

Assessing genetic variability and heritability in the F₄ population of mutant Bioguma and Gando Keta local Indonesian sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract: This study examined the genetic variability, heritability, and genetic advance in the F₄ generation of sorghum to enhance grain productivity. The study was conducted at Citayam Research Station, Depok Indonesia (March–September 2023), and evaluated 102 progenies of the F₄ population from a Bioguma (a mutant line) × Gando Keta (a local variety) cross using an Augmented block design with four replications. The results of this experiment indicated that the F₄ generation outperformed the Gando Keta grain yield components, but remained inferior to Bioguma. The yield traits were influenced by the non-additive gene action, with genetic factors playing a significant role in grain weight variation. The top 20% of F₄ progenies exhibited improved yield characteristics, including thicker stems, larger panicles, and increased grain weight.

Keywords: cluster analysis; F₄ generation; genetic advancement; genotypic coefficient of variation (GCV)

Sorghum (*Sorghum bicolor*) is a multipurpose crop used in food, livestock feed, and bioindustry applications (Stefoska-Needham et al. 2015; Rashwan et al. 2021; Wang et al. 2023). Sweet sorghum juice is valuable in the food and beverage industry due to its high glucose and fructose content, making it ideal for fermentation and bioethanol production (Pandebsie et al. 2019; Santoso et al. 2021). Sorghum grain is rich in protein, fibre, vitamins, and essential

minerals, serving as a nutritious, gluten-free alternative for flour blends (Tanwar et al. 2023; Suvarna et al. 2024). The crop requires less water than other cereal crops such as corn and wheat and can withstand heat and drought, making it resilient to climate change (Hadebe et al. 2017; Baloch et al. 2023).

Breeding programmes aimed at developing high-yielding and nutrient-rich crop varieties are essential for ensuring national food security, particularly in the

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context of climate change and population growth (Gyawali et al. 2018). Sorghum breeding efforts have primarily focused on enhancing the yield, stress tolerance, and nutritional quality, however, consumer preferences for soft-textured grains remain largely unaddressed (Boatwright et al. 2021). To address both yield and grain texture preferences, a cross breeding approach was employed, combining the high-yielding, but hard-textured Bioguma variety with Gando Keta a soft-textured, but low-yield landrace from West Nusa Tenggara (Rhezandy et al. 2023). Gando Keta is traditionally utilised in rice blends, tortillas, and various processed foods (Fitrahtunnisa et al. 2020). Selection in the F_2 population revealed substantial genetic variation and high heritability for key agronomic traits, leading to the development of promising selection lines (Lestari et al. 2023).

Understanding genetic variability, heritability, and genetic gain is essential for selecting superior plant traits (Tadesse et al. 2018; Rai et al. 2021). Genetic variability provides the basis for effective selection, while heritability measures the proportion of phenotypic variation attributable to genetic factors. Genetic gain reflects the improvement achieved through selective breeding (Mustafa et al. 2015; Abebe et al. 2017; Korsu et al. 2024). High heritability suggests that trait expression is largely governed by genetic factors rather than the environment, making such traits reliable targets for breeding (Islam et al. 2015; Herniwati et al. 2024). Heritability is classified into broad-sense (total genetic variance: additive, dominance, epistatic) and narrow-sense (additive variance only) types (Lado & Muthomi 2017; Bhatt et al. 2024). Traits with high heritability are especially favourable for selection, due to their stability across environments.

This study aimed to evaluate the genetic variability and heritability in sorghum (*Sorghum bicolor* L.) derived from a cross between the Bioguma mutant and the local Gando Keta variety. The analysis was conducted to identify superior traits for targeted improvement in sorghum breeding programmes.

MATERIAL AND METHODS

Field of experiment. The experiment was conducted from March to September 2023 in the Experimental Field at Citayam Depok, Indonesia, situated at an elevation of 100 m above sea level, on latosol soil type during the dry season.

Plant material. A total of 102 F_4 lines derived from a hybridisation between of the Bioguma ×

Gando Keta variety were evaluated, along with the two parental cultivars as the genetic material. The Bioguma variety as a female parent, was developed from gamma irradiation (50 Gy) of the Indonesian commercial sorghum variety Numbu (Lestari et al. 2020). Meanwhile, Gando Keta, the male parent, is a traditional landrace from West Nusa Tenggara.

