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PHYTIC ACID CONTENT IN BIOFORTIFIED RICE LINES AND ITS ASSOCIATION WITH MICRONUTRIENT CONTENT AND GRAIN YIELD OF RICE

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SUMMARY

Iron and zinc are essential micronutrients for human growth, development, and immune system maintenance. Iron (Fe) and zinc (Zn) are necessary for psychomotor development, sustained physical activity and work capacity, infection resistance, and various metabolic functions. Currently, rice, preferred for being high in Fe and Zn, is also a choice for being low in anti-nutritional compounds, namely, phytic acid. The presented study sought to identify the phytic acid content in several biofortified rice lines and determine the correlation of phytic acid with Fe and Zn content and yield characters. The research ran during the dry season (DS) from January to September 2022, with an alluvial soil type at the Sukamandi Experimental Station of ICRIST – Subang, West Java, Indonesia (altitude of 40 masl). Testing of 10 rice (*Oryza sativa* L.) genotypes comprising six BC_4F_3 biofortified rice lines for high Zn and four check cultivars (Inpari IR Nutri Zinc, Inpari 23, Ciherang, and Sintanur) used a randomized complete block design with three replications. Correlation analysis determined breeding strategies for high Zn/Fe rice with low phytic acid. The results revealed that phytic acid did not significantly correlate with Zn content, 1000-grain weight, and yield per plant. However, phytic acid had a strong positive correlation with Fe content. The rice line WR10 occurred as the best line because it had the highest Zn content (33.80 mg kg^{-1}) and lower phytic acid and Zn ratio (PA: Zn) compared with all check cultivars, giving the highest yield potential and better yield than the Ciherang cultivar.

Keywords: *Oryza sativa*, iron and zinc content, essential micronutrients, phytic acid, correlation, grain yield

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Key findings: The promising line WR10 with high Zn and lower PA: Zn molar ratio compared with the check varieties emerged. Noting phytic acid having a significant positive association with Fe and having no significant association with Zn, the breeding program for getting high Fe rice with low phytic acid becomes more challenging than breeding for acquiring high Zn rice with low phytic acid. Therefore, an advanced breeding approach may help obtain rice cultivars with high Fe and low phytic acid.

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INTRODUCTION

Iron and zinc are essential nutrients that help in protein synthesis, cell growth and development, and immune system functions (Roohani *et al.,* 2013). Zinc also has antiviral properties against several virus species that commonly attack humans (Read *et al.* 2019). Iron is vital in living organisms' metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport (Abbaspour *et al.,* 2014). Therefore, sufficient intake of Fe and Zn nutrients is necessary in the body.

Lack of Fe and Zn in childhood causes health problems in developing countries. Worldwide, the overall prevalence was 29.1% for stunting, 6.3% for wasting, and 13.7% for underweight (Ssentongo *et al.,* 2021). Iron and zinc are mostly participants in varied metabolic functions of the human body. Therefore, inadequate Fe and Zn intake can lead to cell-mediated immune dysfunction, cognitive impairment, and, most prominently, growth retardation. Iron deficiency causes a decrease in circulating hemoglobin and erythrocyte production in the body, resulting in anemia. Iron deficiency symptoms can appear as fatigue, decreased immunity, shortness of breath, hypothermia, and an increased risk of infection (Lopez *et al*., 2016).

Anti-nutritional compounds, such as phytic acid, also create problems and can interfere with the food nutrients absorption by the human body. Phytic acid (myo-inositol hexa-phosphoric acid, IP6) is a crucial phosphorus storage compound and natural antioxidant found in cereals (Vashishth *et al.,* 2017). Phytic acid is also an anti-nutrient because it interferes with mineral absorption (Silva and Bracarense, 2016), with its ability to chelate with minerals, such as iron, copper, zinc, and calcium, inhibiting their absorption in the gastrointestinal tract (Bhagyawant *et al.,* 2018). Phytic acid is an influential chelator of minerals in rice grains, reducing their bioavailability (Kumar *et al.* 2017). Phytic acid in cereal food becomes a critical concern regarding essential micronutrient deficiency in developing countries (Vashishth *et al.,* 2017). The low phytic acid characteristic of grains will increase the "global" bioavailability of minerals (zinc, calcium, magnesium) in the human diet, which will ultimately improve their health (Raboy 2020).

