ASSESSMENT OF GENETIC PARAMETERS IN SEGREGATING POPULATIONS OF SORGHUM (*SORGHUM BICOLOR* L.)

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SUMMARY

The development of sorghum breeding must continue with an assessment of genetic parameters in developing sorghum lines. Hence, the presented study sought to estimate the genetic variation, heritability, and gain in the selected F3 populations obtained by crossing the sorghum parental genotypes 'Bioguma-1' and 'Gando keta.' The study transpired from June to October 2022 in the experimental field of Citayam Depok, Indonesia. Plant materials consisted of 160 lines from 18 selected genotypes in F2 using an augmented experimental design, with 'Bioguma-1' and 'Gando keta' as checks and parents. Based on performance, the selection of sorghum lines G01 (4-1), G02 (1-5), G03 (3-3), G04 (3-4), G05 (3-6), and G06 (3-8) proceeded for evaluation in the next generation for seed weight traits and similarity traits to cultivar Bioguma-1. The predicted genetic gain in the F4 populations will further enhance seed weight compared with the F3 populations and parental lines by 33.3% and, consequently, the superior sorghum genotypes for releasing as new cultivars with high yield components, broader stem and panicle diameter, and shorter plant.

Keywords: Bioguma-1, Gando keta, genetic gain, segregating populations, *Sorghum bicolor*

Key findings: The sorghum's promising F3 lines revealed wide genetic variations and high heritability for plant height, wet and dry panicle weights, and seed weight traits. Several F3 lines selected for further studies in the next generation were on seed weight traits and their similarity to the parental cultivar Bioguma-1. In F4 populations, the expected genetic gain will further enhance seed weight compared with the F3 populations and parental lines and release them as superior sorghum cultivars.

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is an essential grain crop grown for human consumption and as fodder for livestock worldwide (Prasad and Biradar, 2017; Ananda *et al*., 2020; Chadhary *et al*., 2020; Pinho *et al*., 2022). In developing countries, sorghum is a chief source of energy, minerals, proteins, vitamins, phytochemicals, phenolic, and gluten-free compounds that considerably benefit human health (Alina *et al*., 2017; Jafar *et al*., 2023).

Sorghum comprises a high phenotypic variation for various traits, eventually influencing grain yield quantitatively and qualitatively (Gichile, 2022). Therefore, obtaining more seeds with high nutrition and quality necessitates developing cultivars according to the needs of society and industry (Abdelhalim *et al*., 2019). Several sorghum cultivars intended for food succeeded in Indonesian release, including Numbu, Kawali, Bioguma, Mandau, Super, Samurai, Pahat, and Soper. However, the farming community and industries highly prefer the sorghum cultivar 'Bioguma-1' because it has the properties of a compact and large panicle size and huger white seeds (Lestari *et al*., 2019, 2023). The characteristics of the cultivar 'Bioguma-1' are pure white grains, and the flour is highly suitable for processed pastries and cakes. However, this cultivar is dense, making sorghum rice products still unacceptable among Indonesian people's taste. Therefore, genetic improvement of the cultivar Bioguma-1 must include the character of hard to fluffy seed texture.

Genetic improvement in sorghum can progress by crossing the potential parental cultivars under the well-planned hybridization program. Sorghum genetic resources are often immediately applicable to produce improved sorghum cultivars (Barmawi *et al*., 2013;

Ananda *et al*., 2020). One of the parental lines with fluffy and waxy potential is the Gando keta, the most widely used local cultivar of NTT with fluffy seed characteristics (Fitrahtunnisa *et al*., 2020). Crosses between the cultivars could produce a population that has high productivity with fluffy rice quality because character differences were quite different. The diverse traits of seed color and panicle shape may produce high diversity (Sutaryo, 2014). Such hybridization and crossing activities have progressed and reached the F3 generation. Therefore, evaluating segregating populations is crucial, considering the high heritability of the target character expected in further selection (Yunandra *et al*., 2017; Napitupulu and Damanhuri, 2018).

