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GENETIC VARIABILITY ON AGRONOMIC TRAITS AND GRAIN IRON CONTENT OF INDONESIAN RICE (*ORYZA SATIVA* L.)

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SUMMARY

Iron (Fe) deficiency remains a major problem of nutrient disorder worldwide. Increasing genetic variability is crucial for breeding efforts to enhance Fe content in rice (*Oryza sativa* L.) grains. The presented study sought to identify genetic variability of Indonesian rice (*Oryza sativa* L.) genotypes based on agronomic traits and grain Fe content, consisting of local varieties from Aceh (12), Riau (19), and Java (5). Likewise, other samples were two improved lines and two released biofortified varieties as checks. The study materialized in Central Java, Indonesia during the dry season of 2023, using a randomized complete block design with two replications. The Fe content measurement in brown rice samples used the Atomic Absorption Spectrophotometer (AAS) method in the laboratory of the BRIN, Jakarta, Indonesia. The results indicated the tested genotypes had wide genetic variability. The Fe content revealed low heritability, and the other traits had medium to high heritability. The group of Java local variety had a higher Fe content than the other groups. The discovery of genotypes with high grain Fe content and acceptable yield performance emerged from this study, suggesting the prospect of their utilization in future rice biofortification efforts.

Keywords: Rice (*O. sativa* L.), agronomic traits, biofortification, Fe, genetic variability

Key findings: Rice (*O. sativa* L.) genotype Menor and Padi Malang-2 had the combination of high Fe content, shorter growth duration, and high yield. These genotypes were prospective for further utilization in future biofortification efforts.

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INTRODUCTION

Iron (Fe) deficiency is one of the highest prevalence in developing countries (Zemrani and Bines, 2020), including Indonesia (Sitaresmi *et al*., 2023a). Fe deficiency affected 40% of children aged 6–59 months, 37% of pregnant women, and 30% of women aged 15–49 years (WHO, 2023). The global climate change decreases micronutrient uptake and translocation in crop plants, which becomes an additional challenge in the future (Maqbool *et al*., 2020).

Rice (*Oryza sativa* L.) biofortification seemed to be an efficient and effective method to combat hidden hunger (Swamy *et al*., 2016), where initial efforts have progressed in recent decades (Bouis and Saltzman, 2017). Currently, global efforts have released 37 Fe, Zn, protein and provitamin-A biofortified varieties with high polished grain Fe (>10 ppm), Zn (>24 ppm), and protein (>10%) content (Senguttuvel *et al*., 2023).

Increasing of genetic variability is crucial in breeding efforts to increase Fe content. Indonesia had plenty of local rice varieties adapted to various ecoregions, such as, swampy, flood, acidic, rainfed, and dry lands, and the presence and absence of micronutrients due to pedological conditions (Karimah *et al*., 2021). Evaluation had been ongoing (Susanto *et al*., 2017; Rohaeni and Susanto, 2021); but still, much more germplasm requires further observation.

This study aimed to determine the genetic variation of local rice varieties originating from different ecological regions, especially from Aceh (12 – upland), Riau (19 – swampy), and Java (5 – irrigated), in comparison with two improved lines and two biofortified released varieties as check genotypes. The basis of study is on grain Fe content (so-called Fe content) and agronomic traits' performance. This information will be useful to support the strategy and efforts made for Fe biofortification, especially in Indonesia and globally, in general.

MATERIALS AND METHODS

Experimental design

The study comprised 40 rice (*O. sativa* L.) genotypes for testing. They included local varieties from Aceh (12), Riau (19), and Java (5), along with two improved lines and two Zn biofortified varieties as controls (Table 1). The Aceh local varieties were mostly adapted to upland conditions, while the genotypes from Riau were favorable with swampy, upland, and irrigated lowland areas. Meanwhile, the ones from Java were mostly accustomed to irrigated lowland conditions. The genotypes' planting in irrigated field commenced during the dry season of 2023 in Cilacap Regency, Central Java, Indonesia.

Transplanting ensued 21 days after sowing into 3 m × 4 m plots, following a randomized complete block design (RCBD) and two replications, with a planting distance of 25 cm × 25 cm. The rice plants' set up engaged local recommendations considering integrated crop management practices.

