CHAPTER 3

Effects of iron and zinc foliar application on rice grain quality

3.1 Introduction

In chapter 2, results showed that cropping season had influence on quality of the rice grain. In the summer season, grain Fe was higher in brown rice, but not found in white rice; while grain Zn was lower in both brown and white rice. In addition, milling quality of rice in the summer season was very poor compared with the other seasons. Therefore, improving nutritional and milling quality of rice grain in summer season which is the normal off season rice growing in the irrigated areas might be the advantage for farmers and consumers.

Rice is a poor source of Fe and Zn. It has been reported that the Fe and Zn concentrations in brown rice among different varieties varied from 4 to 24 mg Fe/kg and 13.5-58.4 mg Zn/ kg (Gregorio, 2002). White rice, the form most commonly consumed, generally has much lower Fe and Zn, at 2 to 11 mg Fe/kg (Prom-u-thai et al., 2007a) and 9.7–26.5 mg Zn/kg (Prom-u-thai *et al.*, 2010; Sellappan *et al.*, 2009) due to the removal of high concentration of Fe and Zn bran fraction in the milling process (Prom-u-thai *et al.*, 2007b). There are several potential approaches to increase Fe and Zn in rice grain including conventional breeding (Graham *et al.*, 1999; Welch and Graham, 2004), nutrient fortification (Prom-u-thai *et al.*, 2008; Prom-u-thai *et al.*, 2010)) and biofortification (Vasconcelos *et al.*, 2003). Currently, foliar nutrient application was reported to be promising cost effective and sustainable strategy to overcome improving plant micronutrient deficiency. The considerable progress has been made on impact of foliar Zn fertilizer in wheat (Cakmak, 2008; Wissuwa *et al.*, 2008). Moreover, foliar spray Zn at the early milk stage and dough stage appeared an effective inversing grain Zn in wheat (Cakmak *et al.*, 2010). In rice, it has been reported that foliar Zn fertilizer has also been improved grain Zn (Fang *et al.*, 2008; Phattarakul *et al.*, 2012; Wissuwa *et al.*, 2008). Positive responses to foliar Fe have also

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been reported in some rice varieties (Wei *et al.*, 2012). However, there is no information on the effectiveness of foliar Fe or Zn application on the improvement of grain nutritional qualities together with milling quality.

In previous studies it has been reported that N status of the plant can influence enrichment grain Fe and Zn. Positive correlation has been found between grain Fe and N concentration in wheat (Cakmak *et al.*, 2010) and rice (Prom-u-thai *et al.*, 2007a). In wheat this has been supported by studies that found increasing soil N application significantly improved shoot and grain Fe concentrations (Cakmak *et al.*, 2010; Kutman *et al.*, 2010). Similarly, foliar spray of urea enhanced wheat grain Fe (Aciksoz *et al.*, 2011; Kutman *et al.*, 2010). Nonetheless, influence of foliar N application on Fe and Zn in rice is still to be tested.

Nitrogen fertilizer was reported to be able to improve milling quality such as head rice yield when soil applied at booting stage of flowering stage (Alcantara and Cassman, 1996; Leesawatwong *et al.*, 2005; Perez *et al.*, 1996) by increased N concentration and protein fraction in rice grain especially in peripheral region (Leesawatwong *et al.*, 2005). In addition, nitrogenous compound had been reported to be able to increased Fe and Zn concentration in rice grain when apply to rice plant. Yuan *et al*. (2012) sprayed Fe fertilizer with nicotianamine (NA) at the flowering stage and found Fe concentration in brown rice of most varieties was increasing. Wei *et al*. (2012) reported that addition amino acid (AA) to Fe foliar application increased grain Fe in some varieties. Nevertheless, there is still lack of information whether the use of nitrogen fertilizer together with Fe and Zn by foliar spraying will be able to improve grain Fe and Zn as well as milling quality.

Therefore, the present study aims to evaluate the effects of foliar Fe and Zn and combined Fe or Zn fertilizers with nitrogen fertilizer (urea) on milling quality and nutritional quality of rice grain in the summer cropping season.

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3.2 Materials and Methods

3.2.1 Rice varieties and culture

To investigate the effects of foliar spraying of combined urea and Fe or Zn on rice grain quality, a field experiment was conducted at the Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand, between February-June, 2010. The same 4 popular photoperiod insensitive rice varieties used in the previous chapter (SPR1, CNT1, PTT1 and RD21) were used in this experiment. The experiment was laid out as a completely randomized block design with 4 replications. Seedling preparation and supplementary fertilization were as described previously (Chapter 3). The following foliar spraying treatments were applied:

1. Distilled water (Control)
2. 0.5% (w/v) $ZnSO_4.7H_2O$ (Zn)
3. 0.5% (w/v) $FeSO_4.7H_2O$ (Fe)
4. 1% (w/v) urea
5. Combined spray of 1% (w/v) urea and 0.5% (w/v) $ZnSO_4.7H_2O$ (urea-Zn)
6. Combined spray of 1% (w/v) urea and 0.5% (w/v) $FeSO_4.7H_2O$ (urea-Fe)

The surfactant was added into spray solutions to increase efficiency of the foliar treatments. Foliar sprays were applied using fine mist hand sprayer once in the evening of day 7 after flowering to 6 plants per treatment per replicate plot. Plants were sprayed thoroughly until the solution started to run-off the panicles and flag leaves. To prevent cross contamination, the surrounding plants were covered with a plastic sheet while spraying. The volume of sprayed solution was applied about 45 to 55cc. per replication (The spray solution volumes were recorded before and after spraying.) Shoots were harvested at maturity, and the yield and grain quality were assessed.

