

Effects of Iron Deficiency Stress on Plant Growth and Quality in Flowering Chinese Cabbage and Its Adaptive Response

Xian Yang (✉ yangxian@scau.edu.cn)

Research article

Keywords: Flowering Chinese cabbage, Fe deficiency, Growth, Quality, Adaptive response

Posted Date: December 1st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1110263/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Iron (Fe) plays an important role in the growth and development of the human body and plants. The effects of different Fe concentrations, 1-aminocyclopropane-1-carboxylic acid (ACC), and cobalt chloride (Co^{2+}) treatments on plant growth, quality and the adaptive response to Fe deficiency stress were investigated in flowering Chinese cabbage. The results revealed that Fe deficiency stress inhibited plant growth. The content of vitamin C (Vc), soluble protein, and soluble sugar in leaves and stalks were significantly reduced under Fe deficiency stress, while the content of cellulose and nitrate was increased. Meanwhile, Fe deficiency stress obviously reduced the net photosynthetic rate and nitrate reductase (NR) activity of leaves. The balance system of active oxygen metabolism was destroyed due to Fe deficiency, resulting in the decrease of catalase (CAT) activity, superoxide dismutase (SOD) activity of roots and leaves, and peroxidase (POD) activity of leaves, while POD activity in roots and malonaldehyde (MDA) content were significantly increased. The treatments of Fe deficiency and ACC significantly reduced pH value of the root medium, promoted release of ethylene, and increased Fe^{3+} reductase activity, while Co^{2+} treatment showed the results opposite to those of Fe deficiency and ACC treatments. Thus, Fe deficiency stress could induce nitrogen metabolism, photosynthesis, reactive oxygen metabolism, pH of root medium, and Fe^{3+} reductase activity that was related to physiological adaptive response and tolerance mechanisms. We also found that ethylene could involve in regulating the adaptive response to Fe deficiency stress and improve the availability of Fe in flowering Chinese cabbage.

Main Conclusion

Fe deficiency stress could induce nitrogen metabolism, photosynthesis, reactive oxygen metabolism, pH of root medium, and Fe^{3+} reductase activity that was related to physiological adaptive response and tolerance mechanisms.

1. Introduction

Iron is an essential mineral nutrient element for human health and plant growth and development, not only is an essential element for all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport and deoxyribonucleic acid synthesis, but also play significant roles in the physiological processes of photosynthesis, respiration, nitrogen metabolism, redox system of the plasma membrane, protein and nucleic acid synthesis in plant (Connorton et al. 2017). Although Fe is a relatively abundant element in soil, the contents of the soluble Fe are remarkably poor, and hence poor solubility impairs its effectiveness, which often becomes a limiting nutrient element for plant growth and development. Indeed, one-third of the cultivated areas in the world are made of calcareous soils, which are the primary cause of Fe deficiency and about 40% of soil occur Fe deficiency. Thus, Fe deficiency is a common problem affecting the yield of crop (Mori 1999). Fe deficiency had a detrimental effect on plant growth and development, inducing various metabolic disorders, resulting in the abnormalities of chloroplast morphology and structures, reducing chlorophyll contents and photosynthetic rate, and diminishing respiratory of plants, which severely reduced plant yield and quality (Dey et al. 2020; Riaz et al. 2021).

The adaptive mechanisms of higher plant activation of Fe are divided into strategy I (Reduction Strategy) and strategy II (Chelation Strategy) (Romheld et al. 1986). For example, cucumbers, tomatoes, and soybeans are dicotyledons, their adaptive mechanisms induced by Fe deficiency all belonged to the strategy I. However, their specific responses to Fe deficiency stress are different. Cucumber and tomato with Fe deficiency mainly show enlargement and coarseness near the root tip, increased root hairs, developed into a characteristic of metastatic cells, actively excrete large amounts of H^+ that significantly enhanced the Fe^{3+} reduction ability of roots, which improved the availability of Fe in the rhizosphere. Soybean reduced the insoluble Fe^{3+} mainly by the swelling and thickening of root tips and the accumulation of a large number of phenolic substances in the epidermis and cortex (Yi et al. 1998). Therefore, different plants show different response mechanisms to Fe deficiency stress.

Brassica campestris L. ssp. *chinensis* var. *utilis* Tsen et Lee, known as flowering Chinese cabbage, belongs to the genus *Brassica*, which is a popular cruciferous vegetable commercially distributed in China, especially is one of the annually-produced vegetables with the largest cultivation scale and consumption in South China (Kang et al. 2014). The lack of Fe nutrition gradually becomes the key factor limiting the yield of flowering Chinese cabbage due to the continuous increase of multiple cropping index and the higher and higher degree of soil alkalization. Our previous study indicated that mineral nutrition has obvious effects on the growth, yield, quality, and disease resistance of flowering Chinese cabbage (Chai et al. 2013; Yang et al. 2000, 2004, 2012).

However, the effects of Fe deficiency on the growth and development of flowering Chinese cabbage and its related physiological characteristics are still lacking in an investigation. In this study, we aim to investigate the effects of Fe deficiency stress on the growth, yield, and quality, and to investigate its adaptive physiological mechanism to Fe deficiency stress through different Fe concentrations, ACC, and Co^{2+} treatments in flowering Chinese cabbage.

2. Materials And Methods

2.1. Materials and reagents

This experiment was carried out in greenhouse of South China Agricultural University with flowering Chinese cabbage '60-day' as experimental cultivar, Guangzhou, China. The seeds were sown in the nutrient bowl containing perlite on April 13, with 1/4 Hoagland nutrient solution were sprayed every 3 days at the seedling stage. They were transplanted in a plastic box (61 cm×42 cm×15 cm) with 20 L Hoagland nutrient solution for hydroponic cultivation on May 2, while the seedlings had 3-4 true leaves. Every 14 seedlings with a plant spacing of 12.5×11 cm were in each plastic box. Five treatments of 1Fe (5.6 mg·L⁻¹), 1/2Fe (2.8 mg·L⁻¹), 0Fe (0 mg·L⁻¹), 0Fe (0 mg·L⁻¹) + ACC (1 μM) and 0Fe (0 mg·L⁻¹) + Co^{2+} (10 μM) were carried out based on the Hoagland nutrient solution in this experiment. Each treatment was repeated 3 times. Each plastic box was put on one oxygen pump for ventilation (ventilation 15 minutes per hour). The nutrient solutions were renewed once a week.

2.2. Determination of growth and quality index

The plants were collected on May 25. The plant height, stem diameter, fresh weight of roots, overground part of the plant, and whole plant as well as the quality of flower stalk of each 10 plants as a treatment group were measured. The flower stalk was the part above the third leaves of the plant. The stalk was the leafless part of the flower stalk. The quality index of the flower stalk was determined after separating it into the leaves and the stalks. Vc content was measured by molybdenum blue colorimetric method. Soluble sugar content and soluble protein content were determined by following the method of Chai et al. (2013) and Bradford (1976) respectively. Nitrate content was determined by the derivate spectrophotometric method (Lastra 2003), while cellulose content was determined by the anthrone colorimetric method (Gao 2006). Active Fe content was measured by the method of Pierson et al. (1984).

2.3. Determination of photosynthetic characteristics

The chlorophyll content was determined by the method of Arnon et al. (1949). The photosynthetic rate, transpiration rate, intercellular CO_2 concentration, and stomatal conductance were performed on a portable automatic photosynthesizer (LI-6400, LI-COR, USA).

