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Association between iron, zinc and protein concentration in the embryo and endosperm regions of rice grain

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Abstract

This study evaluated the relationships between iron (Fe), zinc (Zn) and protein concentrations in the embryo and endosperm regions of rice grain and its relationship to the total concentrations of these nutrients in brown rice. This study used 12 genotypes of brown rice with wide variations in nutrient concentrations. All genotypes were grown in the same condition and management to avoid environmental effects on nutrients concentration in rice grain. Brown rice was separated into two regions (embryo and endosperm intact with the pericarp) for the nutrient concentration analysis. Nutrient concentrations varied widely, ranging from 47 to 166 mg kg^{-1} for Zn, 26 to 80 mg kg^{-1} for Fe and 13 to 25% for protein in the embryo region; and from 13 to 37 mg kg^{-1} for Zn, 4 to 19 mg kg^{-1} for Fe and 7 to 13% for protein in the endosperm region. Zn, Fe and protein concentrations were 4.7, 6.5 and 2.0 times higher, respectively, in the embryo than the endosperm. Different relationships between nutrients were found within the embryo and endosperm regions, with correlations found between concentrations of Fe and Zn, Fe and protein and Zn and protein in the embryo, but only between Fe and protein in the endosperm. The concentrations of all nutrients in the endosperm and embryo significantly influenced their total concentration in brown rice, with the endosperm contributing the most. This study confirmed the possibility of the embryo and endosperm binding nutrients differently. In selecting genotypes of brown rice for high levels of nutrients, either for consumption and/or breeding purposes, nutrient levels in both the embryo and endosperm should be considered, as concentrations there, particularly in the starchy endosperm, significantly influenced the overall nutrient concentrations in brown rice.

Introduction

Iron (Fe) and Zinc (Zn) are essential mineral nutrients for humans. Deficiencies in both contribute to severe cases of malnutrition. People in South Asia and Africa, where rice is the main staple and access to Fe and Zn rich sources, such as animal products, is limited, are particularly susceptible, especially in rural areas, with their lower purchasing power compared with metropolitan areas (Mannar and Gallego, 2002; Black *et al.*, 2008). Deficiencies of both elements are known to have serious adverse effects on human health, especially in children, such as impairing growth, the immune system, and learning, with serious consequences for adulthood (Keen and Gershwin, 1990; Ho *et al.*, 2003). Breeding programs have been suggested as a promising strategy to increase Fe and Zn concentrations in rice grain, and improving Fe and Zn intake among rice consumers over the long run (Welch and Graham, 2002; Cakmak, 2008). This requires understanding the distribution of these micro nutrients in relation to each other among different rice grain tissues.

Nutrient distribution gradients across grain tissues of rice have been reported. For example, Fe is relatively abundant throughout the aleurone, scutellum and embryonic tissues of rice grain, while little Fe is present in the endosperm (Hansen *et al.*, 2009). In addition, while Fe and Zn concentrations (16.4-18.5%) were similar in the embryo, they varied in the aleurone (40.8% of Fe, but only 12.5% of Zn) and endosperm (69.0% of Zn, but only 42.8% of Fe) (Saenchai *et al.*, 2012). The variation in the distribution of nutrients across grain regions implies different complex forms of nutrients between the grain tissues. A recent study on localization of Fe in rice grain using overexpression of OsNAS genes, synchrotron X-ray fluorescence microscopy and high resolution secondary ion mass spectrometry suggested that nicothiamine synthase may complex to

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Fe as well as Zn in rice grain (Kyriacou *et al*., 2014). Another investigation of rice grain suggested that Fe and Zn complexed with phytic acid, as the molar ratio of phytic acid to Zn or Fe is often an indicator of the bioavailability of these minerals (Prom-u-thai *et al*., 2008; Ryan *et al*., 2008; Simic *et al*., 2009). However, the above studies provided no information of the nutrients association on specific grain tissues. Specific grain tissues have been examined in other cereals. For example, zinc has been shown to bind with peptide in the embryo region of barley; but the endosperm is more complicated, and the binding form of Fe and Zn has not yet been reported (Persson *et al.*, 2009). In wheat, the concentrations of Zn and Fe correlated positively with the concentration of N, in both the embryo and endosperm (Kutman *et al*., 2011).

Protein is not only an essential nutrient for human health, but also an important storage vacuole for Fe and Zn in different regions of the rice plant, such as the embryo and aleurone of the rice grain (Prom-u-thai *et al.*, 2008). Hence, it is worth studying the association between each nutrient in rice grains, as it may have a strong effect on the amount of nutrients allocated in the grains, and consequently the total concentration of nutrients in brown rice.