Functional food products are the chief and indispensable foods with high health values. A supplementation strategy appears promising to prevent and combat the negative consequences of Fe and Zn deficiencies (Abdollahi *et al.,* 2019). However, staple food crops like rice cannot provide sufficient Fe and Zn to meet daily food needs because these vital elements will chelate with phytic acid in food grains, resulting in low Fe and Zn bioavailability for the human body (Wang *et al.,* 2021). Developing rice cultivars with high Fe and Zn content continues in several international and national institutions, such as FAO, IRRI (International Rice Research Institute), ICRR (Indonesian Center of Rice Research), ICAR (Indian Council of Agricultural Research), and other national institutions in Asia-Africa in collaboration with partner countries to address the problems of malnutrition globally (HarvestPlus 2018; Swamy *et al.,* 2021).

Rice (*Oryza sativa* L.) is no longer targeted for high Fe and Zn content and low phytic acid (Wang *et al.,* 2021). Plant breeding programs aim to enhance micronutrient and antioxidant concentrations and keep low phytic acid concentrations (Bhagyawant *et al.,* 2018). Thus, an increase in Fe and Zn content followed by a decrease in phytic acid is a valuable way to increase the bioavailability of both primary rice elements (Ha *et al*., 2020; Mukhina *et al*., 2022; Tumanyan *et al*., 2022). Correlation is a statistical method that can identify the association level among traits. This analysis could provide insights regarding combining several characters into one genotype, such as genotypes with high iron and zinc contents and low phytic acid in rice.

Progress in breeding or genetically engineering high-yielding stress-tolerant low-phytate crops continues, i.e., on rice (Wang *et al.,* 2021), maize (Chomba *et al.,* 2015), barley (Raboy *et al.,* 2015), and other cereal crops (Rose *et al.,* 2013). In the pertinent study, assessing several BC_4F_3 rice lines resulting from crosses of aromatic and high Zn parental genotypes with popular Indonesian cultivars proceeded selection. Cultivar Inpari IR Nutri Zinc, a high Zn parental genotype, received crossing with two other cultivars, Inpari 23 and Sintanur, the existing popular aromatic cultivars with fluffier and flavorful rice texture. The presented study aimed to (1) determine the content of Fe and Zn, phytic acid, and grain yield potential in several BC_4F_3 lines, (2) verify the correlation among these characters, and (3) identify potential rice lines with high Fe and Zn and lower phytic acid content than the existing parental rice cultivars.

MATERIALS AND METHODS

Experimental site

The research (field and laboratory work) transpired during the dry season from January to September 2022 at the Sukamandi Experimental Station, Indonesian Center for Rice Instrument Standard Testing (ICRIST) – Subang, West Java, Indonesia (with an altitude of 40 masl). The field was alluvial soil irrigation with a pH (4.6) and texture of sand (20%), dust (43%), and clay (37%) (based on soil analysis results).

Plant material and growth conditions

Ten rice genotypes comprising six BC_4F_3 biofortified rice lines for high Fe and Zn content and four check cultivars underwent the study. The used rice lines came from the ICRIST – Indonesian Ministry of Agriculture collection (a collaboration with Harvest Plus IRRI). The rice lines were the backcross results with information presented in Table 1. The check cultivars used were superior and existing rice cultivars popular in Indonesia, including Ciherang, Sintanur, Inpari 23, and Inpari IR Nutri Zinc.

The experiment comprised 10 rice genotypes in a randomized complete block design (RCBD), with three replications. Planting of seedlings aged 21 DAS (days after sowing) included as much as one seedling per hill in an experimental unit plot size of 3 m × 5 m with a spacing of 25 cm × 25 cm. Fertilization and pest control application were optimal, according to the operational standards of the experimental station. Fertilizer dosages were 250 kg urea/ha, 100 kg SP36/ha, and 100 kg KCl/ha. The whole SP36 and KCl dosage application ensued seven days after transplanting with 1/3 urea. Applying the second 1/3 urea followed three weeks after transplanting, and the remaining 1/3 urea application was at the panicle initiation stage.

The harvest of rice genotype samples happened after physiological maturity. A total of 10 sample plants per plot became sample plants. Sample drying in an oven at 50 °C ran for three days. The rice grains' dried samples, cleaned from the empty grain, underwent observations. Using as much as 25 g of filled grain per sample continued for further measurement of the Fe and Zn content. Measuring phytic acid content ran on samples of composite brown rice taken from three replications after previously measuring Fe and Zn content.

