdot \text{L}^{-1} \text{Fe}^{3+}\text{-EDTA}$ ; and A4, control with  $100 \text{ mol} \cdot \text{L}^{-1} \text{Fe}^{3+}\text{-EDTA}$ . Factor B comprised two maize cultivars: B1, 'Chuandan 418'; and B2, 'Zhenghong 2'.

Maize seeds were rinsed with distilled water to remove the original coating, sterilized with 70% ethanol for 30 seconds, washed again, and soaked in distilled water for 10 hours. The seeds were then sown evenly on moist quartz sand (1-2 mm particle size) and covered with a thin layer of sand. Distilled water was applied twice daily. Seeds were germinated in an artificial climate chamber with a 14 h light/10 h dark photoperiod at  $26 \text{ }^\circ\text{C}/22 \text{ }^\circ\text{C}$ , light intensity of 12,000 lx, and 60% relative humidity.

After 9 days, seedlings at the 2-leaf-1-heart stage were removed from endosperm and transferred to modified Hoagland nutrient solution. The macronutrient composition was:  $945 \text{ mg} \cdot \text{L}^{-1} \text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $506 \text{ mg} \cdot \text{L}^{-1} \text{KNO}_3$ ,  $80 \text{ mg} \cdot \text{L}^{-1} \text{NH}_4\text{NO}_3$ ,  $136 \text{ mg} \cdot \text{L}^{-1} \text{KH}_2\text{PO}_4$ , and  $493 \text{ mg} \cdot \text{L}^{-1} \text{MgSO}_4$ . Micronutrients included:  $4.15 \text{ } \mu\text{g} \cdot \text{L}^{-1} \text{KI}$ ,  $31.0 \text{ } \mu\text{g} \cdot \text{L}^{-1} \text{H}_3\text{BO}_3$ ,  $111.5 \text{ } \mu\text{g} \cdot \text{L}^{-1} \text{MnSO}_4$ ,  $43.0 \text{ } \mu\text{g} \cdot \text{L}^{-1} \text{ZnSO}_4$ ,  $1.25 \text{ } \mu\text{g} \cdot \text{L}^{-1} \text{Na}_2\text{MoO}_4$ ,  $0.125 \text{ } \mu\text{g} \cdot \text{L}^{-1} \text{CuSO}_4$ , and  $0.125 \text{ } \mu\text{g} \cdot \text{L}^{-1} \text{CoCl}_2$ . Iron was supplied as  $100 \text{ mol} \cdot \text{L}^{-1} \text{Fe}^{3+}\text{-EDTA}$  ( $27 \text{ mg} \cdot \text{L}^{-1} \text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  and  $37.3 \text{ mg} \cdot \text{L}^{-1}$  disodium EDTA, pH 5.5).

When seedlings reached the 3-leaf-1-heart stage, they were subjected to different low-iron stress treatments using modified Hoagland solution with varying  $\text{Fe}^{3+}$  concentrations. The experiment consisted of 8 treatments (4 iron levels  $\times$  2 cultivars) with three replications, totaling 24 containers. Each container was covered with a 2-cm-thick foam board with 20 holes, with one seedling per hole fixed by sponge-wrapped stems. The nutrient solution pH was adjusted daily to approximately 5.8 using  $0.1 \text{ mol} \cdot \text{L}^{-1}$  HCl or NaOH, and the solution was replaced every 4 days with continuous aeration.

### 1.3 Sample Collection and Measurement

Representative plants from each treatment were sampled at 14 and 28 days after treatment initiation for measurement of the following indicators, with three replicate measurements per treatment.

**1.3.1 Root Morphology** At 14 and 28 days after treatment, root length and volume were measured. Root length was determined by measuring the longest root per plant with a ruler, while root volume was measured using the water displacement method.

**1.3.2 Dry Matter Accumulation** At 14 and 28 days after treatment, maize seedlings were harvested and separated into shoots (stems and leaves) and roots. Plant materials were deactivated at  $105 \text{ }^\circ\text{C}$  for 40 minutes, then dried at  $80 \text{ }^\circ\text{C}$  to constant weight before weighing.

