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Improving nutritional quality through foliar iron application of rice grown in Northeastern Thailand

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ABSTRACT: Iron deficiency is one of the major health problems which can overcome by consuming more iron rich foods. Enriching iron content in stable food such as rice may eventually help to supply adequate iron for human health. This study was to find the way to increase grain iron content in rice. The field experiments were done at two locations (low and high soil iron content) to ascertain the effect of foliar application of iron at different growth stages in two rice cultivars. The experiments were setup in a split-plot design. In the main plots, $FeSO_4$ was sprayed at different growth stages (panicle initiation, flowering, milking, and panicle initiation+ flowering+ milking, including without iron application). The sub-plot consists of two rice cultivars Khao Dawk Mali 105 (poor iron concentration 5.7 mg/kg) and Riceberry (rich iron concentration 15.5 mg/kg). The foliar spray of $FeSO_4$ three times at panicle initiation + flowering + milking stages resulted in tend to give highest iron concentration of brown and polished rice grain in both locations, excepted the Fe concentration in polished rice grain for KDML 105 cultivar. Riceberry produced higher iron concentration in grain than Khao Dawk Mali 105 in both locations. The brown rice iron concentration was positively correlated with polished rice iron concentration. The correlations were different for different experimental sites. The iron concentration in brown and polished rice was positively correlated when grown in low iron concentration soil, but negatively correlated when grown in high iron concentration soil. The combined application at panicle initiation + flowering + milking stages significantly increased higher grain yield than a single application at panicle initiation, flowering and milking in low soil iron concentration filed, but not in high soil iron concentration field. Khao Dawk Mali 105 produced significantly higher grain yield than Riceberry in both locations.

Keywords: $FeSO_4$; foliar spray of iron; rice iron concentration; rice yield; rice nutrition

Introduction

Half of the world's population suffers from mineral deficiencies, primarily iron and zinc (Horton et al., 2008). Iron deficiency causes impairments in mental and psychomotor development in children, and diminished productivity in adults and represents the most common cause of anemia (Neumann et al., 2002). One of the reasons is due to low iron concentration in staple food crops (Cakmak, 2002).

Rice is one of the staple foods for more than half of the world's population, but it contains low content of micronutrients such as iron (Boonyaves et al., 2017). In addition, the micronutrient-rich layers of the grain, for instance aleurone, bran, and husk, are removed during the polishing of the rice grain (Boonyaves et al., 2017; Prom-u-thai et al., 2007). Increasing the iron concentration in rice is still a difficult task for several reasons, especially in the inner grain layers. Firstly, plants cannot utilize largely insoluble Fe (III) compounds from soil (Miller et al., 1984). Among

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the micronutrient, iron (Fe) deficiency is quite predominated particularly on alkaline, calcareous and upland soil of semiarid regions (Miller et al., 1984). Secondly, soils cultivated have a high pH (Bashir et al., 2010). Thirdly, Fe can readily accept and donate electrons, making high concentration of Fe toxic as it can lead to the production of reactive oxygen species (Bashir et al., 2010). Thus, soil application of most Fe sources is generally ineffective, and unavailable forms to plants (Fageria et al., 2009).

Biofortification is one of the ways to provide an increased level of micronutrients in crop (Huang et al., 2020). There are three major approaches to biofortification: conventional plant breeding, genetic engineering, and agronomic (Garg et al., 2018). It has been adequately documented that certain plant genotypes within species exhibit genetic variation in the mobilization, absorption and translocation of soil Fe (Hacisalihoglu et al., 2003; Nogiya et al., 2016). Genotypic differences in Fe nutrition of rice are known (Fageria et al., 2002; Zuo and Zhang, 2009; Maganti et al., 2020). Agronomic seems to be the simplest biofortification as it can be done by supplying micronutrients to the plants via soil mineral application and/or foliar fertilization.

Foliar fertilizer sprays have proven to be a sustainable, effective, and low-cost method to improve Fe concentration in edible parts of staple food crops (Rengel et al., 1999). Several studies have demonstrated that foliar application of $FeSO_4$, increased iron concentration in grain (Zhang et al., 2009; Wei et al., 2012; Yuan et al., 2012). Foliar applied, micronutrients motion into the plant tissues seems to involve dissemination through the cuticle and uptake by the leaf cells (Lahijani et al., 2020). Foliar nutrients can improve the potential yield and physiological and biological traits of crops. Foliar sprayed Fe-EDTA at active tiller, panicle initiation and milking stages increased yield components and yield of rice plants (Sudhagar Rao et al., 2019). In addition, foliar $FeSO_4.7H_2O$ at booting, anthesis and early filling stages increased the thousand kernel weight, kernels per spike and grain yield of winter wheat (Niyigaba et al., 2019).

