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

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Abstract: Iron (Fe) plays an important role in the growth and development of 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 contents of vitamin C, 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. Fe deficiency stress clearly reduced the net photosynthetic rate and nitrate reductase activity in the leaves. The balance system of active oxygen metabolism was destroyed due to Fe deficiency, resulting in the decrease in catalase activity, superoxide dismutase activity of roots and leaves, and peroxidase (POD) activity of leaves, while POD activity in roots and malonaldehyde content in roots and leaves were significantly increased. The treatments of Fe deficiency and ACC significantly reduced the pH value of the root medium, promoted the release of ethylene, and increased Fe^{3+} reductase activity, while Co^{2+} treatment showed results that were the opposite to those of Fe deficiency and ACC treatments. Thus, Fe deficiency stress affected nitrogen metabolism, photosynthesis, reactive oxygen metabolism, pH of root medium, and Fe^{3+} reductase activity, which was related to physiological adaptive response and tolerance mechanisms. We also found that ethylene could be involved in regulating the adaptive response to Fe deficiency stress in flowering Chinese cabbage.

Keywords: flowering Chinese cabbage; Fe deficiency; adaptive response

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1. Introduction

Iron (Fe) is an essential mineral nutrient element for plant growth and development and an essential element for all living organisms as it participates in a wide variety of metabolic processes [1]. Although Fe is a relatively abundant element in soil, the contents of soluble Fe are remarkably poor, and thus a poor solubility impairs its effectiveness [2]. Indeed, one-third of cultivated areas in the world are made of calcareous soils, which are the primary cause of Fe deficiency, and Fe deficiency occurs in about 40% of soil [3]. Fe deficiency induces various metabolic disorders, resulting in abnormalities of chloroplast morphology and structures, reduced chlorophyll contents and photosynthetic rate, and the diminished respiratory ability of plants, which severely reduces plant yield and quality [4,5]. Thus, Fe deficiency is a common problem affecting the yield of crops.

The adaptive mechanisms of higher plant activation of Fe are divided into strategy I (reduction strategy) and strategy II (chelation strategy) [6]. For example, cucumbers, tomatoes, and soybeans are dicotyledons; their adaptive mechanisms induced by Fe deficiency all belong 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 and increased root hairs, which are characteristics of metastatic cells, actively excreting large amounts of H^+ that significantly enhance the Fe^{3+} reduction ability of roots, which improves the availability of Fe in the rhizosphere. Soybean reduces 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 [7]. Therefore, different plants show different response mechanisms to Fe deficiency stress. In addition, Fe deficiency can promote ethylene biosynthesis and signal transduction, while ethylene can enhance Fe transport and distribution in rice [8,9]. However, the response of ethylene to Fe deficiency in Chinese cabbage has not been explored.

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, the stalk is tender, easy to cook, and has a pleasant taste [10]. This crop is one of the annually produced vegetables with the largest cultivation scale and consumption in South China [10]. The lack of Fe nutrition gradually becomes the key factor limiting the yield of flowering Chinese cabbage due to the continuous increase in multiple cropping index and the increasing degree of soil alkalization. Our previous study indicated that mineral nutrition had clear effects on the growth, yield, quality, and disease resistance of flowering Chinese cabbage [11–14]. However, the effects of Fe deficiency on the growth and development of flowering Chinese cabbage and its related physiological characteristics are still lacking an investigation. In this study, we aim to investigate the effects of Fe deficiency stress on the growth, yield, and quality of flowering Chinese cabbage, and to investigate its adaptive physiological mechanism to Fe deficiency stress through different Fe concentrations, a precursor of ethylene biosynthesis (1-aminocyclopropane-1-carboxylic acid (ACC)), and an inhibitor of ACC synthase (Co^{2+}) treatments in flowering Chinese cabbage.

2. Materials and Methods

2.1. Materials and Reagents

This experiment was carried out in greenhouse at South China Agricultural University, Guangzhou, China, with flowering Chinese cabbage '60-day' as the experimental cultivar. The seeds were sown in a nutrient bowl containing perlite on 13 April 2020, and 1/4 Hoagland nutrient solution was sprayed every 3 days at the seedling stage. The seeds were transplanted in a plastic box (61 cm × 42 cm × 15 cm) with 20 L Hoagland nutrient solution for hydroponic cultivation on 2 May, while the seedlings had 3–4 true leaves. Every 14 seedlings with a plant spacing of 12.5 × 11 cm were placed 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 contained three biological replicates. Each plastic box was put on one oxygen pump for ventilation (ventilation: 15 min per hour). The nutrient solutions were renewed once a week.

