PHOTOSYNTHETICA
International Journal for Photosynthesis Research

DOI 10.32615/ps.2022.020 PHOTOSYNTHETICA 60 (2): 293-303, 2022

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

S.R. CHENG[*,**,†], S.S. LI[*,†], Z.W. LIANG[***,†], F.C. HUANG[*], X.Q. WU[*], Z.Y. HAN[*], X.B. HUANG[*], X.M. HUANG[*], and Y. REN[*,**,+]

College of Biology and Pharmacy of Yulin Normal University, Yulin, China[*]
Guangxi Key Laboratory of Agricultural Resources Chemistry and Biotechnology, Yulin, China[**]
Research Office of Yulin Normal University, Yulin, China[***]

Abstract

At a three-leaf stage, two Fe treatments [0 mg kg^{-1} (Fe–) and 20 mg·kg^{-1} (Fe+) in the form of $FeCl_3$] 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

Received 10 January 2022
Accepted 31 March 2022
Published online 12 April 2022

[+]Corresponding author
e-mail: renyong20190076@163.com

Abbreviations: C – cultivar; C × T – the interaction of cultivar and treatment; C_i – intercellular CO_2 concentration; DAS – days after spraying; DM – dry mass; E – transpiration rate; FM – fresh mass; g_s – stomatal conductance; P_N – net photosynthetic rate; T – treatment.
Acknowledgments: This work was supported by grants from the Research Start-up Fund for High-level Talents of Yulin Normal University (G2019ZK41 and G2019ZK42), Natural Science Foundation of Guangxi (2021GXNSFBA196084), and College Students Innovation and Entrepreneurship Training Program (202010606045 and 202110606160). The authors would like to thank TopEdit (https://topeditsci.com/) for its linguistic assistance during the preparation of this manuscript.
[†]These authors have contributed equally to this work.
Conflict of interest: The authors declare that they have no conflict of interest.

and Roychoudhury 2019). And many physiological traits regulate the seedling vigor in rice. Therefore, related physiological traits involved in the seedling vigor in rice are important to investigate.

α-ketoglutaric acid, an important intermediate metabolite of the tricarboxylic acid (TCA) cycle, is largely produced by the transamination of L-glutamic acid and has been implicated in the formation of amino acids and proteins, nitrogen transport, and oxidation reactions (Sun *et al.* 2013, 2020; Chen *et al.* 2019). Previous studies reported that certain abiotic stresses, such as high temperature, heavy metals, drought, and salinity, can affect the content of α-ketoglutaric acid in plants (Zhang *et al.* 2016, Kováčik *et al.* 2017, Su *et al.* 2017, Zhu *et al.* 2020). The significance of α-ketoglutaric acid in plant growth is highlighted by its dual roles as a protector and a regulator in plant growth and development. For example, Bayliak and Lushchak (2021) reported that α-ketoglutaric acid can increase stress resistance in model organisms. Morgunov *et al.* (2017) reported that α-ketoglutaric acid could directly inhibit the growth of phytopathogenic fungus (*Fusarium napiforme*) and nematodes (*Ditylenchus destructor*). Li *et al.* (2012) and Sun *et al.* (2014) reported that the application of α-ketoglutaric acid could relieve the negative influence of drought and low nitrogen stress on wheat. Similarly, Du *et al.* (2021) showed that the exogenous application of α-ketoglutaric acid improved the 2-acetyl-1-pyrroline content in grains and enhanced the quality of fragrant rice. In addition, the interaction of high nitrogen and α-ketoglutaric acid significantly affected the agronomic traits and greatly increased the grain yield and 2-acetyl-1-pyrroline content in fragrant rice (Fu *et al.* 2021).

Iron (Fe), an essential microelement required for the growth and development of plants, is mostly available as ferric oxide in the Earth's crust (Verbon *et al.* 2017). Fe participates in multiple metabolic processes of plants, such as electron transport, chlorophyll synthesis, photosynthesis, DNA synthesis, and energy production. Moreover, it is an important constituent of several vital enzymes that are involved in photosynthesis, metal homeostasis, nucleic acids synthesis and repair, and maintaining the structural and functional integrity of chlorophyll and proteins (Rout and Sahoo 2015, Mahender *et al.* 2019). However, Fe deficiency or excess Fe is known to negatively regulate the growth and development of plants. For example, Schmidt *et al.* (2020) reported that Fe deficiency resulted in interveinal chlorosis in young leaves and inhibited the growth of roots. In addition, the consumption of Fe-deficient plants and their products may cause Fe deficiency-induced anemia in humans. Similarly, excess Fe can lead to low pH, low redox potential, infertility of paddy soil, and reduced absorption of essential nutrients, leading to decreased rice yields (Fageria *et al.* 2008). Moreover, Li *et al.* (2021) suggested that the Fe, which OsNRAMP2 transports from the vacuole to the cytosol, plays a pivotal role in seed germination. And also, iron can regulate the growth and development of rice through interaction with other heavy metals (Zhou *et al.* 2018, Ghorbani *et al.* 2021).

