BIODIVERSITAS
Volume 24, Number 1, January 2023
Pages: 208-214

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240125

Genetic diversity, heritability, and accumulation patterns of Zn content in biofortified rice

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Manuscript received: 19 October 2022. Revision accepted: 4 January 2023.

Abstract. *Rohaeni WR, Suwarno WB, Susanto U, Trikosoemaningtyas, Ghulamahdi M, Aswidinnoor H. 2023. Genetic diversity, heritability, and accumulation patterns of Zn content in biofortified rice. Biodiversitas 24: 208-214.* The study on zinc accumulation patterns in various rice organs is valuable information to support effective biofortified breeding. Research reports related to zinc accumulation have only been limited to seeds, while the pattern of zinc accumulation in each part of the plant has not been reported, especially for Indonesian rice germplasm. The study aims to provide information on the pattern of accumulation and genetic diversity of Zn content in rice plants. The experiments used a randomized complete block design (RCBD) with 3 replications and genotypes as a treatment. The genotypes were 29 lines of BC4F3 Inpari IR Nutri Zinc////Inpari 23, 5 lines of BC4F3 Inpari IR Nutrizinc////Sintanur, and 1 line of BC4F3 Inpari IR Nutrizinc////Inpari 35. A total of 6 varieties were used as checks. The Zn content of dehulled (brown rice) grains sample was measured using the Oxford Supreme8000 XRF machine with the Rice15 method that had been validated by the Inductively Coupled Plasma (ICP) method, while the analysis of the pattern of Zn content of several organs (seeds, leaves, stems, and roots) used the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-IOS) method. The coefficient of genetic variation of the grain zinc content (GZn) was moderate and had high heritability criteria (H^2_{bs} = 0.83). Yield characters have a narrow coefficient of genetic variation and low heritability. There were differences in the pattern of Zn accumulation in plant organs between high GZn and low GZn genotypes. The distribution of Zn content in high GZn genotypes showed 26.4% in seeds, 13.7% in leaves, 39.3% in stems, and 20.6% in roots. The distribution of Zn content in low GZn genotypes showed 14.2% in seeds, 10.9% in leaves, 31.0% in stems, and 44.0% in roots. The results of this study indicate that a different breeding strategy is needed to develop new high-yielding rice varieties based on zinc accumulation patterns in cross-parents.

Keywords: Biofortification, genetic diversity, rice, Zn accumulation

INTRODUCTION

Malnutrition and chronic disorders in developing countries are the main obstacles to achieving the Millennium Development Goals (Dipti et al. 2012). Malnutrition, such as stunting, wasting, and being underweight in childhood, is reported to cause more than 1.0 million deaths, 3.9% of life years lost, and 3.8% of life years lost. The prevalence estimation value of malnutrition in West African, South Asian, and Southeast Asian countries are thought to be much higher than the global average (Ssentongo et al. 2021). Improving nutrition by increasing the nutrient content of staple foods, such as rice, will be more effective because it is consumed daily by the people of Indonesia. Rice breeding programs can focus on developing new varieties with higher Fe, Zn, or β-carotene content. Indonesia, one of the developing countries prone to stunting, has pursued a biofortified rice breeding program for zinc to overcome stunting.

Biofortification is defined as an effort to increase the nutritional value of food commodities through genetic engineering approaches (biotechnology and conventional breeding), agronomic approaches (microbe-mediated, mineral-rich fertilization, growth regulators, and cultivation systems) (Garg et al. 2018; Olson et al. 2021), and post-harvest product supplementation (Siwela et al. 2020). Biofortification through a plant breeding approach is reported to be more effective and efficient than agronomy and supplementation approaches, especially to reach target areas that have limited access to factory-made nutrient-rich products (Olson et al. 2021).

Biofortification of micronutrients, vitamins, and other important substances, including Zn is possible to be carried out in rice commodities through conventional breeding approaches (Swamy et al. 2016; Nakandalage et al. 2016; Rao et al. 2020). The effectiveness of selection is largely determined by genetic diversity and the heritability value of the target characters (Keerthana et al. 2019). The higher the genetic diversity, the higher the selection effectiveness. The selection of characters with moderate to high heritability values will be effective in the early generations. Conversely, selection for characters with low heritability may be more effective in later generations (>F7).