Traits observations

Observations occurred on plant height (PH), tiller number (TN), days to heading (DTH), days to maturity (DTM), yield (t/ha at 14% moisture content), and 1000-grain weight (TGW). The measurement of Fe content followed the method by Analytic Jenna ContrAA300 – Atomic Absorption Spectrophotometer (AAS) using a Xenon cathode lamp (Wasim *et al*., 2019). Grain samples' dehulling used a Satake mini dehulling machine. Approximately one g of clean healthy brown rice samples, ground in a Teflon vessel, sustained digestion with a concentrated HNO_3 (65%) and H_2O_2 (30%) on a hot plate to obtain a clear solution. The cooled solution proceeded filtering using the Whatman paper before pooling in a volumetric flask. The absorbance value measurement was at 248.6 nm based on a calibration curve of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/L of Fe standard solution.

Table 1. List of 40 Indonesian rice genotypes used in this study.

Genotypes	Group	Remark	Genotype	Group	Remark
Sigupai Wangi	Aceh	Local, upland	Karan Duku	Riau	Local, swampy
Sigupai Abaya	Aceh	Local, upland	Napal Merah	Riau	Local, upland
Tengku Rubiah	Aceh	Local, upland	Kalpatali	Riau	Local, upland
Arias Peunaron	Aceh	Local, upland	Super Biru	Riau	Local, swampy
Babulon	Aceh	Local, upland	Putri Malu	Riau	Local, swampy
Bumirah	Aceh	Local, upland	Napal Putih	Riau	Local, upland
Padi Acong	Aceh	Local, upland	Saiya Kuning	Riau	Local, lowland
Sikuneng	Aceh	Local, upland	Cupak Putio	Riau	Local, lowland
Ramos Lamteuba 1	Aceh	Local, upland	Kuning	Riau	Local, lowland
Ramos Lamteuba 2	Aceh	Local, upland	Cupak Onda	Riau	Local, lowland
Ramos Lamteuba 3	Aceh	Local, upland	Sikampau	Riau	Local, lowland
Ramos Seulawah	Aceh	Local, upland	Sidenok	Java	Local, lowland
Super Madu	Riau	Local, swampy	Segreng Handayani	Java	Local, upland
Kuok	Riau	Local, upland	Menor	Java	Local, lowland
Karya Putih	Riau	Local, swampy	Sembada Hitam	Java	Local, lowland
Sikuning	Riau	Local, upland	Sembada Merah	Java	Local, lowland
Lantik Bamban	Riau	Local, swampy	Padi Malang 1	Improved genotype	Lowland
Solo	Riau	Local, swampy	Padi Malang 2	Improved genotype	Lowland
Panama	Riau	Local, swampy	Inpago 13 Fortiz	Improved genotype	Released variety, Lowland
Jangkar Durian	Riau	Local, upland	Inpari IR Nutri Zinc	Improved genotype	Released variety, Lowland

Statistical analysis

Anderson and Darling normality of frequency data distribution and Pearson correlation analyses conducted using the MINITAB® Release 14.12.0. Analysis of variance (ANOVA) and LSD values employed the CropStat Ver 7.2 (IRRI, 2009), while analyzing the HSD difference among the group means used the SPSS ver. 20.

Broad sense heritability (BSH) calculation engaged the method described by Akhtar *et al*. (2007). BSH values received classification as high (>60%), medium (30%–60%), and low (<30%) (Khan *et al*., 2023). The genetic coefficient of variation (GCV) computation proceeded by dividing the square root of the genotypic variance by the population mean and multiplying by 100. The GCV values had categories, such as, low (0%–10%), moderate (10%–20%), and high (>20%) (Khan *et al*., 2023). Dendrogram analyses used the PBSTAT-CL 2.2 (available at https://apps.pbstat.com/reports/pbstat-cl/), following Gower dissimilarity calculation method, with the dendrogram generated using the WARD method.

RESULTS

Descriptive analysis

All the rice (*O. sativa* L.) observed traits had normal distribution, except Fe content and days to maturity (Table 2). Fe content ranged from 7.91 ppm (Cupak Unda) to 43.22 ppm (Segreng Handayani), with an average of 16.63 ppm (Table 3). Menor (31.16 ppm), Padi Malang 2 (30.18 ppm), Sembada Hitam (28.20 ppm), Ramos Lamteuba-3 (26.97 ppm), and Ramos Lamteuba-1 (22.50 ppm) had the highest Fe content. However, Inpari IR Nutri Zinc and Inpago 13 Fortiz had Fe content of 17.12 ppm and 13.60 ppm, respectively. It indicates the newly released Zn biofortified rice varieties of Indonesia had a relatively low Fe content. Combining high Fe and Zn content is a suggestion for further biofortification efforts in Indonesia. The high Fe content genotypes mentioned above were prospective to benefit this purpose.