Table 1. Genetic material used in the study.

No.	Genotype Code	Rice lines and cultivars	Pedigree
Lines			
1	WR1	BP 35645-3-4	Inpari IR Nutri Zinc////Inpari IR Nutri Zinc///Inpari IR Nutri Zinc//Inpari IR NutriZinc/Sintanur
2	WR5	BP 35650-1-3	Inpari IR Nutri Zinc////Inpari IR Nutri Zinc///Inpari IR Nutri Zinc//Inpari IR NutriZinc/Inpari 23
3	WR10	BP 35650-12-1	Inpari IR Nutri Zinc////Inpari IR Nutri Zinc///Inpari IR Nutri Zinc//Inpari IR NutriZinc/Inpari 23
4	WR13	BP 35650-16-1	Inpari IR Nutri Zinc////Inpari IR Nutri Zinc///Inpari IR Nutri Zinc//Inpari IR NutriZinc/Inpari 23
5	WR1	BP 35650-23-2	Inpari IR Nutri Zinc////Inpari IR Nutri Zinc///Inpari IR Nutri Zinc//Inpari IR NutriZinc/Inpari 23
6	WR26	BP 35650-32-1	Inpari IR Nutri Zinc////Inpari IR Nutri Zinc///Inpari IR Nutri Zinc//Inpari IR NutriZinc/Inpari 23
Cultivars			
7	CHR	Ciherang	Popular
8	SNTR	Sintanur	Aromatic
9	Inp. 23	Inpari 23	Aromatic
10	NZ	Inpari IR Nutri Zinc	High Zn

Zinc (Zn) and iron (Fe) measurement

The Fe and Zn content (mg kg^{-1}) of dehulled (brown rice) grain samples' measurement used an XRF machine (Oxford Instrument X-Supreme8000) that had validation by the ICP method. The machine is available in the Plant Breeding Laboratory of ICRIST (Indonesian Center for Rice Instrument Standard Testing) in Subang District, West Java, Indonesia. Dehulling approximately 25 g grain samples from each subplot engaged the *Satake THU* Testing Husker. Brown rice samples' sorting ensured only healthy and completely filled grains, then used for Fe and Zn content measurement using the XRF machine set up for Rice15 (a 15-second reading time per sample).

Phytic acid measurement

Phytic acid measurement occurred at the Food Technology and Agricultural Product Laboratory, Faculty of Agriculture, Gajah Mada University (UGM), Indonesia. A total of 25 g of the sample of brown rice obtained from each replication underwent phytic acid analysis, following the methodology of Davies and Reid (1979) and also described by Pramitha *et al.* (2019), with some modifications, according to laboratory operational standards of the UGM, Indonesia.

The molar ratios of PA:Zn and PA:Fe in rice grains calculations were as follows (Saha *et al.,* 2017):

$$PA{:}Zn\ molar\ ratio = \frac{(Phytic\ acid\ content\ in\ \mathrm{mg\,kg^{-1}})/660}{(Zn\ content\ in\ mg\ kg^{-1})/65}$$

$$PA{:}Fe\ molar\ ratio = \frac{(Phytic\ acid\ content\ in\ \mathrm{mg\,kg^{-1}})/660}{(Fe\ content\ in\ mg\ kg^{-1})/56}$$

Data analysis

Analysis of variance among the rice genotypes was for the traits, i.e., filled grain weight per plant, 1000-grain weight (WG1000), Fe (mg kg^{-1}), Zn (mg kg^{-1}), and the phytic acid content (PA) (mg g^{-1}) in brown rice. Data analysis used the SAS software. The analysis of variance (ANOVA) proceeded, followed by the least significant difference (LSD) to compare the means of various rice genotypes when the F-test was significant, considering means as significantly different if the p-value is less than 0.05. The Pearson correlation analysis helped analyze the correlation with the R version 4.1.1. Descriptive analysis of the phytic acid compound and scatter plot visualization of Fe and Zn characters with phytic acid continued with the help of the Minitab 18.1 software.

Table 2. Mean value of phytic acid (PA), Fe, Zn, 1000-grain weight (1000GW), and grain yield per plant in rice lines and cultivars.