Experimental design and trial management. An augmented block design (ABD) was employed with four blocks to accommodate all the plant material. The two parental lines served as the check varieties. Each experimental plot consisted of three metres of four rows per lines spaced at 75×25 cm. The crop maintenance followed management practices including watering, weeding, and earthing-up. Fertilisation was applied twice, at 15 and 42 days after sowing (DAS) using 150 kg/ha of urea (46% N), 100 kg/ha of SP-36 (36% P_2O_5) (super phosphate), and 100 kg/ha of KCl (60% K_2O). The urea was split into two applications: half at 15 DAS, and the remaining applied at 42 DAS. The pest and disease control included the application of carbofuran at sowing and foliar spraying with deltamethrin.

Data collection and quantitative traits. The observed traits included the plant height (cm), sugar Brix content (%), stem width (mm), panicle length (cm), panicle width (cm), wet panicle weight (g), dry panicle weight (g), and grain weight (g). Harvesting was performed at physiologically maturity, indicated by the presence of black spots at the panicle base, and hardened grains.

Statistical analysis. Variance components and heritability estimates were calculated using the single-plant basis method as follows:

- (a) σ^2_p = phenotypic variance (variance of all the individuals in the F_4 population)
- (b) σ^2_e = environmental variance based on the average method of variance of the two cultivars with the formula:

$$\sigma^2_e = \sqrt{(\sigma^2_{P1})(\sigma^2_{P2})}$$

where:

P1 – female parental line;

P2 – male parental line.

- (c) σ^2_g = genetic variance: $\sigma^2_g = \sigma^2_p - \sigma^2_e$
- (d) Genotypic coefficient of variation:

$$GCV = \frac{\sqrt{\sigma^2_g}}{\bar{x}}$$

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The genetic variability was categorised as follows: $\leq 10\%$ (narrow), $10\text{--}20\%$ (medium), $\geq 20\%$ (wide). Broad sense heritability ($H_{bs}^2 = \sigma^2g/\sigma^2p$) with the heritability level description: low ($0 < H_{bs}^2 < 20\%$), moderate ($20 \leq H_{bs}^2 < 50\%$), and high ($50 \leq H_{bs}^2$). A cluster analysis was performed to group the genotypes based on morphological traits similarities. Selection in the F_4 generation was conducted using a weighted index, targeting on the top 20% lines.

All the trait data were subjected to an analysis of variance (ANOVA) following the procedure described by Gomez and Gomez (1984). The genetic parameters, including the variance components, broad sense heritability, and genetic gain, were analysed using Microsoft Excel 2010, SAS (Ver. 9.0), Minitab (Ver. 18.0). The PB-Stat CL 2.1.1 programs were employed to perform the clustering analysis.

RESULTS AND DISCUSSION

Table 1 presents the analysis of variance from eight traits observed in the F_4 generation of the Bioguma $\times$ Gando Keta hybrids. A significant genotype effect on the stem diameter was observed, indicating notable differences among the progenies. The coefficient of variance (CV) ranged from 3.13% (panicle diameter) to 19.53% (wet panicle weight). A lower CV suggests reduced variability and higher experimental precision, which is essential for attributing the observed differences to uncontrolled environmental factors (Berry et al. 2022). A CV below 20% indicates measurement stability across the data subsets enhancing the reliability of the field experiments (Agranov et al. 2020).

The parental varieties, Bioguma and Gando Keta, as checks, showed variation in the genetic variability of most of the studied characteristics. Meanwhile, their F_4 population showed variations in the genetic variability solely on the stem diameter characteristic. The mean value and standard deviation of the tested F_4 sorghum population characteristics are displayed in Table 2.

The F_4 population showed a mean value of the plant height and Brix content higher than Bioguma, but lower than the Gando Keta, ranging from 224.4 to 291.13 cm for the plant height, and ranged from 11.40 to 20.40 for the Brix content. The stem diameter ranged from 16.91 to 23.59 mm, with a mean value higher than Gando Keta, but lower than Bioguma. The panicle length ranged from 16.54 to 23.77 cm, exceeding Gando Keta and, but the panicle diameter (49.66 to 76.35 mm) was lower than both parents. The yield components of the F_4 population-including wet panicle weight (77.99 to 191.17 g), dry panicle weight (68.82 to 140.42 g), and grain weight (57.05 to 113.51 g) were higher than Gando Keta, but lower than Bioguma.