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3.2.2 Data collection

1) Grain yield

Rice was harvested at physiological maturity. The grain of the 6 treated plants per plot were air-dried to 11-12% moisture content then weighed.

2) Grain quality

Rough rice were dehusked with a laboratory husker machine (model P-1, Ngek Seng Huat, Thailand) then 30 g of the resulting brown rice were milled for 30 s with a laboratory milling machine (model K-1, Ngek Seng Huat, Thailand) to yield white rice. The husker and miller were Teflon-coated for all containers and handles to avoid Fe contamination during the process (Prom-u-thai *et al.*, 2007a; Prom-u-thai *et al.*, 2007b). White rice was separated into head rice (≥ 4/5 whole grain length) and broken rice and weighed separately for calculating the percentage of head rice yield, percentage of broken grain and degree of milling (DOM). About 5 g of white rice were determined grain whiteness and transparency by a Satake Milling Meter (model MM 1D, Japan).

Brown and white rice were analyzed for the concentration of nitrogen (N), iron (Fe) and zinc (Zn). Samples were oven dried at 75°C for 72 h and dry-ashed in a muffle furnace at 535 °C for 8 h. The ash was dissolved in HCl (1:1; HCl:deionized water and the concentration of Fe and Zn were determined using an Hitachi Z-8230 atomic absorption spectrophotometer (AAS) (Allan, 1961), N was analyzed by the Kjeldahl method (Jackson, 1967). Soybean leaf was used for reference material in all samples to check the quality of Fe, Zn and N analyses.

3) Statistical analysis

Analysis of variance was conducted to detect the differences of grain yield and grain qualities by using Statistic 8 (analytical software, SXW).

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Data on proportion were arcsine transformed before analysis. The least significant difference (LSD) at $P< 0.05$ was applied to compare the means for significant differences between variety and spray treatment.

3.3 Results

3.3.1 Grain and milling quality

Foliar applications had no effect on the weight of filled grain per plant and total grain weight per plant (Table 3.1 and 3.2). However, thousand filled grain weight was affected foliar application differently by the rice varieties by foliar application interaction ($P<0.001$). RD21showed a slight depression in thousand filled grain weight with foliar sprayed of Fe and Zn with and without urea, while there was on effect in other three varieties (Table 3.3).

Head rice yield was depressed by some foliar treatments but unaffected by others, with varieties responding to the foliar treatments in the same way (Table 3.4). Generally, urea and urea with Zn had no affected on percent head rice when compared to the control. Head rice yield was depressed when sprayed with Zn alone and Fe with and without urea. Among rice varieties, SPR1 (57.5%) had the highest head rice yield followed by CNT1 and RD21 (47.6% and 48.2%, respectively) and the lowest was PTT1 (38.0%).

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Table 3.1 Weight of filled grain of 4 rice varieties sprayed with 6 fertilizer treatments.

Treatment	Weight of filled grain (g/plant)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	25.7	31.0	22.3	25.1	26.0
Zn	31.1	25.2	28.0	18.0	25.6
Fe	24.4	19.3	23.0	22.7	22.3
Urea	24.4	28.4	27.9	23.0	25.9
Urea-Zn	27.4	25.9	27.0	24.9	26.3
Urea-Fe	27.3	27.6	28.2	22.0	26.3
Mean	26.7	26.2	26.1	22.6	
	Variety	Treatment	Variety x Treatment		
F-test	ns	ns	ns		

Table 3.2 Total grain weight of 4 rice varieties sprayed with 6 fertilizer treatments.

Treatment	Total grain weight (g/plant)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	29.9	32.7	24.6	26.2	28.3
Zn	32.5	26.5	30.1	20.2	27.3
Fe	27.1	22.7	26.3	24.8	25.2
Urea	25.8	29.6	29.8	24.5	27.4
Urea-Zn	28.9	27.2	29.1	26.3	27.9
Urea-Fe	29.9	28.9	30.4	24.1	28.3
Mean	29.0	27.9	28.4	24.3	
	Variety	Treatment	Variety x Treatment		
F-test	ns	ns	ns		

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Table 3.3 Thousand filled grain weight of 4 rice varieties sprayed with 6 fertilizer treatments.