Genotypes	WG1000 (g)	Grain Yield (g)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	PA (mg g^{-1})
WR1	23.10^{NS}	45.07 +	14.30^{NS}	22.10^{NS}	21.42 -
WR5	23.20^{NS}	37.40^{NS}	14.10^{NS}	26.90^{NS}	17.90 -
WR10	23.60^{NS}	35.23^{NS}	13.90^{NS}	33.80 +	19.50 -
WR13	25.10 +	35.37^{NS}	15.60^{NS}	29.80 +	25.98 +
WR21	22.00^{NS}	36.90^{NS}	16.20^{NS}	28.00^{NS}	25.33 +
WR26	22.40^{NS}	32.00^{NS}	16.80 +	22.20^{NS}	26.38 +
Inpari IR Nutri Zinc	22.80^{NS}	41.90 +	15.40^{NS}	26.50^{NS}	22.84 +
Inpari 23	24.90^{NS}	33.27^{NS}	16.80^{NS}	22.50^{NS}	25.07 +
Sintanur	27.20 +	31.03^{NS}	14.90^{NS}	22.30^{NS}	24.01 +
Ciherang	23.60	29.23	14.00	23.20	22.34
Mean	23.79	35.74	15.20	25.73	23.08
CV (%)	3.18	15.42	8.89	11.64	0.89
LSD 5%	1.33	9.91	2.36	5.15	0.47

Notes: $^{+, -}$ = significantly higher and lower than the cultivar Ciherang by LSD ($p < 0.05$), respectively. NS = not significant than Ciherang. LSD 5% = the least significant difference at 5%. CV = coefficient of variant. WG1000 = 1000 grain weight. Grain Yield = filled grain yield per plant. PA = phytic acid content.

RESULTS

Variance analysis and PA:Zn and PA:Fe molar ratios

The analysis of variance showed significant differences among the rice genotypes for the traits, i.e., 1000-grain weight, grain yield per plant, Fe, Zn, and phytic acid contents. The rice cultivar Sintanur as a check was visible with the largest grain size (1000-grain weight = 27.20 g), whereas the smallest grain size came from cultivar Inpari IR Nutri Zinc (1000-grain weight = 22.80 g) compared with three check cultivars. The cultivar Inpari IR Nutri Zinc had the highest filled grain weight per plant (41.90 g). The cultivar Inpari 23 revealed the highest Fe content (16.80 mg kg^{-1}), while the rice cultivar Inpari IR Nutri Zinc as a check cultivar emerged with the highest Zn content (26.50 mg kg^{-1}). Cultivars Inpari IR Nutri Zinc and Ciherang had equal low phytic acid content. The measurement results also showed that Inpari Nutri Zinc and Ciherang contained a phytic acid content of 22.84 mg g^{-1} and 22.34 mg g^{-1}, respectively.

According to the LSD test, the rice line WR 1 resulted in having the highest grain weight per plant (45.07 g) and was significantly higher than the standard cultivar Ciherang (29.23 g) (Table 2). The maximum Fe content was notable in the rice WR 26 (16.80 mg kg^{-1}), which was also significantly higher than Ciherang (14.00 mg kg^{-1}). The peak Zn content was with the rice lines WR10 (33.80 mg kg^{-1}), which was also considerably more than Ciherang (23.20 mg kg^{-1}). The lowest phytic acid content was evident in the rice WR5 (17.90 mg g^{-1}), also having a significantly lower content than the check cultivar Ciherang (22.34 mg g^{-1}).

Three potential rice lines, observed with the lowest phytic acid, i.e., WR1, WR5, and WR10, were also significantly lower than the check cultivar Ciherang, a popular rice cultivar grown in Indonesia. Rice line WR1 had the minimum phytic acid (21.42 mg g^{-1}) and the highest grain yield (45.07 g $plant^{-1}$); however, the Zn content was also low (22.10 mg kg^{-1}). Rice line WR5 had a low phytic acid (17.90 mg g^{-1}), and its seed size was equivalent to Ciherang, with a 1000-grain weight of 23.20 g, while the Fe and Zn content was equivalent to cultivar Inpari IR Nutri Zinc. Line WR10 has a 1000-grain weight equal to Ciherang (1000-grain weight = 23.6 g), better yield potential per plant (35.23 g $plant^{-1}$) than Ciherang, and the highest Zn content (33.80 mg kg^{-1}), which was also significantly higher than the best check cultivar Inpari IR Nutri Zinc (26.50 mg kg^{-1}).