2.2. Determination of Growth and Quality Index

The plants were collected on 25 May 2020. The plant height, stem diameter, fresh weight of roots, shoot fresh weight, and plant fresh weight, as well as the quality of flower stalks of each 10 plants, were measured as a treatment group. The flower stalk was the part from the third node to the growing point 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. Vitamin C (Vc) content was measured by molybdenum blue colorimetric method [15]. Soluble sugar content and soluble protein content were determined by following the methods of Chai et al. [11] and Tan et al. [15], respectively. Nitrate content was determined using the derivate spectrophotometric method [16], while cellulose content was determined by the anthrone colorimetric method [17]. Active Fe content was measured using the method of Wang et al. [18].

2.3. Determination of Photosynthetic Characteristics

The 10 plants from each treatment were randomly selected to determine photosynthetic characteristics. Three replicates were performed for each measurement. The chlorophyll content was determined by the method of Zhao et al. [19]. The photosynthetic rate, transpiration rate, intercellular CO_2 concentration, and stomatal conductance were performed on a portable automatic photosynthesizer (LI-6400, LI-COR, Lincoln, NE, USA).

2.4. Analysis of Nitrate Reductase Activity

The 10 plants from each treatment were randomly selected to determine nitrate reductase (NR) activity. Three replicates were performed for each measurement. 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 $\mu g \cdot NO^{-2} \cdot h^{-1} \cdot g^{-1}$ FW [20].

2.5. Determination of Reactive Oxygen Metabolism

The 10 plants from each treatment were randomly selected to determine the reactive oxygen metabolism. Three replicates were performed for each measurement. The reactive oxygen metabolism was determined using our previously reported method [12]. In brief, each 0.5 g sample was precooled in a mortar, and 5 mL of 0.05 $mol \cdot L^{-1}$ pH 7.0 phosphate-buffered solution and 0.3 g polyvinylpyrrolidone were added and ground, then centrifugated at 4 °C with 3000 $r \cdot min^{-1}$ rates for 10 min. The supernatant was collected for the determination of the POD (EC 1.11.1.7), catalase (CAT) (EC 1.11.1.21) and superoxide dismutase (SOD) (EC 1.15.1.1) activities and malonaldehyde (MDA) content. All spectrophotometric analyses were conducted using a SHIMADZU UV-2410PC spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

2.6. Determination of Ethylene Release from Roots

The 5 plants from each treatment were randomly selected to determine the ethylene release. Three replicates were performed for each measurement. The content of ethylene release from roots was measured for 6 consecutive days using the modified method [21]. Briefly, each of the five roots (0.5 g) and 200 µL water were added in a 25 mL tube, sealed and kept in dark conditions for 2 h, then 1 mL gas from the tube, for the determination of ethylene release, was extracted using a Shimazu 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 6.26 and treated with different Fe concentrations, ACC and Co^{2+}; then, the pH of the root medium was measured for 6 consecutive days (at 9 a.m. each day) using a Leici PHS-3B precision pH meter (Shanghai Jinke Leici, Shanghai, China). Three replicates were performed for each measurement. Each replicate contained 14 plants.

2.8. Determination of the Fe^{3+} Reductase Activity of Roots

The Fe^{3+} reductase (EC 1.16.1.7) activity was evaluated for 6 consecutive days using a 2,2-bipyridine method [22]. In brief, the samples were put in the saturated $CaSO_4$ solution and soaked for 5 min, washed using deionized water, then placed in a nutrient solution containing 0.1 mM Fe (III)-EDTA and 0.4 mM 2,2′-bipyridine with Fe (II). After a 2 h reaction under continuous ventilation with light illumination, the roots were weighed, and the absorbance of the reactive liquid at 520 nm was measured. All spectrophotometric analyses were conducted in a SHIMADZU UV-2410PC spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The 5 plants from each treatment were randomly selected to determine the Fe^{3+} reductase activity of roots. Three replicates were performed for each measurement.