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% H_2O_2 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 $g \cdot kg^{-1}$) 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 $FeCl_3$ solution (Zhang *et al.* 1998); 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 seeding 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_2O_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 and E 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+A– and Fe–A+ treatments (Fig. 1). However, the interaction between Fe and α-ketoglutaric acid significantly reduced the P_N. In addition, following the Fe–A+ treatment, P_N at 1 and 2 DAS showed the maximum value, whereas the maximum value following the Fe+A– treatment was reported for 3 and 4 DAS. The lowest P_N was recorded following the Fe+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+A– treatment, whereas the highest g_s at 3 and 4 DAS were recorded after the Fe–A+ treatment. Similarly, the lowest g_s was recorded with the Fe+A+ treatment at each sampling stage, except the Yuxiangyouzhan showed the lowest g_s at 4 and 5 DAS with Fe–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+A– treatment and at 2, 3, and 4 DAS following the Fe–A+ treatment. Like P_N, the interaction between Fe and α-ketoglutaric acid resulted in the lowest transpiration rate values following the Fe+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–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+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–A+ treatment (Fig. 5*A–D*). For Meixiangzhan, the highest shoot fresh mass, shoot dry mass, root fresh mass, and root dry mass were recorded after the Fe+A– treatment. For Yuxiangyouzhan, the highest shoot fresh mass, shoot dry mass, root fresh mass, and root dry mass were recorded after the Fe–A+ treatment. Compared to the Fe–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

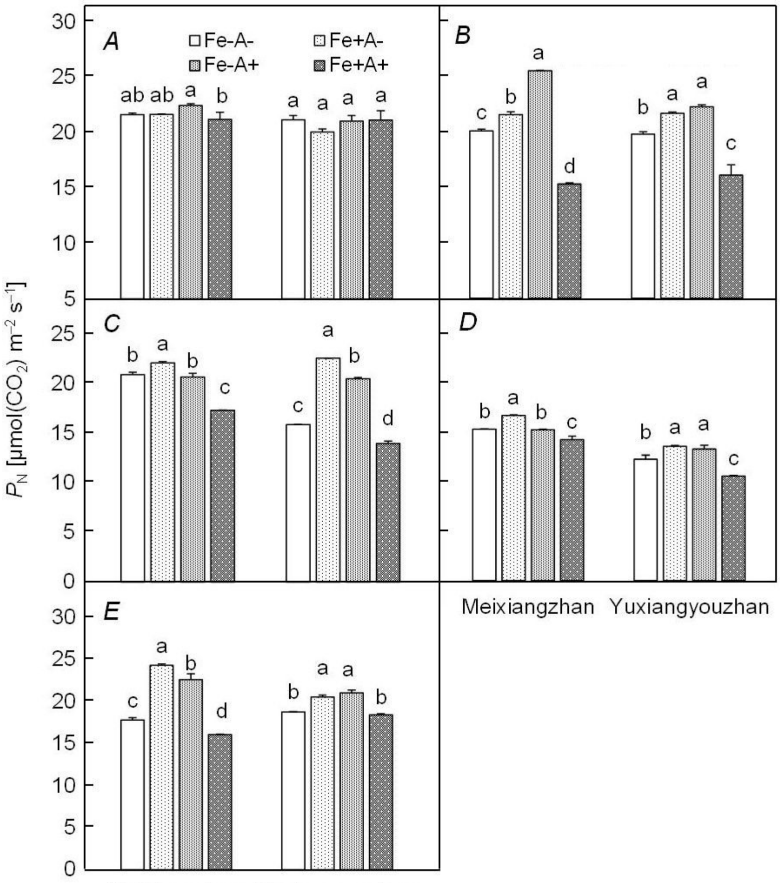

Fig. 1. Effect of Fe and α-ketoglutaric acid on the net photosynthetic rate (P_N) at (*A*) 1 DAS, (*B*) 2 DAS, (*C*) 3 DAS, (*D*) 4 DAS, and (*E*) 5 DAS for Meixiangzhan and Yuxiangyouzhan. Vertical bars with *different lowercase letters* above are significantly different at $P<0.05$ by LSD tests. Capped 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.

