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Foliar zinc application improved grain zinc accumulation and bioavailable zinc in unpolished and polished rice

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ABSTRACT

This study examined the effect of foliar zinc (Zn) application on grain yield, Zn and phytate concentrations and its impact on the phytate:Zn molar ratio, an indicator for Zn bioavailability in human digestive tracts, in unpolished and polished rice between two rice cultivars across two cropping years. The modern improved rice cultivar CNT1 and the traditional improved cultivar KDK were foliar applied with five rates of ZnSO₄ in two cropping years. In 2016, 0.3% ZnSO₄ increased the Zn concentration of unpolished rice in KDK by 21% over the nil Zn, while the effect was not found in CNT1. In polished rice, 0.2–0.4% ZnSO₄ increased the Zn concentration by 11.2–20.0% in CNT1 and by 8.3–24.1% in KDK, and decreased the phytate concentration by 5.2–16.9% in KDK but not in CNT1. In 2017, 0.4% ZnSO₄ increased the Zn concentration by 18.2–26.2% and 32.4–42.6% in unpolished and polished rice, respectively, in both cultivars. Application of 0.4% ZnSO₄ decreased the phytate:Zn molar ratio in polished rice from 29.7 to 18.3 and from 26.4 to 17.9 in CNT1 and KDK, respectively in 2016, and from 15.7 to 12.6 in KDK in 2017. Foliar Zn application decreased the phytate:Zn molar ratio in unpolished rice from 27.9 to 22.7 and from 21.9 to 17.2 in CNT1 and KDK, respectively in 2017, but had no effect in 2016. Thus, foliar Zn application can improve grain Zn concentration and decrease the phytate:Zn molar ratio in both unpolished and polished rice but the response can vary with cropping year and cultivar.

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Foliar Zn fertilizer; Zn concentration; phytate concentration; bioavailable Zn



Introduction

Zinc deficiency is a major cause of impairments in growth development, immune system function and learning disabilities in human health (Hotz & Brown, 2004). Enhancing

grain Zn concentration in staple food crops has been suggested as a sustainable strategy to solve the problem of Zn deficiency in humans, especially in rice (*Oryza sativa* L.) which is consumed in more than half of the world (Cakmak, 2008). Applying Zn fertilizer to the soil

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and/or foliage is the most common agronomic practices reported to improve Zn uptake and partitioning into different plant parts, and consequently potentially improving grain yield and nutritional quality in rice grain (Li et al., 2015). Foliar Zn application can mitigate against nutrient deficiencies in crop plants and increase Zn accumulation in rice grain (Wang et al., 2014). Pot and field experiments have shown that foliar application of 0.2–0.5% ZnSO₄ had no effect on grain yield, but greatly improved grain Zn concentration in unpolished rice, from 35% to 55% compared with no foliar Zn application (Boonchuay et al., 2013; Phuphong et al., 2018). Therefore, foliar Zn application is suggested as a promising way to increase grain Zn concentration in rice and nutritional benefits to consumers.

However, it is only useful to increase the Zn concentration in rice if the Zn is bioavailable in human diets. Anti-Zn nutrients in human diets are a major cause of Zn deficiency as they reduce Zn absorption in the digestive tract (Lönnerdal, 2002). Antinutrients are substances that reduce the degradation, absorption, or utilization of nutrients, of which phytate is the key compound in grains especially in cereals such as rice (Coulibaly et al., 2011). Phytate (IP₆ or PA) is a cation salt of phytic acid with 12 hydrogens on the 6 phosphate groups, and phytic acid is a strong chelator with cations such as Fe^{2+/3+}, Zn²⁺, Mg²⁺, Ca²⁺, K⁺ and Cu²⁺ forming insoluble salts (Kumar et al., 2010). Phytate is an important storage form of phosphorus and is metabolized during seed germinating and seedling growth (Doria et al., 2009). The accumulation of storage forms of phosphorus not only stimulates Zn deficiency in plant tissues but it is also an antinutrient in human diets (Huang et al., 2000). Reducing phytate in cereal grains is expected to increase Zn availability in both the plant and in human diets (Cakmak, 2008). Foliar Zn application with 0.5% ZnSO₄ combined with soil Zn application in calcareous soil has been reported to reduce the phytate:Zn molar ratio in rice grain by 35% compared with the control (Imran et al., 2015). However, the phytate:Zn molar ratio has yet to be evaluated under field conditions over cropping seasons. A lower phytate concentration was observed after foliar Zn application with 0.5% ZnSO₄ in both unpolished and polished rice, but only the single rate of foliar Zn and growing season was evaluated, while a various foliar Zn rates and molar ratio between phytate:Zn which indicating the bioavailability of Zn in the rice grain across the cropping seasons has not been explored (Jaksomsak et al., 2018). Therefore, this study was undertaken to investigate the response of grain yield, Zn and phytate to rates of foliar ZnSO₄ over 2 cropping seasons in two rice cultivars representing modern and traditional rice types. The results should be useful for biofortification programs to increase grain Zn concentration and Zn availability for human diets.

