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SORGHUM (*SORGHUM BICOLOR* L. MOENCH) GERMPLASM WITH VARIATIONS IN FE AND ZN CONTENTS AND THEIR CORRELATION WITH GRAIN YIELD

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SUMMARY

Sorghum (*Sorghum bicolor* L.) serves as an alternative food crop to rice in addressing food shortages and malnutrition. Breeding programs aimed at increasing yield and biofortification in sorghum require accessions with superior agronomic traits, as well as high iron (Fe) and zinc (Zn) contents. This study sought to assess the variation in agronomic characteristics and Fe and Zn contents among Indonesian sorghum germplasm. The genetic materials evaluated consisted of 20 IPB breeding lines, six introduced accessions, five local varieties, and seven national varieties arranged in an augmented design. The observed traits included agronomic characters and grain micronutrient contents (Fe and Zn). Leaf number, green leaf index, days to flowering and maturity, panicle weight, and thousand-seed weight exhibited high heritability. Fe content showed the highest broad-sense heritability, whereas Zn content displayed relatively low variation and heritability. All observed traits, except green leaf index and panicle length, revealed a positive correlation with seed weight per panicle. Fe and Zn contents indicated nonsignificant correlations with panicle weight and seed weight per panicle. Based on mean values and heritability estimates, promising germplasm for further development includes national varieties for yield improvement and local varieties for biofortification purposes.

Keywords: Sorghum (*S. bicolor* L.), biofortification, Fe and Zn, germplasm, Pearson's correlation, XRF

Key findings: Among 38 Indonesian sorghum (*S. bicolor* L.) germplasm evaluated, the local variety Pulut-3 exhibited the highest seed iron (Fe) content, reaching 54.5 ppm. This trait shows very high broad-sense heritability and a significant positive correlation with seed zinc (Zn) content. This genotype holds strong potential for further biofortification efforts aimed at enhancing micronutrient concentrations.

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INTRODUCTION

Globally, one in three people is malnourished based on 2015–2017 data, and by 2030, one in two may face malnutrition if no action takes place (Fanzo *et al*., 2017). Widespread micronutrient deficiencies (MNDs) lead to severe and potentially fatal health issues (WHO, 2024). Micronutrients are essential nutrients needed by the human body in small quantities to maintain optimal health and functioning. They consist of vitamins and minerals, including iron, vitamin A, iodine, folate, and zinc (Inzaghi *et al*., 2022).

Micronutrient deficiencies, particularly Fe and Zn, remain a significant public health concern in Indonesia, especially during fetal development and childhood. Iron is essential for protein metabolism, hemoglobin synthesis, red-blood-cell formation, and immune function, and its deficiency can lead to anemia and impaired growth (Honestdocs, 2024). Zinc is critical for immune function and the activity of over 100 enzymes; prolonged deficiency can result in poor health, stunted growth, and increased susceptibility to infections (Sanna *et al*., 2018; Hallosehat, 2024). National data indicate that 13.4% of Indonesian children have low ferritin levels (iron), and 19.7% have inadequate zinc status (Ernawati *et al.,* 2023). Additionally, 40.5% of 2,061 children aged 6–23 months do not meet recommended zinc intakes (Chirivi and Betti, 2023). Dietary interventions, including supplementation and nutritional support, can help alleviate these deficiencies (WHO, 2024). Both iron and zinc are available in animal-derived foods, as well as plant-based sources such as grains and vegetables (Gaddameedi *et al.,* 2022).

Sorghum could serve as a promising staple food, with macronutrient composition almost similar to rice and protein content approaching that of wheat. It is also rich in phenolic compounds, which exhibit antioxidant, anti-inflammatory, and anti-carcinogenic properties (Ofosu *et al*., 2021). The high levels of protein, fat, and fiber contribute to a low glycemic index, making sorghum a suitable dietary option for individuals with diabetes (Taylor and Duodu, 2018). Research has revealed considerable genetic variations among 220 sorghum accessions, with Fe contents ranging from 47.54 to 75.7 ppm and Zn contents from 21.8 to 46.2 ppm (Xiong *et al*., 2019). Iron is primarily prevalent in the scutellum, whereas Zn predominantly exists in the embryonic axis, aleurone layer, and endosperm (Gaddameedi *et al*., 2022).