The study of the genetic diversity of Indonesian rice germplasm for the character of zinc content in grain is still limited. The Indian Institute of Rice Research (IIRR) initiated efforts to biofortify rice for Zn by characterizing the Zn content in various dehulled rice using energy-dispersive X-ray fluorescence spectroscopy (XRF). The results reported that the zinc content in rice ranged from 7.3 to 52.7 mg/kg (Rao et al. 2020). So far it has been known that *Oryza australiensis* is a species with high nutritional content. Meanwhile, breeding efforts for Zn-rich rice in Indonesia have succeeded in obtaining seven lines that have higher grain Zn content (range 30.0-34.2 mg/kg) than the Ciherang line. These five lines have productivity comparable to Ciherang (Susanto et al. 2017).

The nutritional content of a grain of rice is influenced by the morphology and histological characteristics of cariopsis (Roy and Shil 2020), both of which are genetically controlled. It is not enough to know the genetic diversity of zinc in grains, the exploration of genetic diversity related to Zn content in other organs is also important, because physiologically, zinc is translocated from roots to all plant organs, including rice grains. Therefore, information on the accumulation pattern of Zn content and its genetic diversity in various plant organs will be very useful as a basis for determining future rice biofortification breeding programs for Zn, both conventionally and using biotechnology. Estimation by combining heritability values and hereditary propels is thought to be very helpful in predicting genetic progress compared to relying solely on heritability estimation values (Cooper et al. 2021). Information on genetic diversity and character control genes related to zinc accumulation in rice is needed to see the effectiveness of selection methods in biofortified rice breeding programs. The present investigation aims to provide information on genetic diversity and accumulation patterns of Zn content in several rice organs and to obtain potential lines with high yields and high Zn content in the grain.

MATERIALS AND METHODS

Study area

The research was conducted at two locations (an irrigation agroecosystem and rainfed lowland) in March - September 2022. The site 1 irrigation agroecosystem was located at The experimental station of the Indonesian Center of Rice Research (ICRR), Subang, West Java - Indonesia (6°21'10.0"S:107°38'54.1"E with an altitude of 10m above sea level, the soil type was Alluvial). The site 2 rainfed lowland was located at 6°31'08.4"S:107°31'05.3"E with an altitude of 92.7m above sea level and the soil type was Latosol.

Genetic diversity and heritability of agronomic characters and Zn content

A total of 35 promising lines were used in this study, including 29 lines BC4F3 Inpari IR Nutri Zinc////Inpari 23, 5 lines BC4F3 Inpari IR Nutrizinc////Sintanur, and 1 line BC4F3 Inpari IR Nutrizinc////Inpari 35. The check varieties used were 6 varieties, namely Inpari IR Nutri Zinc, Inpari 23, Sintanur, Inpari 35, Ciherang, and Inpari 46 TDH. Research at each location using a randomized complete group design with 3 replications. Seedlings aged 21 hss were transplanted with a spacing of 25cm x 25cm in a 4m x 5m plot. Fertilizer applications followed a recommendation from The experimental station of ICRR (equal to the results of the paddy soil rapid test kit with a recommendation of 300 kg/ha of urea, 100 kg/ha of SP-36, and 50 kg/ha of KCl). Pest and disease management according to Integrated Crop Protection principles.

Several agronomic characteristics that were measured were plant height (cm), the number of tillers, panicle length (cm), panicle weight per clump (g), total grain weight per clump (g), and filled grain weight per clump (g). The targeted traits were the Zn content in brown rice (mg/kg) and yield (t/ha at 14% moisture content), and the supported biofortified trait was the Fe content in brown rice (mg/kg).

Measurement of zinc content for grains (GZn - Grains zinc) according to the standard procedure described by Rohaeni et al. (2016) and Susanto et al. (2017). The Zn content (mg/kg) of the peeled brown rice grain sample was measured using the Oxford Supreme 8000 XRF machine with the Rice15 method which has been validated by the ICP method. The zinc measurement to study accumulation patterns in plant organs was carried out using the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) method at the SIG-Bogor Company Laboratory. Analysis of the pattern of zinc accumulation was carried out on representatives of low and high GZn genotypes taken from experimental harvest samples at site 1. A total of 7 genotypes were used for this analysis. Samples of plant organs were taken from 3 replicates.