The yield of the evaluated genotypes ranged from 2.82 t/ha (Segreng Handayani) to 8.13 t/ha (Padi Malang 2). Three genotypes had higher yields than the Inpari IR Nutri Zinc

Table 2. Normality, variance, broad sense heritability, and genetic variability of 10 agronomic traits and grain Fe content of 40 Indonesian rice genotypes.

Trait	Normality	MSG	MSE	BSH	Class of BSH	GCV (%)	Class of GCV
Fe	NN	109.23^{ns}	88.00	10.77	Low	19.59	Moderate
PH	N	1048.99**	21.93	95.90	high	18.02	Moderate
TN	N	17.61**	6.08	48.65	Moderate	17.46	Moderate
DTH	N	200.56**	10.96	89.63	High	10.46	Moderate
DTM	NN	244.21**	9.74	92.33	High	9.15	Low
TGW	N	16.49**	2.77	71.26	High	11.03	Moderate
YLD	N	3.94**	0.23	88.85	High	25.45	High

MSG = means square of genetics, MSE = means square error, BSH = broad sense heritability, GCV = genotypic coefficient of variability, SdGV= Standard deviation of the genetic variance; Fe = grain Fe content (ppm); PH = plant height; TN = tiller number/plant; DTH = days to heading (das); DTM = days to maturity (das); TGW = 1000-grain weight (g); YLD = yield (t/ha); N = Normally distributed, NN = Not normally distributed; ns = no significant difference existed; ** = significant differences existed among the tested genotypes at α – 0.01.

	Fe	PH	TN	DTH	DTM	TGW	YLD
Fe	1						
PH	-0.19	1					
TN	-0.09	**-0.5**	1				
DTH	**-0.47**	0.2	**0.21**	1			
DTM	**-0.5**	0.23	0.19	**0.9**	1		
TGW	**0.47**	0.02	-0.19	**-0.46**	**-0.48**	1	
YLD	0.07	**-0.55**	**0.51**	-0.09	-0.25	0.15	1

-1	-0.5	0	0.5	1

Figure 1. Pearson correlation among grain Fe content and agronomic traits of 40 Indonesian rice genotypes.

Remark: Printed in bold = significant at α = 5%; Fe = grain Fe content (ppm); PH = plant height; TN = tiller number/plant; DTH = days to heading (das); DTM = days to maturity (das); TGW = 1000-grain weight (g); YLD = yield (t/ha).

(6.38 t/ha), i.e., Padi Malang 2 (8.13 t/ha), Padi Malang 1 (7.72 t/ha), and Menor (7.50 t/ha). Their Fe contents were 30.18, 20.54, and 31.16 ppm, respectively. It agreed with the correlation analysis, showing no correlation between Fe content and yield (Figure 1).

The tested genotypes had plant height ranging from 82.40 cm (Putri Malu) to 175.20 cm (Tengku Rubiah), with an average of 125.79 cm. The tiller number ranged from 8.30 tillers (Napal Putih) to 19.30 tillers (Super Madu), with the mean of 13.75 tillers.

Analysis of variance

Analysis of variance revealed the existence of variation in all observed traits, except Fe content (Table 2). Based on the LSD test to Inpari IR Nutri Zinc, the tested genotypes were varied, from a significantly ($P \leq 0.05$) lower to a higher than the Inpari IR Nutri Zinc for days to heading, days to maturity, 1000-grain weight, and yield (Table 3). The tested genotypes had a plant height comparable and higher than the Inpari IR Nutri Zinc. The tested

Table 3. Mean performance of grain Fe content and agronomic traits of 40 Indonesian rice genotypes.