2.4. Analysis of NR activity

The NR (EC 1.7.99.4) activity was determined by using p-aminobenzene sulfanilic acid α-naphthylamine as a display agent and expressed in $\mu\text{g}\cdot\text{NO}^{-2}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$ FW (Wang et al. 2010).

2.5. Determination of reactive oxygen metabolism

The reactive oxygen metabolism was determined using our previously reported method (Yang et al. 2000). In brief, each 0.5 g sample was precooled in a mortar, and 5 mL of 0.05 mol·L⁻¹ pH 7.0 phosphate buffer solution and 0.3 g polyvinylpyrrolidone were added and grind, then centrifugated at 4°C with 3000 r·min⁻¹ rates for 10 min, and the supernatant was collected for the determination of the POD (EC 1.11.1.7), CAT (EC 1.11.1.21) and SOD (EC 1.15.1.1) activities and MDA content.

2.6. Determination of ethylene release from roots

The content of ethylene release from roots was measured for consecutive 6 days using the modified method (Romera et al. 1999). Briefly, each of five roots and 200 μL water was added in a 25 mL tube, sealed and kept in dark conditions for 2 h, then 1 mL extracted gas from the tube for the determination of ethylene release were carried out using a Shimadzu gas chromatography (GC-14C).

2.7. Measurement of pH of root medium

First, the pH value of the initial solution of the root medium was adjusted to be 6.26 and treated with different Fe concentrations, ACC and Co^{2+} , then the pH of the root medium was measured for consecutive 6 days (at 9 am each day) using a Leici PHS-3B precision pH meter.

2.8. Determination of the Fe^{3+} reductase activity of roots

The Fe^{3+} reductase (EC 1.16.1.7) activity was evaluated for consecutive 6 days using a 2,2-bipyridine method (Gogorcena et al. 2000). In brief, the samples were put in the saturated CaSO_4 solution and soaked for 5 min, then washed using deionized water, then placed in a nutrient solution containing 0.1 mM $\text{Fe}(\text{III})$ -EDTA and 0.4 mM 2,2'-bipyridine with $\text{Fe}(\text{III})$. After a 2-hour reaction under continuous ventilation with light illumination, the roots were weighted and the absorbance of the reactive liquid at 520 nm was measured.

2.9. Data processing and analysis

Data from experiments with triplicate or sextuplicate were statistically analyzed using SAS 13.0 software (SAS Institute, Inc, Cary, NC, USA) by Duncan's multiple range. The difference was considered to be statistically significant at $P < 0.05$. The figures were generated using Origin 2018c.

3. Results

3.1. Effects of Fe deficiency stress on the plant growth

Our results indicated that Fe deficiency stress obviously inhibited the plant growth (Table 1 and Figure 1). From the plant phenotype, Fe deficiency treatment had poor growth, dwarf, and yellowed heart leaves. The 1/2Fe or 1Fe treatment showed plant fresh weight, shoot fresh weight, stem height, and stem diameter significantly better than those of Fe deficiency treatment, Compared with the 1Fe treatment, the plant fresh weight in the Fe deficiency stress and 1/2Fe treatments decreased by 33.6% and 9.2%; the root fresh weight in the Fe deficiency stress and 1/2Fe treatments decreased by 43.7% and 39.8%; the plant height in the Fe deficiency stress and 1/2Fe treatments decreased by 21.4% and 1.9%; the stem diameter in the Fe deficiency stress and 1/2Fe treatments decreased by 22.8% and 6.4%; while the 1Fe treatment displayed the plant fresh weight, root fresh weight, stem height, and stem diameter slightly better than that of 1/2Fe treatment with no significant difference. The root fresh weight, and root shoot ratio of 1Fe treatment were significantly higher than those of 1/2Fe or Fe deficiency treatment, while those of 1/2Fe and Fe deficiency treatments showed no significant difference. Thus, these results indicated that Fe deficiency stress could significantly inhibit the growth of aboveground and roots and the expansion of flower stalk, which resulted in a significant decrease in fresh weight of flowering Chinese cabbage.

Table 1 Effects of Fe deficiency stress on the plant growth

Treatment	Plant fresh weight (g.plant ⁻¹)	Aboveground fresh weight (g.plant ⁻¹)	Root fresh weight (g.plant ⁻¹)	Root shoot ratio	Plant height (cm)	Stem diameter (mm)
1 Fe	20.34±0.47 a	18.04±1.08 a	2.54±0.66 a	0.15±0.04	17.33±3.22	8.91±0.74
1/2 Fe	18.47±0.81 a	16.92±0.58 a	1.53±0.20 b	0.11±0.01	17.00±2.65	8.34±0.37
0 Fe	13.51±2.30 b	11.24±1.45 b	1.43±0.12 b	0.10±0.02	13.63±1.89	6.88±0.91

3.2. Effects of Fe deficiency stress on the quality of flower stalk

Fe deficiency stress obviously reduced the contents of Vc, soluble protein, and soluble sugar in leaves and stalks of the flower stalk, while increased the content of cellulose, and promoted the accumulation of nitrate in leaves and stalks of the flower stalk. Compared with the 1Fe treatment, the Vc content in leaves of the flower stalk in the Fe deficiency stress and 1/2Fe treatments decreased by 22.5% and 14.6%; the soluble protein content in leaves of the flower stalk in the Fe deficiency stress and 1/2Fe treatments decreased by 15.1% and 3.5%; the cellulose content in leaves of the flower stalk in the Fe deficiency stress and 1/2Fe treatments increased by 24.5% and 2.4%; the nitrate content in leaves of the flower stalk in the Fe deficiency stress and 1/2Fe treatments increased 49.8% and 17.8%. The contents of nitrate and cellulose of flower stalk in the 1/2Fe treatment were higher than those in the 1Fe treatment, while the Vc content of the 1/2Fe treatment was lower than that of the 1Fe treatment in stalks of the flower stalk. Compared with the 1Fe treatment, the Vc content in stalks of the flower stalk in the Fe deficiency stress and 1/2Fe treatments decreased by 56.2% and 30.3%; the soluble protein content in stalks of the flower stalk in the Fe deficiency stress and 1/2Fe treatments decreased by 11.7% and 5.0%; the cellulose content in stalks of the flower stalk in the Fe deficiency stress and 1/2Fe treatments increased by 43.1% and 11.9%; the nitrate content in stalks of the flower stalk in the Fe deficiency stress and 1/2Fe treatments increased 14.9% and 11.5%. There was no significant difference in the contents of soluble protein, soluble sugar, and Vc in leaves and stalks of the flower stalk between 1/2Fe and 1Fe treatment. Thus, it could be seen that Fe deficiency stress affected the formation of the quality of the flower stalk, leading to the decline of the quality of flowering Chinese cabbage (Table 2).

