Effect of application of iron (Fe) and α-ketoglutaric acid on growth, photosynthesis, and Fe content in fragrant rice seedlings

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Abstract

At a three-leaf stage, two Fe treatments [0 mg kg–1 (Fe–) and 20 mg·kg–1 (Fe+) in the form of FeCl3] were used in the soil of the pot and then two concentrations of α-ketoglutaric acid [0 mg L–1 (A–) and 50 mg L–1 (A+) were sprayed to the rice plants of Meixiangzhan and Yuxiangyouzhan cultivars. We showed that seedlings exhibited an increased length and fresh and dry mass of shoots and roots with treatments Fe+A– and Fe–A+, as well as the Fe content increased greatly. Both treatments increased the morphological characteristic values of roots and promoted photosynthesis. Interestingly, Fe+A+ notably affected the photosynthesis of fragrant rice seedlings; however, it exerted no significant differences on other parameters. Overall, Fe and α-ketoglutaric acid had the potential for improving the growth of fragrant rice seedlings. The interaction between Fe and α-ketoglutaric acid regulated photosynthesis in seedling leaves, which provided evidence for further improvement of rice cultivation.

Keywords: net photosynthetic rate; plant mass; root morphological characteristics; seedling height.

Introduction

Rice (Oryza sativa L.) is an important cereal crop worldwide and a source of food for billions of people. The cultivated area of rice is more than 150 million hectares, mainly located in Asia and Latin America. Rice production is intricately linked to global food security (Farooq et al. 2009, Mahajan et al. 2010, Aslam et al. 2015). Good quality rice seedling is the first critical factor for a good harvest (Najeeb et al. 2020). The vigor of rice seedlings is strongly influenced by environmental factors, such as light, temperature, water management, fertilizer, heavy metals, and growth regulators, changing the physiological traits of the growth and development of rice seedlings (Mishra and Salokhe 2008, Han et al. 2009, Guo et al. 2011, Srivastava et al. 2014, Ma et al. 2015, Banerjee

Highlights

- Fe and α-ketoglutaric acid application strongly promoted the growth of rice seedlings
- Fe content increased greatly following Fe and α-ketoglutaric acid treatment
- Fe and α-ketoglutaric acid combinations regulated photosynthesis in seedling leaves

Abbreviations: C – cultivar; C × T – the interaction of cultivar and treatment; C i – intercellular CO2 concentration; DAS – days after spraying; DM – dry mass; E – transpiration rate; FM – fresh mass; g – stomatal conductance; Pn – net photosynthetic rate; T – treatment.

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Numerous studies have reported the effects of interaction between plant regulators, such as the interaction between water regimes and nitrogen application, carbon dioxide and temperature, calcium and silicon, cadmium and mineral nutrients, α-ketoglutaric acid and nitrogen, and Fe and zinc (Zn) on the growth of rice (Ma and Takahashi 1993, Liu et al. 2003, Yuan et al. 2013, Wang et al. 2016, Ren et al. 2017, Mo et al. 2019, Fu et al. 2021). For instance, an interaction between α-ketoglutaric acid and Zn has been reported to regulate rice growth, gas-exchange attributes, and chlorophyll fluorescence in rice seedlings (Liu et al. 2020). However, rare studies are exploring how the interaction of α-ketoglutaric acid and Fe would affect plant growth. To understand this, we performed experiments to detect the Fe content, seedling height, fresh and dry masses of shoots and roots, morphological indexes of roots, and photosynthetic parameters to evaluate the effects of the foliar application of α-ketoglutaric acid and Fe on the seedlings of fragrant rice, which would provide theoretical basis to further improve the cultivation of rice. In addition, it is worth exploring whether α-ketoglutaric acid as an organic carbon nutrient can be discovered and utilized for sustainable agricultural practices.