In F3 populations, the assessment can apply a quantitative genetic approach using the pedigree selection method of conventional breeding (Meriaty *et al*., 2021). This approach will further explore the genetic potential of segregating populations and improve selection efficiency. Genetic improvement for quantitative and qualitative traits depends on the nature and amount of genetic variability found in the genetic stock pool. If desirable traits have high heritability, then there are more chances of improvement through selection (Arifiana and Sjamsijah, 2017; Bhagasara *et al*., 2017). Information on the genetic variability chiefly resulted from the genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, and genetic gain for individual quantitative traits and through equilibrium distance over the parameters (Bhagasara *et al*., 2017). Therefore, genetic variability is a prerequisite for any breeding program seeking to improve yield-related traits (Prasad and Biradar, 2017). The relationship between yield and its components is also necessary for a breeder to ensure the superiority of direct and indirect selection (Prabha *et al*., 2022).

The F3 populations still experience considerable segregation, although not as large as the F2 populations, and selection in the F2 generation can continue evaluation in this generation. This advantage will show the potential for systematic genetic inheritance patterns from a crossbreed population for selection in obtaining potential F4 lines with high productivity and good pollen levels that can effectively progress. Therefore, the genetic evaluation of the progenies from crosses between Bioguma and Gando keta in the F3 populations must be precise. Based on the above discussion, the presented study sought to estimate the genetic variation, heritability, and gain in selected F3 populations obtained from crossing the sorghum parental cultivars Bioguma and Gando keta.

MATERIALS AND METHODS

The pertinent experiment ensued from June to October 2022 in the experimental field of Citayam Depok, Indonesia, with a latosol soil type (100 masl). The plant materials comprised 18 F3 sorghum lines of crossbreed Bioguma × Gando keta. Likewise, planting the two parental cultivars Bioguma and Gando occurred for their comparison. The augmented experimental design ensued with three replications. The concept analysis appears in Figure 1.
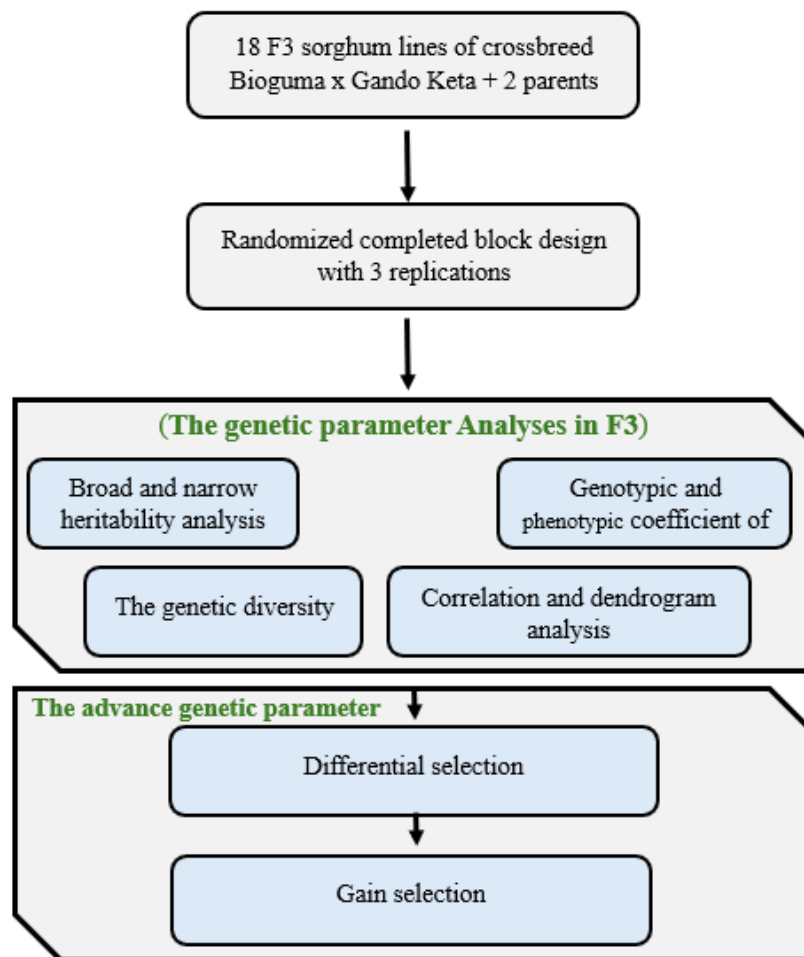

