

Phenotypic Evaluation of Fe and Zn Content and Agronomic Traits in Sorghum Segregating Population

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Abstract

Biofortification is one approach to overcoming micronutrient deficiencies by enhancing the nutritional content of food crops. Biofortification of sorghum's iron (Fe) and zinc (Zn) content is essential to increase sorghum's value as an alternative carbohydrate source. This research aimed to study phenotypic variation in Fe and Zn content and its correlation with agronomic traits and yields in the F₃ population generated from a cross between the 'Demak 4' and 'Soper 6 Agritan' developed using the Single Seed Descent (SSD) method. This research was conducted at the Muara Experimental Field in Bogor, from May to August 2024. The experiment used an augmented design with four replications based on an individual-based segregated design. Fe and Zn content in sorghum grain samples was measured using an X-ray fluorescence machine. The results showed that the Fe content in sorghum grain within the F₃ population ranged from 11.70-21.80 ppm, and Zn content ranged from 16.70-26.25 ppm. The correlation analysis showed that Fe content was not related to agronomic traits or yield. Zn showed a significant positive correlation with flag leaf area ($r = 0.30$, $p < 0.01$) and stem diameter ($r = 0.24$, $p < 0.05$). Fe and Zn content of sorghum grain was observed to be positively correlated ($r = 0.68$, $p < 0.001$). The results of multi-character identification using MGIDI yielded 10 selected genotypes: G1, G2, G6, G18, G20, G63, G82, G108, G122, and G181. Genotypes G1, G2, G181, and G122 are suitable for high yield.

Keywords: augmented, biofortification, genetic diversity, MGIDI, micronutrient, SSD

Introduction

Micronutrient deficiencies, commonly referred to as hidden hunger, are a global health challenge. According to the WHO (2023), the most common micronutrient deficiencies include vitamin A, vitamin D, vitamin B12, iron (Fe), iodine (I), and zinc (Zn). Worldwide, over 1.2 billion people experience iron deficiency anemia (Camaschella, 2019), and 17% of the global population suffers from zinc deficiency, which impairs immune function and increases susceptibility to infectious diseases (Chasapis et al., 2020). According to Weffort and Lamounier (2023), nutritional deficiencies, particularly those involving Fe and Zn, are prevalent in low- and middle-income countries, especially among children under five years of age.

Indonesia faces significant challenges related to micronutrient deficiencies. UNICEF (2022) reported that a quarter of pregnant women and teenage girls in the country suffer from anemia, and according to Ernawati et al. (2023), over 4.5 million children under five years old were affected by stunting. Among these children, the prevalence of Fe and Zn deficiencies were found in 13.4% and 19.7% respectively. Aisyah et al. (2024) identified Fe and Zn deficiency as major contributors to stunting in children. Therefore, enhancing the micronutrient content of plant-based foods through biofortification is an

effective strategy for addressing micronutrient deficiencies.

Biofortification has emerged as a low-cost, sustainable solution to address micronutrient deficiencies (Ashok Kumar et al., 2012; Gaddameedi et al., 2020). In Indonesia, Zn biofortification has been successfully applied to rice, achieving Zn concentrations of 30.0-34.2 ppm (Susanto et al., 2017). Similarly, Rohaeni et al. (2023) developed rice lines with Fe and Zn concentrations ranging from 15.75 to 19.07 ppm and from 30.02 to 33.07 ppm, respectively. Sorghum, in addition to rice, is another promising carbohydrate source in Indonesia. It contains 8%-18% protein, 70%-80% carbohydrates, 19% fiber, and essential minerals. The carbohydrates in sorghum are complex and provide sustained energy due to the slow digestion of starch compared with simple sugars (Tanwar et al., 2023). Sorghum grains also contain bioactive components, including dietary fiber, unsaturated fatty acids, phytosterols, policosanol, iron, and zinc (Ghinea et al., 2021; Istrati et al., 2019). Considering that sorghum is a high water-use efficiency plant (Xie & Xu, 2019) and known for its good adaptability to thrive on marginal land with high salinity and low nutrients (Shan & Xu, 2009; Tu et al., 2023) these factors make it a reliable crop, especially in regions under changing climate conditions.