The present study aims to examine the relationship between Fe, Zn and protein concentrations in the embryo and endosperm regions of rice grains, and how their concentrations contribute to the total concentration in brown rice, potentially useful information in developing breeding programs to select high-nutrient rice genotypes. This has not yet been investigated, due to the difficulty of separating the embryo after shrinkage and sink into the mature rice grain.

Materials and Methods

Plant culture

Twelve Thai rice genotypes, Mei Nong (MN), Jaoleeso (JSL), Buenermu (BNM), Suphanburi 1 (SPR 1), Chainat 1 (CNT 1), Khao Dak Mali 105 (KDML105), Khamhom Morchor (KHC), Buesaklo (BSK), Bieisu (BES), Kham Doi Saket (KDK), Paeiklo (PEK), Homnil (HNL), were grown in a field on Sansai series soil under wetland conditions at Chiang Mai University (18 °47' N, 98 °57' E) during the wet season, the main rice season in Thailand. Four-week-old seedlings of each genotype were transplanted into 3 x 4 m plots at 0.25 x 0.25 m spacing, in four replications. The field was kept flooded under 0.1 – 0.2 m of water, until maturity. Four weeks after transplanting, 25 kg N and 14 kg P2O ha^{-1} were applied, followed by 63 kg N ha^{-1} two weeks later. Rice grain was harvested at physiological maturity.

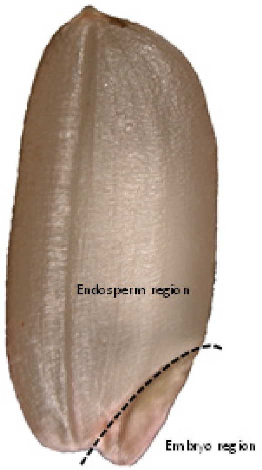

Figure 1. Brown rice (entire caryopsis without husk) manually separated by hand into the embryo and endosperm regions with pericarp intact (dashed line)

Sample preparation

The brown rice samples were prepared by subsample of fifty grams of rough rice (entire caryopsis with husk intact) from each plot, and de-husked by hand to avoid the contamination of Fe and Zn from the husking machine into the brown rice (entire caryopsis without husk). The embryo samples were prepared by subsample of the prepared above brown rice by separating the embryo from the endosperm region using Teflon-coated razor blades to yield embryo and endosperm samples (with pericarp intact in both regions) (Figure 1). Subsamples of brown rice, endosperm and embryo were oven dried at 70^{o}C for 72 h and their dry weight recorded. Iron and Zn concentrations of the samples were determined by Atomic Absorption Spectrophotometer after dry-ashing (Bell *et al*., 1991). Soybean leaves were used as the certified reference material in each batch during analysis. All samples were also analyzed for nitrogen (N) concentrations by Kjeldahl method in order to determine the protein concentration. A nitrogen conversion factor of 6.25 was used to compute the protein value (Zhang *et al*., 2008).

Data analysis

The data were subjected to analysis of variance (ANOVA); means that were significantly different were separated at $p < 0.05$ by the least significant difference (LSD) test. Certain sets of data were also subjected to correlation and regression analysis.