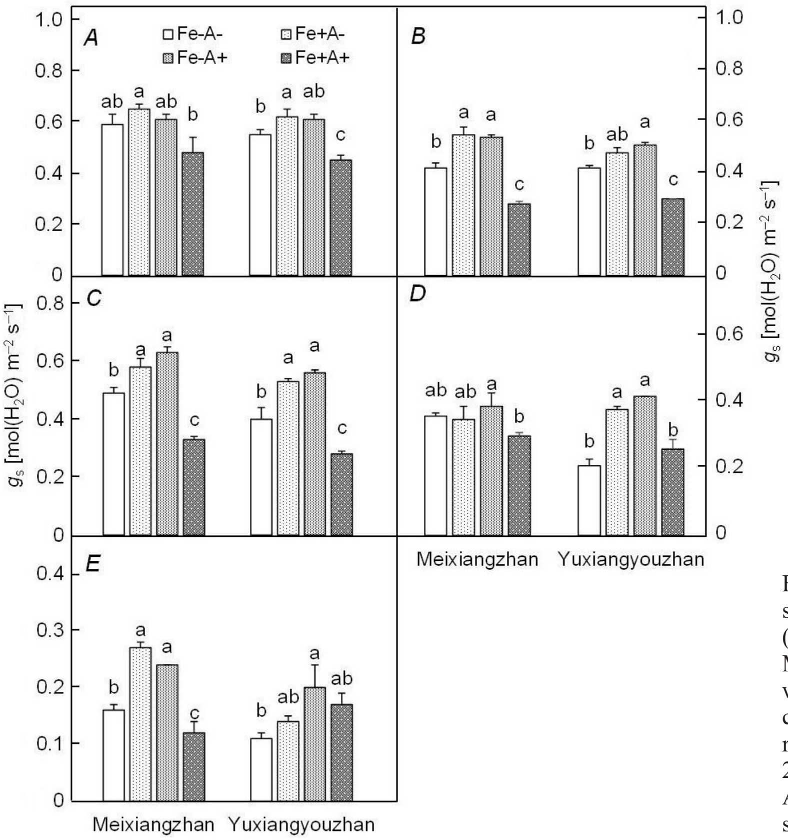

Fig. 2. Effect of Fe and α-ketoglutaric acid on stomatal conductance (g_s) at (*A*) 1 DAS, (*B*) 2 DAS, (*C*) 3 DAS, (*D*) 4 DAS, and (*E*) 5 DAS for Meixiangzhan and Yuxiangyouzhan. Vertical bars with *different lowercase letters* above are significantly different at $P<0.05$ by LSD tests. Capped bars 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.

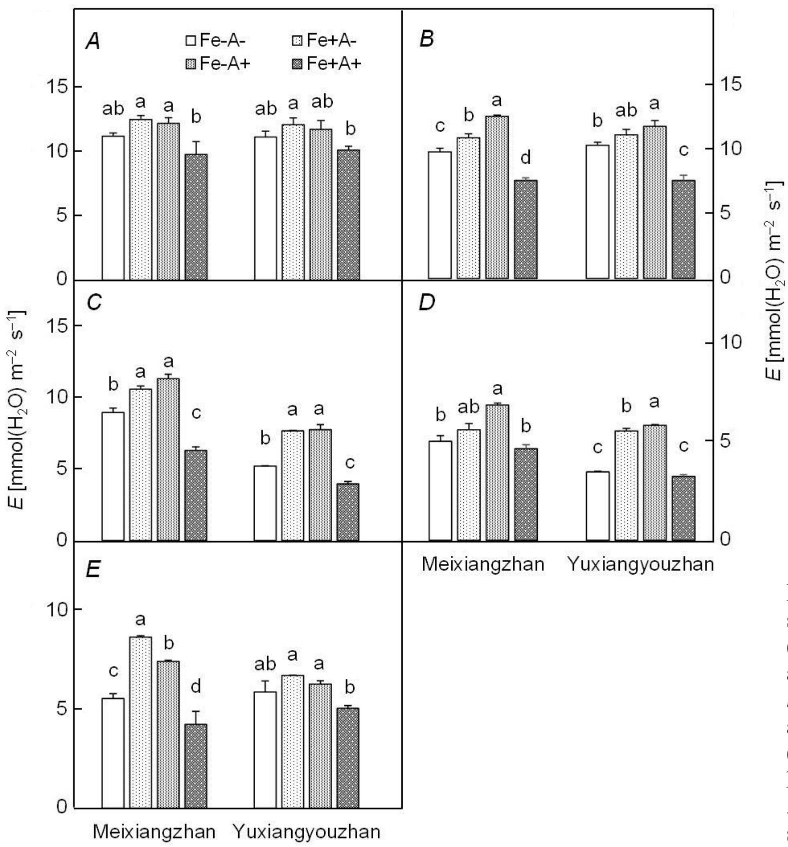

Fig. 3. Effect of Fe and α-ketoglutaric acid on transpiration rate (*E*) at (*A*) 1 DAS, (*B*) 2 DAS, (*C*) 3 DAS, (*D*) 4 DAS, and (*E*) 5 DAS for Meixiangzhan and Yuxiangyouzhan. Vertical bars with different *lowercase letters* above are significantly different at $P<0.05$ by LSD tests. Capped bars 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.