The standard deviation of all the traits in the F_4 population was lower than both parents, indicating greater phenotypic stability. Significant differences were observed between the two parental varieties in the plant height, stem diameter, panicle length, Brix content, wet panicle weight, dry panicle weight, and grain weight. The panicle length and diameter also differed significantly between the candidates' lines and parents.

This phenotypic stability in the F_4 population may result from genetic variability, genotype-environment

Table 1. Variance and mean square of the F_4 sorghum population of the Bioguma $\times$ Gando Keta with eight observed characteristics

Source	DF	Mean square							
		PH	SD	PL	PD	Brix	WPW	DPW	GW
Genotype	101	180.15 ^{ns}	1.85*	2.83 ^{ns}	28.27 ^{ns}	5.34 ^{ns}	351.98 ^{ns}	163.14 ^{ns}	107.63 ^{ns}
Candidate (V)	99	157.81 ^{ns}	1.83*	2.58 ^{ns}	21.94 ^{ns}	5.29 ^{ns}	318.71 ^{ns}	141.56 ^{ns}	99.54 ^{ns}
Check (C)	1	2 451.40**	5.58**	17.79*	146.72 ^{ns}	14.85*	3 829.01*	2 256.58*	1 010.92*
V vs. C	1	121.06 ^{ns}	0.01 ^{ns}	12.84*	536.19*	0.78 ^{ns}	168.47 ^{ns}	184.40 ^{ns}	4.57 ^{ns}
Error	6	164.74	0.38	1.89	42.79	2.47	543.58	172.70	146.51
CV (%)		4.83	3.13	6.77	11.18	10.01	19.53	13.75	15.97

^{ns}not significant at $P < 0.05$; *, **significant at $P < 0.05$, 0.01; CV – coefficient of variance; DF – degree of freedom; PH – plant height; SD – stem diameter; PL – panicle length; PD – panicle diameter; Brix – Brix content; WPW – wet panicle weight; DPW – dry panicle weight; GW – grain weight

Table 2. Mean value and standard error of the tested F_4 generations characteristics resulting from Bioguma $\times$ Gando Keta vs. two parental varieties

Characters	Plant individual ranges	Means and standard error		
		F_4	Bioguma	Gando Keta
Plant height (cm)	224.4–291.13	265.56 $\pm$ 1.26	252.10 $\pm$ 7.40	287.11 $\pm$ 5.25
Stem diameter (mm)	16.91–23.59	19.73 $\pm$ 0.14	20.57 $\pm$ 0.39	18.90 $\pm$ 0.21
Panicle length (cm)	16.54–23.77	20.38 $\pm$ 0.16	20.55 $\pm$ 0.95	17.57 $\pm$ 0.18
Panicle diameter (mm)	49.66–76.35	57.86 $\pm$ 0.47	62.09 $\pm$ 4.23	70.65 $\pm$ 1.86
Brix content (°)	11.40–20.40	15.74 $\pm$ 0.23	14.05 $\pm$ 1.08	16.78 $\pm$ 0.28
Wet panicle weight (g)	77.99–191.17	119.72 $\pm$ 1.79	136.80 $\pm$ 15.70	93.07 $\pm$ 4.94
Dry panicle weight (g)	68.82–140.42	95.78 $\pm$ 1.21	107.86 $\pm$ 8.33	74.27 $\pm$ 4.12
Grain weight (g)	57.05–113.51	75.84 $\pm$ 1.00	86.30 $\pm$ 7.31	63.81 $\pm$ 4.45

interactions, and the cumulative effects of selection during breeding. In contrast, the parental lines exhibited high trait variance. Selecting genetically diverse parents with contrasting phenotypes is critical for developing stable and improved progenies (Madhusudhana et al. 2012). Genetic variation can arise through environmental adaptation or intentional selection across generations.

Genotypic variation, phenotypic variation and heritability in the broad sense of the F_4 population of Bioguma $\times$ Gando Keta. The data in Table 3 indicate genetic variability across most traits. The genetic coefficient variation (GCV) ranged from 4.83 to 24.31%, suggesting a wide spectrum of variability. A similar finding was reported in sorghum by Gebregergs & Mekbib (2020) with the GCV and phenotypic coefficient variation (PCV) values ranging from 3.13 to 37.44% and from 1.44 to 55.58% by Jimmy et al. (2017). Moreover, a previous study by Akshaykumar et al. (2023) also noted that the

GCV value for the panicle weight and grain yield, consistent with this study although their results differed for the plant height, panicle length, and panicle diameter.