Materials and methods

Plant culture

The experimental design was arranged as RCBD with two cultivars, five Zn rates and three replicate plots in each cropping season. The two rice cultivars, Chai Nat 1 (CNT1, modern rice) and Kam Doi Saket (KDK, traditional rice) were grown in the same paddy field at the Faculty of Agriculture, Chiang Mai University, during the wet season (June to November) in 2016 and 2017 on a sandy loam-textured soil of the Sansai series, pH 5.8 and 5.5 (1:1, soil:water), respectively. The average temperatures during the cropping seasons in 2016 and 2017 were similar at 27.5°C, with 80.2% and 77.0% relative humidity, respectively, and the average sunshine duration was 5.2 and 5.7 h, respectively. The average precipitation during grain filling was 4.0 mm and 3.4 mm, respectively (Northern Meteorological Center, 2018). Plant culture was conducted with similar practices in both cropping years. Seedlings (21–25 days old) were transplanted to a single plant per hill, with spacing between hills of 25 × 25 cm, in 2 × 2 m² plots with the total number of 64 plants per plot. Plots were separated by 0.5 m between fallow distances. The Zn fertilizer was foliar applied at 5 rates as an aqueous solution containing 0, 3.5, 7, 10.5 or 14 mM Zn (0%, 0.1%, 0.2%, 0.3% and 0.4% ZnSO₄, respectively), at booting, flowering and milky grain. The volume of foliar Zn application was approximately 900–1000 L ha⁻¹ (Boonchuay et al., 2013). To prevent contamination, each plot was vertically protected with a plastic sheet during spraying. All plants received basal fertilizer to the soil, a total of 85 kg NH₂CONH₂ ha⁻¹, 35 kg P₂O₅ ha⁻¹ and 15 kg K₂O ha⁻¹ which was four split equally at 7 days after planting, tillering, booting and flowering stages (Jaksomsak et al., 2015). The fields were permanently flooded under 0.1–0.2 m of water until maturity. The fungicide Isoprothiolane and insecticide Fipronil were applied at recommended rates to control pests. Weeds were manually removed.

Sample collection and preparation

At maturity, a 1 × 1 m² area from the center of each plot was harvested to determine yield, straw dry weight and yield components (number of tillers hill⁻¹, number of panicles plant⁻¹, number of spikelets panicle⁻¹, thousand seed weight and percentage of filled grain). Grain yield was measured at 14% moisture content and straw dry weight was determined after oven drying at 70°C for 72 h. The unpolished rice and polished rice samples were analyzed for Zn and phytate. The unpolished rice, the caryopsis, was prepared by dehusking paddy rice with

a rice testing machine (model P-1, Ngek Seng Huat Company, Thailand) and 50 g of the unpolished rice samples was polished by a milling machine (model K-1, Ngek Seng Huat Company, Thailand) for 30 s to yield polished rice. The yield of unpolished and polished rice was measured after oven drying at 70°C for 72 h.

Chemical analysis

Analysis of Zn concentrations was carried out with an atomic-absorption spectrophotometer (Z-8230 Polarized Zeeman, Hitachi, Japan) after dry-ashing (Allan, 1961). The phytic acid analysis (phytate) was conducted by the precipitation of ferric phytate and measurement of iron (Fe) remaining in the supernatant (Haug & Lantzsch, 1983). Each batch of Zn and phytate analysis included peach (SRM 1547) and soybean leaves as certified reference materials.

Statistical analysis

Combined analysis of variance (ANOVA) between cropping seasons was carried out using Statistical Analysis System software (Statistic 9, analytical software SX). Grain yield, yield components and quality of the unpolished and polished rice were examined to determine the combined effects of cultivar, foliar Zn treatment and cropping season. Significant differences between treatment means were separated at $p < 0.05$ by the least significant difference (LSD) test. Correlation analysis was used to detect the significance of each relationship by Pearson correlation.

Results

Grain yield and yield components

Foliar Zn application did not affect grain yield or straw dry weight ($p = 0.81$ and $p = 0.72$, respectively), but these parameters differed among rice cultivars depending on

the cropping year ($p < 0.05$) (Figure 1(a,b)). The CNT1 had 67.7% higher grain yield than KDK (Figure 1(a)), while KDK had 52% higher straw dry weight than CNT1 in 2017, but it was not different in 2016 (Figure 1(b)).