Figure 1. The flow process of this study.

Each planted F3 crossbreed line had as many as 160 plants, while 10 plants served as samples per experimental unit for the parental lines. The planting distance was 75 cm × 25 cm. Crop maintenance included fertilizing and pests and disease control. Carbofuran application at planting and spraying used deltamethrin. Fertilization transpired twice when the plant turned 15 and 42 days after planting (DAP). The fertilizer doses used were 150 kg/ha of Urea (N), 100 kg/ha of SP-36 (P), and 100 kg/ha of KCl. The Urea application was twice, the first half at 15 DAP, while the second half at 42 DAP.

Data recorded and statistical analysis

The variables observed in the sorghum F3 lines and their parental genotypes consisted of agronomic characteristics, including plant height (cm), juice brix content of sugar, stem width (mm), panicle length (cm), panicle width (cm), wet panicle weight (g), dry panicle weight (g), and seed weight (g). The data analysis included analysis of variance (ANOVA) to develop genetic variance components, correlation analysis, heritability (broad sense), genetic gain, and cluster analysis. Data analysis used Microsoft Excel 2010, SAS Version 9.0, Minitab 18.0, and PB-Stat CL 2.1.1 programs. The calculation of variance and heritability components employed the following stages of analysis.

$$H^2 = \frac{\sigma^2 G}{\sigma^2 P} \times 100\%$$

Where H^2(%) = Heritability (broad sense) based on a single plant base method, $\sigma^2 p$ = phenotypic variance (variance of all individuals in the F3 population), and $\sigma^2 g$ = Genetic variance. However, in general, determining genetic variation cannot be directly known; thus, determining environmental variation is critical for identifying phenotypic variation. Meanwhile, the formula for various environments is:

$$\sigma^2 e = \frac{\sigma^2 P1 + \sigma^2 P2}{2}$$

Where $\sigma^2 e$ = Environmental variance based on the average method of variance of the two cultivars, P1 = Female parental genotype, and P2 = Male parental genotype. The formula result could count the genetic variance:

$$\Sigma^2 g = \sigma^2 p - \sigma^2 e$$

The categorization of the obtained heritability comprised three classifications, namely, low (0 < H^2 < 20%), moderate (20 ≤ H^2 < 50%), and high (50 ≤ H^2). In addition to heritability, the genetic variants obtained could identify genetic diversity, the genotypic coefficient of variation (GCV), and the phenotypic coefficient of variation (PCV). Calculating genetic diversity applied the method according to Asghar and Mehdi (2010). The criteria of genetic diversity depended on the standards with the following terms:

$\sigma^2 g > 2\sigma(\sigma^2 g)$ = Wide genetic variation

$\sigma^2 g < 2\sigma(\sigma^2 g)$ = Narrow genetic variation

Computing for GCV and PCV could follow the formula:

$$GCV = \frac{\sqrt{\sigma^2 g}}{\bar{X}} \; ; \; PCV = \frac{\sqrt{\sigma^2 p}}{\bar{X}}$$

Where $\bar{X}$ is the population mean. Meanwhile, the expected genetic gain from phenotypic selection (Rutkoski, 2019) used the formula below:

$$R = kh^2 \sigma_p$$

Where
R is the genetic gain,
H^2 is the narrow- or broad-sense heritability,
σ_p is the phenotypic standard deviation, and
k is the selection intensity.