Sorghum naturally contains Fe and Zn, which are essential for heme structure, iron transport, and bioavailability (Abdelhaleim et al., 2019; Oken & Duggan, 2002). Zn is a vital micronutrient involved in more than 3,000 proteins, including enzymes and transcription factors (Costa et al., 2023; March, 2013). Fe and Zn concentrations in sorghum grains generally range from 20 to 30 ppm, similar to those in wheat (Gaddameedi et al., 2022; Zhao et al., 2009).

Despite the nutritional potential of sorghum and its biofortification, the widespread release of biofortified sorghum varieties with significantly enhanced Fe and Zn content has not yet been implemented in Indonesia. To address this situation, we have been working to improve sorghum grain quality by crossing local

Indonesian varieties with national varieties. This study aimed to evaluate the genetic diversity of Fe and Zn content and its correlation with agronomic traits in the F_3 population generated from the 'Demak 4' × 'Soper 6 Agritan' cross. This research is crucial as a foundation for improving the quality of sorghum grain as a food source in Indonesia.

Materials and Methods

Genetic Materials

The genetic material used in this study included 483 F_3 genotypes developed through Single Seed Descent from the cross of 'Demak 4' × 'Soper 6 Agritan'. Five varieties were used as checks: 'Demak 4', 'Soper 6 Agritan', 'Soper 7 Agritan', 'Kawali', and 'Bioguma 1'.

Field Experiment

The experiment was conducted at the Muara Experimental Field in Bogor, Indonesia, from May to August 2024. The plants were spaced 75 cm apart in rows and 15 cm apart within rows. The experiment was designed with an augmented design of four replications to evaluate varieties based on SSD-derived individual plant evaluations. Each plot consisted of 120-121 F_3 plants and five check varieties. Total checks per plot were 100 plants, with 20 plants per check. This design aimed to minimize experimental error and enable genotype-control comparison (Jambormias & Riry, 2009).

Fertiliser doses used were urea (45% N) at 150 kg/ha, KCl (60% K_2O) at 100 kg/ha, and SP-36 (36% P_2O_5) at 100 kg/ha (Wirnas et al., 2021). Urea was applied in two stages: one-third at planting and two-thirds 3-4 weeks after planting or simultaneously with wounding. Meanwhile, KCl and SP-36 were used only once at planting—plant maintenance involved replanting, wounding, and control of pests, weeds, and diseases. Pest and disease control was based on field conditions. Harvesting occurred when the seeds were fully mature, indicated by the emergence of a black layer on the lower part of the seed.

Data Collection

Observations were made on all plants in the F₃ population. For each check variety, 10 plant samples were selected per replication using random sampling to ensure representativeness. Agronomic traits observed during the vegetative phase included plant height (PH), stem diameter (SD), leaf number (LN), and flag leaf area (FLA). After harvest, panicle-related agronomic traits, such as panicle length (PL), panicle diameter (PD), panicle weight (PW), and grain weight per panicle (GWPP), were measured.

Fe and Zn contents were determined in 100 randomly selected F₃ sorghum seed samples. Elemental quantification was performed with an XRF analyzer (Oxford Instruments X-Supreme 8000), and the measurements were validated by Inductively Coupled Plasma (ICP) spectrometry. For each sample, approximately 3 g of seeds were weighed and placed into the XRF sample tube following the protocol described by Rohaeni et al. (2016).

Data Analysis

Data analysis was performed in RStudio (version 2024.12+467; R Core Team, 2024) using the “augmented RCBD package” (version 0.1.7; Aravind et al., 2023). This analysis included variance component estimation, heritability, genetic coefficient of variation (GCV), and phenotypic coefficient of variation (PCV). Pearson’s correlation coefficients were calculated using the “cor()” function in RStudio. Multi-trait genotype selection was conducted

using the Multi-Trait Genotype-Ideotype Distance Index (MGIDI) based on Olivoto and Nardino (2021) with the “metan package” in RStudio.

Results and Discussion

Analysis of Fe and Zn Content Diversity in F₃ Sorghum Population

Analysis of variance showed that genotype had a significant effect on Fe and Zn contents in sorghum seeds. Fe content in the F₃ population ranged from 11.70 to 21.80 ppm, with a mean of 16.81 ppm, and did not differ significantly from that of the parental lines. Zn content ranged from 16.70 to 26.25 ppm, with a mean of 19.83 ppm (Table 1). The maximum Zn content observed in the F₃ population was 26.25 ppm, exceeding the Zn levels of both parental lines, ‘Demak 4’ (21.49 ppm) and ‘Soper 6 Agritan’ (18.47 ppm). This potentially indicates transgressive segregation for Zn content in the F₃ population.