Treatment	Thousand filled grain weight (g)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	25.2 Ab	26.3 Ab	24.6 Ab	31.0 Aa	26.8
Zn	25.5 Ab	26.1 Ab	25.4 Ab	29.9 Ba	26.7
Fe	24.7 Ab	26.1 Ab	24.8 Ab	29.9 Ba	26.4
Urea	25.5 Ab	25.3 Ab	25.7 Ab	30.0 ABa	26.6
Urea-Zn	24.5 Ab	25.3 Ab	26.3 Ab	29.1 Ba	26.3
Urea-Fe	24.8 Ab	26.3 Ab	25.0 Ab	29.6 Ba	26.4
Mean	25.0	25.9	25.3	29.9	

	Variety	Treatment	Variety x Treatment
F-test	***	ns	**
$LSD_{0.05}$	0.4		1.1

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

Table 3.4 Percent head rice of 4 varieties after sprayed with 6 fertilizer treatments.

Treatment	Head rice yield (%)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	58.5	46.0	38.6	60.8	51.0 A
Zn	56.7	54.2	41.7	38.7	47.8 AB
Fe	49.7	40.3	30.7	50.8	42.9 B
Urea	62.3	53.8	39.5	45.1	50.2 A
Urea-Zn	63.6	50.2	41.2	52.8	52.0 A
Urea-Fe	54.3	41.3	36.3	41.2	43.3 B
Mean	57.5 a	47.6 b	38.0 c	48.2 b	

	Variety	Treatment	Variety x Treatment
F-test	***	**	ns
$LSD_{0.05}$	5.0	6.1	

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3.3.2 Nutritional quality

1) Zinc in rice grain

The foliar treatment affected the concentration of Zn in brown and white rice differently among the rice varieties (Table 3.5). Compared to the control (water), the foliar application containing Zn increased Zn concentration in brown rice in all varieties. Spraying Zn alone increased brown rice Zn concentration to a similar extent as urea-Zn in all varieties except in RD21. The Zn concentration was increased by 43% in SPR1, 35% in CNT1 and 45% PTT1. In RD21, the Zn concentration was increased by 21% when sprayed with Zn alone and more (45%) with urea-Zn

Like in brown rice, the Zn concentration in white rice was increased by foliar Zn applications to different extent in the different varieties (Table 3.6). Compared to control, the white rice Zn concentration was increased by foliar Zn by 45% in SPR1, 33% in CNT1, 50% in PTT1 and 21% in RD21, with no significant additional effect of applying Zn with urea. The Zn concentration in brown rice and white rice were closely correlated (r=0.73, $P < 0.001$) (Figure 3.1)

The content of Zn in brown rice showed a similar response to variety, foliar application and variety by foliar application interaction ($P<0.05$) (Table 3.7). Brown rice Zn content was increased foliar with Zn treatments. Foliar Zn and urea-Zn increased brown rice Zn content similarly in all varieties except RD21. The brown rice Zn content was increased by average 39% in SPR1, 31% in CNT1 and 39% in PTT1 while RD21, the Zn content was increased when sprayed with urea-Zn by 44%.

The white rice Zn content was increased by foliar Zn and urea-Zn in all varieties, but RD21 was an exception. Compared to control, the Zn content of SPR1, CNT1 and PTT1 was increased by 27%, 15% and 25%, respectively. For RD21, Zn content was increased 40% when sprayed with Zn and up to 60% when sprayed with urea-Zn (Table 3.8).

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Table 3.5 Zn concentration in brown rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	Zn concentration (mg/kg)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	21.8 Ba	19.4 Ca	20.8 Ca	22.0 Ca	21.0
Zn	31.6 Aa	25.8 Ab	31.3 Aa	26.6 Bb	28.8
Fe	21.4 Ba	19.5 Ca	22.2 BCa	20.2 Ca	20.8
Urea	22.2 Ba	21.6 BCa	23.5 BCa	22.7 Ca	22.5
Urea-Zn	31.1 Aab	26.4 Ac	28.9 Abc	31.8 Aa	29.6
Urea-Fe	22.3 Ba	22.6 Ba	24.0 Ba	22.6 Ca	22.9
Mean	25.0	22.5	25.1	24.3	

	Variety	Treatment	Variety x Treatment
F-test	***	***	**
$LSD_{0.05}$	1.1	1.4	2.8

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Table 3.6 The Zn concentration in white rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	Zn concentration (mg/kg)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	17.3 Bab	15.5 Bb	18.9 Ba	14.8 Cb	16.6
Zn	22.8 Aab	17.1 ABc	24.8 Aa	21.7 Ab	21.6
Fe	18.0 Ba	15.1 Bb	19.9 Ba	18.7 Ba	17.9
Urea	17.9 Bab	16.1 ABb	18.6 Ba	15.2 Cc	16.9
Urea-Zn	20.8 Ab	18.3 Ac	22.4 Aab	24.0 Aa	21.4
Urea-Fe	16.0 Bb	15.4 Bb	18.8 Ba	16.4 BCab	16.7
Mean	18.8	16.2	20.6	18.5	