Biofortification of essential crops provides a cost-effective approach to addressing micronutrient deficiencies (Abebe *et al.,* 2023). Extensive sorghum biofortification programs have had successful implementation in India (Kumar *et al*., 2023), North Africa (Abdelhalim *et al*., 2021), East Africa (Endalamaw *et al*., 2025), and other regions. In Indonesia, biofortification efforts have successfully produced the Inpari IR Nutri Zinc rice varieties, which contain up to 34.5 ppm Zn (Rohaeni and Susanto, 2021; Rohaeni *et al*., 2023a). However, the documentation of similar initiatives for Fe and Zn biofortification in sorghum has not yet taken place in Indonesia. Considering that rice is rich in Zn and sorghum is abundant in Fe, the two crops could complement each other in alleviating micronutrient deficiencies. Developing sorghum varieties with enhanced Fe and Zn contents offers a viable solution. Indonesian sorghum germplasm includes introduced lines, local, and national varieties (Suroya *et al*., 2023), as well as breeding lines. Meeting future needs requires improvements in yield potential accompanied by enhancements in grain quality through biofortification (Wirnas *et al*., 2021; Abebe *et al.*, 2023). Pre-breeding evaluations are therefore essential to analyze the variation in Fe and Zn contents of sorghum grains. This study aimed to assess the agronomic diversity, yield performance, and grain Fe and Zn contents of Indonesian sorghum germplasm, providing a basis for selecting potential parental lines for the development of segregating populations via hybridization or mutation.

MATERIALS AND METHODS

The study comprised two experiments: 1) a field experiment to evaluate variation in

Table 1. Genetic materials used in the study.

No.	Genotype	Status	No.	Genotype	Status
	Lines				
1	NS2-12	Breeding line	21	KP 62R6	Local cultivar
2	NS2-15	Breeding line	22	KP 64R6	Local cultivar
3	NS2-19	Breeding line	23	Demak 2	Local cultivar
4	NS2-21	Breeding line	24	Pulut 3	Local cultivar
5	NS2-22	Breeding line	25	5D x 160	Introduced genotype
6	NS2-24	Breeding line	26	IS 23509	Introduced genotype
7	NS2-107	Breeding line	27	IS 19551	Introduced genotype
8	NS2-108	Breeding line	28	M-4	Introduced genotype
9	NS2-112	Breeding line	29	K905	Introduced genotype
10	NS2-114	Breeding line	30	431	Introduced genotype
11	NS2-115	Breeding line	31	UPCA-SI	National cultivars
12	NS2-116	Breeding line	32	Sorgum Manis	National cultivars
13	NS2-119	Breeding line	33	Soraya3	National cultivars
14	NS2-121	Breeding line			
15	NS2-122	Breeding line		Checks	
16	NS2-123	Breeding line	34	Demak 4	Local cultivar
17	NS2-126	Breeding line	35	Soper 6 Agritan	National cultivar
18	NS2-129	Breeding line	36	Bioguma 1	National cultivar
19	NS2-156	Breeding line	37	Numbu	National cultivar
20	NS2-168	Breeding line	38	Samurai 2	National cultivar

Note: NS2 = Numbu x Samurai 2 breeding line.

Table 2. Climate profile from May to October 2024.

Climate variables	May	June	July	August	September	October
Daily temperature (°C)	27.4	26.9	26.3	26.8	26.8	27.2
Air humidity (%)	84	83	79	77	79	79
Rainfall (mm)	550.6	224.1	139.8	122.5	402	493.6
Rainy days	21	20	13	14	15	14
Wind speed (m/s)	3.4	3.2	3.5	3.8	3.8	3.9

agronomic traits and yield potential and 2) a micronutrient analysis to assess Fe and Zn content in sorghum grains. Both experiments proceeded using an augmented design.

Genetic material and site location

Genetic materials used in this study included sorghum germplasm comprising introduced lines, local varieties, promising IPB lines, and national varieties. Genetic materials totaling 38 underwent evaluation, categorized into 33 lines and five checks according to the augmented design (Table 1). The field experiment commenced from June to October 2024 at the Muara Experimental Field in Bogor, Indonesia. The site trial description is available in Table 2. The conduct of micronutrient analysis was from January to February 2025 at the Laboratory of the Center for Rice Crop Research and Agricultural Modernization, Sukamandi.

Field experiment

Sowing of seeds had a spacing of 70 cm between rows and 15 cm between hills, with three seeds per hill. Two weeks after planting, thinning took place to leave one plant per hill. Dolomite application had a rate of 1 t ha^{-1} one month before planting. Treatment of fertilizers had the following rates: 150 kg urea (45% N), 100 kg KCl (60% K_2O), and 100 kg SP36 (36% P_2O_5). One-third of the urea application was as a basal dose, while applying the remaining two-thirds continued four weeks after planting. The study followed all recommended agronomic and plant protection practices, conducting field observations on agronomic traits. Trait evaluations focused on morphological and agronomic parameters following Wirnas *et al*. (2021). Harvesting ensued when 80% of plants in each row reached physiological maturity, indicated by hardened seeds, a starchy texture upon biting, and the presence of a black layer. Harvested panicles entailed sun-drying for 2–3 days before manual threshing.