Data analysis

The analysis covered ANOVA per site, combined ANOVA, estimation of H^2_{bs} value, and coefficient of genetic variation (CGV) (Table 1). If the genotype effect was significant at the 5% level, then the least significant difference (LSD) test at the 5% level was performed. Statistical analyses were conducted using SAS, Microsoft Excel, and Minitab.

Table 1. Combined analysis and partition of expectation of mean square for estimating genetic variation component

Source	DF	MS	E(MS)
Environment (E)	l-1	M5	
Replication/environment	l(r-1)	M4	
Genotype (G)	g-1	M3	$\sigma^2_e + r\,\sigma^2_{gl} + rl\,\sigma^2_g$
GxE	(g-1) (l-1)	M2	$\sigma^2_e + r\,\sigma^2_{gl}$
Error (E)	l(g-1)(r-1)	M1	σ^2_e

Notes: DF: degree of freedom, MS: mean square, E(MS): expectation of mean square

Estimation of the components of genetic, environmental, phenotype, and GxE variation were referred to in Table 1 and carried out in the following formulas:

σ^2 G = (M3 - M2) / rl
σ^2 GxE = (M2 - M1) / r
σ^2 E = M1
σ^2 P = σ^2 G + σ^2 E / rl + σ^2 GxE / l

Where: l: environment, r: replication, σ^2 E: environment variance, σ^2 G: genotype variance, σ^2 P: phenotype variance, σ^2 GxE = GxE variance

$$H^2_{bs}(broad\ sense\ heritability) = \frac{\sigma^2_G}{\sigma^2_P} \times 100\%$$

H^2_{bs} criteria :

Low ($H_{bs}^2 \leq 20\%$); Moderate ($20\% < H_{bs}^2 < 50\%$); High ($H_{bs}^2 \geq 50\%$).

Coefficient of genetic variation (Singh and Chaudhary 2007)**:**

$$\text{Coefficient of genetic variation (CGV)} = \frac{\sqrt{\sigma^2_G}}{\bar{X}} \times 100\%$$

Where:

$\bar{X}$= general mean

CGV categories: narrow (0-10%), moderate (10-20%), and wide (> 20%)

RESULTS AND DISCUSSION

Genetic diversity and heritability of agronomic characters and Zn content

The estimated value of genetic diversity showed the character of plant height, number of tillers, physiological maturity, number of panicles per hill, number of filled grains per hill, number of empty grains per hill, 1000 grain weight, and grain Zn content (GZn) had a coefficient of genetic variation with moderate criteria. Meanwhile, the maturity age, the weight of filled grain per clump, the Fe content of the grain, and the yield characters had narrow genetic diversity (CGV) (Table 2).

The heritability value with high criteria was owned by GZn (H^2_{bs} = 0.83), 1000 grain weight (H2bs = 0.91), and maturity age (H2bs = 0.65). While the heritability value with moderate criteria was on plant height, number of tillers, number of panicles per clump, number of filled grain per clump, number of empty grains per clump, and grain Fe content (Table 2). These results led to the notion that the GZn trait, which was the target of the study, had a very large potential to be inherited, indicated by a high criterion heritability value. This result is the same as several previous reports showing that GZn has high heritability (Swamy et al. 2016; Velu et al. 2019; Mukamuhirwa et al. 2015).

The GZn character, being the target character of this study, shows a high heritability value. This means that the selection of high Zn lines can be carried out from the early generation. Traits with high heritability predictive value have higher prospects for selection success because most of the variation is caused by genetics. Estimates of heritability provide a better estimate of population genetic diversity and allow prediction of selection gain. Therefore, they are very important in breeding programs (Ribeiro et al. 2019). Sadimantara et al. (2021) reported that a high heritability predictive value helps select superior genotypes based on phenotypic appearance. It has been reported that rice has abundant germplasm variation thus providing the required genetic diversity for rice breeding programs. The existence of genetic variability is a prerequisite for a successful breeding program for any species. Sadimantara et al. (2021) showed that the yield character has a heritability value with high criteria and a moderate coefficient of genetic variation.