	Variety	Treatment	Variety x Treatment
F-test	***	***	**
$LSD_{0.05}$	1.6	1.3	2.6

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

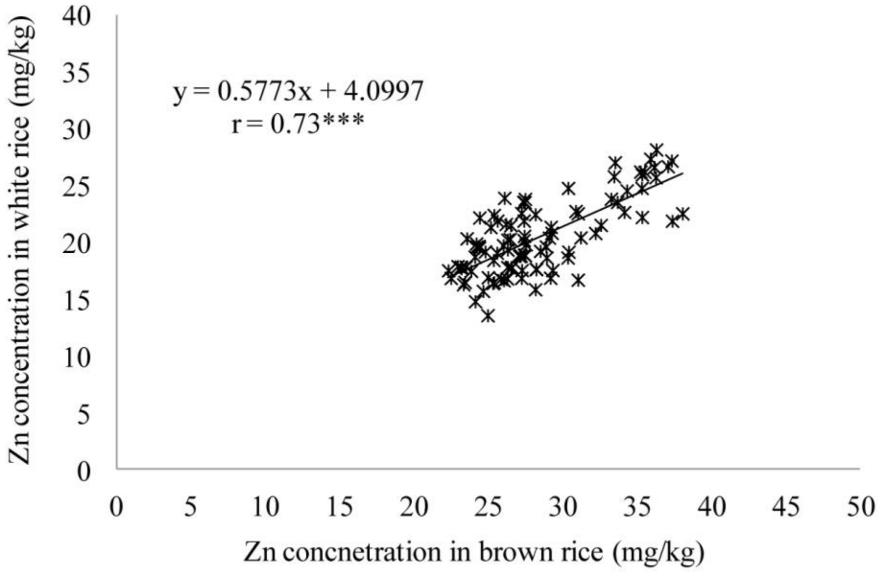

Figure 3.1 Relationships between Zn concentrations in brown and white rice among four rice varieties after sprayed with 6 fertilizer treatments.

Table 3.7 Zn content in brown rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	Zn content (µg/grain)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	0.41 Bb	0.38 CDb	0.39 Bb	0.48 BCa	0.42
Zn	0.58 Aa	0.49 ABb	0.57 Aa	0.53 Bab	0.54
Fe	0.39 Bab	0.36 Db	0.38 Bb	0.45 Ca	0.40
Urea	0.40 Bb	0.40 CDb	0.43 Bb	0.51 BCa	0.44
Urea-Zn	0.56 Ab	0.51 Ab	0.52 Ab	0.69 Aa	0.57
Urea-Fe	0.40 Bb	0.43 BCb	0.42 Bb	0.51 BCa	0.44
Mean	0.46	0.43	0.45	0.53	

	Variety	Treatment	Variety x Treatment
F-test	***	***	*
LSD $_{0.05}$	0.03	0.03	0.07

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Table 3.8 The Zn content in white rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	Zn content (µg/grain)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	0.28 Bab	0.26 BCb	0.30 Ba	0.30 Da	0.28
Zn	0.37 Ab	0.29 ABc	0.39 Ab	0.44 Ba	0.37
Fe	0.29 Bc	0.25 Cd	0.32 Bb	0.37 Ca	0.31
Urea	0.29 Ba	0.27 BCa	0.30 Ba	0.30 Da	0.29
Urea-Zn	0.34 Abc	0.31 Ac	0.36 Ab	0.48 Aa	0.37
Urea-Fe	0.26 Bb	0.26 BCb	0.30 Ba	0.33 Da	0.29
Mean	0.3	0.3	0.3	0.4	
	Variety	Treatment	Variety x Treatment		
F-test	***	***	**		
$LSD_{0.05}$	0.02	0.02	0.04		

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2) Iron in rice grain

The foliar treatment affected the concentration of Fe in brown and white rice differently among the rice varieties (Table 3.9). Compared to the control, foliar Fe alone increase brown rice Fe concentration in SPR1 and PTT1by 19% but not in CNT1 and RD21. Foliar urea containing solutions increased brown rice Fe concentration more than foliar Fe alone in most varieties except PTT1 which had similar Fe concentration with all Fe and urea treatments by 24 %. In SPR1 and CNT1 and RD21, spraying urea alone increased brown rice Fe concentration to a similar extent as urea-Fe. The Fe concentration was increased by 14% in SPR1, 25% in CNT1 and 25% PTT1.