Materials and methods

Plant materials and growth conditions: Two widely used fragrant rice cultivars in south China, namely, Meixiangzhan and Yuxiangyouzhan, were used. These were provided by the College of Agriculture, South China Agricultural University, and used in this study. The seeds of experimental cultivars were sterilized in 30% H2O2 for 20 min, followed by thoroughly rinsing with deionized water thrice. Afterward, the seeds were soaked in deionized water for 24 h, placed in an artificial climate box to germinate for 24 h, and sown in plastic pots (9 cm in height and 15 cm in diameter). For conducting experiments, 50 vigorous seedlings were selected in each pot at the three-leaf stage.

The following experimental soil characteristics were used: pH 5.1, 25.7 g(organic matter content) kg–1, 85.51 mg(available N) kg–1, 25.11 mg(available P) kg–1, 153.2 mg(available K) kg–1, and 20 mg(available Fe) kg–1. Two days before sowing, a compound fertilizer (N:P:K = 15:15:15; 5 g kg–1) was applied to the pots at basal stage to ensure nutrient growth during the seedling stage.

Treatments design: The pot experiments were conducted using a completely randomized design and in three replications. The experiments were initiated at the three-leaf stage. Two Fe application concentrations (Fe–: 0 mg kg–1; Fe+: 20 mg kg–1) were used in the soil and Fe was supplied as FeCl3 solution (Zhang et al. 2017). Two concentrations of α-ketoglutaric acid [A–: 0 mg(α-ketoglutaric acid) L–1; A+: 50 mg(α-ketoglutaric acid) L–1] were sprayed with 3 mL in each pot of each treatment once every 24 h (thrice in total) after the Fe treatment. In addition, the α-ketoglutaric acid solution was mixed with 5% Tween-60.
Photosynthetic parameters: At 1–5 d after spraying (DAS), completely expanded leaves of nine representative seedlings from three pots of each treatment were selected. We measured the net photosynthetic rate ($P_N$), stomatal conductance ($g_s$), intercellular CO$_2$ concentration ($C_i$), and transpiration rate ($E$) using a portable photosynthesis system (LI-6400, LI-COR, USA) attached to live leaf from 9:00 to 11:00 h (Liu et al. 2020). First, the photosynthetic apparatus was preheated and calibrated according to manufacturer’s instructions. Then the fixed flow model was set at 500 μmol s$^{-1}$. The relative humidity was adjusted at about 65%, and the concentration of CO$_2$ in the surrounding environment was about 400 μmol mol$^{-1}$. Leaf temperature was at room temperature.

Seedling height, plant dry and fresh mass determination: We selected ten representative seedlings at 5 DAS from each replicate to measure the seedling height. All sampled seedlings were harvested and washed with deionized water. An absorbent paper was used to clean the surface water. Next, the seedlings were immediately divided into root and shoot parts to detect the fresh mass (FM) and oven-dried at 80°C for 7 d to achieve a constant mass and determine the dry mass (DM) (Hussain et al. 2020).

Root morphological index detection: Six sampled seedling roots were carefully dug out from each treatment pot and rinsed with deionized water. The absorbent paper was used to remove the residual water on the roots. Next, the average diameter of roots, root length, root surface area, and root volume were measured and analyzed using a root analysis instrument (WinRhizo–LA1600, Regent, Canada) (Ruan et al. 2021).

Fe content estimation: Following the method described by Hussain et al. (2020), the oven-dried shoots were pulverized into a fine powder using a multifunctional disintegrator and filtered by a standard sieve (aperture size: 1 mm). The powders (0.2 g) were then placed in digestion tubes and mixed with 6 mL of concentrated HNO$_3$ and 3 mL of H$_2$O$_2$ for 24 h for nitration. Next, the tubes were placed in the digester at 160°C for 90 min. Following nitration, the solutions were cooled down to room temperature and diluted with 25 mL of deionized water. Finally, the diluted solutions were subjected to atomic absorption spectrophotometry (AA-6300C, Shimadzu, Japan) for estimation of Fe content in the shoots. The Fe content was expressed as μg g$^{-1}$(DM).