There were interaction effects between rice cultivar and cropping year for yield components, but foliar Zn fertilizer only affected ($p < 0.05$) 1,000 seeds weight (Table 1). The CNT1 grown in 2016 had 25.9% and 20.3% higher number of tillers and panicles per plant, respectively, than in 2017 but there was no difference between spikelets per panicle, and percent-filled grain between cropping seasons. In KDK, there were no differences between cropping years in the number of tillers per hill, or panicles and spikelets per plant, but the 2017 crop had 8.6% and 12.0% higher 1,000 seeds weight and percent filled grain than in 2016.

Grain Zn and phytate concentration

In unpolished rice, grain Zn concentration was significantly affected by foliar Zn applications and rice cultivars similarly in both years ($p < 0.05$) (Figure 2). In 2016, the Zn concentration of unpolished KDK rice was highest in the 0.3% ZnSO₄ treatment, being 21% higher than in the nil Zn plants. Application of 0.4% ZnSO₄ was ineffective in improving the grain Zn concentration in KDK. However, application of foliar Zn did not affect the grain Zn concentration in CNT1. The concentration of Zn in unpolished rice of both cultivars in 2017 was slightly lower than in 2016 for the nil Zn treatment. In KDK, foliar Zn application of 0.3% and 0.4% ZnSO₄ increased grain Zn concentration by 27.2% and 18.2%, respectively, in 2017. The grain Zn concentration in CNT1 responded most to 0.4% ZnSO₄ treatment with an increase in 26.1% over nil Zn plants.

In polished rice, grain Zn concentration significantly affected by foliar Zn application differently between the cultivars and years ($p < 0.05$) (Figure 2). In 2016, application of foliar ZnSO₄ at 0.2%, 0.3% and 0.4% increased Zn

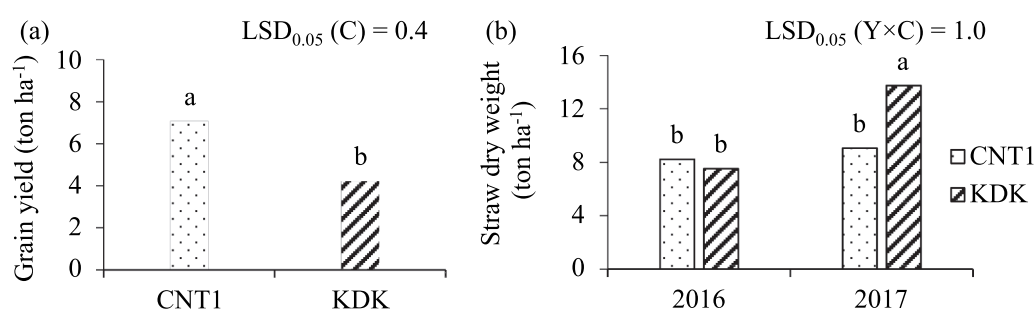


Figure 1. Grain yield (a) and straw dry weight (b) of two rice cultivars (CNT1 and KDK) foliar applied with 5 rates of ZnSO₄ grown at two cropping years (2016 and 2017). The foliar Zn application on grain yield ($p = 0.81$) and straw dry weight ($p = 0.72$). Different letters above bars indicate significant differences by the least significant difference (LSD) at $p < 0.05$.

Table 1. Yield components of two rice cultivars (CNT1 and KDK) foliar applied with five rates of ZnSO₄ grown at two cropping years (2016 and 2017).

Cropping year	Cultivar	Tiller hill ^{-a}	Panicle plant ^{-a}	Spikelet panicle ^{-a}	1,000 seeds weight (g)	Filled grain (%)
2016	CNT	20.9 a	17.8 a	134.3 b	29.7 b	93.8 a
	KDK	9.9 c	8.4 c	202.2 a	30.2 b	83.5 b
2017	CNT	16.6 b	14.8 b	152.1 b	29.9 b	91.5 a
	KDK	8.6 c	7.0 c	196.7 a	32.8 a	93.5 a
Foliar Zn (P-Value) ^a		0.92	0.79	0.21	0.04	0.29

Means in the same column followed by different letters are significantly different by the least significant difference (LSD) at $p < 0.05$.