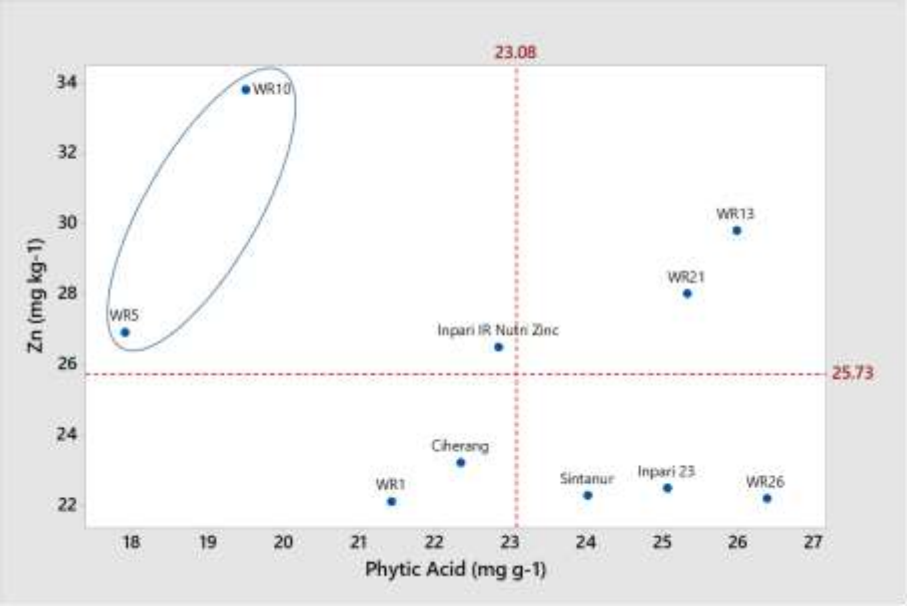


Figure 1. Scatter plot of relationship between phytic acid and Zn of rice lines and cultivars. Red lines X, Y = mean value of phytic acid (mg g^{-1}) and Zn (mg kg^{-1}), respectively.

The scatter plot showed the best rice genotype based on high Zn characters and low phytic acid content (Figure 1). Rice lines WR5 and WR10 appeared promising genotypes with high Zn and low phytic acid. The line WR1 also has low phytic acid but deficient Zn content. Lines WR21 and WR26 had the highest Zn content than the commercial cultivar Inpari Nutri Zinc, yet had a high phytic acid. Promising rice lines, such as WR5 and WR10, were apparently to become the candidate genotypes for new superior cultivars with the advantages of high Zn and low phytic acid.

The biochemical analysis further revealed the molar ratios (PA:Zn and PA:Fe), as presented in Figure 2. The data indicated that the tested genotypes' PA:Zn molar ratio values ranged from 56.82–117.01, while the PA:Fe ratio values ranged from 125.03–164.02. The lowest PA:Zn ratio resulted in rice line WR10, whereas the highest was line WR26. The minimum PA:Fe ratio came from line WR5, and the highest, as shown by WR13. Cultivar Inpari IR Nutri Zinc, an elevated Zn biofortified cultivar released in Indonesia, has PA:Zn and PA:Fe molar ratios of 84.86 and 146.97, respectively. Cultivar Ciherang, a popular cultivar in Indonesia, has PA:Zn and PA:Fe molar ratios of 94.81 and 157.12, respectively.

Correlation of phytic acid with Fe, Zn, 1000-grain weight, and grain yield

Correlation analysis of phytic acid with Zn, Fe, 1000-grain weight, and grain weight per plant appears in Figure 3. The results showed a significant positive correlation of the phytic acid with the Fe content ($r = 0.75$, $p < 0.05$) and a nonsignificant negative correlation with the Zn content in the seed ($r = -0.33$, $P > 0.05$). The 1000-grain weight ($r = 0.22$, $P > 0.05$) and the grain yield per plant ($r = -0.38$, $P > 0.05$) also had a nonsignificant correlation with phytic acid.

The presented study also disclosed that the Zn content had a significant positive correlation with Fe ($r = 0.28$, $p < 0.01$). However, Zn has a significant negative correlation with 1000-grain weight ($r = -0.29$, $p < 0.01$). The Zn content's correlation with the grain yield was insignificant. The trait 1000-grain weight also did not have a substantial association with the grain yield per plant ($r = 0.09$, $P > 0.05$).