The broad sense heritability (H_{bs}^2) ranged from 48.77% for the panicle diameter (moderate) to 95.06% in the stem diameter (high). Traits such as the plant height, stem diameter, panicle length, Brix content, wet panicle weight, dry panicle weight and grain weight exhibited high heritability. These findings align with Ranjith et al. (2017), who reported high broad sense heritability for similar traits. High broad-sense heritability implies that the phenotypic variation is primarily governed by genetic factors rather than environmental influences (Neupane et al. 2020).

The highest GCV was observed in the wet panicle weight, dry panicle weight, and grain weight indicating strong genetic influence. The high heritability values for the characteristics assessed indicate that the genetic factors significantly influence the pheno-

Table 3. Variance components and broad sense heritability of the test characteristics based on the single plant basis heritability method for the F_4 population

No.	Characters	σ^2p	σ^2e	σ^2g	H_{bs}^2 (%)	Criteria	GCV (%)	Criteria
1	plant height	329.58	164.74	164.84	50.01	high	4.83	narrow
2	stem diameter	7.71	0.38	7.33	95.06	high	13.72	medium
3	panicle length	4.24	1.89	2.35	55.49	high	7.52	narrow
4	panicle diameter	83.52	42.79	40.73	48.77	medium	11.03	medium
5	Brix content	8.47	2.47	6.00	70.85	high	15.56	medium
6	wet panicle weight	1 099.15	543.56	555.59	50.55	high	19.69	medium
7	dry panicle weight	624.76	172.70	452.07	72.36	high	22.20	wide
8	grain weight	486.37	146.52	339.86	69.86	high	24.31	wide

H_{bs}^2 – broad sense heritability (%); σ^2e – environmental variance; σ^2p – phenotypic variance; σ^2g – genotypic variance; GCV – genotypic coefficient variance (%)

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typic variation more than the environmental (non-genetic) factors (Vashistha et al. 2013; Korsa et al. 2024). Additionally, both the dry panicle weight and grain weight exhibited considerable genetic variability, while the wet panicle weight showed moderate genetic variance. The classification of medium and high genetic variability suggests that the studied genotypes are diverse, offering more potential for variability and an increased chance of developing the desired lines (Vashistha et al. 2013).

GCV is a crucial parameter for evaluating variability in specific characteristics (Adewumi et al. 2023). Analysing genetic variation within a population provides valuable insights into the potential for effective selection. Genetic variation in a population enhances the likelihood of obtaining desirable phenotypes through selection (Korsa et al. 2024; Reddy & Pitha 2024).

A high H^2_{bs} combined with a narrow GCV in the plant height and panicle diameter characteristics suggests that these traits are genetically controlled and exhibit uniformity within the F₄ population, with minimal environmental influence. Conversely, traits

with both a high H^2_{bs} and a wide GCV indicate a strong genetic effect and greater variability, enhancing their suitability for selection (Neupane et al. 2020).

Estimating the heritability and genetic gain is critical in a breeding programme, as it helps predict the selection efficiency (Bilgin & Kahraman 2010). A previous study reported a low GCV and PCV for the plant height and cob diameter in maize, consistent with the traits governed more by the additive gene action (Neupane et al. 2020; Rai et al. 2021). High genetic variability and heritability are essential for breeding efforts, as they facilitate the identification of desirable traits, contributing to improved yield, adaptability, and resilience (Lestari et al. 2020; Ferdous et al. 2023; Reddy & Pitha 2024).

Clustering analysis of the Bioguma × Gando Keta F₄ population. The clustering analysis based on all the morphology traits is shown in Figure 1. The 100 F₄ tested lines and two parent varieties were grouped into four clusters: C-1, C-2, C-3, and C-4. The female parent, Bioguma variety, was grouped in C-3, while the male parent Gando Keta, was in C-2, indicating that both parents had distinct or clearly different characteristics.