Micronutrient analysis

Grain Fe and Zn contents measurement used an X-ray fluorescence (XRF) analyzer (Oxford instrument X-Supreme 8000), validated against inductively coupled plasma (ICP) analysis. XRF utilizes X-ray excitation to stimulate atoms within the sample, generating element-specific fluorescence. Recorded Fe and Zn concentrations reached automatic displays on the Rice15 analyzer's LCD screen in parts per million (ppm or mg kg^{-1}) (Rohaeni *et al*., 2023b).

Data analysis

Variance analysis for both experiments proceeded using the augmented design model in SAS OnDemand (Federer, 1961). Genotypes showing significant effects received further analysis using Dunnett's t-test and genetic parameter estimation. Applying mean adjustment sought to correct potential biases that could affect analytical results. Variance components and heritability estimates included phenotypic variance (σ^2p), environmental variance (σ^2e), genotypic variance (σ^2g), broad-sense heritability, phenotypic coefficient of variation, genotypic coefficient of variation, and correlation analysis.

RESULTS AND DISCUSSION

Performance of agronomic and yield traits

The analysis of variance (ANOVA) revealed the genotype, line, and check factors had significant effects on leaf number, days to flowering, days to maturity, panicle length, and thousand-seed weight. Variations in green leaf index gained significant influences only from genotype and check varieties, whereas variation in panicle weight had the genotype and line significantly affecting it. The coefficients of variation in this study were relatively low, ranging from 0.85% to 20.96%, indicating good experimental precision.

Post-hoc analysis with Dunnett's *t*-test showed that genotype IS18551 had the highest average leaf number among the evaluated lines, which differed significantly from Demak 4 (Table 3). This genotype falls within the medium leaf category (>12 leaves), which has associations with enhanced photosynthetic efficiency and increased yield (ICAR-IIMR, 2024). Previous studies have shown an increase in leaf number contributes to greater leaf area, improved photosynthetic capacity, and higher biomass accumulation, all of which positively affect crop yield (Mingnan *et al*., 2017).

The genotype K905 exhibited the longest days to flowering, significantly differing from Demak 4 (Table 4). A prolonged flowering period indicates an extended vegetative phase that enhances photosynthetic activity and energy accumulation, potentially increasing seed yield capacity. In contrast, genotypes KP62R6, 431, and UPCA-SI showed early flowering, suggesting they are early-maturing lines. Both IS18551 and K905 displayed the longest days to maturity, allowing a longer grain-filling period that could promote biomass accumulation and higher yield potential. Conversely, genotypes, such as 50x160, KP62R6, and several others, reached earlier maturity, completing their lifecycle faster (Table 3).

Previous studies have reported flowering and maturing durations vary according to genetic origin, with landraces and mutant lines generally maturing earlier (<60 days) than hybrid varieties (Zabuloni *et al*., 2025). Indonesian sorghum germplasm exhibited medium flowering and maturing durations (66–75 days), whereas several introduced lines were under the early category (56–65 days) (ICAR-IIMR, 2024). Reduced flowering and maturity durations may enhance

Table 3. Morphological trait of sorghum germplasm.