**1.3.3 Root Activity** Root activity was measured at 14 days after treatment using the triphenyltetrazolium chloride (TTC) reduction method [19]. Roots were blotted dry, and 0-1 cm root tips were excised. A 0.2 g sample was placed in a test tube for analysis.

**1.3.4 Mugineic Acid Secretion Measurement** 1) **Mugineic acid collection** [20]: On day 14 after treatment, at 9:00 AM, plants were removed from containers and roots were rinsed with tap water followed by distilled water. The plants were then placed in beakers containing 300 mL distilled water and grown under light for 4 hours. After removal, 10 mL of 30%  $\text{H}_2\text{O}_2$  was immediately added to inhibit mugineic acid decomposition. The collected root exudates were stored in a refrigerator for later analysis.

2) **Determination of mugineic acid secretion using the activated  $\text{Fe}_2\text{O}_3$  method** [20]: Fifty milligrams of  $\text{Fe}_2\text{O}_3$  powder was placed in a test tube, followed by addition of 10 mL root exudate and 1 mL of  $1 \text{ mol} \cdot \text{L}^{-1}$  NaCl solution. The mixture was incubated for 24 hours to allow complete reaction and formation of  $\text{Fe}^{3+}$ -PS (mugineic acid-iron complexes). After centrifugation at 2,500 rpm for 10 minutes, 5 mL of supernatant was collected and Fe content was measured directly by atomic absorption spectrophotometry.

**1.3.5 Iron Content** Dried samples were ground into powder after weighing, and total iron content in shoots (stems and leaves) and roots was determined by atomic absorption spectrophotometry [21].

#### 1.4 Data Processing and Analysis

Iron accumulation ( $\mu\text{g} \cdot \text{plant}^{-1}$ ) = Shoot iron content ( $\mu\text{g} \cdot \text{g}^{-1}$ )  $\times$  Shoot dry matter accumulation (g) + Root iron content ( $\mu\text{g} \cdot \text{g}^{-1}$ )  $\times$  Root dry matter accumulation (g) (1)

Iron distribution to shoots (%) = Shoot iron accumulation / Total iron accumulation  $\times$  100 (2)

Relative iron absorption capacity ( $\text{mg} \cdot \text{g}^{-1}$ ) = Iron accumulation (mg) / Root dry weight (g) (3)

Iron physiological efficiency ( $\text{kg} \cdot \text{mg}^{-1}$ ) = Plant dry weight (kg) / Iron accumulation (mg) (4)

Experimental data were processed and analyzed using Microsoft Excel 2007 and DPS 7.05 software.

## Results

### 2.1.1 Effects on Root Morphology

As shown in Table 1, low-iron stress decreased maize root volume and length, with more severe stress causing greater reductions. The two cultivars differed in their response magnitude. At 14 and 28 days after treatment, except under mild low-iron stress, ‘Zhenghong 2’ showed smaller decreases in root volume and length than ‘Chuandan 418’, and its coefficient of variation was also lower. This indicates that ‘Zhenghong 2’ could maintain longer roots and larger root volume under low-iron stress, possessing a more robust root system that facilitates iron absorption under iron-deficient conditions.

### 2.1.2 Effects on Root Activity and Mugineic Acid Secretion

As shown in Table 2, root vitality of both cultivars decreased with increasing low-iron stress severity. Under severe low-iron stress, root vitality was significantly lower than in the control, indicating that low-iron stress inhibits root activity in maize seedlings, with more severe stress causing stronger inhibition. No significant difference in root vitality was observed between cultivars under mild low-iron stress, but ‘Zhenghong 2’ showed significantly higher root vitality than ‘Chuandan 418’ under moderate and severe stress.

Mugineic acid secretion was expressed as iron-dissolving capacity per unit root weight. Table 2 shows that low-iron stress increased mugineic acid secretion in both cultivars, but their responses differed. Under mild, moderate, and severe low-iron stress, the increases in iron-dissolving capacity per unit root

weight for ‘Zhenghong 2’ were 15.5%, 46.6%, and 11.2% at 14 days, and 20.9% at 28 days, respectively, compared to the control. Under mild low-iron stress, ‘Chuandan 418’ showed a significantly greater increase in mugineic acid secretion than ‘Zhenghong 2’, but at all other treatment levels, ‘Zhenghong 2’ exhibited larger increases. This suggests that ‘Chuandan 418’ is more sensitive to low-iron stress, possessing some tolerance under mild stress but lower tolerance than ‘Zhenghong 2’ under moderate and severe stress.

