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Effects of potassium on organic acid metabolism of Fe-sensitive and Fe-resistant rices (*Oryza sativa* L.)

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Abstract

Rice is the world’s number one staple food and feeds half the world’s population. Excessive iron (Fe) is a major abiotic stress factor that contributes to crop yield losses and decline in the nutritional quality of rice worldwide. Effects of potassium on rice plant stature, production, acid metabolism, and content were studied by hydroponic experiments. The plants were subjected to three different potassium levels, i.e., 100, 200, and 400 mg L^{-1}. The Hoagland's solution was applied as base nutrients solution. Excessive Fe^{2+}(250 mg L^{-1}) significantly inhibited the growth of both Fe-sensitive cultivar IIyou838 and Fe-resistant cultivar xieyou9308, including the shoot and root lengths, root and shoot fresh weights, and dry weight. The aconitase and phosphoenolpyruvate carboxylase activity decreased significantly ($p < 0.05$) in both the root and leaf, especially the aconitase activity in the leaves of xieyou9308 and IIyou838, which decreased by 51.85% and 54.55%, respectively. Citrate synthase and malate dehydrogenase activities as well as the malic acid content increased significantly. Citric acid also increased, however no obvious difference among different treatments was observed. The addition of potassium can alleviate iron toxicity, but a certain difference occurs compared with the control. The results indicated that potassium can alleviate iron toxicity to a certain degree. Under iron toxicity, changes in plant height, root length, biomass, organic acid contents, and enzyme activities of IIyou838 were greater than those of xieyou9308, showing its sensitivity to iron toxicity.

Keywords: Fe-sensitive rice; Fe-resistant rice; iron stress; potassium; organic acids.
Abbreviations: PEPCase-Phosphoenolpyruvate carboxylase; FSW-Fresh shoot weight; DSW-Dry shoot weight; FRW-Fresh root weight; DRW-Dry root weight.

Introduction

Although iron (Fe) is an essential microelement for most plants, excessive iron (Fe) is a major abiotic stress factor that contributes to crop yield losses and decline in the nutritional quality worldwide (Yadavalli et al., 2012). Plant iron toxicity is a common symptom worldwide, especially in tropical and subtropical regions. Iron toxicity is common in rice cultivation and seriously affects its production (Kochian et al., 2004). Iron toxicity symptoms include leaf spots, plant dry weight reduction, root growth restrains, and root oxidation (Feng et al., 1996; Bandara and Gunatilaka et al., 1994). The occurrence of iron toxicity in rice is related to the high Fe^{2+} uptake by roots. Fe^{2+} is the formation of free radical-inducing agent, and auto-oxidation can produce the superoxide radicals O_2^-, which participate in Fenton reaction to produce hydroxyl radicals –OH (Becana et al., 1998). These free radicals attack the membrane lipid or enzyme reaction center, leading to membrane leakage or affecting the enzyme activity. The more Fe^{2+} accumulated in roots, the stronger the poison reaction of the free radicals. Consequently, severely inhibited root growth reduces production (Majerus et al., 2007; Pongmanee and Bernard, 2000). Plant can secrete more organic acids when under heavy metal stress (Wang and Shen, 2006; Chen et al., 2009; Gong and Lan, 2006). The reason may be because organic acids have important metabolic functions in plants (Li et al., 2004; López-Bucio et al., 2000; Lu et al., 1999; Zeng et al., 2008; Sun et al., 2002). Organic acids participate in photosynthesis and respiration and can serve as an active metabolic solute, regulating osmotic pressure and balancing excess cation (Sun et al., 2002). Organic acids are also a key component involved in response to nutrient deficiency, metal coercion, root–soil interface, plant–microorganism interactions, and other plant metabolic processes (López-Bucio et al., 2000; Zeng et al., 2008; Sun et al., 2002;). Organic acid metabolism has become one of the interesting areas in current plant physiology and nutrition research. Lu et al. (1999) reported that the organic acids exuded by root can mobilize soil phosphorus and micronutrients and relieve aluminum toxicity under nutrient stress (Lu et al., 1999). Secretion and detoxification mechanisms of root organic acid have been studied (Sun et al., 2002; Li et al., 2004; You and Yang, 2005; Singh and Chauhan, 2011). Chen et al. (2005) showed the organic acid synthesis, degradation, vacuole storage, and other metabolic processes of the physiological and biochemical mechanisms of fruits (Chen et al., 2005). Potassium, which involved in the synthesis of the essential enzymes can enhance the oxidizing force of root, plays an important role in maintaining plant cell physiological function and regulating intracellular osmotic pressure and has an intimate connection with plant growth (Eun et al., 2000; Giertha and Mäser, 2007). Zhou et al. (2005) revealed that high concentrations of K^+ can alleviate the adverse effects of Fe on rice growth, with leaf chlorophyll contents of three rice genotypes increased (Zhou et al., 2005). Therefore studies on the mitigation role of potassium of the organic acid metabolism system in rice under iron stress are