The white rice Fe concentration was increased by foliar treatments with varieties responding to foliar treatments in the same way (Table 3.10). The Fe concentration in white rice was increased with foliar with Fe and containing urea when compared to the control. Among rice varieties, PTT1 (5.8 mg/kg) had the higher white rice Fe than other three varieties which had similar Fe concentration (range 4.3 to 4.6 mg/kg). The Fe concentration in brown rice weakly correlated with Fe concentration in white rice (r=0.24*) (Figure 3.2)

The foliar treatment affected the content of Fe in brown rice differently among the rice varieties ($P<0.05$) (Table 3.11). Brown rice Fe content was increased with foliar Fe and even increased when sprayed with containing urea. Compared to the control, the Fe content was slightly increased by foliar Fe by 5% in SPR1, 13% in CNT11 and 5% in PTT1. While in RD21, foliar Fe had no affected on the brown rice Fe content. There was no significant additional effect of foliar Zn or Fe with urea, the Fe content was increased in average by 30% when compared to the control. Unlike brown rice, the white rice Fe content was increased by foliar treatments with varieties responding to foliar treatments in the same way. Foliar Fe and containing urea increased white rice Fe content similarly when

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compared to the control. PTT1 and RD21 had similar Fe content in white rice which was higher than CNT1 and SPR1, respectively (Table 3.12).

Table 3.9 The Fe concentration in brown rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	Fe concentration (mg/kg)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	9.9 Ca	7.9 Cb	8.3 Cb	8.8 Cab	8.7
Zn	9.7 Ca	8.9 BCa	9.1 BCa	9.9 Ca	9.4
Fe	10.8 BCa	8.7 Cb	9.9 ABa	8.8 Cb	9.6
Urea	11.3 Aba	9.9 Abc	10.0 ABb	11.0 Bab	10.6
Urea-Zn	10.4 BCb	10.3 Ab	10.0 ABb	12.3 Aa	10.8
Urea-Fe	12.1 Aa	10.9 Ab	10.9 Ab	11.6 ABab	11.4
Mean	10.7	9.5	9.7	10.4	
	Variety	Treatment	Variety x Treatment		
F-test	***	***	*		
$LSD_{0.05}$	0.5	0.6	1.1		

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Table 3.10 The Fe concentration in white rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	Fe concentration (mg/kg)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	3.6	4.1	4.7	4.2	4.2 B
Zn	3.6	4.0	5.5	4.4	4.4 B
Fe	5.0	4.7	6.1	4.7	5.1 A
Urea	4.5	5.0	5.6	4.7	4.9 A
Urea-Zn	4.3	5.0	5.7	5.3	5.1 A
Urea-Fe	4.6	4.9	7.1	4.5	5.2 A
Mean	4.3 b	4.6 b	5.8 a	4.6 b	

	Variety	Treatment	Variety x Treatment
F-test	**	***	ns
$LSD_{0.05}$	0.4	0.5	

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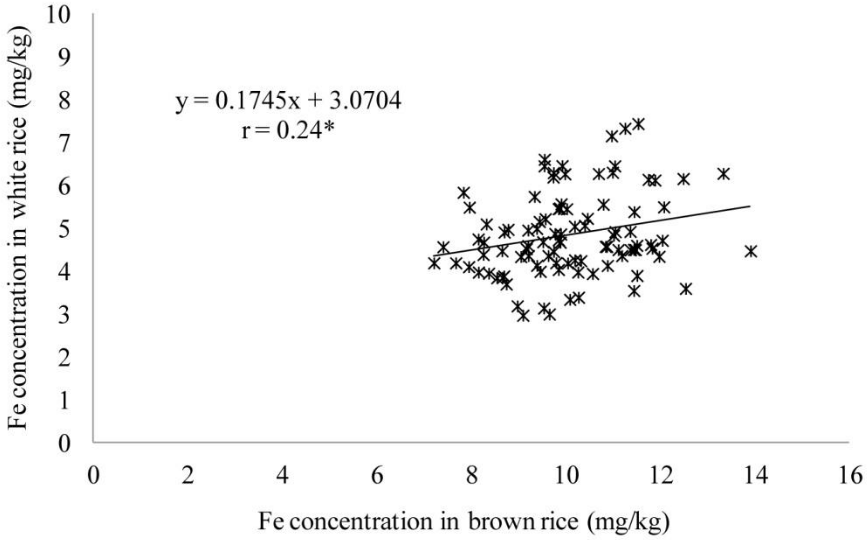

Figure 3.2 Relationships between Fe concentrations in brown and white rice among 4 rice varieties after sprayed with 6 fertilizer treatments

Table 3.11The Fe content in brown rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	Fe content (µg/grain)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	0.19 Ba	0.15 Cb	0.16 Bb	0.20 Ba	0.17
Zn	0.18 Bb	0.17 BCb	0.17 Bb	0.22 Ba	0.18
Fe	0.20 ABa	0.17 BCc	0.17 ABc	0.20 Ba	0.18
Urea	0.21 Ab	0.18 ABb	0.18 ABb	0.25 Aa	0.21
Urea-Zn	0.19 Bb	0.20 ABb	0.18 ABb	0.27 Aa	0.21
Urea-Fe	0.22 Ab	0.21 Ab	0.19 Ab	0.26 Aa	0.22
Mean	0.20	0.18	0.18	0.23	

	Variety	Treatment	Variety x Treatment
F-test	***	***	*
$LSD_{0.05}$	0.01	0.01	0.03