2.9. Data Processing and Analysis

Data from experiments with three biological replicates for each experiment 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

Fe deficiency stress clearly inhibited plant growth (Table 1 and Figure 1). The results showed that the 1Fe treatment significantly increased plant fresh weight, shoot fresh weight, root fresh weight, root shoot ratio, plant height, and stem diameter compared with the 0Fe treatment. The 1/2Fe treatment displayed the plant fresh weight, shoot fresh weight, plant height, and stem diameter significantly more clearly than those of the 0Fe treatment, whereas there was no significant difference in the root fresh weight and root shoot ratio between the 1/2Fe treatment and 0Fe treatment. Compared with the 1Fe treatment, the 1/2Fe treatment significantly decreased the root fresh weight and root shoot ratio, while the 1Fe treatment displayed the plant fresh weight, shoot fresh weight, plant height, and stem diameter slightly more clearly than those of the 1/2Fe treatment, which displayed no significant difference. Thus, these results indicated that Fe deficiency stress could significantly inhibit the plant growth, which resulted in a significant decrease in the plant fresh weight of flowering Chinese cabbage.

Table 1. Effects of Fe deficiency stress on the plant growth of flowering Chinese cabbage.

Treatment	Plant Fresh Weight (g Plant^{-1})	Shoot 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 ^{a}	17.33 ± 3.22 ^{a}	8.91 ± 0.74 ^{a}
1/2 Fe	18.47 ± 0.81 ^{a}	16.92 ± 0.58 ^{a}	1.53 ± 0.20 ^{b}	0.11 ± 0.01 ^{b}	17.00 ± 2.65 ^{a}	8.34 ± 0.37 ^{a}
0 Fe	13.51 ± 2.30 ^{b}	11.24 ± 1.45 ^{b}	1.43 ± 0.12 ^{b}	0.10 ± 0.02 ^{b}	13.63 ± 1.89 ^{b}	6.88 ± 0.91 ^{b}

Note: Values represent the mean ± standard error (n = 3), different letters indicate significant differences among different treatments at a significance level of 0.05 using Duncan's analysis.

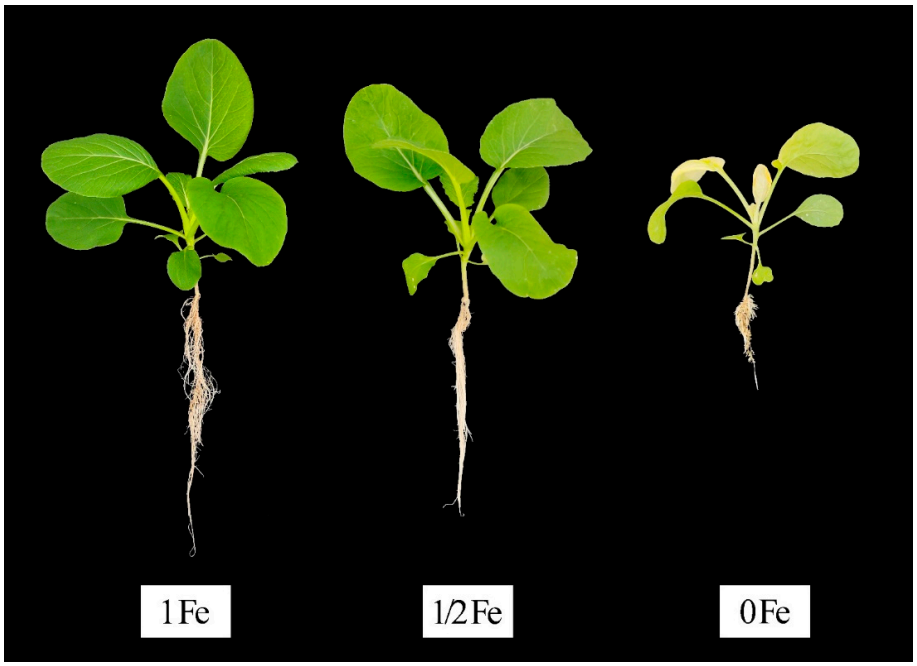

Figure 1. The plant phenotype of different Fe concentration treatments for 9 days. The 1Fe treatment displayed the best plant parameters such as aboveground, root, plant height, and stem thickness; The 1/2Fe treatment showed better plant parameters such as aboveground, plant height and stem thickness; the 0Fe treatment had poor plant growth, dwarfism, and serious yellowing.

3.2. Effects of Fe Deficiency Stress on the Quality of Flower Stalk

Fe deficiency stress clearly reduced the contents of Vc, soluble protein, and soluble sugar, while increased the content of cellulose, and promoted the accumulation of nitrate in leaves and the flower stalks.