Table 1. Effect of exogenous potassium on the growth characteristics of xieyou9308(Al-resistant) and IIyou838(Fe-sensitive) under iron stress.

Cultivar	Treat ment	root lengation (%)	Shoot length (cm plant^{-1})	FSW (g plant^{-1})	DSW (g plant^{-1})	FRW (g plant^{-1})	DRW (g plant^{-1})
xieyou9308	T0	100a	55.43a	1.46a	0.16a	0.26a	0.033a
	T1	59.26d	38.22c	0.44c	0.06a	0.16b	0.020b
	T2	65.42c	40.67ab	0.93b	0.11a	0.21ab	0.026ab
	T3	74.57b	42.12b	0.93b	0.13a	0.23ab	0.029ab
	T4	61.17d	43.17b	1.08b	0.15a	0.19ab	0.020b
IIyou838	T0	100a	51.94a	1.56a	0.17a	0.35a	0.042a
	T1	54.65d	30.08d	0.55c	0.06a	0.15b	0.019c
	T2	60.77c	32.46cd	0.60c	0.08a	0.19b	0.026b
	T3	72.15b	35.02c	0.84b	0.12a	0.21b	0.026b
	T4	71.99b	39.98b	0.96b	0.15a	0.20b	0.022ab

Note: Different letters represent significant difference at $P<0.05$ within the same category. FSW-Fresh shoot weight; DSW-Dry shoot weight; FRW-Fresh root weight; DRW-Dry root weight.

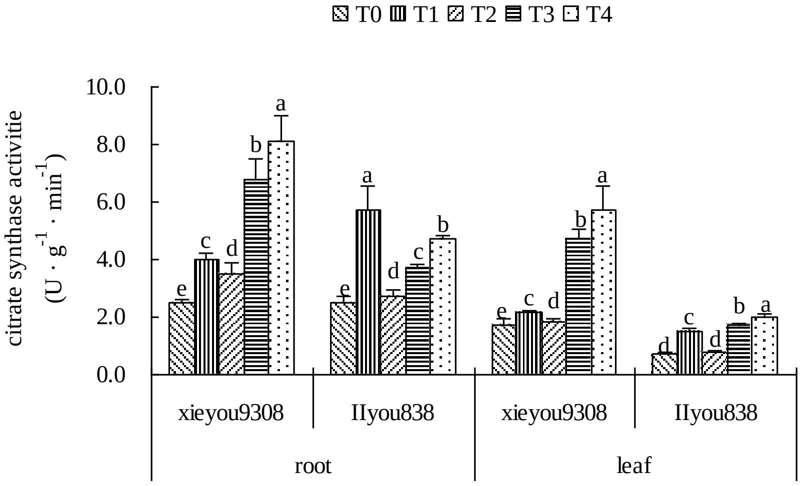

Fig 1. Effects of exogenous potassium on citrate synthase activity of xieyou9308 and IIyou838 under iron stress

needed. In the current study, the authors focused on the role and mechanism of external potassium on organic acid metabolism in rice under iron stress to provide a theoretical basis for the prevention of iron toxicity in rice.