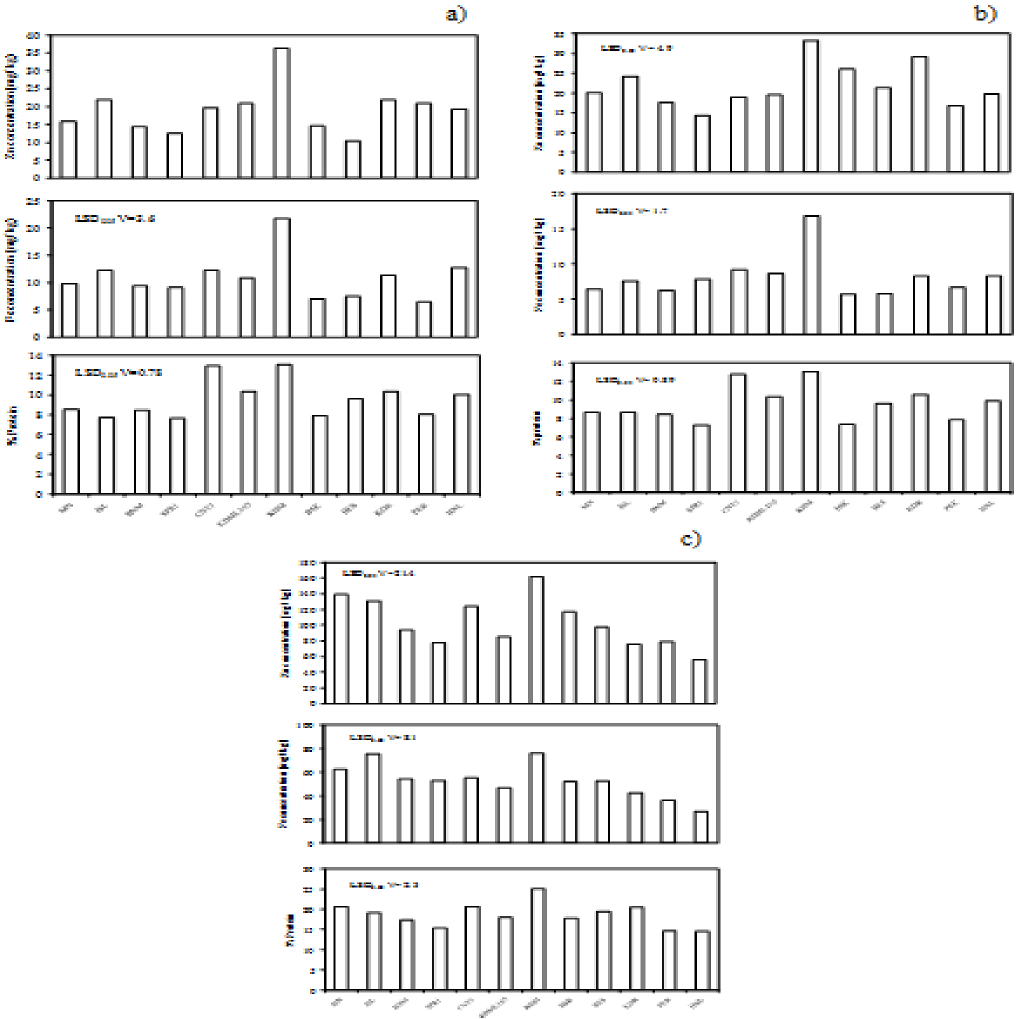

Figure 2. Zinc, Fe and protein concentrations in brown rice (entire caryopsis without husk) (a), endosperm region (brown rice without embryo) (b) and embryo region (separated from brown rice without endosperm) (c) among 12 rice genotypes

Results

A wide variation of Zn, Fe and protein was found in brown rice among the 12 genotypes, ranging from 10-36 mg Zn kg^{-1}, 7-23 mg Fe kg^{-1}and 8-13% protein ($p< 0.05$) (Figure 2a). The KHC genotype had the highest Zn and Fe grain concentrations; KHC shared the highest protein concentration with CNT1. Genotypic variation of Zn, Fe and protein concentrations was also found in the embryo and endosperm regions among the 12 genotypes ($p < 0.05$) (Figure 2b, 2c), with the embryo having 4.7, 6.5 and 2.0 times higher Zn, Fe and protein concentrations, respectively, than the endosperm in all genotypes. The concentration of Zn, Fe and protein ranging from 47-166 mg Zn kg^{-1}, 26-80 mg Fe kg^{-1} and 13-25% protein, respectively in the embryo and ranging from 13-37 mg Zn kg^{-1}, 4-19 mg Fe kg^{-1} and 7-13% protein, respectively in the endosperm. The KHC genotype also had the highest Zn and Fe grain concentrations, while KHC and CNT1 had the highest protein concentration in the embryo region. In the endosperm, the highest Zn and protein concentration was found in KHC, while KHC and JSL had the highest Fe concentration. In the embryo, there were significant positive correlations between Fe and Zn concentrations (R^2= 0.64; P<0.01), protein and Zn concentrations (R^2= 0.47; P<0.01) and protein and Fe concentrations (R^2= 0.43; P<0.01) (Figure 3). The only correlation between nutrients found in the endosperm was Fe and protein concentration (R^2= 0.51; P<0.01) (Figure 4). Multiple regression analysis showed that nutrient concentrations in the endosperm and embryo affected on the total nutrient concentration in brown rice, especially nutrient concentrations in the endosperm region (Table 1).