Genotype	Plant height (cm)	Leaf number	Leaf flag area (cm^2)	Stem diameter (mm)	Green leaf index (CCI units)	Days to flowering (DAS)	Days to maturing (DAS)
431	166.9	7.5	90.8	57.6	11.8	55.2	99.2
5D x 160	191.7	9.5	348.4	55.8	16.9	62.0	94.2
Demak2	187.5	9.4	146.3	53.6	16.7	59.2	96.2
IS18551	165.6	12.6	247.4	56.0	23.6	70.2	111.2
IS23509	193.3	10.1	179.6	53.3	16.2	67.0	100.2
K905	129.8	10.9	116.5	67.8	19.3	74.2	111.2
KP62R6	191.4	8.4	229.7	59.9	15.1	52.0	94.2
KP64R6	153.4	9.2	71.6	55.8	15.2	59.2	96.2
M-4	202.8	9.1	148.6	58.4	13.7	57.2	97.2
NS2-107	204.7	9.9	233.1	52.3	15.4	62.6	101.4
NS2-108	220.2	10.0	269.0	57.9	16.2	60.6	100.4
NS2-112	209.1	10.0	260.8	51.4	16.3	61.4	101.4
NS2-114	212.7	10.2	240.0	53.8	16.6	61.4	101.4
NS2-115	190.3	10.0	240.2	54.4	15.2	61.4	101.4
NS2-116	208.6	10.4	263.8	53.7	17.5	64.4	101.4
NS2-119	220.5	10.4	230.1	54.9	15.1	61.4	101.4
NS2-12	214.3	10.4	241.4	52.1	16.4	62.6	101.4
NS2-121	197.9	9.5	NA	54.6	17.6	64.4	102.4
NS2-122	216.6	9.8	236.0	53.5	15.9	61.4	101.4
NS2-123	215.8	9.2	221.7	52.4	15.3	61.4	101.4
NS2-126	220.9	10.3	181.2	50.7	14.5	62.0	101.2
NS2-129	211.8	9.6	230.6	49.4	18.5	62.0	101.2
NS2-15	219.8	9.8	274.9	53.5	15.8	60.6	100.4
NS2-156	209.1	10.0	226.1	48.7	15.1	62.0	101.2
NS2-168	217.0	10.0	180.9	47.4	14.8	62.0	101.2
NS2-19	234.5	10.7	202.6	56.0	17.1	60.6	100.4
NS2-21	224.9	10.8	191.4	47.8	16.0	60.6	100.4
NS2-22	220.5	10.0	271.4	55.3	13.9	61.6	99.4
NS2-24	225.2	10.4	197.1	53.0	15.3	60.6	99.4
UPCA-SI	184.0	9.1	NA	60.0	14.2	55.2	100.2
Bioguma1	221.7	10.0	250.2	54.0	17.5	67.3	101.0
Demak 4	189.2	8.9	192.6	55.8	14.9	60.3	98.0
Numbu	224.7	10.2	227.9	52.5	16.5	63.8	100.3
Samurai 2	214.0	9.9	223.2	50.0	18.4	63.5	100.5
Soper 6	188.1	11.5	219.7	52.4	19.2	66.0	102.0
LSD value	75.4	1.4	110.2	11.0	4.2	6.2	2.6

seed production efficiency in cropping systems with short rotation cycles but can also reduce productivity. Variations in flowering and maturity times considerably influence seed yield (Chirivi and Betti, 2023).

The genotype IS19551 and genotype 50x160 exhibited substantially longer panicles than all control varieties. Both genotypes received classification as having long panicles, with lengths ranging from 30 to 40 cm (ICAR-IIMR, 2024). Somu *et al*. (2025) reported that panicle lengths varied from 9.2 to 31.4 cm among 103 sorghum germplasm accessions, classified as short to medium. The presence of long panicles suggests improved grain filling, which may contribute to higher yield performance (Otwani *et al*., 2025). These findings indicate Indonesian sorghum germplasm has substantial potential for further improvement. Moreover, genotypes IS23509, K905, KP62R6, and M-4 showed notably longer panicles than Bioguma 1, Demak 4, and

Table 4. Yield-component trait of sorghum germplasm.

Genotype	Panicle length (cm)	Panicle diameter (mm)	Panicle weight (g)	Seed weight per panicle (g)	Thousand-seed weight (g)
431	22.3	44.0	32.6	28.0	30.6
5D x 160	30.9	50.6	53.0	31.0	21.1
Demak2	19.8	56.4	43.4	37.6	27.9
IS18551	32.8	60.1	87.3	61.8	29.5
IS23509	25.4	53.4	39.9	24.9	21.1
K905	26.2	46.6	36.1	19.7	16.6
KP62R6	25.4	42.8	29.8	22.2	21.9
KP64R6	26.4	60.7	65.0	47.3	19.6
M-4	24.5	48.1	42.1	31.7	24.6
NS2-107	18.0	56.6	58.0	43.9	36.3
NS2-108	20.7	56.8	61.4	47.0	36 e
NS2-112	21.3	57.5	49.9	34.8	35.7
NS2-114	21.8	64.1	53.8	39.8	37.7
NS2-115	20.9	56.8	54.4	42.9	41.0
NS2-116	21.0	58.1	78.0	61.4	37.7
NS2-119	21.0	57.2	62.2	48.9	41.0
NS2-12	20.5	61.0	60.7	47.5	33.3
NS2-121	21.2	55.2	72.0	55.1	42.7
NS2-122	20.5	56.6	50.9	37.6	40.0
NS2-123	20.3	49.8	40.1	31.3	35.0
NS2-126	19.3	55.2	51.0	43.0	37.1
NS2-129	21.0	59.3	73.8	61.6	39.1
NS2-15	20.5	55.4	62.3	46.2	33.6
NS2-156	20.9	57.1	63.6	55.5	38.1
NS2-168	21.0	59.7	69.5	58.6	36.6
NS2-19	20.9	64.1	90.9	75.5	37.3
NS2-21	19.7	60.5	83.2	74.9	38.3
NS2-22	20.2	52.4	57.4	44.8	34.6
NS2-24	20.2	59.5	68.4	53.9	33.3
UPCA-SI	21.5	45.7	40.7	29.3	29.9
Bioguma1	21.5	57.7	69.4	51.3	33.7
Demak 4	19.8	54.0	51.6	39.9	30.5
Numbu	20.7	57.6	67.7	54.6	36.3
Samurai 2	26.1	61.2	76.2	62.1	30.3
Soper 6	35.3	56.5	71.6	42.0	28.3
LSD value	2.0	15.7	39.5	36.9	3.98