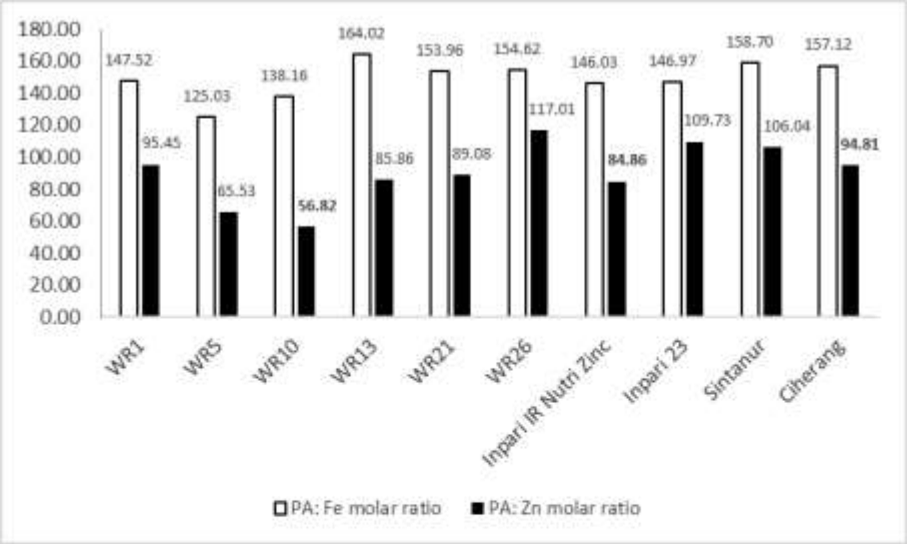
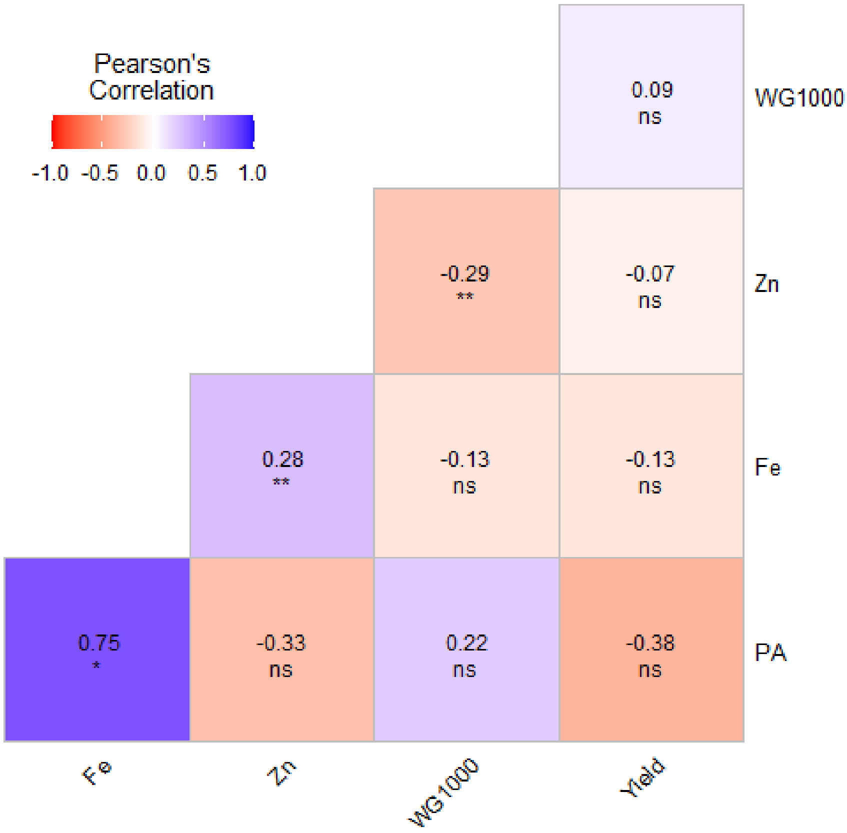


Figure 2. PA:Zn and PA:Fe molar ratios in 10 rice lines and cultivars.



ns p >= 0.05; * p < 0.05; ** p < 0.01; and *** p < 0.001

Figure 3. Pearson's correlation coefficients between phytic acid (PA) characters with Fe, Zn, 1000-grain weight (WG1000), and grain yield per plant in rice lines and cultivars. Significance of correlations indicated as ** *P* < 0.001; **P* < 0.01; ns, not significant.