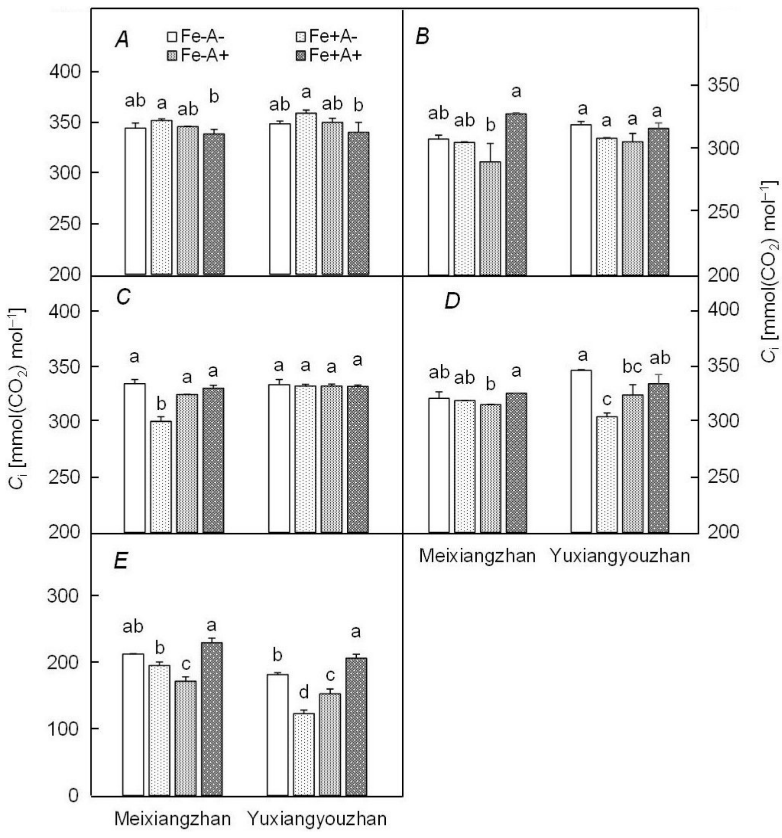

Fig. 4. Effect of Fe and α-ketoglutaric acid on intercellular CO_2 concentration (C_i) at (*A*) 1 DAS, (*B*) 2 DAS, (*C*) 3 DAS, (*D*) 4 DAS, and (*E*) 5 DAS for Meixiangzhan and Yuxiangyouzhan. Vertical bars with *different lowercase letters* above are significantly different at $P<0.05$ by LSD tests. 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.

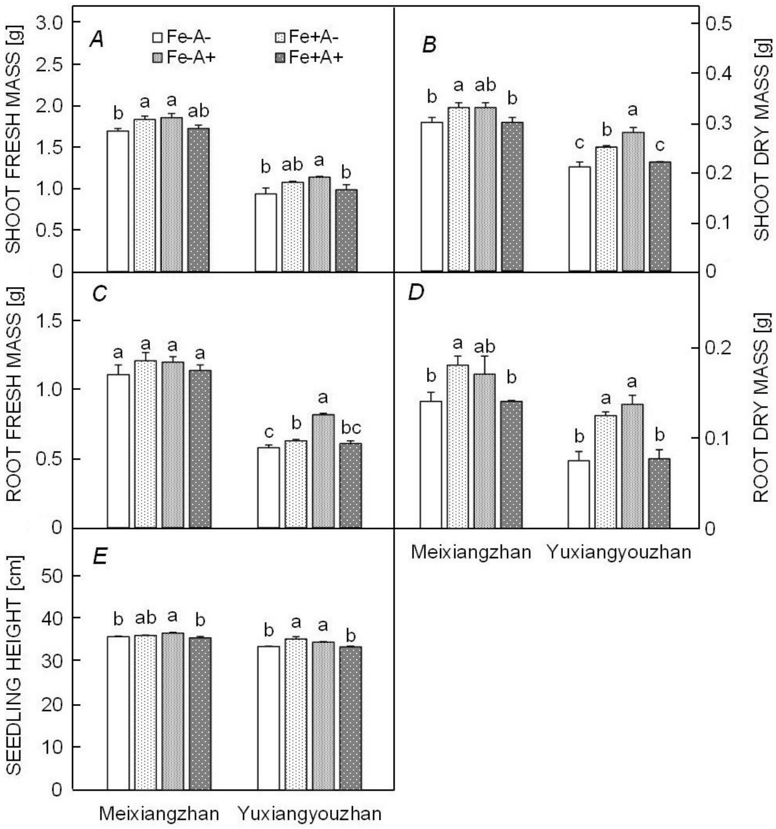

Fig. 5. Effect of Fe and α-ketoglutaric acid on (*A*) shoot fresh mass, (*B*) shoot dry mass, (*C*) root fresh mass, (*D*) root dry mass, and (*E*) seedling height for Meixiangzhan and Yuxiangyouzhan at 5 DAS. Vertical bars with *different lowercase letters* above are significantly different at $P<0.05$ 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. 5*E*). 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. 6*A*). For the Meixiangzhan cultivar, both Fe and α-ketoglutaric acid and their interaction increased the root length, with increments of 3.4, 3.8, and 1.6% under Fe+A–, Fe–A+, and Fe+A+ treatments, respectively. For the Yuxiangyouzhan cultivar, the root length significantly increased only under the Fe+A– treatment (Fig. 6*B*). The root surface area showed the highest value following the Fe+A– treatment, with increments of 3.6 and 47.7%, respectively, for Meixiangzhan and Yuxiangyouzhan. The Fe–A+ treatment significantly increased the root surface area of the Yuxiangyouzhan cultivar. The Fe+A+ treatment exerted no difference on the root surface area for the two cultivars (Fig. 6*C*). We recorded the same root volume for the two varieties, *i.e.*, both Fe+A– and Fe–A+ treatments significantly increased the root volume, whereas the Fe+A+ treatment exerted no significant difference on root volume (Fig. 6*D*). Meanwhile, significant effects of the interaction between cultivar and treatment were reported for shoot dry mass, root fresh mass, root dry mass, seedling height, root average diameter, root length, and root surface area (Table 1S).