Numbu. A longer panicle provides a larger surface area for grain development, enabling more efficient grain filling and enhancing overall yield potential (Table 4).

According to Somu *et al.* (2025), the highest seed weight per panicle among 103 sorghum accessions was 60.37 g, with a maximum thousand-seed weight of 39.8 g. In this study, genotypes NS2-19 and NS2-21 recorded the highest seed weights per panicle (>70 g), indicating strong potential for high-yield production. Conversely, K905 had the lowest seed weight per panicle (19.7 g) (Table 4), which may limit its yield potential. Seed weight per panicle is a critical determinant of production potential in sorghum, as genotypes with heavier seeds generally produce higher yields.

The genotype NS2-121 demonstrated the highest thousand-seed weight (>40 g), substantially exceeding nearly all control varieties, whereas K905 had the lowest value (16.6 g) (Table 4). Genotypes showing significantly higher thousand-seed weights than check varieties received the category as high (>35 g) (ICAR-IIMR, 2024). Thousand-seed weight is a vital indicator of grain quality in sorghum cultivation, as superior grains are

Table 5. The agronomic traits of sorghum grouped into introduced lines, local cultivars, national varieties, and IPB breeding lines.

Characters	Mean		
	Introduced lines	Local cultivars	National varieties and IPB breeding lines
Plant height (cm)	174.35	188.33	210.61
Leaf number	9.65	9.11	10.09
Leaf flag area (cm^2)	179.08	169.44	207.68
Stem diameter (mm)	16.47	15.75	16.54
Green leaf index (CCI units)	58.06	54.69	53.20
Days to flowering (days after sowing)	59.70	62.08	62.42
Days to maturing (days after sowing)	97.08	100.40	100.87
Panicle length (cm)	26.73	19.83	22.78
Panicle diameter (mm)	50.80	55.22	56.68
Panicle weight (g)	48.22	47.50	64.10
Seed weight per panicle (g)	33.33	38.77	49.04
Thousand-seed weight (g)	23.14	29.24	34.45

typically larger and denser. Increased grain weight improves postharvest handling efficiency and enhances the commercial value of grain commodities (Kamal *et al*., 2023).

The evaluated germplasm consisted of introduced lines, local and national varieties, and promising IPB breeding lines. The introduced lines exhibited more leaves, shorter flowering and maturity durations, longer panicles, and lower thousand-seed weights than the local varieties. In contrast, the national varieties and promising IPB breeding lines showed greater leaf counts, as well as increased panicle and thousand-seed weights, than the local varieties.

National varieties and promising IPB breeding lines also demonstrated taller plant heights (210.61 cm) relative to the introduced lines (174.35 cm). The Indonesian sorghum germplasm is generally distinct with medium plant height, ranging from 151 to 225 cm (ICAR-IIMR, 2024). Furthermore, these two groups (national and IPB breeding lines) showed longer durations to flowering and maturity, shorter panicles, higher panicle weight, and greater thousand-seed weight than the introduced lines (Table 5).

The differences in average trait performance among the test lines and control varieties acquired influences from both genetic and environmental factors. Environmental conditions can either suppress or enhance the expression of genetic potential (Sharif *et al*., 2024). The distinct trait variations observed among the tested lines provide an important foundation for plant breeding programs, as they reveal substantial genetic diversity that warrants further evaluation and selection.

The magnitude of genetic influence on genotype performance entailed evaluation by partitioning the expected mean squares for each source of variation, followed by the estimation of variance components. Table 6 presents the genotypic and phenotypic variances, broad-sense heritability, and coefficients of variation for the evaluated traits.