Other cereals, such as wheat and maize, typically contain approximately 30 ppm Fe and 25 ppm Zn, whereas rice grains typically contain 2 ppm Fe and 16 ppm Zn (Ibrahim et al., 2021). Similar variations in Fe and Zn contents in sorghum have been reported, which range from 12.1 to 83.4 ppm for Fe and 6.3 to 51.4 ppm for Zn (Gebre et al., 2024; Hariprasanna et al., 2014; Phuke et al., 2017). Genetic and environmental factors influence this diversity in micronutrient content. Madhusudhana et al. (2023) stated that the interaction between a sorghum genotype and the environment significantly affects the Fe and Zn contents in

Table 1

Mean and Standard Deviation of Fe and Zn Content in ‘Demak 4’, ‘Soper 6’, and F₃ Sorghum Population

Characters	Mean and standard deviation (ppm)				Range of F ₃ (ppm)
	‘Demak 4’	‘Soper 6’	ttest	F ₃	
Fe content	17.03 ± 0.61	17.81 ± 0.80	ns	16.81 ^{ns} ± 1.91	11.70-21.80
Zn content	21.49 ± 0.71	18.47 ± 1.20	**	19.83 ^{aB} ± 1.67	16.70-26.25

Note. ns= $p > 0.05$, *= $p < 0.05$, **= $p < 0.01$, A= Significantly higher than ‘Demak 4’, B= Significantly higher than ‘Soper 6 Agritan’; a= Significantly lower than ‘Demak 4’, b= Significantly lower than ‘Soper 6 Agritan’.

sorghum grain. Kotla et al. (2019) and Thakur et al. (2024) found that candidate-associated genes involved in Fe and Zn accumulation can be modulated by environmental factors, affecting their expression and effects. Satyavathi et al. (2022) reported that several gene families, including multicopper oxidase, major facilitator superfamily, cytochrome P450, zinc-containing alcohol dehydrogenase, ferredoxin-NADP reductase type 1, and CONSTANS, play roles in iron and zinc accumulation in pearl millet. These genes were found to be more active (up-regulated) in genotypes with high Fe and Zn content.

Table 2 summarizes the components of variance and broad-sense heritability (h^2_{bs}) for Fe and Zn content. According to Stanfield (1983), high heritability (>50%) was observed for both Fe (95.93%) and Zn (85.78%), indicating that genetic factors play a dominant role in the variation of these traits. Studies by Motilhaodi et al. (2018) and Andi et al. (2022) also reported high heritability for Fe and Zn content, supporting the conclusion that selection for these traits can be effective (Gonçalves et al., 2013).

The Genetic Coefficient of Variation (GCV) is classified as low (<10%), moderate (10%-20%), and high (>20%) (Sivasubramanian & Madhavamenon, 1973). The GCV for Fe content (11.44%) falls within the moderate range, indicating sufficient genetic variability to enable measurable selection gains. In contrast, the GCV for Zn content (7.83%) is low, suggesting limited allelic diversity and reduced potential for selection. Low GCV values are commonly associated with narrow parental genetic backgrounds, which restrict recombination of

favorable alleles (Magar et al., 2021; Muniz et al., 2025). For Zn biofortification, using more genetically diverse parental crosses or incorporating genomic selection approaches may be more effective than relying solely on phenotypic screening.

Diversity Analysis of Agronomic Traits

Ttests between the 'Demak 4' and 'Soper 6 Agritan' varieties revealed significant differences in stem diameter, leaf number, panicle length, and panicle weight (Table 3). Compared to the 'Demak 4' variety, 'Soper 6 Agritan' exhibited superior traits, including greater stem diameter, more leaves, longer panicle length, and heavier panicle weight. The F_3 population showed a mean plant height of 192 cm, flag leaf area of 253.3 cm², panicle diameter of 6.1 cm, panicle weight of 54.1 g, and grain weight per panicle of 38.3 g, which were similar to parental values. The F_3 population exhibited wide variability, particularly in agronomic traits like plant height (114.3-242.9 cm), flag leaf area (72.1-474.9 cm²), panicle weight (14.4-116.6 g), and grain weight per panicle (6.4-95.3 g). This variability indicates the potential for transgressive segregation, which can lead to the selection of high-yielding genotypes (Rini et al., 2022). Transgressive segregants are inferred from F_3 genotypes at the distributional extremes (i.e., values higher or lower than both parental values by more than the 5% critical difference) (Shreya et al., 2017). According to Maryono et al. (2019), detecting transgressive segregants in early generations enhances the efficiency of the plant breeding process.