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Table 3.12 The Fe content in white rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	Fe content (μg/grain)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	0.06	0.07	0.07	0.08	0.07 C
Zn	0.06	0.07	0.09	0.09	0.08 B
Fe	0.08	0.08	0.10	0.10	0.09 A
Urea	0.07	0.08	0.09	0.10	0.09 A
Urea-Zn	0.07	0.09	0.09	0.11	0.09 A
Urea-Fe	0.07	0.08	0.11	0.09	0.09 A
Mean	0.07 c	0.08 b	0.09 a	0.09 a	

	Variety	Treatment	Variety x Treatment
F-test	***	***	ns
LSD $_{0.05}$	0.01	0.01	

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

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3) Nitrogen in rice grain

The N concentration in brown rice was affected by variety and foliar application (Table 3.13). Compared to the control, foliar urea containing solutions increased brown rice N concentration similarly. RD21 was found to have the highest grain N followed by SPR1 and PTT1 while the lowest was in CNT1.

There was no effect of foliar application in grain N concentration in white rice. The N concentration in SPR1, CNT1 and PTT1 did not differ but lower than that in RD21 (Table 3.14).

Brown rice N content followed the same trend as brown rice N concentration being increased when foliar urea containing solutions when compared to the control. RD21 had higher N content than SPR1 and PTT1 and CNT1 which were similar (Table 3.15). Foliar treatments had no effect on white rice N content. RD21 had the highest white rice N content while others three varieties had similarly N content (Table 3.16).

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Table 3.13 The N concentration in brow rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	N concentration (%)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	1.28	1.23	1.23	1.39	1.28 BCD
$ZnSO_4$	1.26	1.14	1.29	1.30	1.25 D
$FeSO_4$	1.26	1.18	1.27	1.36	1.27 CD
Urea	1.30	1.24	1.31	1.47	1.33 AB
Urea-$ZnSO_4$	1.27	1.30	1.26	1.45	1.32 ABC
Urea-$FeSO_4$	1.37	1.21	1.28	1.55	1.35 A
Mean	1.29 b	1.22 c	1.27 b	1.42 a	

	Variety	Treatment	Variety x Treatment
F-test	***	**	ns
$LSD_{0.05}$	0.05	0.61	

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

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Table 3.14 The N concentration in white rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	N concentration (%)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	1.22	1.12	1.19	1.35	1.22
Zn	1.20	1.12	1.20	1.28	1.20
Fe	1.19	1.14	1.16	1.33	1.21
Urea	1.26	1.20	1.24	1.38	1.27
Urea-Zn	1.21	1.23	1.18	1.37	1.25
Urea-Fe	1.20	1.19	1.17	1.39	1.24
Mean	1.21 b	1.17 b	1.19 b	1.35 a	

	Variety	Treatment	Variety x Treatment
F-test	***	ns	ns
$LSD_{0.05}$	0.06		

The lowercase letter is used for comparison between columns. The different letters are significantly different by LSD (P<0.05).

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Table 3.15 The N content in brown rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	N content (µg/grain)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	0.24	0.24	0.23	0.31	0.25 AB
Zn	0.24	0.22	0.24	0.26	0.24 B
Fe	0.23	0.22	0.22	0.30	0.24 B
Urea	0.24	0.23	0.24	0.33	0.26 A
Urea-Zn	0.23	0.25	0.23	0.32	0.26 A
Urea-Fe	0.25	0.23	0.23	0.35	0.26 A
Mean	0.24 b	0.23 b	0.23 b	0.31 a	

	Variety	Treatment	Variety x Treatment
F-test	***	**	ns
$LSD_{0.05}$	0.02	0.02	

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

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Table 3.16 The N content in white rice (mg/grain) of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	N content (µg/grain)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	0.20	0.19	0.19	0.27	0.21
Zn	0.19	0.19	0.19	0.26	0.20
Fe	0.19	0.19	0.19	0.26	0.21
Urea	0.20	0.20	0.20	0.28	0.22
Urea-Zn	0.20	0.20	0.19	0.27	0.21
Urea-Fe	0.19	0.20	0.19	0.28	0.21
Mean	0.19 b	0.19 b	0.19 b	0.27 a	

	Variety	Treatment	Variety x Treatment
F-test	***	ns	ns
$LSD_{0.05}$	0.02		

The lowercase letter is used for comparison between columns. The different letters are significantly different by LSD (P<0.05).

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4) Phosphorus in rice grain

There was no effect of foliar application on grain P concentration in brown rice (Table 3.17). However, the foliar treatment affected the concentration of P in white rice differently among the rice varieties (*P*<0.01) (Table 3.18). Compared to the control, foliar treatments decreased white rice P concentration in all varieties, excepted foliar with Fe alone. The biggest decrement of white rice P concentration was found when sprayed with containing urea in all varieties except RD21. White rice P concentration of RD21 was increased with all foliar treatment except foliar with urea.