Compared with the 1Fe treatment, the Vc content in leaves of the flower stalk in the Fe deficiency stress and the 1/2Fe treatments decreased by 22% and 14%, respectively; the soluble protein content in leaves of the flower stalk in the Fe deficiency stress and the 1/2Fe treatments decreased by 15% and 3%, respectively; the nitrate content in the leaves of the flower stalks during Fe deficiency stress and the 1/2Fe treatments increased by 49% and 17%, respectively; the cellulose content in leaves of the flower stalk in the Fe deficiency stress and the 1/2Fe treatments increased by 24% and 2%, respectively. The contents of nitrate and cellulose of flower stalks 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 the flower stalks. Compared with the 1Fe treatment, the Vc content in the flower stalks under Fe deficiency stress and the 1/2Fe treatments decreased by 56% and 30%, respectively; the soluble protein content in the flower stalks under Fe deficiency stress and the 1/2Fe treatments decreased by 11% and 5%, respectively; the nitrate content in stalks of the flower stalk in the Fe deficiency stress and the $\frac{1}{2}$Fe treatments increased by 43% and 11%, respectively; and the cellulose content in stalks of the flower stalks under Fe deficiency stress and the 1/2Fe treatments increased by 14% and 11%, respectively. There was no significant difference in the contents of soluble protein, soluble sugar, and Vc in leaves and the flower stalks between the 1/2Fe and 1Fe treatments. Thus, it could be seen that Fe deficiency stress affected the formation of the quality of the flower stalk, leading to a decline in 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 of 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 of 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}

Note: Values represent the mean ± standard error (n = 3), different letters indicate significant differences among different treatments at a significance level of 0.05 using Duncan's analysis.

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 the 1/2Fe and the 1Fe treatments. Compared with the 1/2Fe and 1Fe treatments, the chlorophyll content in the Fe deficiency treatment was significantly decreased by 21% and 20%, respectively, while there was no significant difference between the 1/2Fe and 1Fe treatments.

Fe deficiency did not affect the transpiration rate of leaves but had significant effects on stomatal conductance, photosynthetic rate, and intercellular CO_2 concentration (Table 3). Fe deficiency stress significantly reduced the net photosynthetic rate and stomatal conductance but significantly increased the intercellular CO_2 concentration in leaves. The net photosynthetic rate and stomatal conductance in the 1/2Fe treatment were not significantly different except for the intercellular CO_2 concentration, which 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 of the plant, which resulted in a decrease in the photosynthetic rate in flowering Chinese cabbage.

Table 3. The 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 [b]
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 [c]

Note: Values represent the mean ± standard error (n = 3); different letters indicate significant differences among different treatments at a significance level of 0.05 using Duncan's analysis.

3.4. Effects of Fe Deficiency Stress on the NR Activity

The NR activity of leaves significantly decreased. Compared with the 1Fe treatment, the NR activity of leaves in the Fe deficiency stress and the 1/2Fe treatments decreased by 36% and 24%, respectively (Table 3). These results suggested that Fe deficiency stress caused a decrease in NR activity in the leaves, which resulted in a decrease in nitrogen metabolism and the accumulation of nitrate in the leaves, inhibiting the normal plant growth of flowering Chinese cabbage.

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 had different trends under the different Fe concentrations. Compared with 1Fe treatment, the POD activity of roots in the Fe deficiency stress and the 1/2Fe treatments significantly increased by 6% and 5%, respectively, but there was no significant difference between the Fe deficiency stress and the 1/2Fe treatment. Compared with the 1Fe treatment, the POD activity of leaves in Fe deficiency stress and 1/2Fe significantly decreased by 21% and 35%, 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 the leaves. The increase in 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 the damage of cell membrane system caused by the reactive oxygen, preventing further damage to the plant.

Compared with the 1Fe treatment, the CAT activity of roots in the Fe deficiency and the 1/2Fe treatments was decreased by 28% and 21%, respectively, and the CAT activity of leaves decreased by 66% and 38%, respectively (Figure 2b). In the 1Fe and the 1/2Fe treatments, SOD activity in roots or leaves was significantly higher than that in the Fe deficiency stress treatment, but there was no significant difference between the two treatments (Figure 2c). These results indicated that Fe deficiency stress could reduce the CAT and SOD activities of roots and leaves.