RESULTS AND DISCUSSION

Variance analysis, heritability, and correlation

The traits of plant height, wet and dry panicle weight, and seed weight revealed a wide range

Table 1. Variance components and heritability according to the mean basis for the third population.

No.	Character	σ^2 p	σ^2 e	σ^2 g	2 σ σ^2g	GCV (%)	PCV (%)
1	Plant height	1376.91	886.44	490.47[W]	44.29	8.52	14.28
2	Juice brix content	11.17	4.28	6.89[N]	5.25	25.76	32.80
3	Stem width	5.13	1.95	3.18[N]	3.57	8.86	11.25
4	Panicle length	13.81	10.59	3.22[N]	3.59	7.58	15.69
5	Panicle width	0.49	0.15	0.34[N]	1.17	9.27	11.13
6	Wet panicle weight	1575.84	1061.42	514.42[W]	45.36	16.96	29.69
7	Dry panicle weight	725.34	448.86	276.48[W]	33.26	15.21	24.63
8	Seed weight	458.52	244.44	214.08[W]	29.26	17.14	25.09

Notes: H) High heritability; M) Medium heritability; L) Low heritability; W) Wide genetic variability; N) Narrow genetic variability; h²bs) Heritability of broad sense (%); σ^2e) Environmental variance; σ^2p) phenotype variance; σ^2g) genetic variance, 2 σ σ^2g) Broad-narrow criteria for genetic variance; GCV) Genotipic Coefficient of Variance (%); PCV) Phenotypic Coefficient of Variance (%).

Table 2. Heritability value (broad sense and narrow sense heritability) in the third segregated population.

No.	Character Observation	Broad sense heritability		Narrow sense heritability	
		h² bs *indirect estimate of* ve (%)	h² bs *Plot basis* (%)	h²ns *midparental lines-offspring* (%)	h² ns *Realized* (%)
1	Plant height	83.60[H]	35.62[M]	23.11[M]	87.34
2	Juice brix content	2.82[L]	61.68[H]	5.58[L]	0
3	Stem width	-5.73[(a)]	61.99[H]	20.06[M]	36.41
4	Panicle length	65.35[H]	23.32[M]	0.97[L]	0
5	Panicle width	53.06[H]	69.39[H]	13.26[L]	28.33
6	Wet panicle weight	10.90[L]	32.64[M]	22.86[M]	65.31
7	Dry panicle weight	-20.56[(a)]	38.12[M]	78.16[T]	52.89
8	Seed weight	-50.42[(a)]	46.69[M]	55.55[H]	48.15

Notes: H) High heritability; M) Medium heritability; L) Low heritability; W) Wide genetic diversity; N) Narrow genetic diversity; h² bs) Heritability of broad sense (%); (a) Values of genetic variance and heritability less than 0 are considered σ^2 g = 0 and h² bs = 0%.

of genetic variance based on sorghum F3 populations (Table 1). The wide genetic variability means that genetic factors have significantly influenced these traits. However, the traits juice brix content, stem width, panicle length, and panicle width owned a narrow genetic variability, which means that the variation of these characteristics in the F3 populations was low. These attributes also acquired less environmental effects, as illustrated in the phenotypes and environment. The genetic and phenotype coefficient of variations values were higher for the juice brix content than the stem and panicle width. In breeding for high yield, the genetic variability, heritability, and genetic gain information of the populations are necessary to determine breeding success (Asadi, 2016; Kristamtimi *et al.*, 2016; Prajapati *et al.*, 2021). Therefore, genetic information is crucial for determining the best selection method to obtain the genotypes with the desired traits (Maftuchah *et al.*, 2022; Trikoesoemaningtyas *et al.*, 2024). Information on genetic variability helps the breeder choose the desirable parental genotypes for the breeding program and gene introgression from distantly related genotypes (Prasad and Biradar, 2017; Chadhary *et al.*, 2020).