Table 2

The Estimated Variance Components and Broad-sense Heritability Values in the F_3 Sorghum Population

Characters	σ^2_p	σ^2_g	σ^2_e	h^2_{bs}	h^2_{bs} criteria	GCV	GCV criteria	PCV
Fe content	3.72	3.57	0.15	95.93	high	11.20	moderate	11.44
Zn content	2.83	2.43	0.40	85.78	high	7.83	low	8.46

Note. σ^2_p = phenotype variance, σ^2_g = genotype variance, σ^2_e = environment variance, h^2_{bs} = broad-sense heritability, GCV= genetic coefficient variation, PCV= phenotype coefficient variation.

Table 3

Mean, Standard Deviation, and Range of Parent and F₃ Sorghum Population

Characters	Mean and standard deviation			F ₃	Range of F ₃
	'Demak 4'	'Soper 6 Agritan'	ttest		
PH	199.4 ± 5.2	189.1 ± 6.7	ns	192.1 ^{ns} ± 14.1	114.3 - 242.9
SD	14.7 ± 0.7	18.8 ± 0.6	**	13.9 ^b ± 2.5	5.6 - 22.6
LN	9.5 ± 0.3	11.0 ± 0.4	**	8.9 ^b ± 0.9	6.5 - 11.5
FLA	242.2 ± 31.9	221.4 ± 23.3	ns	253.3 ^{ns} ± 70.8	72.1 - 474.9
PL	19.5 ± 0.8	32.8 ± 1.4	**	19.5 ^b ± 1.8	14.5 - 30.3
PD	6.2 ± 0.3	6.0 ± 0.4	ns	6.1 ^{ns} ± 0.9	2.1 - 9.2
PW	58.7 ± 3.3	84.8 ± 15.7	*	59.0 ^{ns} ± 25.5	14.4 - 116.6
GWPP	41.1 ± 5.1	56.0 ± 7.6	ns	42.0 ^{ns} ± 18.1	6.4 - 95.3

Notes. PH= plant height (cm), SD= stem diameter (mm), LN= Leaf number (blade), FLA= flag leaf area (cm²), PL= panicle length (cm), PD= panicle diameter (cm), PW= panicle weight (g), GWPP= grain weight per panicle (g). ns= $p > 0.05$, *= $p < 0.05$, **= $p < 0.01$. a= significantly lower than 'Demak 4', b= significantly lower than 'Soper 6 Agritan'.

Table 4

The Estimated Variance Components and Broad-Sense Heritability Values in the F₃ Sorghum Population

Characters	σ^2_p	σ^2_g	σ^2_e	h^2_{bs}	h^2_{bs} criteria	GCV	GCV criteria	PCV
PH	297.46	250.59	46.87	84.24	high	8.23	low	8.96
SD	7.04	6.35	0.69	90.13	high	18.10	moderate	19.07
LN	0.97	0.62	0.36	63.49	high	8.78	low	11.02
FLA	5047.61	4423.40	624.22	87.63	high	26.27	high	28.07
PL	3.39	2.60	0.79	76.68	high	8.26	low	9.43
PD	0.87	0.68	0.19	77.74	high	13.57	moderate	15.39
PW	335.94	260.06	75.88	77.41	high	31.76	high	36.10
GWPP	263.23	197.47	65.76	75.02	high	38.45	high	44.39

Notes. PH= plant height (cm), SD= stem diameter (mm), LN= Leaf number (blade), FLA= flag leaf area (cm²), PL= panicle length (cm), PD= panicle diameter (cm), PW= panicle weight (g), GWPP= grain weight per panicle (g), σ^2_p = phenotype variance, σ^2_g = genotype variance, σ^2_e = environment variance, h^2_{bs} = broad-sense heritability, GCV= genetic coefficient variation, PCV= phenotype coefficient variation.