Table 2 Effects of Fe deficiency stress on the quality of flower stalk

Treatment	Organ	Vc content (mg·g ⁻¹ ·FW)	Soluble protein content (mg·g ⁻¹ ·FW)	Soluble sugar content (ug·g ⁻¹ ·FW)	Nitrate content (mg·g ⁻¹ ·FW)	Cellulose content (%·g ⁻¹ ·DW)
1Fe	Leaf flower stalk	0.89±0.10 a	1.72±0.03 a	0.90±0.02 a	9.08±0.27 c	16.64±1.61 b
1/2Fe		0.76±0.04 ab	1.66±0.01 a	0.88±0.02 a	10.7±0.46 b	17.04±1.43 b
0Fe		0.69±0.01 b	1.46±0.08 b	0.89±0.02 a	13.6±0.27 a	20.71±1.25 a
1Fe	Stalk flower stalk	0.89±0.01 a	1.20±0.02 a	1.11±0.01 a	11.61±0.09 c	41.25±0.54 c
1/2Fe		0.62±0.03 b	1.14±0.12 a	1.06±0.02 ab	12.99±0.37 b	45.98±0.09 b
0Fe		0.39±0.04 c	1.06±0.05 b	1.01±0.01 b	16.61±0.28 a	47.41±1.16 a

3.3. Effects of Fe deficiency stress on the photosynthetic characteristics

As shown in Table 3, the chlorophyll content in the Fe deficiency treatment was significantly lower than that in 1/2Fe and 1Fe treatments, compared with the 1/2Fe and 1Fe treatments, the chlorophyll contents were significantly decreased by 21.0% and 20.5%, respectively, while there was no significant difference between the 1/2Fe and 1Fe treatment.

Fe deficiency did not affect the transpiration rate of leaves but had significant effects on stomatal conductance, photosynthetic rate, and intercellular CO₂ concentration (Table 3). Fe deficiency stress significantly reduced the net photosynthetic rate and stomatal conductance but significantly increased the intercellular CO₂ concentration in leaves. The net photosynthetic rate and stomatal conductance in the 1/2Fe treatment were not significantly different except that the intercellular CO₂ concentration was higher than that in the 1Fe treatment. Thus, these results showed that Fe deficiency stress significantly inhibited the formation of chlorophyll and destroyed the photosynthetic characteristics, which resulted in the decrease of photosynthetic rate in flowering Chinese cabbage.

3.4. Effects of Fe deficiency stress on the NR activity

The NR activity of leaves decreased significantly. Compared with the 1Fe treatment, the NR activity of leaves in the Fe deficiency stress and 1/2Fe treatments decreased by 36.9% and 24.2%, respectively (Table 3). These results suggested that Fe deficiency stress caused the decrease of NR activity of leaves, which resulted in the decrease of nitrogen metabolism and the accumulation of nitrate in leaves, and inhibited the normal plant growth of flowering Chinese cabbage.

Table 3 Effects of Fe deficiency stress on the photosynthetic characteristics and NR activity of leaves

Treatment	Chlorophyll content (mg·g ⁻¹ ·FW)	Stomatal conductance (mol·m ⁻² ·s ⁻¹)	Net photosynthetic rate (umol·m ⁻² ·s ⁻¹)	Intercellular CO ₂ concentration (umol·m ⁻² ·s ⁻¹)	Transpiration rate (mmol·m ⁻² ·s ⁻¹)	NR activity (ug·g ⁻¹ ·h ⁻¹)
1Fe	1.438±0.005 _a	0.153±0.0007 _a	17.00±0.82 a	147.5±0.71 c	7.37±0.495 a	12.01±0.52 _a
1/2Fe	1.446±0.019 _a	0.154±0.0093 _a	16.27±1.31 a	174.0±3.00 b	7.74±0.197 a	9.17±0.45 _a
0Fe	1.143±0.093 _b	0.132±0.0055 _b	8.15±0.47 b	252.0±1.00 a	7.75±0.295 a	7.57±0.27 _a

3.5. Effects of Fe deficiency stress on the reactive oxygen metabolism

Fe deficiency stress affected the balance of the reactive oxygen metabolism system. As shown in Figure 2a, POD activity in roots and leaves had different trends under the different Fe concentrations. Compared with 1Fe treatment, POD activity of roots in Fe deficiency stress and 1/2Fe treatments was significantly increased by 6.1% and 5.7%, respectively, but there was no significant difference between Fe deficiency stress and 1/2Fe treatment. Compared with 1Fe treatment, POD activity of leaves in Fe deficiency stress and 1/2Fe was significantly decreased by 21.5% and 35.2%, respectively, but there was no significant difference between the Fe deficiency stress and 1/2Fe treatment. Moreover, we could also see that the POD activity of roots was nearly 100 times higher than that of leaves. The increase of POD activity in roots under the Fe deficiency condition may be due to the root resistance to the production of reactive oxygen caused by Fe deficiency stress and induces a root resistance reaction to damage of cell membrane system caused by the reactive oxygen, which prevents the further damage to the plant.

Compared with the 1Fe treatment, CAT activity of roots in Fe deficiency and 1/2Fe treatments was decreased by 28.5% and 21.0%, respectively, and CAT activity of leaves decreased by 66.1% and 38.8%, respectively (Figure 2b). In the 1Fe and 1/2Fe treatments, the SOD activity in roots or leaves was significantly higher than that in the Fe deficiency stress treatment, but the difference was not significant (Figure 2c). These results indicated that Fe deficiency stress could reduce the CAT and SOD activities of roots and leaves.

The root of MDA content increased significantly with the decrease of Fe concentrations. Compared with the 1Fe treatment, MDA content in the Fe deficiency stress and 1/2Fe treatments increased by 97.1% and 26.8%, respectively. Compared with the 1Fe treatment, the leaves of MDA content in Fe deficiency treatment was increased, but the difference was not significant. While the MDA content of leaves in the 1/2Fe treatment was lower than those in the Fe deficiency and 1Fe deficiency treatments, but there was no significant difference between 1/2Fe and 1Fe treatments (Figure 2d). These results indicated that Fe treatment with appropriate concentration could inhibit membrane lipid peroxidation in leaves and roots, while Fe deficiency stress increased

membrane lipid peroxidation in leaves and roots. In addition, the effect of Fe deficiency stress on membrane lipid peroxidation of roots was different from that on leaves, the effect of Fe deficiency stress on membrane lipid peroxidation in roots was greater than that in leaves. While in the 1Fe treatment, the membrane lipid peroxidation level of leaves increased, which might be due to the stress caused by excessive absorption of Fe in leaves under the condition of rich Fe nutrition.

The above results indicate that Fe deficiency stress could cause the metabolic system of reactive oxygen to be disturbed. Fe deficiency stress significantly increased the POD activity of roots, but the activity of SOD and CAT in roots and leaves and POD in leaves decreased significantly, and MDA content in roots and leaves was greatly accumulated, which indicated that Fe deficiency stress decreased the scavenging capacity of reactive oxygen species and increased the membrane lipid peroxidation level, thus affecting the normal plant growth and development of flowering Chinese cabbage.

3.6. Effects of Fe deficiency stress, ACC and Co²⁺ on endogenous ethylene release from roots

Fe deficiency stress had a great effect on ethylene release from roots (Figure 3a). With the prolongation of treatment time, the release of ethylene in the Fe deficiency stress, 1/2Fe, and 1Fe treatments showed a single peak curve, which first increased and then decreased, and their peak value came out on the fourth day. With the increase of Fe concentrations, the ethylene release from roots decreased continuously. In short, Fe deficiency stress could promote the release of ethylene from roots, which indicated that ethylene was involved in the response to Fe deficiency stress.

ACC is a precursor of ethylene biosynthesis and Co²⁺ is an inhibitor of ACC synthase. To verify the role of ethylene in the response to Fe deficiency stress, ACC and Co²⁺ were added to study the effects of ethylene release from roots based on Fe deficiency stress. The results showed that ACC could obviously improve the release of ethylene in roots, while Co²⁺ led to inhibition under the condition of Fe deficiency (Figure 3b). It is further suggested that ethylene was involved in the regulation of the response to Fe deficiency, and Fe deficiency stress led to increasing the release of ethylene from roots might be an adaptive response to Fe deficiency stress in flowering Chinese cabbage.