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

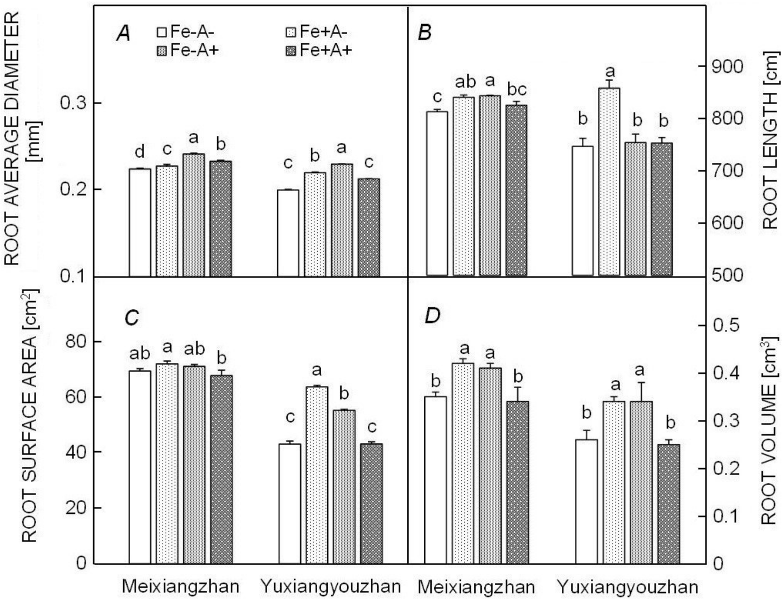

Fig. 6. Effect of Fe and α-ketoglutaric acid on (*A*) root average diameter, (*B*) root length, (*C*) root surface area, and (*D*) root volume for Meixiangzhan and Yuxiangyouzhan at 5 DAS. Vertical bars with *different lowercase letters* above are significantly different at $P<0.05$ by LSD tests. Capped 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}.

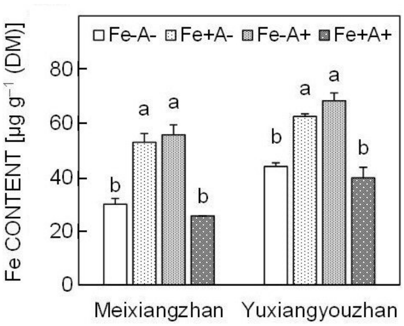

Fig. 7. Effect of Fe and α-ketoglutaric acid on Fe content for Meixiangzhan and Yuxiangyouzhan. Vertical bars with *different lowercase letters* above are significantly different at $P<0.05$ by LSD tests. Capped bars represent SD (n = 3). Fe–: 0 mg($FeCl_3$) kg^{-1}; Fe+: 20 mg($FeCl_3$) kg^{-1}; A–: 0 mg(α-ketoglutaric acid) L^{-1}; A+: 50 mg(α-ketoglutaric acid) L^{-1}. DM – dry mass.

5 DAS, E at 5 DAS and g_s at 2 DAS, g_s at 2 DAS and Fe content, Fe content and P_N at 2 DAS, P_N at 2 DAS and shoot dry mass, shoot dry mass and root average diameter, root average diameter and root dry mass. These significant correlations indicated that changes in photosynthetic parameters could affect the seedling growth and Fe uptake. Fig. 1S (*supplement*) shows the top 25 parameters selected from all investigated parameters that strongly correlated with the shoot fresh mass (Fig. 1S*A*), shoot dry mass (Fig. 1S*B*), root fresh mass (Fig. 1S*C*), root dry mass (Fig. 1S*D*), Fe content (Fig. 1S*E*), and P_N at 2 DAS (Fig. 1S*F*). For example, the root fresh mass and shoot dry mass constituted the top two parameters that correlated with shoot fresh mass. The top two parameters correlating with Fe content were E at 2 DAS and P_N at 5 DAS. Similarly, E at 2 DAS and g_s at 2 DAS comprised the top two parameters correlating with P_N at 2 DAS.

Discussion

Numerous studies have proved that Fe, an essential micronutrient 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. 1S*E*). However, a decrease in intercellular CO_2 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