The heritability (broad sense) analysis based on indirect estimates showed that plant height, panicle length, and panicle width have higher heritability values, meaning that genetic factors considerably influenced these traits versus environmental factors (Table 2). Heritability values that were negative or zero

for stem width, dry panicle weight, and seed weight were due to the values of environmental variance, represented by the variance of parental lines, which was higher than the phenotypic variance of the F3 population. This result aligns with research conducted by Insan *et al*. (2016), Trikoesoemaningtyas *et al*. (2017), and Gebregergs and Mekbib (2020), where the heritability values were high for plant height, panicle length, and width characteristics, while low for the stem width based on the crossbreed populations. In general, heritability analysis helps to estimate the proportion of phenotypic variation in a trait that can refer to genetic factors and supports understanding genetic factors influence a specific trait within a population (Sabiel *et al*., 2016; Jimmy *et al.*, 2017). Higher heritability is also necessary to ensure the proper inheritance of target traits in crop plants (Jafar *et al*., 2023). The heritability value primarily resulted from parental lines' genetic distance, the number of populations, and the test environment conditions. Therefore, the traits with a higher heritability can considerably determine the selection criteria because genetic factors are more influential than environmental ones (Birhan *et al*., 2020).

The narrow sense heritability analysis also revealed that plant height, wet and dry panicle weights, and seed weight have medium to high broad and narrow sense heritability. Narrow sense heritability was near the potential for a pattern of a series of genes for additive inheritance from one generation to the next. The higher the narrow sense heritability value, the greater the effectiveness of selecting a character. The reason is an additive guarantee exists that the trait potential of a genotype will remain stable and come out in the next generation. Therefore, based on the genetic variability and narrow sense heritability values, one can conclude that selection traits for this population were plant height, wet and dry panicle weights, and seed weight. For the next generation, selection targeting can be more for these four traits and the traits that considerably correlate with these four variables. Several studies that worked on the variability and heritability of sorghum have found that plant height and seed weight are qualities with high variation and heritability in crossed and non-crossed populations (Elangovan *et al*., 2014; Maftuchah *et al*., 2021). A different result came from Rini *et al*. (2017), who found that all these features have low variability and heritability for narrow and broad sense.

A correlation analysis helped study the relationships that can continue to determine the correlation between the traits (Table 3). It helps to understand the strength and direction of the association between traits, and the coefficient ranges from -1 to +1. Values closer to +1 or -1 suggest a stronger relationship, while values closer to 0 indicate a weaker or no relationship. A positive correlation showed a relationship between characteristics, where an increase in the trait will incur an increase in the

Table 3. Correlation values between observed characteristics in F_3 (Bioguma × Gando keta) populations.

Character	Plant height	Brix	Stem width	Panicle length	Panicle width	Wet panicle weight	Dry panicle weight
Juice brix content	0.626**						
Stem width	-0.183**	-0.435**					
Panicle length	-0.355**	0.234ns	-0.447**				
Panicle width	-0.181ns	-0.223ns	0.279*	-0.183tn			
Wet panicle weight	-0.081ns	-0.275*	0.594**	-0.393**	0.703**		
Dry panicle weight	0.006 ns	-0.218ns	0.643**	-0.500**	0.693**	0.963**	
Seed weight	0.181 ns	-0.024ns	0.476**	-0.436**	0.638**	0.905**	0.944**

Notes: ns - is insignificant based on Pearson correlation at α = 0.05 level; *) Significant effect based on Pearson correlation at α = 0.05 level; **) Significant effect based on Pearson correlation at α = 0.01 level.

secondary feature correlated with it. However, a negative correlation shows that an increase in one characteristic will bear a decrease in the secondary characteristic negatively correlated. Previous research discovered that seeds per panicle appeared positively connected with various yield-related traits, except plant height and flowering age (Arunkumar, 2013). In agreement with the latest results, seed weight selection tended to increase the stem width, panicle width, and wet and dry panicle weight. However, seed weight selection tends to reduce the panicle length and brix degree (Pugahendhi *et al.*, 2023). Based on these results, morphological characteristics, such as stem and panicle width, can become secondary selection traits or morphological markers in the field to improve seed-related traits.