Results

Effect of exogenous potassium on the growth characteristics of xieyou9308 and IIyou838 under iron stress

As shown in Table 1, the relative root length, plant height, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight of Fe-resistant cultivar xieyou9308 were significantly decreased by 40.74%, 31.05%, 41.90%, 61.26%, 38.71%, 40.36%, respectively in T1 compared to control treatment. The Fe-sensitive cultivar IIyou838 was significantly decreased by 45.35%, 42.09%, 64.82%, 62.69%, 57.52% and 56.37% with T1 compared to T0, respectively. The addition of exogenous potassium can alleviate iron toxicity in different degrees. When the K^+ concentration is 200 mg L^{-1} (T3), the relative root length, fresh root weight, dry root weight of Fe-resistant cultivar xieyou9308 were increased respectively by 25.84%, 45.09% and 46.46% and IIyou838 by 32.02%, 39.55% and 40.00%, comparing with T1 treatment. The two rice cultivars can greatly alleviate the iron toxicity for the relative root length and root weight. When the K^+ concentration is 400mg L^{-1}(T4), the shoot length, fresh shoot weight and dry shoot weight of Fe-resistant cultivar xieyou9308 was increased by 12.95%, 144.50% and 144.59% and IIyou838 increased by 32.91%, 75.08% and 126.54%, comparing with T1 treatment. The results shows that the two rice cultivars can greatly alleviate the iron toxicity in relative root length and root weight when K^+ concentration is 200mg L^{-1}, and they can greatly alleviate iron toxicity in shoot length, fresh shoot weight and dry shoot weight very well when K^+ concentration is 400 mg L^{-1}. Difference analysis showed that there were significant differences between the different treatments.

Effects of exogenous potassium on organic acid enzyme metabolic scheme of rice under iron stress

Citrate synthase activity

Citrate synthase, present in the mitochondria matrix, controls the entrance of the citric acid cycle and catalyses the reaction of oxaloacetate and acetyl-CoA coming from glycolysis or other alienation to synthesize citric acid through the condensation reaction, and becomes the speed-limited step of citric acid cycle. As shown in Fig. 1, high iron stress increased the citrate synthase activity of the both rice cultivars increased. Comparing with T0, T1 treatment increased the activity of citrate synthase in roots and leaves of xieyou9308 increased by 60% and 22.86%, while IIyou838 increased by 130% and 100%. The higher increment by Fe-sensitive cultivar IIyou838 increased much greatly than xieyou9308 shows its iron sensitivity. When exogenous potassium concentration was 100mg.L^{-1}, the enzyme activity of two rice cultivars have decreased, comparing with the T1 treatment. The citrate synthase activity in roots and leaves of xieyou9308 were decreased by 12.5% and 13.95%, and of IIyou838 decreased by 52.17% and 47.33% when compared to T1. IIyou838 decreased much greatly than xieyou9308, and there was significant difference among treatment. With increasing potassium concentration, the citrate synthase activity began to increase and the reason needs further research.

Aconitase activity

Aconitase is involved in glyoxylate cycle. Fatty acids are decomposed to acetyl-CoA through β-oxidation which on reaction with oxaloacetate condencse to citric acid through the catalysis of citrate synthese, and then the aconitase catalyze the formation of isocitrate. Compared with T0 treatment, the aconitase activity of the two rice cultivars showed a downward trend under T1 treatment. The aconitase activity in roots and leaves of xieyou9308 decreased by 39.39% and 51.85%, and IIyou838 by 42.86% and 54.55%, respectively. The addition of potassium made the aconitase activity of the two rice cultivars increased. When the concentration of exogenous potassium is 200mg L^{-1} (T3), the alleviate effects was much better than other groups. When comparison with T1, the aconitase activity in T3 in roots and leaves of xieyou9308 increased by 39.39% and 50%, and the enzyme activity of IIyou838 increased by 39.39% and 120%, respectively. Under T2 treatment, the changes of enzyme activity both in root and leaves of IIyou838 were greater than those of xieyou9308 (fig 2).