Broad-sense heritability estimates for the agronomic traits ranged from 0% to 94.96%. Traits, such as leaf number, stem diameter, days to flowering, days to maturity, panicle size, and thousand-seed weight, exhibited heritability values exceeding 50%, indicating a strong genetic contribution relative to environmental effects (Ardiyanti *et al*., 2019). Contrastingly, flag leaf area, panicle weight, and seed weight per panicle displayed moderate heritability levels (20%–50%), while plant height, green leaf index, and panicle diameter showed low heritability (<20%), suggesting substantial environmental influence on these traits. Such environmentally sensitive traits are often less stable across generations and more challenging to improve through selection (Samudin *et al*., 2023).

Table 6. Genetic parameters of agronomic traits of sorghum germplasm.

Character	Ve	Vg	Vp	h^2_{bs}	CGV	CPV
Plant height	159.52	-74.00	85.52	0	0.00	5.20
Leaf number	0.06	0.13	0.18	69.17	3.56	4.28
Leaf flag area	340.87	311.88	652.75	47.78	8.46	12.24
Stem diameter	3.41	-0.07	3.34	-2.09	0.00	11.06
Green leaf index	0.48	0.57	1.05	54.07	1.40	1.90
Days to flowering	1.07	3.13	4.21	74.48	2.83	3.28
Days to maturing	0.18	2.87	3.05	93.99	1.69	1.74
Panicle length	0.11	2.11	2.23	95.00	6.27	6.43
Panicle diameter	6.86	0.02	6.88	0.22	0.22	4.66
Panicle weight	43.80	16.59	60.39	27.47	6.60	12.60
Seed weight per panicle	38.16	10.36	48.52	21.35	6.86	14.85
Thousand-seed weight	0.44	8.37	8.81	94.96	8.90	9.14

Note: Ve = environment variance, Vg = genetic variance, Vp = phenotypic variance, h^2_{bs} = broad-sense heritability, CGV = coefficient of genetic variance, and CPV = coefficient of phenotypic variance.

Table 7. Mean of Fe and Zn content.

Genotype	Fe (ppm)	Zn (ppm)
Introduced lines	23.49a	19.48
Local cultivars	31.33ab	20.47
National varieties and IPB breeding line	20.01b	19.56

Note: Means followed by the same letter are significantly different based on the contrast test.

Fe and Zn content of sorghum germplasm seeds

Analysis of variance for Fe and Zn concentrations in sorghum germplasm seeds revealed genotypes, lines, and checks significantly influenced Fe content, while Zn content obtained no significant effects from these factors. The coefficient of variation for Fe was relatively low, indicating reliable measurement precision. Local varieties exhibited the highest mean Fe concentration (31.33 ppm), while both introduced lines and national varieties showed comparable Fe levels, ranging from 20.01 to 23.49 ppm. Similarly, the highest mean Zn concentration resulted in local varieties (20.47 ppm), followed by national varieties (19.57 ppm) and introduced lines (19.48 ppm) (Table 7). The lack of significant differences in Zn concentration among genotypes suggests limited genetic variability for this trait within the evaluated accessions.

These findings align with Kamal *et al*. (2023), who reported that smaller sorghum seeds had the highest Fe and Zn concentrations. The Fe and Zn contents of each evaluated genotype appear in Table 8. Previous studies have shown that Fe and Zn concentrations in sorghum grains typically range around 30 and 20 ppm, respectively, comparable to levels observed in wheat (Zhao *et* al., 2009; Gaddameedi *et al*., 2022). In the presented study, the local variety Pulut 3 exhibited the highest Fe concentration (54.45 ppm), exceeding all control varieties. Meanwhile, genotype Soraya 3 showed the greatest Zn concentration (25.28 ppm). Among the control varieties, Fe concentrations ranged from 16.10 to 21.80 ppm, while Zn concentrations varied between 19.70 and 22.30 ppm.