Statistical analysis: The experimental data were analyzed using a one-way analysis of variance (ANOVA) and performed using Statistix version 8 (Analytical software, Tallahassee, FL, USA). The correlation analysis among all indexes was performed using the MetaboAnalyst software (https://www.metaboanalyst.ca/) following the methods of Mo et al. (2019). The graphs were drawn in Microsoft Excel 2010 (Microsoft Corporation, New Mexico, USA). The significant differences between treatments were indicated by using the least significant difference (LSD) test at a 5% probability level.

Results

Photosynthesis and gas exchange: For the two fragrant rice cultivars, certain photosynthesis parameters, except $P_N$ at 5 DAS, $g_s$ at 1 and 2 DAS, $C_i$ at 1, 2, and 4 DAS, displayed notable differences (Table 1S, supplement). Both Fe and α-ketoglutaric acid positively affected the $P_N$ of the two cultivars. $P_N$ either significantly increased or displayed no significant difference following Fe$+\text{A}$– and Fe$–\text{A}$+ treatments (Fig. 1). However, the interaction between Fe and α-ketoglutaric acid significantly reduced the $P_N$. In addition, following the Fe$–\text{A}$+ treatment, $P_N$ at 1 and 2 DAS showed the maximum value, whereas the maximum value following the Fe$+\text{A}$– treatment was reported for 3 and 4 DAS. The lowest $P_N$ was recorded following the Fe$+\text{A}$+ treatment for the two cultivars at all sampling stages. The effects of Fe, α-ketoglutaric acid, and the interaction between Fe and α-ketoglutaric acid on $g_s$ were similar to those on $P_N$ (Fig. 2). However, the highest $g_s$ at 1 DAS was recorded after the Fe$+\text{A}$– treatment, whereas the highest $g_s$ at 3 and 4 DAS were recorded after the Fe$–\text{A}$+ treatment. Similarly, the lowest $g_s$ was recorded with the Fe$+\text{A}$+ treatment at each sampling stage, except the Xuyiaoyouzhan showed the lowest $g_s$ at 4 and 5 DAS with Fe$–\text{A}$–. In addition, Fe and α-ketoglutaric acid promoted the transpiration rate, with the highest transpiration rate values recorded at 1 and 5 DAS after the Fe$+\text{A}$– treatment and at 2, 3, and 4 DAS following the Fe$–\text{A}$+ treatment. Like $P_N$, the interaction between Fe and α-ketoglutaric acid resulted in the lowest transpiration rate values following the Fe$+\text{A}$+ treatment for the two cultivars at each sampling stage (Fig. 3). For $C_i$ at 1 DAS, no significant difference was observed under all treatments compared with Fe$–\text{A}$– (Fig. 4). Both Fe and α-ketoglutaric acid exerted a negative effect on $C_i$ at 2, 3, 4, and 5 DAS and significantly reduced $C_i$ at 4 and 5 DAS. However, $C_i$ increased due to the interaction between Fe and α-ketoglutaric acid, with the highest values reported at 2, 3, and 5 DAS.

Morphological characteristics of rice seedlings: The morphological characteristics of fragrant rice cultivars significantly differed and were significantly affected by all the treatments (Table 1S). Both Fe and α-ketoglutaric acid increased the fresh and dry mass of shoots and roots. The average increment in the shoot fresh mass, shoot dry mass, root fresh mass, and root dry mass was 11.6, 14.5, 8.8, and 50.0%, respectively, following the Fe$+\text{A}$– treatment. Similarly, the average increment in the shoot fresh mass, shoot dry mass, root fresh mass, and root dry mass was 15.3, 21.7, 24.7, and 60.7%, respectively, following the Fe$–\text{A}$+ treatment. For Xuyiaoyouzhan, the highest shoot fresh mass, shoot dry mass, root fresh mass, and root dry mass were recorded after the Fe$–\text{A}$+ treatment. Compared to the Fe$–\text{A}$– treatment, the above values were significant except for the root fresh mass in Meixiangzhan. However, the interaction between Fe and α-ketoglutaric acid only exerted
**Fig. 1.** Effect of Fe and α-ketoglutaric acid on the net photosynthetic rate ($P_n$) at (A) 1 DAS, (B) 2 DAS, (C) 3 DAS, (D) 4 DAS, and (E) 5 DAS for Meixiangzhan and Yuxiangyouzhan. Vertical bars with different lowercase letters above are significantly different at $P<0.05$ by LSD tests. Capped bars represent SD ($n = 3$). Fe–: 0 mg(FeCl$_3$) kg$^{-1}$; Fe+: 20 mg(FeCl$_3$) kg$^{-1}$; A–: 0 mg(α-ketoglutaric acid) L$^{-1}$; A+: 50 mg(α-ketoglutaric acid) L$^{-1}$. DAS – days after spraying.