Malate dehydrogenase activity

Malate dehydrogenase, one of enzyme in the citric acid cycle, catalyses the dehydrogenation of L-malic acid and change into oxaloacetate. With different concentrations of iron and potassium treatments, the changes trend of roots and leaves of the two rice cultivars were inconsistent. Comparing with T0, malate dehydrogenase activity of xieyou9308 and IIyou838 in root were increased by 29.63% and 228.57% whereas in leaf were decreased by 29.41% and 58.82%, respectively under T1 treatment. The changes of enzyme activity of xieyou9308 were significantly less than those of IIyou838 in T1. When the exogenous potassium concentration was 200 mg L^{-1}, the root malate dehydrogenase activity of xieyou9308 and IIyou838 decreased by 51.43% and 65.22%, respectively, while increased by 33.33% and 114.29% in leaves compared with T1 treatment (Fig 3). The results show that potassium concentration can greatly alleviate iron stress.

phosphoenolpyruvate carboxylase activity

Phosphoenolpyruvate carboxylase (PEPCase), a CO_2 fixation enzyme, catalyzes the response of phosphoenolpyruvate (PEP) and bicarbonate anhydride (CA) to generate oxaloacetate (OAA), and plays multiple roles in energy and biosynthesis metabolism. Fig. 4 shows that the PEPCase activity of the two rice cultivars decreased under T1 treatment. The PEPCase activity of the roots and leaves of xieyou9308 were decreased by 33.33% and 34.43%, while in IIyou838 decreased by 28.57% and 23.08%, respectively. After potassium being added, the enzyme activities of both two rice cultivars increased gradually with increasing potassium concentration. When the concentration of exogenous potassium was 400 mg L^{-1}, PEPCase activity of the roots and

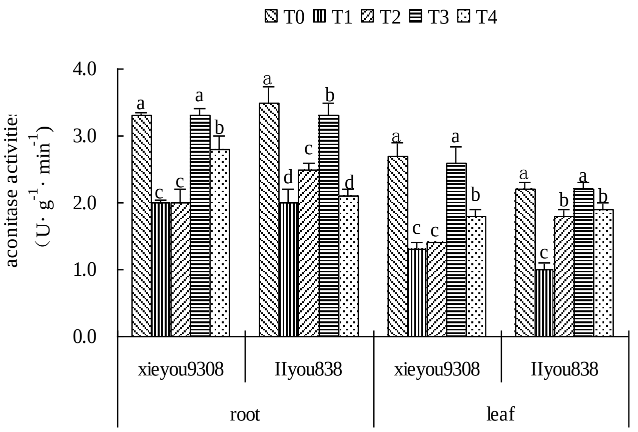

Fig 2. Effects of exogenous potassium on aconitase activity of xieyou9308 and IIyou838 under iron stress.

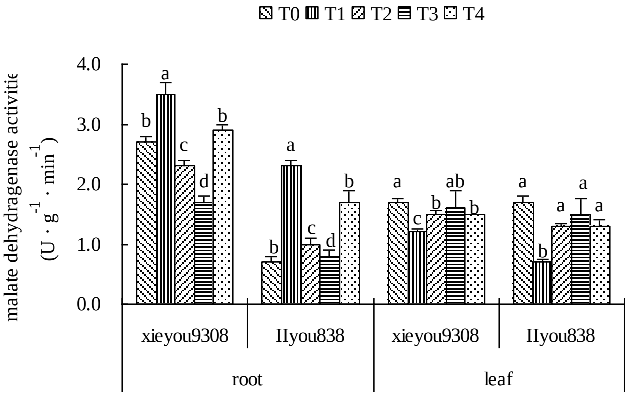

Fig 3. Effects of exogenous potassium on malate dehydrogenase activity of xieyou9308 and IIyou838 under iron stress.