## 2.2 Effects on Seedling Dry Matter

As shown in Table 3, low-iron stress significantly affected dry matter accumulation in various organs of maize seedlings, reducing both shoot and root dry weight. More severe stress caused greater reductions, and under moderate and severe stress, the magnitude of reduction increased with stress duration. Plant dry weight showed a significant positive correlation with iron concentration. The regression equations for ‘Chuandan 418’ between individual plant dry weight ( $y$ ) and iron concentration ( $x$ ) were  $y_{14d} = 0.07 + 0.004118x$  ( $R^2 = 0.9500$ ) and  $y_{28d} = 0.36 + 0.025197x$  ( $R^2 = 0.8878$ ) at 14 and 28 days, respectively. For ‘Zhenghong 2’, the corresponding equations were  $y_{14d} = 0.46 + 0.003197x$  ( $R^2 = 0.9851$ ) and  $y_{28d} = 2.04 + 0.023495x$  ( $R^2 = 0.9752$ ). These results indicate that ‘Zhenghong 2’ was less affected, showing smaller decreases in individual plant dry weight with declining iron concentration (as reflected by the regression slopes) than ‘Chuandan 418’ at both time points. Additionally, the coefficients of variation for shoot dry weight, root dry weight, and total dry weight of ‘Zhenghong 2’ under different low-iron stresses were lower than those of ‘Chuandan 418’.

## 2.3 Effects on Iron Content, Accumulation, and Distribution

As shown in Table 4, low-iron stress significantly decreased root iron content, while shoot iron content showed relatively smaller changes. At 14 days, ‘Chuandan 418’ had higher shoot iron content than ‘Zhenghong 2’, but at 28 days, both cultivars had similar levels, suggesting that ‘Chuandan 418’ may have higher iron demand during early growth, which could be an important reason for its low-iron sensitivity at the seedling stage. The magnitude of iron content reduction under low-iron stress varied by cultivar and growth stage. Averaged across the three low-iron treatments, ‘Chuandan 418’ showed average reductions in shoot iron content of 4.5% and 8.7% at 14 and 28 days, respectively, and root iron content reductions of 61.6% and 42.0%. In contrast, ‘Zhenghong 2’ showed corresponding shoot iron content reductions of 0.5% and 18.2%, and root reductions of 55.2% and 38.9%. Thus, ‘Chuandan 418’ experienced greater reductions in root iron content, though smaller reductions in shoot iron content at 28 days.

Individual plant iron accumulation decreased with iron concentration in both cultivars, showing a significant positive correlation. The regression equations for ‘Chuandan 418’ between iron accumulation ( $y$ ) and iron concentration ( $x$ ) were

$y_{14d} = -31.11 + 4.4085x$  ( $R^2 = 0.9557$ ) and  $y_{28d} = 1.98 + 13.5925x$  ( $R^2 = 0.9771$ ) at 14 and 28 days, respectively. For ‘Zhenghong 2’, the corresponding equations were  $y_{14d} = 59.13 + 6.4911x$  ( $R^2 = 0.9650^*$ ) and  $y_{28d} = 383.38 + 13.2359x$  ( $R^2 = 0.9811^{**}$ ). These results indicate that iron concentration strongly influenced iron accumulation in both cultivars. At both time points, iron accumulation in ‘Zhenghong 2’ was significantly higher than in ‘Chuandan 418’ across all treatments, and the coefficient of variation with concentration changes was lower for ‘Zhenghong 2’.

The proportion of iron distributed to shoots increased with stress severity, indicating that low-iron stress enhances iron translocation to shoots to meet leaf growth requirements, which may represent an important physiological adaptation mechanism. With extended stress duration, ‘Zhenghong 2’ showed increased iron allocation to shoots, while ‘Chuandan 418’ showed significant decreases.