**Fig. 2.** Effect of Fe and α-ketoglutaric acid on stomatal conductance ($g_s$) at (A) 1 DAS, (B) 2 DAS, (C) 3 DAS, (D) 4 DAS, and (E) 5 DAS for Meixiangzhan and Yuxiangyouzhan. Vertical bars with different lowercase letters above are significantly different at $P<0.05$ by LSD tests. Capped bars represent SD ($n = 3$). Fe–: 0 mg(FeCl$_3$) kg$^{-1}$; Fe+: 20 mg(FeCl$_3$) kg$^{-1}$; A–: 0 mg(α-ketoglutaric acid) L$^{-1}$; A+: 50 mg(α-ketoglutaric acid) L$^{-1}$. DAS – days after spraying.
Fig. 3. Effect of Fe and α-ketoglutaric acid on transpiration rate ($E$) at (A) 1 DAS, (B) 2 DAS, (C) 3 DAS, (D) 4 DAS, and (E) 5 DAS for Meixiangzhan and Yuxiangyouzhan. Vertical bars with different lowercase letters above are significantly different at $P<0.05$ by LSD tests. Capped bars represent SD ($n=3$). Fe–: 0 mg(FeCl$_3$) kg$^{-1}$; Fe+: 20 mg(FeCl$_3$) kg$^{-1}$; A–: 0 mg(α-ketoglutaric acid) L$^{-1}$; A+: 50 mg(α-ketoglutaric acid) L$^{-1}$. DAS – days after spraying.

Fig. 4. Effect of Fe and α-ketoglutaric acid on intercellular CO$_2$ concentration ($C_i$) at (A) 1 DAS, (B) 2 DAS, (C) 3 DAS, (D) 4 DAS, and (E) 5 DAS for Meixiangzhan and Yuxiangyouzhan. Vertical bars with different lowercase letters above are significantly different at $P<0.05$ by LSD tests. Capped bars represent SD ($n=3$). Fe–: 0 mg(FeCl$_3$) kg$^{-1}$; Fe+: 20 mg(FeCl$_3$) kg$^{-1}$; A–: 0 mg(α-ketoglutaric acid) L$^{-1}$; A+: 50 mg(α-ketoglutaric acid) L$^{-1}$. DAS – days after spraying.
a slight promoting effect on the fresh and dry mass of shoots and roots, with no significant difference compared to the Fe–A– treatment.

The Fe+A– treatment increased the seedling height by 0.8 and 5.2% for Meixiangzhan and Yuxiangyouzhan, respectively, reaching a significant level in Yuxiangyouzhan (Fig. 5E). The seedling height of the two cultivars significantly increased following the Fe–A+ treatment and exhibited the highest values in Meixiangzhan. Compared with Fe–A– treatment, the Fe+A+ treatment resulted in no difference in the seedling height.

The average root diameter increased following the Fe+A– treatment and reached a significant level for the two fragrant rice cultivars. The Fe–A+ treatment greatly promoted the average root diameter, showing the highest values for the two cultivars. The Fe+A+ treatment exerted promoting effect on the average root diameter, reaching a significant level in Meixiangzhan as compared to the Fe–A– treatment (Fig. 6A).