In plants, it needs iron for several biological processes, including photosynthesis and chlorophyll biosynthesis. Iron deficiency in plants causes chlorosis and decrease root length (Muller and Schmidt, 2004; Schmidt, 1999; Jones and Olson-Rutz, 2016). However, the high reactivity of iron can generate toxic hydroxy radicles that can damage plant cells (Briat et al., 2007; Halliwell, 2006). However, there are not many available information on finding the suitable growth stages for foliar application to increase the grain iron concentration, growth, and yield of rice. Hence, these field experiments were set up to assess the effect of foliar iron application at different growth stages on grain iron concentration, growth, and yield of two rice cultivars in the rainy season of Northeastern Thailand.

Materials and methods

Field locations

The experiments were conducted during the rainy season of 2018 in Khon Kaen province, Northeastern Thailand. One location was a low soil iron concentration field in Mueang district (latitude; 16°57'02.9"N, longitude; 102°69'49.9"E) (experimental site 1), and another was a high soil iron concentration field in Namphong district (latitude; 16°72'54.0"N, longitude; 102°98'03.6"E) (experimental site 2) (Table 1). The site's climate is semi-humid, which has rain in May – October, and dry season in November – April (Goto et al., 2008). The total rainfall during the trial period (June – December) was 894.6 mm and 615.3 mm in the experimental site 1 and the experimental site 2, respectively. The experimental site 1, the total rainfall levels during the PI and grain filling stages were 299.7 mm and 77.6 mm, respectively. The experimental site 2, the total rainfall levels during the PI and grain filling stages were 212.3 mm and 50.5 mm, respectively. The average temperature during the PI stage was 28.7 °C, while during the grain filling stage was 28.2 °C. The light intensity during the PI and grain filling stages were 6.3 h/day and 8.1 h/day, respectively.

Experimental design and treatments

A split-plot design with four replications was employed in the present study. The main plots consist of different growth stages; panicle initiation (PI), flowering (F), milking (M), and PI+F+M which folia Fe applied in comparison to without Fe application. The two rice cultivars namely Khao Dawk Mali 105 (KDML 105, low Fe concentration) and Riceberry (high Fe concentration) were assigned as sub-plots.

Crop culture

Thirty days old rice seedlings were transplanted to the plots by using a single seedling per hill with spacing of 25 cm x 25 cm. Fertilizer formular 16-8-8 (N – P_2O_5 – K_2O) was applied at the rate of 156 kg/ha. The first half of fertilizer was applied at 30 days after transplanting (DAT), and the other half at PI stage. Foliar solution of Fe 1.5 g Fe/L was applied for all single growth stage treatment, and 0.5 g Fe/L was applied for the treatment with the combination between growth stages. The application was done by dissolving iron sulfate powder [$FeSO_4 \cdot 7H_2O$ (1% Fe)] in distilled water, then sprayed on the plants with a hand-held pump sprayer at a rate of 1,000 L/ha in the morning around 10 am. Hand weeding was done at 45 and 75 DAT. Pesticides were not applied throughout the growing season. The transplanting took place in June, and harvesting was in December.

Sampling and measurement

The plant height, tiller number, leaf area and above ground dry weight were recorded from five hills in a sampling area of 10 m^2 from each plot at 90 DAT. For leaf area, the leaf samples were measured with leaf area meter (AAC-400, Hayashi Denkoh, Japan) and then computed for leaf area index (LAI) by leaf area per hill divided by ground area covered (Ekanayake, 1994). The above ground dry weight was measured after drying the samples at constant temperature of 80°C for 72 hours. Yield components such as panicle number, grain number per panicle and 1,000 grain weight were recorded from the sampling area of each plot at harvest. Grain yield was measured from the 3 x 2 m^2 of samples area of each plot. The grain samples were dried to decrease the moisture content and calculated for grain yield at 14% by the following formula:

$$\text{Grain yield (kg/plot, 6m}^2\text{)} = \frac{100 - \mathit{sample\ moisture\ content}\ (\%) \times \mathit{sample\ weight}\ (kg)}{100 - \text{standard moisture content (14\%)}}$$

Subsequently, gain yield was computed to kg/ha.

The iron concentration measurement was done by sampling the grains from each plot at harvest. Then, the husks were removed using a laboratory-scale dehulling machine. One gram of brown and polished rice grain were analyzed for iron concentration. The analysis was done by extracting rice grain flour of each sample with hydrochloric acid (HCl) until the solution became colorless and clear, then diluting the remainder with distilled water. The samples were read for Fe concentration by the Atomic Absorption Spectrophotometer (AAS), then compared with the concentration of standard solution of iron (Zarcinas et al., 1987).

Statistical analysis

All collected data were subjected to analysis of variance (ANOVA) for the split-plot design. The means were tested using the least significant difference (LSD) at P $\leq$ 0.05 levels (Gomez and Gomez, 1984). The data from each experimental site was analyzed separately.

Regression analysis is a statistical method that shows the relationship between two or more variables. It tests the relationship between a dependent variable (Y) against independent variables (X). Typically, the independent variable(s) changes with the dependent variable(s) and the regression analysis attempts to answer which factors matter most to that change (Chansuvarn, 2018).