The results of this study indicated that there were considerable challenges in combining the yield character with the GZn character. The heritability value of the GZn was high, while the yield character was low. The selection for high GZn can be performed in early generations, but selection for high yield will perhaps be more effective when carried out in the later generations. GZn selection in the early generations might allow accumulating the additive genes regulating GZn, but there will be erosions of additive genes controlling yield.

Table 2. Estimated variance components and broad-sense heritability of biofortified rice genotypes based on field trials from two agroecosystems (irrigation and rainfed lowland)

Characters	Mean	CV %	σ^2g	σ^2_{gxe}	σ^2_e	σ^2p	CGV %	CGV criteria	H^2_{bs}	H^2_{bs} criteria
PH	105.72	11.85	147.69	2.10	157.07	306.86	11.50	Moderate	0.48	Moderate
NT	18.02	16.19	5.21	0.50	8.52	14.23	12.66	Moderate	0.37	Moderate
MA	113.32	1.20	5.34	1.04	1.86	8.24	2.04	Narrow	0.65	High
NP	18.02	16.19	5.21	0.50	8.52	14.23	12.66	Moderate	0.37	Moderate
NPG	1928.32	15.76	50065.88	3190.44	92351.59	145607.91	11.60	Moderate	0.34	Moderate
NEG	404.49	24.16	2411.04	217.43	9552.56	12181.03	12.14	Moderate	0.2	Moderate
WPG	44.31	16.6	5.85	0.81	54.14	49.10	5.46	Narrow	0.12	Low
1000GW	23.25	3.66	7.96	0.02	0.72	8.70	12.13	Moderate	0.91	High
FE	16.54	9.50	1.31	0.05	2.47	3.82	6.91	Narrow	0.34	Moderate
ZN	29.45	6.20	23.95	1.44	3.33	28.73	16.62	Moderate	0.83	High
Yield	6.67	12.30	0.13	0.16	0.67	0.96	5.41	Narrow	0.13	Low

Notes: PH: plant height (cm), NT: number of tillers, MA: maturity age, NP: number of panicles per clump, NPG: number of filled grains per clump, NEG: number of empty grains per clump, WPG: weight of filled grains per clump, 1000GW: 1000 grains weight, FE: grains Fe content (mg/kg), ZN: grains Zn content (mg/kg), CV: coefficient of variation (experimental error), CGV: coefficient of genetic variation, H^2_{bs}: Broad-sense heritability

Table 3. The best eight genotypes based on higher GZn than the best check (Inpari IR Nutri Zinc)

GEN	PH	NT	MA	NP	NPG	NEG	WPG	1000GW	FE	ZN	Yield
G17	104.47	18.85	111.83	17.33	1771.56	392.61	42.17	22.97	19.07+	**33.07+**	6.66
G32	105.46	21.22+	112.80	17.20	1912.40	463.00	43.39	22.98	16.60	32.04	6.88
G13	101.38	18.43	111.50	18.11	1851.17	388.44	44.54	24.18+	16.33	31.65	7.17
G5	99.57	19.72	111.50	18.61	1772.39	392.72	41.87	23.80+	15.75	31.02	6.76
G11	108.77+	19.28	114.33+	17.61	1869.72	384.22	42.62	22.93	16.28	30.87	6.71
G20	104.40	18.07	113.50	16.28	1605.50	385.94	38.23	23.77+	16.62	30.82	6.96
G4	104.82	18.55	113.00	18.11	2184.83+	316.94	48.00	22.20	16.30	30.10	7.00
G6	101.63	17.75	113.00	18.72	2024.11+	436.44	45.19	22.38	16.13	30.02	7.46
Inpari IR Nutri Zinc	103.68	19.15	112.50	18.33	2183.44+	391.22	47.99	22.38	16.62	29.98	6.61
Mean	105.72	19.02	113.32	18.02	1928.33	404.48	44.34	23.25	16.54	29.45	6.67
CV %	11.85	13.75	1.20	16.23	15.76	24.16	16.57	3.66	9.50	6.20	12.30
LSD 5%	2.99	0.94	1.56	3.34	347.01	111.61	8.39	0.97	1.79	2.08	0.94