The MDA content significantly increased with the decrease in Fe concentrations in root. Compared with the 1Fe treatment, the MDA content of roots in the Fe deficiency stress and the 1/2Fe treatments increased by 97% and 26%, respectively. Compared with the 1Fe treatment, the MDA content of leaves 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 the 1Fe deficiency treatments, but there was no significant difference between the 1/2Fe and the 1Fe treatments (Figure 2d). These results indicated that Fe treatment with an appropriate concentration could inhibit membrane lipid peroxidation, while Fe deficiency stress increased membrane lipid peroxidation in leaves and roots. In addition, the effect of Fe deficiency stress on the 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. The membrane lipid peroxidation level of leaves increased under the 1Fe treatment, which might be due to

the stress caused by the excessive absorption of Fe in leaves under the condition of rich Fe nutrition.

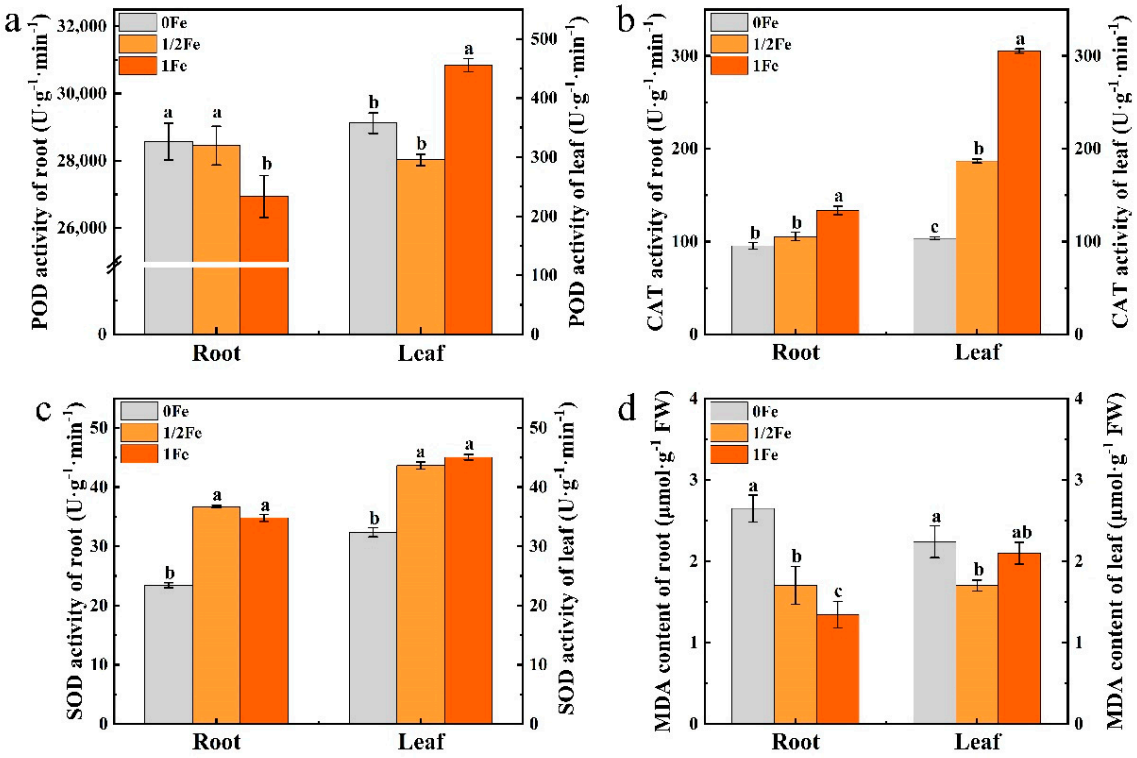

Figure 2. The activities of POD (**a**), CAT (**b**), SOD (**c**) and content of MDA (**d**) in flowering Chinese cabbage treated with Fe deficiency stress. Data are mean ± SE (n = 3). Different letters above the columns show significant differences ($p < 0.05$) between treatments.

The above results indicated that Fe deficiency stress could cause an abnormality in the metabolic system of reactive oxygen. 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, significantly decreased. MDA content in roots and leaves was greatly accumulated, indicating 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 Co^{2+} 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 the peak values of the treatments were reached on the fourth day. With the increase in Fe concentrations, the ethylene release from roots continuously decreased. 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, but Co^{2+} is an inhibitor of ACC synthase. To verify the role of ethylene in the response to Fe deficiency stress, ACC and Co^{2+} were added to study the possible effects of ethylene on Fe deficiency stress. The results showed that ACC could clearly improve the release of ethylene in roots, while Co^{2+} led to an 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, which increased the release of ethylene from roots, might be an adaptive response to Fe deficiency stress in flowering Chinese cabbage.