Regression analysis was calculated by the following formula;

$$y = a + bx$$

Y = dependent variable

X = independent variables

a = intercept

b = slope

The correlation coefficient is a value that shows how much the x variable influences the y variable. The correlation coefficient is represented by r where 0 ≤ r ≤ 1, which can be summarized as follows (Chansuvarn, 2018).

If r is close to 1, then the two variables are highly related. And have the same direction, that is, if x is large, y is also large.

If r is close to -1, then the two variables are highly related. But in opposite directions, that is, if x is large, y is small, or x is small, y is high.

If r approaches 0, then x and y are less correlated.

Correlation was calculated by the following formula;

$$r = \frac{\sum_{i=1}^{n}(x^i - \bar{x})(y^i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x^i - \bar{x})^2}\ \sqrt{\sum_{i=1}^{n}(y^i - \bar{y})^2}}$$

Table 1 Soil physico-chemical properties of the two experimental sites at 0 to 30 cm depth from soil surface

Parameters	Experimental site 1	Experimental site 2	Analysis method

pH (1:1 soil:water)	5.52	6.01	pH meter
Total N (%)	0.036	0.057	Kjeldahl nitrogen method
Available P (mg/kg)	7.65	2.88	Bray II
Exchangeable K (mg/kg)	90.82	151.86	Flame Photometer
Exchangeable Ca (mg/kg)	338	1881	Flame Photometer
Available Fe (mg/kg)	52.90	148.46	Atomic Absorption Spectrophotometer
Extractable Zn (mg/kg)	0.55	0.43	Atomic Absorption Spectrophotometer
Organic matter (%)	0.463	1.015	Wet oxidation
CEC (c mol (+)/kg)	7.74	18.86	Ammonium acetate extract
Sand (%)	46.30	45.64	Hydrometer method
Silt (%)	40.40	25.14	Hydrometer method
Clay (%)	13.30	29.22	Hydrometer method
Textural class	Loam	Sandy loam	Hydrometer method

Results and discussion

Iron concentration

The present study revealed a significant increase in iron concentration in brown rice and polished rice grain via foliar iron application treatment at all growth stages (**Table 2**). The foliar application of iron increased the Fe concentration in cereal crops as previously reported in the studies of Fang et al. (2008), Wei et al. (2012) and Yuan et al. (2012). In general, foliar application of iron at flowering (F), milking (M), and panicle initiation (PI) +F+M had no significant effect on brown rice grain Fe concentration in both experimental sites **(Table 2)**. However, iron application at PI+F+M tended to give higher iron concentration in brown rice grain than those of applications at F stage or M stage. This was mainly due to the roots continued uptakes iron from soil throughout the growing period (Jiang et al., 2007). The plant receives Fe from foliar application is accumulates in the shoot tissues especially leaves, and then translocate to the grain (Jiang et al., 2008). The foliar application of iron at PI+F+M+dough stages led to significantly higher rice grain iron concentration as reported by Kamali et al. (2020).

According to rice cultivar, Riceberry (high Fe concentration) gave a significantly higher brown rice grain iron concentration than Khao Dawk Mali 105 (low Fe concentration) in the experimental site 2 (high soil iron content), but that was not significantly different from experimental site 1 (low soil iron content). This indicates that Riceberry responded better to iron fertilizer application than Khao Dawk Mali 105 cultivar. Saenchai et al. (2015) reported that the IR68144 cultivar (high Fe concentration) had higher Fe concentration in brown rice grain than those of the RD7 and Khao Dawk Mali 105 cultivars (low Fe concentration).

In the present experiment, an interaction effect between cultivar and growth stage was observed for the Fe concentration in brown and polished rice grain in the experimental site 1 (**Figure 1**). The foliar application of iron at the flowering (F) stage, milking (M) stage, and panicle initiation (PI)+F+M stages had no significant effect on the brown rice grain iron concentration of Riceberry. For KDML 105, foliar application of iron at the at PI+F+M stages significantly increased Fe concentration in brown rice grain compared to the foliar application of iron at the flowering stage alone. However, the effect was not significantly different between foliar iron application at PI+F+M stages and foliar application of iron at milking stage alone **(Figure 1a)**.

Regarding the iron concentration of polished rice grain, foliar application of iron at PI+F+M stages was not significantly different from foliar application of iron at the flowering stage of Riceberry, but it was significantly higher than foliar application of iron at the flowering stage of Khao Dawk Mali 105 **(Figure 1b)**.