The foliar treatments had no effect on brown rice P content (Table 3.19). However, the foliar treatment affected P content in white rice differently (*P*<0.05) (Table 3.20). Compared to the control, foliar with urea-Zn and urea-Fe clearly decreased white rice P content in SPR1, CNT1 and PTT1 by average 19%, 12% and 12%, respectively. While foliar urea-Zn and urea-Fe had no effect on white rice P content in RD21.

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Table 3.17 The P concentration in brown rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	P concentration (%)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	0.34	0.34	0.34	0.37	0.35
Zn	0.34	0.34	0.35	0.37	0.35
Fe	0.35	0.34	0.35	0.36	0.35
Urea	0.34	0.36	0.33	0.38	0.35
Urea-Zn	0.34	0.35	0.35	0.39	0.36
Urea-Fe	0.34	0.36	0.36	0.38	0.36
Mean	0.34 b	0.35 b	0.35 b	0.38 a	

	Variety	Treatment	Variety x Treatment
F-test	ns	***	ns
$LSD_{0.05}$		0.01	

The lowercase letter is used for comparison between columns. The different letters are significantly different by LSD (P<0.05).

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Table 3.18 The P concentration in white rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	P concentration (%)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	0.22 Abc	0.23 Ab	0.25 Aa	0.21 Dc	0.23
Zn	0.20 BCb	0.21 BCb	0.23 Ba	0.24 Aa	0.22
Fe	0.22 Ac	0.22 Abc	0.26 Aa	0.24 Ab	0.23
Urea	0.21 Aba	0.21 Bca	0.22 Bca	0.21 Da	0.21
Urea-Zn	0.19 Cc	0.20 Cbc	0.21 Cb	0.23 Bca	0.21
Urea-Fe	0.17 Dc	0.20 Cb	0.22 BCa	0.22 Cda	0.20
Mean	0.20	0.21	0.23	0.22	

	Variety	Treatment	Variety x Treatment
F-test	***	***	**
LSD $_{0.05}$	0.01	0.01	0.02

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

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Table 3.19 The P content in brown rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	P content (μg/grain)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	0.064	0.067	0.061	0.086	0.069
Zn	0.064	0.065	0.063	0.074	0.066
Fe	0.064	0.064	0.060	0.079	0.067
Urea	0.063	0.066	0.061	0.086	0.069
Urea-Zn	0.061	0.068	0.063	0.085	0.069
Urea-Fe	0.061	0.067	0.063	0.085	0.069
Mean	0.063 c	0.066 b	0.062 c	0.082 a	
	Variety	Treatment	Variety x Treatment		
F-test	***	ns	ns		
$LSD_{0.05}$	0.003				

The lowercase letter is used for comparison between columns. The different letters are significantly different by LSD (P<0.05).

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Table 3.20 The P content in white rice of 4 varieties after sprayed with 6 fertilizer treatments

Treatment	P content (µg/grain)				Mean
	SPR1	CNT1	PTT1	RD21	
Water	0.035 Ac	0.038 Abc	0.039 ABab	0.042 Ba	0.038
Zn	0.033 Abc	0.035 ABbc	0.037 BCb	0.048 Aa	0.038
Fe	0.035 Ac	0.037 ABc	0.041 Ab	0.048 Aa	0.040
Urea	0.034 Ab	0.035 ABb	0.035 CDb	0.042 Ba	0.036
Urea-Zn	0.030 BCc	0.034 BCb	0.033 Dbc	0.045 ABa	0.035
Urea-Fe	0.027 Cd	0.033 Cc	0.035 CDb	0.042 Ba	0.034
Mean	0.032	0.035	0.036	0.044	

	Variety	Treatment	Variety x Treatment
F-test	***	***	*
$LSD_{0.05}$	0.001	0.002	0.004

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD ($P<0.05$).

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3.4 Discussion

Chapter 3 found that both nutritional and milling quality of rice grain was influenced by the season under which the crop was grown. In this chapter I found that that foliar application can increase rice grain nutritional quality in both of grain Fe and Zn, however, it cannot improved milling quality of the summer season rice.

The nutrient solutions were directly sprayed to rice panicle, however, there was no negative effect on grain fertilization that shown on the filled grain number and total grain weight per plant of rice was similar to the control. It might due to foliar application was done when rice was fertilized completely. This was in agreement with the previous studies that foliar application of mineral elements after flowering did not affect grain yield of rice (Zhang *et al.*, 2008). Nevertheless, the 1000- grain weight of RD21 was slightly increased when sprayed with urea plus Zn but there was no effect on 1000-grain weight of other varieties in all treatments.

Foliar Fe alone or Fe plus urea did not improve head rice yield. In contrast, head rice yield was decreased when sprayed with Fe containing solutions. This result was the same as in spraying with Zn alone. However, the effect of foliar Zn on head rice yield was disappeared when sprayed urea plus Zn. It has been reported that applying soil nitrogen fertilizer at flowering stage could improved milling quality by increased protein bodies in rice grain which may increased the hardness of rice grain and resistant to breakage during milling (Leesawatwong *et al.*, 2005). However, this present study showed that foliar nitrogenous compound urea did not improve head rice yield in both sprayed alone or together with Fe or Zn fertilizers. It might due to the amount of urea (1% w/v) was too small to have the impact on head rice.