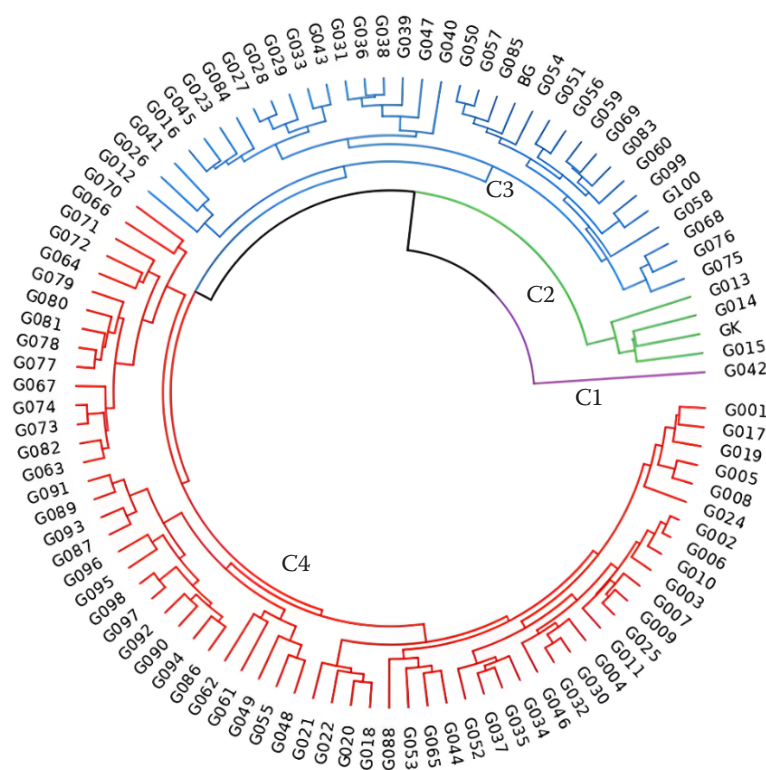


Figure 1. Dendrogram of the F₄ population based on the quantitative characteristics

BG – Bioguma; GK – Gando Keta; G001–G100 – F₄ progeny of Bioguma × Gando Keta cross; C1 – purple path; C2 – green path; C3 – blue path; C4 – red path; analysed and drawn with PBStat application for clustering (PBStat-CL; <https://apps.pbstat.com/reports/pbstat-cl/>)

This result is consistent with previous clustering based on nutritional traits of the Bioguma and Gando Keta lines, although, in that study, both parents were grouped in the same cluster (Lestari et al. 2025). Figure 1 shows, in this study, 62 lines were grouped in C-4, 35 in C-3, four lines were in C-2 and one line in C-1. Notably, G042 exhibited the greatest phenotypic divergence from both parents and the other progenies.

Clustering simplifies large datasets by grouping genotypes with similar traits, facilitating the interpretation of the relationship between the offspring

and parents and providing the genetic structure of the population (Gilbert 2016; Bedboudi et al. 2017).

The results showed that 62% of the F₄ population had a relatively different phenotype compared to the two parental comparison varieties. The selected lines were spread across all the clusters. The F₄ progeny line G042 had the most obvious difference compared to its two parents and the other tested progenies.

The appearance of desirable traits in cross-bred progenies is a key indicator of success in plant breeding programmes (Hao et al. 2021). In this study, the F₄ population exhibited a wide range of variation

Table 4. Mean value and genetic advance of 20 selected lines of the F₄ population based on the weighted index selection method

No.	Components		PH	PL	SD	PD	Brix	WPW	DPW	GW
			(cm)		(mm)		(°)		(g)	
Selected lines										
1	G012	2-5-1-12	238.67	22.37	22.19	64.62	13.60	146.60	108.37	80.79
2	G026	3-4-6	260.21	22.89	20.42	62.66	11.60	152.80	112.51	89.60
3	G036	3-6-4	282.73	21.47	20.53	55.65	15.40	140.57	109.54	89.41
4	G040	3-6-8	281.93	23.40	20.55	59.71	14.80	162.80	122.25	97.27
5	G041	3-6-9	243.00	23.54	20.17	62.72	13.20	170.68	122.07	97.49
6	G042	3-6-10	245.60	23.77	21.00	64.92	15.20	191.17	140.42	113.51
7	G047	3-8-4	282.47	20.14	20.39	56.95	14.40	138.62	117.28	93.90
8	G050	3-8-7	272.53	20.39	20.94	58.90	16.00	132.60	110.94	85.43
9	G051	5-2-1	275.60	20.45	22.15	58.55	15.80	118.50	103.16	82.77
10	G057	14-1-2	256.73	20.49	21.05	58.87	16.00	137.61	109.83	85.49
11	G059	14-1-4	269.47	19.66	20.83	62.39	16.00	131.68	105.52	83.40
12	G060	14-1-5	264.00	19.87	21.95	65.73	15.40	137.71	105.19	80.13
13	G066	14-1-11	269.80	19.71	22.59	66.91	18.80	123.00	103.85	79.97
14	G068	14-4-2-1	256.27	19.73	21.84	65.71	15.20	134.20	119.10	96.60
15	G069	14-4-2-2	262.67	19.50	20.25	64.11	15.40	117.50	103.70	83.38
16	G070	14-4-2-3	247.75	20.16	23.59	63.54	20.00	110.14	97.53	70.92
17	G074	14-4-2-7	256.93	18.99	21.00	61.57	19.60	121.30	103.56	81.13
18	G075	14-4-2-8	259.67	19.97	20.42	63.09	18.40	133.16	115.58	93.77
19	G076	14-4-2-9	257.67	19.48	22.11	63.45	17.60	138.32	120.21	94.80
20	G085	14-4-2-18	267.93	20.64	21.01	55.46	14.40	134.38	108.83	87.69
Selection mean ($\bar{x}_1$)			262.58	20.83	21.25	61.78	15.84	138.67	111.97	88.37
F ₅ mean ($\bar{x}_0$)			265.56	20.38	19.73	57.86	15.74	119.72	96.05	75.84
Differential selection (S)			2.98	0.45	1.52	3.91	0.10	18.95	15.92	12.53
Gain/cycle (G)			1.49	0.25	1.45	1.91	0.07	9.58	11.52	8.76
% Genotypic gain			0.56	1.23	7.34	3.30	0.46	8.00	11.99	11.55
F ₆ means prediction			264.07	20.63	21.17	59.77	15.81	129.30	107.57	84.60