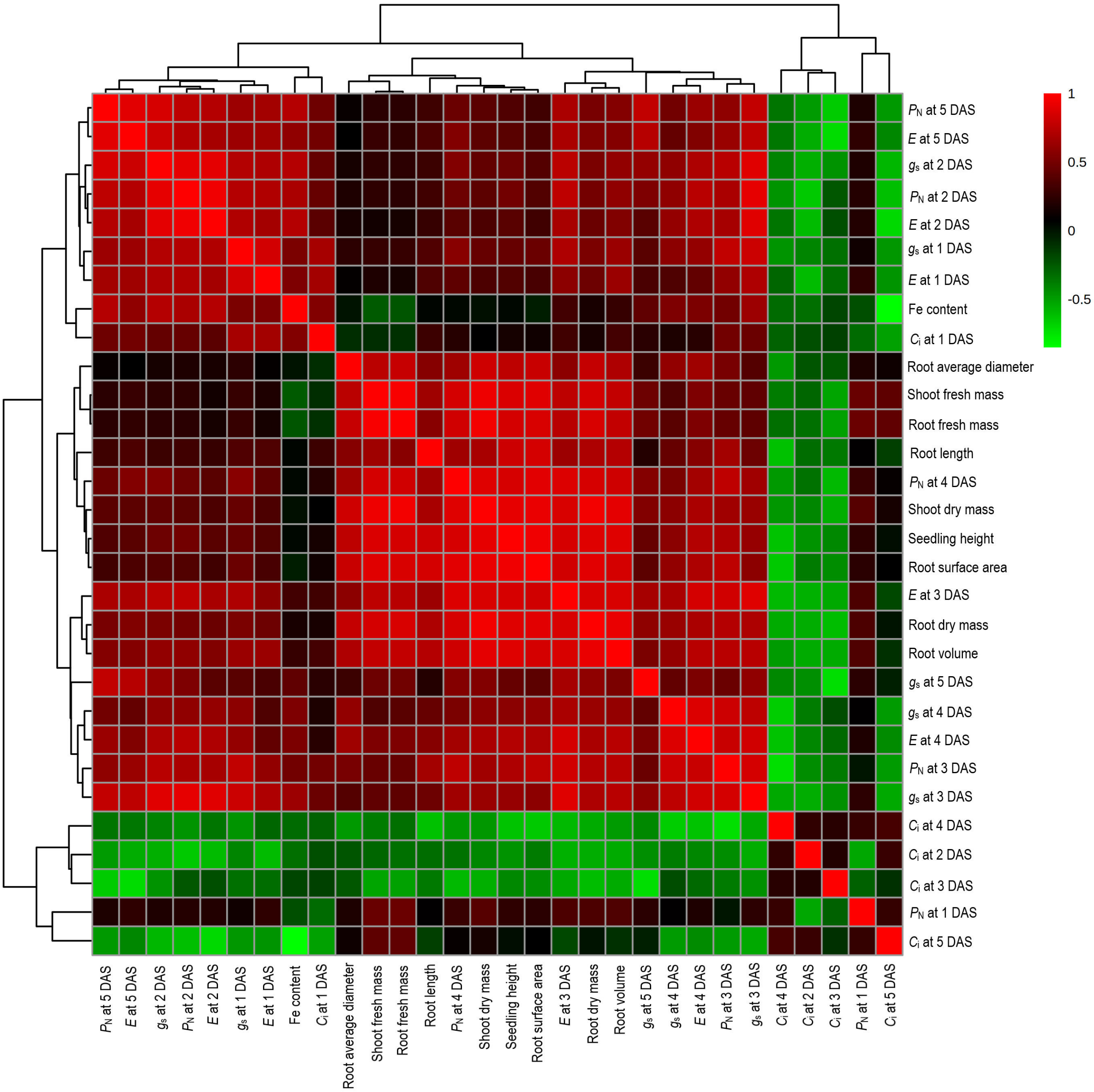

Fig. 8. Correlation heatmap of the investigated parameters. C_i – intercellular CO_2 concentration; DAS – days after spraying; E – transpiration rate; g_s – stomatal conductance; P_N – net photosynthetic rate.

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 CO_2. 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* Kimura et 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_i) 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 CO_2 concentration at 2 and 5 DAS, and a decrement in net photosynthetic rate, stomatal conductance, and transpiration rate at 2–5 DAS (except the g_s 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 Fe^{2+} 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 (P_N, g_s, 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

Araújo T.O., Freitas-Silva L., Santana B.V.N. *et al.*: Tolerance to iron accumulation and its effects on mineral composition and growth of two grass species. – Environ. Sci. Pollut. R. **21**: 2777-2784, 2014.

Aryal J.P., Sapkota T.B., Krupnik T.J. *et al.*: Factors affecting farmers' use of organic and inorganic fertilizers in South Asia. – Environ. Sci. Pollut. R. **28**: 51480-51496, 2021.

Aslam M.M., Zeeshan M., Irum A. *et al.*: Influence of seedling age and nitrogen rates on productivity of rice (*Oryza sativa* L.): A review. – Am. J. Plant Sci. **6**: 1361-1369, 2015.

Banerjee A., Roychoudhury A.: Melatonin application reduces fluoride uptake and toxicity in rice seedlings by altering abscisic acid, gibberellin, auxin and antioxidant homeostasis. – Plant Physiol. Bioch. **145**: 164-173, 2019.

Bayliak M.M., Lushchak V.I.: Pleiotropic effects of alpha-ketoglutarate as a potential anti-ageing agent. – Ageing Res. Rev. **66**: 101237, 2021.

Chen J., Le X.C., Zhu L.: Metabolomics and transcriptomics reveal defense mechanism of rice (*Oryza sativa*) grains under stress of 2,2',4,4'-tetrabromodiphenyl ether. – Environ. Int. **133**: 105154, 2019.

Du B., Wu Q., Jiang S. *et al.*: Effects of exogenous α-ketoglutaric acid on 2-acetyl-1-pyrroline, yield formation and grain quality characters of aromatic rice. – Phyton (B Aires) **90**: 437-447, 2021.

Fageria N.K., Santos A.B., Barbosa Filho M.P., Guimarães C.M.: Iron toxicity in lowland rice. – J. Plant Nutr. **31**: 1676-1697, 2008.

Farooq M., Basra S.M.A., Wahid A. *et al.*: Rice seed invigoration: a review. – In: Lichtfouse E. (ed.): Organic Farming, Pest Control and Remediation of Soil Pollutants. Pp. 137-175. Springer, Dordrecht 2009.

Fu X., Gui R., Li W. *et al.*: Nitrogen and α-ketoglutaric acid application modulate grain yield, aroma, nutrient uptake and physiological attributes in fragrant rice. – J. Plant Growth Regul. **40**: 1613-1628, 2021.

Ghorbani A., Tafteh M., Roudbari N. *et al.*: *Piriformospora indica* augments arsenic tolerance in rice (*Oryza sativa*) by immobilizing arsenic in roots and improving iron translocation to shoots. – Ecotox. Environ. Safe. **209**: 111793, 2021.