Location	Genotype	Fe	PH	TN	DTH	DTM	TGW	YLD
Aceh	Sigupai Wangi	16.90	115.50	14.00	81.00	109.00	24.96	6.99
	Sigupai Abaya	16.77	131.70	12.10	106.00	123.00	19.29	3.99
	Tengku Rubiah	18.51	175.20	13.20	88.00	123.00	23.79	2.88
	Arias Peunaron	13.12	122.20	16.00	92.00	109.00	23.00	6.53
	Babulon	16.43	160.10	15.10	98.00	123.00	26.54	5.64
	Bumirah	11.44	160.00	15.70	103.00	123.00	24.85	5.80
	Padi Acong	8.62	98.00	19.00	106.00	134.00	18.43	5.03
	Sikuneng	14.08	142.00	8.40	101.00	123.00	26.52	3.88
	Ramos Lamteuba-1	22.50	133.10	10.40	88.00	109.00	23.69	4.75
	Ramos Lamteuba-2	20.91	135.50	10.80	84.50	109.00	24.02	5.30
	Ramos Lamteuba-3	26.97	138.20	11.40	88.00	109.00	24.55	4.47
	Ramos Seulawah	12.17	135.50	10.80	89.00	109.00	21.37	4.51
Riau	Super Madu	17.46	123.20	19.30	103.50	128.50	22.70	6.40
	Kuok	9.00	151.80	9.00	95.00	123.00	25.99	3.82
	Kaya Putih	13.23	122.10	15.40	106.00	134.00	20.54	5.79
	Sikuning	10.62	147.70	9.80	95.00	123.00	21.70	3.54
	Lantik Bamban	9.45	157.10	16.00	103.50	134.00	24.57	3.10
	Solo	9.56	118.90	17.50	102.00	123.00	22.19	6.41
	Panama	13.42	113.70	14.40	103.00	123.00	22.37	5.92
	Jangkar Durian	10.91	157.20	9.70	95.00	123.00	19.47	3.16
	Karan Duku	21.10	143.70	15.10	88.00	111.00	19.84	6.27
	Napal Merah	11.37	140.50	16.00	89.00	123.00	26.82	4.35
	Kalpatali	19.25	158.40	8.90	95.00	123.00	24.62	3.51
	Super Biru	9.59	97.00	15.40	106.00	134.00	23.28	4.68
	Putri Malu	17.93	82.40	18.90	91.50	123.00	22.58	6.35
	Napal Putih	20.73	152.00	8.30	88.50	116.00	27.29	3.66
	Saiya Kuning	16.40	115.10	13.40	97.00	134.00	22.68	5.64
	Cupak Putio	12.61	111.50	13.90	106.00	134.00	18.35	6.18
	Kuning	11.29	102.70	15.70	95.00	123.00	21.24	6.87
	Cupak Onda	7.91	129.30	14.30	95.00	123.00	21.65	6.67
	Sikampau	12.55	120.10	13.60	104.00	128.50	21.51	5.57
Java	Sidenok	19.53	108.40	14.20	81.00	102.50	25.99	6.36
	Segreng Handayani	43.22	93.70	13.20	71.00	101.00	25.99	2.82
	Menor	31.16	109.30	15.90	81.00	101.00	28.33	7.50
	Sembada Hitam	28.20	110.60	15.90	106.00	128.50	25.86	6.15
	Sembada Merah	9.08	99.30	12.10	73.50	101.00	25.73	5.17
	Inpago 13 Fortiz	13.60	115.00	13.10	73.50	101.00	23.76	6.21
	Inpari IR Nutri Zinc	17.12	85.20	18.50	81.00	101.00	24.01	6.38
	Padi Malang 1	20.54	110.80	13.30	88.00	105.00	30.14	7.72
	Padi Malang 2	30.18	107.80	12.20	84.50	106.50	29.95	8.13
CV (%)		56.4	3.7	17.9	3.6	2.6	7.0	9.0
LSD (α=5%)		18.97	9.47	4.99	6.7	6.31	3.36	0.98
Group	Aceh	16.54[b]	137.25[b]	13.08[ns]	93.71[a]	116.92[b]	23.42[b]	4.98[b]
	Riau	13.39[b]	128.65[a]	13.93[ns]	97.79[a]	125.47[a]	22.60[b]	5.15[b]
	Java	26.24[a]	104.26[b]	14.26[ns]	82.50[b]	106.80[c]	26.38[a]	5.60[b]
	Improved Genotype	20.36[ab]	104.70[b]	14.28[ns]	81.75[b]	103.38[c]	26.97[a]	7.11[a]
	Grand Mean	16.18	126.66	13.80	93.41	119.03	23.42	5.22
	P of group	0.04	0.00	0.70	0.00	0.00	0.00	0.01

Fe = grain Fe content (ppm); PH = plant height; TN = tiller number/plant; TGW = 1000-grain weight (g); YLD = yield (t/h).