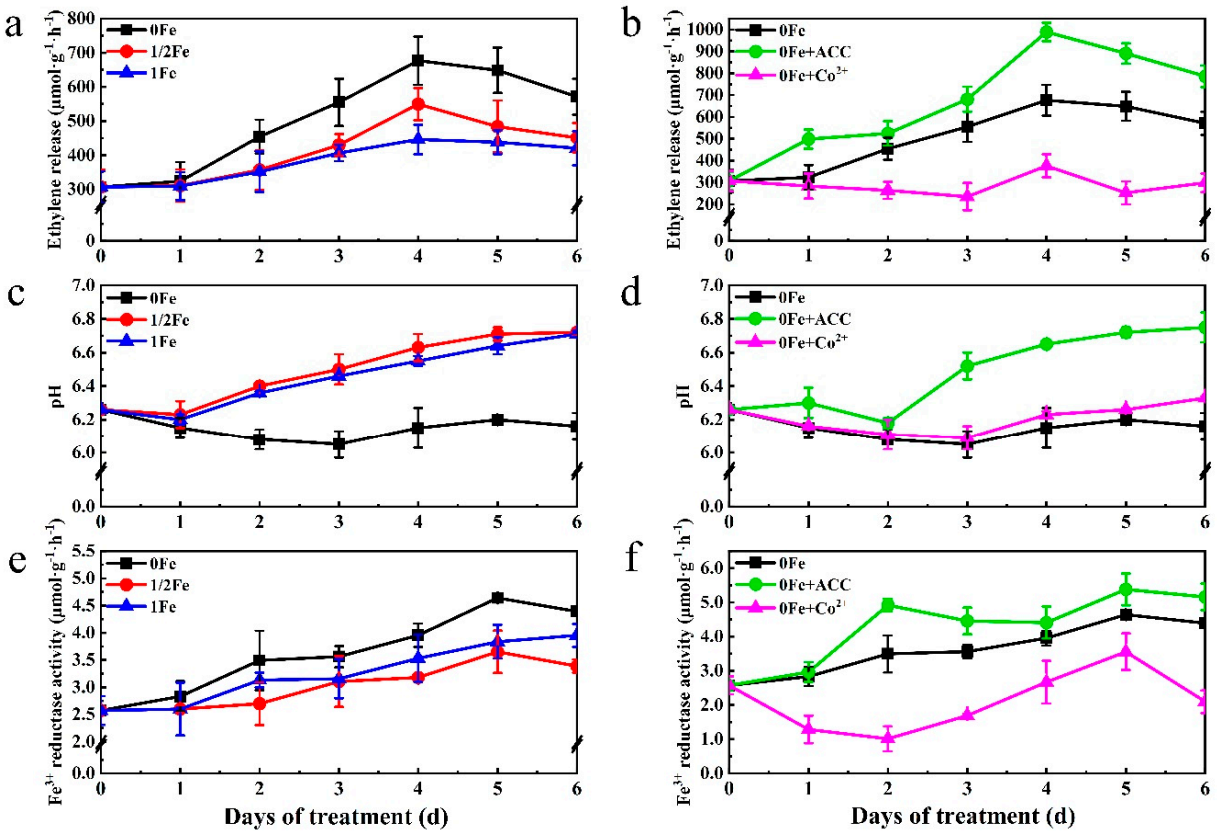

Figure 3. Effects of ACC and Co^{2+} treatments and Fe deficiency stress on the ethylene release (**a**,**b**), pH value (**c**,**d**) and Fe^{3+} reductase activity (**e**,**f**) in roots of flowering Chinese cabbage. Data are mean ± SE (n = 3).

3.7. Effects of Fe Deficiency Stress and ACC and Co^{2+} Treatment on the pH Value in Root Medium

Fe deficiency stress had a significant effect on the pH value of the root medium (Figure 3c). After Fe deficiency stress treatment, the pH value of root medium gradually decreased, reached a low peak on the third day, and then increased gradually. The pH value of the root medium in the 1/2Fe and the 1Fe treatments decreased on the first day and then linearly increased. In general, the pH value of the root medium with the 1/2Fe treatment was the highest, followed by the 1Fe treatment, and the Fe deficiency treatment was the lowest. This indicated that the acidification capacity of the plant roots 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 its lowest point on the third day, before gradually increasing and then decreasing again on the fifth day. Moreover, under the condition of Fe deficiency, Co^{2+} treatment also decreased the pH value of the root medium with the extension of treatment time, and it decreased to its lowest point on the third day and then kept gradually increasing. Among the different treatments, the pH value of the root medium with the ACC treatment was the lowest, while that of Co^{2+} treatment was the highest (Figure 3d). The results suggested that the ACC treatment based on Fe deficiency stress could enhance the acidification capacity of the root medium, while the Co^{2+} treatment showed the 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 Co^{2+} on the Fe^{3+} Reductase Activity in Roots