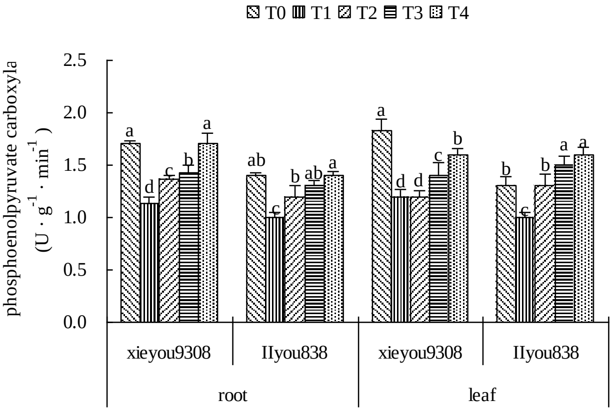

Fig 4. Effects of exogenous potassium on phosphoenolpyruvate carboxylase activity of xieyou9308 and IIyou838 under iron stress.

leaves of xieyou9308 increased by 50% and 33.33%, while IIyou838 increased by 28.57% and 60%, respectively, comparing with the T1 treatment. The results indicate that exogenous potassium plays a certain role in mitigating iron toxicity.

Effects of exogenous potassium on organic acid contents of rice under iron stress

Malic Acid contents

Malic acid, an intermediate body citric of acid cycle, can reduce the pH value and result in chelating and suppressing phenol enzyme activity to prevent enzymatic browning. Under the T1 treatment, the malic acid contents of both rice cultivars increased (Fig. 5). The malic acid contents of roots and leaves of xieyou9308 increased by 17.45% and 18.39%, while IIyou838 increased by 89.3% and 20.18%, respectively. Under T2 treatment, when the concentration of exogenous potassium was 100 mg L^{-1}, the mitigation effect was better. The malic acid contents of roots and leaves of xieyou9308 were decreased by 7.7% and 26.23%, while IIyou838 decreased by 5.43% and 41.71% comparison with the T1. Difference analysis showed that there was obvious difference among different treatments. When the concentration of exogenous potassium was 200 mg L^{-1}, the malic acid contents of both rice cultivars had increased sharply. When the concentration of exogenous potassium increased to 400 mg L^{-1}, it has better mitigation effect in IIyou838 than that in xieyou9308 in both root and leaf.

Citric acid contents

The citric acid cycle, also known as the tricarboxylic acid cycle (TAC) and Krebs cycle, is a circulatory system of enzymatic reaction for acetyl-CoA oxidized to CO_2. The first step of the cycle is that the enzyme acetyl-CoA and oxaloacetate react and condense to citric acid through the catalysis of citrate syntheses. Under different concentrations of iron and potassium treatment, the citric acid content changed (Fig. 6), but there was no significant difference. Under T1 treatment, the citric acid contents of roots and leaves of xieyou9308 increased 18.98% and 24.15% compared with T0, while those of IIyou838 increased by 35.68% and 42.11%, respectively. The exogenous potassium can alleviate the iron toxicity. When the potassium concentration was 200 mg L^{-1}, the mitigation effect was the best and the citric acid contents of roots and leaves of xieyou9308 were decreased by 18.98% and 24.15%, while that of IIyou838 were decreased by 35.68% and 42.11% compared with T1 group. The changes of citric acid contents of IIyou838 were greater than those of xieyou9308, showing its sensitivity to Fe.

Discussion

We observed that the growth of both Fe-sensitive cultivar IIyou838 and Fe-resistant cultivar xieyou9308, including the shoot and root lengths, root and shoot fresh weights, and dry weight, was decreased significantly ($p < 0.05$). The inhibition of the growth of rice shoot and root lengths, root and shoot fresh weights, and dry weight by Fe^{2+} stress was decreased with the addition of exogenous potassium, similar to the results of Zhou et al. (2005). The amelioration of K^+ was more pronounced on the sensitive genotype (IIyou838) than that on the tolerant genotype (xieyou9308), indicating that different genotypes exist in the amelioration of K^+ on Fe^{2+} stress. The metabolism and regulation of organic acid are a complex physiological process. During the plants respiratory process, the citric acid cycle produces a series of organic acids that oxidize coenzyme NAD^+ and FAD and produces NADH, $FADH_2$, and CO_2. This series of organic acids include pyruvic acid, citric acid, isocitric acid, α-ketoglutaric acid, succinic acid, fumaric acid, malic acid, oxaloacetate, and so on. A component of the organic acids, produced mainly in the mitochondria, takes part in the glyoxylate cycle as glyoxysome (Singh and Chauhan, 2011; Peng et al., 1998). Citric and malic acids are the intermediate products in the citric acid cycle (TCA), and their synthesis and degradation are closely related with the TCA cycle (Schulze et al., 2002). Our results confirm that iron stress influenced the contents of citric and malic acids of the two rice cultivars, both malic acid and citric acid contents were increased significantly ($p < 0.05$) under iron stress. The exogenous potassium has a role in alleviating iron stress to a certain degree, and malic acid content was decreased to a comparable value of the control group when the exogenous potassium was 100 mg L^{-1}. However, the citric acid content was decreased to a comparable value of the control when exogenous potassium was increased to 200 mg L^{-1}. Malic and citric acid contents of the Fe-sensitive varieties of IIyou838 are greater than those of xieyou9308, showing its sensitivity to iron toxicity. Previous studies showed that the activity of aconitase has a certain relationship with the accumulation of citric acid, but does not have a significant relevance due to low aconitase