Notes: PH: plant height (cm), NT: number of tillers, MD: maturity age, NP: number of panicles per clump, NPG: number of filled grains per clump, NEG: number of empty grains per clump, WPG: weight of pithy grains per clump, 1000GW: 1000 grains weight, FE: grains Fe content (mg/kg), ZN: grains Zn content (mg/kg), Yield: ton/ha. +: significantly higher than check (Inpari IR Nutri Zinc)

Several strategies are needed to combine 2 traits that have different heritability criteria (low and high) such as GZn and yield. First, do pre-breeding for trait enrichment in prospective crossbreeding parents. Second, increasing the number of populations and various types of crosses. Third, using a new plant breeding technique approach by inserting the GZn control gene from super high zinc parents into the genome of high-yielding varieties with high yield potential.

Fortunately, this study resulted in some new promising lines with superior GZn content and higher yield potential than the check variety. The G17, G32, G13, G5, G11, G20, G4, and G6 were the promising lines that had higher GZn than the best GZn parents (Inpari IR Nutri Zinc, GZn 29.98 mg/kg) and had better yield potential (Table 3). Lines G6 (GZn = 30.02 mg/kg; Yield = 7.46 tons/ha), G13 (GZn = 31.65 mg/kg; Yield = 7.17 tons/ha), and G4 (GZn = 30.10 mg/kg; Yield = 7.00 tons/ ha) showed yield potential with better GZn content than the high GZn parent Inpari IR Nutri Zinc (GZn 29.98 mg/kg; Yield = 6.61 mg/kg). Furthermore, these promising lines need to be tested for further yields in several locations to see the stability of the GZn content and potential yields (Table 3).

Conventional breeding for the combination of biofortified and high-yield rice traits requires more energy for several reasons. First, the correlation between the GZn trait and yield properties is inconsistent. Some reports show a negative correlation between grain zinc trait and yield components in rice (Inabangan-Asilo et al. 2019; Rao et al. 2020) and wheat (Khokhar et al. 2020), but several other studies show the opposite results (Paramesh et al. 2020; Calayugan et al. 2020). Second, selection for high GZn may be effective in early generations, whereas selection for a high-yield trait is effective in later generations. GZn has high heritability but the yielding character has low heritability. Third, in the present study, the genetic diversity for GZn was included in the moderate criteria but the genetic diversity for the yield components was included in the low category.

A breeding strategy involving crossing combinations is needed to increase the chances of obtaining high GZn genotypes as well as high yields. Breeding high-yield varieties that are responsive to zinc-rich fertilization can increase the GZn content of high-yield genotypes. Muthukumararaja and Sriramachandrasekharan (2012) showed that rice plants are very responsive to the addition of extracellular zinc. Shahzad et al. (2021) reported that plant biofortification could be achieved through conventional approaches or new breeding techniques (NBTs). Conventional approaches to biofortification include mineral fertilization through foliar or soil applications (Cakmak and Kutman 2018), as well as microbial-mediated nutrient uptake enhancement (Krithika and Balachandar 2016).

Zinc distribution pattern on rice plant organs

Analysis of variance showed that replication had no significant effect on Zn content in all rice plant organs. Genotype has a very significant effect on the diversity of zinc content in each organ (Table 4). Further tests in this study showed that there were 3 classes of genotypes based on GZn content. The classes were genotypes containing high, moderate, and low GZn. The high GZn was possessed by the Pulut Timuru genotype (34.0 mg/kg) and was significantly different from the moderate and low genotypes. Genotypes included in the moderate GZn category were Inpari IR Nutri Zinc (19.6 mg/kg), G5 (21.2 mg/kg), Inpari 23 (18.2 mg/kg), and G27 (18.4 mg/kg). The genotypes included in the low GZn category were Inpari 46 GSR TDH (16.5 mg/kg) and G34 (13.9 mg/kg) (Table 5).