The components of variance, broad-sense heritability, genetic coefficient of variation (GCV), and phenotypic coefficient of variation (PCV) are presented in Table 4. All traits showed high h^2 (>50%) under the conditions of this augmented design study, though unreplicated test entries likely inflated estimates by underestimating environmental variance (σ^2_e). The error variance of unreplicated genotypes was estimated using only replicated controls. H^2 from single-trial

augmented design was possibly overestimated relative to multi-environment trials, due to genetic variance that includes inseparable genotype-by-environment interactions (You et al., 2016). Somu et al. (2024) also reported high h^2 values for all agronomic traits observed in sorghum using the incomplete block design. High heritability values indicate that genetic factors, rather than environmental factors, play a greater role. High heritability enhances the effectiveness

of plant breeding processes. The stem diameter trait exhibited the highest heritability (90.13%) among the traits. A study by Dare et al. (2024) on an F₂ sorghum population also demonstrated high h² values for traits such as plant height, stem diameter, leaf greenness, leaf area, and grain weight per panicle.

The GCV for traits such as flag leaf area, panicle weight, and grain weight per panicle was high (>20%), indicating substantial genetic variability. This variability is critical for the success of breeding programs aimed at enhancing these traits. Stem diameter and panicle diameter showed moderate GCV, while leaf number had the lowest GCV. PCV for all traits was higher than GCV. This indicates the influence of environmental factors on each trait. The narrow difference between GCV and PCV values suggests that genetic influence remains dominant, with environmental effects considered negligible (Chacko et al., 2023).

Correlation Between Agronomic Traits and Fe and Zn Content

Correlation analysis is essential for estimating the strength of the relationships between agronomic traits. Positive correlation values indicate that an increase in one trait is associated with an increase in another. The

characteristics of stem diameter, flag leaf area, panicle length, panicle diameter, and panicle weight are positively correlated with grain weight per panicle (Figure 1). The trait panicle weight showed the highest correlation with grain weight per panicle, at 0.97. This positive relationship suggests that enhancing morphological dimensions, such as panicle and stem size, can improve grain production. The number-of-leaves trait showed a positive correlation, but the correlation was low and not significant.

The positive correlation between agronomic and yield-related traits enables simultaneous selection for both. Wirnas et al. (2021) explained that stem diameter, leaf number, flag leaf area, leaf greenness, percentage of green leaves, and panicle diameter all positively contribute to yield improvement. Flag leaf area plays a crucial role in supporting the plant's photosynthetic capacity, even though its correlation with seed weight per plant is not significant. Larger flag leaf areas enable the plant to optimize carbohydrate production during the seed-filling phase, ultimately enhancing the harvest. Santhiya et al. (2021) found a significant positive association between flag leaf area and yield in sorghum.

The correlation analysis indicates a positive correlation between the Fe and Zn content in sorghum seeds. A correlation coefficient of 0.68 suggests that an increase in Fe concentration

Table 5

Selection Differential Using MGIDI Selection

Characters	Factor	X _o	X _s	SD	SDperc (%)
PD	FA1	6.3	7.0	0.70	11.2
PW	FA1	53.6	84.9	31.3	58.4
GWPP	FA1	39.1	67.3	28.2	72.0
Zn	FA2	19.9	23.0	3.09	15.6
Fe	FA2	16.9	19.3	2.42	14.3
SD	FA3	14.1	16.8	2.63	18.6
FLA	FA3	244.0	339.0	94.9	38.9
PL	FA3	19.4	20.4	1.0	5.1
PH	FA4	194.0	178.0	-16.3	-8.4

Notes. PH= plant height (cm), SD= stem diameter (mm), LN= Leaf number (blade), FLA= flag leaf area (cm²), PL= panicle length (cm), PD= panicle diameter (cm), PW= panicle weight (g), GWPP= grain weight per panicle (g), X_o= mean value before selection, X_s= mean value after selection, S= selection differential, % SD= selection differential percentage.

in the seeds is likely to be accompanied by an increase in Zn concentration. Similar findings were reported in previous studies by Hariprasanna et al. (2014), Kotla et al. (2019), and Gaddameedi et al. (2020). The positive and significant correlation between Fe and Zn content provides an opportunity for the simultaneous selection for both micronutrients. This will make the biofortification process in sorghum more efficient.