Selection response and genetic gain in F4 populations

The results enunciated that the mean values and the selection gain in F3 populations showed an increase for all the traits. The plant height, wet and dry panicle weights, and seed weight were characteristics with a wide average range. The mean value of seed weight rose by 133.93%, which could be a selection trait because of the higher range of F3 values. Selection in F2 populations succeeded in the average seed weight increase of the F3 populations. In F3 populations, the rise in the mean value of stem width was about 16.57%, panicle length (17.67%), panicle width

(0.48%), wet panicle weight (89.08%), and dry panicle weight (84.95%) (Table 4).

The curve probability plot showed the normal distribution of the seed weight traits, with a P-value = 0.08 or Pr > 0.05 (Figures 2a and b). The sorghum lines, i.e., G01 (1-4), G02 (1-5), G03 (3-3), G04 (3-4), G05 (3-6), and G08 (3-8) were choices because of their higher seed weight than the total F3 population means (Table 5). There was a difference of about 13.96 and 23.79 g between the average weight values of selected F3 lines against all other F3 populations and the parental cultivars, respectively. The selected sorghum lines had significantly lower plant height and higher stem width, panicle width, and yield components than the cultivar Gando keta. Selection lines reduced the seed weight diversity due to a decline in standard deviation values compared with the parental cultivars and other F3 populations. The total average of selected lines for plant height, brix degree, and panicle length were lower than the parental cultivars and F3 populations. The performance of the chosen lines has the criteria of high yield components, broader stem and panicle widths, and short stature plants, and therefore, had the potential to be more resistant to lodging. Gomez *et al*. (2017) found lodging-susceptible genotypes had greater internode volume and stiffness, while lodging-resistant sorghum genotypes were shorter and had a lower stem slenderness ratio. Another study found that internode length influenced plant height, as affected by the action of the *dw1* gene (Yamaguchi *et al*., 2016).

Table 4. Second and third population mean values, the interval between generations, and the selection gains.

Character	F_2** Mean	F_2**Interval	F_3 Mean	F_3 Interval	Selection Gain
Plant height (cm)	176.70	126.0 - 256.0	258.45	117.8 - 355.0	-81.75
Stem width (mm)	17.44	11.8 - 23.4	20.33	13.0 - 29.3	2.89
Panicle length (cm)	19.69	5.8 - 27.0	23.17	7.7 - 50.9	3.48
Panicle width (mm)	6.28	3.5 - 10.5	6.31	2.8 - 9.7	0.03
Wet panicle weight (g)	71.53	20.7 - 151.7	135.25	55.4 - 282.5	63.72
Dry panicle weight (g)	60.01	20.7 - 122.4	110.99	7.6 - 237.3	50.98
Seed weight (g)	36.90	0.3 - 90.0	86.32	8.1 – 141	49.42

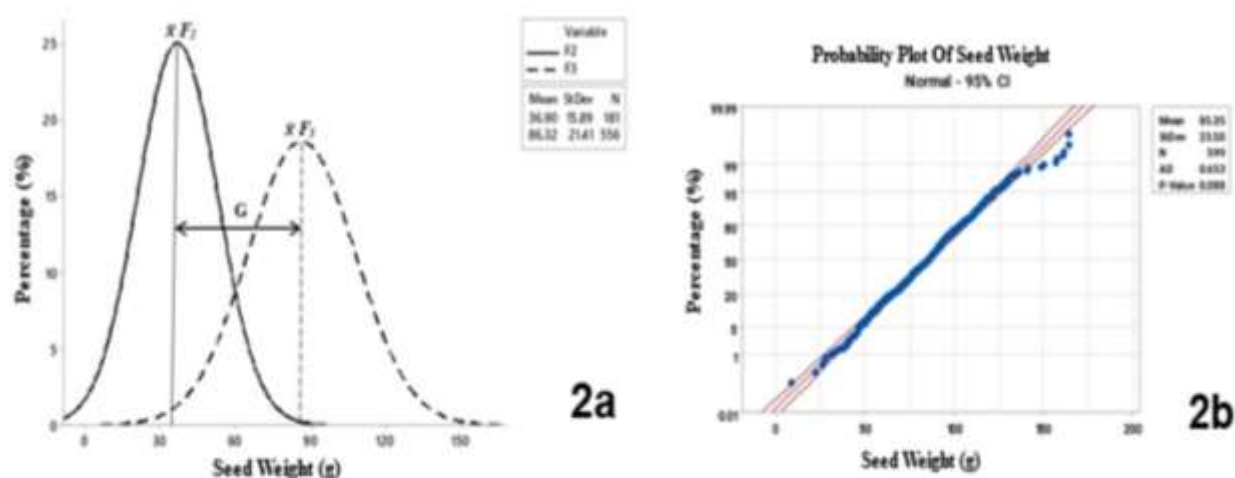Note: ** cited from Lestari *et al*. (2023).