^alevel of significance was set at $p < 0.05$.

concentration by 11.2%, 13.0% and 20.0% in CNT1 and by 8.3%, 23.2% and 24.1% in KDK, respectively, but 0.1% ZnSO₄ had no effect. In 2017, 0.3% and 0.4% ZnSO₄ increased the Zn concentration in CNT1 by 22.2% and 32.4%, respectively, while 0.1%, 0.2%, 0.3% and 0.4% ZnSO₄ increased the Zn concentration in KDK by 17.4%, 31.4%, 40.0% and 42.6%, respectively.

The phytate concentration was significantly affected by foliar Zn application difference between the cultivars and cropping years ($p < 0.01$) (Figure 3). The phytate concentration in 2017 was lower than in 2016 in unpolished and polished rice of both cultivars. In unpolished rice in 2016, the phytate concentration decreased by

5.2% and 11.5% in KDK when applied with 0.2% and 0.3% ZnSO₄, respectively, compared with nil Zn, but the phytate concentration was not affected by foliar Zn treatments in CNT1. In 2017, the phytate concentration in KDK decreased by 20.9% in the 0.3% ZnSO₄ treatment, while it was not affected in CNT1. Compared to the nil Zn treatment, the application of 0.4% ZnSO₄ decreased phytate concentration in polished rice of CNT1 and KDK in 2016 by 26.2% and 16.2%, respectively. In 2017, the concentration of phytate increased with 0.4% ZnSO₄ in KDK by 13.6%, while it was not affected in CNT1.

The phytate:Zn molar ratio was used as an indicator for bioavailable Zn in the digestive tract of human diets. The phytate:Zn molar ratio was significantly affected by foliar Zn applications differently between rice cultivars in both years ($p < 0.01$) (Figure 4). In unpolished rice in 2016, foliar Zn application rates of 0.2% and 0.3% ZnSO₄ decreased the phytate:Zn molar ratio in KDK by 9.7% and 26.0%, but the higher rate of foliar Zn had no effect. However, there was no effect of foliar Zn application on the phytate:Zn molar ratio in CNT1 in 2016. In the 2017 crop, the phytate:Zn molar ratio decreased by 18.5% in the 0.4% ZnSO₄ treatment in CNT1 and by 21.5% in KDK. In polished rice, 0.4% ZnSO₄ decreased the ratios in CNT1 and KDK in 2016 by 38.5% and 32.0%, respectively. However, in 2017, the same treatment decreased the ratio in KDK by 19.8%, but there was no change in CNT1.

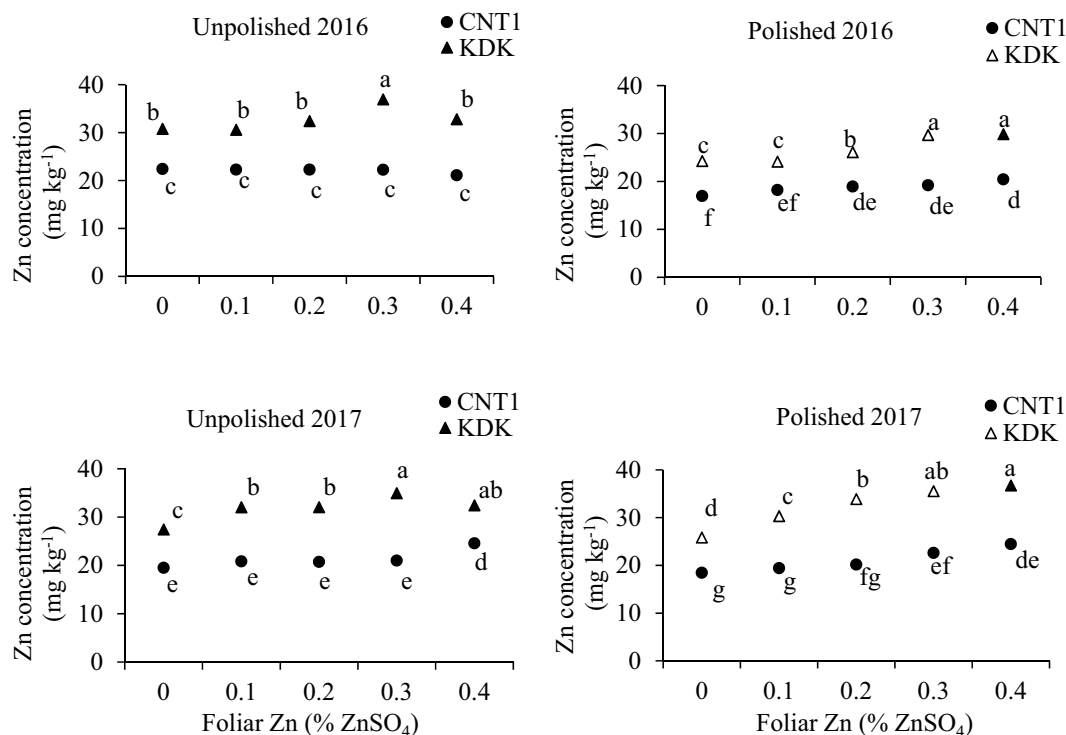


Figure 2. Zn concentration in unpolished and polished rice in 2 rice cultivars foliar applied with 5 rates of ZnSO₄ grown at two cropping years (2016 and 2017). Different letters above lines indicate significant differences by the least significant difference (LSD) at $p < 0.05$.