In addition, an interaction effect between cultivar and growth stage was also observed for brown and polished rice grain iron concentrations in the experimental site 2 **(Figure 2a)**. Riceberry brown rice iron concentration was not different between the foliar application at the PI+F+M stages and the foliar application at the F stage. Nevertheless, the differences in Fe concentration from those treatments were significant for KDML 105. The iron concentration in polished grain rice was not significantly different between the foliar application at PI+F+M stages and at PI or F stage for the Riceberry cultivar. The application of iron at all growth stages including without application (control) did not show a significant difference in the iron concentration of polished rice grain for Khao Dawk Mali 105 **(Figure 2b)**. This indicated that rice cultivars performed variable in response to high and low soil iron content prior to foliar application of iron. Partitioning of Fe and Zn into rice grain depends on soil uptake and remobilization efficiency in different rice genotypes (Saenchai et al. 2015). Foliar spraying with micro-fertilizer during spike differentiation is more effective for improving micronutrient content in rice grain (Prasad et al., 2017). Foliar sprayed of fertilizer at maximum tillering, pre-flowering and flowering improved the grain Fe concentration (Yadan et al., 2013).

The brown rice grain Fe concentration was positively correlated with the polished rice grain Fe concentration for Riceberry and Khao Dawk Mali 105 in experimental site 1, the Fe concentration in brown rice grain increased, the Fe concentration in polish rice grain increased **(Figure 3)**. For experimental site 2, the brown rice grain Fe concentration was negatively correlated with the polished rice grain Fe concentration for the Riceberry cultivar ($r = 0.3397$) (Figure 4b). Meanwhile, the brown rice grain Fe concentration was positively correlated with the polished rice grain Fe concentration for Khao Dawk Mali 105 cultivar ($r = 0.7157$) **(Figure 4a)**. This indicated that Fe accumulation in brown rice grain is similarly associated with Fe accumulation in the polished rice grain when both cultivars are grown in low soil iron content. Each rice cultivar had different Fe accumulation in brown rice grain and polished rice grain when grown in soils with high iron content. The positive correlation between iron and zinc content of brown and polished rice grains was previously reported by Maganti et al. (2020) and Saenchai et al. (2014).

Table 2 Brown rice grain and polished rice grain iron concentration as affected by foliar application with iron at different growth stages and cultivars

Treatment	Experimental site 1		Experimental site 2	
	Brown rice	Polished rice	Brown rice	Polished rice
	(mg /kg)		(mg /kg)	
Growth stages (G)				
Non-application	10.14 c	7.03 c	10.22 c	7.74 c
Panicle initiation (PI)	12.97 b	10.38 a	15.66 b	10.07 a
Flowering (F)	13.10 ab	9.80 a	17.72 a	9.68 ab
Milking (M)	14.25 a	8.67 b	17.90 a	9.01 b
PI + F + M	14.28 a	10.42 a	18.30 a	10.55 a
Cultivars (C)				
Khao Dawk Mali 105	12.37	8.15 b	14.90 b	9.12 b
Riceberry	13.52	10.78 a	16.62 a	9.55 a
F-test				
G	**	**	**	*
C	ns	**	*	**
G x C	**	**	*	*
CV (a) %	12.62	6.30	2.29	5.85
CV (b) %	7.60	7.30	7.62	12.39

*, ** = significant at 0.05 and 0.01 level, respectively; ns = non-significant. Mean in the same column with different letters are significantly different at $p < 0.05$, as determined by LSD.

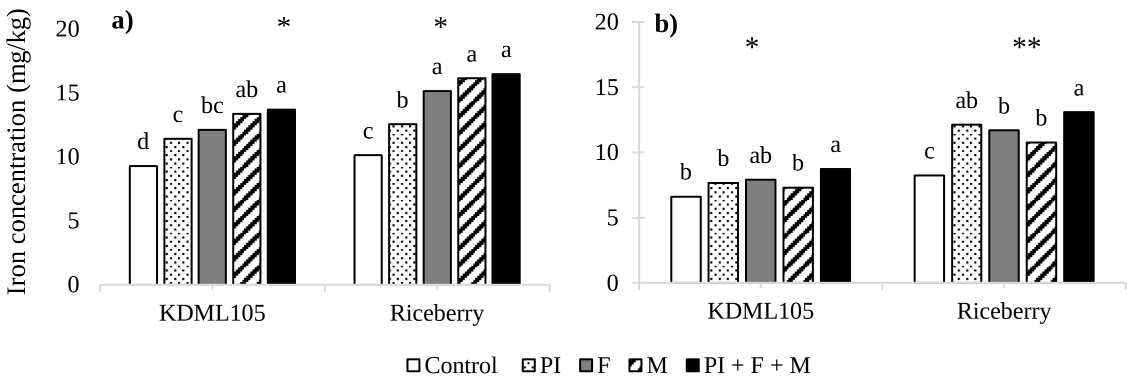

Figure 1 The interaction between growth stages and cultivars for brown (a) and polished rice grain iron concentration (b) as affected by foliar Fe application in the experimental site 1. The different growth stages were sprayed of Fe fertilizer at panicle initiation (PI), flowering (F), milking (M), and the combination treatments (PI+F+M). * and ** = significant at 0.05 and 0.01 level, respectively. Mean in the same column with different letters are significantly different at $p < 0.05$, as determined by LSD.