DISCUSSION

The results indicated a significant effect of genotypes in managing traits' variations, i.e., 1000-grain weight, grain yield, Fe, Zn, and phytic acid content. Three rice genotypes obtained have low phytic acid, namely, WR1, WR5, and WR10. These three genotypes were significantly lower in phytic acid than the check cultivar Ciherang. Rice line WR1 has a lower phytic acid and equivalent seed size to cultivar Ciherang, an excessive yield but low Zn. Line WR5 has a low phytic acid content and seed size and was at par with cultivar Ciherang, while Fe and Zn contents were the same as in cultivar Inpari IR Nutri Zinc, with a high Zn cultivar in Indonesia. Rice line WR10 has a seed size corresponding to cultivar Ciherang but a better yield potential, with the highest Zn content versus other rice genotypes and the best check cultivar Inpari IR Nutri Zinc.

Phytic acid behaved like an inhibitor compound to nutrient absorption. Thus, the food sources are expected with low phytic acid, so nutrients in cereals and others could have better absorption than food sources with high phytic acid (Bhagyawant *et al.,* 2018; Wang *et al.,* 2021). However, Silva and Bracarense (2016) explained the chief chemical and biological aspects mentioned that phytic acid (IP6) is a natural antioxidant found mainly in cereals and vegetables, which is also beneficial in preventing several diseases. The association of phytic acid characteristics must be known in breeding materials for biofortifying specific nutrients, such as Fe and Zn, in rice seeds.

The present-day study showed that Zn has an insignificant correlation with the phytic acid content. For a selection, a nonsignificant correlation between PA and Zn is more beneficial than a positive correlation. The seeds' higher and lower Zn content has no association with high and low phytic acid. Therefore, it is possible to produce high Zn and less phytic acid cultivars through conventional breeding programs by selecting the segregants possessing these traits. In line with a previous study, Yatou *et al.* (2018) also revealed a nonsignificant correlation between phytic acid and Zn content.

Phytic acid content had no significant correlation with 1000-grain weight, yield per plant, and Zn content. Based on this study, high-yielding rice cultivars could contain low or high phytic acid content. Genotypes with slender seed shapes could have either a low phytic acid content (such as W10) or a high phytic acid content (such as WR21). However, the Zn content has a significant negative correlation with 1000-grain weight. It indicated that the larger the seed size, the smaller the Zn content in the seeds; conversely, the smaller the seed size, the higher the seed's Zn content. It was a conjecture that there was an unstable relationship between Zn/Fe and the 1000-grain weight and other yield traits. Findings by Anusha *et al.* (2021) showed that the Fe and Zn contents of the rice grain for the parental lines indicated a negative association with grain yield under two contrasting growing conditions. Rohaeni and Susanto's (2021) results showed a nonsignificant correlation between the Zn/Fe ratio and 1000-seed weight occurred. Calayugan *et al.* (2020) reported a negative correlation between grain zinc traits and yield components in rice. The inconsistency of the relationship between Zn/Fe with 1000-grain weight has a report by Kanatti *et al.* (2014), with the same genetic material tested under diverse environments.

This study provided an interesting result and a challenge that phytic acid content significantly positively correlates with Fe content. It means the higher the seeds' Fe content, the higher the phytic acid content. These results were in analogy with past findings, which showed a relationship between phytic acid content and the bioavailability of the Fe content (Bhagyawant *et al.* 2018). It will be an obstacle for the high Fe biofortified rice breeders because it was a suspicion that inbred and hybrid lines with high Fe possibly have a high phytic acid. The ideal genotypes should have high Fe content with low phytic acid so that the proper absorption of Fe minerals in rice can be easier for the human body.

In the presented study, Zn content had a significant positive correlation with Fe content. These results agree with those of Rohaeni and Susanto (2021), who observed a significant positive correlation between Zn and Fe content in rice grains studied in 180 rice genotypes. These results also align with that of Anusha *et al.* (2021), which showed a positive correlation between Fe and Zn content in the parental genotypes and in hybrids under irrigated conditions. In addition, Liu *et al.* (2014) studied 320 cultivars procured from China, which showed a significantly positive correlation between the Zn and Fe content. The correlation between Fe and Zn attributes may depend on the species studied. In the family *Leguminosae* (Diaz *et al.,* 2022), findings showed that Zn had a substantial positive correlation with Fe in three biparental common bean (*Phaseolus vulgaris* L.) populations. Some studies disclosed contradictory results, and Kumar *et al.* (2019) reported that there was no correlation between the iron and zinc content in both F_3 and F_4 populations.