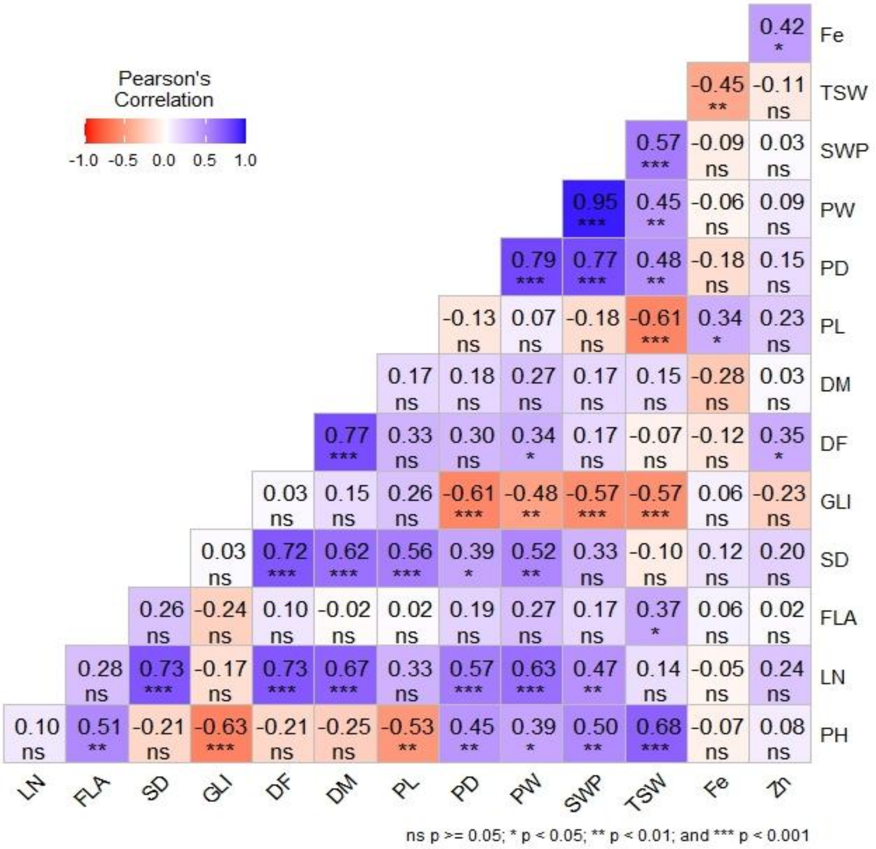
Enhanced Fe accumulation in sorghum grains holds considerable potential for biofortification efforts aimed at improving nutritional quality and addressing micronutrient deficiencies. Local or regional varieties with elevated mineral content represent valuable genetic resources for developing nutrient-enriched sorghum cultivars. The observed

Table 8. Fe and Zn seed content of sorghum germplasms.

Genotype	Fe (ppm)	Zn (ppm)
431	17.80	15.90
5D x 160	25.75	20.10
Demak 2	23.45	19.85
IS 18551	23.25	19.10
IS 23509	24.65	23.85
K905	19.35	19.60
KP62R6	30.45	18.10
KP64R6	24.35	19.85
M-4	22.35	19.35
NS2-107	13.10	16.30
NS2-108	25.80	17.40
NS2-112	18.85	17.90
NS2-114	16.75	18.85
NS2-115	15.95	16.60
NS2-116	20.15	18.20
NS2-119	18.70	20.75
NS2-12	18.90	18.95
NS2-121	19.80	20.95
NS2-122	17.40	18.75
NS2-123	18.25	18.60
NS2-126	23.15	22.55
NS2-129	19.85	19.40
NS2-15	31.00	20.20
NS2-156	23.40	24.90
NS2-168	21.00	19.60
NS2-19	19.35	17.25
NS2-21	19.85	17.45
NS2-22	16.95	18.00
NS2-24	20.40	19.35
Pulut 3	54.45	21.30
Soraya 3	20.37	25.28
Sorgum Manis	19.05	13.40
UPCA-SI	18.65	16.50
Bioguma 1	21.82	19.68
Demak 4	16.10	20.25
Numbu	20.77	21.33
Samurai 2	21.28	20.40
Soper 6	20.00	22.27

genetic variability in Fe and Zn concentrations underscores the opportunity to identify alleles associated with superior nutritional profiles, contributing to both germplasm conservation and the strategic utilization of genetic resources for food security and sustainable agricultural production (Kebede *et al*., 2023).

The study also revealed a high broad-sense heritability value for Fe content (94.46%), indicating strong genetic control and a high likelihood of successful transmission to subsequent generations. Such high heritability suggests that Fe concentration can serve as an effective selection criterion in sorghum breeding programs. Consequently, genotypes exhibiting superior Fe content—such as Pulut 3—represent promising parental materials for biofortification-oriented breeding. A comprehensive exploration of available germplasm could further uncover valuable genetic resources for enhancing the nutritional quality of sorghum (Babiker *et al*., 2024).