Fe content in seedlings: The application of Fe and α-ketoglutaric acid significantly increased the Fe content. In addition, similar performance trends were recorded for the two varieties, namely, the highest Fe content was recorded following the Fe–A+ treatment with increments of 85.7 and 55.6%, respectively, for Meixiangzhan and Yuxiangyouzhan. A higher Fe content was detected following the Fe+A– treatment, resulting in a 76.7 and 42.4% increase as compared to nontreated (Fe–A–) cultivars. However, a slight but insignificant decrease in the Fe content was reported following the Fe+A+ treatment (Fig. 7).

Correlation analyses: The correlation analyses revealed possible relationships between the investigated parameters as shown in a heatmap (Fig. 8). Significant correlations were observed in the groups of $P_N$ at 5 DAS and $E$ at...
Discussion

Numerous studies have proved that Fe, an essential micro-nutrient for plants, is required for proper plant growth and human health (Schmidt et al. 2020, Hanikenne et al. 2021). For instance, Pavlovic et al. (2013) reported that the application of 50 μM Fe could significantly improve the SPAD value, dry biomass of roots and shoots, and leaf Fe concentration in cucumber (Cucumis sativus L. cv. Chinese long). Similarly, Araújo et al. (2014) reported that 7 mM Fe-EDTA enhanced the development of root length and increased the root volume of two grass species [S. parviflora (Poir.) Kerguélen and P. urvillei Steudel (Poaceae)]. Moreover, Valentinuzzi et al. (2020) showed that the supplied iron was preferentially translocated to leaves and the root was supplied after leaves have been sufficiently supplied. Compared with Fe–A–, the Fe+A– treatment significantly improved several characteristics of seedlings of both fragrant rice cultivars including the dry mass of shoots and roots, the length and volume of roots, and the Fe content in shoot (Figs. 5, 6, 7). In addition, Vigani et al. (2013) implicated that Fe is involved in photosynthesis by regulating the biosynthesis of chloroplasts and pigments and activating the photosynthetic enzymes and the electron transport. Rizwan et al. (2019) showed that the foliar application of iron oxide nanoparticles can increase the gas-exchange characteristics. Thus, we observed improvements in net photosynthetic rate, stomatal conductance, and transpiration rate for both cultivars following the Fe+A– treatment at 2–5 DAS (Figs. 1, 2, 3). The top two parameters that correlated with the Fe content were the transpiration rate at 2 DAS and the net photosynthetic rate at 5 DAS (Fig. 1E). However, a decrease in intercellular CO₂ concentration for both cultivars was found at 2–5 DAS with Fe+A– treatment (it significantly decreased for Meixiangzhan at 3 DAS and Yuxiangyouzhan at 4 and
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5 DAS (Fig. 4). This finding could be attributed to Fe which promoted the photosynthesis of rice seedlings by increasing net photosynthetic rate, stomatal conductance, and transpiration rate and simultaneously consuming the intercellular CO2. These findings are consistent with the study of Ma et al. (2019) that showed Fe is helpful to increase the rates of photosynthesis.