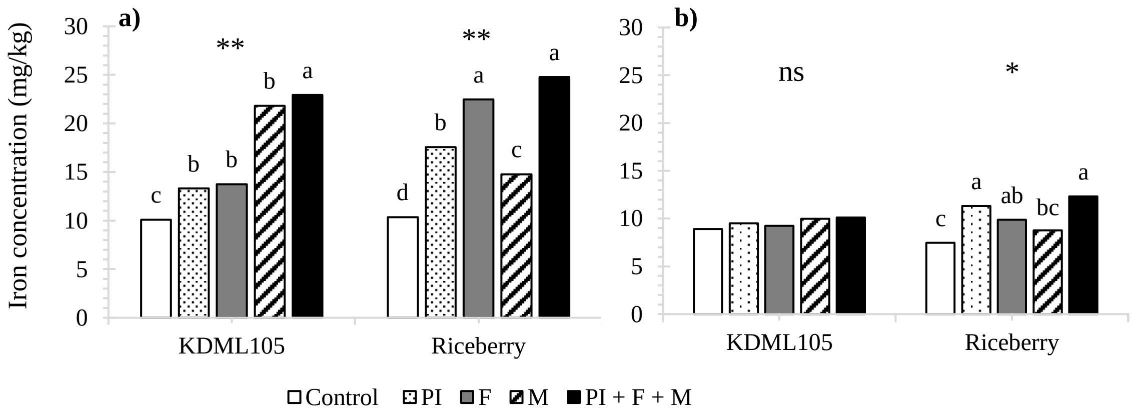

Figure 2 The interaction between growth stages and cultivars for brown rice grain iron concentration (a) and polished rice grain iron concentration (b) as affected by foliar Fe application in the experimental site 2. The different growth stages were sprayed of Fe fertilizer at panicle initiation (PI), flowering (F), milking (M), and the combination treatment (PI+F+M). * and ** = significant at 0.05 and 0.01 level, respectively, ns = non-significant. Mean in the same column with different letters are significantly different at $p < 0.05$, as determined by LSD.

Growth and yield

The foliar iron application at different growth stages had no significant effect on plant height, tiller number, leaf area index, or above-ground dry weight of rice in experimental site 1 **(Table 3 and 4)**. This was probably due to foliar iron fertilizer application at PI, F, M and PI+F+M in the present experiment which were sprayed after the maximum vegetative growth stage. The application of iron at different growth stages had a significant effect on grain number per panicle, and filled grain percentage, but not on panicle number or 1,000 grain weight **(Table 5)**. The highest grain number per panicle and filled grain percentage obtained in the foliar application of iron at PI+F+M stages. These findings are in agreement with Sudhagar Rao et al. (2019). Grain yield was significantly affected by the foliar application at different growth stages **(Table 5)**. The highest grain yield was attained in the foliar iron application at the PI+F+M stages. This was probably due to the higher grain number per panicle and filled grain percentage in the present study. The foliar $FeSO_4$ application at panicle initiation + flowering + milking + dough stages produced the maximum rice grain yield as reported by Kamali et al. (2020). Foliar $FeSO_4$ application could have increased chlorophyll content, antioxidant enzymes and their activities which could result in increasing yield components and grain yield. This finding agrees with the previous studies by Shaygany et al. (2012); Singh et al. (2013) and Kamali et al. (2020) who have found that $FeSO_4$ foliar spray at various growth stages of rice crops increased grain yield through effective translocation of Fe to economic parts. Foliar sprays are usually short lived because iron is not translocated to the new growth, they must be applied at least several times during the growing season (Wysocki and Hopkins, 2015).

For cultivar, application of iron had a significant effect on plant height and leaf area index of rice, but was not significantly different on tiller number or aboveground dry weight **(Table 3 and 4)**. The cultivar Khao Dawk Mali 105 had higher plant height and leaf area index than Riceberry in this experiment. Khao Dawk Mali 105 produced significantly the higher grain yield than Riceberry (Table 5) which might be due to higher grain number per panicle and 1,000 grain weight **(Table 5)**.

For experimental site 2, foliar application of iron at different growth stages had no significant effect on plant height, tiller number, leaf area index, above ground dry weight, yield components and grain yield of rice **(Table 3, 4 and 6)**. This was probably due to the fact that the rice plant grew in high iron content soil in the experimental site 2 **(Table 1)**. Then, the rice plant continued its uptake of Fe mainly from the soil, the growth period. Regarding rice cultivars, grain yield, and grain number per panicle were significantly affected by foliar iron application, but there was no effect on panicle number and 1,000 grain weight **(Table 6)**. Khao Dawk Mali 105 produced a higher grain number per panicle and grain yield than Riceberry. The previous research reported that Khao Dawk Mali 105 (poor Fe grain concentration) gave a significantly higher grain yield than IR68144 (rich Fe grain concentration) (Saenchai et al. 2015). Grain yield was higher in experimental site 1 than experimental site 2. This was mainly due to a higher amount of rainfall and a better distribution of the rainfall during the growing season.