Gui P., Chen X., Liao Z. *et al.*: [Effect of organic carbon on carbon and nitrogen metabolism and the growth of water spinach as affected by soil nitrogen levels.] – Acta Pedol. Sin. **53**: 746-756, 2016. [In Chinese]

Guo Y.S., Gu A.S., Cui J.: [Effects of light quality on rice seedlings growth and physiological characteristics.] – J. Appl. Ecol. **22**: 1485-1492, 2011. [In Chinese]

Han F., Chen H., Li X.J. *et al.*: A comparative proteomic analysis of rice seedlings under various high-temperature stresses. – BBA-Proteins Proteom. **1794**: 1625-1634, 2009.

Hanikenne M., Esteves S.M., Fanara S., Rouached H.: Coordinated homeostasis of essential mineral nutrients: a focus on iron. – J. Exp. Bot. **72**: 2136-2153, 2021.

Huang N., Li Q.X., Ran D.L.: [Effect of spraying α-ketoglutarate on *Dendrobium officinale* quality and nutrient content.] – J. Zhejiang Agr. Sci. **62**: 2165-2168, 2021. [In Chinese]

Hussain B., Li J., Ma Y. *et al.*: Effects of Fe and Mn cations on Cd uptake by rice plant in hydroponic culture experiment. – PLoS ONE **15**: e0243174, 2020.

Kováčik J., Klejdus B., Babula P., Hedbavny J.: Ascorbic acid affects short-term response of *Scenedesmus quadricauda* to cadmium excess. – Algal Res. **24**: 354-359, 2017.

Li Y., Li J., Yu Y. *et al.*: The tonoplast-localized transporter OsNRAMP2 is involved in iron homeostasis and affects seed germination in rice. – J. Exp. Bot. **72**: 4839-4852, 2021.

Li Y., Wang Z., Ma C. *et al.*: [Effects of exogenous α-oxoglutarate on grain filling and yield formation of wheat under drought stress.] – J. Triticeae Crops **32**: 249-253, 2012. [In Chinese]

Liao Y., Wu W.L., Meng F.Q. *et al.*: Increase in soil organic carbon by agricultural intensification in northern China. – Biogeosciences **12**: 1403-1413, 2015.

Liu J., Li K., Xu J. *et al.*: Interaction of Cd and five mineral nutrients for uptake and accumulation in different rice cultivars and genotypes. – Field Crop. Res. **83**: 271-281, 2003.

Liu X., Huang Z., Fan P. *et al.*: Zinc and α-ketoglutaric acid modulates plant growth, gas exchange attributes, chlorophyll fluorescence and Zn content in rice. – Int. J. Agric. Biol. **23**: 155-163, 2020.

Lu C., Tian H.: Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: shifted hot spots and nutrient imbalance. – Earth Syst. Sci. Data **9**: 181-192, 2017.

Ma J., Zhang M., Liu Z. *et al.*: Effects of foliar application of the mixture of copper and chelated iron on the yield, quality, photosynthesis, and microelement concentration of table grape (*Vitis vinifera* L.). – Sci. Hortic.-Amsterdam **254**: 106-115, 2019.

Ma J.F., Takahashi E.: Interaction between calcium and silicon in water-cultured rice plants. – Plant Soil **148**: 107-113, 1993.

Ma X., Lin C., Qi L. *et al.*: [Effect of different lighting quality and intensities on quality of rice seedling by greenhouse stereoscopic nursing.] – Trans. Chin. Soc. Agr. Eng. **31**: 228-235, 2015. [In Chinese]

Machado P.V.F., Farrell R.E., Deen W. *et al.*: Contribution of crop residue, soil, and fertilizer nitrogen to nitrous oxide emissions varies with long-term crop rotation and tillage. – Sci. Total Environ. **767**: 145107, 2021.

Mahajan G., Sekhon N.K., Singh N. *et al.*: Yield and nitrogen-use efficiency of aromatic rice cultivars in response to nitrogen fertilizer. – J. New Seeds **11**: 356-368, 2010.

Mahender A., Swamy B.P., Anandan A., Ali J.: Tolerance of iron-deficient and -toxic soil conditions in rice. – Plants-Basel **8**: 31, 2019.

Mishra A., Salokhe V.M.: Seedling characteristics and the early growth of transplanted rice under different water regimes. – Exp. Agr. **44**: 365-383, 2008.

Mo Z., Tang Y., Ashraf U. *et al.*: Regulations in 2-acetyl-1-pyrroline contents in fragrant rice are associated with water-nitrogen dynamics and plant nutrient contents. – J. Cereal Sci. **88**: 96-102, 2019.

Morgunov I.G., Kamzolova S.V., Dedyukhina E.G. *et al.*: Application of organic acids for plant protection against phytopathogens. – Appl. Microbiol. Biot. **101**: 921-932, 2017.

Najeeb S., Ali J., Mahender A. *et al.*: Identification of main-effect quantitative trait loci (QTLs) for low-temperature stress tolerance germination- and early seedling vigor-related traits in rice (*Oryza sativa* L.). – Mol. Breeding **40**: 10, 2020.

Pavlovic J., Samardzic J., Maksimović V. *et al.*: Silicon alleviates iron deficiency in cucumber by promoting mobilization of iron in the root apoplast. – New Phytol. **198**: 1096-1107, 2013.