The development of science and technology has witnessed a sharp increase in the use of low-cost nitrogen fertilizers to enhance crop production (Liao et al. 2015). However, the surfeit use of these fertilizers has severely hampered the environment, such as reduced fertilizer efficiency and soil organic carbon content, an increased rate of soil acidification and degradation, and created economic problems (Lu and Tian 2017, Zhao et al. 2018, Aryal et al. 2021). Moreover, these fertilizers contribute to the emission of nitrous oxide—a contributor to global warming (Machado et al. 2021). Therefore, the focus has shifted to the utilization of organic fertilizers and nutrients as plant growth regulators, especially α-ketoglutaric acid, which is thought to be an important ‘node’ linking carbon and nitrogen metabolism (Wang et al. 2020, Wu et al. 2021).
Gui et al. (2016) found that the fresh mass, dry mass, and the content of total carbon and total nitrogen of water spinach significantly increased by foliar application of α-ketoglutaric acid. Huang et al. (2021) revealed that the content of Fe, Zn, and total soluble sugar of *Dendrobium officinale* Kunnert and Migowas was also promoted obviously under the α-ketoglutaric acid treatment. Similarly, we found that the treatment of rice seedlings with Fe–A+ remarkably improved the fresh mass of shoots, seedling height, the average diameter and volume of roots, and Fe content (Figs. 5, 6, 7). In addition, photosynthetic parameters (except C) increased by the Fe–A+ treatment at 2–5 DAS (Figs. 1, 2, 3). The correlation analysis revealed that changes in photosynthesis regulated the seedling growth and Fe uptake (Fig. 8). These results implied that α-ketoglutaric acid could be used as a nitrogen-free organic fertilizer to promote the growth of fragrant rice seedlings. Yang et al. (2022) suggested that it is important to understand whether and how α-ketoglutaric acid is involved in the spatial and temporal distribution of amino acid contents and components in rice grains during the grain-filling stage for further revealing the synergistic relationship and regulatory mechanism of carbon and nitrogen metabolism in rice grains during the grain-filling stage. Therefore, it is of fundamental significance to understand the effects of α-ketoglutaric acid on rice growth.

In line with previous studies on the effects of interactions among several plant regulators on the growth and development of plants (Liu et al. 2020, Fu et al. 2021), we studied the effect of the interaction between α-ketoglutaric acid and Fe on the seedling growth of fragrant rice. The Fe+A+ treatment resulted in an increment in intercellular CO₂ concentration at 2 and 5 DAS, and a decrement in net photosynthetic rate, stomatal conductance, and transpiration rate at 2–5 DAS (except the gₛ of Yuxiangyouzhan at 4 and 5 DAS) in both cultivars (Figs. 1, 2, 3, 4). Compared with Fe–A–, the fresh and dry mass of shoots and roots, seedling height, Fe content, and morphological indexes of roots displayed no significant differences following the Fe+A+ treatment (Figs. 5, 6, 7, 8). These results indicated that the interaction between Fe and α-ketoglutaric acid modified photosynthesis in rice seedling leaves, with little effect on the fresh and dry mass of roots and shoots, seedling height, Fe content, and morphological characteristics of roots. Recent work has revealed that moderate inputs of N-rich biochar can increase the grain yield in rice and the Fe²⁺ concentration in the soil as well as moderate soil cumulative carbon emissions (Yin et al. 2021). Hence, the study of the interaction of Fe and α-ketoglutaric acid (nitrogen-free organic fertilizer) on plant growth and environmental impacts needs further exploration to process toward C-neutral agriculture.

**Conclusion:** The application of both Fe+A– and Fe–A+ treatments increased the height of seedlings, the fresh and dry masses of shoots and roots, and significantly improved the Fe content in seedlings of fragrant rice. In addition, Fe+A– and Fe–A+ treatments improved the root morphological characteristics and photosynthesis (*Pₛ*, *gₛ*, and *E* at 2–5 DAS). The Fe+A+ treatment regulated the photosynthesis of fragrant rice seedlings, whereas the fresh and dry masses of shoots and roots, seedling height, Fe content, and root morphological characteristics values displayed no significant differences as compared with the Fe–A– treatments. Our results indicated the potential use of Fe and α-ketoglutaric acid as a plant regulator fertilizer in promoting the growth of fragrant rice seedlings. Furthermore, the interaction between Fe and α-ketoglutaric acid modified photosynthesis in rice seedling leaves. We believe the findings of this study will provide a theoretical foundation for the use of fertilizers for sustainable agriculture. For revealing the mechanism of the interaction of Fe and α-ketoglutaric acid on plant growth, photosynthesis characteristics, and environmental impacts, much work should be done at a molecular and physiological level.

**References**


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