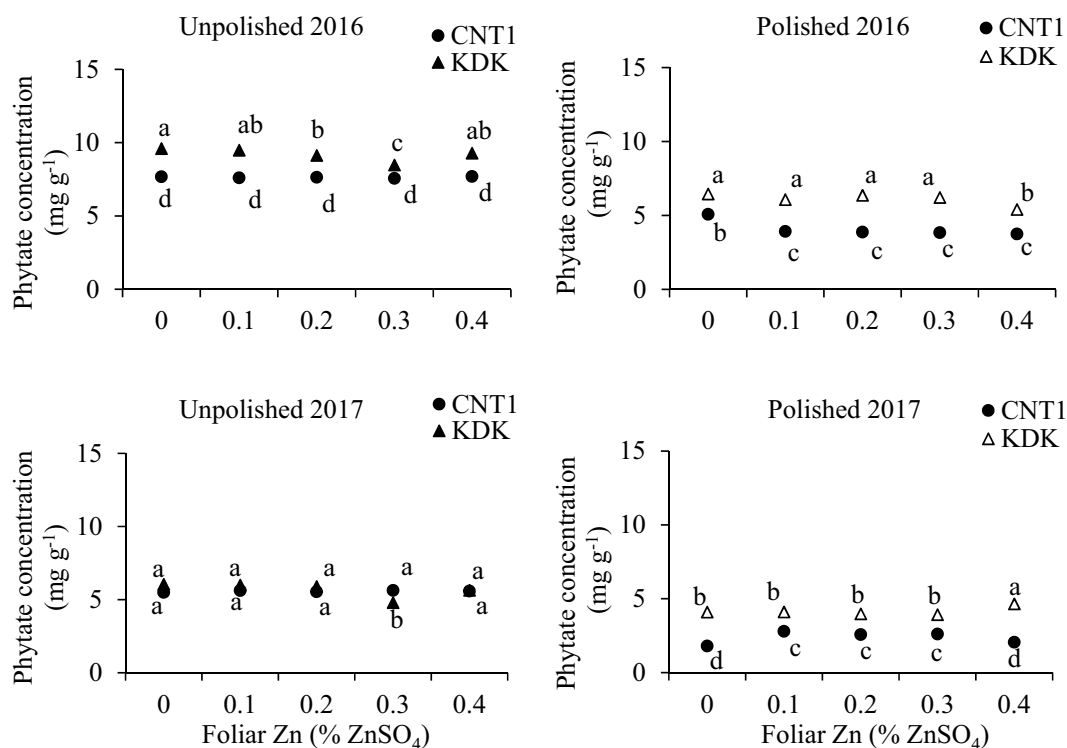


Figure 3. Phytate concentration in unpolished and polished rice in 2 rice cultivars foliar applied with 5 rates of ZnSO₄ grown at two cropping years (2016 and 2017). Different letters above lines indicate significant differences by the least significant difference (LSD) at $p < 0.05$.

Relationship between phytate:Zn molar ratio and Zn concentration

Relationships between phytate:Zn molar ratio and Zn concentration in unpolished rice and polished rice in the rice cultivars were evident in 2016 and 2017 (Figure 5). The phytate:Zn molar ratio was significantly decreased with increasing Zn concentration in unpolished rice in CNT1 ($r^2 = 0.69$, $p < 0.05$) and KDK ($r^2 = 0.92$, $p < 0.05$) in 2016, and in CNT1 ($r^2 = 0.88$, $p < 0.05$) and KDK ($r^2 = 0.69$, $p < 0.05$) in 2017 (Figure 5(a,b)). In addition, in 2016 the phytate:Zn molar ratio was reduced when the Zn concentration increased in polished rice in CNT1 ($r^2 = 0.40$, $p < 0.05$), but this relationship was not observed in polished rice in KDK (Figure 5(c)). In 2017, the phytate:Zn molar ratio decreased with an increase in Zn concentration in polished rice in KDK ($r^2 = 0.65$, $p < 0.05$), but not in CNT1 (Figure 5(d)).