Ren Y., Ashraf U., He L.X. *et al.*: Irrigation and nitrogen management practices affect grain yield and 2-acetyl-1-pyrroline content in aromatic rice. – Appl. Ecol. Env. Res. **15**: 1447-1460, 2017.

Rizwan M., Noureen S., Ali S. *et al.*: Influence of biochar amendment and foliar application of iron oxide nanoparticles on growth, photosynthesis, and cadmium accumulation in rice biomass. – J. Soils Sediments **19**: 3749-3759, 2019.

Rout G.R., Sahoo S.: Role of iron in plant growth and metabolism. – Rev. Agr. Sci. **3**: 1-24, 2015.

Ruan S., Wu F., Lai R. *et al.*: Preliminary application of vermicompost in rice production: effects of nursery raising with vermicompost on fragrant rice performances. – Agronomy **11**: 1253, 2021.

Schmidt W., Thomine S., Buckhout T.J.: Iron nutrition and interactions in plants. – Front. Plant Sci. **10**: 1670, 2020.

Srivastava R.K., Pandey P., Rajpoot R. *et al.*: Cadmium and lead interactive effects on oxidative stress and antioxidative responses in rice seedlings. – Protoplasma **251**: 1047-1065, 2014.

Su J., Ye M., Lou Y. *et al.*: Low-molecular-mass organic acid and lipid responses of *Isochrysis galbana* Parke to high temperature stress during the entire growth stage. – Algal Res. **26**: 93-103, 2017.

Sun H., Wang X., Li H. *et al.*: Selenium modulates cadmium-induced ultrastructural and metabolic changes in cucumber seedlings. – RSC Adv. **10**: 17892-17905, 2020.

Sun Q.L., Jia L., Wang Z.Q. *et al.*: [Effects of exogenous α-oxoglutarate on yield traits of wheat under low water potential and low nitrogen stress.] – J. Anhui Agric. Sci. **42**: 671-676, 2014. [In Chinese]

Sun Z., Qi X., Wang Z. *et al.*: Overexpression of *TsGOLS2*, a galactinol synthase, in *Arabidopsis thaliana* enhances tolerance to high salinity and osmotic stresses. – Plant Physiol. Bioch. **69**: 82-89, 2013.

Valentinuzzi F., Pii Y., Carlo P. *et al.*: Root-shoot-root Fe translocation in cucumber plants grown in a heterogeneous Fe provision. – Plant Sci. **293**: 110431, 2020.

Verbon E.H., Trapet P.L., Stringlis I.A. *et al.*: Iron and immunity. – Annu. Rev. Phytopathol. **55**: 355-375, 2017.

Vigani G., Zocchi G., Bashir K. *et al.*: Signals from chloroplasts and mitochondria for iron homeostasis regulation. – Trends Plant Sci. **18**: 305-311, 2013.

Wang D.R., Bunce J.A., Tomecek M.B. *et al.*: Evidence for divergence of response in *Indica*, *Japonica*, and wild rice to high CO_2 × temperature interaction. – Glob. Change Biol. **22**: 2620-2632, 2016.

Wang J.K., Wang Y.L., Chen H.Z. *et al.*: [Mechanism of high temperature affecting carbon and nitrogen metabolism of rice grain at the early stage of grain filling.] – Chin. J. Agrometeorol. **12**: 774-784, 2020. [In Chinese]

Wu T.T., Chen J.B., Yuan H.W. *et al.*: [Effects of foliar spraying organic carbon on carbohydrate metabolism and Fe, Zn content of *Dendrobium officinale*.] – Ecol. Sci. **40**: 31-36, 2021. [In Chinese]

Yang J., Li C., Jiang Y.: [Contents and compositions of amino acids in rice grains and their regulation: a review.] – Acta Agron. Sin. **48**: 1037-1050, 2022. [In Chinese]

Yin X., Peñuelas J., Sardans J. *et al.*: Effects of nitrogen-enriched biochar on rice growth and yield, iron dynamics, and soil carbon storage and emissions: A tool to improve sustainable rice cultivation. – Environ. Pollut. **287**: 117565, 2021.

Yuan L., Wu L., Yang C., Lv Q.: Effects of iron and zinc foliar applications on rice plants and their grain accumulation and grain nutritional quality. – J. Sci. Food Agr. **93**: 254-261, 2013.

Zhang J., Yang D., Li M., Shi L.: Metabolic profiles reveal changes in wild and cultivated soybean seedling leaves under salt stress. – PLoS ONE **11**: e0159622, 2016.

Zhang X., Zhang F., Mao D.: Effect of iron plaque outside roots on nutrient uptake by rice (*Oryza sativa* L.): Zinc uptake by Fe-deficient rice. – Plant Soil **202**: 33-39, 1998.

Zhao Y., Wang M., Hu S., Shi X.: Economics- and policy-driven organic carbon input enhancement dominates soil organic carbon accumulation in Chinese croplands. – P. Natl. Acad. Sci. USA **115**: 4045-4050, 2018.

Zhou H., Zhu W., Yang W.T. *et al.*: Cadmium uptake, accumulation, and remobilization in iron plaque and rice tissues at different growth stages. – Ecotox. Environ. Safe. **152**: 91-97, 2018.

Zhu B., Xu Q., Zou Y. *et al.*: Effect of potassium deficiency on growth, antioxidants, ionome and metabolism in rapeseed under drought stress. – Plant Growth Regul. **90**: 455-466, 2020.