	LN	FLA	SD	GLI	DF	DM	PL	PD	PW	SWP	TSW	Fe	Zn
Fe													0.42 *
TSW												-0.45 **	-0.11 ns
SWP											0.57 ***	-0.09 ns	0.03 ns
PW										0.95 ***	0.45 **	-0.06 ns	0.09 ns
PD									0.79 ***	0.77 ***	0.48 **	-0.18 ns	0.15 ns
PL								-0.13 ns	0.07 ns	-0.18 ns	-0.61 ***	0.34 *	0.23 ns
DM							0.17 ns	0.18 ns	0.27 ns	0.17 ns	0.15 ns	-0.28 ns	0.03 ns
DF						0.77 ***	0.33 ns	0.30 ns	0.34 *	0.17 ns	-0.07 ns	-0.12 ns	0.35 *
GLI					0.03 ns	0.15 ns	0.26 ns	-0.61 ***	-0.48 **	-0.57 ***	-0.57 ***	0.06 ns	-0.23 ns
SD				0.03 ns	0.72 ***	0.62 ***	0.56 ***	0.39 *	0.52 **	0.33 ns	-0.10 ns	0.12 ns	0.20 ns
FLA			0.26 ns	-0.24 ns	0.10 ns	-0.02 ns	0.02 ns	0.19 ns	0.27 ns	0.17 ns	0.37 *	0.06 ns	0.02 ns
LN		0.28 ns	0.73 ***	-0.17 ns	0.73 ***	0.67 ***	0.33 ns	0.57 ***	0.63 ***	0.47 **	0.14 ns	-0.05 ns	0.24 ns
PH	0.10 ns	0.51 **	-0.21 ns	-0.63 ***	-0.21 ns	-0.25 ns	-0.53 **	0.45 **	0.39 *	0.50 **	0.68 ***	-0.07 ns	0.08 ns

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

Figure 1. Phenotypic correlation coefficients for 14 traits. Zn = Zn grain content, Fe = Fe grain content, TSW = thousand-seed weight, SWP = seed weight per panicle, PW = panicle weight, PD = panicle diameter, PL = panicle length, DM = days to maturing, DF = days to flowering, GLI = green leaf index, SD = stem diameter, FLA = flag leaf area, LN = leaf number, and PH = plant height.

Traits correlation

Correlation analysis disclosed a highly significant positive correlation between seed weight per panicle and both plant height (r = 0.50) and leaf number (r = 0.47) (Figure 1). Increased plant height often implies an association with a higher leaf number, which expands the photosynthetically active surface area and enhances assimilate production, thereby supporting more efficient grain development (Mingnan *et al*., 2017). Among yield-related traits, seed weight per panicle showed strong positive correlations with panicle diameter (r = 0.77), panicle weight (r = 0.95), and thousand-seed weight (r = 0.57). These findings align with Thant *et al.* (2021) and Rohila *et al.* (2022), who reported that plant height, panicle diameter, and panicle weight exert substantial positive effects on both seed weight per panicle and thousand-seed weight. Such strong inter-trait relationships indicate simultaneous improvement of these traits could effectively enhance sorghum yield potential.

A significant positive correlation (r = 0.42) was also evident between Fe and Zn concentrations, suggesting a shared genetic basis for their co-expression. According to Thakur *et al.* (2024), Fe concentration in sorghum received influences from both additive and non-additive gene actions, indicating stable inheritance across generations and clear expression in early segregating progeny. The positive association between Fe and Zn content is consistent with findings by Qureshi *et al.*

(2022), supporting the feasibility of simultaneous improvement of these traits through breeding—particularly within biofortification programs. Enhancing Fe concentration could, thus, concurrently increase Zn levels, simplifying selection strategies by allowing one micronutrient to serve as an indirect selection proxy for the other.

Interestingly, thousand-seed weight exhibited a significant negative correlation with Fe concentration, whereas panicle length showed a positive association. Genotypes with heavier seeds generally contained lower Fe and Zn concentrations, corroborating the results of Kamal *et al.* (2023), who reported smaller sorghum seeds tend to have higher levels of Fe and Zn. The negative correlation between agronomic traits and seed Fe and Zn content refers to sink capacity and activity. As energy and nutrients reach an increasing allocation for vegetative growth and seed development, the accumulation of micronutrients in seeds becomes limited (Otwani *et al*., 2025).

CONCLUSIONS

The evaluated sorghum (*Sorghum bicolor* L.) germplasm exhibited considerable genetic variability across agronomic and nutritional traits. Leaf number, flag leaf area, green leaf index, days to flowering and maturity, panicle diameter, panicle weight, and thousand-seed weight demonstrated moderate to high heritability. This indicates strong genetic control and potential for selection in breeding programs. Among micronutrients, Fe content showed broad variability and high heritability, while Zn content displayed relatively low variation and heritability. All evaluated traits, except green leaf index and panicle length, signified a positive correlation with seed weight per panicle. Fe and Zn contents expressed no significant correlation with panicle weight or seed weight per panicle. Based on mean performance and heritability estimates, national varieties emerged as promising candidates for yield improvement, whereas local varieties possess potential for biofortification breeding to enhance Fe and Zn concentrations.

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