Discussion

Rice cultivars responded differently between cropping years to foliar Zn application for grain yield, Zn and phytate concentrations, with consequences for the phytate:Zn molar ratio, an indicator of Zn bioavailability in human diets. The modern improved rice cultivar CNT1 had higher

grain yield than KDK, the traditional rice cultivar, in both years. Even though grain yield was not affected by foliar Zn application, the yield components were influenced by interaction effects between cultivar x cropping year (Table 1). Higher relative humidity (RH) (4.2%) and lesser sunshine duration (8.8%) in 2016 during the cropping season (June to November) may have resulted in higher numbers of tillers and panicles per plant, but it did not influence the number of spikelets per panicle, 1000 grain weight or the percent filled grain in CNT1. In KDK, the higher relative humidity may have reduced the supply of photosynthate for grain filling leading to a lower percentage of filled grain and less individual seed weight. In Indica and Japonica rice cultivars, spikelet fertility and percentage of filled grain decreased with increasing RH (Weerakoon et al., 2008). Furthermore, lower hours of sunlight after heading reduced the number of filled grains and 1,000 seed weight (Liu et al., 2014). No data were available for cloud cover in our study area but the lower sunshine in 2016 is most likely to have been due to increased cloud cover. Kisimoto and Dyck (1976) reported that during the rainy season, cloudy weather and high humidity encourage the survival and multiplication of the gall midge. We observed but did not quantify that stem borer pest infestation in 2016 was more prevalent in KDK than in 2017.

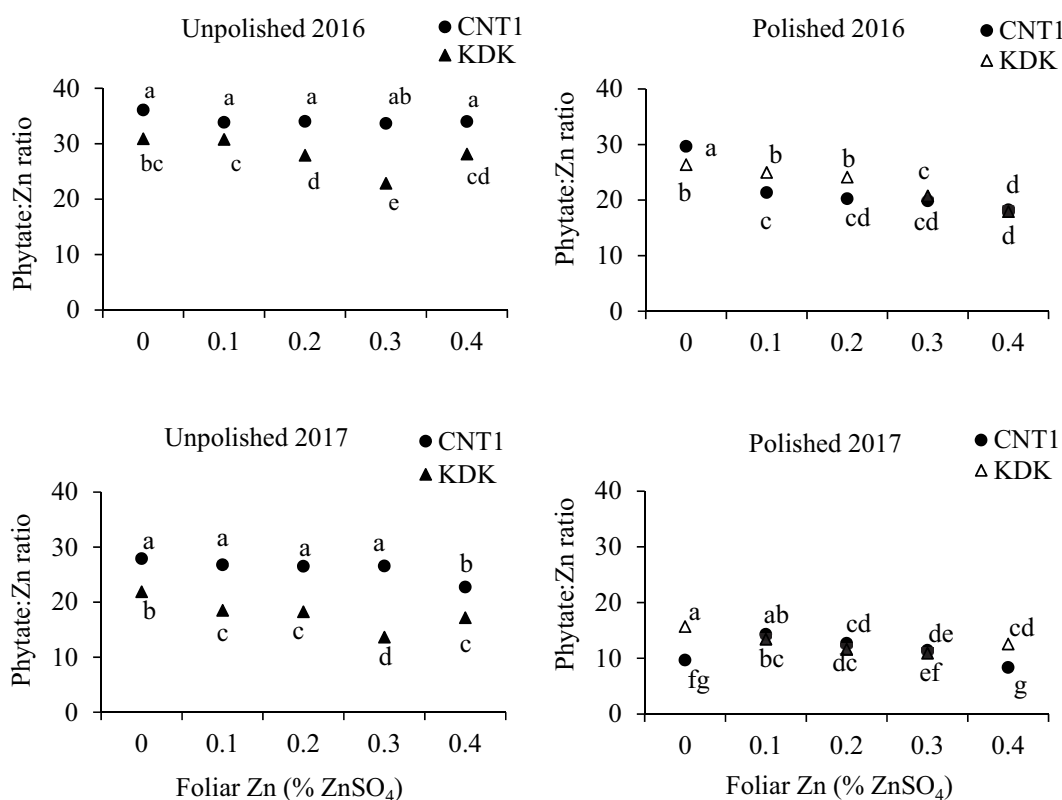


Figure 4. Phytate:Zn molar ratio in unpolished and polished rice in 2 rice cultivars foliar applied with 5 rates of ZnSO₄ grown at two cropping years (2016 and 2017). Different letters above lines indicate significant differences by the least significant difference (LSD) at $p < 0.05$.