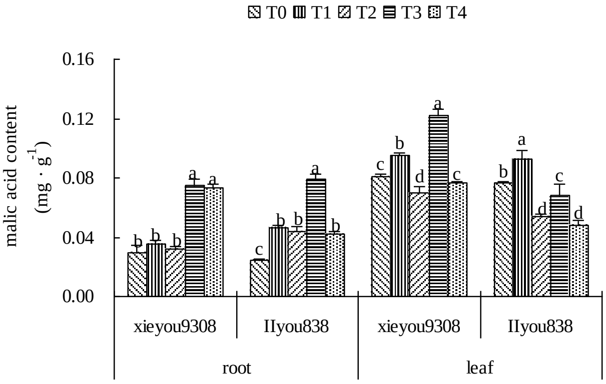

Fig 5. Effects of exogenous potassium on malic acid contents of xieyou9308 and IIyou838 under iron stress.

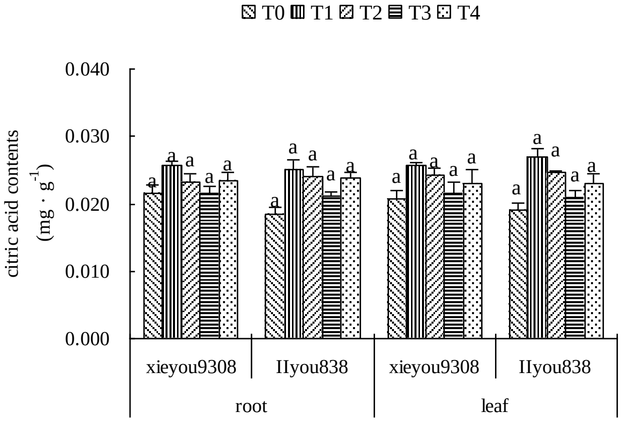

Fig 6. Effects of exogenous potassium on citric acid contents of xieyou9308 and IIyou838 under iron stress

activity (Sadka et al., 2000). In our experiments, the four kinds of organic acid metabolic enzyme activities changed under iron stress. The exogenous potassium alleviated iron stress to a certain degree, with obvious differences in different treatments. This result shows that iron stress affects rice organic acid metabolic enzymes. The effects of exogenous potassium on organic acid contents and enzyme activity under iron stress in the molecular mechanism need to be further studied. Organic acids metabolism is an extremely complex process in plant, being regulated by a variety of enzymes. Organic acids are involved in the process of photosynthesis and respiration in plants, and interact with nitrogen metabolism. The organic acids secreted into the rhizosphere have an important role in the adaptation to nutrient deficiency and metal stress in plants (You and Yang, 2005; Oburger et al., 2009). The formation, accumulation, and secretion of organic acids in plants are mutual effects of inherent genetic characteristics, external natural environmental factors, and cultivation measures. The formation and transfer of organic acids in plant cells, the activity and characteristics of organic acid metabolism enzyme, and the secretion character of root organic acids have been studied (Ma et al., 2001; Mani-Lόpez et al., 2012). However, a number of problems, such as the role of key enzymes, the trans-membrane transport of organic acid, and the gene identification, which decides the formation and secretion of organic acid, have not been resolved yet. Rice organic acid metabolism, a complex process regulated by various enzymes, which is greatly affected by external environments.