Zinc and Fe content sources are cereals, nuts, legumes, fruits, and vegetables, including non-heme/vegetable Zn and Fe (Singh *et al.,* 2016). Several previous studies reported that the uptake of Zn and Fe from non-heme sources was lower than heme (animal) Zn and Fe. Hurrell and Egli's (2010) findings revealed that the absorption of vegetable iron was much lower than that of animal iron, which was 2%–20%, and the vegetable iron's absorption has strong influences from the presence of other food components. The nutrient absorption level also has gender managing and influencing it (Dainty *et al.* 2014). One of the compounds that inhibit nutrient uptake (antinutrients) are phytic acid compounds (Silva and Bracarense, 2016; Bhagyawant *et al.,* 2018; Wang *et al.,* 2021; Ibrahim *et al.,* 2022). Therefore, it seems necessary for pyramiding breeding to combine high nutritional character with low anti-nutritional compound character. Wang *et al.* (2021) suggested pyramid breeding combined with high Zn density and low phytic acid as a practical and helpful approach to increase Zn bioavailability in rice. It will be more effective in helping malnutrition problems than when focused on raising Zn quality in a particular cultivar.

Zinc's accumulation in grains resulted in a significant loss of Fe and phytic acid. The bioavailability of Zn and Fe nutrients increases if the phytic acid content decreases. Saha *et al.* (2017) studies have shown a significant reduction in the molar ratio PA:Zn (from 112.3 in control to 43.7 in SFtf - foliar supply at maximum tillering and flowering stages) in Zn applications due to reduced phytic acid and increased Zn content in seeds. These results showed that the rice line WR10 emerged with the lowest PA:Zn molar ratio. It may indicate that line WR10 has better bio-accessibility of Zn minerals than all the check cultivars.

The findings of Hama-Salih *et al.* (2021) showed that the rice samples in their study had poor bioavailability of Fe and Zn because of high PA:Fe and PA:Zn molar ratios. It can be due to the high PA content in the studied rice, which impacted Fe and Zn content bioavailability in these foods. Although the samples had high Fe and Zn content, they also had a high PA content, which could decrease the bioavailability of Fe and Zn in the body. The high-moderate PA:Zn and PA:Fe molar ratios were <15. In the presented study, the rice samples showed an availability of >15. Thus, the breeding program on low phytic acid in rice required the new plant breeding technique approach (NPBT). Ali *et al*. (2013) showed that the low-phytate rice seeds accumulated 1.8-fold more iron in the endosperm due to the decreased phytic acid levels, and the study provided evidence that silencing of the *IPK1* gene can mediate a substantial reduction in seed phytate levels without hampering the growth and development of transgenic rice plants.

Developing rice cultivars containing high Zn and Fe content with a higher bioavailability level (low in phytic acid) is a high recommendation for developing countries, such as Indonesia. This approach is optional in a sustainable, targeted, and food-based manner as it is more cost-effective and efficient in reducing malnutrition due to zinc and iron deficiency. However, these relevant results displayed that breeding high Zn biofortified rice with low phytic acid would be easier than generating high Fe rice with low phytic acid. Rice breeding to produce cultivars with high yields and low phytic acid is possible because the association of phytic acid with harvest is not significant. The contemporary research identified a potential high Zn rice line WR10 (Zn = 33.80 mg kg^{-1}; better yield potential per plant than Ciherang; PA:Zn 56.82 ratio was lower than all check cultivars).

New plant breeding technique (advanced breeding) approaches, such as CRISPR, is a better alternative for developing rice genotypes with low phytic acid content. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) is an advanced breakthrough technology for genome editing. For example, the *GmIPK1* gene mutation using CRISPR/Cas9 reduced phytic acid content in soybean seeds (Song *et al.,* 2022). The PA content of rice grain can decline by down-regulating the *IPK1* gene (Kumar *et al.,* 2022).

CONCLUSIONS

The results revealed that phytic acid showed nonsignificantly correlated with Zn, 1000-weight grain, and yield per plant. However, phytic acid exhibited a strong positive correlation with Fe. The rice line WR10 was notable, with the highest Zn content (33.80 mg kg^{-1}), lower PA:Zn molar ratio than all the check cultivars, the highest yield potential, and better yield than the Ciherang cultivar. Based on this research, an advanced breeding approach is highly recommendable for getting rice cultivars with low phytic acid.

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