#### 2.4 Effects on Relative Iron Absorption Capacity and Physiological Efficiency

The amount of iron absorbed per unit root weight (individual plant iron accumulation divided by root dry weight) reflects the relative iron absorption capacity of roots. As shown in Table 5, the relative iron absorption capacity (relative absorption efficiency) of both cultivars decreased with increasing low-iron stress. Except for the mild iron deficiency treatment at 14 days, no significant differences in relative absorption capacity were observed between ‘Zhenghong 2’ and ‘Chuandan 418’ across other treatments. This suggests that the higher iron accumulation in ‘Zhenghong 2’ compared to ‘Chuandan 418’ was primarily due to differences in root system development rather than differences in relative iron absorption capacity.

The production capacity of absorbed iron (iron physiological efficiency) in maize seedlings was also significantly affected by low-iron stress (Table 5). Iron physiological efficiency increased with low-iron stress severity, which may represent one mechanism for adaptation to iron-deficient environments. Highly significant differences were observed between cultivars, and the interaction between cultivar and iron concentration reached extremely significant levels. Under low-iron stress, ‘Zhenghong 2’ exhibited higher iron physiological efficiency, particularly at 28 days, indicating stronger adaptation to iron deficiency than ‘Chuandan 418’—an important physiological mechanism underlying its superior low-iron tolerance.

#### 2.5 Correlation Analysis Between Root Traits and Iron Uptake/Utilization

Correlation analysis between main root traits and iron uptake/utilization indicators in maize seedlings under 14 days of low-iron stress (Table 6) revealed that iron accumulation was highly significantly correlated with root length, root volume, and root dry weight, indicating that a well-developed root system consti-

tutes an important morphological basis for enhanced iron absorption and accumulation. Iron accumulation was also highly significantly positively correlated with relative iron absorption efficiency, which in turn was significantly positively correlated with root vitality. Thus, enhancing root vitality can improve iron absorption capacity and increase iron accumulation. Iron physiological efficiency was highly significantly positively correlated with iron distribution to shoots, which was positively correlated with root mugineic acid secretion capacity (correlation coefficient = 0.64). Therefore, increasing mugineic acid secretion can enhance iron allocation to shoots and consequently improve iron physiological efficiency.

## Discussion

Iron deficiency stress induces a series of morphological and physiological changes in plant roots that affect iron absorption. Zhang et al. [22] reported that peanut (*Arachis hypogaea*) root volume and vitality decreased significantly under iron deficiency. Hong et al. [23] observed that maize roots developed club-shaped swellings at the tips with distinct red spots and increased lateral roots under iron deficiency stress. After 40 days of iron deficiency treatment, mitochondrial cristae began to disintegrate and vacuolization occurred in most root mitochondria, suggesting that loss of mitochondrial function may contribute to reduced root vitality. Yin et al. [24] found that iron-deficient nutrient solution enhanced  $\text{Fe}^{3+}$  reduction capacity and rhizosphere acidification in wheat (*Triticum aestivum*) but decreased shoot iron content. Wu et al. [25] reported that iron deficiency significantly reduced root iron content and biomass in strawberry (*Fragaria ananassa*) seedlings. Our study demonstrated that low-iron stress significantly decreased maize seedling root length, volume, vitality, root dry matter, root iron content, and iron accumulation, while increasing mugineic acid secretion—findings consistent with previous research. Low-iron stress enhanced iron distribution from roots to shoots, with greater impacts on roots than shoots. This contrasts with Wu et al. [25] who reported greater effects on leaf than root iron content, likely due to differences in iron acquisition strategies among crops. Maize is a Strategy II plant that actively secretes mugineic acid phytosiderophores to chelate  $\text{Fe}^{3+}$  in the rhizosphere, with the  $\text{Fe}^{3+}$ -chelate complex taken up by specific transporters. In contrast, Strategy I plants like strawberry rely on enhanced secretion of  $\text{H}^+$  and reductants or increased root ferric reduction system activity to absorb iron as  $\text{Fe}^{2+}$  ions. High  $\text{Fe}^{2+}$  can be toxic, so Strategy I plants maintain relatively stable root iron content. As a Strategy II plant, maize (*Zea mays*) internalizes iron as  $\text{Fe}^{3+}$  chelates, protected by intracellular tolerance mechanisms [26], resulting in much higher root iron content than Strategy I plants like strawberry and greater susceptibility to external iron concentration fluctuations.