The brown rice Fe concentration was negatively correlated with grain yield in experimental site 1 (r = -0.6742) **(Figure 5a)**. A similar tendency was observed (r = 0.3330) between the brown rice grain Fe concentration and grain yield in experimental site 2 **(Figure 5b**. The Fe concentration in brown rice grain increased, grain yield decreased in both experimental sites.

The polished rice Fe concentration was negatively correlated with grain yield in the experimental site 1 (r = - 0.5343) (Figure 6a). While, the polished rice grain Fe concentration was positively correlated with grain yield in the experimental site 2 (r = 0.5176) **(Figure 6b)**.

Table 3 Plant height and tiller number of rice as affected by foliar application with iron at different growth stages and cultivars at 90 days after transplanting (DAT)

Treatment	Experimental site 1		Experimental site 2	
	Plant height (cm)	Tiller (no./ hill)	Plant height (cm)	Tiller (no./ hill)
Growth stages (G)				
Panicle initiation (PI)	96.98	11.58	93.32	7.22
Flowering (F)	96.50	10.80	94.85	7.12
Milking (M)	93.48	11.22	98.85	7.25
PI + F + M	95.35	10.62	96.25	6.80
Non-application	91.90	9.35	94.90	6.08
Cultivars (C)				
Khao Dawk Mali 105	101.27 a	10.82	102.49 a	6.58
Riceberry	88.41 b	10.61	89.05 b	7.21
F-test				
G	ns	ns	ns	ns
C	*	ns	**	ns
G x C	ns	ns	ns	ns

*, ** = significant at 0.05 and 0.01 level, respectively; ns = non-significant. Mean in the same column with different letters are significantly different at $p < 0.05$, as determined by LSD.

Table 4 Leaf area index (LAI) and above ground dry weight (ADW) of rice as affected by foliar application with iron at different growth stages and cultivars at 90 days after transplanting (DAT)

Treatment	Experimental site 1		Experimental site 2	
	LAI	ADW (g /plant)	LAI	ADW (g /plant)
Growth stages (G)				
Panicle initiation (PI)	7.75	25.30	5.51	24.10
Flowering (F)	7.39	25.89	6.14	21.04
Milking (M)	7.75	24.83	6.00	23.03
PI + F + M	8.12	26.60	5.82	18.97
Non-application	6.85	25.30	5.14	23.79
Cultivars (C)				
Khao Dawk Mali 105	7.97 a	25.82	6.04	22.30
Riceberry	7.07 b	25.35	5.40	22.07
F-test				
G	ns	ns	ns	ns
C	*	ns	ns	ns
G x C	ns	ns	ns	ns

* and ns = significant at 0.05 level and non significant, respectively. Mean in the same column with different letters are significantly different at $p < 0.05$, as determined by LSD.

Table 5 Yield and yield components of rice as affected by foliar application with iron at different growth stages and cultivars in experimental site 1

Treatment	Panicle (no./plant)	Grain (no./panicle)	1,000 grain weight (g)	Filled grain (%)	Grain yield (t/ ha)
Growth stages (G)					
Panicle initiation (PI)	10.52	180.43 a	29.46	80.45 b	2.703 b
Flowering (F)	9.55	180.03 a	30.92	86.89 a	2.749 b
Milking (M)	8.5	176.10 a	28.85	87.24 a	2.654 b
PI + F + M	10.68	183.40 a	31.81	87.82 a	3.179 a
Non-application	8.38	162.13 b	28.55	86.00 a	2.512 b
Cultivars (C)					
Khao Dawk Mali 105	9.81	195.55 a	31.04 a	87.26	3.067 a
Riceberry	9.24	157.28 b	28.79 b	84.1	2.451 b
F-test					
G	ns	**	ns	**	**
C	ns	**	*	ns	**
G x C	ns	ns	ns	**	ns

*, ** = significant at 0.05 and 0.01 level, respectively; ns = non-significant. Mean in the same column with different letters are significantly different at $p < 0.05$, as determined by LSD.

Table 6 Yield and yield components of rice as affected by foliar application with iron at different growth stages and cultivars in experimental site 2

Treatment	Panicle (no. /plant)	Grain (no./panicle)	1,000 grains weight (g)	Filled grain (%)	Grain yield (t/ ha)
Growth stages (G)					
Panicle initiation (PI)	8.45	185.11	25.76	81.45	2.142
Flowering (F)	8.84	190.55	26.55	83.26	2.292
Milking (M)	8.20	180.09	27.21	82.96	2.170
PI + F + M	8.77	175.15	27.10	83.86	2.197
Non-application	9.04	186.63	24.75	81.45	2.153
Cultivars (C)					
Khao Dawk Mali 105	8.68	189.80 a	26.11	83.16	2.527 a
Riceberry	8.64	177.22 b	26.44	82.50	1.854 b
F-test					
G	ns	ns	ns	ns	ns
C	ns	**	ns	ns	**
G x C	ns	ns	ns	ns	ns

** and ns = significant at 0.01 level and non significant, respectively. Mean in the same column with different letters are significantly different at $p < 0.05$, as determined by LSD.