3.7. Effects of Fe deficiency stress and ACC and Co²⁺ treatment on the pH value in root medium

Fe deficiency stress had a significant effect on the pH value of root medium (Figure 3c). After Fe deficiency stress treatment, the pH value of root medium decreased gradually, reached a low peak on the third day, and then increased gradually. The pH value of root medium of 1/2Fe and 1Fe treatments decreased on the first day and then increased linearly. In general, the pH value of root medium with 1/2Fe treatment was the highest, followed by 1Fe treatment, and the Fe deficiency treatment was the lowest. It indicated that the acidification capacity of the plant root was significantly improved under the condition of severe Fe deficiency, thus improving the availability of Fe in the root medium.

Under the condition of Fe deficiency, ACC treatment could decrease the pH value of the root medium with the extension of treatment time, and the pH value decreased to the trough on the third day and gradually increased, and then decreased again on the fifth day. Moreover, under the condition of Fe deficiency, Co²⁺ treatment also decreased the pH value of root medium with the extension of treatment time, and it decreased to the trough on the third day and then kept rising gradually. Among different treatments, the pH value of the root medium with ACC treatment was the lowest, while that of Co²⁺ treatment was the highest (Figure 3d). The results suggested that ACC treatment based on Fe deficiency stress could enhance the acidification capacity of the root medium, while the Co²⁺ treatment showed an opposite result, which might be because the proton pump controlled by ATPase on the protoplasm membrane of the root cells was induced by ACC, and the number of protons pumped out of the plasma membrane increased, resulting in a significant decrease in the pH value of the root medium of flowering Chinese cabbage.

3.8. Effects of Fe deficiency stress and ACC and Co²⁺ on the Fe³⁺ reductase activity in roots

Different Fe concentration treatments had significant effects on the Fe³⁺ reductase activity of roots (Figure 3e). With the increases in treatment time, the Fe³⁺ reductase activity treated different Fe concentrations showed an increasing trend. On the fifth day of treatment, the Fe³⁺ reductase activity in the Fe deficiency stress and 1/2Fe treatments decreased, but continued to increase in the 1Fe treatment. In a word, Fe deficiency stress or rich Fe nutrition could increase the Fe³⁺ reductase activity of roots in flowering Chinese Cabbage, especially the Fe³⁺ reductase activity in the Fe deficiency stress treatment was the highest.

Based on Fe deficiency stress, ACC and Co²⁺ treatments also had a great impact on the Fe³⁺ reductase activity in roots (Figure 3f). ACC treatment could rapidly increase the Fe³⁺ reductase activity in roots, and the line shows an "M" shape in the process of treatment. Co²⁺ treatment could rapidly decrease the Fe³⁺ reductase activity in roots, which decreased a small trough on the second day, then gradually increased, and reached a peak on the fifth day, and gradually decreased at last. In the process of treatment, Fe³⁺ reductase activity of roots in the ACC treatment was always the highest, while in the Co²⁺ treatment was always the lowest. Thus, the result indicated that ACC could induce the increase of Fe³⁺ reductase activity of roots in flowering Chinese cabbage, while Co²⁺ was the opposite.

In summary, the above results showed that Fe was related to the Fe³⁺ reductase activity of roots in flowering Chinese Cabbage. And Fe deficiency stress led to the improvement of the Fe³⁺ reductase activity in roots, ACC was further induced to increase the Fe³⁺ reductase activity of roots, while Co²⁺ was inhibited.

3.9. Effects of Fe deficiency stress and ACC and Co²⁺ on active Fe content

Fe deficiency stress, ACC, and Co²⁺ treatments had significant effects on the active Fe content (Table 4). The active Fe content in both roots and leaves increased significantly. Based on Fe deficiency stress with the increase of Fe concentration, ACC treatment could significantly increase the active Fe content in plants, while the Co²⁺ treatment reduced the active Fe content. These results indicated that Fe deficiency stress or Co²⁺ treatment inhibited the Fe³⁺ reductase activity, leading to the decrease of active Fe content, while ACC treatment increased the Fe³⁺ reductase activity, thus increasing the supply of available Fe of flowering Chinese cabbage.

Table 4 Effects of Fe deficiency stress and ACC and Co²⁺ treatment on active Fe content (mg·kg⁻¹ FW)

Treatment	Root	Leaf
0Fe	16.18±0.27 d	3.76±0.42 d
1/2Fe	35.77±0.51 b	8.35±0.38 b
1Fe	58.13±3.22 a	10.57±0.56 a
0Fe+ACC	22.78±1.75 c	7.42±0.73 c
0Fe+Co ²⁺	12.43±0.51 e	2.35±0.61 e

4. Discussion

4.1. Effect of Fe deficiency stress on the growth, yield, and quality

Fe deficiency stress was one of the most important abiotic stresses influences factors that cause a reduction in the yield and quality of fruits and vegetables (Abadia et al. 2011). The plant became yellow and growth was inhibited under the Fe deficiency

stress (Dey et al., 2020; Riaz et al. 2021), which caused a significant yield reduction in leaf vegetable, as well as affected quality, such as color, hardness, and acidity (Alvarez-Fernandez et al. 2003). Ding et al. (2007) found that Fe deficiency stress inhibited the growth of pakchoi and reduced the content of nutrient, such as soluble protein and Vc, leading to an undesirable quality. Fe acted as an activator of sucrose phosphate synthase and was involved in sucrose synthesis, thus the Fe deficiency also led to a reduction in sucrose content and inhibition protein synthesis (Yarnia et al. 2008). Our present results showed that Fe deficient plants with yellowing of heart leaves, dwarf, significantly decreased in plant height, stem thickness, aboveground weight, plant weight, leaf chlorophyll content, and decreased in the content of Vc, soluble protein, and soluble sugar in the leaves and stalks of the flower stalk, while the contents of cellulose and nitrate in the leaves and stalks of the flower stalk were significantly increased. Therefore, Fe deficiency stress inhibited the plant growth, which resulted in a significant decrease in the quality of flowering Chinese Cabbage.

4.2. Effect of Fe deficiency stress on nitrogen assimilation and photosynthesis

About 80% of the Fe in plants is found in photosynthetic cells. Fe deficiency negatively affected the constituent proteins of chloroplasts such as Cyt/b6f protein complex and ferredoxin, reducing photosynthetic pigment synthesis and chlorophyll content, making plant leaves chlorotic and reducing photosynthetic efficiency (Roncel et al. 2016). We found that Fe deficiency stress resulted in a significant decrease in chlorophyll content and an obvious impact on photosynthetic performance, with a significant decrease in photosynthetic rate and stomatal conductance, and an increase in intercellular CO₂ concentration in flowering Chinese cabbage. Additionally, Fe is also an important component of NR and nitrite reductase. NR is a core enzyme in plant nitrogen metabolism regulated by NO³⁻, has a great impact on plant nitrogen assimilation and utilization (Faure et al. 1991). Kaya et al. (2020) suggested that Fe deficiency caused an increase in NR activity, but Zou et al. (1998) insisted that NR activity was reduced in maize when it was deficient. Our present results indicated that Fe was closely related to the nitrogen metabolism of flowering Chinese Cabbage, and Fe deficiency stress caused a decrease in NR activity, resulted in weakened nitrogen metabolism of leaves, caused a large accumulation of nitrate in leaves, and affected the availability and utilization of nitrogen nutrients, which in turn inhibited plant growth. Our present results agreed with the findings of the peanut investigated by Zou et al (1998) and Song et al. (2016). It could be seen that Fe deficiency stress had different effects on nitrogen assimilation in different plants.