Different Fe concentration treatments had significantly different effects on the Fe^{3+} reductase activity of roots (Figure 3e). With the increases in treatment time, the Fe^{3+} reductase activity that treated different Fe concentrations showed an increasing trend. On the fifth day of treatment, the Fe^{3+} reductase activity in the Fe deficiency stress and 1/2Fe treatments decreased but continued to increase in the 1Fe treatment. In short, Fe deficiency stress or rich Fe nutrition could increase the Fe^{3+} reductase activity of roots in flowering Chinese cabbage; in particular, the Fe^{3+} reductase activity in the Fe deficiency stress treatment was the highest.

Based on Fe deficiency stress, ACC and Co^{2+} treatments also had a great impact on Fe^{3+} reductase activity in roots (Figure 3f). ACC treatment could rapidly increase the Fe^{3+} reductase activity in roots, and the line showed an "M" shape in the process of treatment. Co^{2+} treatment could rapidly decrease the Fe^{3+} reductase activity in roots, which decreased to a small trough on the second day, then gradually increased, reaching a peak on the fifth day, before finally decreasing gradually. In the process of treatment, Fe^{3+} reductase activity of roots in the ACC treatment was always the highest, while in the Co^{2+} treatment, it was always the lowest. Thus, the results indicated that ACC could induce an increase in the Fe^{3+} reductase activity of roots in flowering Chinese cabbage, while Co^{2+} showed the opposite effect.

In summary, the above results showed that Fe was related to the Fe^{3+} reductase activity of roots in flowering Chinese cabbage. Additionally, Fe deficiency stress led to the improvement of Fe^{3+} reductase activity in roots, ACC was further induced to increase the Fe^{3+} reductase activity of roots, while Co^{2+} showed the opposite effect.

3.9. Effects of Fe Deficiency Stress and ACC and Co^{2+} on Active Fe Content

Fe deficiency stress, ACC, and Co^{2+} treatments had significant effects on the active Fe content (Table 4). With the increase in Fe concentration, the active Fe content in both roots and leaves significantly increased. Based on Fe deficiency stress, ACC treatment could significantly increase the active Fe content in roots and leaves, while the Co^{2+} treatment reduced the active Fe content.

Table 4. Effects of Fe deficiency stress and ACC and Co^{2+} treatment on active Fe contents (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}

Note: Values represent the mean ± standard error (n = 3), different letters indicate significant differences among different treatments at a significance level of 0.05 using Duncan's analysis.

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 and influencing factors that caused a reduction in the yield and quality of fruits and vegetables [23]. The flowering Chinese cabbage plant became yellow, and growth was inhibited under Fe deficiency stress [4,5], which caused a significant yield reduction in leaf vegetables, as well as affecting the quality of its characteristics, such as color, hardness, and acidity [24]. Ding et al. [25] found that Fe deficiency stress inhibited the growth of pak choi and reduced its content of nutrients, 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; therefore, Fe deficiency also led to a reduction in sucrose content and inhibition protein synthesis [26]. Our present results showed that Fe-deficient plants, as well as showing