A larger root surface area under low-iron stress is the primary reason for stronger iron absorption capacity in tolerant cultivars. Dasgan et al. [27] reported that low-iron-tolerant tomato parents had more lateral roots than sensitive genotypes,

increasing root absorption surface area. Shi et al. [28] demonstrated that differences in iron absorption among six wheat genotypes were mainly attributable to variations in root surface area. Han et al. [29] showed that the iron-efficient apple species *Malus xiaojinensis* had higher iron affinity and absorption capacity. Our results revealed highly significant positive correlations between iron accumulation and root volume, length, and vitality in maize seedlings under low-iron stress. Under moderate and severe low-iron conditions, the tolerant cultivar ‘Zhenghong 2’ exhibited significantly greater root length, volume, and dry matter than ‘Chuandan 418’, resulting in a more developed root system and significantly higher individual plant iron accumulation. Therefore, in arid or semi-arid calcareous soil regions, cultivars with well-developed root systems should be prioritized, or management practices should be implemented to enhance root growth to improve soil iron acquisition.

Significant genotypic variation exists among cultivars of the same species in response to nutrient deficiency stress. Tolerant cultivars exhibit not only strong mineral element absorption capacity but also high utilization efficiency. Zhang et al. [30] reported that under low phosphorus conditions, low-P-tolerant maize inbred lines 99239T, 99180T, and 99327T had high phosphorus absorption efficiency, while lines 99186T and 99184T showed high phosphorus utilization efficiency; sensitive lines 99152S and 99270S had low efficiency in both aspects. Clark et al. [31] found that the iron-efficient maize cultivar ‘WF9’ had significantly higher root weight, greater iron translocation to shoots, and higher root concentrations of citric, aconitic, and malic acids than the iron-inefficient cultivar ‘ys1/ys1’. Our study also demonstrated that low-iron stress enhanced iron allocation to shoots, with mugineic acid secretion positively correlated with iron distribution to shoots ( $r = 0.64$ ), and iron distribution to shoots highly significantly correlated with iron physiological efficiency. This suggests a potential link between mugineic acid secretion and enhanced iron translocation to shoots and physiological efficiency under low-iron stress. Previous research has shown that three molecules of S-adenosylmethionine are converted to nicotianamine by nicotianamine synthase, which then undergoes amino group transfer and keto acid reduction by nicotianamine aminotransferase, followed by hydroxylation or deoxygenation catalyzed by dioxygenases to generate mugineic acid siderophores [32]. Nicotianamine, the precursor for siderophore synthesis, is considered the most likely intracellular iron chelator. Increased mugineic acid secretion necessarily enhances nicotianamine activity in cells, thereby increasing iron transport capacity within the plant, raising iron allocation to shoots, and improving iron physiological efficiency. Therefore, increasing mugineic acid secretion under low-iron stress can enhance maize iron physiological efficiency. With increasing stress severity, ‘Zhenghong 2’ showed greater increases in mugineic acid secretion than ‘Chuandan 418’ under moderate and severe low-iron stress. With extended stress duration, ‘Zhenghong 2’ enhanced iron allocation to shoots while ‘Chuandan 418’ showed decreased allocation. Consequently, ‘Zhenghong 2’ exhibited significantly higher iron physiological efficiency than ‘Chuandan 418’—the primary reason for its ability to adapt to low-iron environments.

## Conclusion

Low-iron stress significantly reduced root length, root volume, root vitality, dry matter, iron content, iron accumulation, and relative iron absorption capacity in maize seedlings, but increased mugineic acid secretion from roots, enhanced iron translocation to shoots, and improved iron physiological efficiency. These responses represent important physiological mechanisms for maize adaptation to low-iron stress.

Iron accumulation in maize seedlings was highly significantly positively correlated with root length, root volume, root dry weight, and root vitality. Under moderate and severe low-iron stress, the low-iron-tolerant cultivar maintained greater root length, volume, dry weight, and vitality than the sensitive cultivar, which was the primary reason for its higher iron absorption and accumulation.

Mugineic acid secretion was positively correlated with iron distribution to shoots, which was highly significantly positively correlated with iron physiological efficiency. Under moderate and severe low-iron stress, the tolerant cultivar showed significantly greater increases in mugineic acid secretion than the sensitive cultivar, which was the main reason for its higher iron physiological efficiency.

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