4.3. Effect of Fe deficiency stress on the antioxidant

When plants faced with stress, reactive oxygen metabolism is dysregulated and the free radical balance is disrupted, resulting in the accumulation of reactive oxygen species, which triggers and exacerbates membrane lipid peroxidation and may lead to the destruction of cell membrane integrity (Shabala et al. 2014). The plant could balance reactive oxygen metabolism by increasing the activity of antioxidant system. Antioxidant enzymes such as POD, CAT, and SOD, which played a role in scavenging free radicals and preventing free radical formation, they were contributing to reducing peroxidative damage, stabilizing membrane structure and its function, and to improving cellular resilience (Yang et al. 2004). Additionally, Fe was a component of SOD, POD, and CAT, that affects their biological activities. In the Fe deficient plants, the SOD activity slightly increased in leaves and roots, but POD and CAT activities in roots decreased by 52.0% and 34.5%, respectively, as compared to the control, MDA content increased by 94.9% and 99.0% in leaves and roots as compared to the control (Song et al. 2016). Dey et al. (2020) pointed out that Fe deficiency caused an increase in SOD activity and MDA content, while CAT activity was decreased in black gram. However, Jia et al. (2018) concluded that SOD, POD, and CAT activities were reduced in both roots and stems under low Fe deficiency stress in three apple stocks. Thus, the antioxidant enzyme activities of different plants differed in response to Fe deficiency. Our present research showed that Fe deficiency stress led to disruption of the reactive oxygen metabolic system in flowering Chinese cabbage, causing a significant increase in POD activity of roots, but a significant decrease in SOD and CAT activity of the roots and leaves, and POD activity in the leaves, and resulting in a large accumulation of MDA in plant. These findings indicated that Fe deficiency stress caused a decrease in the ability to scavenge reactive oxygen species and led to an increase of membrane lipid peroxidation, thus affected the normal growth of flowering Chinese cabbage.

4.4. The role of ethylene in the adaptive response to Fe deficiency

Fe deficiency stress in plants will cause a series of physiological adaptive responses, such as morphological structure alteration of the root system, causing inter-root acidification and increasing capacity of Fe reduction. Under Fe deficiency stress, the increased acidification capacity of the root system and the increased Fe^{3+} reductase activity of roots are important mechanisms for plants to improve Fe uptake in soil (Schmidt 2003). The dicotyledonous plants, cucumber, tomato, and soybean, their adaptive mechanisms induced by Fe deficiency all belong to the strategy I, but their specific responses to adapt to Fe deficiency stress were different. In cucumber and tomato, Fe deficiency was mainly showed as enlargement and thickening near the root tip, root hairs increased and developed the features of transfer cells, and automatically secreted large amounts of H^+ , which resulted in a significant increase of Fe^{3+} reduction ability in the root systems, and increased the effectiveness of Fe in roots (Yi et al. 1998). Our present research also confirmed that Fe deficiency caused acidification of the inter-root medium, meanwhile, increased the Fe^{3+} reductase activity of the root system in flowering Chinese cabbage.

Ethylene is one of the main plant hormones, which plays an important role in regulating plant metabolism, such as inhibiting growth, inducing fruit ripening, accelerating the abscission of leaves, flowers, and fruits, and so on. The relationship between ethylene and Fe uptake of the plant is mainly manifested in two aspects, one is to influence the redistribution of Fe by participating in the metabolism; the other is to act as a signalling substance to regulate the adaptive response to Fe deficiency. Plant with Fe deficiency induced the accumulation of ethylene in roots (Sabrina et al. 2009). We found that ethylene was involved in regulating the Fe deficiency response of flowering Chinese cabbage, and Fe deficiency stress caused a significant increase of ethylene release in root. These results were in accordance with the findings of the investigation carried out by Romera et al. (2004). However, to study if the release of ethylene is an adaptive response to Fe deficiency stress, researchers have been gradually carried out on the ethylene biosynthesis precursor ACC and the ACC synthase inhibitor Co^{2+} . Waters et al. (2007) showed that the addition of ACC after 1 day of Fe deficiency treatment significantly enhanced the Fe^{3+} reduction ability, whereas the addition of Co^{2+} significantly reduced the Fe^{3+} reduction ability, which indicated that ethylene was involved in the regulation of the adaptive response to Fe-deficiency. Our present study also found that ethylene was involved in regulating the adaptive response to Fe deficiency in flowering Chinese cabbage. ACC treatment promoted the ethylene synthesis in roots, reduced the pH of the root medium, enhanced Fe^{3+} reductase activity in roots as well as increased the active Fe content in roots and leaves under the Fe deficiency stress, while the Co^{2+} treatment did the opposite results, which indicated that the inter-root environmental conditions were improved and the effectiveness of root Fe nutrition by ethylene induction in flowering Chinese cabbage. However, there are different views on the role of ethylene in Fe deficiency stress. The role of Co^{2+} in the adaptive response to Fe deficiency depended on the concentration of Fe and the genotype of plant. Fe^{3+} reduction ability of Fe efficient mutant of pea under Fe deficiency conditions decreased significantly when the concentration of Co^{2+} was $100 \mu\text{mol}\cdot\text{L}^{-1}$, while the Fe^{3+} reduction ability in roots of parental increased gradually, and there was no obvious change under the concentration of Co^{2+} was $1 \mu\text{mol}\cdot\text{L}^{-1}$ or $10 \mu\text{mol}\cdot\text{L}^{-1}$ (Cui et al. 1999). There was no regular change of Fe^{3+} reduction ability in roots when using ethephon, and the results of direct ethylene collection assay also showed no direct correlation between root Fe^{3+} reduction ability and ethylene release rate (Romheld et al. 1986). The reduction of Fe could stimulate the synthesis of ethylene, but there was no correlation between this stimulation and the Fe status of the cell. The Co^{2+} reduced the synthesis of ethylene but did not affect the Fe reduction (Malerba et al. 1996). Consequently, the physiological adaptations in response to Fe deficiency are different in different crops, and more in-depth studies on the relationship between ethylene and Fe reduction are needed.

5. Conclusion

Our present study showed that Fe deficiency stress treatment induced a series of physiological adaptive responses, caused a decrease in nitrogen metabolism, photosynthesis, and pH value of root media, promoted the release of ethylene and the increase in Fe^{3+} reductase activity, and improved the effective supply of Fe nutrients to plant, but disrupted the balance of the reactive oxygen metabolism system, led to a decrease of CAT in roots and leaves, SOD and POD activities in leaves, and membrane lipid peroxidation product MDA accumulated in large quantities, all the changes led to poor plant growth and decreased yield and quality. ACC treatment significantly improved the pH environmental of roots, promoted the release of ethylene, increased the Fe^{3+}

reductase activity and effective Fe supply, and increased the adaptation to Fe deficiency stress, while Co²⁺ treatment inhibited the release of ethylene, decreased the Fe³⁺ reductase activity, and led to a decrease in the active Fe content of the plant, which indicated the ethylene was involved in regulating the adaptive response to Fe deficiency stress in flowering Chinese cabbage.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by Guangdong Provincial Special Fund for Modern Agriculture Industry Technology Innovation Teams [No. 2021KJ122]; Natural Science Foundation of Guangdong Province [No.2018A0303130316]; Key-Area Research and Development Program of Guangdong Province [No. 2018B020202010].