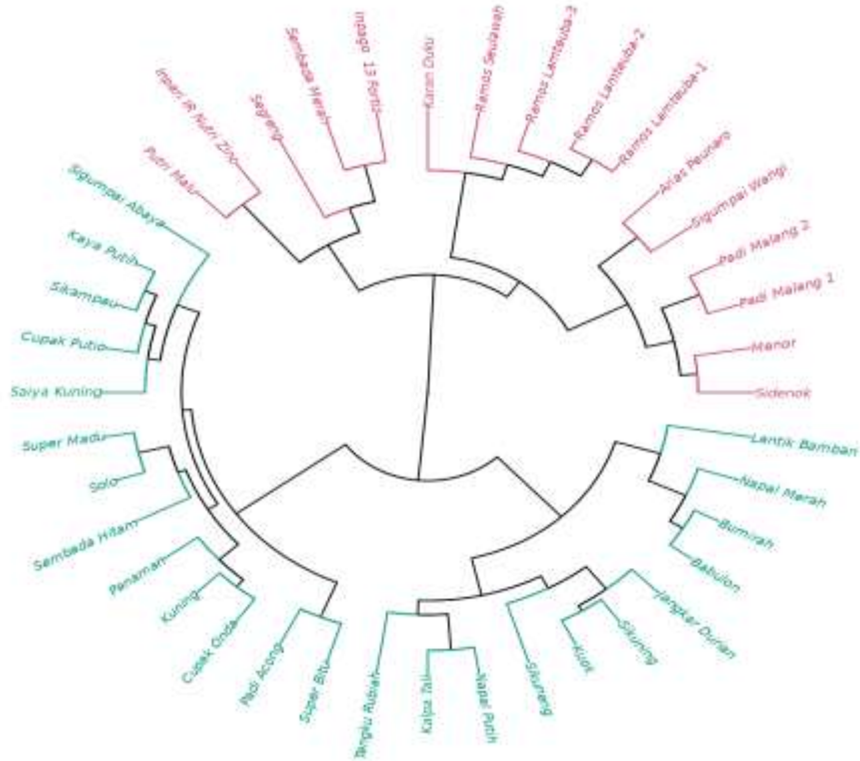

Figure 2. Dendrogram of 40 Indonesian rice genotypes based on grain Fe content and agronomic traits.

genotypes had a similar or lower tiller number and seed set than the Inpari IR Nutri Zinc. This indicates the tested genotypes, which were mostly local varieties, tended to have longer growth periods and a higher plant stature.

Grouping the genotypes, based on the origin and improved genotypes as an additional separate group, showed the Java group had the highest Fe content, followed by improved rice genotypes, Aceh and Riau (Table 3). For yield, the group of improved rice genotypes had the maximum, followed by Java, Riau, and Aceh genotypes.

The broad sense heritability of plant height, days to heading, days to maturity, 1000-grain weight, and yield received a high category. On the other hand, tiller number had a medium broad sense heritability, while the Fe content had a low broad sense heritability (Table 2). Higher GCV was noteworthy for yield, but, low for days to maturity. The other traits had moderate GCV values.

Correlation analysis

Correlation analysis revealed Fe content has a positive correlation with 1000-grain weight, while a negative correlation with days to heading and days to maturity. It had no correlation with yield. The plant height showed negatively correlated with the tiller number (Figure 1). The days to heading gave a positive association with the days to maturity. In addition, the 1000-grain weight had a negative connection with days to heading and days to maturity. Finally, the yield indicated negatively associated with plant height; however, it positively correlated with tiller number.

Cluster analysis

Cluster analysis divided the genotypes into two big clusters (Figure 2). The cluster I comprised the two check varieties (Inpari IR Nutri Zinc and Inpago 13 Fortiz), two improved lines

(Padi Malang 1, Padi Malang 2), four Java local varieties (Segreng Handayani, Sembada Merah, Sidenok, and Menor), six Aceh local varieties (Ramos Seulawah, Ramos Lamteuba 1, Ramos Lamteuba 2, Ramos Lamteuba 3, Arias Peunaron, and Sigupai Wangi), and two Riau local rice varieties (Putri Malu and Karan Duku). The second cluster consisted of the rest of the genotypes, i.e., 17 Riau, six Aceh, and one Java local varieties.