a yellowing of heart leaves and dwarfism, significantly decreased in plant height, stem diameter, shoot fresh weight, plant fresh weight, leaf chlorophyll content; decreased in the content of Vc, soluble protein, and soluble sugar in the leaves and flower stalks; and the contents of cellulose and nitrate in the leaves and flower stalks significantly increased. Therefore, Fe deficiency stress inhibited plant growth, which resulted in a significant decrease in the plant fresh weight and 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, making plant leaves chlorotic and reducing photosynthetic efficiency [27]. We found that Fe deficiency stress resulted in a significant decrease in chlorophyll content and a clear impact on photosynthetic performance, with a significant decrease in photosynthetic rate and stomatal conductance, and an increase in intercellular CO_2 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 NO^{3-}, and it has a great impact on plant nitrogen assimilation and utilization [28]. Kaya et al. [29] suggested that Fe deficiency caused an increase in NR activity. 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, resulting in the weakened nitrogen metabolism of leaves and causing a large accumulation of nitrate in leaves. This affected the availability and utilization of nitrogen nutrients, which in turn inhibited plant growth. Our present results agreed with the findings from the study of peanuts by Song et al. [30], where 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 are 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 [31]. Plants can 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, contributed to reducing peroxidative damage, stabilizing membrane structure and its function, and improving cellular resilience [13]. Additionally, Fe is a component of SOD, POD, and CAT, which affects their biological activities. In Fe-deficient plants, the SOD activity slightly increased in leaves and roots, but POD and CAT activities in roots decreased by 52% and 35%, respectively, as compared to the control. MDA content increased by 95% and 99% in leaves and roots as compared to the control [30]. Dey et al. [4] 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. [32] 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 a 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, resulting in a large accumulation of MDA in the plant. These findings indicated that Fe deficiency stress caused a decrease in the ability to scavenge reactive oxygen species and led to an increase in membrane lipid peroxidation, thus affecting 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 causes a series of physiological adaptive responses, such as a morphological structure alteration of the root system, leading to inter-root acidification

and increasing the capacity of Fe reduction. 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 under Fe deficiency stress [33]. The dicotyledonous plants—cucumber, tomato, and soybean—have adaptive mechanisms that are induced by Fe deficiency and all belong to strategy I, but their specific responses to adapt to Fe deficiency stress are different. Fe deficiency was mainly shown as an 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 in the Fe^{3+} reduction ability in root systems, increasing the effectiveness of Fe in roots of cucumber and tomato [7]. Our present research also confirmed that Fe deficiency caused an acidification of the inter-root medium; meanwhile, it increased the Fe^{3+} reductase activity of the root system in flowering Chinese cabbage.

Ethylene is one of the main plant hormones that plays important roles in regulating plant metabolism. The relationship between ethylene and Fe uptake is mainly manifested in two aspects in plants: one is to influence the redistribution of Fe by participating in the metabolism; the other is to act as a signaling substance to regulate the adaptive response to Fe deficiency. Plants with Fe deficiency induced the accumulation of ethylene in roots [34]. We found that ethylene was involved in regulating the Fe deficiency response of flowering Chinese cabbage, and Fe deficiency stress caused a significant increase in ethylene release in roots. These results were in accordance with the findings of the investigation carried out by Romera et al. [35]. However, to study if the release of ethylene is an adaptive response to Fe deficiency stress, research has gradually been carried out on the ethylene biosynthesis precursor ACC and the ACC synthase inhibitor Co^{2+}. Waters et al. [36] showed that the addition of ACC after 1 day of Fe deficiency treatment significantly enhanced Fe^{3+} reduction ability, whereas the addition of Co^{2+} significantly reduced Fe^{3+} reduction ability, indicating 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 ethylene synthesis in roots, decreased the pH of the root medium, enhanced Fe^{3+} reductase activity in roots, and increased the active Fe content in flowering Chinese cabbage under Fe deficiency stress. Meanwhile, the Co^{2+} treatment demonstrated the opposite results, showing that the inter-root environmental conditions and effectiveness of root Fe nutrition were improved by ethylene induction in flowering Chinese cabbage. The role of Co^{2+} in the adaptive response to Fe deficiency depended on the concentration of Fe and the genotype of the plant. The Fe^{3+} reduction ability of Fe-efficient mutants of pea plants under Fe deficiency conditions significantly decreased when the concentration of Co^{2+} was 100 $\mu mol \cdot L^{-1}$, while the Fe^{3+} reduction ability in roots of parental peas gradually increased, and there was no clear change under the concentration of Co^{2+}, which was 1 $\mu mol \cdot L^{-1}$ or 10 $\mu mol \cdot L^{-1}$ [37]. There was no regular change in the 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 [6]. The reduction in 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 Fe reduction. 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. Conclusions

Our present study showed that Fe deficiency stress treatment induced a series of physiological adaptive responses, causing a decrease in Vc, soluble protein and sugar, nitrogen metabolism, photosynthesis, and the pH value of root media, promoting the release of ethylene and an increase in Fe^{3+} reductase activity. This improved the effective supply of Fe nutrients in the plant, but disrupted the balance of the reactive oxygen metabolism system, leading to a decrease in CAT in plants. The SOD and POD activities,

and MDA content accumulated in large quantities, and all of these changes led to poor plant growth and decreased yield and quality. ACC treatment significantly improved the environmental pH 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 Co^{2+} treatment inhibited the release of ethylene, decreased Fe^{3+} 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.

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