Authors' Contributions

XY and XX designed the research; YP and YY performed the experiments and wrote the paper; LZ and XR analyzed the data; MZ reviewed the manuscript.

Acknowledgements

Not applicable.

References

- Abadía J, Vázquez S, Rellán-Álvarez R, El-Jendoubi H, Abadía A, Álvarez-Fernández A, López-Millán AF (2011) Towards a knowledge-based correction of iron chlorosis. *Plant Physiology and Biochemistry*. 49, 471-482. <https://doi.org/10.1016/j.plaphy.2011.01.026>.
- Álvarez-Fernández A, Paniagua P, Abadía J, Abadía A (2003) Effects of Fe deficiency chlorosis on yield and fruit quality in peach (*Prunus persica* L. Batsch). *Journal of Agricultural and food chemistry*. 51, 5738-5744. <https://doi.org/10.1021/jf034402c>.
- Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *beta vulgaris*. *Plant Physiology*. 24, 1-15. <https://doi.org/10.1104/pp.24.1.1>.
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72, 248-254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3).
- Chai XR, Kang YY, Li XX, Yang X, Zhang XL (2013) Effect of foliar application of sucrose on yield and soluble sugar content of flowering Chinese cabbage. *China Vegetables*. 20, 61-66. <https://doi.org/10.3969/j.issn.1000-6346.2013.20.009>.

- Connorton JM, Balk J, Rodriguez-Celma J (2017) Iron homeostasis in plants - a brief overview. *Metallomics*. 9, 813-823. <https://doi.org/10.1039/c7mt00136c>.
- Cui XY, Cao YP, Zhang FS (1999) Effect of cobalt on root responses to iron deficiency in peas (*Pisum sativum* L.). *Scientia Agricultura Sinica*. 32, 105-107. <https://doi.org/10.3321/j.issn:0578-1752.1999.02.019>.
- Dey S, Chowardhara B, Regon P, Kar S, Saha B, Panda S.K (2020) Iron deficiency in blackgram (*Vigna mungo* L.): redox status and antioxidant activity. *Plant Biosystems*. 1-16. <https://doi.org/10.1080/11263504.2020.1866093>.
- Ding F, Wang XF, Shi QH, Wang EL, Feng JF (2007) Effects of iron deficiency on photosynthetic characteristics and quality of Chinese cabbage (*Brassica Chinensis* L.). *Shandong Agricultural Sciences*. 6, 51-53. <https://doi.org/10.3969/j.issn.1001-4942.2007.06.016>.
- Faure J, Vincentz M, Kronenberger J, Caboche M (1991) Co-regulated expression of nitrate and nitrite reductases. *The Plant Journal*. 1, 107-113. <https://doi.org/10.1111/j.1365-313X.1991.00107.x>.
- Gao JF (2006) Instruction for plant physiology experiments. Beijing: Higher Education Press.
- Gogorcena Y, Abadía J, Abadía A (2000) Induction of *in vivo* root ferric chelate reductase activity in fruit tree rootstock. *Journal of Plant Nutrition*. 23, 9-21. <https://doi.org/10.1080/01904160009381993>.
- Jia XM, Zhu YF, Hu Y, Cheng L, Zhao T, Wang YX (2018) Tolerance to iron-deficiency stress of three apple rootstock species in hydroponic system. *Agricultural Science & Technology*, 19, 21-30. <https://doi.org/10.16175/j.cnki.1009-4229.2018.03.003>.
- Kang YY, Liu B, Yang X, Chai XX (2014) Effects of glucohexaose on sheath blight, pathogenesis-related proteins and endogenous hormones in flowering Chinese cabbage. *Acta Horticulturae Sinica*, 41, 1125-1132.
- Kaya C, Ashraf M, Alyemini MN, Ahmad P (2020) Nitrate reductase rather than nitric oxide synthase activity is involved in 24-epibrassinolide-induced nitric oxide synthesis to improve tolerance to iron deficiency in strawberry (*Fragaria × annassa*) by up-regulating the ascorbate-glutathione cycle. *Plant Physiology and Biochemistry*. 151, 486-499. <https://doi.org/10.1016/j.plaphy.2020.04.002>.
- Lastra OC (2003) Derivative spectrophotometric determination of nitrate in plant tissue. *Journal of AOAC International*. 86, 1101-1105. <https://doi.org/10.1093/jaoac/86.6.1101>.
- Malerba M, Bianchetti R (1996) Trans-plasma membrane reduction of Fe³⁺-EDTA or hexachloroiridate IV by cultured cells of *acer pseudoplatanus* L. induces an ethylene response. *Journal of Plant Physiology*. 149, 753-756. [https://doi.org/10.1016/S0176-1617\(96\)80102-7](https://doi.org/10.1016/S0176-1617(96)80102-7).
- Mori S (1999) Iron acquisition by plants. *Current Opinion in Plant Biology*. 2, 250-253. [https://doi.org/10.1016/S1369-5266\(99\)80043-0](https://doi.org/10.1016/S1369-5266(99)80043-0).
- Pierson EE, Clark RB (1984) Ferrous iron determination in plant - tissue. *Journal of Plant Nutrition*. 7, 107-116. <https://doi.org/10.1080/01904168409363178>.
- Riaz N, Guerinot ML (2021) All together now: regulation of the iron deficiency response. *Journal of Experimental Botany*. 72, 2045-2055. <https://doi.org/10.1093/JXB/ERAB003>.
- Romera FJ, Alcantara E, (2004) Ethylene involvement in the regulation of Fe-deficiency stress responses by strategy I plants. *Functional Plant Biology*. 31, 315-328. <https://doi.org/10.1071/FP03165>.
- Romera FJ, Alcantara E, De La Guardia MD (1999) Ethylene production by Fe-deficient roots and its involvement in the regulation of Fe-deficiency stress responses by strategy I plants. *Annals of Botany*. 83, 51-55. <https://doi.org/10.1006/anbo.1998.0793>.