Materials and methods

The experiments were carried out from March to December 2010 at the glasshouse of our university. IIyou838 (Fe-sensitive rice), with 7.92% root elongation, and xieyou9308 (Fe-resistant rice), with 45.07% root elongation were chosen as experimental materials by measuring the relative root elongation rate. The nutrient solution was made according to Yoshida et al (1976). Full and healthy rice seeds were chosen, and then cleaned with water and disinfected with 0.1% H_2O_2 for 30 minutes. After disinfection, the seeds were rinsed with tap water and distilled water for three times, respectively. Seeds were cultivated in 30℃incubators for germination. A week after germination, seedling were transplanted into 48 L plastic tanks one week later and cultivated first with 1/4 complete culture nutrient solution and then 1/2 complete nutrient solutions for one week, then cultivated rice with full nutrient solution culture for two weeks. pH was adjusted to 5.0 every day and nutritional solution was changed once every three days. After one month, the rice then was continued to be cultivated with treatments as follows, T0 (complete nutrient solution, Fe^{2+} 2 mg L^{-1}, K^+ 40 mg L^{-1}); T1 (Fe^{2+} 250 mg L^{-1}); T2 (Fe^{2+} 250 mg L^{-1} + K^+ 100 mg L^{-1}); T3 (Fe^{2+} 250 mg L^{-1} + K^+ 200 mg L^{-1}); and T4 (Fe^{2+} 250 mg L^{-1} + K^+ 400 mg L^{-1}). The iron was supplied in the form of EDTA-Fe^{2+} (EDTA chelated with $FeSO_4 \cdot 7H_2O$) and the potassium was supplied in the form of KCl, pH was adjusted to 5.0 every day and nutritional solution was changed once every three days. The plant growth characteristics, organic acid mechanism, and organic acid content were measured after 7 d and 14 d treatments. Fe^{2+} and K^+ concentration were chosen by the preliminary experiments, and 250 mg L^{-1} Fe^{2+} concentration was found to result in obvious iron stress on rice.

Enzyme extraction and assay

0.1 g plant leaves and root tips were taken and placed in 4℃ ice mortar and lap with 1 mL homogenate extracts, including 0.1 mol/L Tris-HCl buffer (pH 8.0), 0.1% (v/v) Triton X-100 (Triton -100), 2% (w/v) PVP, and 10 mmol/L iso-ascorbic acid. The extracts were centrifuged for 5 min at 15000 rpm (4℃) and the supernatant was measured for enzyme activity. Enzyme activities (Citrate synthase, Aconitase, Malate dehydrogenase, and Phosphoenolpyruvate carboxylase) were extracted and determined by method according to Chen et al. (2009), with some modifications (Chen et al., 2009a; Chen et al., 2009b). Absorption was measured by UV-8500 ultraviolet-visible spectrophotometer immediately after reactants being added. Absorbance changes were recorded every 10 s, and repeated for three times. Separation and determination of organic acids was according to Chen (2005).

Extraction and assay of organic acids

Malate and citrate were extracted according to Chen et al. (2002). Citrate was assayed according to the methods of Delhaize et al. with some modifications (Delhaize et al., 1993). One milliliter of reaction mixture contained 100 mM Tris–HCl (pH 7.6), 0.2 mM NADH, 7 unit lactate dehydrogenase, 14 unit NAD-malate dehydrogenase, 0.5 unit citrate lyase, 2 mM EDTA and 200 µL supernatant.

Data Analysis

All the values indicated in this investigation are the mean of four replicates. The data were analyzed with the help of SPSS statistical software package (Version 11.0). For statistical analysis, one-way analysis of variance (ANOVA) and t-test were used to determine the significance at $p < 0.05$.

Conclusion

Root and shoot growth, plant fresh and dry biomass, citrate acid, malate acid, organic acid, and organic acid metabolism enzyme activity are affected by iron stress. Exogenous potassium has a certain mitigation effect on iron stress. Therefore, the molecular aspects of some other enzymes catalyzing organic acid synthesis and metabolisms via Fe-induced are needed to find effective measures to resist Fe toxicity.

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