The present study showed that there were differences in the pattern of Zn accumulation in plant organs between high GZn and low-moderate GZn genotypes. The distribution of Zn content in the high GZn genotype, Pulut Timuru, showed higher content of zinc (26.4%) in seeds than in stems (39.3%) while zinc content in leaves was 13.7%, and 20.6% in roots.

Table 4. Analysis of variance on the character of Zn content on seeds, leaves, stems, and roots

Source of variation	F value				
	Zn grain	Zn leaf	Zn stem	Zn root	Zn total
Replication	0.07 ns	0.72 ns	2.81 ns	0.42 ns	1.85 ns
Genotype	33.64 **	12.89 **	10.97 **	3.67 *	4.68 *

Note: *, **: significant at p < 0.05 and p < 0.01, respectively; ns: not significant

Table 5. Zn content (mg/kg) in grain, leaf, stem, and root on low, moderate, and high GZn genotype

Genotype	Zn grain	Zn leaf	Zn stem	Zn root	Zn total
High GZn genotype					
Pulut Timuru	34.0[a]	17.7[a]	50.6[a]	26.6[c]	**128.9[a]**
Moderate GZn genotypes					
Inpari IR Nutri Zinc	19.6[bc]	14.6[b]	18.4[c]	38.0[bc]	90.6[c]
G5	21.2[b]	13.5[bc]	20.5[c]	50.8[ab]	106.0[bc]
Inpari 23	18.2[bc]	14.0[b]	24.7[bc]	37.1[bc]	94.0[c]
G27	18.4[bc]	14.3[b]	24.1[bc]	42.1[ab]	98.9[bc]
Low GZn genotypes					
Inpari 46 TDH GSR	16.5[cd]	11.4[d]	32.0[b]	39.5b[c]	99.4[bc]
G34	13.9[d]	11.8[cd]	34.2[b]	54.5[a]	**114.4[ab]**

Note: Means followed by the same letter in the same column are not significantly different. GZn: grain zinc content (mg/kg)

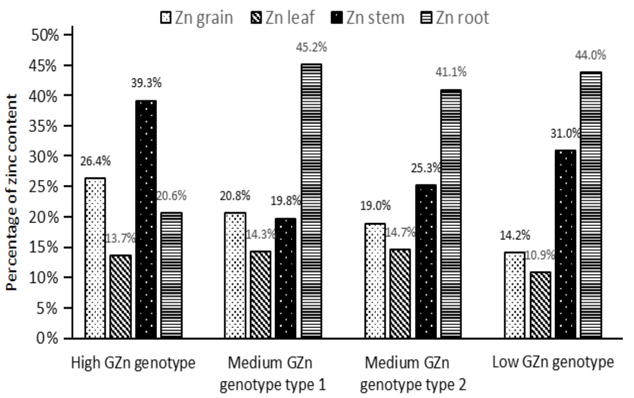

Figure 1. Percentage of zinc content in grains, leaves, stems, and roots in high, moderate, and low GZn genotypes

Conserve, the distribution pattern of Zn content in low GZn genotype, such G34 and Inpari 46 TDH, showed 14.2% in seeds, less than zinc accumulation in stem (31.0%), while zinc accumulation on leaves and root, were 10.9% and 44.0%, respectively. The unique pattern of zinc accumulation was found in the moderate GZn genotype. The first pattern was followed high GZn genotype (20.8% in seeds, 19.8% in stems, 14.3% in leaves, and 45.2% in roots), called genotype type I, and the second pattern was according to a low GZn genotype (19.0% in seeds, 25.3% in stems, 14.7% in leaves, and 41.1% in roots), namely genotype type II (Figure 1).

The G34 line was the lowest GZn, but the total zinc value in all organs (114.4 mg/kg) was equivalent to the highest GZn genotype (Pulut Timuru = 128.9 mg/kg). This indicates that G34 line can be increased in GZn content by genetic engineering to relocate the zinc content from the stem to the seed. The gene overexpression technology approach can be applied to genotypes with accumulation patterns, such as G34 (Table 5). Meanwhile, zinc accumulation in Pulut Timuru, a zinc-rich genotype, was concentrated in the stems, whereas in the moderate and low GZn genotypes, zinc accumulation was generally found in the root organs, as reported by Impa et al. (2013) and Stomph et al. (2014).