- Romheld V, Marschner H (1986) Evidence for a specific uptake system for iron phytosiderophores in roots of grasses. *Plant Physiology*. 80, 175-180. <https://doi.org/10.1104/pp.80.1.175>.
- Roncel M, González-Rodríguez AA, Naranjo B, Bernal-Bayard P, Lindahl AM, Hervás M, Navarro JA, Ortega JM (2016) Iron deficiency induces a partial inhibition of the photosynthetic electron transport and a high sensitivity to light in the diatom *Phaeodactylum tricornutum*. *Frontiers in Plant Science*. 7, 1-14. <https://doi.org/10.3389/fpls.2016.01050>.
- Sabrina Z, Stefano C, Zeno V, Roberto P, Stefania A (2009) Sulphur deprivation limits Fe-deficiency responses in tomato plants. *Planta*. 230, 85-94. <https://doi.org/10.1007/s00425-009-0919-1>.
- Schmidt W (2003) Iron solutions: acquisition strategies and signaling pathways in plants. *Trends in Plant Science*. 8, 188-193. [https://doi.org/10.1016/S1360-1385\(03\)00048-7](https://doi.org/10.1016/S1360-1385(03)00048-7).
- Shabala S, Shabala L, Barcelo J, Poschenrieder C (2014) Membrane transporters mediating root signalling and adaptive responses to oxygen deprivation and soil flooding. *Plant Cell and Environment*. 37, 2216-2233. <https://doi.org/10.1111/pce.12339>.
- Song YL, Dong YJ, Tian XY, Kong J, Bai XY, Xu LL, He ZL (2016) Role of foliar application of 24-epibrassinolide in response of peanut seedlings to iron deficiency. *Biologia Plantarum*. 60, 329-342. <https://doi.org/10.1007/s10535-016-0596-4>.
- Wang XY, Han SL, Guo SH (2010) The measurement system optimization study on the nitrate reductase activity of apple leaf. *Northern Horticulture*, 6, 52-55.
- Waters BM, Lucena C, Romera F J, Jester GG, Wynn A N, Roja CL, Alcántara E, Pérez-Vicentec R (2007) Ethylene involvement in the regulation of the H⁺-ATPase CsHA1 gene and of the new isolated ferric reductase CsFRO1 and iron transporter CsIRT1 genes in cucumber plants. *Plant Physiology and Biochemistry*. 45, 293-301. <https://doi.org/10.1016/j.plaphy.2007.03.011>.
- Yang X, Cheng XY, Feng HX (2004) The effects of nitrogen nutrition and inoculation with *Colletotrichum higginsianum* on anthracnose resistance physiology in flowering Chinese cabbage. The effects of nitrogen nutrition on anthracnose and cell protective enzymes in flowering Chinese cabbage. *Journal of South China Agricultural University (Natural Science Edition)*. 25, 26-30. <https://doi.org/10.3969/j.issn.1001-411X.2004.02.007>.
- Yang X, Cheng XY, Liu ZC (2000) Effects of boron and molybdenum nutritions on curd yield and active oxygen metabolism in Broccoli (*Brassica oleracea* var. *italica*). *Acta horticulturae sinica*. 27, 112-116. <https://doi.org/10.3321/j.issn:0513-353X.2000.02.007>.
- Yang X, Gu MM, Kang YY, Feng XF (2012) Contribution of N : P ratio and endogenous phytohormones during development of phosphorus toxicity in *Brassica campestris* spp. *parachinensis*. *Journal of Plant Nutrition and Soil Science*. 175, 582-594. <https://doi.org/10.1002/jpln.201100237>.
- Yarnia M, Benam M, Arbat HK, Tabrizi E, Hassanpanah D (2008) Effects of complete micronutrients and their application method on root yield and sugar content of sugar beet cv. Rassoul. *Journal of Food Agriculture & Environment*. 6, 341-345.
- Yi CL, Wang H, Zhang FS, Li CJ (1998) The difference of iron deficiency induced adaptable reaction among cucumber, tomato and soybean. *Chinese Journal of Plant Ecology*. 22, 80-86.
- Zou CQ, Zhang FS, Mao DR (1998) Effect of iron supply on nitrogen metabolism of corn. *Journal of China agricultural university*. 3, 45-49.