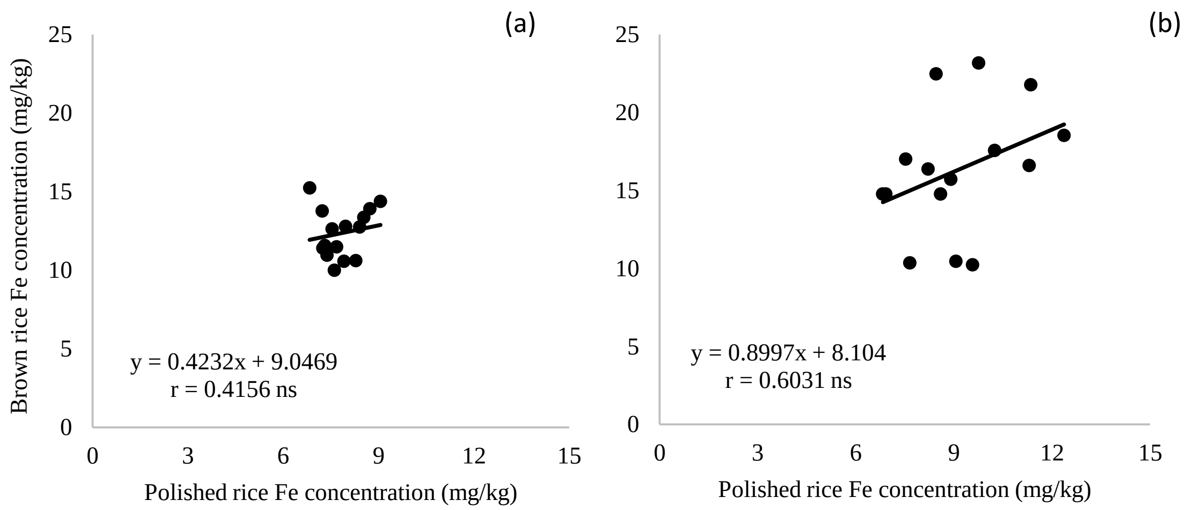

Figure 3 The relationship between brown rice grain and polished rice grain Fe concentration of Khao Dawk Mali 105 (a), and Riceberry (b) in the experimental site 1.

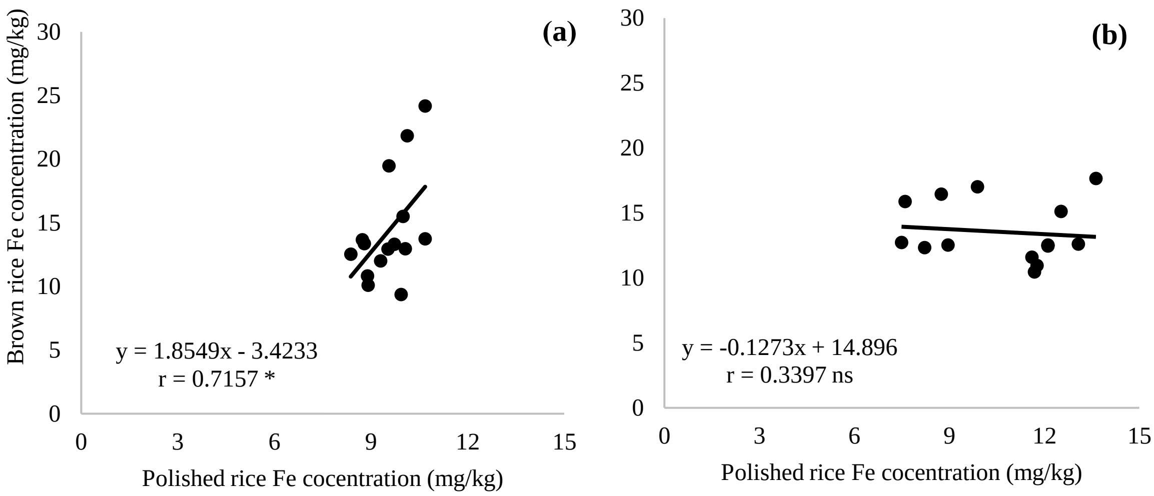

Figure 4 The relationship between brown rice grain and polished rice grain Fe concentration of Khao Dawk Mali 105 (a), and Riceberry (b) in the experimental site 2.