Figure 2. (a) Data Distribution of F2 and F3 for seed weight characteristics, **(b)** Probability curve of seed weight, G= gain selection.

Table 5. Mean values of seed weight characteristic of the selected lines in the F3 population.

Selected Lines	PH	Brix	SW	PL	PW	WPW	DPW	SD
G01 (1-4)	264.2	9	2.09	22.54	6.51	168.94	129.66	99.43
G02 (1-5)	260.24	9.78	2.01	21.56	6.2	148.8	118.22	94.05
G03 (3-3)	255.27	10.33	2.03	21.9	6.26	154.37	119.31	95.78
G04 (3-4)	256.14	10.18	1.91	21.68	6.2	159.2	125.63	101.95
G05 (3-6)	251.06	9.53	2.05	22.3	7.5	193.14	141.87	109.74
G06 (3-8)	220.05	7.01	2.24	22.84	7.05	189.11	146	100.85
Average total	251.16	9.3	2.06	22.14	6.62	168.93	130.11	100.3
The average of F_3 population lines	258.49	9.98	2.03	23.17	6.31	135.28	110.84	86.34
Average parental lines	273.09	12.12	1.83	28.33	6.16	119.71	94.35	76.51

Notes: PH=Plant Height; Brix= brix degree; SD= Stem Width; PL= Panicle Length; PW= Panicle Width; WPW= Wet Panicle Weight; DPW= Dry Panicle Weight; SW= Seed Weight.

The percentage of response selection varied from -4.47% to 23.39% (Table 6). Plant height has a positive genetic gain percentage even though the differential selection value was negative because of its negative direction. The potential genetic gain of the yield component characteristics was more than 10%, which revealed that seed weight as a selection target characteristic could increase the values of yield components, with the same also supported by a robust correlation coefficient value (r > 0.80). However, the chosen F3 lines reduced the panicle length and brix degree. Prediction of mean seed weight value for F4 populations increased by 26.82% and 12.38% compared with the parental cultivars and the F3 populations, successively, until the seed weight of the F4 populations can be superior (Figure 3).

Table 6. Potential genetic gain and predicted mean value of F4 population based on the method, *single plant basis heritability*.

No.	Characteristics	xi	x0	S	h² bs	G	G (%)	BeforexF4
1	Plant height (cm)	251.16	258.49	-7.33	0.97	7.12	2.75	251.37
2	Juice Brix content (°)	9.30	9.98	-0.67	0.66	-0.45	-4.47	9.53
3	Stem width (cm)	2.06	2.03	0.02	0.80	0.02	0.89	2.05
4	Panicle length (cm)	22.14	23.17	-1.03	0.97	-1.00	-4.32	22.17
5	Panicle width (mm)	6.62	6.31	0.31	0.51	0.16	2.56	6.47
6	Wet panicle weight (g)	168.93	135.28	33.65	0.94	31.64	23.39	166.92
7	Dry panicle weight (g)	130.11	110.84	19.27	0.84	16.12	14.54	126.96
8	Seed weight (g	100.30	86.34	13.96	0.77	10.69	12.38	97.03