Figures

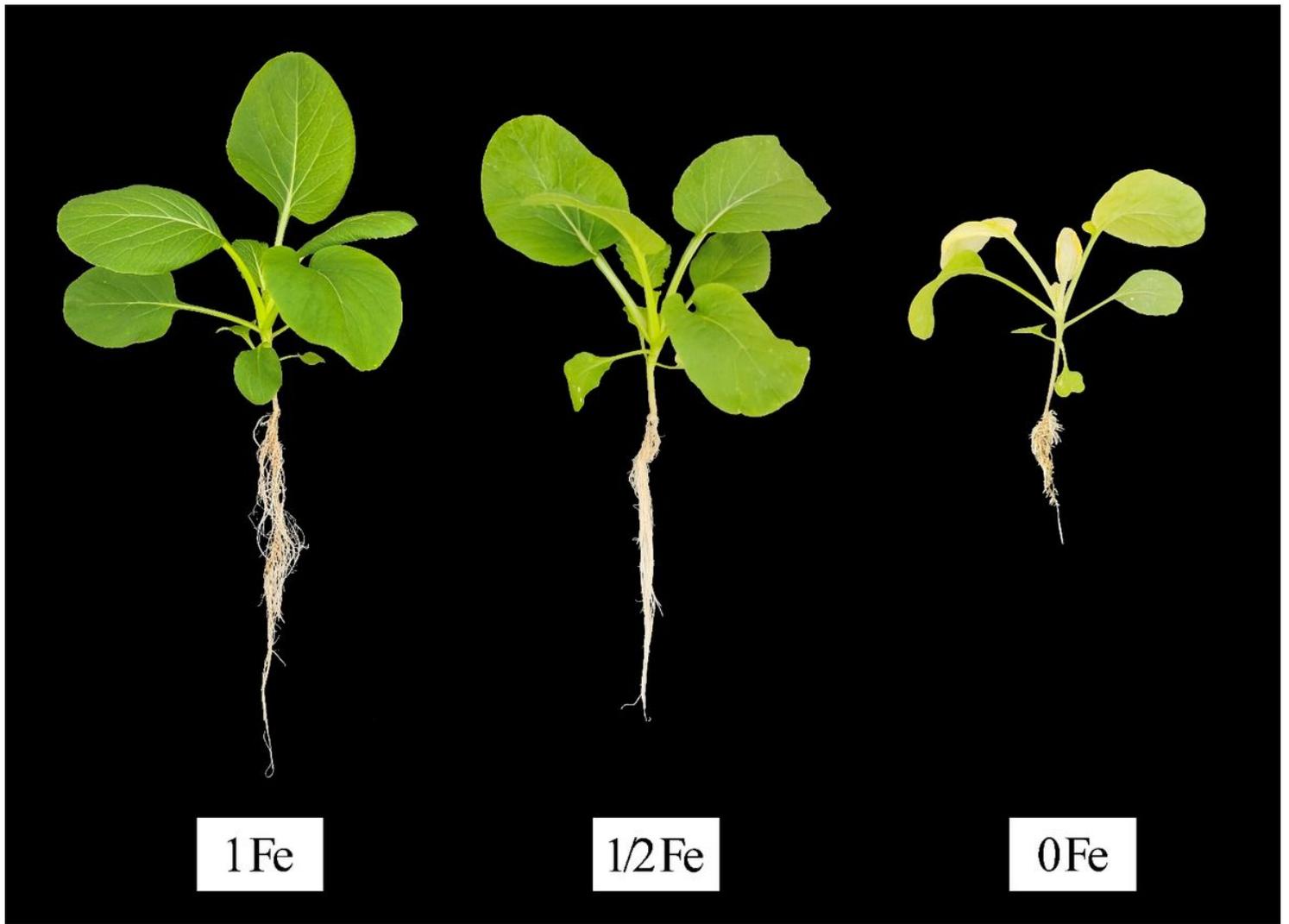


Figure 1

The plant phenotype of different Fe concentration treatments for 9 days

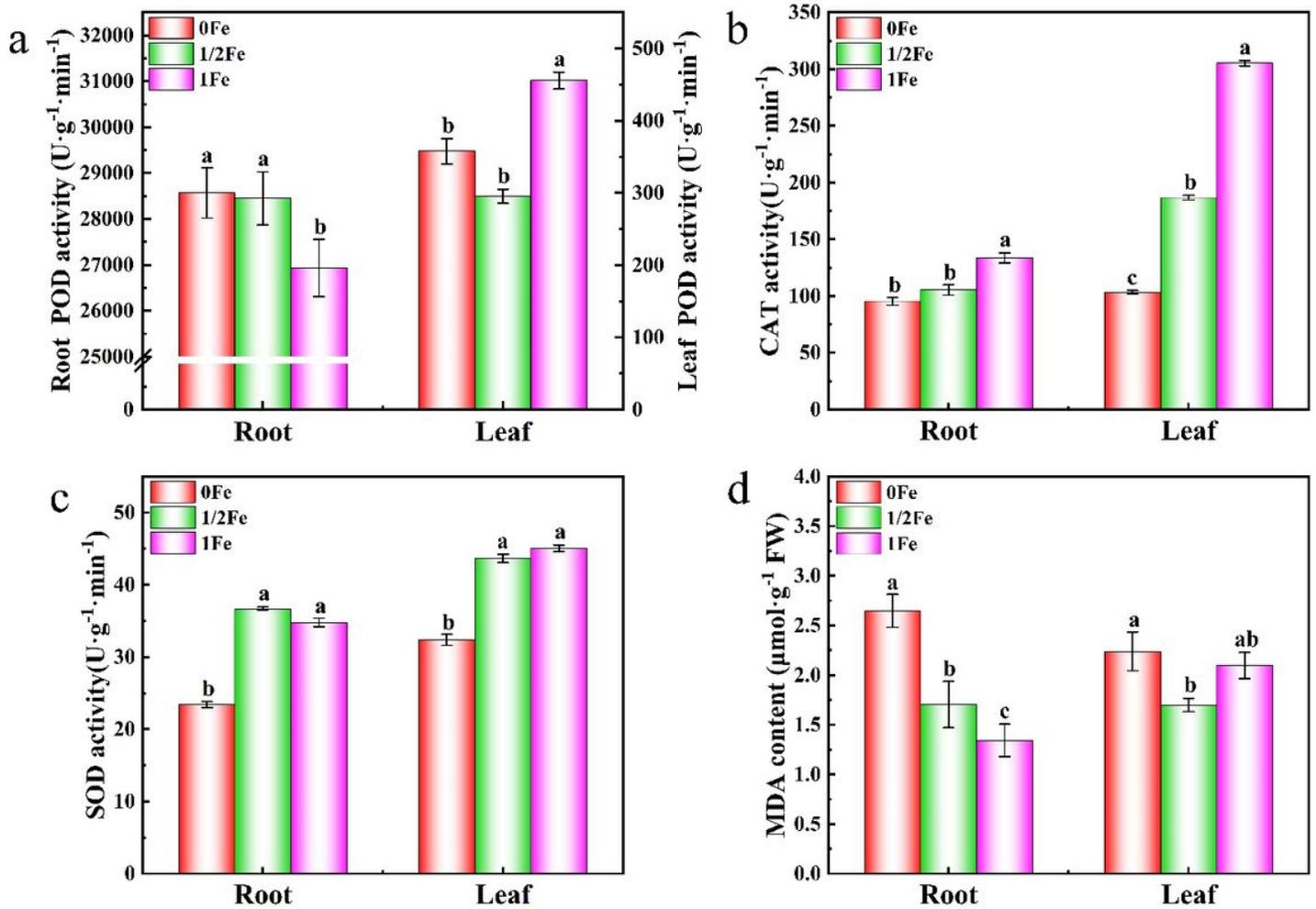


Figure 2

The activities of POD(a), CAT(b), SOD(c) and content of MDA(d) in flowering Chinese cabbage treated with Fe deficiency stress. Data are mean ± SE (n=3). Different letters above the columns show significant differences (p<0.05) between treatments

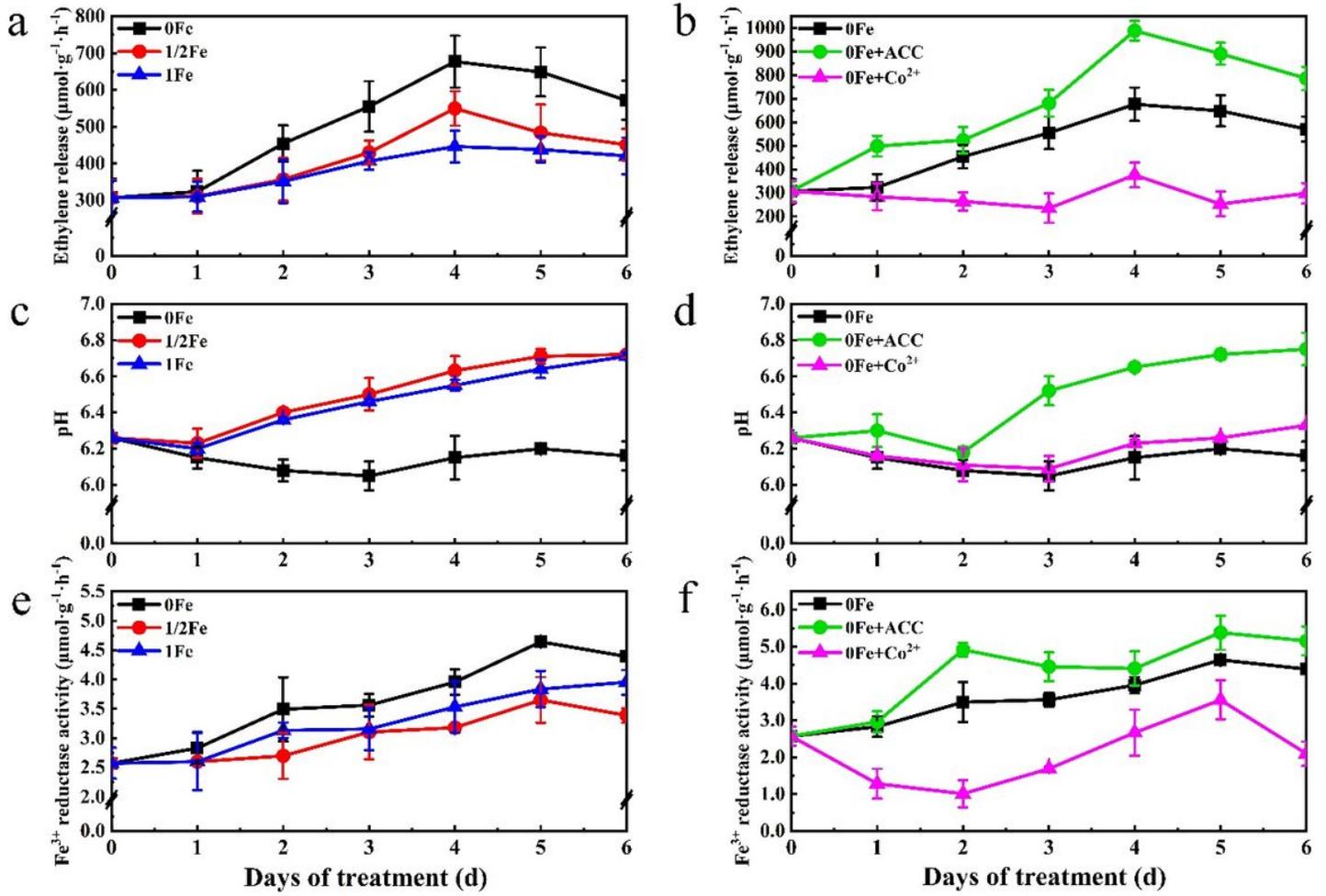


Figure 3

Effects of ACC and Co²⁺ treatments and Fe deficiency stress on the ethylene release (a, b), pH value (c, d) and Fe³⁺ reductase activity (e, f) in roots of flowering Chinese cabbage. data are mean ± SE (n=3)