The application of low foliar Zn rates (0.1% to 0.4% ZnSO₄) increased the Zn concentration in unpolished and polished rice in both cropping years, except for unpolished rice of CNT1 in 2016. An increase in grain Zn concentration in unpolished and polished rice has been reported as a potential tool to raise the Zn concentration in human diets, particularly in rice consumption countries (Cakmak, 2008). Foliar micronutrient application is effective in reducing deficiencies in crop plants (Wasaya et al., 2017) and is suitable under wetland conditions. However, Liu et al. (2019) have been reported that grain Zn concentration is influenced by environmental factors such as precipitation resulting in differences between cropping years. Whether the small differences in precipitation during grain filling in our study could have influenced grain filling is unknown. Thus, improving grain Zn concentration by foliar Zn fertilizer application may also require attention to the seasonal climate conditions in each growing region. For optimum effects, it may be necessary to vary the foliar application rate for Zn depending on both the rice cultivar and prevailing climatic conditions. This area requires further field evaluation.

This study established that rates of foliar Zn application lower than 0.5% ZnSO₄, the level generally applied

(Boonchuay et al., 2013), have the potential to improve grain Zn concentration in rice. This is the first study to explore the effects of lower Zn foliar rates on rice grain phytate and nutritional quality. This study showed that foliar application of 0–0.4% ZnSO₄ resulted in Zn concentrations ranging from 19.5 to 34.9 and 17 to 36.8 mg kg⁻¹ in unpolished and polished rice, respectively, in both cultivars and cropping years. This compares to 18.0 and 23.0 mg kg⁻¹ Zn in unpolished and polished rice, respectively, in CNT1 given 0.5% foliar ZnSO₄ (Jaksomsak et al., 2018). This suggests that lower rates of foliar Zn can be effective depending on the cultivar and cropping season. The remobilization of Zn from vegetative parts via the phloem to the developing grain after foliar spraying is a key process in the effectiveness of the treatment protocol. This remobilization is influenced by factors such as the plant cultivar and physiological characteristics, including phenological stage and/or environmental conditions (Kutman et al., 2010). However, the grain Zn concentration was varied by yield between the two cultivars. The cultivar CNT1 had grain yield higher than KDK, while grain Zn concentration in both the unpolished and polished rice was found in the opposite which might be a possible mechanism of the dilution effect. This can be confirmed