Rice is reported to be capable of accumulating large amounts of heavy metals in its seeds (Uddin et al. 2021). This accumulator property is used to enrich the nutritional content of rice with useful minerals. However, knowledge of the physiological processes in mineral absorption needs to be considered. In rice, zinc is absorbed by the roots and can be mobilized to all parts of the plant (Impa et al. 2013; Stomph et al. 2014). In contrast, iron is local, stored in certain cellular components, and has a certain physiological role. Post-anthesis Zn uptake by shoots increased and Zn translocation from roots to shoots decreased as the Zn application rate increased through fertilization. Zn accumulation rate in grain resulting from pre-anthesis vs. post-anthesis remobilization depends on the availability of Zn in the soil. Pre-anthesis remobilization occurs at lower soil Zn content (Liu et al. 2019).

The OsZIP1 and OsZIP3 genes have been shown to be associated with Zn uptake in rice roots and Zn homeostasis in rice shoots, whereas OsZIP4 transports Zn intracellularly or between rice tissues (Ishimaru et al. 2007). The mechanism of Zn accumulation in each rice organ can be grouped into two categories: Zn is absorbed and retained in the root tissue, or remobilization of Zn from root to shoot (Impa et al. 2013). Genotypes with concentrated Zn accumulation patterns in roots require a genetic engineering approach to increase Zn accumulation in seeds. On the other hand, genotypes capable of accumulating zinc up to the stem are also indicated to be able to translocate high amounts of zinc to the seed organs, such as in Pulut

Timuru. The type of Zn accumulation pattern of the Pulut Timuru genotype is highly expected by breeders because it has a double advantage. Rice grains are a source of human food, while zinc-rich stem parts are a source of fodder for animals.

One of the conveniences of assembling zinc-rich rice varieties compared to Fe, Ca, Mn, and K is that zinc minerals are mostly stored in the endosperm, while the minerals Fe, Ca, Mn, and K are abundant in the aleurone layer. Unfortunately, many of the aleurone layers were lost due to the rice polishing process. Lu et al. (2013) reported that around 56.6% Zn was contained in the endosperm based on RGB-based mineral visualization. Sadeghzadeh (2013) reported that some plant genotypes could absorb trace minerals and accumulate them in the grain, thus enabling an increase in the concentration of Zn in the edible parts without negatively impacting the yield.

Selective breeding is one of the most powerful techniques used for the biofortification of staple crops, which involves crossing micronutrient-enriched varieties. However, certain limitations in selective breeding, such as low heritability, lack of genetic diversity for micronutrients, and linkage barriers, make genetic engineering a more deliberate approach to fortifying staple crops (Malik and Maqbool 2020). Genetic engineering techniques can be used to produce new cultivars with desired attributes such as GZn. Introduction of new genes from wild rice cultivars to superior varieties, overexpression of existing genes, or interfering with the synthesis pathway of zinc accumulation inhibitory genes can be alternative breeding methods that are more effective in directing the mechanism of zinc mineral accumulation in the seed organs of a genotype.

In conclusion, genotype has a highly significant effect on GZn characters. The coefficient of genetic variation for GZn-related traits was moderate and the estimated broad-sense heritability was high (H^2_{bs} = 0.83). Meanwhile, the yield has a low broad-sense heritability (H^2_{bs} = 0.13). There are differences in Zn accumulation patterns in plant organs between high GZn and low GZn genotypes based on the proportion of Zn content in the seeds and stems. The distribution of Zn accumulation in genotypes with moderate Zn content can follow high GZn or low GZn. The results of this study recommend different strategies for breeding new high-yielding rice varieties with different patterns of zinc accumulation.

ACKNOWLEDGEMENTS

WRR was supported by the Indonesian Agency for Agricultural Research and Development scholarship of the Ministry of Agriculture of Indonesia. This study was partially supported by the Directorate General of Research of the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia, through the scheme of the Doctoral Dissertation Research funding program, contract number 082/E5/PG.02.00.PT/2022.

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