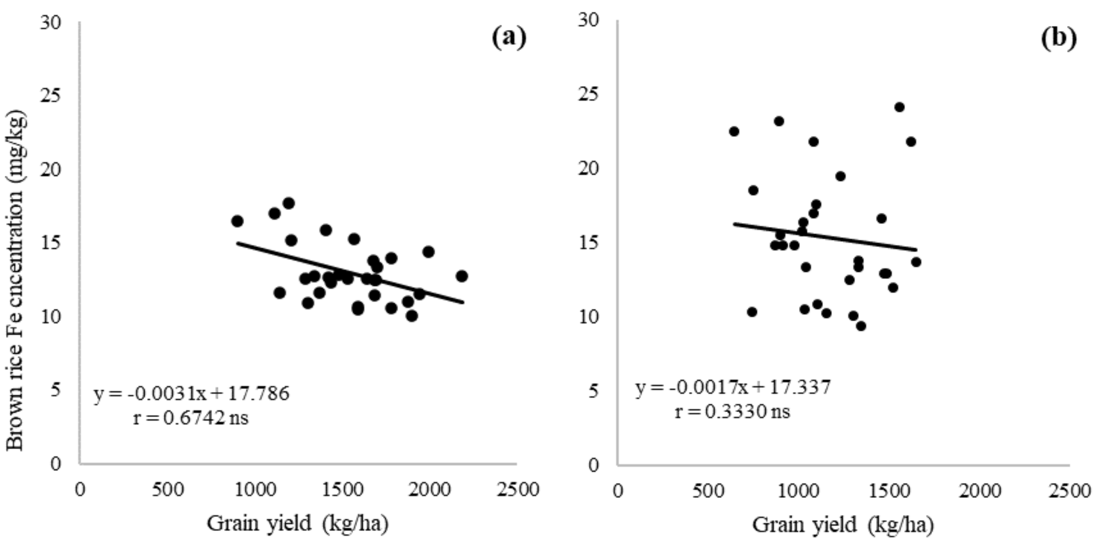

Figure 5 The relationship between brown rice grain Fe concentration and grain yield of two rice cultivars in the experimental site 1 (a), and the experimental site 2 (b).

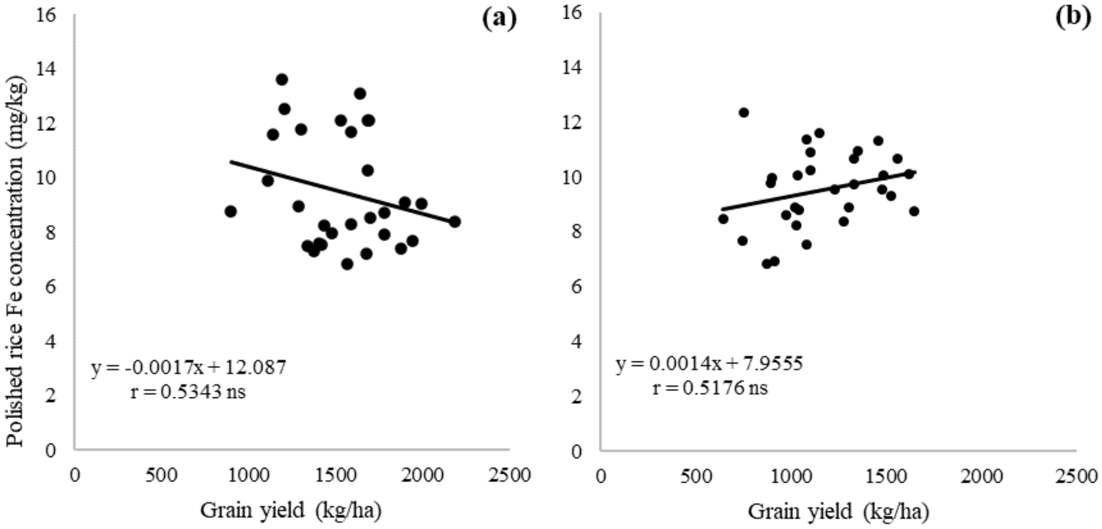

Figure 6 The relationship between polished rice grain Fe concentration and grain yield of two rice cultivars in the experimental site 1 (a), and the experimental site 2 (b).

Conclusion

Application of iron fertilizer at different growth stages significantly enriched brown and polished rice grain iron concentrations for Riceberry (high iron concentration in grain), similarly for KDML105 (low iron concentration in grain) in brown rice grain excepted the Fe concentration in polished rice grain. The foliar Fe application at the PI+F+M stages was the most effective methods to increase iron concentration in rice grain. The responsiveness to foliar Fe fertilizer application at different growth stages is different between rice cultivars. Riceberry cultivar seemed to be more responsive to iron fertilizer application than Khao Dawk Mali 105, especially when grown under high soil iron content. The maximum grain yield was obtained when Fe fertilizer was applied at PI+F+M stages, especially rice grown under low soil iron content. Khao Dawk Mali 105 cultivar produced higher grain yield than Riceberry both locations.

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