Fe content shows a weak correlation with all observed agronomic traits and yield. Meanwhile, Zn shows positive correlations with stem diameter (0.24) and flag leaf area (0.30), both of which are positively correlated with grain weight per panicle. Simultaneous selection for these traits can enhance yield and is expected to indirectly increase Fe and Zn content. Watters et al. (2009) reported that the flag leaf plays an important role in the accumulation of Fe and Zn content in wheat seeds. To the best of our knowledge, no previous studies have directly examined the relationship between flag leaf area and Fe or Zn accumulation in seeds. However, Deshpande et al. (2018) reported that the expression of ZIP family proteins in the

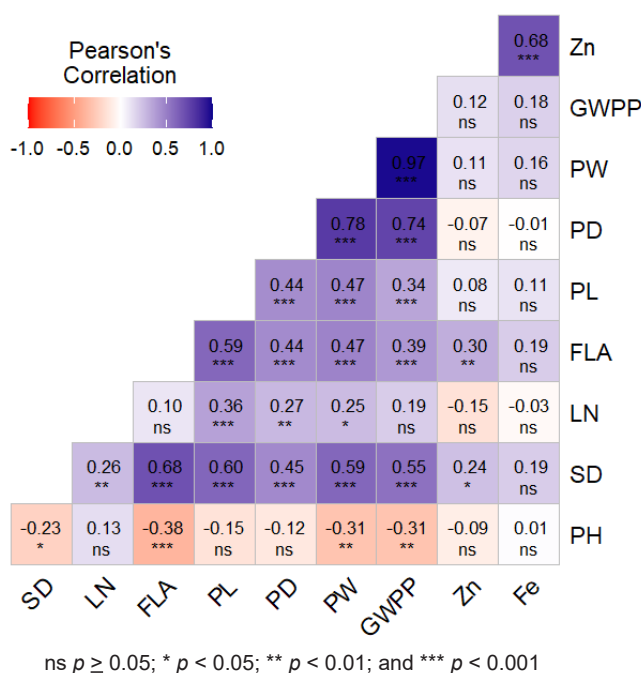
flag leaf, which are key transporters involved in metal uptake and translocation, was significantly associated with Zn content in wheat grains. The accumulation of Fe and Zn in seeds is highly dependent on the flag leaf's ability to store and then remobilize metals during senescence.

Identification of Potential Genotypes Using the Multi-trait Genotype-Ideotype Distance Index

The SSD method allows breeders to maintain high genetic diversity from the F₂ generation onward. Selection is generally carried out in the F₅ or F₆ generations to increase homozygosity (Gajghate et al., 2018). This study aims to identify genotypes with high yield and high Fe and Zn content in early generations using the Multi-trait Genotype-Ideotype Distance Index (MGIDI). The MGIDI method simplifies multi-trait selection by grouping related traits into several factors using Principal Component Analysis (PCA). These factors help identify genotypes based on various characteristics, minimizing redundancy and multicollinearity (Debnath et al., 2024; Olivoto & Lucio, 2020).

Figure 1

Correlation Between Agronomic Traits and Fe and Zn Content



For instance, traits associated with panicle characteristics form one factor, while Fe and Zn contents form another. Genotypes are then ranked by how closely their combined trait profile matches the ideal genotype. Figure 2A visually summarizes this process and highlights the top-selected genotypes (red dots). Identification was carried out on 100 genotypes tested for Fe and Zn content, resulting in the selection of 10 genotypes: G1, G2, G6, G18, G20, G63, G82, G108, G122, and G181.