Foliar Fe fertilizer alone increased Fe concentration in brown rice of PTT1 but not in other varieties, while spraying with urea containing solutions, Fe concentrations were increased significantly when compared to the control. Similar to this study, Aciksoz *et al.* (2011) reported that Fe concentration in wheat grain was increased by foliar Fe fertilizer together with 1% urea. Iron and urea could be act synergistically in improving the grain Fe concentration; urea might promote nitrogenous compounds, the Fe transporter such as NA and YSl to transported Fe to the outer layer of rice grain.

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In white rice, foliar Fe fertilizer or urea containing solution slightly increased grain Fe but there are no different effects on rice varieties. The results resemble to Prom-u-thai *et al*. (2003) who reported that applying N fertilizer could improved grain Fe in brown rice but not in white rice. Most of Fe in rice grain is stored in protein bodies which concentrated in the aleurone layer and embryo but not in the endosperm. The developing grain is connected to the maternal plant by a single vascular bundle (Zhang *et al.*, 2007). This vascular bundle ends at the seed coat and is not connect to the endosperm or embryo. It has been suggested that nutrients are delivered to the maternal tissues surrounding the seed and eventually effluxes appoplastically into the grain tissue (Waters and Sankaran, 2011). Phom-u-thai *et al.* (2007a) noted that the variation of Fe concentration among grain tissue component (husk, caryopsis) and cell layer across the caryopsis (bran and endosperm) might be the key role in determining Fe concentration in white rice rather than total amount of Fe transported into endosperm. Therefore, the Fe supply to the grain might be limited by Fe transfer rate from the husk to the grain.

Foliar Zn containing solutions significantly increased the grain Zn of brown and white rice in all varieties whereas urea did show the influence on grain Zn in all varieties except RD21 which Zn concentration in brown rice was higher when sprayed Zn together with urea, however, the influence of urea was disappeared in white rice. Previous studied note that nitrogenous compounds promote Zn allocated in wheat grain (Kutman *et al.*, 2010). Most of the Zn in the cereal grain is thought to localize in protein bodies in the form of globoid crystals. Grain proteins may contribute to the accumulation of Zn by increasing the storage capacity of the grain Zn. In addition, a potential transported of metal in phloem such as NA, oligopeptide and amino acid that all certain N. However, this present study shown that spraying urea did not increase grain Zn. The similar response of grain Zn to N fertilizer has been study by Zhang et al. (2008) who reported that rice grew in no soil N supply gave highest Zn concentration in rice grain. To our study, however, it is possible that urea might not be the suitable from to transports Zn into rice grain like other nitrogenous compounds such as NA or AA which were found efficiency to transport Zn into rice grain (Ling *et al.*, 2012; Wei *et al.*, 2012). A further impossible factor determining grain Zn response to foliar urea would be the application of nitrogen to the soil. It might be expected that the response

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of rice to foliar urea would be reduced by the increasing of N availability in soil solution.

There was genotypic difference for response to foliar Zn, such as CNT1 showed less response to foliar Zn than other varieties. Therefore, increasing grain Zn by foliar application might be selected varieties with high ability in absorption and translocation Zn to rice grain. It has been suggested that that rice genotypes differ greatly in their response to foliar Zn to increasing grain Zn (Phattarakul *et al.*, 2012; Wissuwa *et al.*, 2008)

The accumulation of N and P in rice grain was not shown significant different among spraying treatments although it has been reported that Fe and Zn densities were related with the chemical composition in rice grain such as phytate and protein. However, it was found the relationship among Fe and N in brown rice (r=0.44***, data not show). This results similar to Prom-u-thai *et al*. (2007a) who studied on the variation of partitioning of Fe in rice grain, noted that Fe is stored in the nitrogenous compounds such as protein bodies and the intensity of protein bodies in the embryo and aleurone layer, but not in the endosperm, is positively correlated with Fe concentrations in brown rice. The previous study suggested that P in rice grain are most stored in phytate form which complex with Fe and Zn (Raboy, 1997), however, there did not found the relationship between Fe or Zn with P in this study (data not show).

In conclusion, improving dry season rice grain qualities by foliar Fe and Zn application had been success in term of improving nutritional quality. The grain Fe and Zn increased by foliar Fe and Zn fertilizers. In addition, urea fertilizer can promote rice grain Fe but not grain Zn. Nevertheless, there was negative impact on head rice yield when sprayed with Fe and Zn fertilizer, but effect of foliar Zn can be reduced by foliar Zn together with urea. Although, grain breakage cannot reduced by nutrient spraying method, however, the previous study reported that broken rice grain had more Fe than those in the full grain (Prom-u-thai *et al.*, 2009). This can be an economic advantage of broken rice. However, there is no available information on the Fe and Zn composition of different broken rice components. Therefore, in chapter 4, the distribution of Fe and Zn in rice grain was examined.

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