Discussion

In this study, the substantially higher concentrations of Zn, Fe and protein in the embryo region compared with the endosperm confirmed the large gradient differences in these nutrients among different grain tissues, as has been observed

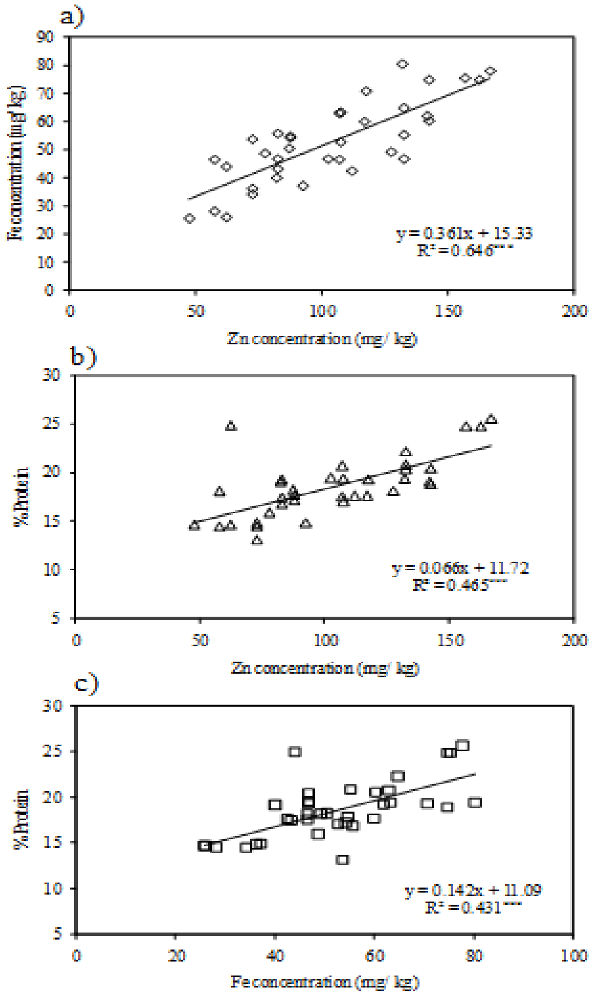

Figure 3. The relationship between nutrient concentrations (Zn, Fe and protein) in the embryo region of 12 rice genotypes

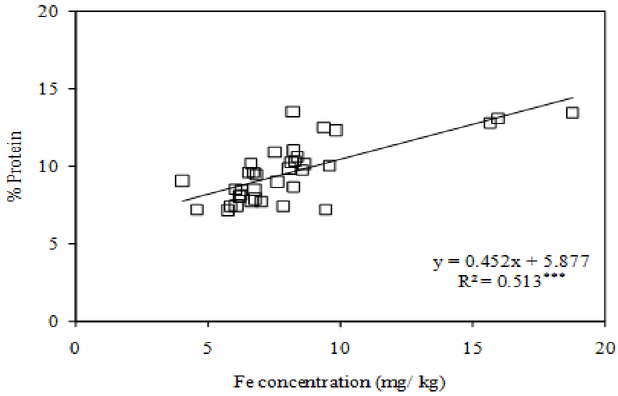

Figure 4. The relationship between Fe and protein concentrations in the endosperm region of 12 rice genotypes

Table 1. Multiple regressions between different grain parts (brown rice, endosperm and embryo) of each nutrient concentration (Zn, Fe and protein)

Nutrient concentration	Multiple regression
Zn	$y= 2.0+0.54x_1+0.05x_2$ ($P< 0.001$)
Fe	$y=-0.92+1.08x_1+0.06x_2$ ($P<0.001$)
Protein	$y=2.0+0.53x_1+0.05x_2$ ($P<0.001$)

y= brown rice, x_1=endosperm, x_2=embryo

previously in rice (Hansen *et al*., 2009; Saenchai *et al*., 2012), wheat (Kutman *et al*., 2011; Lu *et al*., 2013) and barley (Lombi *et al*., 2011). Lombi *et al.* (2011) reported a relationship between the ratio of Zn and Fe concentrations in brown rice grain as seen in semi-quantitative data using mega pixel imaging. In contrast, Saenchai *et al.* (2012), found no correlation between degree of milling and milling loss of both Fe and Zn in the starchy endosperm. These results indicated the different associations of Fe and Zn between the whole brown rice grain and when it is partitioned into embryo and endosperm regions. Furthermore, this study found a relationship among nutrients (Zn, Fe and protein) in the embryo, with its high density of nutrients, but only a relationship between Fe and protein in the endosperm, a low nutrient density region. The only correlation in the endosperm region could also be about the intact pericarp layer, which has been reported to have higher concentrations of Fe (40.8%) than Zn (12.5%) (Saenchai *et al*., 2012). The relationship between the nutrients should be further evaluated in the tissues with and without pericarp intact.