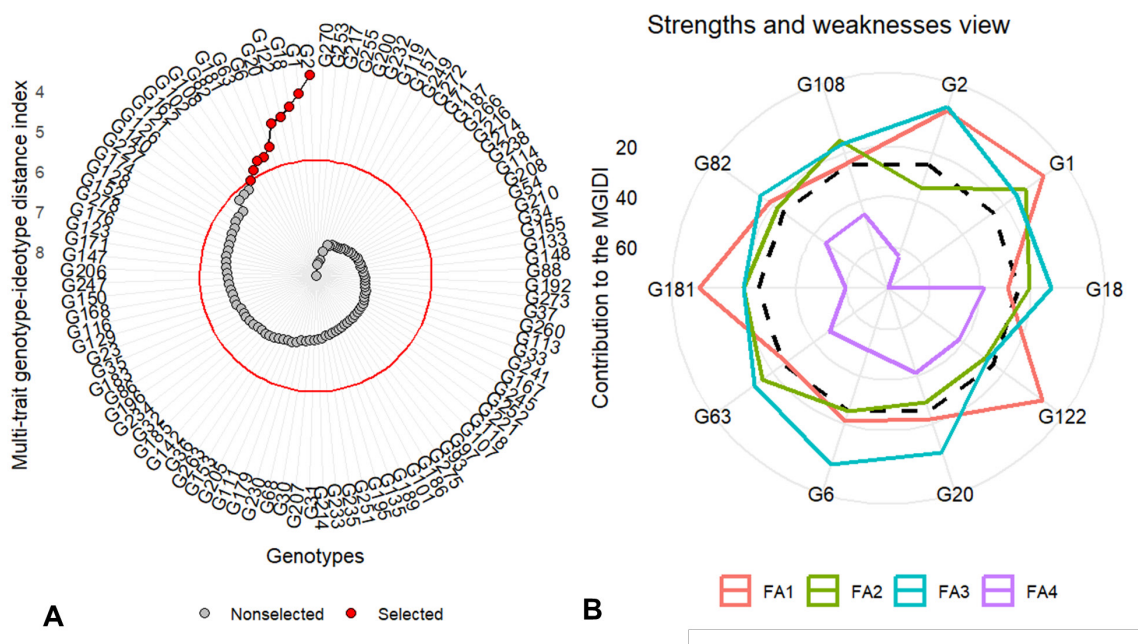
The strengths and weaknesses of each genotype are shown in Figure 2B. The factor lines moving toward the outer edge indicate a smaller proportion explained by that factor, suggesting that the traits within that factor are closer to the ideotype (Olivoto & Nardino, 2021). Yield-related traits are located in FA1, while Fe and Zn content are positioned in FA2. Genotypes G1, G2, G181, and G122 are superior for high yield. The contribution of Fe and Zn (FA2) to the MGIDI index is similarly low among selected genotypes, indicating that Fe and Zn values do not strongly differentiate the selected lines.

Genotypes G2, G6, G18, G20, G63, and G82 excel in the FA3 traits of stem diameter, flag leaf area, and panicle length. Based on the strengths and weaknesses of all genotypes, genotypes G1, G2, G181, and G122 are suitable for high yield.

The desired ideotype is the genotype that exhibits higher values for all traits, except for plant height, as taller plants are more prone to lodging (Wang et al. 2020). All traits exhibit positive selection differentials, except for plant height. Multiple studies show that it is possible to identify or breed sorghum lines that combine high micronutrient content with competitive or even high grain yield (Djanaguiraman et al., 2024; Hariprasanna et al., 2014, Ng'uni et al., 2016). However, some genotypes, such as G18, G63, and G108, exhibit low grain yield. Upadhyaya et al. (2016) reported that certain sorghum accessions with high Fe and Zn content also have low grain yields. Similarly, Andiku et al. (2022) demonstrated a negative association between Fe and Zn content and yield.

Figure 2

A) Genotype Ranking in Ascending Order for the MGIDI Index; B) Visualization of Strengths and Weaknesses of Selected Genotypes in the F₃ Population



Note. FA1: PD, PW, and GWPP; FA2: Fe and Zn content; FA3: SD, FLA, and PL; FA4: PH.

Conclusions

The Fe and Zn content in the F₃ sorghum population ranged from 11.70 to 21.80 ppm and 16.70 to 26.25 ppm, respectively. In the present study, all observed traits exhibited high broad-sense heritability. This may result from unreplicated test entries, which inflate estimates by underestimating environmental variance (σ^2_e) in augmented designs. Sorghum grain weight per panicle was positively correlated with stem diameter, flag leaf area, panicle length, panicle diameter, and panicle weight, suggesting that simultaneous selection for these traits is feasible. While Fe content showed a weak correlation with agronomic traits, Zn content exhibited positive correlations with flag leaf area (0.30) and stem diameter (0.24). The positive, significant correlation between Fe and Zn enables simultaneous selection of both micronutrients, thereby improving the biofortification process. Using MGIDI led to identifying the superior genotypes (G1, G2, G6, G18, G20, G63, G82, G108, G122, and G181). Genotypes G1, G2, G181, and G122 are suitable for high yield.

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