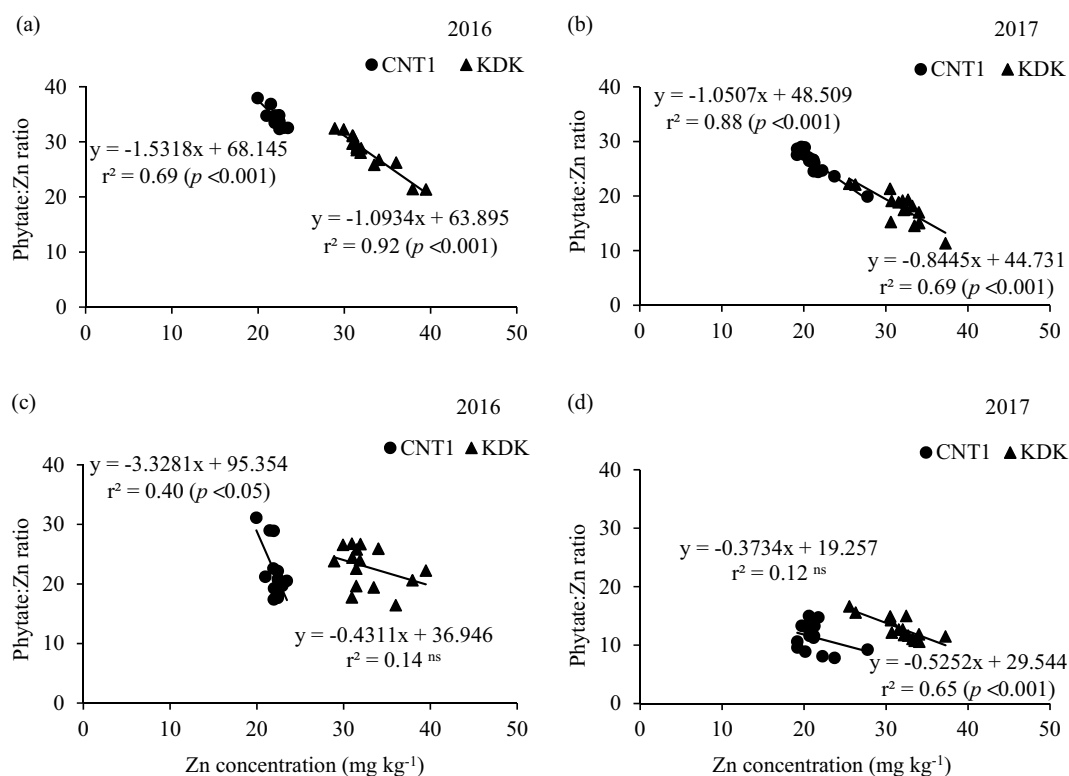


Figure 5. Relationship between phytate:Zn molar ratio and Zn concentration in unpolished rice grown in 2016 (a) and 2017 (b) and relationship between phytate:Zn molar ratio and Zn concentration in polished rice grown in 2016 (c) and 2017 (d) in 2 rice cultivars foliar applied with 5 rates of ZnSO₄ ($n = 15$).

by increasing the number of rice cultivars with a diverse range of grain Zn concentration and yield potential in the future study. The success of foliar Zn application has been reported in other cereals. In China, foliar application with 0.4% ZnSO₄ increased whole grain Zn concentration in wheat by 58%, while foliar application of 0.2%, 0.4% and 0.5% ZnSO₄ increased the Zn concentration in flour by 60%, 76% and 76%, respectively, compared with the control treatment (Zhang et al., 2012). Also, in wheat, increasing the grain Zn concentration by foliar Zn application decreased the concentration of anti-nutrients such as phytate (Cakmak & Kutman, 2018). In field pea (*Pisum sativum* L.), the phytate concentration in raw grains decreased with foliar Zn application (0.25% and 0.5%) (Poblaciones & Rengel, 2016). Likewise, in our study 0.2% and 0.3% ZnSO₄ corresponded with a decrease in phytate concentration in unpolished rice of KDK and in polished rice of cultivar CNT1. That foliar Zn did not affect the phytate concentration in polished rice in KDK suggests that there could be differences in the distribution of phytate across grain tissues among rice varieties. Genotypic variation in phytate distribution was found among rice cultivars with phytate mostly distributed in the aleurone layer and embryo and rarely present in the endosperm (Prom-U-Thai et al., 2008). Thus, the degree of loss of the aleurone layer and

embryo during the polishing process as well as grain morphological traits, such as the thickness of aleurone layer, may also contribute to differences in phytate concentrations in polished rice.

Reduction in the phytate concentration as reflected in the phytate:Zn ratio in the second crop differed between rice genotypes, indicating G x E effects on the accumulation of phytate in unpolished and polished rice. In KDK, the traditional-improved rice cultivar, foliar Zn application improved Zn concentrations and corresponded with decrease phytate concentrations in unpolished rice, but not in CNT1, the modern-improved rice cultivar. Variation in phytate concentration has been reported among rice cultivars (Wang et al., 2011), but there is limited information available on G x E effects. The decrease in the phytate concentration in the polished rice in 2017 across the genotypes might be also associated with the several factors such as time and polishing machine use in the polishing process and grain morphological characteristic as mentioned in the above paragraph.

The molar ratio of phytate:Zn is an indicator used to examine the bioavailability of Zn in the digestive tract in human diets (Poblaciones & Rengel, 2016). Molar phytate:Zn ratios greater than 15 can decrease Zn bioavailability, but lower molar ratios of 4 to 8 can also decrease Zn absorption (Wang et al., 2014). As phytate is a major

inhibitor of Zn absorption (Ma et al., 2007), the phytate:Zn ration is a useful tool for predicting the bioavailability of Zn in human diets. In the study (Figure 4), the ratio in unpolished rice of CNT1 and KDK ranged from 23 to 36 in 2016. Moreover, foliar Zn application decreased the phytate:Zn ratio of both cultivars in Figure 4. Similarly, Hussain et al. (2012) found that the foliar Zn application increased the estimated Zn bioavailability and decreased the molar ratio of phytate:Zn in the whole grains of wheat.

In conclusion, foliar Zn application is one of the promising ways to improve grain Zn concentration and to decrease the phytate:Zn ratio in rice grain. This study revealed that modern and traditional improved rice cultivars respond differently to foliar Zn application and the effects can vary with the cropping year. Foliar Zn application had no effect on grain yield, but increased the Zn concentration in unpolished and polished rice, and consequently decreased the phytate:Zn ratio in rice grain. Foliar Zn application is suitable for producers to improve grain Zn concentration as well as improving Zn bioavailability for human consumption. However, further studies are needed to explore optimum foliar Zn rates for the main commercial rice cultivars in a wider range of growing conditions. In addition, the effectiveness of decreasing the phytate:Zn ratio in rice on human Zn nutrition needs investigating in both *in vitro* and *in vivo* programs.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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