Notes: *xi*) The mean value of the population resulting from selection; *x0*) Mean value of F3 population lines; S) Differential selection; h²bs) Heritability of broad sense; Ĝ) Estimation of selection gain; PredxF4) Prediction of the mean value of the F4 population.
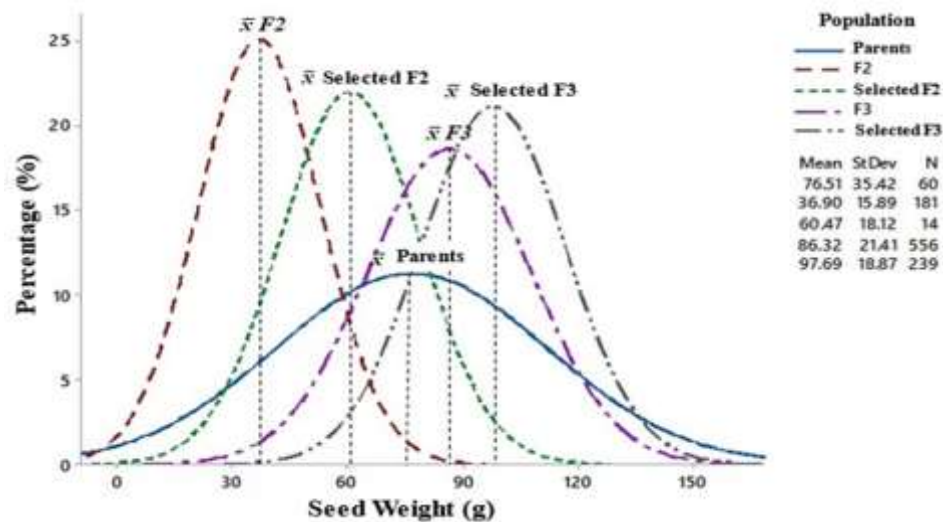
Figure 3. Normal distribution curve between the F2 populations, selected F2 populations, F3 populations, selected F3 populations, and parental lines based on seed weight data.

The population genetic gain for seed weight in each generation and the selected generation shifted positively (Figure 3). The parental cultivars' average seed weight was lower (±75 g) than F3 populations (±90 g), increasing by about 20%. The average weight of selected F3 populations was higher than all other populations (±100 g), gaining about a 33.3% rise. Therefore, one can conclude that the F4 populations will gain higher seed weight for the following future selection. The heritability of a target character mainly determines genetic gain (Xu *et al*., 2017). In the study results, the seed weight served as a target feature, resulting in positive selection progress. Although the increase in seed weight was moderate compared with the seed weight of the parental cultivar Bioguma; however, this addition is the most important because variations in the positively correlated traits, such as broader stem diameter and shorter plants, immediately followed; in the future, the genotypes will have to be more resistant to lodging, strong winds, and easier to harvest (Gardiner *et al*., 2016; Wang *et al*., 2020). The predicted seed weight of the F4 populations was around 100 g per panicle, which means a 33.3% increase from the average seed weight of its parental lines (Figure 3).

CONCLUSIONS

The results revealed that the traits of plant height, wet and dry panicle weights, and seed weight owned the broad genetic variation and high heritability. The six sorghum lines, G01 (4-1), G02 (1-5), G03 (3-3), G04 (3-4), G05 (3-6), and G06 (3-8), were choices for further studies in the F4 generation based on seed weight traits and quantitative characteristics similar to parental cultivar Bioguma. The selection based on the seed weight trait showed that the F3 populations had been options for high-yield components, broader stem and panicle diameter, and shorter plants, with the potential to be resistant to lodging and faster to harvest. The predicted selection gain in the F4 populations will enhance the seed weight compared with the F3 populations and parental cultivars and could get some superior sorghum genotypes.

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