PH – plant height; PL – panicle length; SD – stem diameter; PD – panicle diameter; Brix – Brix content; WPW – wet panicle weight; DPW – dry panicle weight; GW – grain weight

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in the yield components and a significantly higher mean for several traits compared to one of the parent check varieties, indicating the potential for further selection.

The genotype selection was conducted using a weighted selection index where the relative importance of each trait was determined based on the characteristic associations and breeder-defined ideotype preferences. The selection index weights were as follows: plant height = -1 ; stem diameter = $+3$; panicle length = $+1$; panicle diameter = $+3$; Brix = $+1$; wet panicle weight = $+3$; dry panicle weight = $+3$, and grain weight = $+5$. The characteristic weighting selection index depends on the parameters' heritability and economic value (Gazal et al. 2016). The grain weight receives the highest weight due to its importance as a primary target trait (Hadianto et al. 2023), which is influenced by the high heritability and genetic diversity.

The preferred plant ideotype is characterised by a short stature, sturdy stems, long and wide panicles, and high yields. Accordingly, the plant height was negatively weighted, while all the other traits received positive weights with the breeding goals.

The data in Table 4 show that grain weight in 20 selected F_4 lines ranged from 70.92 g/panicle (G070) to 113.51 g/panicle (G042), averaging 16.53% higher than the mean of the entire F_4 population (75.84 g). The wet and dry panicle weights increased by 15.83 and 16.57%, respectively, reaching 138.67 and 111.67 g in the selected lines. The panicle length and diameter also improved by 2.22 and 6.76%, respectively, compared to the overall F_4 average.

The selected lines showed a slightly higher average Brix content (15.84 units), 0.65% above the total F_4 population mean, indicating the potential for use in bioethanol and liquid sugar production. For the plant height and stem diameter, 20 selected lines recorded averages of 262.58 cm and 21.25 mm, with genetic gains of 0.56 and 7.34%, respectively. These results indicated that the selection achieved a genetic gain for the ideotype traits, particularly in the yield components, reflecting superior agronomic performance over the base population.

Selection based on a weighted index identified 10 out of 20 selected lines (50%) with the grain weight exceeding that of both parental varieties (Bioguma and Gando Keta). The combination of high selection differential and heritability led to substantial genetic progress, particularly in the grain weight and dry panicle weight. The mean grain weight of the selected

lines increased to 88.37 g, with reduced variability, as indicated by a lower standard deviation value compared to total the F_4 population. Overall, the selected lines outperformed the parents, supporting the effectiveness of an ideotype-based selection (Hadianto et al. 2023; Behera et al. 2024).

CONCLUSION

In this study, sorghum F_4 progenies exhibited superior yield components compared to the Gando Keta parent. This improvement was likely influenced by non-additive gene interactions. The grain weight variation was significantly determined by genetic factors. The application of a weighted index selection at the top 20% yield intensity effectively enhanced the key yield traits, including a higher grain weight, longer and wider panicles, and an increased stem diameter.

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