DISCUSSION

Rice cultivars Menor and Padi Malang 2 had the combination of high Fe content and yield with shorter plant growth duration. Menor had 31.16 ppm of Fe content with the yield of 7.5 t/ha and a growth duration of 101 days. Padi Malang 2 had 30.18 ppm Fe content with the yield of 8.13 t/ha and 106.5 days to maturity. These genotypes had higher Fe contents and yield with comparable growth duration versus Inpari IR Nutri Zinc, which had 17.12 ppm Fe content, 6.38 t/ha yield, and 101 days growth duration. These genotypes gave double advantage for Fe content and yield compared with the check variety. Those rice genotypes match with the characteristics of popular varieties and elite lines available in Indonesia (Sitaresmi *et al*., 2023b). It indicated the chance of adoption of the varieties, and thus, a prospective to be beneficial for further biofortification efforts.

This study revealed no correlation between yield and Fe content (Figure 1). However, some consequences implied the possible combination of high yield and high Fe content. It may segregate independently; thus, simultaneous selection for both traits is advisable. Application of molecular markers linked to yield and Fe content may help in selecting the breeding material since the early generation stage. Several molecular studies on yield (Zhong *et al*., 2023) and Fe content (Dhanyalakshmi *et al*., 2024) had progressed, revealing abundant information useful for initiating molecular studies and applications for specific breeding populations.

The target of Fe content is 13 ppm in polished rice, which is essential to fulfill 30% of the estimated average requirement of humans (Bouis *et al*., 2011). Fe content of popular rice varieties is approximately 2 ppm in polished grain (Trijatmiko *et al*., 2016). Previous study identified Fe content ranged from 3.53 to 16.70 ppm among 328 Indonesian local varieties (Widyastuti *et al*., 2024) and ranged from 9.70 to 17.40 ppm among 176 Indonesian released varieties of polished rice samples (Rohaeni and Susanto, 2021). A study in swampy areas had identified a big variation in Fe and Zn content among local varieties (Khairullah *et al*., 2021) and improved genotypes (Sabran *et al*., 2022). Variations among local varieties were wider than among improved lines and varieties (Khairullah *et al*., 2021). It indicated the potential of Indonesian rice germplasm for Fe biofortification efforts. Nevertheless, data on milled rice samples are not always available. A study reported Fe loss due to polishing ranged from 16% to 97.4% (Maganti *et al*., 2020). Investigating milled rice Fe content is then advisable in the pre-breeding and breeding processes.

This study revealed local rice varieties from Java tend to have higher Fe content than Riau and Aceh. It signifies local varieties may become good sources of higher Fe content (Nugraha *et al*., 2016; Anuradha *et al*., 2012). Therefore, continuous efforts are urgently necessary to screen the new rice genotypes from various regions and identify more potential genotypes with higher Fe content in grains.

Dissecting the genetic mechanism controlling the accumulation of Fe in rice grains of the selected genotypes may be useful to determine if the genes and mechanisms of the genotypes were unique and different from previously identified genes. Furthermore, combining the genes from these various resources may be effective for increasing the genetic variability of the donor gene, contributing to high Fe content. Pyramiding genes in rice have ongoing studies for some traits; however, a lack of research on micronutrient content existed (Isnaini *et al*., 2023).

This study discovered the yield had a high broad sense heritability, high GCV, and wide genetic variability (Table 2). It is in accordance with previous studies, which obtained high broad sense heritability (Debsharma *et al*., 2023) and high GCV (Okasa *et al*., 2021) in rice. It specifies the chance of developing genetic study and breeding populations for enhancing yield.

Cluster analysis indicated rice genotypes with shorter growth duration tend to belong in cluster 1 with days to maturity ranging from 101 to 123 days, with an average of 106.69 days. Meanwhile, cluster 2 ranged from 116 to 134 days, with an average of 126.15 days. Genotypes in cluster 1 tend to have higher Fe and Zn content, tiller number/plant, and yield than cluster 2, and vice versa for other traits. Fe content had a negative correlation with plant growth duration, which is negatively correlated with yield. It seems conformable with the clustering results. More variations could result from the crossing of genotypes with larger variations (Chemutai *et al*., 2016; Tam *et al*., 2021). The presented study indicated the chances of developing breeding populations for the targeted traits.

CONCLUSIONS

The evaluated rice (*O. sativa* L.) genotypes had wide genetic variability with the heritability ranging from medium to high for all observed traits, except for Fe content. The Java rice group tends to have higher Fe content than other groups. Cluster analysis showed genotypes with early maturity, high Fe, and high yield tend to group together in cluster I. Menor and Padi Malang 2 had a combination of relatively higher Fe content and yield with early maturing characteristics, which were suitable for further biofortification efforts.

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