However, the concentration of each nutrient in rice grain may affects differently by environmental factors, especially grain Zn which is more sensitive to the environment than the others. Grain Zn concentration was reported to be affected by both genotype and altitude with higher the concentration was found when grown in the lowland than that grown at the highland, but with the difference between altitudes ranging from 16–50% among the genotypes (Rerkasem *et al*., 2015). Rice genotypes growing in soil with high Zn status e.g., slight acid and wetland soils have been found with grain Zn that was double to many times the concentration when compared to rice genotypes grown in soil with less available Zn e.g., alkaline and aerobic soils (Phattarakul *et al*., 2012; Saenchai *et al*., 2012). On the other hand, insensitivity to the environment of grain Fe was indicated by the Fe concentrations in both the brown rice and the endosperm which were within the same range in aerobic soil and wetland soil (Saenchai *et al*., 2016), while application of N fertilizer has been reported to increase protein content in rice grain (Gu *et al*., 2015). Therefore, environmental management such as growing condition should be carefully considered together with genotypic variation in order to improve the concentration of nutrients in rice grain.

Considerable variation in grain Zn, Fe and protein in brown rice has been found among different rice genotypes in this study. Ranges of 13.5-58.4 mg Zn kg^{-1}, 7.5-24.4 mg Fe kg^{-1} and 6.2-13.4% protein in brown rice grain have been reported in a large germplasm at IRRI and Japan (Welch and Graham, 2002; Tsukaguchi *et al.*, 2016). The genotype KHC was found to have high in all nutrients concentration with CNT1 sharing the similar level of grain protein in brown rice. These genotypes can be useful as the rich source of nutrient in the diets and as a source of genetic material in breeding program for high nutrient concentration in rice. The similar trend of genotypic variation in nutrient concentration was also observed in the embryo and endosperm regions. High nutrient concentrations among different genotypes and grain regions have been discussed as the difference in their transport ability including uptake, translocation, remobilization and accumulation mechanisms which involves in many physiological and biochemical processes (Prom-u-thai *et al.*, 2007). These mechanisms should be further investigated among different grain tissues across genotypes with low to high nutrient concentration, especially during the nutrient transports from the plant into the individual tissue of rice grain.

Brown rice starts to develop externally on the day after fertilization, and it elongates mainly longitudinally, reaching its full length 5-6 days after touching the inner ceiling of the husk; internal development begins with increasing the reserve substances from the embryo and continuing to the endosperm until maturity (Hoshikawa, 1993) which nutrients transport into brown rice through vascular bundles run along the grain (Oparka and Gate, 1981). Even though nutrients concentrations among grain tissues show a large gradient, it has been reported that nutrients accumulate inside the storage vacuole of the embryo at concentrations 5-6 times higher than in the endosperm (Prom-u-thai *et al.*, 2008; Saenchai *et al.*, 2012). This previous result that accumulation of Fe and Zn in the embryo region depends on the storage protein was confirmed by our study, which observed a significant correlation between both nutrients and protein. It has been reported that the protein storage vacuoles (PSVs) of the embryo and aleurone of rice are important storage sites for nutrients and enzymes required for germination (Wada and Lott, 1997; Yoshida *et al.*, 1999). The accumulation of Fe and Zn in the storage protein body of the embryo was also reported by Prom-u-thai *et al.* (2008). However, Persson *et al.* (2009) reported another possibility, having found that Fe mainly binds with P, while Zn binds with peptide ligand in the embryo tissue of barley grains. However, fewer reports exist on nutrient relationships in endosperm tissue. The only nutrient correlation this study found in the endosperm was between Fe and protein indicated the further complicate complex of Fe and Zn in this region. Thus, Zn and Fe bind differently with embryo and endosperm tissues; this should be examined further in a future study. Multiple regression analysis showed a relationship between the nutrient concentrations in both the embryo and endosperm indicating the contribution of these nutrients to the total nutrient concentration in brown rice caryopsis, especially the nutrients in the endosperm, which by weight is much more significant than in the embryo, even though the nutrients are more concentrated in the embryo.

This study indicated significant positive correlations between Fe and Zn concentrations, protein and Zn concentrations and protein and Fe concentrations in the embryo region, while the only correlation between nutrients found in the endosperm was Fe and protein concentration. Additionally, nutrient concentrations in the endosperm and embryo were found to be affected on the total nutrient concentration in brown rice, especially nutrient concentrations in the endosperm region. Meanwhile, selection for rice genotypes with high concentration of Fe, Zn and protein in the endosperm is suggested as the way to meet high nutrient concentration in whole brown rice grain to address nutrient deficiency among populations that rely primarily on rice as a food source. However, the relationship among nutrients and its binding form in the starchy endosperm, the part primarily consumed by humans, has yet to be elucidated as well as the environmental effects on the correlation among nutrients in rice grain.

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